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Congenital Heart Defects and Twinning

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Abstract. A preliminary analysis of twins or triplets with heart defects, ascertained in five centres, confirms earlier suggestions that monozygotic (MZ) twins are over represented among twins with heart defects, even after excluding persistent ductus arteriosus and conjoined twins. An MZ twin individual has a risk of cardiovascular malformation approximately twice that of DZ twins and singletons. It is suggested that the twinning process itself affects one of the pair. Disturbance of laterality ('mirror imaging') is probably a more important mechanism than twin-twin transfusion. Inappropriate use of the twin method in the past has caused the importance of genetic factors in the etiology of congenital heart defects to be underestimated. Neverthless, twins do provide a useful illustration of the likely importance of epigenetic factors in heart development.

Key words: Congenital heart defects, Twinning, Laterality, Mirror imaging

INTRODUCTION

In 1960, McKeown and Record [16] commented on an apparent excess of heart malformations among their population of like sex twins. Using the same data, Leck [15] in his doctoral thesis suggested that the monozygotic (MZ) twinning process itself might account for this excess. In 1961, Campbell [2] suggested a higher incidence of heart defects among MZ twin pairs with only one of the pair affected, based on 16 twin pairs, 14 of which were of like sex. Several subsequent reports of heart defects in twins show a similar excess. However, the difficulty of obtaining adequate data has meant that, to date, it has not been possible to establish with certainty whether MZ twins are at increased risk, and if so, to what degree.

If MZ twinning does have an adverse effect on formation of the heart, an exami-

nation of the nature of these defects might shed light on the twinning process itself, and be of interest to obstetricians and pediatricians involved in the management of multiple pregnancy. Cardiovascular malformations are the largest category of major congenital defects, and yet understanding of their pathogenesis is limited. If specific malformations are associated with the twinning process, this would represent an 'experiment of nature' of particular interest to the cardiac pathologist. Finally, the geneticist traditionally has made use of twins to estimate genetic variance, using the twin method first put forward by Francis Galton in 1876 [7]. When applied to heart malformations, the 'twin method' has in the past given very low estimates of heritability. However, if in some cases, the twinning process itself has produced a heart malformation, and if the malformation affects only one of the pair, the result would be to 'dilute' the concordance figure for MZ twins.

Table 1 summarises 'limited ascertainment' series of twins identified among patients with heart defects. The report by Anderson [1] is of particular interest; among over 100 twin pairs, 58% were adjudged MZ using the similarity method. Even allowing for the rise in relative proportion of MZ twins which has resulted from the decline in DZ twinning, this Caucasian population should have no more than 35% MZ pairs.

Table 2 lists 'unlimited ascertainment' studies based on total population samples. As in the hospital series, these are marred by the problems of establishing zygosity and diagnosis and collecting a sufficient number of affected twins. Ascertainment bias is again an acute problem because this type of study typically derives malformation data from perinatal records. As a means of identifying patients with heart defects, these records are hopelessly inadequate. Innocent murmurs may cause some to be included, while a majority of significant lesions are not identified.

Most studies do not give sufficient details of cardiac diagnoses and do not exclude persistent ductus arteriosus. The ductus arteriosus connects the pulmonary artery to the aorta to permit blood to bypass the lungs in utero. Failure to close may be due to congenital abnormality of the duct lining, but in the preterm infant patency is very common owing to lack of duct maturity. Since preterm delivery is more common among twin deliveries, an excess of this anomaly is to be expected. It is necessary to consider persistent ductus arteriosus separately, in order to establish whether other structural defects are more frequent among twins.

Conjoined twins often share a heart and cardiac malformation is common. There can be little doubt that such cardiovascular anomalies are consequent upon the 'twinning' process; strictly speaking, conjoined 'twins' do not constitute a multiple pregnancy. Again, these infants must be distinguished in the analysis.

Table 2 contains two large studies reported recently. The report by Myrianthopoulos [21] would seem, at first sight, to confirm absolutely the excess of defects among MZ twins. However, their figure of 17.57/1000 results from the inclusion of several cases of cardiac enlargement. A degree of cardiac hypertrophy is characteristic in the recipient of a twin-to-twin transfusion. This is transient and does not constitute a malformation. Exclusion of these, a pair of conjoined twins, and a case of persistent ductus arteriosus, lowers the overall figure to just over 10/1000. Three other MZ twins had trivial lesions. Removal of these means there is no demonstrable excess. The data from Layde et al [13] is contained in a larger report on malformations with good ascertainment and five-year follow-up. Again, removal of persistent ductus arteriosus cases and conjoined twins

reduces the significance of the excess, but, as with the other studies, there is a trend towards more structural cardiac defects among like-sex, and so presumably MZ twins.

The general assessment of the literature to date is that, while the studies are compatible with the suggestion that MZ twinning is associated with an excess of cardiac malformations, none to date has combined adequate proof of zygosity with unbiased ascertainment and precise cardiac diagnoses in a sufficiently large series to provide confirmation.

METHODS

This study combines data from five centres: Newcastle, London, Aberdeen, Birmingham, and Liverpool. The first two are hospital series of twins identified among children referred for investigation of heart murmurs, and so use the 'limited ascertainment' approach. When it became apparent that MZ twins were overrepresented in these two series, the study was extended to include the remaining three, whose twin or malformation registries, based on defined populations, permit an 'unlimited ascertainment'.

The Pediatric Cardiology unit of Freeman Hospital, Newcastle on Tyne provides a service to England's Northern region (Cumbria, Northumberland, Durham, Tyne Wear, and Cleveland). Children with heart defects come under the care of two consultants. Over a two-year period, all twins seen by them or their staff were noted and invited to take part in the study. The twins were seen and examined by JB. Zygosity determination was supported by the examination of bloodgroup and serum polymorphisms in the Department of Human Genetics, University of Newcastle.

The notes of all children admitted to The Hospital for Sick Children, Great Ormond Street, carry the question 'TWIN, YES/NO', by which means records of 1500 twin individuals were examined for cases of heart defect. In addition, all new referrals to the hospital during the course of the study have been included. These families have been approached, with the permission of their general practitioner. Diagnosis of zygosity has been supported, where possible by examination of bloodgroup and serum polymorphisms, by the MRC Blood Group Unit and MRC Human Biochemical Genetics Unit, London.

The great majority of twins born to mothers in the Grampian region of North East Scotland are delivered in Aberdeen, where detailed records are kept. Since 1968, these have included analysis of bloodgroup and serum polymorphisms at the Galton Laboratory London, and placental diagrams. A registry of all congenital malformations is also kept, based on autopsy reports and perinatal hospital records. Children with congenital heart defects are referred to the regional cardiac clinic. By comparing the names of over 700 twin pairs with the cardiac clinic records index, twins found to have heart murmurs in later childhood were identified. All records of twins with heart murmurs were reviewed. Those with significant defects were visited where possible and cotwins examined. If zygosity had not been established, bloodgrouping was performed.

A registry of twins and malformations has been kept in Birmingham since 1950. The present study uses data from the Department of Social Medicine, University of Birmingham, where a malformation registry obtains details of twin births and malformations from maternity units. Follow-up is by surveillance of hospital admissions and from health visitor reports. For all but four of the twins listed as having heart defects, diagnoses have been confirmed by personal examination of the clinical records. The list of twins has also been compared to the records of Dr Hugh Cameron, Birmingham Children's Hospital, who has carried out detailed pathological examination of twin placentae from a number of maternity units since the early 1960s.

The Liverpool malformations registry is based on infants born to women resident in Liverpool, with recent extension to adjacent areas, and is maintained by follow-up of hospital records. Again, the records of twins listed as having heart defects have been examined by one of us (JB).

RESULTS

Table 3 contains a preliminary analysis of the two limited ascertainment studies. Together, these involve 216 twins or triplets with heart defects in 193 multiple births. A total of 74 families have been seen at home and 50 in hospital. Of these, blood has been

TABLE 1 - Limited Ascertainment Series

Authors	Year	Twin Pairs (N)	Percentage MZ	Criticism			
				P	A	D	S
Uchida & Rowe	1957	45	↑	+	–	–	–
Lamy et al	1957	19	↑	+	+	–	–
Ross	1959	37		+	–	–	–
Campbell	1961	16	↑	–	–	–	–
Nora et al	1967	43		+	–	+	–
Jorgensen	1970	50	↑	–	+	+	–
Mori	1975	113		+	–	+	+
Anderson	1977	107	↑	–	+	+	+

TABLE 2 - Unlimited Ascertainment Series

Authors	Year	Total twin individuals (N)	Heart defects			Criticism			
			Total	Like-sex	MZ	P	A	D	S
McKeown & Record	1960	1550		↑		–	+	–	–
Stevenson et al	1966	4847	↑			–	–	–	–
Edwards	1968	not given	↑			+	+	–	–
Hay & Wehrung	1970	199,700		↑		–	–	–	+
Mitchell et al	1971	1204	↑			–	+	–	–
Kenna et al	1975	4886			↑	–	+	+	–
Myrianthopoulos	1975	1195			↑	–	+	+	–
Layde et al	1980	4490		↑		–	+	+	–
Cameron et al	1983	2848			↑	+	+	–	–

LEGEND: ↑ indicates a figure greater than expected for the population group under study; +/–, published data adequate/inadequate; P, proof of zygosity; A, ascertainment; D, diagnosis of heart defect; S, sample size.

collected from 77 like-sex pairs and, where possible, their parents, in order to confirm zygosity. Information has been provided using a questionnaire by a further 30 families; 28 twin pairs are untraced, 2 have declined, and 9 remain to be seen.

Cases of conjoined twinning, isolated persistent ductus arteriosus, biased referral, chromosome anomalies, syndromes and acquired or secondary cardiac defects are excluded to give a study group of twins with structural heart defects, analogous to those used typically in concordance studies. The proportion of MZ twins is significantly in excess of that expected.

The proportion of MZ twins is calculated in two ways; the first excludes the group where zygosity is uncertain, while the second 'corrected' figure is obtained by distributing the unknown zygosity group in the same ratio as the MZ to like-sex DZ numbers. This correction is based on the assumption that the unknown zygosity group will contain a disproportionate number of MZ twins. For the purpose of statistical analysis, these results from London and Newcastle (Table 3) are compared with recently published British data [5], in which MZ twins constitute 34.8% of the known zygosity group, and 37.8% when those of unknown zygosity are apportioned as described above.

TABLE 3 - Limited Ascertainment

	London		Newcastle
	Retrospective	Prospective	
Twin pairs	128	22	41
Triplets	2	0	0
MZ	40	12	22
DZ	51	8	14
TZ	2	0	0
ZU	37	2	5
Exclusions			
PDA	13	3	4
Conjoined	3	0	0
Other	14	4	3
Total twin pairs after exclusions	100	15	34
MZ	33	9	19
DZ	42	5	11
ZU	25	1	4
% MZ	47.2 **		63.3 **
% MZ 'Corrected for ZU'	52.2 *		64.7 **

LEGEND: MZ, monozygotic; DZ, dizygotic; TZ, trizygotic; ZU, zygosity uncertain.
Significance levels: *0.05, **0.02.

It might be argued that some of the excess in the Newcastle study is the result of biased ascertainment, based as it is on staff recall. Given the care with which the ascertainment was carried out, this was probably not a major influence. The London data was not open to this bias because the twins were ascertained through the routine recording system. There was no evidence to suggest preferential referral of MZ twins, since in many case notes there was little or no reference to the multiple birth and remarkably few discussed possible zygosity. The most obvious conclusion is that MZ twins suffer an excess of heart defects. There are two alternative explanations; DZ twins may have fewer heart defects, or MZ twins may suffer a more severe clinical effect. It could be argued that the impact of MZ twinning on physical development might increase the likelihood of a child with a heart defect being referred to the pediatric cardiologist.

These alternative hypotheses may be tested using data from the unlimited ascertainment studies in Aberdeen, Liverpool and Birmingham. These contain an excess of heart murmurs in twins, but analysis is not yet sufficiently advanced for the findings to be published in detail. The need for diagnostic review is illustrated by the data from Aberdeen; among 48 twin children with heart murmurs noted in the postnatal period or referred to the heart clinic, 22 were adjudged innocent murmurs, 13 had persistent ductus arteriosus, all but one being preterm deliveries, leaving only 13 with structural cardiovascular malformation.

The incidence of heart defects in singletons in Aberdeen, and the total number of twins born in the Liverpool registry area remain to be determined, but the records of most 'affected' twins and triplets listed in the three centres have now been reviewed. Even after excluding persistent ductus arteriosus, innocent murmurs and conjoined twins, there is a higher incidence of heart defects among twins and triplets compared to singletons. In the Birmingham data this is confined to like-sex twins, while the unlike-sex twins have approximately the same incidence as singletons. Statistical analysis of the available data suggests these excesses are significant, adding further weight to the belief that MZ twinning is associated with an excess of structural cardiovascular malformations.

DISCUSSION

In 1950, Bronson Price [25] concluded that the twin method was likely to give an underestimate of the importance of genetic factors as a cause of congenital malformations. He believed that influences such as twin-to-twin transfusion, unequal division of maternal cytoplasm, and disturbance of laterality, were liable to result in one of an MZ twin pair being malformed. He noted that Galton himself avoided the use of the 'twin method' for malformations, because he too was of similar belief.

Preliminary analysis of our data from five centres in Britain would appear to support this hypothesis in respect to heart defects. Of interest to the gemellologist, is which, if any, of the forces peculiar to twinning might account for this excess.

Melnick and Myrianthopoulos [17] proposed the 'two hit' hypothesis, which accounts for the malformed twin by suggesting that one insult causes twinning, leaving one or both twins susceptible to the action of a second teratogen. However, while occasional clusters of conjoined twins have been described, there is little evidence of environmental insults playing a major role in the etiology of MZ twinning in humans, given the remarkable constancy of the just under 4/1000 deliveries in all populations studied. A similar criticism may be levelled at the second 'hit'. While teratogens such as rubella, alcohol and maternal diabetes are important, the evidence does not favour 'hidden teratogens' as a cause for the majority of heart malformations. The incidence of 4-10/1000, depending on diagnostic criteria and methods of ascertainment, is reamarkably uniform in different populations with little or no seasonal variation [30] and a steady annual incidence in long term studies [10, 26].

It is more likely, therefore, that MZ twinning is largely or entirely a chance event and that the mechanisms alluded to by Price cause cardiovascular malformations either as 'nongenetic' additive factors in a polygenic threshold model or as the sole influence in a 'genetically normal' fetus. Given the low level of concordance, it might be assumed that this influence is typically on only one of the pair.

Circulatory imbalance due to the twin-twin transfusion syndrome is the first choice of most authors as the likely mechanism for the excess heart defects. That cardiac hypertrophy may occur in the recipient is well known. There is also evidence for cardiovascular growth being in part dependent on flow. Newborn children have a narrowing of the aortic arch proximal to the ductus arteriosus, the 'physiological coarctation', which resolves when the duct closes and flow through the arch increases. Two MZ twins, one from Aberdeen and a second from Newcastle, were donors in a major twin-twin transfusion and both suffered severe pulmonary stenosis. While reduced flow may have been a factor in these two cases, the paucity of reports of circulatory imbalance in the series as a whole is

not in favour of this being the major mechanism. Twin to twin transfusion probably cannot occur in the first 4 to 6 weeks of development when most heart defects, particularly the more severe varieties, have their genesis.

Heart development seems particularly sensitive to disturbance of situs or laterality. For example, in the iv/iv mouse, homozygotes for a recessive allele lack the ability to determine situs; 20% have complex heart defects, probably involving those mice in which the critical step of formation of the dextral heart loop is most disturbed [14]. In man, complex cardiac malformations are associated with the isomerism sequence. Isomerism sequence, (Ivemark syndrome, asplenia/polysplenia syndrome) involves the body having either two left or two right sides, a feature most apparent in bronchial and atrial morphology [18]. Noonan [22] noted a close similarity between the defects in isomerism and those seen in conjoined twins. When conjoined twins have distinct hearts, it is not uncommon for one, typically the 'right half', to have dextrocardia. This has been used as evidence in favour of the left side of the body determining laterality [4]. One inference from these observations is that twinning, particularly if it occurs late, might cause the right half to become separated from its 'point of reference', so interfering with formation of the precisely angled dextral heart loop which in turn might result in heart malformation.

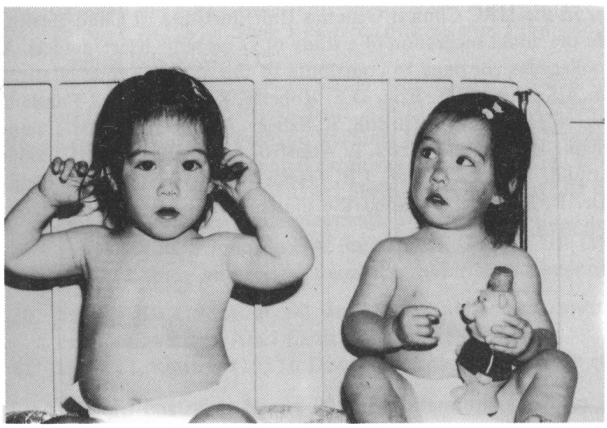

Fig. 1a - Monoamniotic twins, mirror imaged for tooth eruption, position of hair whorl, and hand preference. The twin on the right has tetralogy of Fallot.

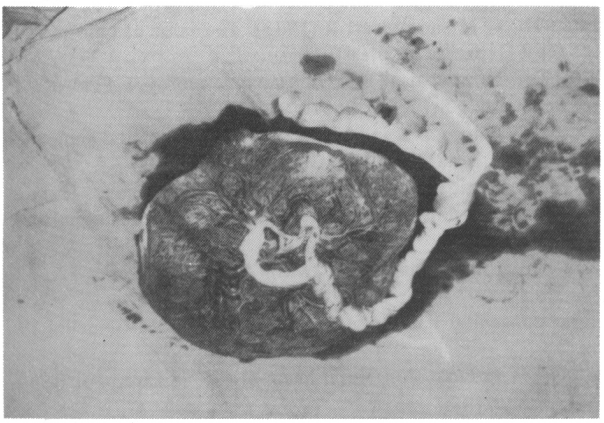

Fig. 1b - The placenta with the two umbilical cords coiled around each other.

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The twins shown in Fig. 1a are the MZ twin daughters of a midwife. They were monoamniotic and had their cords coiled together (Fig. 1b). The child shown on the right has tetralogy of Fallot. Mother reported mirror imaging of tooth eruption and thumb sucking. On examination, it was noted that the affected child with earlier eruption of right sided teeth, also had an occipital hair whorl displaced to the right of centre, whereas her 'left' twin had a left-sided whorl. Studies of hair whorl mirror imaging in twins have concentrated on the direction of rotation [11]. However, position is perhaps of greater interest in this context as it is said to be determined by outgrowth of the occiput at 16 weeks, being displaced if one side, usually the left, grows more quickly [28]. To date, among 43 twin pairs examined for this feature, 12 had evident contrast of hair whorl position; 9 of these were MZ, and in all cases the heart defect occurred in the twin with the rightsided hair whorl.

Among twin pairs discordant for handedness, no clear association between side of preference and malformation is emerging, but there is evidence of an overall excess of contrast for handedness among MZ twins with heart defects when compared to published data from healthy MZ twins [24], which adds some support to the impression that disturbance of laterality is an important 'epigenetic factor' in the etiology of heart malformations.

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