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Evaluation of Zygosity Diagnosis in Twin Pairs Below Age Seven by Means of a Mailed Questionnaire

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Abstract. The mothers of a consecutive series of 125 same sexed pairs of twins aged 6 months to 6 1/2 years completed a mailed questionnaire concerning the physical similarity of the twin partners. Zygosity diagnoses were first determined blindly on the basis of the answers given by the mothers, who did not know the result of the blood group test when fulfilling the questionnaire. Next, the results were compared with the zygosity determination based on examination of erythrocyte-, serum- and enzyme-groups. It turned out that a few simple questions distinguished well enough between MZ and DZ pairs, with a frequency of misclassification of 4%, leaving 5% as unclassifiable. It is concluded that twin zygosity can be estimated by a simple questionnaire with sufficient accuracy even in very young twin pairs. This has relevant implications for more extensive twin studies where the use of a wide spectrum of serological characters would imply too high expenses.

Key words: Twin, Zygosity, Questionnaire

INTRODUCTION

The determination of twin zygosity type is essential in twin research. The most accurate method separating monozygotic (MZ) from dizygotic (DZ) twins is based on the increasing number of erythrocyte-, serum- and enzyme-group polymorphisms which have been and are being disclosed [6,4]. When a reasonable selection of these are used, the accuracy of classification exceeds 99%.

In epidemiological research based on large samples, however, these comprehensive blood group analyses are generally too expensive and time-consuming. For such studies, simpler methods have been used, based on the degree of similarity between twin partners as stated in a simple mailed questionnaire [1,5,7,8,9,11]. Very few studies have applied this similarity method to children [2,10], and only the last mentioned study included twin pairs as young as 4-5 years.

The purpose of the present study was to examine the validity of a short, simple, mailed questionnaire in zygosity assessment of twin pairs aged 1/2 to 6 1/2 years.

MATERIAL AND METHODS

Nearly all same-sexed twin pairs born in the Department of Obstetrics and Gynecology, University Hospital of Odense, from 1 January 1980 to 31 December 1985 participated in the study. Only in a few cases an emergency situation prevented cord blood sampling. In the period mentioned, samples were obtained from 132 same-sexed twin pairs. Out of these, 2 pairs were excluded because one member in each pair had a cleft lip (which influenced the external similarity of the twins). Two twin pairs were excluded because the parents already had a knowledge of the result of the blood group tests. A mailed questionnaire was sent to the mothers of the remaining 128 twin pairs in June 1986, when the twins were between 6 months and 6 1/2 years old. None of these mothers knew the result of the blood group test. The questionnaire was completed and returned by 125 of the mothers (97.7%).

The questionnaire, which is shown in Appendix I, asked for information concerning similarity of the twin partners and experiences of mixing up their identity. For all twins, zygosity was determined by means of 17 serological marker systems (see Appendix II). The tests were performed in the Institute of Forensic Medicine, University of Copenhagen.

The classification of zygosity according to the answers given in the questionnaire was made without knowledge of the blood groups. The key decision rules for the questionnaire zygosity diagnosis are shown in Table 1. Difference with respect to hair and/or eye colour was taken as proof of dizygosity. In cases of identity of hair and eye colour, description of more than ordinary family likeness and histories of mixed identity were considered indications of monozygosity. These rules are the same as those adopted by the Danish Twin Registry when classifying adult twin pairs [5].

Without knowing the result of the zygosity classification based on questions no. 1-5 (see Appendix I), we evaluated the effect of adding the information from question no. 6. If the mother stated that she hardly ever could tell the two twins apart or that she often made mistakes at the time when the twins were 6 months old, the pair was classified as MZ. If, on the other hand, the mother found that she could always tell them apart at the age of 6 months, they were classified as DZ. All answers in between implied that the pair in question was considered unclassifiable (XZ).

Table 1 - Guidelines to the zygoty classification according to the questionnaire

	N	Quest. diagnosis	Question number		No. of misclassifications
I	32	DZ:	3	Different hair and/or eye colour	0
II	7	DZ:	1	No, not as two peas in a pod	0
			2	Ordinary family likeness	
			3	Same hair and eye colour	
			4	No mixed identity	
			5	No external marks	
III	38	MZ:	1	Yes, as two peas in a pod	0
			2	More than ordinary family likeness	
			3	Same hair and eye colour	
			4	Mixed identity	
			5	Need for external marks	
IV	9	MZ:	1	Yes, as two peas in a pod	0
			2	More than ordinary family likeness	
			3	Same hair and eye colour	
			4	Mixed identity	
			5	No external marks	
V	6	MZ:	1	No, not as two peas in a pod	0
			2	More than ordinary family likeness	
			3	Same hair and eye colour	
			4	Mixed identity	
			5	Need for external marks	
VI	27	MZ:	1	No, not as two peas in a pod	5
			2	More than ordinary family likeness	
			3	Same hair and eye colour	
			4	Mixed identity	
			5	Need for external marks	
VII	6	XZ:		Mixed answers - unclassified	
Total	125				5
Unclassified	6/125 = 4.8%				
Misclassified	5/125 = 4.0%				

RESULTS

The results of the serological tests which are taken as leading to the "true" zygoty type showed 78 pairs to be MZ (with complete blood group concordance) and 47 to be DZ (exhibiting one or more differences). When only the answers to the questionnaire were used, 5 of 125 pairs (4%) were misclassified, ie, labelled in disagreement with the results of blood group determinations. Mistakes were seen exclusively in DZ pairs. The ages of these misclassified twin pairs were 6, 14, 18, 37 and 37 months, respectively. Six twin pairs of 125 (5%) were unclassifiable on the basis of the questionnaires when the rules described in Table 1 were followed.

The answers were in all these cases ambiguous such as: "Not as two peas in a pod, only ordinary family likeness, same hair and eye colour and mixed identity". One of these (an MZ pair) needed, however, external marks. Three of these six unclassifiable pairs were MZ according to the blood groups, the remaining being DZ.

Table 2 - Comparison of classification methods

Questionnaire method	Blood groups	
	MZ	DZ
MZ	75	5
DZ	0	39
XZ	3	3
Total	78	47

Sensitivity $75/80 = 93.8\%$ (test for likeness)
 Specificity $39/39 = 100\%$ (test for unlikeness)

Table 3 - Relation between answers to the individual questions and zygosity according to blood groups

Answers of the mothers	Zygosity according to blood groups			
	MZ		DZ	
	N	%	N	%
1 Yes, as two peas in a pod	45	57.7	0	0.0
No, not as two peas in a pod	31	39.7	47	100.0
Unanswered	2	2.6	0	0.0
2 Ordinary family likeness	5	6.4	36	76.6
More than ordinary family likeness	68	87.2	9	19.2
Unanswered	5	6.4	2	4.3
3 Same hair and same eye colour	78	100.0	15	31.9
Different hair and/or different eye colour	0	100.0	32	68.1
4 Yes, mixed identity	78	100.0	20	42.6
No mixed identity	0	0.0	26	55.3
Unanswered	0	0.0	1	2.1
5 Need for external marks	45	57.7	1	2.1
No need for external marks	33	42.3	46	97.9
6 Mothers' impression of zygosity				
Right	45	57.7	46	97.9
Wrong	19	24.4	0	0.0
Don't know	14	18.0	1	2.1

A comparison of the classifications made according to blood groups and to questions no. 2, 3 and 4, respectively, is shown in Table 2. This reveals a sensitivity

of 94% and a specificity of 100% of the questionnaire method as used here. No gain was achieved when question no. 6 was included. On the contrary, this resulted in 10 misclassifications (8%). Eight of these were made in pairs who had to be classified as DZ as the mothers reported that they could always tell the twins apart when six months old (although they were MZ). In addition, the group of XZ pairs increased to 18 (14%).

It was furthermore found that questions no. 1 and 5 can be omitted without losing precision in the classification.

The relation between answers to the individual questions and zygosity according to blood groups is shown in Table 3, and the number of correctly classified twin pairs according to the individual questions is shown in Table 4. It should finally be mentioned that no single question could classify more than 83% of the pairs correctly.

Table 4 - Twin pairs correctly classified according to each individual question

Question no.	Correctly classified	
	N	%
1 As to peas in a pod	92	73.6
2 Family likeness	104	83.2
3 Hair and eye colour	32	25.6
4 Mixed identity	104	83.2
5 External marks	91	72.8
7 Mother's impression	91	72.8

DISCUSSION

Previous studies in adults have shown that a reliable zygosity diagnosis can be obtained by means of questionnaires using only a few simple questions [1,2,5,7-11]. However, the validity of this method when applied to infants has not previously been evaluated. We found that the same few simple questions offer an equally acceptable basis for zygosity classification of children aged 6 months to 6 1/2 years. By this method, 5% were unclassifiable, and 4% were misclassified. This corresponds to a sensitivity of 94% and a specificity of 100%, as five pairs of true DZ twins were classified as MZ.

Using the information from an additional question (no. 6 in Appendix I) did not improve the accuracy, as more cases were misclassified and a greater number left unclassified. Furthermore, questions no. 1 and 5 could be omitted without any loss of precision. Thus, questions 2, 3 and 4 were found to give sufficient information for establishing the zygosity diagnosis, as in adult twin pairs.

The frequency of misclassification according to the results of the blood group examinations is very small (considerably less than 1%), and consequently, not more than one pair, if any, in the present material is likely to have been misclassified.

In some previous studies [2,7,8] more questions have been used, eg, on height, weight, hair texture and by whom the twins were mistaken. Such questions are not very suitable for young children. A discrepancy in weight or height might be due to differences at birth which would probably be reduced later in life, at least in MZ pairs. When dealing with adult twins, answers are collected from both twin partners, if possible. This may require more advanced computer programs [7,9]. In the present study, we found no need for more complex statistical methods, as three answers per twin pair were sufficient for a reliable zygosity classification.

No single question could by itself separate completely between MZ and DZ twins. Answers to questions on family likeness and mixed identity had the highest discriminating power as they could both place 83% of the twin pairs correctly. It is worth noting that 40% of MZ twins are described as "not as alike as two peas in a pod". This may be because some of the MZ twin pairs are really not all that identical. However, this is a difficult question to answer for a mother of twins. She might well eagerly be looking for any small differences to tell her twins apart so that she will no longer consider them or, perhaps, accept them as "two peas in a pod". All MZ twins showed histories of mixed identity, but as much as 42% of DZ twins had also been mixed up, partly perhaps due to their young age. Mothers of younger twins seem to be less able to determine zygosity correctly (point 7 in Table 3). Magnus [7] found that 91% of adult twins could classify themselves correctly. It is important to note that nearly all mistakes of the mothers' impression of zygosity were found in the group of true MZ pairs. This may lead to an underestimation, especially of discordant MZ pairs.

The anthropological variables used in the present study are hair and eye colour. They have been shown to be the most valuable anthropological characters in differentiating between MZ and DZ twin pairs [3]. Hair and eye colour were found useful also in the present sample, which, however, included Danes only. The rules accepted here may not retain their value when applied to groups with less variation in hair and eye colour.

The conclusion of our study is that the zygosity of infant twin pairs can be determined with acceptable accuracy if the same few simple questions as in adult pairs are used. Thus, this method is fully applicable from an early age. This will save time and reduce expenses in studies involving any higher number of pairs. Furthermore, population-based twin registers may confidently include twin pairs who have survived the first year or so, as long as the mother is available to provide the information needed.

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Appendix I

TRANSLATION OF THE QUESTIONNAIRE

- 1 Are the twins much alike in general appearance (as two peas in a pod)? —Yes —No
- 2 Is the likeness between the twins as ordinary family likeness? —
 More than ordinary family likeness (remarkably alike) —
- 3 Do the twins have the same hair colour —Yes —No
 same eye colour —Yes —No
- 4 Have the twins been mixed up by family and friends? —Yes —No
- 5 Have the twins ever been so much alike that external marks have been needed to tell them apart (bracelets, nail polish, ink marks, different clothes, etc)? —Yes —No

When we ask the mother of the twins if she could tell the twins apart (question no. 6), we mean when watching them at some distance. Ignore external marks which you yourself have given the twins (eg, ignore bracelets, nail polish, ink marks, different clothes, etc).

- 6 Could the mother of the twins tell them apart when they were

	Yes, always	Yes, near- ly always	Often made a mistake	No, could near- ly never tell them apart
about 1 month old	—	—	—	—
about 6 months old	—	—	—	—

(Tick off the right answer)
- 7 Do you yourself think that the twins are *(Tick off)*
 - identical (from one egg) —
 - fraternal (from two eggs) —
 - I don't know —

Appendix II

SEROLOGICAL MARKERS

ABO, MNSs, Rhesus (4 sera), Kell, Duffy, Haptoglobin (Hp), Group-specific component (Gc), Transferrin (Tf), Phosphoglucomutase (PGM-1), Acid phosphatase (AcP), Glutamate pyruvate transaminase (GPT), Esterase D (EsD), Glyoxalase (GLO), Adenyladenylatkinase (AK), 6-Phosphogluconate dehydrogenase (PGD), Adenosine deaminase (ADA), Galactose-1-phosphate uridylyltransferase (GLT/Gt).