Monozygotic twinning, cerebral palsy and congenital anomalies

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Background: The majority of cases of cerebral palsy (CP) have their pathogenesis during fetal development and are a form of congenital anomaly, the aetiology of which is uncertain. Anomalous development of other organs evident at birth is also a congenital anomaly. A small proportion of these are known to be caused by chromosomal or gene abnormalities, environmental tetratogens and dietary deficiencies. The majority are of unknown aetiology.

Methods: A review of monochorionic (MC) monozygotic (MZ) placentation in the pathogenesis of congenital anomalies and CP was conducted using the PubMed, MEDLINE, EMBASE and Cochrane databases.

Results: Zygote division and MC placentation have serious implications for the development of both conceptuses. Most reports observe predominantly cerebral abnormalities in one or both conceptuses. These cerebral abnormalities often present as CP or other disabilities attributable to central nervous system impairment. In addition to the anomalies in central nervous system development, anomalies in the fetal development of a wide variety of other organs have been reported with MC MZ twinning.

Conclusions: CP and congenital anomalies share a common pathogenic mechanism attributable to MZ twinning. These abnormalities in singletons are coincident with very early loss of one conceptus. The quantitative contribution of monozygosity and monochorionicity to the genesis of CP and congenital anomalies needs to be made.

Key words: monozygosity / monochorionicity / cerebral palsy / congenital anomalies

Introduction

Congenital anomalies (CA) are an important cause of fetal and infant death and disability. In England and Wales, 2006, there were 669,601 total births of which 3602 were stillbirths and 2325 were neonatal deaths. CA were registered as the main or subsidiary cause of death in 19.4% of the fetal and 36.9% of the neonatal deaths (Office for National Statistics, 2008). The birth prevalence of cerebral palsy (CP) is 2.0–2.5 per 1000 (Stanley and Watson, 1992; Pharoah et al., 1998; Hagberg et al., 2001). However, this is a serious underestimate of the true extent of the problem because those with severe cerebral impairment that would have presented as CP do not survive sufficiently long for the diagnosis to be made.

Twin compared with singleton gestations are at increased risk for both CA and CP. Among twins, zygosity is an important feature...
because the high levels of concordance in monozygotic (MZ) and discordance in dizygotic (DZ) twins have implications for many clinical conditions with a genetic component (Machin, 2005). However, in addition to the genetic implications, there are significant non-genetic abnormalities attributable to twinning. In particular, several clinical abnormalities are more common in MZ than in DZ twins but show low concordance in MZ twins thus indicating that a gene segregation process is not responsible (Knox and Lancashire, 1991). The difference is mainly attributable to monochorionic (MC) placentation that is found in approximately two-thirds of MZ twins. MC placentation with inter-fetal arterial and venous anastomoses have important pathogenic implications for one or both fetuses.

The aim of this review is to summarize the data examining the role of a multiple conception and, in particular, zygosity and chorionicity in the pathogenesis of CA and CP. Initially the review will deal with the possible pathogenic mechanism for CP and follow this with the possible pathogenic mechanism for CA. The hypothesis for a unified pathogenic pathway for CP and CA will finally be considered.

Methods

PubMed was searched, initially from January 1966 to December 2008 for evidence relating to CP, CA and multiple pregnancies. The original search was extended using MEDLINE, EMBASE from 1950 to February 2009 and the Cochrane Library (Issue 1, 2009) to identify additional studies. These studies were supplemented by hand searching the reference lists of relevant systematic reviews. The search terms for this expanded review included a combination of index terms (MZ twins, multiple pregnancy, CP, congenital abnormalities, fetal death) and free text terms (congenital malformations, multiple births, chorionicity, brain damage). Search strategy did not include filters that would limit results to specific publication types or study designs. Systematic reviews, cohort studies, case–control studies, case series or case reports reporting on the association of MZ, specifically monochorionic multiple gestations with CA and/or fetal damage presenting as CP were included. Studies reporting CP of post-natal origin were excluded. No formal quality assessment of the studies was attempted although several general issues relating to both study design and methodological quality were considered when reviewing the data.

Results

Literature search

The searches identified 1825 titles and abstracts. Both authors scanned these and identified potentially relevant articles to be retrieved. Included in the bibliography were 2 systematic reviews, 2 other reviews, 26 cohort studies, 23 case series/case reports and 2 case control studies.

<table>
<thead>
<tr>
<th>Register</th>
<th>Singletons</th>
<th>Twins</th>
<th>Triplets</th>
<th>Quadruplets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern California (Grether et al., 1993)</td>
<td>1.1</td>
<td>6.7</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Western Australia (Petterson et al., 1993)</td>
<td>1.6</td>
<td>7.3</td>
<td>27.9</td>
<td>na</td>
</tr>
<tr>
<td>Japan, Kinki University (Yokoyama et al., 1995)</td>
<td>na</td>
<td>8.5</td>
<td>31.4</td>
<td>111.1</td>
</tr>
<tr>
<td>UK, Merseyside (Pharoah and Cooke, 1996)</td>
<td>2.3</td>
<td>12.6</td>
<td>44.8</td>
<td>na</td>
</tr>
<tr>
<td>UK, North East Thames (Williams et al., 1996)</td>
<td>1.0</td>
<td>7.4</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>China (Liu et al., 2000)</td>
<td>1.5</td>
<td>9.7 All multiples</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Europe: multicenter (Topp et al., 2004)</td>
<td>1.8</td>
<td>7.6</td>
<td>na</td>
<td>na</td>
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</table>

CP = cerebral palsy; na = not available.

The role of MZ twinning in the pathogenesis of CP

Twinning, as a risk factor for CP, was noted over a century ago (Freud, 1897). Early reports of case series, before the establishment of population-based registers of CP, allowed a comparison of proportions of twins and singletons in the series with that in the general population. These reported a 5- to 10-fold risk in twins compared with singletons (Asher and Schonell, 1950; Illingworth and Woods, 1960; Russell, 1961; Alberman, 1964). Population-based registers of CP and multiple birth registers have confirmed these levels of increased risk of CP among multiple births (Grether et al., 1993; Petterson et al., 1993; Yokoyama et al., 1995; Pharoah and Cooke, 1996; Williams et al., 1996; Liu et al., 2000; Topp et al., 2004) (Table I).

The prevalence of CP in singletons and multiples increases sharply with decreasing gestational age at birth. Comparison of crude prevalence rates of CP in singletons and multiples ignores the confounding effect of immaturity as multiples are more likely to be born prematurely. Ideally, gestational age prevalence data are required but such data, even from population-based CP registers, are scarce. However, birthweight specific prevalence rates may be substituted and the confounding effect of birthweight be taken into account.

Table II compares the birthweight specific prevalence of twins and singletons. The striking observation is that for the low birthweight groups <2500 g, there are no statistically significant differences between twins and singletons. In contrast, twins compared with singletons show a highly significant increased prevalence of CP in birthweight group ≥2500 g. It can be concluded that, although some of the increase prevalence of CP in twins is attributable to immaturity, the major part of the twin singleton difference is among infants of normal birthweight. An explanation other than immaturity must explain the difference.

The numerous definitions and classifications that have been proposed for CP are testimony to its complexity (Ingram, 1984). A classification that is relevant to the role of twinning in the causation of CP, is the timing of the cerebral impairment. It may occur pre-partum, intra-partum or post-partum.
Intra-partum CP
Cerebral impairment occurring during labour, usually ascribed to birth asphyxia, accounts for <10% of CP (Nelson and Ellenberg, 1986; Blair and Stanley, 1988; Yudkin et al., 1995; Sugimoto et al., 1995; Jaw et al., 1998). Although twins compared with singletons may be at increased risk of birth asphyxia, it is only of marginal relevance to the contribution of twinning to the aetiology of CP.

Post-partum CP
Cerebral impairment occurring in the neonatal period is predominantly associated with immaturity. In a small proportion, about 10%, the cerebral impairment occurs later in infancy or early childhood as a consequence of conditions such as cerebral infections, trauma, drowning and severe dehydration (Paneth and Kiely, 1984; Pharoah et al., 1986; Burke, 1990; Fusi and Gordon, 1990; Adgebite et al., 1993).

Difficulty in maintaining oxygenation and perfusion in the immature infant may lead to cerebral impairment from periventricular haemorrhage or leukomalacia. Preterm birth is significantly more common in multiple than singleton births and this confers a greater risk of CP as shown in Table I. Post-partum CP accounts for <20% of all CP so the differential contribution of twins and singletons to the overall prevalence of CP is small. Nevertheless, within this subset, it is worth considering whether MZ or DZ twins differ in their contribution. To examine this, the birthweight frequency distribution of MZ and DZ twins has to be compared. Population-based data are not routinely available because zygosity is not usually recorded at birth.

Pre-partum CP
In approximately 70–80% of cases of CP, the cerebral impairment occurs during fetal development. These cases are, therefore, a form of congenital anomaly. As the population prevalence of CP is 2–2.5 per 1000 births, it can be inferred that CP as a congenital anomaly has a prevalence of approximately 1.5–2.0 per 1000 live births. The wide range of motor, cognitive and sensory disabilities that present clinically as CP, are indicative of the extent and spatial distribution of the cerebral impairment that has occurred.

Twinning and CP
It is clear that CP in twins is predominantly associated with an insult that is pre-partum in timing.

Numerous case reports and case series (Melnick, 1977; Yoshioka et al., 1979; Embom, 1985; Yoshida and Soma, 1986; Szymonowicz et al., 1986; Burke, 1990; Fusi and Gordon, 1990; Adgebite et al., 2004) and population-based registers CP draw attention to the high risk of CP when the co-conceptus dies in utero. In another series of 11 cases, three of the live born co-twins died of multiple infarctions of liver and spleen, renal aplasia and CP. Seven of the eleven live births had abnormal cerebral ultrasound images (Van Heteren et al., 1998). Antenatal origin of neurologic damage in multiple gestations was noted particularly in MC gestations with intrauterine death of one twin (Bejar et al., 1990). The feto-fetal transfusion syndrome is, per se, associated with an increased neurological morbidity irrespective of whether or not the infants survived (Cincotta et al., 2000; Dickinson et al., 2005).

Two population-based CP registers observed four and six cases of CP among 33 and 63 live born, respectively, from live born/stillborn twin pairs (Grether et al., 1993; Pharoah and Cooke, 1996). Thus the combined prevalence in these two studies found a prevalence of 104 CP per 1000 births. This is a 40–50-fold increase compared with the population prevalence of 2–2.5 CP per 1000 births. Unfortunately zygosity was not recorded. However, almost all case reports implicate MZ twinning with MC placentaion as the causative factor.

A national population study of the surviving twin whose co-twin suffered intrauterine death reported a CP prevalence of 95/1000 live births when the twin were of the same sex and 29/1000 live births when the twins were of opposite sex thereby confirming the high risk associated with in-utero fetal death of one twin (Pharoah and Adi, 2000).

A systematic review of studies of the co-twin following single twin death found 18% with neurological abnormality in MC and 1% in dichorionic gestations (Ong et al., 2006).

Causal mechanism for the pathogenesis of CP in twins
A proposed explanation for the causal mechanism whereby fetal death of a twin compromised cerebral development of the co-twin, was the development of disseminated intravascular coagulation following the transfer of thromboemboli from the dead to the surviving twin via placental anastomoses (Benirschke, 1961, 1993; Moore et al., 1969). However, this explanation has not been confirmed by the demonstration of thromboemboli. It was subsequently suggested that acute haemodynamic and ischaemic changes resulting from acute twin–twin transfusion at the time of intrauterine death rather than late onset disseminated intravascular coagulation provided the pathogenic mechanism.

An alternative proposal was that fetal death of a twin provided a low pressure sump into which drainage from the surviving twin occurred, thereby compromising its development (Fusi and Gordon, 1990).

Table II Comparison of birthweight-specific cerebral palsy (CP) prevalence rates in twins and singletons (Petterson et al., 1993)

<table>
<thead>
<tr>
<th>Birthweight</th>
<th>Twins</th>
<th>Singletons</th>
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<tbody>
<tr>
<td>No. of</td>
<td>No. of</td>
<td>CP prevalence per</td>
</tr>
<tr>
<td>CP prevalence per</td>
<td>infant</td>
<td>1000 survivors</td>
</tr>
<tr>
<td>survivors</td>
<td></td>
<td>survivors</td>
</tr>
<tr>
<td>No. CP</td>
<td>No. of infants</td>
<td>CP prevalence per</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 survivors</td>
</tr>
<tr>
<td>&lt;1500 g</td>
<td>29</td>
<td>260</td>
</tr>
<tr>
<td>1500–1999 g</td>
<td>16</td>
<td>626</td>
</tr>
<tr>
<td>2000–2499 g</td>
<td>8</td>
<td>1470</td>
</tr>
<tr>
<td>&gt;2500 g</td>
<td>11</td>
<td>2602</td>
</tr>
</tbody>
</table>
1990; Fusi et al., 1991). Explicit in both proposals is that the demise of one twin is causal of pathological abnormality in the other. However, neither proposal adequately accords with all the facts. When both twins are live born and one dies in infancy, the survivor is at high risk of CP and that risk is greater in same-sex than opposite-sex pairs. In a population-based national survey of survivors whose co-twin died in infancy, for those of birthweight <1500 g, the CP prevalence in the survivors of same and opposite sex pairs was 205 and 125 per 1000 live births, respectively. Among infants of birthweight \( \geq 1500 \) g, the prevalence of CP among same sex twins was 32/1000 live births but there were no cases of CP in the 52 opposite sex pairs (Pharoah, 2001). It is highly likely that the differences in CP prevalence among same and opposite sex pairs are in part due to the greater probability of MZ twins being born preterm. However, in addition to this, among twins that are not significantly preterm, MZ compared with DZ twins are at significantly increased risk of CP. These results have been confirmed using data from a Regional Perinatal Mortality survey (Glinianaia et al., 2002). Even among very preterm infants, monochorionicity is a risk factor for CP (Burgueto et al., 1999).

Furthermore, neither of the proposed pathogenic mechanisms explains the observation that there is a high prevalence of congenital anomaly in the demised twin but the surviving co-twin is unaffected (Kilby et al., 1994).

A further observation negating the role of fetal death of a twin as causal of CP or other abnormality in the surviving co-twin, are reports of CP when both twins survive. In a national population-based survey, among all birthweight groups, the prevalence of CP was higher in same compared with opposite sex twins (Pharoah et al., 2002). Data from a Regional CP register observed that when both twins are live births, there is a 1 in 56 probability that one has CP and a 1 in 430 probability that both have CP (Pharoah and Cooke, 1996).

These observations indicate that fetal death of one twin is not a prerequisite for the pathogenesis of CP and other anomalies in multiple births. Chronic feto-fetal transfusion leading to discordance in amniotic fluid volume and fetal growth and with inter-twin haemoglobin disparity is a well recognized complication of MC, MZ placentation. Severe cases, with dangers for the well-being of both conceptuses, are generally associated with a dominant arterio-venous anastomosis. In these cases the fetofetal transfusion is unidirectional. However, bi-directional fetofetal transfusion is also possible and has been proposed to lead to a different form of abnormality (Larroche et al., 1990; Gonen, 1991; Grafe, 1993). This hypothesis denies that fetal death of one twin is causal of abnormality in the surviving co-twin. Instead it proposes that both the fetal death and the abnormality in the co-twin survivor are the result of perturbations in inter-fetal blood flow dynamics associated with placental vascular anastomoses.

It is accepted that clinically recognized and recorded fetal death is associated with CP in the co-twin. This contributes to a small proportion of CP but fails to explain CP in singletons who contribute the majority of pre-partum cases. Following the use of sonography in early gestation, it became clear that multiple conceptions were considerably more common than originally thought and that loss of a fetus in the first trimester of pregnancy was frequently observed (Schneider et al., 1979), a phenomenon referred to as the ‘vanishing’ twin (Landy and Keith, 1998; Anderson et al., 1990). Second trimester loss of a fetus generally presents as a fetus papyraceus. Less common than first trimester loss, nevertheless, it contributes a significant proportion to the problem of fetal loss in multiple conceptions.

Although the implications of late fetal loss on the development of the co-twin have long been recognized, first and second trimester losses were considered to be of no serious concern, a view that now needs to be reconsidered. First trimester loss of a conceptus associated with CP in the surviving infant has been reported (van Bogaert et al., 1996; Weiss et al., 2004). Other reports describe a variety of anomalies in addition to CP following second trimester (Saier et al., 1975; Baker and Doening, 1982; Anderson et al., 1990; Larroche et al., 1990; Bordairier and Robain, 1992; van Bogaert et al., 1996; Jelin et al., 2008) and first trimester (Hoyme et al., 1981; Kapur et al., 1991; Baker et al., 1996) loss.

If first or second trimester fetal loss has potentially serious implications for a co-conceptus, it becomes of paramount importance to record all instances of such fetal deaths if quantification of the contribution of MC MZ twinning to the prevalence of CP and other anomalies is to be achieved. First trimester loss as a vanishing twin needs not only formal recording but also its chorionicity noted and follow-up to birth and beyond into early infancy to enable all possible abnormalities to be diagnosed.

Second trimester loss or a fetus papyraceus is usually recognized on clinical inspection of the placenta and recorded in the obstetric records. However, it is contentious whether it is a legal requirement for fetus papyraceus to be registered as a stillbirth (Pharoah, 2002). There are inter- and intra-national variations in the definitions of fetal death that require legal registration. These variations may be determined by the gestational age at fetal death, the gestational age at expulsion from the womb or by the birthweight. As a result of interest in fetal death registration among multiple births and its association with abnormality in the surviving infant, within a decade there has been a 3-fold increase in the registration of fetus papyraceus and other second trimester fetal deaths and a 6-fold increase in feticides at \(<24\) weeks gestation (Pharoah, 2006). Such variation affects investigation of fetal loss on the prevalence of abnormalities in co-conceptuses. A population-based register of CP observed that, in 6 of 18 fetal death/CP survivor twin pairs noted in the medical records, only the singleton survivor was registered (Pharoah and Cooke, 1997a). This led to the hypothesis that, in apparently singleton infants, the cerebral impairment could be attributed to twinning accounting for a significant proportion of all pre-partum CP (Pharoah and Cooke, 1997b).

The role of twinning in the pathogenesis of CA

In about 20% of fetal and 40% of neonatal deaths, CA are registered as the main or subsidiary cause of death (Office for National Statistics, 2008). Among spontaneously aborted embryos and fetuses, 80% were abnormal. Of the twin fetuses, 21% were abnormal, many with cardiac anomalies (Livingstone and Poland, 1980). Recognized causes of some CA are chromosome and gene abnormalities, environmental teratogens and some nutritional deficiencies. However, these causes account for a minor proportion of CA and in the majority, the cause is not known.

Twins are at greater risk of CA than singletons (Myrianthopoulos, 1976; Layde et al., 1980; Windham and Bjerkedal, 1984; Kallen,
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1986; Doyle et al., 1990; Mastroiacovo et al., 1999; Scher et al., 2002; Li et al., 2003). Among twins, MZ are at greater risk than DZ twins. However, MZ twins are more likely to be discordant than concordant for the anomaly (Cameron et al., 1983; Knox and Lancashire, 1991). The prevalence of congenital anomalies in MC is almost twice that in dichorionic twins (Glinsky et al., 2008). Even conjoined twins are frequently discordant for several abnormalities in non-shared organs (Ornoy et al., 1980). The discordance of CA in MZ twins is poorly understood and it has been argued that epigenetic factors contribute to the discordance (Singh et al., 2002). Nevertheless, genetic and epigenetic factors do not provide a sufficient explanation and other possible aetiologic factors need to be established.

CP from cerebral impairment during fetal development is a form of congenital anomaly that in many instances, perhaps the majority, is attributable to perturbations of feto-fetal transfusion in MC placentation with its vascular anastomoses. Although the brain during fetal development is disproportionately large compared with other organs, nevertheless, it would be irrational to assume that other organs also are not prone to haemodynamic insult. Within this context, a three-dimensional concept of possible fetal impairment has been proposed (Pharoah, 2005).

One dimension is the extent or severity of damage that is caused. This may lead to a range of effects from early embryonal or fetal death through late fetal death, a live birth that dies in infancy, an infant with a congenital anomaly to a normal unaffected infant.

A second dimension is spatial and will determine what organ is affected. Cerebral impairment frequently presents as CP but may present in other ways such as microcephaly with isolated cognitive disability or cortical blindness. Any organ would be at risk leading to cardiac, renal, facial, skeletal etc. abnormalities. Indeed, more than one organ may be impaired.

During the period of fetal development, every organ is undergoing rapid development and change. The timing of the insult provides the third dimension and will determine the type of congenital anomaly that is manifest. Variation in the timing of the effects of an insult may be best illustrated when considering the pathogenesis of cardiac and cerebral anomalies.

The manifestation of the anomaly will be crucially dependent on the stage of embryonic development when the insult occurs. For example, the cardiac anomalies must occur very early in gestation as completion of semilunar valves in cardiac development is complete by 9 weeks (Larsen, 2001). In contrast, cerebral development continues throughout fetal life and anomalies may be produced by an insult at any time during gestation. In early gestation, neuronal migration abnormalities may occur, presenting as CP or other clinical syndromes. In mid-gestation, porencephalopathy or multicystic encephalopathy, and late in gestation, subcortical leuencephalopathy may occur.

An infinite variety of anomalous development is possible and may be envisaged as any combination of points between these dimensions of severity, space and time between the twin fetuses. This would explain the majority of adverse outcomes that are attributable to monochorionicity. Furthermore, the model is able to explain the role of monochorionicity in the pathogenesis of apparently singleton births. In these cases it is possible that very early and unrecognized death of one conceptus has occurred and the CP and/or congenital anomaly is observed in the singleton birth. The model would also explain CP and/or a congenital anomaly in dizygous twins. In a triplet conception comprising MZ twins and a singleton, the unrecognized early loss of one of the MZ pair would lead to the birth of DZ twins one of which may have CP and/or a congenital anomaly. This scenario has been reported. Among 17 triplet conceptions with twin registered births following early loss of one conceptus, there were three live born infants with a congenital anomaly (Pharoah et al., 2009).

**Pathogenic mechanisms for CP and CA**

Different possible pathogenic pathways have been proposed to account for some CA and CP of prenatal origin that may be attributable to MZ twinning (Pharoah, 2007). The pathogenic abnormality of the brain as a result of MZ MC twinning vary considerably and include migration abnormalities and cystic encephalopathies. These may present clinically as CP or as other clinical neurological syndromes. Figure 1 is a simplified representation of these pathways.

**Cardiac anomalies and twinning**

MZ twins are more likely to have congenital cardiac anomalies than DZ twins and MZ twins and are generally discordant for the anomaly (Knox and Lancashire, 1991). A disturbance of the fetal circulation to the affected twin because of a single placenta seems to be the most likely cause (Campbell, 1961; Cameron et al., 1983; Bahtiyar et al., 2007). Disorders of organ laterality frequently present as cardiac anomalies such as dextrocardia and atrial isomerism and the twinning process itself has been implicated in the pathogenesis of cardiac looping abnormalities (Berg et al., 1989).

These are part of a wider variety of anomalies that include situs inversus and polysplenia/asplenia syndromes. The anomalies occur very early in the development of the embryo when zygote division rather than feto-fetal transfusion provides the pathogenic mechanism (Burn and Corney, 1984; Burn, 1991). Relevant to the role of MZ twinning in the pathogenesis of CA is that the heterotaxias and other cardiac anomalies are frequently found in conjoined twins and are influenced by the site of union of the twins (Cunniff et al., 1988; Gerlis et al., 1993; Levin et al., 1996). Also attributable to the division of the zygote, but of lesser clinical importance, is the mirror imaging phenomenon of hair whorls, birth marks and tooth eruption patterns that are frequently observed in MZ twins. Zygote division or splitting of the morula may give rise to these abnormalities of laterality.

![Figure 1 Proposed pathogenic pathways in the aetiology of cerebral palsy (CP) and congenital abnormalities (CA).](image-url)
Acute episodes of feto-fetal transfusion following development of a MC placenta can be expected to affect fetal organ development. In the context of cardiac development, the resulting anomaly will be determined by the stage of organ development. The primitive cardiac tube undergoes looping, modelling and septation in weeks 5–7 that leads to the formation of the cardiac chambers (Larsen, 2001). Perturbation of ectomesenchymal tissue migration, as a consequence of an episode of feto-fetal transfusion, could affect development of the cardiac outflow tract and produce conotruncal septation defects such as double outlet right ventricle and truncus arteriosus (Kirby and Waldo, 1995). Blood flow through the developing heart is laminar and perturbation of this laminar flow, together with interference of endocardial cushion development, would lead to sepal and cardiac valve defects and coarctation of the aorta (Clark, 1996).

Thus, cardiac development may be under double jeopardy, both from the division of the zygote and from episodes of feto-fetal transfusion. It is pertinent that anomalies of laterality frequently co-exist with biliary atresia and cardiac valve stenosis and atresias (Rose syndromes frequently co-exist with biliary atresia and cardiac valve stenosis and atresias (Rose et al., 1975). This could be indicative of different spacial and timing effects.

Cardiac anomalies may also be used to illustrate the potential variation in severity of organ impairment. At one extreme is the acardiac twin, a phenomenon that is observed only in MC MZ twins. At the other extreme are the mild pulmonary valve stenoses or septal defects.

**Cerebral anomalies and twinning**

Dependent on the timing of an insult to the developing fetal central nervous system (CNS), a variety of cerebral anomalies may result. The indentation of the neural plate and its subsequent folding to form the neural tube, which is the precursor of the CNS, occurs during the 4th week (Larsen, 2001). The formation of the cerebral cortex results from a combination of neuroblast proliferation, neuronal migration from the ventricular zone to the cortex and further organization of the cortex. Cerebral anomalies such as the lissencephalies, non-lissencephalic cortical dysplasias and heterotopias may occur. Early timing of an insult may affect this phase of fetal brain development. Fetal death of a twin before 12 weeks was associated with bilateral anterior polymicrogyria in the survivor (Baker et al., 1995). In another case, fetal death at between 13 and 16 weeks was associated with death of the surviving co-twin at 3 days in which the autopsy revealed heterotopias and polymicrogyria (Barth and van der Harten, 1985). Late fetal demise of a twin was associated with multicystic encephalomalacia in the survivor (Weig et al., 1995; Lin et al., 1999).

CP, though probably the commonest, is only one of the possible clinical presentations due to cerebral impairment in MZ twins. Other manifestations will include learning disability, epilepsy and cortical blindness. These may accompany CP but need to be considered possibly as isolated manifestations of cerebral impairment.

**Other CA and twinning**

Variations in the timing of an insult may account for different pathologies in other organs. Horseshoe kidney, unilateral and bilateral renal agenesis, renal dysplasias and renal cortical necrosis may be the result of impairment at different phases of fetal development. They have been reported as more common in twins than singletons (Myrianthopolous, 1976) and have been associated with fetal death of the co-twin (Hoyne et al., 1981; Reisman and Pathak, 1996). First trimester fetal loss as a vanishing twin has been associated with sirenomeilia with persistently opposed lower extremities and absent fibulae and severe renal dysgenesis (Kapur et al., 1991). In late gestation congenital renal abnormalities have been attributed to the twin to twin transfusion (Chang et al., 1997; De Paepe et al., 2003).

**Multiple CA and twinning**

Fitting within the three-dimensional model of fetal damage as a consequence of feto-fetal transfusion is the possibility that more than one organ may be affected. Numerous case and series reports have documented anomalous development of more than one organ. The acronym VACTERL represents Vertebral defects, Anal atresia, Cardiac defects, Tracheo-Esophageal fistula, Renal dysplasia and Limb anomalies. It is unusual for all the abnormalities to be present in one infant but subsets suggest that there is heterogeneity within the association (Botto et al., 1997). Some of the subsets of VACTERL may be attributable to haemodynamic instability in a MZ MC conception. It is pertinent that VACTERL has been reported in the surviving twin whose co-twin was a macerated stillbirth (Evans et al., 1999).

Multiple organ anomalies may be illustrated with reference to CP. Compared with the general population of children, those with CP are at significantly greater risk of having a coincidental congenital anomaly (Miller, 1989; Coorssen et al., 1991, Palmer et al., 1995, Croen et al., 2001, Ozaras et al., 2005; Blair et al., 2007, Garne et al., 2008). Similarly, children with a major birth defect are at increased risk of developmental disabilities of which CP is one (Decouflé et al., 2001). These reports were for the generality rather than specific anomalies. Nevertheless, the data supported a prenatal aetiology for CP.

**Coincidence of CA and CP**

Analysis of specific coincident CA in a population-based register of CP compared with the general population of children observed highly significant relative risks for cardiac, eye, cleft lip and palate and oesophageal and other intestinal atresias in the children with CP. Sub-analysis of the cardiac anomalies revealed significant increased relative risks for malformations of the cardiac chambers, the septa, the pulmonary and tricuspid valves and the aortic and mitral valves. Some of the children had undergone cardiac surgery to correct the anomaly and it was presumed that adverse events during surgery may have contributed to the cerebral impairment, particularly in those children with cyanotic heart disease and polycythaemia. However, this was not a sufficient explanation for the majority of cases (Pharoah, 2007).

In a series of 104 consecutive unselected children who underwent open-heart surgery, CP occurred in 22% and was associated with the type of cardiac lesion and the depth of hypothermia during surgery (Miller et al., 1996). Surgical treatment for a univentricular heart was subsequently found to be associated with intellectual and neurologic deficits including one child with CP (Sarajuuri et al., 2007). Undoubtedly the rigorism of the surgery contributes to the pathogenesis of CP in a significant proportion of cases but it may be unwise to assume that the cerebral impairment was not present prior to the surgery. In support of this, the corollary has also been reported. In 32% of
cases, magnetic resonance imaging of term newborns with congenital heart disease were found to have evidence of widespread white matter injury before they underwent cardiac surgery (Miller et al., 2007).

The timing of congenital cardiac anomalies attributable to MZ twinning with feto-fetal transfusion must have occurred very early in gestation. Coincident cerebral impairment occurring at this time would be expected to manifest as neuronal migration abnormalities. This is exemplified by the report of cases of agenesis of the corpus callosum and holoprosencephaly associated with hypoplastic left heart syndrome (Glauser et al., 1990).

Quantifying the role of MZ twinning in the pathogenesis of CP and the CA

Although it is generally accepted that MZ twinning is associated with increased prevalence of both CP and many CA, quantifying the contribution of MZ twinning is fraught with difficulty. Early reports were limited to the co-twin survivors of a late fetal death. The development of the use of ultrasound assessment of pregnancy has enabled a quantification of the proportion of twin conceptions that result in singleton births. However, routine ultrasound assessment is usually performed towards the end of the first trimester or early in the second trimester. Early first trimester sonography generally is not a routine procedure but is performed on women undergoing assisted reproduction therapies or because of early vaginal blood loss. Neither group will provide an unbiased estimate of fetal loss in a multiple conception. The former group will yield an estimate of fetal loss in DZ but not MZ conceptions. The latter will be biased because the clinical presence of bleeding may itself be indicative of loss of a conceptus.

Among natural conceptions it has been estimated that, for every live born twin pair, there appear to be at least six singletons who are sole survivors of twin conceptions and that the majority of these losses occur before 8 weeks gestation (Boklage, 1990). After early loss (<16 weeks gestation) of a fetus from a twin conception, there was a greater than 2-fold relative risk of a congenital anomaly in the surviving singleton birth compared with twin births from a twin conception and a 4-fold relative risk compared with singleton birth from a singleton conception. Similarly, following early loss of a fetus from a triplet conception, there was a 5-fold relative risk of a congenital anomaly among the twin survivors compared with singleton births from singleton conceptions (Pharoah et al., 2008). The observation that there is a significant increase in risk among twin births from a triplet conception with early loss of a fetus assumes importance because it provides an explanation for the pathogenesis of CA in apparently DZ twin births.

As many CA, notably cardiac, neuronal migration and central fusion abnormalities, originate very early in gestation, routine sonographic examination may fail to detect very early loss of a conceptus. Quantification of the role of a multiple conception in the pathogenesis of CA and CP will require specific targeted research.

Conclusions

CA and CP are important causes of fetal and infant death and childhood disability. By definition, a congenital anomaly arises as an aberration of fetal development. CP has a multifactorial pathogenesis but about 70% of cases are attributable to cerebral impairment occurring during fetal development. In these cases, the mal-development of the fetal brain presents clinically as CP but is only one of a variety of possible clinical manifestations of the congenital cerebral anomaly. Multiple births are at greater risk of CA and CP than singleton births. Among multiples, monozygous are at greater risk than dizygous conceptions. Furthermore, monozygous conceptions are more often discordant than concordant for CA and CP, thereby indicating that gene segregation is not a dominant factor. Twin–twin feto-fetal transfusion imbalance among MC conceptions explains some of the pathogenesis. Singletons with a congenital anomaly or CP may be attributable to a multiple conception in which one conceptus has been lost early in gestation. Quantification of the role of twinning in the pathogenesis of CA and CP needs to be established.

References


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