Review Article

Genetic and teratological considerations in the analysis of concordant and discordant abnormalities in twins

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Summary

Results from monozygotic (MZ) and dizygotic (DZ) twin research are often used in an attempt to gain a clearer understanding of the 'nature v. nurture' dilemma. Discordance between MZ twins has been considered to be environmental, and greater concordance in MZ compared with DZ pairs to be genetic. Current genetic and teratological theories considerably complicate the interpretation of concordance and discordance of abnormalities. The high rate of discordant intra-uterine death recently demonstrated in twins may profoundly influence the value of epidemiological studies usually performed in later life. Furthermore, indirect zygosity estimations based on sex ratios in DZ twins may be flawed because it is now recognized that increasing numbers of conditions are genetically heterogeneous.

Emphasis is laid on problems of interpretation of discordance and concordance for developmental abnormalities in twins, and some possible mechanisms for their induction are discussed. Basic genetic concepts relevant to the expression of abnormalities in twins are outlined.

Two inferences have traditionally been drawn for the determination of the relative roles of genetic and environmental factors in twin studies: discordance between monozygotic (MZ) twins has been taken to indicate the presence of environmental determinants, and greater concordance in MZ compared with dizygotic (DZ) pairs to be genetic.

With appropriate attention to recognized sources of potential bias, twin studies remain one of the most powerful means of analysing genetically and environmentally related variation in man.

Analysis of MZ twin data has, however, become increasingly complex because of the development of teratological and genetic theories which influence interpretation of abnormalities in discordance and concordance studies. Some of the problems relating to dysmorphogenesis in twins are discussed.

Is embryonic cleavage due to a teratogenic influence?

MZ twins are generally considered to be formed as a result of early embryonic midline cleavage, which some workers think is a teratogenic process. In 1921 Stockard suggested that MZ twinning was a teratogenic event, and that associated early malformation sequences could be induced by the same insult. Recently Schinzel et al. Jüberg et al. and Smith also proposed that the twinning process and associated structural aberrations were due to a common cause.

It appears that the twinning rate and the intra-uterine mortality rate of twins have both been underestimated. According to one major study, 32% of twin pregnancies were converted to singletons by 40 weeks of gestation. However, only 3.7% of presumed MZ twins had a history of a diseased co-twin. Future inclusion of such embryopathological study results in twin data analysis may profoundly influence estimations of incidence, prevalence and therefore also those of discordance and concordance.

Furthermore, familiarity with abnormalities unique to MZ twinning is required for consideration of the differential diagnosis of a malformation pattern in the twin surviving in cases of discordant intra-uterine death.

The excess of structural defects found in twins is composed of three categories:

1. Defects which are part of the twinning process, such as conjoined and some amorphous twins. In addition, all embryonic malformations and malformation complexes are increased in MZ twins. Concordance for anomalies in MZ twins is uncommon. Abnormalities secondary to a shared circulation. Placental anastomoses seem to be the rule rather than the exception among monochorionic MZ twins. In a series of 60 di-amniotic monochorionic placenta, Benirschke and Driscoll found that 85% contained vascular anastomoses between the fetal circulations. Depending on their nature, various types of vascular interchange can be found. The resulting abnormalities are listed in Table I.

3. Deformities arriving through fetal constraint in late gestation. This problem is found in both MZ and DZ twins. Fetus papyraceus refers to an unusual condition found in MZ twins only, in which one member of a twin pair dies in utero. This usually occurs during the second or third trimester of fetal life and the dead fetus becomes compressed by the growing co-twin. It has recently been proposed that the underlying cause for (discordant) fetus papyraceus is transient hydrops in the surviving twin, with polyhydramnios because of vascular or other abnormalities.

It is possible that a fourth category, namely tissue disruption, could result from the sharing of a single amniotic cavity. Such twins are rarely born alive.

Statistical importance of cleavage-associated phenomena

In 1960 Smith reappraised earlier non-chromosomal research data on Down syndrome in twins and indicated that a conclusion about its cause was incorrect. He found that it was necessary to decide in advance whether diseased-determining events occurred before or after twin cleavage.

In some large-scale studies, investigators have not always established the zygosity of individual twin pairs and many have
of MZ twins introduce additional complexities to concordance/fetuses and high rate of discordant intra-uterine death documented. This approach to the genetic/environmental question was abandoned, as defects of incomplete differentiation (mostly closure defects) cause of growth retardation in the individual twin set. In this manner, early growth retardation associated with early malformation sequence associated with growth retardation; twin placental vascular interchange; or deformities arising through intra-uterine fetal constraint. In the Kansas Fetal Growth Study, 5 of 34 sets of full-term MZ twins were born to mothers with no known growth-retarding factors other than multiple pregnancy, yet displayed significant intrapair growth differences. In 1 MZ twin pair, the difference was 41.5% for birth weight. Many causes of growth retardation or intrapair growth differences can be listed for MZ twins. These include possible effects of the hypothetical teratogenic insult responsible for the twinning process in the first instance; the presence of an early malformation sequence associated with growth retardation; twin placental vascular interchange; or deformities arising through intra-uterine fetal constraint. If serial ultrasound measurements of various fetal growth parameters are made, 'timing of the error' can be used to evaluate the cause of growth retardation in the individual twin set. In this manner, early growth retardation associated with early malformation complexes can be distinguished from growth retardation due to fetal constraint, which in twin pregnancies occurs only after a combined weight of 4 kg has been reached, usually at 30 - 34 weeks' gestation. Since chromosomal errors are associated with growth retardation, heterokaryosis may in unusual instances contribute to a genetic basis for unequal growth patterns in MZ twins. When chromosomal non-dysjunction coincides with twinning, the resulting twins may share the two lines of cells in highly unequal proportions. With the exception of some heterokaryotes in which 'genetically identical' individuals are discordant. Discordance is a striking but not invariable feature of midline defects in MZ twins. Whether twinning is initiated by the occurrence of a malformation or whether a common factor predisposes to both twinning and malformation is uncertain.

**Evaluation of discordant growth in MZ and DZ twins**

Although MZ twins might be expected to react similarly to a given environmental insult, this does not seem to be the rule. MZ twins provide an excellent model for appreciating the spectra of particular malformation complexes since different gradations of severity, to the extent of discordance, can be found. This may be important in the analysis of intra-uterine growth retardation for which 'genetically identical' individuals are discordant.

Consequences of the fact that MZ twinning could represent a 'midline developmental defect'

It has been widely assumed that MZ twinning occurs after about the 4th day of embryonic life when implantation normally occurs; cleavage in the midline results in MZ twins. The embryonal midline represents a developmental field 'the morphogenetic properties of which are particularly poorly buffered.' As developmental field defects are causally nonspecific, one or more common environmental determinants requiring a high threshold for expression could be involved in the twinning process. The reason for this is the markedly uniform frequency of MZ twinning throughout the world (1:286). It appears that specific factors can periodically disturb the 'twinning developmental field' in a dramatic way, as when epidemics of conjoined twining occur.

It is generally thought that there is a continuum in the formation of MZ twins. This formation process includes the whole spectrum running from dichorionic to monochorionic, di-amniotic to mono-amniotic and conjoined pairs. A predisposition to anencephaly appears to be slightly raised in dichorionic pairs, but very high in certain types of conjoined pairs. Although MZ twins, especially mono-amniotic and conjoined pairs, are particularly liable to anencephaly they are not particularly liable to spina bifida. According to James: Other midline sequence abnormalities such as defects of incomplete differentiation (mostly closure defects) will, in twins, have to be carefully distinguished from pleiotropic parental germ cell mutations, which exert their effect predominantly on the midline.

Hemifacial microsomia
Disseminated intravascular coagulation
Horseshoe kidney
Transverse myelia
Renal cortical necrosis
Gastrochisis
Aplasia cutis
Hydrocephaly, hydranencephaly
Hemifacial microsomia
Gastroschisis
Clubfeet
Deformation patterns, bowing of limbs
Fetus papyraceus

**TABLE I. STRUCTURAL DEFECTS IN MZ TWINS**

<table>
<thead>
<tr>
<th>Cause</th>
<th>Resulting defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teratogenic cause of MZ twinning</td>
<td>Early disruptions or disruption sequences related to twin-inducing teratogen</td>
</tr>
<tr>
<td>Incomplete twinning</td>
<td>Conjoined twins</td>
</tr>
<tr>
<td>Vascular shunts</td>
<td>Disruption, including acardiac and amorphous twins with cardio-megaly, liver dysfunction, hypoalbuminaemia, oedema (and sometimes hydrops) in survivor</td>
</tr>
<tr>
<td>Artery-artery</td>
<td>Discrepancy in size with oligohydramnios in donor twin sac and polyhydramnios in recipient amniotic sac</td>
</tr>
<tr>
<td>Death of one twin</td>
<td>Surviving twin may display:</td>
</tr>
<tr>
<td>with thromboplastin or embolic release to survivor</td>
<td>Disseminated intravascular coagulation</td>
</tr>
</tbody>
</table>

*Modified from Smith and Van Allen. MZ = monozygotic.

used an indirect approach such as the Weinberg differential method. Selection of study material may have been based on the presence of abnormality as opposed to non-randomly (irrespective of health status) selected twins. The validity of the Weinberg method depends on an unlike/like sex ratio among DZ pairs of 1:1. This ratio becomes distorted in sex-linked recessive inheritance. This consideration has become important because of the 'splitting' of many genetic conditions previously considered to be single entities and now known to be heterogeneous.

Marshall and Knox devised a method in which a direct approach to the genetic/environmental question was abandoned and one was chosen that depends on timing of the determining embryological events, irrespective of their nature. This model permits investigation of twin data without an over-restrictive set of prior assumptions. Knowledge of cleavage-associated abnormalities is also a prerequisite for the successful application of this approach, however. Continued interchange between developmental biologists and mathematicians involved in twin research data analysis is required. The excess intra-uterine mortality in twin feruses and high rate of discordant intra-uterine death documented for MZ twins introduce additional complexities to concordance/discordance models.
Such heterokaryotic twins are rare and unlikely to have influenced the interpretation of growth data in twins. They do, however, illustrate the importance of accurate zygosity demonstration.

Marked phenotypic discordance can also be expected in monochorionic DZ twins because of polar body twinning.10 The events that give rise to such twins could occur at, or even before, the time of conception or fertilization. Evidence for this was forwarded by Nance,10 who also emphasized that human monochorionic twins should generally be considered monovular rather than monozygotic in origin.

In his study10 on polar body twins in which one member had acardia and the other was normal, pericentric chromosomal heteromorphisms were utilized as a marker for polar body twining. (First polar body twins should be discordant for all maternal centromeric heteromorphisms while second polar twins should be concordant for all such markers.) Superfetation is another possible mechanism for marked weight discordance in DZ twins. Finally, marked weight discordance in DZ twins appears to be transmitted as a genetic trait through the maternal or paternal line in some instances.10

Possible importance of twin fetoplacental haemodynamics

A theoretical possibility remains that some blood-borne mutagens or teratogens may result in dissimilarly affected twins when unequal blood distribution could be responsible for a dosage effect in the abnormal twin. It may be of interest to link such data to the study of various abnormalities for which twins are most often discordant. The theory is not new and has been rejected, for instance in the case of anencephaly,19 where brain development is abnormal before a blood supply develops. However, the importance of vascularization in normal limb development20 indicates that blood-borne noxious substances could be similarly responsible for anomalous development.

An intra-uterine hyperthermic insult can inhibit fetal brain growth,21 but the effect may not be evident clinically, as a slightly smaller brain need not necessarily be inferior to a large brain. Even if such individuals function normally they may not achieve their full genetic potential.

Heat dissipation is very important in the normal metabolically active fetus.22 Fetoplacental haemodynamics play an important role in this regard; excess intrinsic heat arising through vascular shunting in MZ twin pregnancies might decrease the safety margin for the introduction of heat-related dysmorphogenesis22 in a twin member transfused with blood from its co-twin. The transfused twin would then have problems with excess heat dissipation as opposed to the 'pump' twin supplying blood 0.6°C warmer than that usually supplied by the mother.

The possible association between independent findings of heat-induced arthrogryposis multiplex congenita in experimental animals23 and an excess of MZ twins reported to be discordant for this abnormality and in which the smaller (or transfused) twin was invariably abnormal24 appears especially attractive as an explanation for the excess of twins with discordant arthrogryposis.

Genetic concepts relevant to the expression of abnormalities in twins

Gene penetrance in twins

Even with identical genetic endowment the developmental patterns of members of an MZ twin pair are not structured similarly. MZ twins 'are remarkably similar, but never completely identical'.25 Opitz26 has recently drawn attention to the fact that identical twins can be likened to the right and left members of paired organs in the individual. This view has implications for estimating penetrance from the concordance/discordance of a laterally paired organ affected with a genetic trait.

Schinzel27 pointed out in 1945 that MZ twin data used in penetrance studies are mostly inaccurate, since data on pairs in which neither twin was affected are usually lacking. One should therefore be able to demonstrate a significant difference between the 'corrected' penetrance and the 'observed' penetrance, the second (wrongly) based only on data of concordantly and discordantly affected twin pairs.28

The influence of unilaterally expressed host resistance as a multifactorial threshold character with high heritability has been proposed as an explanation for expression and localization of retinoblastoma in successive generations.27 This appears attractive as an explanation for the laterality preference of many developmental abnormalities (such as Poland's anomaly) and will have to be studied for a possible role in discordance of a genetic trait in antimeric or mirror-image individuals (twins).

The existence of such a phenomenon can perhaps be deduced from the observed 12.5% penetrance ratio in type of autosomal recessive trait — Kartagener's syndrome.28 As long ago as 1943 Dahlberg29 wrote about 'genotypic asymmetries'. The high rate of discordance among MZ twins with respect to Kartagener's syndrome, and studies on paucipenetrant autosomal recessive status in the mouse appear to support the earlier concept that genes coding for genotypic asymmetry could be responsible for this phenomenon. The main differential diagnosis in asymmetrical involvement is from vascular disruptive anomalies.30

Contributions by cytoplasmic inheritance

Another explanation for discordancy in MZ twins derives from anencephaly studies where a genetic contribution is indicated by a 4.5% risk of recurrence for offspring of MZ twin siblings. An increased prevalence of anencephaly and spina bifida appears to be confined to matrilineal relatives. Mutations of cytoplasmic genes, important mechanisms in lower forms of life, have been invoked by Nance31 as a cause of cytoplasmic inheritance of nongenetic mutations in this instance. These findings have been carried through to observe the prevalence of malformations, including midline neurological defects, in the offspring of normal twin parents. In this instance the MZ half-sib approach,32 documented as useful for resolving genetic, maternal and environmental influences on quantitative traits in man, confirmed the impression of a matrilineal factor.33 For both black and white patients the highest figures for midline neurological defects were associated with offspring of female, like-sexed twin parents (as opposed to male, like-sexed twin parents or when a sex difference indicated dizygous twin parents).

Can a post-cleavage mutation occur in only one twin?

When phenotypic variation causes problems in deciding whether a single pleiotropic mutant gene or genetic heterogeneity is involved, the answer could be looked for in twins, since they should display variable expression of a single gene when dissimilar syndromic findings are encountered.34 Discordance in which one twin displays a dominant gene condition and the other appears normal could theoretically be due to a post-cleavage mutation in only one of the pair. A late-appearing somatic mutation in one of a twin pair seems unlikely, but has been suggested. Cohen et al.35 reported MZ twins discordant for central core myopathy, usually regarded as an autosomal dominant condition; one had a central core myopathy while the other was normal. The fetus with myopathy also had multiple congenital contractures.

The findings of central cores in muscle fascicles has also been described as a nonspecific maturational phenomenon.36 A teratogenic, mitosis-inhibiting mechanism could have been responsible for a phenocopy of central core myopathy in this instance. This underlines the subtlety by which both environmental and genetic factors seem to produce similar results in an organ system with a limited repertoire of response. Should multiple allelism37 be seen as the cause for variability of penetrance and expressivity, one twin could theoretically be affected so mildly as to lead to acceptance of normality and discordance for a given disorder. Late-appearing mutations may take on different guises:

Some important problems inherent in the interpretation of twin data relate to malignancy. The two-mutation hypothesis of Knudson38 implies that hereditary cancer is found when the first mutation affects the germ cells. All progeny of these germ cells
will carry the first mutation; all that is then needed is for the environment to supply the second noxious influence for cancer formation through a second hit, either a somatic mutation or an error in differentiation caused by loss of normal suppressive mechanisms. 19

It is conceivable that MZ twins could share a first mutation whereas with differing intra-uterine response or postnatal environment only one would have the second mutation. These twins could then be seen as MZ twins discordant for a specific type of cancer, although genetically they are concordant for the first mutation conferring cancer proneness.

Such examples have been recorded in instances where the first mutation causes physical abnormalities. In one report, both MZ twin members were discordant with regard to the anhidrotic syndrome with psychomotor retardation, but the often associated third part of the triad, Wilms' tumour, was found in only one. 20

In another report, 21 one female MZ twin with neurofibromatosis developed an optic glioma which was surgically removed at 2 years of age. The fact that neurofibromatosis is a causally nonspecific although often autosomal dominant dysplasia with high spontaneous mutation rate indicates the tremendous complexity encountered when studying hereditary versus environmental concepts.

Careful scrutiny of parents and older members of the family pedigree is required before autosomal dominant conditions in twins with 'normal' parents are assigned to a new parental germ cell mutation. It has been stated that reduced segregation in parental generations could be an attribute of a mutant gene itself through 'premutation'. The term 'phenotrance' is used for this concept 22 but, apart from delayed mutation, many other mechanisms may influence gene expression in different generations.

Conclusion

Experts are agreed on the tautological nature of the 'principle' that all variation is either genetic or environmental. 23,24 Murphy 25 has stated: 'Repeated failures to establish sound propositions concerning them [tautologies] will sooner or later lead to their being abandoned.' This may be especially true for the study of twins.

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REFERENCES