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Definitive Methods of Zygosity Determination in Twins: Relevance to Problems in the Biology of Twinning

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Abstract. Many studies of embryogenesis and fate of twin pregnancies are invalidated because zygosity is not determined definitively, or is assumed on the basis of inadequate criteria. This paper briefly reviews methods of zygosity determination. It reports published results and a new series of twins in which zygosity was determined by DNA fingerprinting. Implications for methods of prenatal diagnosis of zygosity are discussed in the context of the occasional need for intervention in twin transfusion syndrome or in twins discordant for major malformations. Definitive zygosity and placental anatomy (number of chorions and amnions) is discussed as the firm substrate for studies of normal and abnormal twin development.

Key words: Diseases of twins, MZ twins, Zygosity, Malformations, DNA fingerprinting

INTRODUCTION

Concerning congenital anomalies in twins, there are three main discussion points: 1) Are anomalies more common in monozygotic (MZ) than dizygotic (DZ) twins? (Anomalies should not be confused with malformations). 2) If anomalies are more common in MZ than DZ twins, are there special types of anomalies? 3) How can concordance/discordance for anomalies in MZ twins best be used to investigate the etiology and pathogenesis of these anomalies, eg, between genetic and extrinsic teratogenic causes?

These questions are only being clarified rather slowly for several reasons, including: 1) the relatively small numbers of cases of anomalous twins that can be investigated by any given institution; 2) the need to classify accurately the different types of anomalies

found in twins; and 3) the need to determine clearly the zygosity of twins, based on placental anatomy and direct zygosity testing of like-sexed (LS) dichorionic (DC) twins.

Approximation of assumed rates of MZ among LS,DC twins, using Weinberg's method, is probably not adequate [4,68]. This paper reports a small series of twins in which zygosity was determined by DNA fingerprinting [2,16,32]. The paper also reviews critically some of the recent literature concerning concordance/discordance for anomalies in supposed MZ twins, and the relative prevalence of anomalies in MZ and DZ twins.

METHODS

A survey was carried out in which prenatal and perinatal complications of multiple pregnancies were recorded at Victoria General Hospital, Victoria, British Columbia, for the period, January 1984 to June 1988. Major morbidity and mortality was largely confined to the monochorionic (MC) twins, principally as a result of twin-to-twin transfusion. During the latter part of the survey, DNA fingerprinting was used to determine the zygosity of all LS twins, both MC and DC. Cord blood from both twins was collected into EDTA at delivery; placental tissue was also used in a few cases. DNA was prepared by standard methods, and the restriction enzyme 3'-HVR was used both at low and high stringency for the production of restriction fragments. Southern blots were made, and zygosity was reported without prior knowledge of chorionicity.

RESULTS

A total of 129 twins pairs, and one set of triplets were born during the study period (261 births). Perinatal mortality in the 258 twins is shown in Table 1.

DNA fingerprinting was carried out in 16 LS pairs (Fig. 1, Table 2). All MC twins were confirmed as MZ. Use of 3'-HVR at high stringency required several alleles to ex-

Table 1 - Perinatal mortality, Victoria General Hospital, 1983-1987

	Births (%)	Perinatal deaths (%)	Perinatal deaths/1000 in each category
Total	13,365 (100)	169 (100)	12.6
Singletons	13,104 (98)	142 (84)	10.9
Twins	258 (02)	27 (16)	104.7
Triplets	3	0	0.0
Dichorionic			41
Monochorionic			233

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clude monozygosity in some DC cases, whereas low stringency gave satisfactory results in all cases. Faint tracks were obtainable even in cord blood samples from stillborn fetuses.

Table 2 - DNA fingerprinting studies of twin pairs, Victoria General Hospital

LS,DC		MC
10		6
DZ	MZ	MZ
9	1	6

During the same time period, 7 pairs of ULS,DC twins were born. In total, 23 twin pairs yielded 16 DZ and 7 MZ.

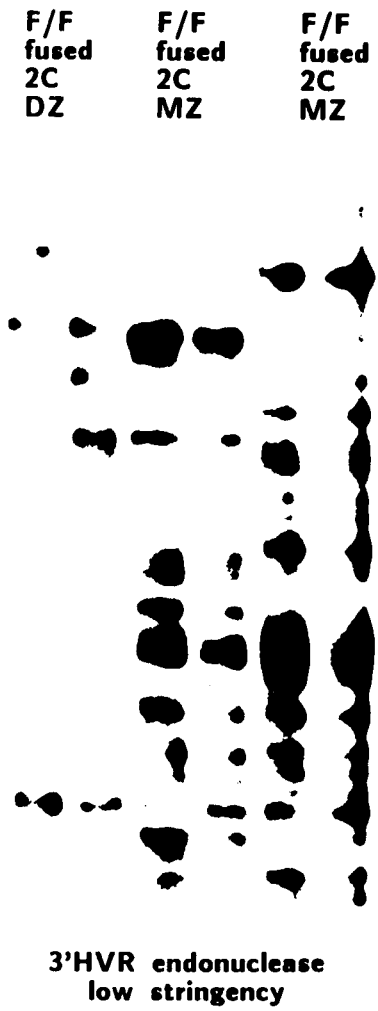

Figure. 3′-HVR at low stringency was applied to DNA extracts of cord blood from 3 pairs of LS,DC twins. In the track on the left, adequate DNA was obtained from a stillborn fetus to diagnose zygosity.

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LITERATURE REVIEW

Reports were reviewed in which supposedly MZ twins were analyzed for concordance/discordance for a variety of developmental anomalies (Table 3). MZ status was accepted on the basis of MC placenta, DNA fingerprinting or a sufficient statistical confidence based on analysis of multiple gene products. MZ status was accepted with qualification if the significance of gene product determination was not given; MZ status was doubted or discounted if analysis was insufficient. Of 78 cases, 43 (55%) were accepted, 14 (18%) provisionally accepted, and 21 (27%) were discounted. A significant number of cases were accepted on the basis of placental monochorionicity.

Literature on prevalence of anomalies in twins by zygosity was also reviewed.

Table 3 - Literature review of concordance/discordance for anomalies in MZ twins

Author	Anomaly	Zygosity method	Results
		A. Monozygosity determination accepted	
[15]	Congenital hypothyroidism	Placenta MC	C normal by neonatal screening, D evolution – (masked by MC?)
[13]	Heterokaryotypia	Multiple antigens, HLA	Triplets: 45,X; 46,XY; 46,XY
[61]	Heterokaryotypia	Multiple antigens	D 45,X/46,XX; 46,XX
[7]	HPE	Multiple antigens to 0.99999	C for HPE, D for severity
[48]	HPE, 13 trisomy	Placenta MC	C for HPE, 13 trisomy, D for omphalocele
[45]	Turner syndrome	Placenta MC	C for Turner, D for congenital heart disease
[8]	DMD	Multiple antigens to 0.99998	D (Unequal lyonization of X-linked allele)
[35]	DMD		C
[41]	Factor IX deficiency	Blood group antigens, ×18	D (Unequal lyonization of X-linked allele)
[33]	Neural tube defect	Placenta MC	D
[28[	Amyoplasia	Placenta MC Placenta MC Placenta MC Placenta MC Placenta MC	Case 1D Case 2D Case 6D Case 8D Case 10D
[14]	ABS	Placenta MC	C
[17]	ABS	Placenta MC	C

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Table 3 - *Continued*

Author	Anomaly	Zygosity method	Results
[18]	ABS	Placenta MC	D
[29]	ABS	Placenta MC	C
[31]	ABS	Placenta MC	D
[38]	ABS	Placenta MC	D
[39]	ABS	Placenta MC	D
[40]	ABS	Placenta MC	D
[47]	ABS	Placenta MC	C
[77]	ABS	Placenta MC	D
[80]	ABS	Placenta MC	C
[62]	ABS	Placenta MC	D
[46]	BWS	Placenta MC RFLP	D
[6]	BWS	Placenta MC, blood groups, HLA	D
[3]	BWS	Blood groups to 0.995	D
[51]	DH	Placenta MC	C
[53]	Fraser syndrome	Placenta MC	D (fetus papyraceus)
[78]	G syndrome	DNA fingerprint	C for disease, D for severity
[22]	Gastroschisis	Placenta MC	C
[5]	GS	Multiple antigens to 0.9936	D
[67]	GS	DNA fingerprint	C for syndrome, D for severity
[63]	Gonadal dysgenesis	Multiple antigens, HLA	C for 45,X/46,XY, D for genital sex
[44]	Idiopathic thrombocytopenic purpura	Multiple antigens	C
[71]	Islet cell antibodies	Multiple antigens, HLA	Case 1:D (triplets) Case 2:C
[64]	Liver calcification	Placenta MC	C
[79]	Thanatophoric dysplasia	DNA fingerprint	C
[25]	UTM	Multiple antigens to 0.96	D for prune belly syndrome
[19]	UTM	Placenta MC	C for multicystic kidneys
[36]	UTM	Placenta MC	D for obstruction
[49]	WT	Multiple antigens to 0.999	Case 1: D, Case 2: D

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Table 3 - *Continued*

Author	Anomaly	Zygosity method	Results
		B. Monozygosity determination probably acceptable	
[74]	NF	Multiple antigens, HLA	C
[11]	NF	Multiple antigens, HLA	C
[56]	HPE, 13 trisomy	Some antigens	D for HPE
[69]	Trisomy 18	HLA, not specified D for severity of phenotype	C for trisomy 18,
70	HD	Placenta "2 membranes", some antigens	Case 1: D, Case 2: D
[52]	HD	Multiple antigens, HLA	D
[30]	HD	Multiple antigens, HLA	D
[66]	Anorchia	Blood groups to 0.93	C
[58]	BWS	Multiple antigens	Case 1 D, 2 D, 3 D
[75]	DH	Blood groups	C
[60]	Rett syndrome	Multiple antigens, HLA	C
[23]	Terminal transverse limb defect	Blood groups, not stated how many	C
[24]	UTM	Some antigens	C for posterior urethral valves
		C. Monozygosity not accepted	
[28]	Amyoplasia	Blood groups, not specified	Case 3 D
		Placenta MC?	Case 4 D
		Placenta MC? blood groups, not specified	Case 7 D
		Blood groups, not specified	Case 9 D
		Blood groups, not specified	Case 11 D
[37]	Chromosomal del (10) (p11-15)	Placentas separate, but said to be MC	C
[34]	Cockayne syndrome	Blood groups, not specified	C
[1]	Dermatopathology: epidermolytic hyperkeratosis	Not stated	D
[9]	Dermatopathology: cutaneous mastocytosis	Not stated	D
[65]	Endocardial fibroelastosis	Not stated	C
[43]	Gestational trophoblastic neoplasia	Not stated	C

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Table 3 - *Continued*

Author	Anomaly	Zygosity method	Results
[73]	Gonadal dysgenesis	Not stated	C
[72]	HPE	Some antigens	D
[59]	Jejunal atresia, type IV	Not stated	C
[42]	Mesangial sclerosis of kidney	Not stated	C
[21]	Multiple endocrine neoplasia type 2b	Not stated	C
[26]	Omphalocele/sirenomelia	Not stated	C (may have been conjoined)
[27]	Ovarian carcinoma	Not stated	Case 1: C; Case 2: C; Case 3: C

C: Concordant; D: Discordant; ABS: Amniotic band syndrome; BWS: Beckwith-Wiedemann syndrome; DMD: Duchenne muscular dystrophy; DH: Diaphragmatic hernia; GS: Goldenhar syndrome; HD: Hirschsprung's disease; HPE: Holoprosencephaly; NF: Neurofibromatosis RFLP: Restriction fragment length polymorphism; UTM: Urinary tract malformation; WT: Wilms' tumor.
Of 79 reported cases, 44 (56%) were accepted; of these, 28 had MC placenta, 4 had DNA fingerprinting and 12 had statistically significant gene product analysis.

DISCUSSION

The pathogenesis of congenital anomalies in twins has been the basis for many publications. It has great potential for distinguishing "environmental" from "genetic" causes. However, the present situation appears rather confused and in need of clarification. For instance, there is widespread acceptance that malformations are more prevalent in MZ than in DZ twins [68]. However, the authors of this paper admit that "among the limitations of this study was the inability to be totally secure about zygosity in most twin pairs. ...However, all cases of evident dizygosity were excluded..." Others have questioned the results of this and other papers [4,12,50], both on the grounds that the origins of MZ and DZ twins may not be as profoundly different as generally supposed, and because the documentation of zygosity was not always sufficiently rigorous.

Inspection of the supposed excess of "malformations" in MZ twins shows that many may not be true malformations, but rather anomalies that may be explicable by vasculogenic events occurring in the context of monochorionicity (Table 3). (Conjoined twins should obviously be excluded from these considerations). Table 4 shows that the prevalence of pure malformations in MZ twins may not be significantly higher than in DZ twins. However, it is possible that certain subtypes of malformations, especially of the midline, may be more common in MZ twins, but there are rather small numbers of

Table 4 - Prevalence of pure malformations and of anomalies in twins by zygosity

A. US Collaborative Perinatal Project [50,54,55]

	Major malformations by zygosity			
	MZ	DZ	Not known	Total
No	40	48	25	113
%	11	8	12	9.4
	MZ,MC	MZ,DC		
No	39	16		
%	33	29		
Total	117	56		

B. Birmingham/Ghent Prospective Survey [10]

	Anomalies by zygosity	
	DZ	MZ
No	50	33
%	2.6	3.7
Total	1956	892

Complete data are not given by chorionicity. Many of these anomalies do not appear to be true malformatios.

cases in the published literature. The individual malformations in MZ,MC twins in the US Collaborative Perinatal Project [50] were more severe and of midline-type than in the MZ,DC twins (eg, anencephaly, tracheoesophageal fistula, biliary atresia and congenital heart disease).

A study of the prevalence of neural tube defects in twins analyzed the twins into like-sexed and unlike-sexed pairs, and therefore only offered derivative (Weinberg-type) data on likely differences between MZ and DZ twins [76]. An ultrasound study of congenital anomalies in twins [57] reported a miscellany of anomalies in four MZ,MC twin pairs, including one fetus papyraceus, a heterokaryotypic pair (45,X and 46,XX), a twin pair concordant for multiple midline malformations, and a pair of conjoined twins. Another report of five MZ pairs was equally miscellaneous [20]; there was a pair discordant for esophageal atresia, and another discordant for neural tube defect, together with an acardiac fetus and two pairs markedly weight-discordant, presumably secondary to twin transfusion syndrome.

The collection of larger numbers of malformations in twins is clearly necessary to resolve these issues, and it is suggested that zygosity and chorionicity must be determined with high accuracy if new data are to be helpful. This author advocates DNA fingerprinting of like-sexed DC twins for the investigation of all aspects of twin pregnancy.

Table 5 gives a suggested provisional classification of congenital anomalies in MZ twins, taking account also of chorionicity and amnionicity. The collection of accurate data on zygosity and chorionicity in the principal centres for twin research would allow the accumulation of sufficient numbers of twins to allow the testing of several hypotheses concerning these congenital anomalies.

Table 5 - A provisional classification of anomalies in MZ twins

Anomaly	DC	MC, DA	MC, MA
1. MZ twins would be expected to be concordant for autosomal mutant gene disorders	Yes	Yes	Yes
2. But are MZ twins sometimes discordant for autosomal mutant gene disorders because of postzygotic somatic cell mutation? If so:	Yes	Yes	Yes
3. Interfetal transfer of metabolites and hormones in MC,MZ twins may cause fetal and neonatal " pseudoconcordance ", eg, congenital hypothyroidism [15]	No	Yes	Yes
4. MZ twins are usually concordant for structural chromosme disorders	Yes	Yes	Yes
5. But heterokaryotypia (for whole chromosomes) occurs in MZ twins, and could invalidate RFLPs	Yes	Yes	Yes
6. MZ twins concordant for aneuploidy may be discordant for the severity of phenotype	Yes	Yes	Yes
7. Unequal lyonization for X chromosome mutant gene disorders can result in discordance in severity of phenotypic expression in MZ twins	Yes	Yes	Yes
8. " True " malformations, especially of midline type, may be more prevalent in MZ than DZ twins. MZ twins may be discordant for severity of these malformations	Yes	Yes	Yes
9. But some cardiac malformations in MZ twins might actually result from early changes in blood flow caused by twin-twin transfusion	No	Yes	Yes
10. " Micro " vasculogenic disruptional events are proposed as causing hypoxic cell and tissue damage in MC,MZ twins, eg, amyoplasia, Hirschsprung's disease	No	Yes	Yes
11. " Large scale " vasculogenic disruptional events in discordant MC,MZ twins, resulting from arterio-venous anastomoses, often in the context of fetal death of one twin, may cause organ infarction, eg, microcephaly	No	Yes	Yes
12. " Large scale " vasculogenic disruptional events in discordant MC, MZ twins, resulting from reversed arterio-arterial perfusion, eg, acardiac fetuses	No	Yes	Yes
13. " Special " anomalies unique to MC, MA, MZ twins, ie, conjoined twins	No	No	Yes

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