Eurocat

European Surveillance of Congenital Anomalies

Supported by the EU-Commission Public Health Directorate Programme of Community Action on Rare Diseases
WHO Collaborating Centre for the Epidemiology Surveillance of Congenital Anomalies

Special Report:

Special Report:

A Review of Environmental Risk Factors for Congenital Anomalies

Edition 1
(uploaded to website 29 April 2004)

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ISBN 1 85923 187 X

ACKNOWLEDGEMENTS

This review of the literature has been supported to date by the DH/DETR/Environment Agency Joint Research Programme on the Possible Health Effects of Landfill Sites Project LSHTM/00/1 and by the European Commission Rare Diseases Programme.

NOTES TO THE USE OF THIS REVIEW

This literature review will be expanded and updated over time. When quoting the review, please quote as follows e.g. Little J (2002) Smoking. In: EUROCAT Special Report. The environmental causes of congenital anomalies: a review of the literature. [online] www.eurocat.ulster.ac.uk/pubdata [accessed date].

It is not the aim of this review to be systematic in all parts, but to be an informative starting point for access to the literature, and for understanding what factors may underlie variations in prevalence of congenital anomalies in time or space.

We set the evidence regarding congenital anomalies in the context of evidence related to other pregnancy outcomes, mainly spontaneous abortion (miscarriage), low birthweight and prematurity. Our review in relation to those outcomes is not exhaustive, and relates only to those risk factors for which there is evidence regarding the risk of congenital anomalies. We mention research on neurodevelopmental outcomes in relation to specific prenatal chemical exposures, but do not touch on wider areas such as neurodevelopmental outcomes in relation to nutrition.

This review is mainly concerned with epidemiologic studies. The assessment of environmental causes of congenital anomalies also draws on toxicological data, data from animal studies, and detailed exposure data. A (future) chapter will discuss these sources of evidence, but we do not review them systematically, nor do we attempt risk assessments for any of the environmental exposures reviewed.

FURTHER RESOURCES

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For information on the genetics of congenital anomalies, refer to www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

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Overview of Principles & Methods in Identifying the Causes of Congenital Anomalies

CHAPTER I.1 GENETIC CAUSES OF CONGENITAL ANOMALIES AND THEIR INTERACTION WITH ENVIRONMENTAL FACTORS

Mary Seller, January 2004

1. Congenital anomalies

The term 'congenital anomaly' is used for all types of structural defects with which a baby can be born. Overall, these abnormalities can be classified broadly into malformations, malformation syndromes, disruptions and deformations, which provides some insight into possible underlying aetiology.

A malformation is a localised error of normal development, a primary structural defect occurring during the morphogenesis of an organ or tissue. Examples are isolated cleft lip, cleft palate, spina bifida or a ventricular septal defect in the heart. Such an isolated malformation occurs in an otherwise normal child. Single malformations are relatively common and usually have a multifactorial aetiology: that is, both genes and environment are important (see later).

A malformation syndrome is the occurrence together in one individual of several different malformations as primary events arising from the same underlying cause. There is a specific recognisable pattern of abnormalities according to each causative factor. The cause is usually a single gene mutation or a chromosome abnormality, or occasionally an environmental agent.

A disruption is where there is a major destruction or alteration of a body part that had previously formed, or which had the intrinsic potential to form, quite normally. An example is a missing limb, or part thereof. Usually the causes of disruptions are not genetic, but environmental, such as drugs, maternal illness or infections, or strands of amnion becoming detached and encircling a limb or digit.

A deformation is an alteration of shape or structure imposed on a body part after, or during, its normal formation, usually by mechanical forces. The cause may be external or internal to the fetal body. For example talipes (club foot) can be produced by an external factor, oligohydramnios (lack of amniotic fluid), or be secondary to open spina bifida, an intrinsic cause, where damage to the spinal cord causes paralysis of leg muscles and then positional abnormalities of the foot. Thus, deformations may have a genetic or part genetic cause, or an environmental one. But as often happens, the situation may not be

clearly categorised, such as when the oligohydramnios producing the talipes is secondary to renal agenesis in the fetus, a multifactorial condition.

It is generally estimated that around 14% of babies are born with a single minor malformation, for example, a skin tag that can easily be removed, and is of little consequence. However, around 2-3% of neonates have a single major malformation like spina bifida that will require more extensive medical treatment, or perhaps even be lethal. Just under 1% of neonates have multiple malformations. These are the prevalence figures, the number observed at birth. However, the occurrence of malformations is much higher, but many affected pregnancies are aborted spontaneously, especially during the first trimester of pregnancy.

The precise cause of congenital malformations is not known for as many as 50-60% of the total. It is believed that overall, multifactorial aetiology accounts for 20-25% of all abnormalities; 6-8% are monogenic, that is, caused by mutations in a single gene; 6-8% by chromosome abnormalities; and 6-8% by environmental factors such as maternal illness, infections, drugs, radiation and alcohol.

2. Monogenic disorders

In monogenic disorders, a mutation in a single gene results in malformations, usually a spectrum of abnormalities: eg Meckel syndrome, in which there is encephalocoele, polydactyly and polycystic kidneys. All known diseases resulting from single gene mutations are listed in OMIM (Online Mendelian Inheritance in Man at http://www3.ncbi.nlm.nih.gov/Omim/searchomim.html). Such disorders are inherited, and the mode of inheritance may be autosomal dominant, autosomal recessive or sex-linked. Within families, each type of inheritance has a characteristic pedigree (family tree).

In autosomal dominant inheritance, manifestation of the disease occurs in heterozygotes when only one copy of the disease gene is present. An affected individual usually has an affected parent, and has a 50% chance of passing the gene on to each of his/her offspring. The pedigree thus shows affected individuals in each generation, there is a ‘family history’, and both males and females are equally likely to be affected.

Autosomal recessive conditions manifest only when an individual has two copies of the mutant gene. One is received from each parent who are both carriers, but completely unaffected, for one copy of the abnormal gene in this case is not sufficient to cause the disease. Usually there is no family history, an affected individual occurs solely in one generation, although there may be more than one case within a single sibship. An exception occurs when there is consanguinity in the family.

In sex-linked inheritance, the disease causing mutation is carried on one of the sex chromosomes. In the most common form, X-linked recessive inheritance, males only are affected because they have only one X chromosome. Their daughters have a 50% chance of receiving the mutant gene from them, but if they do so, they are unaffected as they have two X chromosomes. However, they are carriers and have a 50% chance of passing the defective gene on to their own sons who, if they receive it, will be affected. Once more, there is a family history, but the pattern is different from that of autosomal dominant inheritance. Males alone are affected, comprising affected grandfathers and grandsons with the intermediate generation unaffected, and there is no male-to-male transmission of the disease.

In dominant conditions, the genetic background might influence the phenotype (the clinical features), giving variable expressivity between different affected individuals or causing incomplete penetrance of the gene. Variable expressivity is precisely what its name suggests. An example occurs in neurofibromatosis Type I, in which the clinical manifestation may involve hundreds of cutaneous neurofibromata, that can be extremely disfiguring, or just a few, which may be hardly noticeable. In incomplete penetrance, an individual carries the mutant gene but is completely devoid of any clinical symptoms. Both these phenomena may be explained by specific 'modifier' genes in the genome, the products of which influence either the expression of, or the product of, the mutant gene, or more non-specifically by the products of many other genes in the genome.

In a few dominant conditions, there are instances where an affected individual does not have an affected parent. One way this can occur is when new mutations arise in the gene relatively frequently. This is found in achondroplasia, so that often the affected individual has arisen as a new mutation of the gene in one of the parental germ cells. Another way is when there is germ-line mosaicism in one parent, in which a proportion of the cells of the gonad bear the mutation while the rest do not.

Occasionally, a specific environmental factors is required for the mutant gene to be manifest. An example is the gene that causes malignant hyperthermia. The mutation is manifest only if the individual undergoes halothene anaesthesia with succinylcholine as the muscle relaxant, when muscle rigidity and hyperthermia occur that can be lethal. But under normal circumstances, and with other types of anaesthesia, the individual is unaffected and blissfully unaware that they carry that particular mutation.

3. Molecular pathogenesis in single gene malformation syndromes

As the genes that cause specific malformation syndromes are identified, and the molecular structure of their protein product is determined, so we are beginning to understand the molecular pathogenesis of congenital malformations which underlies the developmental pathogenesis. A few examples only can be given. An interesting point emerging, which was totally unexpected, is that sometimes, different mutations in the same gene cause different syndromes. This is allelic heterogeneity. Three distinct skeletal dysplasias arise from a mutation in the fibroblast growth factor receptor-3 (FGFR-3) gene. They are achondroplasia (marked limb shortening and relatively large head), thanatophoric dysplasia (severe limb shortening, small chest, lethal at birth) and hypochondroplasia (less severe limb shortening). A different domain of FGFR-3 is affected in each case. In achondroplasia, the mutation affects the transmembrane domain (Rousseau et al, 1994); in thanatophoric dysplasia, there is an amino acid substitution in the link between the second and third immunoglobulin-like extracellular domains (Tavotmina et al, 1995), and in hypochondroplasia, the mutation affects the intracellular proximal tyrosine kinase domain (Bellus et al, 1995).

Mutations in another of the fibroblast growth factor receptor genes, the FGFR-2 gene, does not produce a skeletal dysplasia but either, Crouzon syndrome (Reardon and Winter, 1996), a craniosynostosis with normal stature, or, Apert syndrome (Wilkie et al, 1996), a craniosynostosis with severe syndactyly of the hands and feet. There are a number of fibroblast growth factor receptors known and they are ligands for fibroblast growth factors, of which again, there are several. They function at a very early stage of embryogenesis and are involved in cell growth, differentiation, spatial patterning and programmed cell death.

Another example of allelic heterogeneity involves the GLI3 gene, a zinc finger transcription factor. Greig cephalopolysyndactyly, where there is a high forehead with frontal bossing, macrocephaly, broad thumbs and postaxial polydactyly and syndactyly of the hands as well as preaxial polydactyly of the feet, is produced by large deletions in the GLI3 gene (Vortkamp et al, 1991), while smaller frameshift mutations in this gene produce Pallister Hall syndrome (Kang et al, 1997), which has central polydactyly and hamartoma, ear abnormalities, macrognathia and anal defects.

Holoprosencephaly (failure of division of the cerebral hemispheres) is a malformation that can be produced by mutations in a number of different genes. This is locus heterogeneity. The first gene to be discovered in humans was the sonic hedgehog gene (Roessler et al, 1996). The secreted product of this gene is a signalling molecule that affects ventral patterning of the early embryo (Echelard et al, 1993), being expressed in the notochord, the floorplate of the neural tube, and ventral midline pre-somitic mesoderm (Chiang et al, 1996). The sonic hedgehog protein promotes the expression of other

developmental genes. The membrane receptors named 'patched' and 'smoothened' exist as a complex whereby normally, patched prevents smoothened from being expressed. But when the sonic hedgehog protein binds to patched, this repression is released, allowing smoothened to signal, resulting in Gli protein function (Ingham, 1998). Other genes which, when mutated, cause holoprosencephaly, include SIX3, a homeobox containing transcription factor (Wallis et al, 1999), and TGIF, a transcription factor that competitively inhibits the binding of the retinoic acid receptor to a retinoid-responsive promoter (Bertolino et al, 1995).

In many instances, the fact that multiple abnormalities affecting several different body parts occur when there is only one mutation in a single gene, a phenomenon referred to as 'pleiotropy' and hitherto enigmatic, can now be explained by our contemporary molecular knowledge. The genes concerned are those involved in early developmental processes and their control. The expression of these genes is seldom restricted either temporally or spatially and these genes are usually expressed several times over at different times and in different organs.

4. Mutations

A mutation is a change in the DNA. Throughout life, mutations are constantly occurring in the DNA of both somatic and germ-line cells as a result of factors arising both internally or externally. Every time a cell divides, DNA replication has to precede it and this process is prone to error. Also, we are often exposed, usually unknowingly, to environmental agents, such as radiation and chemical agents that can damage the DNA. Most of the errors and some of the damage are detected and corrected by an intrinsic cellular DNA repair mechanism. But those faults that escape can have far-reaching effects. If they are in germ-line cells, then their consequences can extend even further, for they may be inherited.

One far-reaching effect of mutations in somatic cells, arising spontaneously or as a result of environmental insults, is that they may accumulate during life, and eventually cause cancer. The mutations often affect the tumour suppressor genes and oncogenes that are involved in the normal control and regulation of cell division and cell proliferation. Chromosome breakage has long been known to be associated with tumour cells and early studies of some of these led to the identification of some of the cancer causing genes; genes involved in normal cell function were disrupted by the chromosome breakage.

Mutations can occur anywhere in the entire genome, but since only 3% of our DNA comprises protein-coding regions, that is, genes, a mutation is usually only of consequence to the individual when it occurs in these stretches of DNA or in the associated gene regulatory and controlling elements. The malformation syndromes mentioned above have arisen in this way and are said to be the result of 'spontaneous mutations' because the cause of the mutation is unknown.

Mutations may involve a single purine or pyrimidine base change in the DNA, or the deletion or addition of a few or many bases. While occurring throughout the genome, mutations do not happen uniformly, certain areas, such as those rich in CG dinucleotide sequences and known as 'hotspots', are more prone than others. Large genes are more likely to have mutations than smaller genes. Also, mutation rates vary with age, and there is a paternal age effect in some dominant disorders. The risk of a father producing a child with achondroplasia or Marfan's syndrome or Apert's syndrome is markedly increased after the age of 40 years. This is explained by the fact that the progenitor cells of the sperm divide continuously during life, and DNA replication errors accumulate with advancing age. This contrasts with the situation in females where cell division in the egg is suspended during fetal life and is not resumed until ovulation. In females, increase in maternal age is associated with the other major class of mutation where there are observable numerical changes to the chromosomes, as distinct from minute changes in the DNA at the molecular level (see below). The addition or the loss of a whole chromosome, aneuploidy, is the result of the lack of separation of the two chromatids at mitosis. Such non-disjunction seems to happen more commonly the older a woman is, implying that the longer cell division is suspended, the more likely the disjunction process is to be faulty.

Radiation and an uncertain number of chemicals can cause mutations. Ionising radiation from either nuclear fallout or X-rays can break the bonds between the double stranded DNA and also alter the bases within the strands. In addition, they can cause chromosome breakage. The degree of genetic damage is directly related to the amount of exposure and the effects are cumulative. This type of radiation penetrates deep into the body tissues and can reach the gonads, so potentially, both somatic and germ-line mutations can occur. There is evidence on both counts. Survivors of the Japanese atomic bombings and also of the explosion at the Chernobyl nuclear power plant, developed cancers, showing that the expected somatic mutations occur. Dubrova et al (2002) used minisatellites for mutation detection and studied Ukrainian people exposed to the Chernobyl fallout. They found a near twofold increase in the rate of mutations being passed on by exposed males to their offspring, providing evidence that the germ-line is also affected.

Non-ionising UV radiation does not penetrate the body further than the skin. It causes the formation of covalent bonds between neighbouring pyrimidine bases, so that when the DNA replicates these pyrimidine dimers are unable to pair properly with purines. The ultimate result is skin cancer.

As previously mentioned, errors in DNA replication constantly occur naturally, and the body has an innate and complex DNA repair system that rectifies these mistakes and minor environmental damage. Mutations in the genes encoding the enzymes involved in this system can have severe effects and render individuals hugely sensitive to radiation, and even to normal exposure to daylight. Xeroderma pigmentosum is one of several disorders arising in this way, where skin damage and cancers occur in childhood, and neurological impairment.

An enormous number of chemicals, including even commonly used substances like caffeine, are mutagenic in experimental animals. It is tempting to extrapolate this information to humans, but there is good evidence for some chemicals. Many of these substances alter the DNA helical structure or component bases, or interfere with DNA replication. Somatic cells are primarily affected. If a chemically induced mutation on a germ cell does have a deleterious effect on the embryo, it usually leads to death at an early cleavage stage. But exogenous chemicals and drugs may not necessarily cause mutations. Instead, they may directly or indirectly affect the embryo during development and produce congenital malformations (see below). In this case they are called 'teratogens'. They influence crucial developmental processes in a variety of ways.

In addition to innate DNA repair systems, the body also has some complex systems of enzymes that metabolise and detoxify certain environmental chemicals and drugs when they enter the body. A mutation in any of the genes encoding these enzymes, for instance in the family of P450 genes, can lead to increased susceptibility to diseases and cancers. In addition, many of these genes are polymorphic, the various alleles producing different isozymes that have different properties. These allelic variants are responsible for the variation within the population in the response and reaction to individual drugs and chemicals.

5. Chromosome disorders

Chromosome abnormalities are another form of mutation, and there are many different types: duplication of an entire chromosome, or of the entire set, or of only part of a chromosome, or similar deficiencies of chromosomal material. If the chromosome involved is an autosome (all the chromosomes except the two sex chromosomes), then the consequences of such duplications or deficiencies are usually severe. Many affected individuals are lost shortly after conception. Around 60% of all miscarriages in the first trimester have a chromosome abnormality. Those that survive to

be born usually have mental retardation, intrauterine and postnatal growth retardation, dysmorphic features, and there may often be major congenital malformations too.

The most common type of chromosome anomaly is trisomy, where there is an extra version of a single chromosome. Trisomy can arise for any of the chromosomes, but it affects some more frequently than others. Trisomy for chromosome 16 is the most common, but no real embryo forms in the conceptus and none survive more than a few weeks post-conception. Trisomy 1 is extremely rare. Although most of the trisomies 13, 18 and 21 that are conceived are aborted prenatally, these particular trisomies are the only instances where a few cases do survive to be born at term. Trisomy 21 alone has the ability to achieve adulthood. Each of these three trisomies has its own distinct pattern of abnormalities. Trisomy 13 (Patau syndrome) is characterised by microcephaly, scalp defects, microphthalmia, ocular hypotelorism, cleft lip and palate, flexed fingers, polydactyly, prominent heels, heart defects and severe developmental delay, with death usually within the first few months of life. Trisomy 18 (Edwards syndrome) has an elongate skull, small, lowset ears, micrognathia, flexed and overlapping fingers, prominent heels, heart defects, horseshoe kidney and severe developmental delay, few surviving longer than the first year. Trisomy 21 (Down syndrome) has brachycephaly, ocular hypertelorism, upslanting and short palpebral fissures, epicanthic folds, small nose and flat face, small, lowset ears, single transverse palmar crease, stubby fingers with short incurved fifth finger, broad feet, heart defects and learning difficulties.

Sex chromosome aberrations have less severe effects than autosomal abnormalities and in some types, affected individuals can go through life unaware of their condition. Some prenatal loss occurs, but postnatal survival is usually not compromised. The two that may have overt clinical features are Klinefelter syndrome where there is an extra X chromosome in a male (47, XXY), and Turner syndrome, where an X chromosome is missing in a female (45,X). The rarer 47, XXX and 47, XYY conditions usually do not have any phenotypic effects except occasional educational or behavioural problems in childhood and possible infertility in adulthood. In Klinefelter syndrome there is hypogonadism with sterility, tall stature, and gynaecomastia. In Turner syndrome exceptionally, 99% of cases die in utero. Survivors exhibit short stature, webbing of the neck, broad chest, increased carrying angle of the arms, co-arctation of the aorta and sterility.

As mentioned earlier, extra, or missing chromosomes largely arise because of faulty separation or disjunction of the two chromatids during cell division. The other cause is anaphase lag, where one chromosome fails to be incorporated within the newly forming nuclear membrane at the end of mitosis, and is ‘lost’. Non-disjunction in the female is the most common cause of the common autosomal trisomies, and as already described, is often related to raised maternal age, but, young

mothers do have Down's babies and a proportion of cases arise because of non-disjunction in the male, and this is not necessarily related to age.

Less commonly, malformation syndromes may be associated with chromosome translocations. These are structural rearrangements of the chromosomes. The most common form is a reciprocal translocation in which there is a break in each of two unrelated chromosomes of the set. The pieces do not rejoin in the same way as before, but differently. There is exchange of genetic material and two new chromosomes result. The translocations can exist as 'balanced', when there is no overall loss or gain of DNA, and 'unbalanced', where there is either a loss or gain of genetic material that usually has severe clinical consequences. Individuals who bear a balanced translocation are usually normal and unaware of their situation, but they have a high risk of producing offspring with the translocation in the unbalanced form who may be abnormal. This is because segregation of translocated chromosomes at meiosis during formation of the gametes often results in unequal products and consequent chromosome imbalance. Such imbalance can be highly detrimental resulting in congenital malformations. Translocations can occur between any chromosomes, although some are more commonly involved than others.

A few microscopically visible partial chromosome deletions have long been known to be associated with malformation syndromes. Examples are: Wolf-Hirschhorn syndrome where the deletion is on the short arm of chromosome 4 (4p-), and Cri du chat syndrome where the deletion is on 5p. But new cytogenetic techniques, such as high resolution banding on extended chromosomes, and FISH analysis (in situ hybridisation using specific fluorescently labelled DNA probes), have revealed that some rare syndromes with multiple abnormalities that have apparently normal chromosomes actually have a sub-microscopic deletion of a chromosome hitherto too small to be detected. Examples are: DiGeorge, Williams and Rubenstein-Taybi syndromes. The deletion involves the loss of several, or possibly many, genes, and the disorders are called the micro-deletion syndromes.

If only a few genes at adjacent loci are deleted, the indivdual may exhibit the features of several genetic diseases. These are the contiguous gene syndromes. For instance, Duchenne muscular dystrophy is often caused by a deletion of a large part of the dystrophin gene. Occasionally however, an affected boy also has adrenal hypoplasia and glycerol kinase deficiency because the deletion extends to the adjacent DNA where the loci for those genes occur.

6. Genomic imprinting

As a general rule, a specific allele of a particular gene produces the same phenotype regardless of whether that allele is derived from the father or the mother. But there are instances where an allele from one parent is not expressed because it is transcriptionally inactive. This is called 'genomic imprinting'. Only a few genes in only a few areas of some chromosomes appear to be subject to this phenomenon. It involves inactivation of the genes during gametogenesis. At one stage in the maturation of both the ova and sperm, the existing imprinting is erased, and new gamete-specific imprinting is established. The precise mechanism is not known but methylation of the DNA around the gene seems to occur. This is an epigenetic effect, no mutations of the DNA are involved, rather, DNA modification.

In the group of diseases referred to as imprinting disorders, the clinical picture that arises depends on which parent the specific allele comes from. An example of such a disorder is Beckwith-Wiedemann syndrome. Normally, only the paternal allele of the insulin growth factor gene 2 (IGF2) on chromosome 11 is expressed, that on the chromosome 11 derived from the mother being imprinted. In the rare instance when both copies of chromosome 11 are derived from the father (a phenomenon known as uniparental disomy) both IGF2 alleles are expressed, there is no imprinted allele. Double the normal amount of gene product occurs, and Beckwith-Wiedemann syndrome arises, the features of which are obesity, omphalocoele and a tendency to develop Wilms' tumour (a kidney cancer).

The converse situation, absent gene product, occurs when micro-deletions occur in the region of imprinted genes. The consequences differ according to parental origin. A region of the long arm of chromosome 15 is susceptible to macroscopic and micro-deletions involving the removal of many genes. Normally within that region, a cluster of genes is paternally imprinted while another set of genes is maternally imprinted. If the maternally imprinted genes are present, but since they are imprinted are inactive, and the paternally derived, but normally active genes, are and are deleted, Prader-Willi syndrome results. The absence of the paternal gene products has detrimental phenotypic effects: short stature, hypogonadism, obesity and learning difficulties. Where the deletion instead involves the maternally active genes, Angelman syndrome arises, where there are profound learning difficulties, a happy disposition, ataxic gait and epilepsy. These two rare conditions can also occur when the relevant parental genes are missing because there is uniparental disomy.

Recently, an unexpectedly high incidence of Beckwith-Wiedemann syndrome has been noted amongst children born as a result of assisted conception techniques (ACT) in three different countries, the UK (Maher et al, 2003), France (Gicquel et al, 2003) and the United States (Debaun et al, 2003). Earlier, an association between Angelman syndrome and one particular type of ACT, intra-

cytoplasmic sperm injection (ICSI) was found (Cox et al, 2002). No direct causal link between ACT and these imprinting disorders has yet been established, but concern has been expressed that some elements of the procedures, perhaps the female hormone priming, or sperm manipulation, or early embryo culture, interferes with the establishment of the genomic imprints. There is some evidence from animal studies to support this idea, but proof in human subjects is awaited.

7. Multifactorial disorders

Many of the common congenital abnormalities that occur as isolated abnormalities rather than as part of a syndrome, such as spina bifida and cleft lip and palate, have a multifactorial aetiology. Each disorder occurs more frequently within families than in the general population, but there is no recognisable pattern of Mendelian inheritance discernable. Many of the common acquired diseases of adult life too, like diabetes mellitus, coronary artery disease and hypertension show familial clustering, but also do not follow regular inheritance patterns. It is believed that many factors contribute to the cause of all these disorders, both multiple genetic and environmental factors. Neither group of factors alone is sufficient to cause the disorder, all components interact and contribute to its occurrence. This is multifactorial inheritance, about which much still remains to be elucidated.

The basis for ideas about the nature of the genetic components comes from earlier genetic theory on the inheritance of normal variation. Many normal human characteristics such as height, blood pressure and intelligence that are measured on a numerical scale are regarded as quantitative traits. Within the general population, a range of height for example, is found, from a few very small individuals to a few very tall people, with most people being of average height. The population distribution of height is continuous and follows a normal, bell-shaped, curve. Many years ago, it was suggested that such quantitative characters are determined by many genes, probably hundreds, all at different loci, each of which exerted a small and additive effect, cumulatively producing the phenotype. Each gene of small effect was termed a ‘polygene’, and quantitative traits were said to arise by polygenic inheritance. However, it is well known that height is also influenced by environmental factors such as nutrition and exercise, so is really multifactorial. In fact, the only human trait that is nearest to being truly polygenic is the finger ridge count. Finger ridges (fingerprints) are genetically determined and are laid down in the first trimester of pregnancy when environmental influences are minimal. The total number on all ten fingers shows a near 100% correlation between monozygotic twins and a near 50% correlation between first degree relatives, as they should do if they are polygenic. But the fact that the correlation is not absolute shows that even in this trait, there are minor environmental influences as well.

This old idea of hundreds of polygenes being involved in quantitative characters was transferred to the aetiolology of multifactorial diseases. However, this concept is now having to be modified in the light of contemporary genetic research. Particularly in some of the common adult onset diseases, a start is being made in the identification of some of the genetic components by the use of high density genetic maps and highly polymorphic markers. Now, rather than hundreds of genes, fewer are implicated. Further, it appears that they do not necessarily all have an equal, small effect. Although still perhaps quite a number of genes are involved, a few loci seem to contribute a significant proportion of the variance. For example, in insulin dependent diabetes mellitus (type I), it is possible that around 40% of the genetic contribution may come from only two genes. The specific human leucocyte antigen (HLA) class II alleles, DR3 and/or DR4 possibly account for around 30% of the genetic susceptibility (Mein et al, 1998), while allelic variation in a specific polymorphism located 5' to the insulin gene (Bell et al, 1984) may contribute a further, say, 10%. However, there are at least another 20 regions of the genome being investigated as they are considered to contain additional susceptibility genes.

Congenital malformations that have a multifactorial aetiology are explained on the basis of the liability-threshold model (Falconer, 1965). It is accepted that each disorder is caused by both multiple genetic and environmental factors. Together, these factors are considered to create a liablity to the condition. For a particular disorder, the liability of the whole population is normally distributed. Most people have an average number of liability factors, a few people have a small number, and a few people have many. For the disease to be expressed, a certain number of liability factors are needed and this is called the threshold. Below the threshold, individuals do not have the disorder, even those close to the threshold also do not have it, but once the threshold has been crossed, the disease phenotype is manifest. The genetic component alone cannot cause the disease, but it creates a predisposition that, if enough of the environmental factors too are present, together, they push the individual over the threshold of liability and an abnormal phenotype results.

There are many multifactorial congenital abnormalities, but very little is yet known in any of them about the identity of either the genetic components or the environmental factors. Congenital dislocation of the hip is a possible exception, and a folic acid deficiency, as yet unspecified, is implicated in the aetiology of neural tube defects (see below). In congenital dislocation of the hip (Carter, 1969), three of the genetic factors involved are a) having the autosomal dominant gene for familial generalised joint laxity, b) being over the threshold for the multifactorial condition acetabular dysplasia resulting in a shallow acetabulum, and c) being female. Two of the contributory environmental factors are position of the legs in utero, and position of the legs after birth. Other, as yet unknown, factors may also contribute. Further, in multifactorial conditions, there is generally no

knowledge of the relative contribution that individual genetic and environmental components make to the cause. However, the relative contribution that the composite genetic and environmental factors make overall to the causation can be calculated from a comparison of the occurrence of the disorder in monozygotic and dizygotic twins. This is the heritability. It is expressed as a percentage that represents the proportion of the genetic contribution. For instance in cleft lip and palate, the heritability is 76%, while in congenital heart disease, it is 35%. Thus genes are more important than environmental factors in the causation of cleft lip and palate, but the reverse is the case in congenital heart disease.

8. Folates and the prevention of neural tube defects

Long ago it was noted that women who had given birth to infants with neural tube defects (NTD) (referred to as NTD women) had low serum folate levels (Hibbard and Smithells, 1965). Another study showed that NTD women generally had a poor diet compared with their sisters who had normal children (Laurence et al, 1980). An intervention study which administered periconceptional multivitamins including folate to NTD women resulted in a significant reduction in recurrence of the NTD, and for the first time showed that primary prevention of a major congenital malformation was possible (Smithells et al, 1980). A four arm, double blind, placebo trial, again in "at risk" women, proved conclusively that folate was the preventive agent (MRC Vitamin Study Research Group, 1990); and a Hungarian population study showed that first occurrences of an NTD in a family as well as recurrences, were prevented by such therapy (Czeizel and Dudas, 1992).

There is some evidence that other congenital malformations may be prevented to some extent by folate treatment too, notably cleft lip with or without cleft palate (Tolarova, 1982) and also, less convincingly, obstructive urogenital defects and limb reduction defects (Czeizel, 1993). Encouragingly, data continue to come forth suggesting that congenital malformations other than NTD are prevented by maternal periconceptional therapy (for example, de Walle et al, 2003).

The mechanism for the preventive effect of folate on NTD is not yet known. Is it correcting a defect in the embryo or in the mother? Simplistically, it is assumed that the administered folate overcomes a genetically determined defect in folate metabolism that is important in neural tube closure.

Folate metabolism is complicated (see for example, Kelly et al, 1997). Folate occurs in plasma and other body fluids as 5-methyl-tetrahydrofolate (5-methyl-THF) and diffuses into cells in this form, but if it is to be retained intracellularly it must be metabolised by methionine synthase, a vitamin B_{12}

dependent enzyme, to tetrahydrofolate (THF). Folate has a number of functions. Intracellular folate has one-carbon units attached. These are used in the biosynthesis of purines and pyrimidines. Folate is also essential for the action of the enzyme thymidylate synthase. Thus folate is crucial for the *de novo* synthesis of DNA, a process which is extremely important to a rapidly dividing embryo undergoing embryogenesis. In addition, folate participates in the methylation cycle. Through the action of methylene tetrahydrofolate reductase (MTHFR), 5-methyl-THF is formed from which a methyl group is transferred to methionine. The end point of this methyl chain reaction is homocysteine, which can be broken down by cystathionine β-synthase to cysteine and pyruvate, which can be used as a source of energy.

A genetic variant of MTHFR inherited in an autosomal recessive manner produces a thermolabile form of the enzyme that is only partially active. Homozygotes have low folate levels, increased homocysteine levels and an increased risk for NTD and ischaemic heart disease (van de Put et al, 1995). The allele frequency varies in different populations (Schneider et al 1998), in Europeans it is 24-40% (van der Put et al, 1997). In one study, homozygosity was found to be 3 times higher in a group of NTD individuals than in a normal study group (Whitehead et al, 1995). In Ireland, it is regarded as a significant risk factor and is estimated to account for 12% of the genetic risk (Kirke et al, 1996). However, as not every affected individual is of this genotype, and in some countries where NTD are found the allele is uncommon, this can only be one risk factor among many others, and in some individuals it is not a factor.

The possibility that other variants in MTHFR and/or inherited deficiencies in other enzymes involved in folate pathways could be contributory to the cause of NTD are being investigated. Methionine synthase is of especial interest because a low maternal serum vitamin B_{12} level is an independent risk factor for NTD (Kirke et al, 1993). There are no significantly positive results and we are still far from knowing how a folate deficiency causes NTD.

9. Other environmental effects in congenital malformations

More subtle genetic effects that more obviously involve the environment as well, occur through some maternal illnesses. Insulin-dependent diabetes mellitus is, as previously mentioned, a multifactorial condition. If a pregnant mother has this disease and it is not well controlled, an unfavourable environment for the developing embryo arises, and malformations may occur, typically, caudal regression syndrome, heart defects and neural tube defects. The severity of the defects is related to the level of diabetic control.

Many forms of epilepsy have a genetic or part-genetic cause. Maternal epilepsy too, is associated with an increased risk of malformations, although here, the medication can also be teratogenic and it can be difficult to separate the two effects. Several anti-convulsants, for example, phenytoin, demonstrate another important principle of teratogenesis. The genotype of the embryo may be crucial in determining the susceptibility of the embryo to a particular teratogen. It is known that only around half of all fetuses exposed to phenytoin show any adverse effects, 5-10% having severe malformations, 40% having only growth retardation and mental delay (Hanson et al, 1976). Which response occurs, or whether there is no response at all, depends on the fetal genotype. The deleterious effects of phenytoin arise, not directly from the drug itself, but indirectly from the toxic oxidative metabolites that occur during its biotransformation in the body (Marlz et al, 1977). The fetal response is determined by which combinations of two alleles the fetus has of the gene for the enzyme epoxide hydrolase, that detoxifies the phenytoin metabolites (Buehler et al, 1990). The various isozymes differ in the efficiency with which they carry out this reaction.

Another anti-convulsant, valproic acid, known to cause spina bifida, provides an example of how contemporary research is revealing the molecular pathways underlying the teratogenic action. In the mouse, Wlodarczyk et al, (1996) have shown that during neural tube closure in the embryo, valproic acid causes the over expression of certain transcription factor genes and cell cycle checkpoint genes. Organogenesis is brought about by hundreds of genes acting in an elaborately controlled and regulated cascade and transcription factors regulate the expression of downstream developmental genes. The fact that valproic acid alters the expression of transcription factors means that its effects are far-reaching. Indirectly, many other genes are altered too.

Vitamin A, in the form of retinoic acid, is an effective treatment of teenage acne. However, retinoic acid is a potent teratogen causing microcephaly, microtia, micrognathia, hydrocephaly, cardiac, aortic arch and other abnormalities. Retinoic acid is itself a morphogen, and there also exists a complex system of retinoic acid receptors. In particular, retinoic acid regulates the Hox genes, an important family of transcription factors arranged in four gene clusters. Amongst other things they are involved in patterning and conferring axial identity. And so are crucial in development.

Phenylketonuria is a purely genetic condition, being inherited in an autosomal recessive manner. The enzyme phenylalanine hydroxylase is absent, and unless controlled by a special diet, excess phenylalanine accumulates in the blood. This is toxic, especially to the brain, and irreversible mental retardation results. If an affected woman is untreated in pregnancy, the excess phenylalanine reaches the embryonic environment. As a result, and even if, as is usual, the baby is genotypically normal, the phenylalanine-rich environment will produce microcephaly, heart defects and mental retardation in the baby.

10. Associations

There are a number of spectra of malformations that occur together more frequently than can be accounted for by chance, but their aetiology is unknown. This group of disorders is called 'associations'. Each one is usually referred to by an acronym embracing the main abnormalities involved, for example, OEIS: omphalocoele, exstrophy of the bladder, imperforate anus and spinal defect, and VATER: vertebral defects, anal atresia, tracheo-oesophageal fistula and radial and renal defects. There is no clustering of these disorders in families and no environmental agents appear to be responsible. The causes of these non-random associations remain a mystery.

11. Evidence for genetic differences leading to geographical heterogeneity in the prevalence of congenital malformations in the UK today

Data on the geographical heterogeneity in the prevalence of congenital malformations with respect to NTD in the past, are well known, and summarised by Elwood, Little and Elwood (1992). There are geographical variations in NTD prevalence within the British Isles. Basically, there is a gradient of increasing prevalence from the south-east to the north-west. This suggests a high risk of NTD for people of Irish, Scottish or Welsh origins, which could support the idea that genetic factors are important. However, the gross geographic pattern also correlates with the socioeconomic status, the prosperous south-east and the deprived north-west, which lends support to the theory that it is environmental factors that are important, rather than genetic ones.

Worldwide, there are marked geographic differences in NTD prevalence between and within countries. Within China, for instance, the north has a high NTD prevalence while the south has a low prevalence (Berry et al, 1999). In Africa, the fact that poverty is gross and NTD are generally uncommon, could suggest that genetic factors are important.

It is particularly complex to unravel the effects of genetic and environmental factors in geographical variation. A study of migrant populations may help, but ethnic groups usually transport their dietary patterns and lifestyle with them wherever they go. Elwood, Little and Elwood (1992) conclude that NTD prevalence depends on both geographic and ethnic factors, but the precise contribution of genetic factors cannot be determined. Within the UK, certain migrant populations do have an increased prevalence of malformations overall for whatever reason, for instance, the Sikhs in the Birmingham region, and particular immigrant groups in East London. This must be borne in mind in any study that is undertaken.

12. What can clinical studies tell us concerning the specificity of action of chemicals for certain types of congenital abnormality, or certain pregnancy outcomes such as birth weight, prematurity, and fetal death?

Most environmental factors that have a deleterious action on the developing embryo produce both non-specific and specific effects. The non-specific effects, common to most agents, are an increase in intrauterine death, intrauterine growth retardation, decreased birth weight, and general dysmorphic features. The specific effects are a spectrum of congenital abnormalities that appears consistently with a particular agent, which characterises that particular agent, and which is different from that produced by another environmental agent. It is important to remember that a specific malformation may result from the action of a number of different environmental agents. For example, spina bifida can be produced by the drug valproic acid and by maternal insulin-dependent diabetes. There is no difference between the two in the clinical appearance of the spina bifidas, but it is the other abnormalities found in addition to the NTD that together comprise the specific spectrum of abnormalities that characterises the particular environmental agent.

Clinical studies of affected individuals, together with pregnancy and family history analyses, have allowed the delineation of specific patterns of defects associated with particular environmental agents, for example, the Rubella virus and the sedative drug, thalidomide. The fact that different environmental agents produce different spectra of abnormalities shows that the mechanisms of action of the various agents must differ. However, only rarely is the pathogenesis known.

Clinical studies have also shown that, as in animals, a specific teratogen may produce different malformations, or no malformations at all, according to the time of exposure of the embryo during its development. This reflects the critical periods of development of each organ or body part: namely that there is only a short time, just before and during its genesis, that it is vulnerable to malformation by an environmental agent. Before and after that time it will not be affected. For example, exposure to the Rubella virus before 8-10 weeks of gestation causes cataracts and heart defects; from 10-16 weeks, hearing loss and retinopathy; and after 16 weeks has no effect. Thalidomide only produced devastating phocomelic effects if embryonic exposure was in a very specific and narrow time band. Different defects arise at other times of gestation.

Overall, the environmental factors known to produce malformations can be classified into:- a) infectious agents, b) maternal illnesses and deficiency states, c) physical agents including radiation, hyperthermia, and d) drugs and alcohol. Clinical studies highlight the following malformation spectra for each, in addition to generalised low birth weight and dysmorphism, and increased risk of fetal loss:-

A. Infectious Agents

1. Rubella: before 10 weeks gestation, cataracts and heart defects; 10-16 weeks, hearing loss and retinopathy.

2. Varicella: limb hypoplasia, scarring, microcephaly, chorioretinitis.

3. Cytomegalovirus: hydrocephalus, periventricular calcification, neurological problems.

4. Toxoplasmosis: hydrocephalus, microcephaly, cerebral calcification, neurological problems.

B. Maternal Illnesses

1. Insulin-dependent diabetes: macrosomia, caudal regression syndrome, neural tube defects, congenital heart disease especially VSD and transposition of the great vessels. These defects do not occur in children of mothers who develop gestational diabetes.

2. Phenylketonuria (if not controlled by diet): microcephaly, micrognathia, heart defects, mental retardation.

3. Unspecified folate deficiency (see separate section): neural tube defects and possibly cleft lip and palate.

4. Epilepsy: effects cannot be separated from the teratogenic effect of medication (see above).

C. Physical Agents

1. Radiation: very high doses to the fetus in mid to late gestation can produce microcephaly.

2. Hyperthermia: neural tube defects especially anencephaly, microcephaly, microphthalmia, cleft lip and palate; but it is difficult to dissociate the effects of viral or other agents producing pyrexia from the effects of hyperthermia. The data from hot baths and saunas are not convincing. However, evidence for these abnormalities arising with pure hyperthermia exists in animals.

D. Drugs

1. Thalidomide: phocomelia and other limb defects, cardiac defects, gut atresia, renal agenesis.

2. Diethylstilboestrol: females – vaginal adenosis. Males – micropenis, hypospadias, cryptorchidism.

3. Warfarin: hypoplastic nose and bone dysplasia like Conradi disease, choanal atresia, microcephaly, hydrocephaly.

4. Valproic acid: spina bifida, midface hypoplasia, long philtrum, small mouth, cardiac defects.

5. Phenytoin: typical facies of brachycephaly, high forehead, marked eyebrows, long philtrum, depressed nasal bridge, metopic ridging, bowed upper lip; cleft lip and palate; hypoplastic nails and distal phalanges; short neck; hirsutism.

6. Aminopterin/methotrexate: microcephaly, broad nasal bridge, micrognathia, short limbs, talipes, hypodactyly, syndactyly.

7. Retinoic acid (vitamin A congeners): hydrocephalus, microcephaly, cardiac defects especially conotruncal malformations, aortic arch hypoplasia; microtia/anotia, micrognathia, urogenital anomalies, thymic hypoplasia.

8. Alcohol: microcephaly, long face, smooth philtrum, thin vermilion border to upper lip, abnormal palmar creases, short distal phalanges, heart defects, mental retardation.

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CHAPTER I.2 EPIDEMIOLOGICAL EVIDENCE REGARDING ENVIRONMENTAL CAUSES OF CONGENITAL ANOMALIES: INTERPRETATIONAL ISSUES

Helen Dolk, January 2004

Published versions of most of this chapter are available in:

Dolk H (2004) Epidemiologic Approaches to Identifying Environmental Causes of Birth Defects. Am J Med Gen Part C (Semin Med Gen), 125C, 4-11

Dolk H, Vrijheid M (2003) The impact of environmental pollution on congenital anomalies. British Medical Bulletin, 68,1-21

Contents

1. What is an "environmental cause"?

In its widest sense, an "environmental cause" is any non-genetic factor which increases the risk of a congenital anomaly for the exposed individual. Such factors include nutritional excesses and deficiencies (e.g. folic acid) (MRC 1991, Rothman 1995), maternal illness or infection (e.g. diabetes, rubella) (Gregg 1941, McLeod & Ray 2002), drugs taken during pregnancy (e.g. thalidomide, valproic acid) (Lenz 1961, Schardein 2000), chemical exposures in the workplace or home (e.g. to solvents or pesticides) (Cordier et al 1997, Garcia 1998), and radiation (e.g. medical Xrays, and atomic bomb irradiation) (Lione 1987, Otake & Schull 1984). Reviews of many of these environmental risk factors are given in Part II. There is considerable interest in the possible role of chemical contaminants in air, food and water and some authors restrict the term "environmental" to such factors. Contaminants which have been the focus of particular recent interest include byproducts of drinking water chlorination (Niewenhuijsen et al 2000), endocrine disrupting chemicals,

particularly in relation to hypospadias and cryptorchidism, (Toppari et al 1996) and unspecified releases from landfill sites (Vrijheid 2000). A review of environmental pollution as a cause of congenital anomalies is given in Part III.

An environmental cause can broadly have preconceptional mutagenic action (maternal or paternal) or postconceptional teratogenic action (maternal). Preconceptional effects may include chromosomal anomalies and syndromes as a result of new mutations. Postconceptional action (the main focus of this review) can generally be assumed to be during the first trimester of pregnancy when most organogenesis occurs, although relevant exposures may have occurred earlier if their effects are indirect (e.g. effects on endocrine function) or if a chemical has a long biological half life in the body (e.g. PCBs). The precise timing of exposure to the fetus is important – each normal developmental process occurs during a specific period of a few days or weeks, and it is during this "sensitive period" that exposure to a teratogenic agent my lead to a specific anomaly. Where a child has more than one anomaly ("multiply malformed") this may be because exposure has covered a number of sensitive periods for different congenital anomalies, or because exposure at one developmental stage has a number of different effects on organogenesis. The development of the brain remains subject to adverse influences well into the second trimester and beyond (Evrard et al 1989, Otake & Schull 1984).

All congenital anomalies can be presumed to be caused by a combination or interaction of genetic and environmental factors. The epidemiologist is interested in whether it is genetic or environmental factors or both which distinguish individuals with and without a congenital anomaly (epidemiology cannot investigate factors within the causal mechanism that are uniform within the population). At one extreme of the spectrum are the single gene or chromosomal syndromes where individuals with and without the syndrome are distinguished by the genetic mutation alone. Nevertheless, the example of phenylketonuria reminds us that even with a purely "genetic" condition, environmental factors may be involved in the causal mechanism and indeed may provide the basis for therapeutic intervention. At the other end of the spectrum, one can place in the "environmental" category cases with environmental exposures known to carry a high relative and absolute risk of congenital anomaly, such as maternal rubella. Many environmental exposures significantly raise the risk of congenital anomaly, but only the minority of exposed individuals are affected. For example, valproic acid is now a well established risk factor for spina bifida, but most fetuses exposed to maternal valproic acid intake are not born with spina bifida (Robert & Rosa 1982). It is a logical sequence in aetiological research first to identify a factor which raises the risk, and then subsequently identify the other factors that distinguish why only some of those exposed are affected. These other factors may be genetic susceptibility factors (in mother or fetus), and elucidation of these factors would for example help target therapies such as anticonvulsant therapies more safely for pregnant women. These factors may

also be co-existing environmental exposures. A low absolute risk associated with exposure may also indicate exposure misclassification i.e relevant aspects of exposure such as timing or dose or the presence of a specific component of a complex exposure have not been properly identified and measured so that those classified as exposed include fetuses without relevant exposure.

When dealing with "cause", we do not simply have a horizontal array of different biological, chemical or physical agents, but causal pathways and networks which determine exposure to these proximate agents. For example, maternal rubella infection and rubella vaccination policy are at different levels in this causal network, as are folic acid intake and social class or economic prosperity. Preventive strategies use knowledge at more than one level in the causal network. "Risk factor", as used in this chapter, is a looser term than "cause", referring to any factor associated with increased risk of congenital anomaly, whether or not it is an established or agreed "cause", including indicators of causal agents. For example recent immigration from countries without rubella vaccination may be a risk factor for congenital rubella syndrome, and such a risk factor can be used to target vaccination information. The age and reproductive history of the mother may be an indicator of endocrine or other biological factors, or an indicator of lifestyle or exogenous exposures.

Knowledge of socioeconomic inequalities can give clues to the proximate causal agents. For example, early case-control studies of neural tube defects finding strong social class gradients in risk were part of the evidence that finally implicated nutritional factors and then more specifically folic acid in neural tube defect aetiology (Elwood et al 1992). If the proximate causal agents are known, knowledge of social class gradients can help target preventive efforts. Whether the proximate causal agents are known or not, studies of socioeconomic inequalities can suggest ways in which economic and structural changes can be effective as a preventive strategy in addition to focusing directly on the proximate agents, and moreover can achieve a wider range of health benefits. There is surprisingly little evidence regarding socioeconomic inequalities in congenital anomaly prevalence, particularly in relation to congenital anomaly subgroups. Existing evidence suggests that most non-chromosomal anomalies increase in prevalence with increasing socioeconomic disadvantage (Vrijheid et al 2000). Exceptions to this may prove particularly interesting as aetiologic clues (Dolk et al 1998).

The factors determining who gets a congenital anomaly within a population may not be the same as those determining why some subgroups or populations have higher congenital anomaly rates than others. For example, the demonstration that insufficient folic acid intake is a strong risk factor for neural tube defects within populations does not mean that differences in folic acid intake necessarily explain the huge differences in neural tube defect prevalence that exist between populations, geographically or over time (Elwood et al 1992).

While further research continues into the environmental causes of congenital anomalies, there is also evidence that existing knowledge is not being effectively incorporated into health care in all communities, and thus poor health care becomes in this sense a "cause" of the congenital anomaly. Even ten years after randomised controlled trials demonstrated that folic acid prevents neural tube defects, a minority of women were taking periconceptional folic acid supplements, and only a few countries have recently responded to this by introducing folic acid fortification of staple foods, albeit at low levels (EUROCAT Working Group 2003, Honein et al 2001). Studies of epileptic women have also shown that they are not always receiving optimal care for the prevention of congenital anomalies (Fairgreave et al 2000). Thalidomide itself has continued to cause congenital anomalies in some countries where its use was continued to treat leprosy (Orioli & Freire 2000). The epidemiologic approach is not used just to elucidate environmental causes but to track progress towards prevention by the reduction of known environmental causes.

2. The prevalence of congenital anomalies

The first epidemiologic question to be asked is generally how frequent congenital anomalies are in the population, and how this compares to other populations. "Incidence" strictly refers to the number of new cases arising in a population during a defined time period, while prevalence refers to the number of existing cases in a population over a time period. "Prevalence" is a function of both incidence and survival. Embryos and fetuses with congenital anomalies are selectively lost as spontaneous abortions (miscarriages) during pregnancy. Among recognized pregnancies, 33-55% of spontaneous abortions have chromosomal anomalies (Roman 1983), many of these incompatible with continuing in utero life which are therefore never seen at birth. Use of the term "prevalence" is seen as appropriate for the number of cases diagnosed (surviving) in a population of births (Schulman et al 1988). It should be remembered that differences in prevalence may reflect differences in survival of affected fetuses during pregnancy rather than differences in incidence.

Registries of congenital anomaly report 2-4% of births with congenital anomaly, depending on inclusion criteria and ascertainment methods. Cardiac defects account for over one quarter of all cases, limb anomalies one fifth, chromosomal anomalies and urinary system anomalies each around 15%, central nervous system anomalies including neural tube defects 10% and oral clefts 7% (EUROCAT Working Group 2002).

The problem of measuring frequency has been exacerbated in the last few decades by the practice of prenatal screening and termination of pregnancy. For example, in 32 European regions 1995-99, 53% of spina bifida cases and 33% of Down Syndrome cases were prenatally diagnosed leading to

termination of pregnancy (EUROCAT Working Group 2002). These are averages in a range from 0% (in regions where termination is illegal) to over 75% for both of these conditions in four of the 32 regions. In order to compare prevalence rates between populations in relation to possible underlying environmental causes, it is necessary to calculate a "total" or "adjusted" prevalence rate including terminations of pregnancy. However, the inclusion of terminations, especially if they occur relatively early in pregnancy and relate to congenital anomalies with a high spontaneous fetal death rate (like Down Syndrome) can artificially inflate prevalence rates compared to those based on populations without terminations, since they include affected fetuses which would otherwise have been lost as unrecorded spontaneous abortions.

Registries of congenital anomalies are a principal source of prevalence data. An important principle underlying most registries is a well defined geographical population-base of resident mothers. Basing a registry on a single hospital or selected hospitals can create selection bias, where high risk mothers are referred to or from the hospital for specialist services, thus resulting in prevalence rates which are biased upwards or downwards compared to the general population. Prenatal screening has increased the potential for such selective flow between hospitals, and emphasised the need for population-based studies.

The interpretation of differences in prevalence between populations based on registry data needs to take a number of factors into account, some related to diagnostic practice within the region, some related to how the registry gathers and codes its information (EUROCAT Working Group 2002). Factors related to diagnostic practice include variations in autopsy rates on terminations, stillbirths and neonatal deaths, particularly for the detection of malformations not externally visible; whether autopies are performed by specialized fetopathologists; variations in rates of karyotyping and DNA typing, and indications for karyotyping, prenatally or postnatally. Factors related to registration practice include variation in the age limit for inclusion of newly diagnosed cases (some registries use sources of information which cover only the neonatal period, thus missing diagnoses of cardiac and other anomalies made later in infancy); variation in the reporting and classification of component malformations of syndromes and sequences (Jones 1997) - for example, whether Meckel Syndrome is included in the reported prevalence of encephalocele, or whether hydrocephaly secondary to spina bifida is included in the prevalence of hydrocephaly.

Two of the most important sources of differences in reported prevalence of congenital anomalies between populations are a combination of diagnostic and registration practice. The first is variation in diagnosis and reporting of more minor anomalies. Most registries employ exclusion lists of "minor anomalies" which although they may be of relevance to teratogenic exposures are too inconsistently diagnosed and reported to be useful as routinely collected population data (EUROCAT Working

Group 2002, Rasmussen et al 2003). Remaining difficulties lie where malformations range from minor to major forms (such as microphthalmia, microcephaly, polydactyly or syndactyly), since thresholds for diagnosis and reporting may vary, severity is often not reported and definition and coding schemes for severity are lacking. In general, less severe forms are more common, and thus thresholds of severity for inclusion can have a considerable impact on prevalence rates. Recently, assessment of increasing trends in the prevalence of hypospadias has been of particular interest in relation to hypotheses regarding population exposure to endocrine disrupting chemicals, but the potential for variable recording of the more minor distal forms of hypospadias have made this assessment very difficult, particularly as surgery practice for distal forms seems to differ between regions and over time (Dolk 2003).

The second is variation in screening practice. With the increased use of ultrasound prenatally and in early postnatal life, the detection of many non-externally visible anomalies (such as cystic kidneys and some cardiac anomalies) can be brought forward to a much earlier age. Thus, for registries with an early age limit for reporting, cases are being reported that would otherwise have been diagnosed too late for inclusion among registrations. This has led to increases in reported prevalence of anomalies in many areas over the last two decades (EUROCAT Working Group 2003).

Thus, it is not a simple matter to interpret differences in congenital anomaly prevalence between populations, and to progress beyond possible artefacts related to diagnostic and registration practice to environmental hypotheses.

3. The design of an epidemiological study

The epidemiological approach can be contrasted to case reports or case series where one or more cases are described where the mother took a certain drug (for example) and had a child with a congenital anomaly. The more common the drug exposure in the population and the more common the malformation, the more likely that this may be a chance association. Reporting the case may elicit case reports from other clinicians, improving the assessment of whether this is a chance association. However, there is then the danger of what Smithells described as the "me-too phenomenon……as capable of confirming a myth as a truth" (Smithells 1974). This is not to suggest that case reports do not have a very important role. The "alert clinician" reporting case series has been at the origin of many hypotheses subsequently further investigated epidemiologically. These include rubella (Gregg 1941) and thalidomide (Lenz 1961), the most instrumental epidemics in focusing attention on the potential for maternal exposures during pregnancy to affect the fetus. The study of the teratogenic effects of alcohol also had its origin in the reporting of case series (Jones et al 1974).

The ideal epidemiologic design to investigate whether factor F leads to congenital anomaly D is to organise an experiment whereby all factors other than F are held constant between the groups i.e. a randomised trial. For example, randomised trials were carried out to determine whether periconceptional folic acid supplementation could prevent neural tube defects (MRC 1991). An experimental study is rarely practical or ethical when considering most environmental exposures. There are a number of main designs for an observational epidemiological study investigating environmental aetiology. Case-control designs select a group of "cases" with congenital anomaly D, and a group of "controls" without congenital anomaly D, and then set about determining the presence or strength of a set of hypothesised risk factors in each group. The question is whether a greater proportion of cases than controls have a certain risk factor present. Cohort designs identify an "exposed cohort" where a risk factor F is present (e.g an IVF cohort, an occupational cohort, or a cohort of pregnant women exposed to anticonvulsants), and a control cohort where risk factor F is absent, and then follow-up these pregnancies to ascertain congenital anomalies in each cohort. The question is whether a greater proportion of pregnancies with a certain risk factor/exposure have a diagnosed congenital anomaly than without that risk factor/exposure. Such a design is used when the exposure is rare, or needs to be prospectively recorded. More needs to be done to link exposure cohorts with congenital anomaly registries, but confidentiality problems have limited this approach.

An Ecological study considers population subgroups (for example defined by geographical region of residence) rather than individuals as its units of observation. In each population subgroup, the frequency of one or more risk factors is measured, as well as the frequency of one or more congenital anomalies among births. The question then is whether population subgroups which have higher levels of a particular risk factor also have higher proportions of affected births. This design is more common for community exposures, for example a study correlating anencephalus mortality with drinking water composition in 36 Canadian cities (Elwood 1977).

The two measures most frequently reported are odds ratios (mainly from case-control studies) and relative risks (mainly from cohort and ecological studies). The odds ratio is the ratio of the odds of congenital anomaly among the exposed to the odds of congenital anomaly among the unexposed. For rare outcomes like congenital anomalies, it is equivalent to (and can be interpreted as) the much more readily understandable measure relative risk. Relative risk in this context is equivalent to the prevalence of congenital anomalies among the exposed divided by the prevalence among the unexposed ie. how many times higher the prevalence is among the exposed.

4. Sources of bias and confounding in epidemiologic studies

The design and interpretation of observational studies is centrally concerned with assessing the potential for bias i.e. unrecognised differences between the groups being compared and how these may be influenced by the method of selection of those groups. These differences are of three types:

(i) *in the way that congenital anomalies have been diagnosed or ascertained*

Bias can occur in a cohort study if congenital anomaly status is ascertained differently for exposed and unexposed cohorts, for example if congenital anomalies rates from a population register are compared with the results of sending a questionnaire to an occupational cohort of pregnant women, or with the results of a follow-up of pregnant epileptic women with special paediatric examinations for their children. If maternal report is used for ascertainment of congenital anomalies, mothers of differing exposure status may be more or less likely to report more minor congenital anomalies, especially if they are aware of the possible relation between exposure and outcome.

It should be clear from the previous section on Prevalence, that there are numerous artefactual reasons why ecological studies may find congenital anomaly prevalence varying geographically and temporally.

Bias can result in a case-control study from selection of cases and controls according to clinic attendance, rather than with reference to a population base. For example, if the study is looking at pesticide exposure in relation to cleft palate risk, it may be that the clinic serves a wide surrounding urban and rural area for cleft palate, but only a relatively small urban surrounding population for other (control) conditions, and thus pesticide exposure of the mother in agricultural occupation may be spuriously associated with risk of cleft palate.

Bias can occur in studies limited to liveborn cases and controls, where difference in exposure may relate to the probability of prenatal diagnosis and termination (such as social status or maternal age) rather than causal risk factors for the congenital anomaly. Similarly, if cases are limited to survivors of the neonatal period, then one might discover risk factors for severity, multiple congenital anomalies or survival, rather than causal factors for the congenital anomaly itself. Of course, this also applies to survival during pregnancy, as previously mentioned.

(ii) *in the way that exposure has been measured*

Maternal recall bias may occur in a case-control study if mothers of children with congenital anomalies recall exposures during their early pregnancy differently from mothers of unaffected children, either because they are more motivated to remember or because they feel guilty about early

exposures. However, circumstances leading to severe bias are probably uncommon (Khoury et al 1994, Drews & Greenland 1990).

Most case-control studies do not achieve a complete response rate, and bias may result if the characteristics of non-respondents differ between cases and controls, if those characteristics are also related to the probability of exposure.

(iii) *in the presence of confounding factors*

A "confounder" is a factor associated with both the health outcome (congenital anomalies) and the exposure). Social class is related to many exposures, and also to the risk of congenital anomalies, leading to potential socioeconomic confounding. While social class differences in neural tube defect prevalence were a clue to the possibility of nutritional aetiologic factors, social class also acted as a "confounder" when differences in neural tube defect between groups with high and low folic acid intake were being compared. Was it folic acid or any of the numerous difference in exposure between the social classes that was causally associated with neural tube defects?

Typically, people of lower social status are more highly exposed to pollution, either because they move where housing prices are lowest, have less power or advocacy skills to prevent exposure, have less access to environmental health information, or because aspects of lifestyle associated with greater deprivation (such as ability to buy bottled water) lead to higher exposure. Although information on the extent to which congenital anomaly prevalence is linked to social status is rather limited, current evidence does suggest that more socio-economically deprived groups have higher non-chromosomal congenital anomaly rates (Vrijheid et al 2000), and part of this may be explained by nutritional status. Thus we have to take this into account when interpreting an association between, for example, residence near an incinerator and a raised prevalence of congenital anomaly, as a causal effect of incinerator releases. Since, when assessing environmental pollution, we are usually interested in fairly small increases in risk affecting large numbers of exposed people, socio-economic confounding is a particular problem.

Chromosomal anomalies such as Down Syndrome are strongly related to maternal age, and since at present average maternal age increases with social status, higher social status is associated with a greater risk of a Down Syndrome affected pregnancy, leading to inverse socio-economic confounding.

Many potential risk factors are correlated and it can be difficult to disentangle confounding effects e.g. distinguishing the effects of drugs from the disease or indication, or distinguishing the effects of different nutrients in the diet.

5. Exposure assessment and exposure misclassification bias

The sources of bias illustrated above can lead to either a lower or higher estimate of relative risk compared to the "true" relative risk. "Exposure misclassification" is a form of bias that usually results in a lower estimate of relative risk than the true relative risk i.e. obscures a true exposure related risk. For example, this obscuring effect can occur in studies of drug exposure when timing or compliance are not precisely known, in occupational studies when only occupational titles are available, or in environmental studies of proximity to pollution sources when the dispersion pattern of relevant exposures is not known and the migration of residents between organogenesis and birth is not taken into account, or in studies of drinking water exposures which allocate exposure according to water source of residence without information on other sources of drinking water or fluctuation of water contamination over time in relation to the organogenetic period.

Biological markers of individual exposure (such as cotinine or arsenic in urine, or PCB levels in blood serum) can improve exposure assessment, but since cost usually dictates that they must be taken within the framework of a case-control study, there can be problems with relating measurements after birth to early pregnancy. For example, cotinine levels in urine relate to recent exposure, but PCB levels in serum indicate long term buildup of exposure in the body. Where the exposure is complex, such as a hazardous waste landfill site, it may be difficult to identify the key chemicals to measure.

Where teratogenic (malformation causing) effects are concerned, it is usually assumed that there is a threshold of exposure below which the exposure is insufficient to overcome the natural regulatory and repair mechanisms during fetal development and therefore will not lead to a major malformation (Wilson 1977). This contrasts with the stochastic model for cancer initiation, where ever-smaller doses are simply associated with ever-smaller probabilities of effect and a linear dose-response curve is often adequate. Especially where there is evidence from occupational or animal studies that high dose exposures can be teratogenic, the relevant question is whether chronic low-level pollution reaches the threshold for effect. Biologically, it is reasonable to assume that each individual has their own threshold which an exposure would need to exceed to lead to major disturbance of development. Epidemiologically, it is reasonable to assume that individuals have different thresholds, depending on co-existing or previous environmental exposures as well as genetic susceptibility. Epidemiologic studies determine whether the environmental exposure is sufficient to exceed the thresholds of a significant proportion of the population. Under this model it is important not to engage in the "averaging" of exposure used for cancer epidemiology, i.e. the distribution of exposure dose in the population matters, and duration may have importance independently of total dose. Thus, the effects in a population with uniform medium exposure may differ from the effects in a population with the same average exposure but where some are unexposed and others highly exposed. This is relevant to

the type of exposure modelling one might undertake. For example, wind direction may be important in determining which areas near a factory have the highest average exposure (it may be that further residents downwind experience higher average exposure than nearer residents upwind), but maximum exposure may occur on windless days near the factory and affect all those living closest to the source regardless of wind direction.

6. Statistical power

Generally, the rarer the congenital anomaly, the rarer the exposure, and the smaller the risk among the exposed relative to that among the unexposed, the greater will be the population sample size needed to have a study of adequate statistical power to detect a risk of clinical or public health significance. Choice of appropriate epidemiological study designs are on the one hand about minimising bias, and on the other hand about maximising statistical power for a given number of study subjects. For a rare congenital anomaly , one might choose a case-control study, and for a rare exposure such as anticonvulsants or occupation as a dry cleaner one might choose a cohort study. For example, every 10,000 pregnanies in the general population will yield only three or four cases of major congenital malformation born to epileptic women, and a combination of cohort and case-control approaches have been used. Some of the apparent "inconsistency" between studies is simply because some are too small to precisely determine a relative risk, as revealed by wide confidence intervals around estimates of risk. It is therefore important to either proceed with large multicentric studies, or to report data from smaller studies in such a way that eventually meta-analysis of all published studies combined will be possible. A problem with the latter approach can be publishing bias, where positive associations are more likely to be published than negative studies. A problem of the former approach is that the larger the study in terms of number of study subjects, the more expensive it is liable to be, and the more likely that one will forego detail or consistency in congenital anomaly or exposure classification.

A device commonly used to increase numbers and statistical power is to group different types of congenital anomaly or exposure together. Major congenital anomalies as a whole, diagnosed prenatally or neonatally, affect approximately 2% of all births. Specific anomalies may affect one in a thousand births (e.g. neural tube defects) or one in ten thousand (e.g. gastroschisis). Since little is known about the aetiology of the majority of congenital anomalies, it is not always clear whether and how to group different anomalies together (Rasmussen et al 2003). By grouping, one might miss risks confined to specific types of congenital anomaly. Some efforts have therefore been made to define more pathogenetically homogeneous groups, such as defects related to vascular disruption, or to cranial neural crest cells. There is also discussion as to whether isolated defects and multiple

malformations are aetiologically distinct. For example, there is conflicting evidence as to whether isolated neural tube defects and neural tube defects with multiple malformations are aetiologically distinct subgroups as revealed by their epidemiological characteristics (Dolk et al 1991, Khoury et al 1982).

It is important to decide which subgroups of congenital anomalies are to be combined before looking at the data, since spurious statistical significance can be created by grouping together defects on the basis of similar relative risks or odds ratios.

7. Association or causation?

Most studies report results which are statistically "significant" at the conventional 5% level i.e. the probability of a difference arising as great or greater than the one observed when there is no true underlying association is less than 5 in 100. If one hundred environmental exposures are investigated, one would expect five statistically "significant" results purely by chance. This problem of multiple testing has led to a distinction being made between hypothesis generating studies (where there is no prior evidence about the exposures), often called "fishing expeditions", and hypothesis testing studies (where previous studies provide some evidence, and the new study is providing independent confirmation). Systematic reviews of all studies and meta-analyses are important to protect against overinterpretation of single study results.

We have many reported "associations" between a risk factor and a congenital anomaly in the literature which may be due to chance, bias or confounding. Bradford Hill in his influential 1965 paper asked "What aspects of that association should we especially consider before deciding that the most likely interpretation of it is causation?". (Bradford Hill 1965). His list included the strength of the association (a high relative risk is less likely to be explained by bias), consistency (in different populations under different circumstances), specificity (a cause leads to a single effect), a biologic gradient (presence of a dose-response effect) and coherence (between different types of evidence). These aspects of an association help us to assess strength of evidence for causation, and also reveal why it is sometimes difficult to infer causation. For example, environmental pollution is usually present at levels predicted to lead to a small excess risk, if any, though widespread and therefore of potential public health significance. Since the strength of the association observed is low, it is more difficult to put together convincing evidence of causation.

8. "Clusters" and the environmental contamination of air, food and water

Reports in the media of a "cluster" of congenital anomalies, often associated with suspected local contamination of air or water, are relatively frequent. A random distribution of cases in space and time is not a regular distribution, and there will be patches of denser concentration of cases. A community may become aware of an aggregation of cases in their area, and seek the nearest reason such as a waste site or power line. The problem has been likened to the "Texan sharpshooter" who draws his gun and fires at the barn door, and only afterwards goes and draws the target in the middle of the densest cluster of bullet holes. Since random "clusters" are expected to occur and there are usually relatively few cases for investigation, some argue that the likelihood of finding a common causal factor is so low that it may often be better not to investigate but instead to "clean up the mess" of the suspected contaminant without demanding causal proof (Rothman 1990). Others have tried to derive guidelines for deciding which clusters are worth investigating, often containing some reference to the size in number of cases and the nominal "significance" of the difference between the observed and expected number of cases, but increasingly also containing some appraisal of the type of concerns expressed locally. Multisite studies can be a useful line of investigation in response to local clusters, for example investigating all communities with municipal incinerators, rather than just the one where a cluster of congenital anomalies was observed (Dolk 1999).

Most of the well documented instances in the literature where a cluster was observed which was subsequently established as due to environmental contaminants have been related to food exposures, involving both high numbers of cases and high relative risk, including the Minnamata incident in Japan where fish and shellfish were contaminated with methylmercury (Harada 1986), incidents of PCB contamination of cooking oil in Taiwan and Japan (Rogan 1986), and pesticide over-use at a fish farm in Hungary (Czeizel et al 1993). These are discussed in Part III.

9. Genetic epidemiology

Genetic epidemiology is a term used now to refer to "the study of the role of genetic factors and their interaction with environmental factors in the occurrence of disease in human populations" (Khoury et al 1993). Genes currently the focus of research are genes involved in folate metabolism (Botto and Yang 2000) and genes involved in detoxification of xenobiotics (Shaw et al 2003, Van Rooij et al 2002). This approach holds considerable potential for the further elucidation of environmental factors for several reasons. Firstly, if genetic susceptibility to an environmental exposure is relatively uncommon in the population, then by being able to identify the genetic susceptibles we may be able to study environmental factors more effectively in the relevant subpopulation. Secondly, when we are uncertain about whether a statistical association between an environmental exposure and a congenital

anomaly represents a causal association, the finding of a specific relationship with a genetic factor may allay concerns about confounding or other forms of bias if it is possible to suppose that those with and without a specific genetic variant allele are unlikely to remember environmental exposures differently, or differ in the relevant lifestyle factors. Thirdly, knowledge of genetic factors can help elucidate the biological mechanism and thus the potential effect of different environmental agents.

However, the joint assessment of genetic and environmental factors also carries with it problems. The number of statistical tests being made are multiplied, so increasing the number of chance false positive associations found which need to be confirmed or refuted with follow-up studies (Shaw et al 2003, Khoury et al 1993). Also, sample sizes needed for study are greater if combinations of environmental and genetic factors are being assessed (Shaw et al 2003), although this depends on the balance between relative risk and frequency of exposure.

Another approach which has been used in genetic epidemiology with potential application to finding the environmental causes of congenital anomalies is "Mendelian randomisation" (Little & Khoury 2003, Davey Smith and Ebrahim 2003). Recognizing the problems of bias and confounding in observational epidemiologic studies of environmental exposures, proponents of mendelian randomisation recommend studying the association between gene variants of known function and disease, where those gene variants can be considered markers of altered exposure to an environmental factor.

10. The state of the epidemiologic evidence

Pregnant women may with reason expect that every attempt has been made to establish the safety of drugs or new reproductive assistive technology techniques or byproducts of agricultural or industrial processes. In fact, after approval or licensing, epidemiologic studies of the effects of exposure of pregnant women in the population are largely ad-hoc rather than part of a concerted strategy of research or surveillance. Thus the evidence base for policy making and clinical practice continues to be poor in many areas.

It is important to remember that "absence of evidence" is not "evidence of absence". When reviewing large amounts of literature as in this Report, one tends to concentrate on the evidence rather than its absence. Given the observational nature of most epidemiologic studies, it is easy to find concerns relating to potential bias or confounding for any one study, or to find apparent inconsistencies between studies relating to sources of bias, multiple statistical testing, or qualitative or quantitative differences in the exposure analysed. We have taken the approach in chapters to come of presenting

the evidence. Synthesising the evidence in terms of determining the weight of evidence for a causal association, or its magnitude, is beyond the scope of this Report.

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PART II

Selected Environmental Risk Factors for Congenital Anomalies

CHAPTER II.1 NUTRITION

Julian Little, May 2002

1. Issues in the analysis and interpretation of dietary intake data

There are a number of issues in the analysis of nutritional epidemiology studies. First, in the context of congenital anomalies, the most commonly used design is the case-control study. Therefore, control selection is an important issue (part 1.1). Second, between the late 1970s and the early 1990s, there appears to have been considerable international variation in maternal use of vitamin supplements in the periconceptional period and during pregnancy. For example, this varied between 3% and 92% in one international study (Preston-Martin et al., 1998). Following publication of the randomised trial showing that folate reduces the recurrence risk of neural tube defects (NTDs) by about two thirds (MRC Vitamin Study Research Group, 1991), various national recommendations about periconceptional folate acid intake to prevent NTDs were instituted in the early 1990s. These varied in terms of target group, recommended intake and method of achieving this intake (Cornel & Erickson, 1997). There is some evidence from the UK and The Netherlands that the proportion of women taking supplements incorporating folic acid has been increasing, but still less than half start to take these before conception, partly because so many pregnancies are unplanned (Wild et al., 1997; De Walle et al., 1999). From the point of view of investigating possible associations between nutrient intake and congenital anomalies, variation in intake is not solely between those who take supplements and those that do not. Therefore, the question arises as to whether the variation in diet among those who do not take supplements is important in terms of risk of adverse reproductive outcome. However, there have been relatively few studies of diet and congenital anomalies. Third, in regard to diet, validation of the method of assessment is required. It is important that the assessment method takes account of food fortification, e.g. breakfast cereal with folic acid. Fourth, the need for adjustment for total energy intake has been discussed extensively in the cancer literature. Such adjustment enables systematic differences in the completeness of reporting of dietary intake between cases and controls to be taken into account. The most commonly used method of adjusting for energy intake in the reproductive epidemiology field appears to be the standard multivariate model (e.g. Shaw et al., 1999a) (Shaw et al., 1999a). However, when many nutrients which are inter-correlated are included, the model may become unstable. In addition, inclusion of a specific nutrient together with energy changes the biological meaning for energy. For example, inclusion of fat would result in the term for energy indicating the relationship with intake of carbohydrate plus protein (Willett, 1990). Among other methods of adjustment for energy intake that have been proposed, the nutrient residuals technique, which allows assessment of the coefficient effects of total energy intake and intake of a specific nutrient (Willett, 1990), appears to be the easiest to interpret. Fifth, the inter-correlations between intakes of specific nutrients makes it difficult to identify the independent effects

of any one nutrient. This problem arises because people eat foods rather than nutrients, and because supplements tend to contain several vitamins and minerals. Randomised controlled trials are the definitive method of evaluating the effect of a single nutrient. Insight may also be gained from investigations of genetic polymorphisms affecting nutrient metabolism (Little, 1999; Clayton & McKeigue, 2001). So far, the most intensively investigated such polymorphism in relation to congenital anomalies is the C677T mutation in the *MTHFR* gene (Botto & Yang, 2000). In regard to possible gene-nutrient interaction, this has as yet been little studied in relation to congenital anomalies. A further issue is statistical power to detect effects of specific nutrients, especially when there is a need to adjust for other nutrients. Finally, most dietary hypotheses are formulated in terms of nutrients which are included in nutrient databases or are present in supplements. However, other biologically active constituents may be relevant.

2. Nutrients and congenital anomalies

As congenital anomalies are aetiologically heterogeneous, the association with nutrients will be discussed for specific classes of congenital anomalies. It is recognised that in some studies, all malformations or major malformations have been considered as a group. These studies will not be covered in this review, except for those relating to vitamin A, because of the concern about a possible teratogenic effect of this vitamin in humans (Rosa, 1983; Lammer et al., 1985). In the UK in 1985, concern was expressed about a possible increase in self medication with vitamin A (Lancet, 1985) and, more recently, it was pointed out that a possible consequence of recommendations to women of reproductive age to consume a minimum daily amount of folic acid to prevent NTDs might be an increased intake of supplemental vitamin A (Oakley & Erickson, 1995). Vitamin A is an established teratogen in experimental animals (Cohlan, 1953; Shenefelt, 1972; Geelen, 1979; Nau et al., 1994). Observations of specific types of anomalies among the offspring of mothers who took isotretinoin during pregnancy, and in studies in experimental animals, suggested that vitamin A may have a specific effect on cranial neural crest cells (Teratology Society, 1987). Orofacial clefts are the most frequent anomalies of structures thought to be derived from cranial neural crests cells. The available studies of reported intake of dietary and supplemental vitamin A during pregnancy and both (a) congenial anomalies involving structures thought to be derived from cranial neural crest cells and (b) major malformation (Table 1) are inconsistent (Miller et al., 1998; Dolk et al., 1999).

2.1 Neural tube defects

In regard to the epidemiology of NTDs, secular and seasonal variation, and variation by social class are the features most readily attributed to a dietary cause (Elwood et al., 1992a). Most dietary hypotheses have not attempted to explain other features of the epidemiology of these defects, such as the variations by ethnic group, maternal parity, or the sex of the infant. A supplement of 4mg of folic acid per day in the periconceptional period was shown to reduce by two thirds the recurrence risk of NTDs by about two thirds in a randomised trial published in 1991 (MRC Vitamin Study Research Group, 1991). A year later, a multivitamin and mineral supplement containing 0.8 mg of folic acid was found to reduce the risk of first occurrence of NTDs in a Hungarian randomised trial (Czeizel & Dudás, 1992). These findings were consistent with the results of an earlier non-randomised trial of a multivitamin supplement containing 0.4 mg of folic acid (Smithells et al., 1980; Smithells et al., 1983). These results were supported by cohort studies (Smithells et al., 1976; Milunsky et al., 1989), most case-control studies (Schorah et al., 1983; Winship et al., 1984; Molloy et al., 1985; Yates et al., 1987; Mulinare et al., 1988; Bower & Stanley, 1989; Mills et al., 1989; Werler et al., 1993; Friel et al., 1995; Shaw et al., 1995a; Suarez et al., 2000) and two further small randomised trials (Laurence et al., 1981; Kirke et al., 1992). These studies have been reviewed by Little (1995) (Little, 1995) and by Botto et al. (2000) (Botto et al., 2000a) and there have been several reviews of the trials (Lumley et al., 2000; Moore, 2001; Turner et al., 2001). The observational studies suggest a halving of risk associated with periconceptional use of multivitamins or supplements containing folic acid. Blood folic acid levels are lower in mothers of NTD cases than controls (Daly et al., 1995; Wald et al., 1996).

In a community based intervention study in China in areas of low risk (southern) and high risk (northern) in China, a daily 0.4 mg folic acid supplement reduced the risk of NTDs (Berry et al., 1999). A 70% reduction in risk occurred among the fetuses or infants of women resident in northern China who took folic acid, compared with a 16% reduction in the south. The reduction in risk in the subgroup of women who had the highest level of compliance with the instruction to take the daily supplement from the time of their premarital examination until the end of the first trimester of pregnancy, i.e. who took folic acid more than 80% of the time, was 85% in the northern region and 40% in the southern region.

In March 1996, the US Food and Drug Administration (FDA) authorised the addition of folic acid to enriched grain products, and made compliance mandatory by January 1998 (Honein et al., 2001). Substantial increases in serum and red blood cell folate levels of among women of reproductive age in the US population have been reported following fortification (Centers for Disease Control, 2000). Using national birth certificate data on live births in the United States for the period 1990 to 1999, the

prevalence at birth of NTDs declined from 3.7 per 10,000 live births in the period October 1995 to December 1996, i.e. before fortification, to 3.05 per 10,000 live births in the period October 1998 to December 1999, i.e. after mandatory fortification (Honein et al., 2001). This represents a 19% decline. The decline for spina bifida was 23%, and for anencephalus 11%. There are two important limitations of using birth certificate data. First, fetal deaths and still births are not included. Both are common in pregnancies complicated by NTD (Little & Elwood, 1992). Second, birth certificate data excludes pregnancies terminated following prenatal diagnosis of NTD, and this complicates the interpretation of time trends (Elwood & Little, 1992; Mills & England, 2001). In an attempt to address this problem, Honein et al. (Honein et al., 2001) examined the changes in NTD prevalence in the sub-set of women who received prenatal care only in the third trimester of pregnancy or who did not receive any prenatal care this suggested a 17% decline. Overall, these data suggest that the effect of folic acid fortification is less than the reduction suggested on the basis of case-control studies (Mills & England, 2001).

In a multi-centre case-control study in the United States and Canada in which data on 1242 infants or fetuses with NTDs were compared with 6660 infants or fetuses with malformations not related to vitamin supplementation, the odds ratio (OR) of NTD associated with use folic acid antagonists during the first or second months after the last menstrual period was 2.8 (95% CI, 1.4-4.6) (Hernández-Diaz et al., 2001). The prevalence of exposure to folic acid antagonists was low – 2.2% in cases and 1% in controls. The major types of anomaly included in the control group were hypertrophic pyloric stenosis, indeterminate sex for pseudo-hermaphroditism, musculo-skeletal anomalies of the skull or face, foot deformities, anomalies of the diaphragm, gastroschisis/omphalocoele, oesophageal stenosis, stenosis of the large intestine or anus, congenital dislocation of the hip and hypospadias. A comparison was also made with 1626 non-malformed infants and 2138 infants with chromosomal or Mendelian anomalies. The use of the alternative control groups had little effect on the results observed.

In view of the importance of folate in the aetiology of NTDs, and because some studies suggest that some women who had a pregnancy complicated by NTD also had abnormal homocysteine metabolism (Steegers-Theunissen et al., 1991; Steegers-Theunissen et al., 1994; Mills et al., 1995), there has been considerable interest in the possible role of genetic variation in the metabolism of folate and homocysteine. The most studied genetic variant has been the C677T allele of the gene coding for 5, 10-metheylenetetrahydrofolate reductase (MTHFR). Homozygotes for this allele have reduced enzyme activity and higher plasma homocysteine levels in persons whose folate status is not optimal (Botto & Yang, 2000). Compared to infants without the allele, infants homozygous for the C677T allele are at a moderately increased risk of NTDs, and mothers homozygous for the allele also appear to have an increased risk of having a pregnancy complicated by NTD.

In general, homocysteine levels in mothers of infants/fetuses with NTDs tend to be only mildly elevated above normal, in contrast to levels 2-4 times higher in persons homozygous for the MTHFR C677T variant (Lucock, 2000).

It has been suggested that there is an inverse association between NTDs and serum vitamin B_{12} that is independent of the association with blood folate levels (Kirke et al., 1993). However, this was not observed in the analysis of serum B_{12} in women who participated in the MRC trial of prevention of recurrence of NTDs (Wald et al., 1996).

There has been interest in possible effects of zinc on the occurrence of NTDs since zinc deficiency in the rat during pregnancy induces malformations of the fetus and in particular the central nervous system (CNS) (Hurley & Swenerton, 1966). In studies on NTDs, zinc status has been assessed in maternal serum, hair, leukocytes, plasma after an oral zinc tolerance test, toenails and amniotic fluid, with conflicting results (Elwood et al., 1992b; Tamura & Goldenberg, 1996; Shah & Sachdev, 2001). Zinc analysis in serum or plasma requires collection of blood into trace-mineral-free containers to avoid falsely high values due to contamination, and also separation or placing under refrigeration as soon as possible after blood collection, again to avoid falsely high values (Tamura et al., 1994). Infection, fever or the intake of a protein rich meal lower plasma zinc levels, whereas long-term fasting gives an analysed value that is higher than normal (Hallberg et al., 2000). Other biochemical indices such as zinc levels in white blood cells, erythrocytes and hair have not so far proved useful in identifying zinc status. Velie et al. (1999) (Velie et al., 1999) reported an OR of NTDs associated with reported preconceptional dietary and supplemental zinc intake of 0.65 (95% CI, 0.43-0.99) for the highest quintile compared with the lowest. This was adjusted for maternal ethnic origin, education, age and body mass index. Reported total intake of zinc was highly correlated with reported intakes of total folate, total iron and total calcium, and dietary protein, methionine and energy. While further adjustment for folate, iron and energy had no marked effect on the ORs observed, after further adjustment for calcium, methionine or protein, the relationship was attenuated (data not shown for calcium; the association was no longer apparent after adjustment for methionine or protein). In the cohort study of Milunsky et al. (1989) (Milunsky et al., 1989), no association with supplemental zinc intake was observed.

In three case-control studies, one in Finland and two in the United States, no association between NTDs and maternal consumption of coffee or caffeine was observed (Golding, 1995). No clear relationship between NTDs and maternal consumption of tea has been found (Elwood et al., 1992b).

In view of the observation that N-nitroso compounds can induce CNS abnormalities in animal models, Croen et al. (2001) (Croen et al., 2001) investigated the association between NTDs and dietary intake of nitrate, nitrite and nitrogen compounds and intake of nitrate from drinking water (Croen et al., 2001). No association was apparent. There was an increase of anencephalus for nitrate levels above the 5mg per litre maximum contaminant level, but not for spina bifida. In addition, there was an increase risk of anencephalus for nitrate levels above the maximum contaminant levels among ground water drinkers but not associated with exposure to mixed water containing nitrate at levels comparable with the concentration in ground water. Previous studies of NTD risk and nitrate concentration in water have been inconsistent. They related to all CNS defects combined or NTDs as a group.

In six studies, five in the United States and one in Sweden, a two-fold or greater increase in the risk of NTDs associated with maternal obesity before pregnancy, based on a BMI of 29 or more, has been observed (Prentice & Goldberg, 1996; Källén, 1998; Shaw et al., 2000; Hendricks et al., 2001). Studies in California, Sweden and Minnesota suggest that maternal weight gain in pregnancy of less than 10kg is associated with an increased risk of NTDs (Shaw et al., 2001). These observations appear to be consistent with reports of elevated risks of these defects following "famine winter" of 1944-5 in the Netherlands and in Germany where food shortages also occurred (Elwood et al., 1992b).

The observational studies and trials assessing the use of multi-vitamin supplements indicate that vitamin A in the doses used in multi-vitamins does not increase the risks of NTDs (Czeizel & Rockenbauer, 1998; Miller et al., 1998; Mastroiacovo et al., 1999). NTDs are not neural crest derived tissues, and are not an important constituent of the isotretinoin embryopathy. However, Turner et al. (2001) (Turner et al., 2001) noted that in the MRC trial, the prevalence of NTDs was higher in the group who received multivitamins in addition to folic acid than in the group which received folic acid alone. While they acknowledge that this could have been due to chance, they also speculate that the difference in prevalence might be the result of some women being exposed to high doses of vitamin A.

2.2 Limb defects

In the Hungarian randomised controlled trial of a multivitamin and mineral supplement during the periconceptional period, one of the groups which contributed to the overall reduction in the prevalence at birth of congenital malformations in the multivitamin supplement group compared with the trace element group was limb deficiencies (Czeizel, 1993). In case-control studies, an inverse association between limb reduction defects and maternal multi-vitamin supplement use in the

periconceptional period of pregnancy has been found (Shaw et al., 1995b; Yang et al., 1997; Werler et al., 1999) (Table 2). Two of these studies presented results according to type of limb deficiency; in one, the effect was confined to limb reduction defects other than longitudinal deficiencies (Yang et al., 1997), while in the other the effect was confined to limb reduction defects other than transverse deficiencies (Shaw et al., 1995b). In a case-control study in Hungary in the period 1980-1991, the OR of limb deficiencies associated with maternal use of a supplement containing folic acid at any time during pregnancy was 0.85 (95% CI 0.63-1.14) (Czeizel et al., 1996). This was similar to the OR for congenital malformations of all types. In an analysis based on this same study during the period 1980-1994, no association between limb deficiencies and use of supplements containing vitamin A was found (Czeizel & Rockenbauer, 1998).

On the basis of two studies, there appears to be no association between limb anomalies and maternal obesity (Waller et al., 1994; Shaw et al., 2000). In one of these studies no relationship with maternal weight gain in pregnancy was observed (Shaw et al., 2000).

2.3 Cardiac anomalies

In the Hungarian randomised control trial of a multivitamin supplement versus a supplement containing trace elements only during the periconceptional period, there were 10 infants with anomalies of the cardiovascular system in the multivitamin group and 20 in the trace element group (relative risk 0.48, 95% CI 0.23-1.03) (Czeizel, 1996). This difference was mainly accounted for by two cases of ventricular septal defect in the multivitamin group and eight cases in the trace element group. The association between congenital cardiovascular anomalies as a group and maternal multivitamin supplement use has been assessed in two case-control studies (Table 3). First, in Hungary during the period 1980-1991, the OR associated with use of a supplement containing folic acid at any time during pregnancy was 0.86 (95% CI 0.77-0.96) (Czeizel et al., 1996). In an analysis of these data over the extended period 1980-1994, no association with use of supplements containing vitamin A was observed (Czeizel & Rockenbauer, 1998). Second, in a study in the Atlanta area, the OR of non-syndromic cardiac defects associated with periconceptional multi-vitamin use was 0.76 (95% CI 0.60-0.97) (Botto et al., 2000b).

As cardiac anomalies are likely to be aetiologically heterogeneous, a number of investigations have been focussed on specific types of heart defects (Table 3). In two studies of cardiac outflow tract (conotruncal) defects, an inverse association with maternal multivitamin supplement use in the periconceptional period was found (Shaw et al., 1995b; Botto et al., 2000b), while in a third no

association was apparent (Werler et al., 1999). In a further study total (i.e. dietary and supplemental) folate intake was inversely related to the risk of cardiac outflow tract defects among those with transposition, but positively related among those with normally related vessels (Scanlon et al., 1998). However, no such difference was apparent for supplemental folic acid use only, which suggests that any effect is associated not with folic acid but with another component of multi-vitamin supplements or a correlate of multi-vitamin supplement use. The results of the two available studies of ventricular septal defects and periconceptional multivitamin use are inconsistent (Werler et al., 1999; Botto et al., 2000b).

In a comparison of data on 3870 infants with cardiovascular defects and 8387 infants with malformations other than cardiovascular, neural tube, urinary tract or limb reduction defects and other than oral clefts, the relative risk of cardiovascular defects associated with maternal use of a folic acid antagonist of any type was 2.1 (95% CI 1.5-3.0; (Hernández-Díaz et al., 2000)). The risks were increased for the three categories of cardiovascular malformation considered – conotruncal, ventricular septal and other defects. Maternal supplementation with multivitamins containing folic acid appeared to reduce the risk associated with dihydrofolate reductase inhibitors but not antiepileptics.

In two recent studies, a higher proportion of homozygotes for the MTHFR C677T variant was found in children or fetuses with congenital heart disease than in controls (Junker et al., 2001; Wenstrom et al., 2001). In one of these studies, subgroup analysis suggested that this was for pulmonary valve stenosis, hyperplastic left heart syndrome, coartion of the aorta, aortic valve stensosis or subaortic stenosis (Junker et al., 2001). In the study of Wenstrom et al., 2001 (Wenstrom et al., 2001), homocysteine levels in amniotic fluid were higher in pregnancies affected by isolated congenital heart disease than in control pregnancies. It is unclear whether this is due to maternal or fetal factors. Both of these studies were small and neither study investigated parental genotypes.

Cardiac outflow tract defects account for a substantial proportion of cases of congenital anomalies of structures with a neural crest cell contribution. In view of the possible association between high intake of vitamin A and anomalies of structures arising from neural crest cells, the association between cardiac outflow tract defects and vitamin A intake has been investigated. In a multi-centre hospital based study in which the controls were infants with malformations of structures not derived from cranial neural crest cells, the prevalence of use of supplements vitamin A during the first trimester of pregnancy was low. The relative risk of conotruncal defects for daily users of vitamin A supplements in lunar months one and two were 3.0 (95% CI 0.8-11.2), and slightly lower for use in the third lunar month (Werler et al., 1990). Subsequently, in a study in California, none of 207 case mothers and 7 of 481 control mothers used single vitamin A supplements (OR 0, 95% CI 0-2.2)

(Shaw et al., 1996). In the Baltimore-Washington area, compared with an average of less than 10,000 IU, retinol intake of 10,000 IU or more from supplements was associated with an increased risk for transposition of the great arteries (OR 9.2, 95% CI 4.0-21.2), but not for outflow tract defects with normally related arteries (OR 0.8, 95% CI 0.1-6.6) (Botto et al., 2001). Similar intakes of dietary retinol and carotenoids were not associated with an increased risk for either type of outflow tract defect. In a Hungarian study, no association between cardiovascular anomalies of all types combined and use of supplements containing vitamin A in the first trimester was observed (Czeizel & Rockenbauer, 1998).

On the basis of data from the National Neonatal Screening Programme in the Netherlands and data recorded in the EUROCAT Central Registry, it has been suggested that there is an increased prevalence of birth of congenital heart disease in babies with phenylketonuria (PKU) (Verkerk et al., 1991). In another study, a relationship between phenylalanine levels during pregnancy and the frequency of congenital heart disease in the offspring was observed (Lenke & Levy, 1980). In a international collaborative study, 416 live born offspring from 412 maternal PKU pregnancies were compared with 100 offspring from 99 control pregnancies (Levy et al., 2001). 34 of 235 offspring from pregnancies in PKU women with a basal phenylalanine level of 900 microM or more and who were not in metabolic control (i.e. phenylalanine level less than 600 microM) by the eighth gestational week had congenital heart disease compared with 1 control offspring. Coarctation of the aorta and hyperplastic left heart syndrome were over-represented compared with expected percentages among those with congenital heart disease in the general population.

In a case-control study in the Atlanta area, severe nausea during pregnancy was associated with a reduced risk of congenital heart defect in the child (OR 0.81, 95% CI 0.67-0.99) (Boneva et al., 1999). This effect was most pronounced when the anti-nausea medication and Bendectin®. In a meta-analysis, Bendectin® was associated with a slight reduction in the risk of congenital heart defects (McKeigue et al., 1994). Boneva et al. (1999) (Boneva et al., 1999) noted that one of the ingredients of Bendectin® is vitamin B_6 (pyridoxine: 10mg) and that vitamin B_6 alone has been used for treatment of nausea and vomiting during pregnancy. The usual recommended dose of Bendectin® was 2-3 tablets a day. Therefore, an intriguing possibility is that high doses of vitamin B_6 might reduce the risk of congenital heart disease.

There appears to be no association between cardiovascular malformations and reported maternal intake of coffee or caffeine (Golding, 1995).

Waller et al. (1994) (Waller et al., 1994), in a case-control study in California and Illinois in 1995-97,

reported a relative risk of transposition of the great vessels associated with maternal obesity of 6.2 (95% CI 1.4-27.4). The corresponding relative risk (RR) for defects of septal closure was 2.1 (95% CI 0.7-6.4) and for other heart defects 0.8 (95% CI 0.2-3.5). In a subsequent case-control study in California in 1987-8 in which conotruncal defects were considered, the RR for α-transformation of the great vessels was 1.4 (95% CI 0.7-3.1), while that for tetralogy of Fallot was 0.5 (95% CI 0.2-1.4) (Shaw et al., 2000). There was no association in the latter study with maternal height or weight gain in pregnancy (Shaw et al., 2000). In a study in the Atlanta area of the USA of infants born in the period 1968-8, the RR of major isolated heart defects associated with maternal pre-pregnancy overweight or obesity (BMI > 26) was 1.36 (95% 0.95-1.93) in comparison with average-weight pre-pregnancy (BMI 19.9 –22.7) (Watkins & Botto, 2001). The corresponding RR for underweight women (BMI < 16.5) was 0.04 (95% 0.43-0.9). In a study in Germany there were elevated RRs for transposition of the great arteries and truncus arteriosis but not cardiovascular anomalies of all types combined, associated with a BMI greater than 30 (Queisser-Luft et al., 1998).

2.4 Gastroschisis/omphalocele

In a study based on 55 cases of gastroschisis and 182 matched controls in which maternal nutrient intake in the trimester before conception was assessed using a food frequency questionnaire, the nutrients most associated with gastroschisis in multivariate analysis were low alpha-carotene, low total glutathione and high nitrosamines (Torfs et al., 1998). In a case-control study of a range of malformations in Hungary, the OR of exomphalos/gastroschisis associated with use of folic acid supplementation at any time during pregnancy was 0.89 (95% CI 0.55-1.43) while that associated with supplements containing vitamin A use was 1.18 (95% CI 0.7-1.9) (Czeizel et al., 1996; Czeizel & Rockenbauer, 1998).

On the basis of one study, there appears to be no association between gastroschisis and reported maternal intake of coffee or caffeine (Golding, 1995).

In one study in which omphalocele and gastroschisis were not separated, the relative risk of both defects combined associated with maternal obesity was 2.5 (95% CI 1.1-6.0) (Waller et al., 1994).

2.5 Orofacial clefts

Evidence as to the possible effects of vitamin supplementation on the occurrence of orofacial clefts is of two kinds (1) trials which are largely non-randomised; (2) case control studies. The largest of the non-randomised trials was carried out in the Czech Republic (Tolarova & Harris, 1995). The

supplement included 10mg of folate, 6,000 international units of vitamin A and other vitamins and minerals. The trial was conducted in women who had a previous child with non-syndromic cleft lip with or without cleft palate, or cleft palate, or if the mother or the mother's partner was affected with a cleft. The relative risk in those who took supplements versus those who did not was 0.4, but this was not statistically significant. Two other trials, which were smaller and involved lower doses of folate, again including other vitamins, showed a reduced recurrence risk, also not statistically significant (Table 4). All three trials are subject to selection bias. One randomised trial of prevention of first occurrence of major malformations has been published (Czeizel & Dudás, 1992). Its results do not suggest an effect of a multivitamin supplement containing 0.8mg folate on the occurrence of orofacial clefts, but this trial was not designed to test this specific hypothesis and the statistical power was inadequate for this purpose.

Case-control studies of orofacial clefts and use of vitamin supplements are summarised in Table 5. Overall, the evidence is inconsistent. This may reflect methodological differences, differences in the type and composition of supplements used in different populations in different time periods, different patterns of gene-nutrient interaction in populations with diverse genetic backgrounds, the play of chance, or some combination of these. The data from Hungary (Czeizel et al., 1996; Czeizel et al., 1999) has been interpreted as suggesting a dose related effect of folic acid supplements.

In contrast to research on NTDs, there appears to be virtually no work on biochemical markers of nutrient status and orofacial clefts. Wong et al. (1999) (Wong et al., 1999) recently reported on orofacial clefts and the effect of a methionine loading test to determine homocysteine levels in 35 mothers of cases and 56 mothers of controls. Levels of plasma total homocysteine both in the fasting state and after methionine loading tests were determined. These suggested statistically significantly higher levels of plasma total homocysteine in both these circumstances. Serum and red cells folate levels were higher in cases than controls. By contrast, serum vitamin B_{12} difference and whole blood pyridoxal-5-phosphate, a marker for vitamin B_6, were higher in controls that in cases. The findings have some similarities with an earlier small study of neural defects by the same group (Steegers-Theunissen et al., 1991) and suggest that derangement of folate metabolism is involved in the aetiology of orofacial clefts.

In view of evidence of the familial aggregation of orofacial clefts and the varied results of the above studies, research is ongoing on the possible role of gene-nutrient interaction in aetiology. A few studies of the MTHFR polymorphism and orofacial clefts have been published (Shaw et al., 1998; Tolarova et al., 1998; Mills et al., 1999; Gaspar et al., 1999; Shaw et al., 1999b), and others are in progress. Possible gene-nutrient interaction has as yet been little studied and so far, the studies have

lacked statistical power to detect departures from multiplicative interaction. Work also is ongoing on biochemical markers of maternal folate status.

In a comparison of data on 1962 infants with oral clefts and 8387 infants with malformation other than oral clefts and neural tube, cardiovascular, urinary tract or limb reduction defects the relative risk of oral clefts associated with maternal use of folic acid antagonist of any type was 2.1 (95% CI 1.4-3.2; (Hernández-Díaz et al., 2000)). In regard to antiepileptic drugs, the inverse risk appeared to be specific for cleft lip ± palate.

Vitamin A has been investigated in relation to the aetiology of clefts primarily in the context of anomalies of structures derived from the neural crest cells. There has been some controversy about the relationship between these anomalies and use of supplements containing vitamin A. One study of anomalies of structures derived from these cells suggested that high levels of dietary and supplemental retinol intake may increase the risk (Rothman et al., 1995). Data specifically on orofacial clefts and use of supplements containing vitamin A are inconsistent (Table 6). No clear relationship with maternal blood retinol or vitamin A levels has been found. However, blood retinol level is under tight homeostatic control (Hunter, 1990), does not clearly reflect dietary intake, and the metabolic factors controlling it are unclear. In the trial of prevention of recurrence of oral clefts in the Czech Republic (Tolarova & Harris, 1995) (Table 4), the multivitamin supplement included 6000 IU of vitamin A. The recurrence risk was reduced in those who took the supplement.

There appears to be no association between oral clefts and reported maternal intake of coffee or caffeine (Golding, 1995).

The relationships between oral clefts and maternal body mass index, or height, or weight gain in pregnancy has been analysed only in one investigation (Shaw et al., 2000). No associations were apparent.

2.6 Hypospadias

In a cohort study of diet and pregnancy outcome in the Bristol area of the UK, there was positive association with maternal vegetarianism in pregnancy (OR 3.53, 95% CI 1.56-7.98), with use of iron tablets in the first 18 weeks of gestation (OR 1.87, 95% CI 1.02-3.46) and with consumption of pulses during pregnancy (OR for use 4 or more times a week compared with no use 7.56, 95% CI 2.25-25-42) (North et al., 2000). In the Hungarian case-control study, the OR for hypospadias associated with maternal folic acid supplementation at any time during pregnancy was 0.88 (95% CI 0.78-1.01), and

that associated with use of supplements containing vitamin A was 0.89 (95% CI 0.8-1.0) (Czeizel et al., 1996; Czeizel & Rockenbauer, 1998).

2.7 Down Syndrome

DNA hypomethylation has been associated with abnormal chromosome segregation, and methyl deficiency can result in DNA hypomethylation (Rosenblatt, 1999). This has led to investigations of the *MTHFR* C677T polymorphism and folate metabolism in mothers of children with Down Syndrome. About a two-fold increase in the risk of Down Syndrome in mothers who were homozygous or heterozygous for the C677T-variant was observed both in an initial study (James et al., 1999) and in an expansion of this study (Hobbs, 2000). In the initial study, a significant increase in plasma homocysteine concentrations and lymphocyte methotrexate cytotoxicity was observed in the mothers of children with Down Syndrome. In the subsequent study, homozygosity for the methionine synthase reductase (MTRR) A66G polymorphism was associated with an increased risk of a having child with Down Syndrome. In a study in Ireland, there was no significant association between maternal MTHFR C677T genotype and Down Syndrome in the offspring, but there was a positive association with homozygosity and heterozygosity for the MTRR A66G variant (O'Leary et al., 2002). The MTRR variant was not associated with increased plasma homocysteine.

In a large case-control study in California, maternal consumption of four or more cups of coffee per day was inversely associated with the risk of Down Syndrome (OR 0.63, 95% CI 0.41-0.96) (Torfs & Christianson, 2000). This was interpreted as suggesting that high levels of caffeine intake may result in miscarriage of a fetus with Down Syndrome. Case and control mothers who had high levels of coffee consumption had previous miscarriage rates that were about 50% higher than mothers who consumed less coffee.

2.8 Urinary tract malformations

In a randomised trial comparing multi-vitamin supplement taken during the periconceptional period with a supplement containing trace elements only in Hungary in Hungary, urinary tract abnormalities appeared to contribute to a reduction in the prevalence at birth of congenital anomalies of all types (Czeizel, 1996). Two cases of congenital anomalies of the urinary tract occurred in the multi-vitamin group, compared with 9 in the trace element group (relative risk 0.22, 95% CI 0.05-0.99). This difference appeared to be due mainly to obstructive defects of the urinary system. In a case-control study in Hungary, the ORs of renal agenesis associated with folic acid supplementation any time during pregnancy was 0.53 (95% CI 0.23-1.26) and for cystic kidney 0.68 (95% CI 0.35-1.31)

(Czeizel et al., 1996). The OR of cystic kidney associated with maternal vitamin A use during pregnancy was 0.70 (95% CI 0.3-1.5) (Czeizel & Rockenbauer, 1998). In two case-control studies in the United States, there was an inverse association between urinary tract defects and maternal multivitamin use in early pregnancy (Werler et al., 1999).

In a comparison of data in 1100 infants with urinary tract malformations and 8387 infants with malformations other than urinary tract, neural tube, cardiovascular or limb reduction defects, or oral clefts, the relative risk of urinary tract malformations associated with maternal use of a folic acid antagonist of any type was 2.1 (95% CI 1.2-3.7; (Hernández-Díaz et al., 2000)). Very few subjects were exposed to dihydrofolate reductase inhibitors. Maternal supplementation with multivitamins containing folic acid did not appear to reduce the risk associated with antiepileptics.

Table 1

Association between maternal intake of vitamin A during pregnancy and broad groups of congenital anomalies

(a) Anomalies of structures derived from cranial neural crest tissue

Study design	Exposure	Timing	RR (95% CI)	Adjusted for	Reference
Case-control, malformed controls	Supplements containing vitamin A	1st month 2nd month 3rd month	2.5 (1.0-6.2) 2.3 (0.9-5.8) 1.6 (0.6-4.5)	No appreciable confounding was observed	Werler *et al.*, 1990
Cohort	Retinol intake (supplemental non-supplemental) >15, 000 IU per day vs. < 5,000 IU per day	1st trimester	3.5 (1.7-7.3)	Maternal age, education, ethnic group; family history of birth defects; folate intake; alcohol consumption; genital herpes infection; treated diabetes; fever; use of antiseizure medicine, retinoids or exogenous hormones	Rothman *et al.*, 1995
Case-control, non-malformed controls	Supplemental vitamin A (mostly < 8,000 IU vs. none)	1 month before conception to 3 months after	1.4 (0.6-3.2)	No appreciable confounding was observed	Khoury *et al.*, 1996
Case-control, non-malformed controls	Supplemental vitamin A, > 8,000 IU per day vs. > 5,000 IU	Periconceptional	1.1 (0.3-3.7)	No appreciable confounding was observed	Mills *et al.*, 1997

Association between maternal intake of vitamin A during pregnancy and broad groups of congenital anomalies

(b) Major malformations

Study design	Exposure	Timing/dose	RR (95% CI)	Adjusted for	Reference
Case series	25,000 – 500,000 IU vitamin A per day	Early pregnancy	Undefined	-	Rosa *et al.*, 1986
Case-control	Use of drugs containing 10,000 IU or more of vitamin A	Pregnancy; < 40,000 IU vs. no use	0.5 (0.1-1.6)	-	Martínez-Frías & Salvador, 1990
		≥ 40,000 IU vs. no use	2.7 (0.8-11.7)		
RCT	4,000 or 6,000 vitamin A, 11 other vitamins, 4 minerals and 5 trace elements	Periconceptional	0.7 (0.5-0.9)	RCT	Czeizel, 1993
Cohort	Retinol intake (supplemental and non-supplemental) > 15,000 IU per day vs. < 5,000 IU	1st trimester	2.1 (1.3-3.4)	Maternal age, education, ethnic group; family history of birth defects; folate intake; alcohol consumption; genital herpes infection; treated diabetes; fever; use of antiseizure medicine, retinoids or exogenous hormones	Rothman *et al.*, 1995
Case-control	Supplemental vitamin A (mostly < 8,000 IU vs. none)	1 month before conception to 3 months after	0.9 (0.4-1.8)	No appreciable confounding was observed	Khoury *et al.*, 1996
Case-control	Supplemental vitamin A, > 8,000 IU per day vs. < 5,000 IU	Periconceptional	1.1 (0.5-2.2)	No appreciable confounding was observed	Mills *et al.*, 1997
Case series (n=120)	50,000-300,000 IU vitamin A per day	1st 9 weeks	0.0	-	Mastroiacovo *et al.*, 1999

Table 2

Association between the first occurrence of limb defects and use of supplements containing folic acid and other vitamins

Area and period of study	Supplements			Cases		RR (95% CI) use vs. non use of supplements	Adjusted for	References
	Source	Type of supplement	Frequency of use by control mothers (%)	Type	N			
US, California, 1987-8	Supplements	Multivitamins containing folic acid in period from 1 month before to 2 months after conception	67.8	All	178	0.7 (0.4-1.1)	Maternal ethnicity, education, age, gravidity, smoking and alcohol use	Shaw *et al.*, 1995b
Hungary, 1980-91	Supplements	Folic acid (1-2 x 3 mg/day) ± other drugs at any time during pregnancy	54.9	All	395	0.9 (0.6-1.1)	Controls matched with are on sex, birth week and district of residence of parents	Czeizel, 1996
USA and Canada, multicentre, 1993-6	Supplements	Multivitamin use between 1 month before LMP and end of 3rd lunar month	88	Limb reduction defects	31	0.5 (0.2-1.1)	Maternal age, education, ethnic group, planned pregnancy, nausea/vomiting; centre	Werler *et al.*, 1999

Table 3

Association between the first occurrence of congenital heart defects and use of supplements containing folic acid and other vitamins

Area and period of study	Supplements			Cases		RR (95% CI) use vs. non use of supplements	Adjusted for	References
	Source	Type of supplement	Frequency of use by control mothers (%)	Type	N			
USA, Atlanta, 1968-80	Supplements	Multivitamin use from 3 months before pregnancy to end of 3rd month of pregnancy	14.2	Non-syndromic cardiac defects	958	0.8 (0.6-1.0)	Maternal ethnic group, maternal diabetes, period of birth	Botto *et al.*, 2000b
US, California, 1987-8	Supplements	Multivitamins containing folic acid in period from 1 month before to 2 months after conception	67.8	Conotruncal heart defects	207	0.5 (.03-0.9)	Maternal ethnicity, education, age, gravidity, smoking and alcohol use	Shaw *et al.*, 1995b
Hungary, 1980-91	Supplements	Folic acid (1-2 x 3 mg/day) ± other drugs at any time during pregnancy	54.9	All types	2976	0.9 (0.8-1.0)	Controls matched with cases on sex, birth week and district of residence of parents	Czeizel, 1996
USA and Canada, multicentre, 1993-6	Supplements	Multivitamin use between 1 month before LMP and end of 2nd lunar month	88	Conotruncal defects	157	1.0 (0.7-1.5)	Maternal age, education, ethnic group, planned pregnancy, nausea/vomiting and centre	Werler *et al.*, 1999
				VSD	186	1.2 (0.8-1.8)		
USA, Maryland, Washington, DC, N Virginia 1987-9	Supplements	Multivitamins containing folic acid (≥ 400 mg vs. < 400 mg per day) in year before conception	17% (≥ 400 mg)	Non-syndromic outflow tract defects	126	1.0 (0.6-1.6)	Parity, marital status, drug use, daily use of ≥ 10,000 IU of supplemental vitamin A	Scanlon *et al.*, 1998

Table 4

Trials of vitamin supplementation and orofacial clefts

Country	Eligibility criteria	Type of supplement	Number supplemented		RR (95% CI) use vs. non use of supplements	Reference
			Total	With clefts		
Trials of prevention of recurrence, non- randomised						
Czech Republic	Previous child with non-syndromic CL(P) or mother or her partner affected	MV, including 10 mg folate and 6000 IU vitamin A	221	3 (CL)	0.4 (0.1-1.1)	Tolarova & Harris, 1995
New York	Previous child with orofacial cleft	MV, including 0.5 mg folate and 12,500 USP vitamin A	59	0	0.0 (p>0.05)	Conway, 1958
Newark, NJ	Previous child with orofacial cleft	MV, including 5 mg folate and 10 mg B_6	176	4 (CL/P)	0.5 (0.2-1.4)	Peer *et al.*, 1964
Randomised trial of prevention of first occurrence						
Hungary	Family planning programme, not currently pregnant	MV, including 0.8 mg folate and 4000-6000 IU vitamin A	2471	4 (CL(P))	1.3 (0.3-5.8)	Czeizel & Dudás, 1992)

Table 5

Association between the first occurrence of orofacial clefts and use of supplements containing folic acid and other vitamins

Area and period of study	Supplements			Cases		RR (95% CI) use vs. non use of supplements	Adjusted for	References
	Source	Type of supplement	Frequency of use by control mothers (%)	Type	N			
Finland, 1967-71	Prescribed and non-prescribed	Iron and/or vitamins	51.5	All CP CL(P)	599 232 232	1.1 (0.8-1.4) 1.0 (0.7-1.4) 1.5 (1.0-2.1)	Cases and controls matched on area of residence and time of pregnancy	Saxén, 1975
England and Wales, 1983-4	Prescribed drugs in first trimester	Vitamins Folic acid	1.6 0.9	All	676	0.9 (0.6-1.4) 1.1 (0.8-1.5)	Cases and controls matched on general practice and birth date ± 3 months	Hill *et al.*, 1988
USA, Atlanta, 1968-80	Supplements	Multivitamin use in periconceptional period or in first postconceptional month	25.9	All (non-syndromic) CP CL(P)	309 87 222	0.6 (0.4-0.9) 0.8 (0.4-1.5) 0.5 (0.3-0.8)	Maternal age, education, sex of baby, smoking status, influenza, epilepsy, diabetes and family history; matched on ethnic group, birth period and hospital of birth	Itikala *et al.*, 2001
US, California, 1987-9	Supplements	Multivitamins containing folic acid	69.1	All CP CL(P)	731 141 348	0.6 (0.4-0.7) 0.7 (0.5-1.2) 0.5 (0.4-0.7)	No appreciable confounding was observed. Effect modification by alcohol observed for isolated CL(P) but not other subgroups	Shaw *et al.*, 1995c

Area and period of study	Source	Supplements		Cases		RR (95% CI) use vs. non use of supplements	Adjusted for	References
		Type of supplement	Frequency of use by control mothers (%)	Type	N			
Hungary, 1980-91	Supplements	Folic acid (1-2 x 3 mg/day)	54.3 55.6	CP CL(P)	435 940	0.7 (0.6-1.0) 0.8 (0.7-1.0)	Controls matched with cases on sex, birth week and district of residence of parents	Czeizel, 1996
Hungary, 1993-6	Supplements	0.8 mg folate and other vitamins and minerals	Cohort study	CP CL(P)	2 4	1.1 (0.1-10.9) 1.1 (0.2-6.6)	Parity and previous pregnancy outcomes	Czeizel *et al.*, 1999
USA and Canada, multicentre, 1988-91	Supplements	Multivitamin use between one month before LMP and end of 4th lunar month	85 (malformed controls)	All CP CL(P)	303 108 195	1.4 (0.8-1.7) 0.9 (0.5-1.6) 1.3 (0.8-2.1)	Maternal education, dietary folate and calorie intake	Hayes *et al.*, 1996
USA and Canada, multicentre, 1993-6	Supplements	Multivitamin use between one month before LMP and end of 4th lunar month	88	CP	46	0.4 (0.2-0.8)	Maternal age, education, ethnic group, planned pregnancy, nausea/vomiting and centre	Werler *et al.*, 1999
		Multivitamin use between one month before LMP and end of 3rd lunar month		CL(P)	114	0.8 (0.5-1.3)		

Area and period of Study	Source	Supplements: Type of supplement	Supplements: Frequency of use by control mothers (%)	Cases: Type	Cases: N	RR (95% CI) use vs. non use of supplements	Adjusted for	References
Brazil, Sao Paulo, 1991-2	Supplements	Use in first four months of pregnancy	73	CP	96	0.7 (0.4-1.1)	Pollution, heredity, maternal hypertension, contraceptive use	Loffredo *et al.*, 2001
				CL(P)	354	0.6 (0.4-0.8)	Pollution, heredity, maternal epilepsy, maternal hypertension, use of anti-inflammatory, anti-hypertension and pain relieving drugs, contraceptive use	

Table 6

Association between maternal intake of vitamin A during pregnancy and orofacial clefts

Study design	Number of cases	Nature of exposure	Timing or dose	RR (95% CI)	Adjusted for	Ref
Case-control, malformed controls	1332	Supplements containing vitamin A	1st month 2nd month 3rd month	2.3 (0.8-6.6) 2.0 (0.6-6.0) 1.3 (0.4-4.6)	No appreciable confounding was observed	Werler *et al.*, 1990
Case-control, non-malformed controls	731	Supplements containing vitamin A	1 month before conception to 3 months after	0.6 (0.2-1.5)	-	Knox, 1972
Case-control, non-malformed controls	CL±P 954 CP 433	Vitamin A supplements	1st trimester	CL±P 1.0 (0.6-1.8) CP 0.3 (0.1-1.4)	Controls were matched with cases on sex, birth week and district of residence of parents	Czeizel & Rockenbauer, 1998
Case-control, non-malformed controls	59	Maternal serum retinol	Postnatal	Similar between mothers of cases and controls	-	Niebyl *et al.*, 1985
Case-control, non-malformed controls	141	Maternal serum retinol	Postnatal	Lower in mothers of cases than controls	-	Munger *et al.*, 1996
Nested case-control, non-malformed controls	CL±P 14	Maternal plasma vitamin A	Early pregnancy	Similar between mothers of cases and controls	-	Stoll *et al.*, 1999

Table 7

Association between the first occurrence of urinary tract malformations and use of supplements containing folic acid and other vitamins

Area and period of study	Supplements			Cases		RR (95% CI)	Adjusted for	References
	Source	Type of supplement	*Frequency of use by control mothers (%)*	Type	N	use vs. non use of supplements		
Hungary, 1980-91	Supplements	Folic acid (1-2 x 3 mg/day) at any time during pregnancy	54.9	Renal agenesis	43	0.5 (0.2-1.3)	Controls matched with cases on sex, birth week and district of residence of parents	Czeizel, 1996
				Cystic kidney	73	0.7 (0.4-1.3)		
USA and Canada, multicentre, 1993-6	Supplements	Multivitamin use between 1 month before LMP and end of 4th lunar month	88	All	184	0.6 (0.4-0.9)	Maternal age, education, ethnic group, planned pregnancies, nausea/vomiting; centre	Werler *et al.*, 1999
USA, Washington State, 1990-1	Supplements	Multivitamin use in 1st trimester	88	All without chromosomal anomalies	118	0.2 (0.1-0.4)	Maternal ethnic group, family income, country of maternal residence, year of birth	Li *et al.*, 1995

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CHAPTER II.2 SMOKING

Julian Little, May 2002

1. Limitations of epidemiologic studies

Issues in the interpretation of associations between smoking and congenital anomalies include potential recall bias, confounding, and the interpretation of dose-response relationships.

There has been considerable discussion about the possible existence of recall bias, but few studies have attempted to demonstrate or to quantify it. In a comparison of prospective and retrospective responses between 27 mothers of cases of sudden infant death syndrome and 25 mothers of control infants, Gibbons et al, (1993) (Gibbons et al., 1993) found good agreement regarding parental smoking habits for both cases and controls. Verkerk et al. (1994) (Verkerk et al., 1994) compared prospective and retrospective responses for 2806 mothers, and compared recall of smoking during pregnancy between mothers of (a) infants with congenital anomalies, (b) stillborn infants, (c) infants small for gestational age, (d) infants born pre-term, (e) infants of low birth weight and (f) control infants. The only statistically significant evidence of recall bias was for infants small for gestational age. Wong and Koren (2001) (Wong & Koren, 2001) found that, compared with information recorded during pregnancy, the mothers of 25 infants with fetal distress decreased their reports of smoking after delivery, whereas the mothers of 95 women who had uneventful deliveries did not. More generally, the available evidence comparing information collected retrospectively with that collected before the pregnancy outcome was known does not demonstrate any severe bias for those who have had an adverse pregnancy outcome compared with those who have had a normal birth (Little, 1992). Furthermore, it has been shown that recall bias can lead to spurious inferences only under extreme conditions (Drews & Greenland, 1990; Swan et al., 1992; Khoury et al., 1994).

As smoking is related to socio-economic status (Whitehead, 1988), and to other aspects of lifestyle, potential confounding is an issue. Potential confounding has been assessed in most studies. However, the identification of new possible risk factors such as maternal obesity and use of vitamin supplements for some types of congenital anomalies raises the question about the adequacy of control for confounding in earlier work.

It is possible that different levels of exposure may lead to different reproductive problems. For example, low levels of intra-uterine exposure might result in a malformed birth, whereas a higher level of exposure to the same toxicant could result in a fetal death. In these circumstances, a non-traditional dose-response relationship might be observed (Selevan & Lemasters, 1987).

2. Neural tube defects

Except in a few small studies and/or studies in which potential confounding by social class was not adequately controlled, no positive association between NTDs and smoking has been observed (Elwood et al., 1992; Källén, 1998).

3. Limb defects

Early studies suggested a positive association with maternal smoking which did not reach statistical significance (Källén, 1997a). In a large case-control study in Hungary, the OR for total limb defects between categories of never, 1-9 cigarettes per day and 10 or more cigarettes per day was 1.68 (95% CI 1.26-2.24) adjusted for education and birth order (Czeizel et al., 1994). In a record linkage based study in Sweden, the OR of limb reduction defects associated with maternal smoking was 1.26 (95% CI 1.06-1.50), adjusted for year of birth, maternal age and parity (Källén, 1997a). For the subset of infants born in 1986 or 1991, additional adjustment for maternal socio-economic index was possible but had little impact on the OR observed, suggesting that the socio-economic status was not a major confounder. A dose-response relationship was apparent. In an analysis of birth defects in risk factor surveillance data from the northern Netherlands, in which combinations of 32 diagnostic categories and 77 risk factors recorded in a birth defects registry were analysed, a positive association between reduction deformities of the upper and lower limbs and maternal smoking was observed, with an OR of 1.73 (95% CI 1.04-2.78) (Cornel et al., 1997). This association was checked against data from the Atlanta area of the USA, and the corresponding OR was 1.31 (95% CI 0.92-1.84). In a study in California, an increased risk of limb reduction defects associated with maternal smoking was not observed in the absence of paternal smoking (Wasserman et al., 1996).

In the study in the Netherlands already described, there was a positive association between maternal smoking and deformities of the foot (OR 1.73, 95% CI 1.27-2.34), which was supported by data from the Atlanta area (OR 1.26, 95% CI 1.04-1.52) (Cornel et al., 1997). In Washington State, the OR for club foot associated with maternal smoking was 2.6 (95% CI 1.6-4.0) for boys and 1.4 (95% CI 0.6-3.2) for girls (Alderman et al., 1991).

4. Cardiac anomalies

The association between maternal smoking and congenital cardiovascular anomalies is inconsistent (Wasserman et al., 1996). In some analyses, the association with conotruncal heart defects specifically has been assessed, but again the results seem to be inconsistent.

5. Gastroschisis

In four out of five studies of maternal smoking and gastroschisis, a positive association has been observed (Haddow et al., 1993; Torfs et al., 1994; Curry et al., 2000). In a combined analysis of three of the studies, the OR associated with maternal smoking was 1.6 (95% CI 1.2-2.2) (Haddow et al., 1993). In the study with the largest number of cases, the association with maternal smoking did not persist after adjustment for maternal education, annual family income, marital status, maternal recreational drug use, and factors associated with the mother's childhood (Torfs et al., 1994).

6. Orofacial clefts

A meta-analysis of the association between maternal cigarette smoking and oral clefts was carried out by (Wyszynski et al., 1997), based on 11 studies. These authors reported combined ORs for both cleft lip and cleft palate of about 1.3, based on 11 studies. In addition, an increased risk of similar magnitude has been observed in more recent studies (Shaw et al., 1996; Beaty et al., 1997; Källén, 1997b; Lieff et al., 1999; Romitti et al., 1999; Lorente et al., 2000; Chung et al., 2000; Beaty et al., 2001).

7. Hypospadias

In a large study in Sweden, maternal smoking was not associated with an increased risk of hypospadias (Akre et al., 1999).

8. Down syndrome

The results of studies of the association between maternal smoking and Down Syndrome are inconsistent (Little & Vainio, 1994; Chen et al., 1999). A number of earlier studies, mostly based on small numbers of infants, reported an inverse association with smoking (Källén, 1997c; Chen et al., 1999). In a meta-analysis published in 1993, a combined OR of 0.84 (95% CI 0.71-0.99) was calculated (Kline et al., 1993). The authors noted that publication bias was a potential explanation for this observation. In recent large studies in Sweden, Washington State and California, no overall association between Down Syndrome overall and smoking was observed (Källén, 1997c; Chen et al., 1999; Torfs & Christianson, 2000). In the study in Washington State, an inverse association was apparent when broad categories of maternal age were adjusted for, but no association was apparent when exact year of maternal age in conjunction with ethnic group and parity were adjusted for (Chen et al., 1999). This suggests that there was substantial potential for residual confounding by maternal age in studies of maternal smoking and Down Syndrome.

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CHAPTER II.3 IONISING RADIATION

Pat Doyle, February 2003

Review of the known causes of congenital malformation

Ionising radiation (IR) includes X rays, gamma rays, and alpha and beta high energy particles. All have the ability to break atomic bonds and alter molecular structures within cells, including DNA. Human populations are exposed to IR from a variety of sources and exposures (in order of proportion of total dose) for the UK population include radon gas from the ground (50%), gamma rays from the ground and buildings (14%), medical sources including x rays (14%), food and drink (12%), cosmic rays (10%), occupational sources (0.3%), fallout from nuclear tests (0.2%), and very small amounts from nuclear discharges and consumer products. For any individual their cumulative dose will depend on age, calendar period, area of residence, medical treatment, and type of work.

Congenital malformations following *in utero* exposure

Ionising radiation at high dose is a known teratogen (Committee on the Biological Effects of Ionising Radiations 1990). Animal work has clearly demonstrated a relationship between increasing dose and an increased likelihood of malformation and fetal death (Mole 1992). In humans, large doses of IR were used to induce therapeutic abortion in the 1920's and 1930's and the high exposures received by pregnant Japanese women as a result of the atomic bombs resulted in a variety of adverse pregnancy outcomes, including stillbirth, microcephaly, mental retardation, retarded growth and altered sex ratio (Neel & Schull 1956; Otake & Schull 1984; Yamazaki & Schull 1990). This work demonstrated that the fetal nervous system is particularly susceptible to damage from IR between 8 and 15 weeks gestation (Mole 1992; Neel & Schull 1956; Otake & Schull 1984; Yamazaki & Schull 1990).

The teratogenic effects of exposure to IR at low levels, usually taken to be cumulative doses less than 100mSv, is less well understood. There has been a considerable amount of epidemiological research on cancer and it is generally accepted that *in-utero* doses in the order of 10mSv is associated with an increased risk of cancer in childhood (Doll & Wakeford 1997). Theoretically, radiation-induced damage to the fetus may also lead to fetal death, or to an excess of surviving pregnancies with chromosomal defects or other malformations, but the epidemiological evidence for such effects is equivocal (Committee on the Biological Effects of Ionising Radiations 1990; Sever 1991; Kline et al 1989).

In the 1960's it became possible to examine the chromosome constitution of fetal material using karyotyping. Since it was already know from animal studies that IR could lead to chromosomal re-arrangement *in vitro,* the observation that spontaneously aborted fetuses had an increased proportion of

chromosomal anomalies led to speculation that IR exposure of the mother before or during pregnancy could lead to genetic damage of the fetus. Although some studies showed an association between maternal x-rays during pregnancy and increased risk of Downs syndrome and other trisomies in offspring (Creasy et al 1976; Boue et al 1975), no clear pattern has emerged (Kline et al 1989). Studies on other anomalies in relation to X- ray exposure have not demonstrated any consistent association with doses below 5 mSv (Brent 1989; NRPB 1993).

The Chernobyl incident of 1986 stimulated renewed interest in possible effects of IR exposure during pregnancy. An increased frequency of trisomy 21 in the former West Berlin, and increases in the frequency of neural tube defects in several small studies in Turkey, have not been confirmed in larger epidemiological studies within Western Europe (Little 1999; Dolk & Nichols 1999). Overall there is no strong evidence to date that the low-level exposures received by pregnant women living in Western Europe at the time of the incident resulted in an increase in prevalence of congenital malformations in their children.

Congenital malformations following pre-conceptual exposure

It is known from animal work that IR at high dose is a powerful mutagen (Committee on the Biological Effects of Ionising Radiations 1990). Since many congenital malformations are thought to have a genetic component it is possible that exposure to IR before conception could result in increased risk of malformation in subsequent offspring. However human evidence for a genetic effect of pre-conceptual irradiation is mixed, and generally negative.

Early studies of the offspring of survivors of the Hiroshima and Nagasaki A bombs did not demonstrate an increase in poor perinatal outcome (Neel & Schull 1956). A more recent re-analysis of "untoward" pregnancy outcome (pregnancy ending in child with a major congenital anomaly and/or stillbirth and/or death within 14 days of birth) in over 70,000 pregnancies over 20 weeks gestation to women in Hiroshima and Nagasaki found a small but non-significant trend of increasing risk with increasing maternal dose before conception (Otake et al 1990).

Another group of women with fairly high exposure consists of those treated with IR for cancer or other conditions in childhood and early adulthood. Studies of reproductive outcome in these cohorts have so far been equivocal, the majority finding no convincing evidence of a link between maternal preconceptual dose and major congenital malformation in offspring (Byrne 1999; Goldberg et al 1998; Kallen et al; Hawkins 1991). However, an increase in fetal deaths observed in two of these studies should be noted (Goldberg et al 1998; Kallen et al; Hawkins 1991).

Most occupational epidemiological research has focussed on the relation between paternal exposure to IR and cancer in their children (Gardner et al 1990; Beral et al 1993; Draper et al 1997; Roman et al 1999; McLaughlin et al 1993; Roman et al 1996). Evidence for a genetic effect of low-dose preconceptional exposure in fathers on the risk of cancer, in particular childhood leukaemia, in their offspring caused considerable concern at the time of publication (Gardner et al 1990). But several other studies since then have not confirmed this finding (Beral et al 1993; Draper et al 1997; Roman et al 1999; McLaughlin et al 1993; Roman et al 1996).

There have been notably fewer studies investigating congenital malformations and occupational exposure to IR, especially for exposure in men, reflecting the difficulty of obtaining data on congenital malformations in a systematic way. A case-control study of congenital malformations conducted in the 1980s in USA examined parental occupational exposure to low-level ionising radiation at the Hanford nuclear facility (Sever 1988). Neural tube defects showed a statistically significant association with both paternal and combined maternal and paternal pre-conceptual radiation exposure but eleven other defects, including Down's syndrome, showed no evidence of such an association. The authors did not interpret the neural tube finding as causal. A further case-control study used record linkage to identify congenital anomalies among liveborn children of workers in Canada's largest nuclear electrical generating company (Green et al 1997). Congenital malformation risk was not increased for children whose parents had been occupationally exposed to ionising radiation before their conception. Similarly, a large study of the health of offspring of nuclear workers in the UK did not find evidence of a link between congenital malformations following maternal or paternal preconceptual exposure to IR (Doyle et al 2000). However the latter study did report an increased risk of late fetal loss for offspring of women exposed to IR before conception, a finding which is currently being investigated by the study team.

Conclusion

Although IR is a know terotogen and mutagen at high dose, most epidemiological studies to date do not support a hypothesis linking in-utero or parental preconceptual exposure at levels normally encountered in medical, occupational or environmental settings and congenital malformations in offspring.

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CHAPTER II.4 MATERNAL DIABETES

Ester Garne, January 2004

In 1989 the St Vincent declaration (World Health Organisation, 1990) set as a goal that "the outcome of diabetic pregnancy should approximate that of the non-diabetic pregnancy".

Maternal diabetes is a known risk factor for congenital malformations (CM). It has also been known for many years that good metabolic control in the preconceptional period decreases the risk of CM (Greene, 1999). Maternal hyperglycemia is a non-specific teratogen imposing the same risk of CM to pregnant women with both type-1 and type-2 diabetes (Schwartz et al, 2000; Farrel et al, 2002).

The prevalence of pregestational diabetic pregnancies among live and stillbirths is reported from 0.28% (Sheffield et al, 2002) to 0.35% (Savona-Ventura et al, 2003) and 0.38% (type 1 diabetes) (Penny et al, 2003). During the last 10 years an increasing number of infants and children are diagnosed with type 1 diabetes and it seems as if the increase is related to birth populations after 1985 (Feltbower et al, 2003; Svensson et al, 1985; Gale, 2002). Therefore the number of pregnant women with pregestational diabetes will increase over the next decades. Further with the increasing number of obese women the rate of type-2 diabetes in pregnancy is also expected to increase within in the next decades.

Data from four recent studies on the risk of CM in pregnancies with pre-existing diabetes is presented in Table 1. The risk of CM in these four populations of diabetic women were 6.0% - 9.7%. The study from Liverpool (Casson et al, 1997) with the highest risk was from a region without written guidelines for the management of pregnancy in diabetic women. Older studies have shown both higher and lower risk of CM (Greene, 1999). It is important to include data from terminations of pregnancy in studies on malformation risk in diabetic pregnancies, as these pregnancies are considered high-risk pregnancies and therefore may be offered more intense prenatal ultrasound investigations to search for CM.

As the definition of CM and the follow-up period for diagnosis vary between studies it is difficult to compare the percentages directly. On the other hand there is no doubt, that the risk of CM for diabetic pregnancies even with optimal metabolic control is at least twice as high as for non-diabetic pregnancies. Further, the relationship between maternal hyperglycemia in early pregnancy and the frequency of CM seems to be linear without any threshold level (Greene, 1999; Schwartz et al, 2000).

The spectrum of CM in diabetic pregnancies differs from non-diabetic pregnancies with a higher proportion of malformations affecting the central nervous system including neural tube defects, the heart

and the kidneys (Schwartz et al, 2000; Sheffield et al, 2002; Becerra et al, 1990). Further the very rare malformation caudal regression syndrome has a very strong relation to maternal diabetes (Greene, 1999; Schwartz et al, 2000; Becerra et al, 1990).

A recent prospective, population based study showed a prevalence of cardiac malformations of 36 pr 1000 births in pregestational diabetic pregnancies compared to 7.4 pr 1000 in the non-diabetic background population (Wren et al, 2003). The spectrum of cardiac malformations was dominated by malformations of the outflow tract and this observation has also been described by others (Becerra et al, 1990; Myer-Wittkopf et al, 1996; Lofredo et al, 2001). For the prenatal ultrasound screening it is important to know, that the malformations of the outflow tract may not be seen by the four-chamber view. Detailed assessment of the outflow tract is necessary and therefore prenatal echocardiography may be considered if prenatal screening for cardiac malformations in the diabetic pregnancies is requested.

One of the problems in achieving good preconceptional diabetic control is the proportion of unplanned pregnancies. Differences in the proportion of unplanned diabetic pregnancies makes it difficult to compare the outcome of diabetic pregnancies between regions and countries. The proportion of planned pregnancies in the European countries varies from 10-20% to 85% (EUROCAT Working Group, 2003). An American study with interview of 85 women with diabetes showed that despite the majority of women were aware of the importance of optimising preconceptional glucose levels only 41% of the women planned their pregnancy (Holing et al, 1998). This difference between regions and countries in the proportion of planned diabetic pregnancies will affect the prevalence of CM in the diabetic pregnancies.

Besides the increased risk of congenital malformations in diabetic pregnancies, these women also have an increased risk of spontaneous abortions (Greene, 1999). Later in pregnancy there is an increased risk of preterm birth or birth complications related to macrosomia. Perinatal mortality and neonatal morbidity is significantly increased for infants from diabetic pregnancies (Greene, 1999; Schwartz et al, 2000). All these pregnancy complications are related to the level of diabetic control during pregnancy.

Prevention of congenital malformations in diabetic pregnancies

To diminish the risk of CM tight preconceptional and first trimester diabetic control with HgA1c values below 8% is important. Further preconceptional folic acid supplementation of 5 mg/day is recommended. Perhaps also antioxidants (vitamin C and E) and multivitamins lower the risk of CM for diabetic women (Schwartz et al, 2000; Zaken et al, 2001; Correa et al, 2003).

Table 1

Region	Method	Period	Total diabetic pregnancies[1]	% Cong malf	% Cong Malf in non-diabetic pregnancies
Malta (6)	Population based	1999-2001	44	6,4%	3,7%
Texas (5)	Hospital based	1991-2000	410	6,1%	1,5%
Scotland (7)	Population based	1998-99	219	6,0%	N.A.
Liverpool (11)	Population based	1990-94	369	9,7%	N.A.

[1]Livebirths, stillbirths and terminations for malformations
N.A.: not available

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CHAPTER II.5 MATERNAL EPILEPSY

Elisabeth Robert, January 2004

Environmental Causes of Congenital Anomalies: Maternal Epilepsy and Anti-epileptics

Approximately 20% of individuals with epilepsy are of childbearing potential and about 3 to 5 births per thousand will be to women with epilepsy. It has been known for several decades that the risk of major and minor malformations in infants of epileptic mothers is twice higher than in the general population. This is partly attributable to the teratogenicity of the drugs used to treat epilepsy, but the disease in itself most probably increases the risk, while no epidemiological technique is ideal to separate the effects of the disease and the drugs on the fetus. Very few epileptic patients can live without treatment, and those who can might be questioned as whether or not they actually are epileptic. Studies have shown a marked increase in malformations amongst infants exposed to first trimester seizures (Lindhout & Omtzigt, 1992). However, most investigators have found that maternal seizures during pregnancy had no impact on the frequency of malformations, or on the development of infant epilepsy or febrile convulsions (Dansky & Finnell, 1991; Canger et al, 1999). There have been reports of maternal seizures during pregnancy being associated, though, with an increased risk of miscarriage, preterm labor, intracranial hemorrhage, fetal hypoxia with bradycardia and possible developmental or learning difficulties. In a recent study 57 children, whose mothers did have a history of epilepsy, but did not have any antiepileptic medication or any seizures during their pregnancies, were evaluated. Compared with a matched control group there was no difference between the two groups of children in either IQ scores or physical features (facial dysmorphia or digit hypoplasia) (Holmes et al, 2000).

Several dysmorphic syndromes have been described, related to classical anti-epileptics, i.e. *phenytoin, valproic acid, trimethadion, carbamazepine, phenobarbital and primidone.* The developmental anomalies occur in about 1/4 of cases, they resemble each other and may be referred to as an anticonvulsant- or epilepsy- syndrome : epicanthal folds, hypertelorism, hirsute foreheads, broad flat nasal bridges, upturned nasal tip, straight thin upper lips, and also distal digital hypoplasia. These children tend to be small for dates, and have an increased risk for psychomotor retardation (Rudd & Freedom, 1979; Bethenod & Frederich, no date).

Whereas maternal epilepsy induces a two to three-fold increase in the risk of any kind of major malformations, four main types of defects are overrepresented in infants of epileptic mothers : orofacial clefts, cardiovascular defects, spina bifida and hypospadias. Preferential associations have been found in

different studies between specific anticonvulsants and malformations. A markedly increased risk is observed after combination therapy with two or more anticonvulsants (Lindhout & Omtzigt, 1992; Kaneko et al, 1999; Samrén et al, 1999). What must be considered in these cases, however, is that the mothers had a particularly severe form of epilepsy.

Phenobarbital and primidone are effective in focal epilepsy and grand-mal seizures. In two recent studies, observed rates of major malformation were about 5% for phenobarbital monotherapy (Canger et al, 1999; Kaneko et al, 1999). They are mainly congenital heart diseases and oral clefts. Van der Pol (Van der Pol et al, 1991) reported that cognitive development of children who had been exposed in utero to phenobarbital alone or in combination with carbamazepine was significantly impaired in comparison with children of non-epileptic mothers.

Diazepam and clonazepam have proven themselves as anti-epileptics. They are never used in monotherapy to treat epilepsy, but benzodiazepine therapy does not seem to have as high a teratogenic risk as the other anticonvulsants.

Phenytoin is the most widely used hydantoin derivative with an anti-epileptic effect. Its teratogenic potential has been known since 1964 (Janz & Fuchs, 1964). Originally, the anomalies observed were called ”fetal hydantoin syndrome” (Hanson & Smith, 1975) : craniofacial dysmorphia, anomalies of the distal phalanges, pre and post-natal growth retardation, and cardiac defects. Limitations in cognitive development have frequently been observed (Vanoverloop et al, 1992; Scolnik et al, 1994). The most prominent of the observed major malformations are ptosis, iris coloboma, cleft lip and palate, microcephaly, short neck, hypoplasia of nails and distal phalanges in fingers and toes, finger-like thumbs and hip dysplasia (Lu et al, 2000). However, in most cases of affected children only a few of these mentioned anomalies are present. The differences in susceptibility to specific malformations is expressed by genetically diverse siblings who shared approximately comparable uterine environments (Phelan et al, 1982; Raymond et al, 1995). Moreover, Buehler and colleagues (Buehler et al, 1990) demonstrated that infants with low detoxification capacities that were exposed to phenytoin *in utero* were at increased risks for the fetal hydantoin syndrome, compared with similarly exposed individuals with normal epoxide hydrolase activity.

Carbamazepine is used for grand-mal epilepsy, focal and psychomotor seizures as well as for trigeminal neuralgia. It is teratogenic in human beings. Carbamazepine increases the risk of spina bifida about 10-fold, that is, the malformation occurs in about 1% of exposed newborns. Apart from this there have been reports of hypospadias, microcephaly, urinary tract anomalies, and some facial dysmorphic features

(Jones et al, 1989; Rosa, 1991; Källén, 1994; Robert & Källén, 1994; Matalon et al, 2002). The risk of cognitive disturbances with carbamazepine monotherapy was retrieved in subsequent studies (Ornoy & Cohen, 1996), and observed in particular among children who also have facial dysmorphia.

Ethosuximide is effective with petit-mal seizures. There are a few reports only of ethosuximide therapy during pregnancy. No typical pattern of birth defects has been observed in the infants of 57 treated women (Ornoy & Cohen, 1996). In a series of 18 fetuses exposed in the first trimester, there were no birth defects (Rosa, cited in Briggs et al, 1998). Although the available reports are in no way sufficient for a differentiated risk assessment, there does not seem to be a teratogenic risk comparable to the other anticonvulsants.

Valproic acid is effective for different forms of epilepsy. Typical for valproic acid is an approximate 20-fold increased risk of spina bifida or other neural tube defects, such that the observed incidence of these defects among exposed children is 1-2% (Robert & Guibaud, 1982). A dose response relationship was found in several recent studies. Women receiving daily doses of 1000 mg or more were at a significantly increased risk for having a child with a major malformation (Kaneko et al, 1999; Samrén et al, 1999). Several cases of radial ray anomalies have been reported after fetal valproate exposure (Seip, 1976; Jaeger-Roman et al, 1986). Children exposed to valproate in utero also appear to be at greater risk for perinatal distress (43 %) and low Apgar scores (28 %), postnatal growth deficiency and microcephaly (Seip, 1976; Robert & Jouk, 1992). Also craniosynostosis, especially trigonocephaly, has been specifically associated with maternal valproic acid treatment (Ardinger et al, 1988). Other major malformations have been associated with valproic acid, but only as case reports (eg gastroschisis, septo-optic dysplasia). A clinical study was reported, describing 57 children with anticonvulsant syndrome, 46 of which having been exposed to valproic acid in utero. Eighty per cent of these children had behavioral problems, including attention deficit and hyperactivity disorder, autistic features or Asperger's syndrome (Lajeunie et al, 2001). Other studies seem to confirm these findings (Moore et al, 2000; Bescoby-Chambers et al, 2001; Williams et al, 2001; Adab et al, 2001). Examining neonatal behavior and later neurologic functions in a study of 40 children, exposed in utero to a single AED, those exposed to valproic acid were most compromised. Valproic acid serum concentrations at birth correlated with the degree of neonatal hyperexcitability and neurologic dysfunction at the age of 6 (Adab et al, 2001).

A number of novel antiepileptic drugs have been marketed in the 90s : *vigabatrin, lamotrigine, felbamate, gabapentin, sultiame, tiagabine, topiramate, levetiracetam, oxcarbamazepine and zonisamide*. While some of these compounds have been available for some time, the clinical experience with pregnant women is extremely limited. Existing experience is insufficient to estimate if any teratogenic risk, comparable to that of the classic anticonvulsants, does exist. The available, still unpublished (Dean et al,

2002), prospective case observations of the manufacturer on 302 prospective reports of pregnancies with first trimester exposure to lamotrigine as monotherapy. Of these pregnancies, nine children had congenital malformations, for a rate of 3%, ie a rate lower than the average frequency of birth defects in women with epilepsy using monotherapy (Samrén et al, 1999). Moreover, prospective and retrospective data collected from 39 women exposed to gabapentin during pregnancy did not suggest a teratogenic risk (Glaxo Wellcome). The limited number of exposed cases, the fact that most of them did not receive monotherapy but a combination with another anticonvulsant, and the spontaneous recording of the exposed pregnant women do not allow for a differentiated risk assessment.

Folic acid deficiency has been discussed as a possible teratogenic pathway for anticonvulsants (Gabapentin, 2003). A study on folic acid antagonists (including anticonvulsants) and the risk of birth defects (Koch et al, 1996) did not show a protective effect of periconceptional folic acid supplementation, but as this supplementation is now recommended for any woman, epileptic patients should be given 4 mg per day, at least through the 8^{th} week of pregnancy.

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