

Retrospective Determination of Chorion Type in Twins Using a Simple Questionnaire

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This study investigates the validity of retrospective determination of chorion type by asking the question to the mother about the number of placentas. In the "East Flanders Prospective Twin Survey" (EFPTS), accurate information on the placentation and zygosity of the multiples was collected prospectively. The mothers of 231 monozygotic (95 dichorionic and 136 monochorionic) twins and 255 dizygotic twins were asked to fill in a simple questionnaire regarding 1) the zygosity and 2) the number of placentas of their twins. The accuracy of the response to the question on "the number of placentas" was 60% for monozygotic twins and 37% for dizygotic twins. The accuracy of the response to the question on the zygosity of the twins was 93% for monozygotic and 95% for dizygotic twins. If the questionnaire was used for the determination of chorion type, a total of 31 monozygotic twins (13%) should have been assigned as dichorionic on the fact that there were two separate placentas. Of these, 10 (32%) are monochorionic and 12 (39%) were falsely reported as having two placentas. We conclude from these findings that this simple questionnaire method is unreliable for the retrospective determination of the chorion type.

The twin design has frequently been applied to estimate the relative contribution of genes and environment to a variety of traits, including body composition and fat distribution, by comparing the concordance or correlation in monozygotic (MZ) and dizygotic (DZ) twins. A greater degree of similarity between members of MZ pairs than between members of DZ pairs suggests the presence of genetic influences. The basic assumption of the twin design is that MZ monochorionic (MC), MZ dichorionic (DC-MZ), and DZ twins experience a similar pre- and postnatal environment. However, this assumption has been questioned in the light of the prenatal programming hypothesis (Corey et al., 1979; Gruenwald, 1970; Phillips, 1992, 1993). As the prenatal environment of MC twins differs substantially from that of DC-MZ and DZ twins, Phillips argued that the classic twin study might be an unreliable method of estimating the genetic component of traits in which the prenatal environment is thought to play a role.

According to the zygosity and the number of the placental membranes, three types of twins that differ substantially in prenatal environment can be distinguished: (1) DZ, (2) DC-MZ and (3) MC. Since the placenta is formed from chorionic tissue, DZ and DC-MZ have two

placentas that can fuse during development. Both members of a MC pair share one placenta, a setting which enables the development of vascular anastomoses between the circulations of the two fetuses and might therefore influence the competition for a limited food supply. Compared with DC-MZ twins and DZ twins, MC twins have a higher perinatal mortality rate (Derom et al., 1995; Loos et al., 1998).

In the present study, we investigated the accuracy of a simple questionnaire method to determine chorion type retrospectively as has been proposed and used earlier (Duffy, 1993; Healy et al., 2001).

Subjects and Methods

Since 1964, the "East Flanders Prospective Twin Survey" (EFPTS) has collected information on the mother, the placenta and the children of 98% of the multiples born in the province of East Flanders, Belgium (Loos et al., 1998). By the end of 2000, the register counted 5371 twin pairs, 196 sets of triplets and 27 sets of higher order multiples. Zygosity was determined through sequential analysis based on sex, fetal membranes, umbilical cord blood groups (ABO, Rh, CcDEe, MNSs, Duffy, Kell), placental alkaline phosphatase and, since 1982, DNA fingerprints (Derom et al., 1985). Unlike-sex twins and same-sex twins with at least one different blood group or, if blood groups were the same, at least two different DNA-markers were classified as DZ; MC twins were classified as MZ. For all same-sex DC twins with the same genetic markers a probability of monozygosity was calculated using a lod-score method (Meulepas et al., 1998). The collaborating obstetricians are informed by mail on the chorion type and zygosity of the twins.

For the present study, we contacted the mother of twins that had participated in two of our previous studies. The first study was of young adults (age range 18–33 years) and aimed to study the Barker-hypothesis in twins (Loos et al., 2001a, 2001b, 2002). The second study was a project on mental retardation in twins. All twins from the EFPTS

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register born between September 1982 and December 1991 (age range 8–14 years) of Belgian ancestry were invited to participate (Jacobs et al., 2001). Only the mothers of the most recent participating twins were asked to fill in the questionnaire: 231 mothers of MZ twins (95 DC and 136 MC; 116 male and 115 female pairs) and 255 mothers of DZ twins (68 male-male, 71 female-female and 116 unlike-sexed pairs). All DC-MZ twins reached a probability of monozygosity of at least 98%.

The mother of the twins was asked 4 questions:

1. Do you know the zygosity of your twin? (MZ/DZ/unknown)
2. Did your obstetrician give you information on the zygosity of the twins? (yes/no/unknown)
3. Do you know “how many afterbirths” (placentas)¹ there were at birth? (one/two/unknown)
4. Did your obstetrician give you information on the number of placentas at birth? (yes/no/unknown)

The contingency chi-square test was used for comparison of the data. The analyses were conducted with a SAS 6.12 computer package. The reported *p* values are two-sided and were considered statistically significant when *p* ≤ 0.05.

Results

Table 1 summarizes the result of the “number of placentas” as recorded by questionnaire and by EFPTS at birth. As there were no differences in the number of correct and wrong answers given by the mother when comparing both studies (data not shown, *p* = 0.8), their results were combined. When comparing DZ with MZ twins, the number of correct and wrong answers to the third question was different (*p* = 0.02). The accuracy, which is computed as the percentage of correct responses, is 60% for MZ twins and 37% for DZ twins. The accuracy of the response to the question on the zygosity was higher: 93% correct answers for MZ twins and 95% for DZ twins. The number of correct and wrong answers to both questions, number of placentas and zygosity, did not differ according to the information given by the obstetrician at birth (data not shown, *p* > 0.3).

Of the 231 MZ twins 41% were DC and 59% MC. Table 2 summarizes the result of unknown, correct and wrong answers according to chorion type in MZ twins. Of the 95 DC-MZ twins 36 had separate placentas and 59 fused placentas. Of the 136 MC twins all but one had one

Table 2
Number of Placentas : Answers Given by the Mothers of MZ Twins

Number of afterbirths	Chorion type	
	MZ-DC	MC
correct	46 (20%)	92 (40%)
false	17 (7%)	11 (5%)
unknown	32 (14%)	33 (14%)
Total	95 (41%)	136 (59%)

Note: MZ : monozygotic
DC : dichorionic
MC : monochorionic

placenta. Only 16 mothers of DC-MZ twins with two placentas reported correctly that there were separate placentas at birth. Seventeen mothers of DC-MZ twins misclassified their twins according to number of placentas; that is, 12 mothers reported two placentas, whereas only one (fused) placenta was present, and 5 mothers reported “one after-birth” when two separate placentas were present. Ten mothers of MC twins reported falsely two placentas and one mother reported only one, where in fact there were two placentas connected with membranes. Overall, the number of unknown answers was high.

Discussion

Two main macroscopic varieties of placentation can be present in DC twins (DZ as well as MZ): two separate placentas (connected with membranes or not) and two fused placentas. In both zygosity groups about half of the pairs have separate placentas. Only one placenta is present in almost all MC twins (Loos et al., 1998). Therefore, if the number of separate placentas can be correctly assigned and the zygosity of the twin pair is known, the chorion type of half of the DC MZ twins (i.e., these with separate placentas) can be retrospectively correctly determined. Of the remaining twins with “one placenta”, 80% will be MC.

Overall, the accurate reporting of the number of placentas by the mother is low, especially in DZ twins. One reason is surely the confounding of number of placentas and chorionic sacs. DC twins are born in two chorionic sacs but only half of them have separate placentas. As a matter of fact, being born in two chorionic sacs does not equal having separate placentas at birth and is not a proof

Table 1
Number of Placentas as Recorded by the Mother and at Birth (EFPTS)

Mother	Monozygotic twins EFPTS		Mother	Dizygotic twins EFPTS	
	1	2		1	2
1	123 (53%)	12 (5%)	1	23 (9%)	9 (4%)
2	15 (6%)	16 (7%)	2	65 (25%)	71 (28%)
unknown	57 (25%)	8 (3%)	unknown	42 (16%)	45 (18%)
Total	195 (84%)	36 (16%)	Total	130 (51%)	125 (49%)

Note: EFPTS: East Flanders Prospective Twin Survey

of dizygosity. MC twins have one common chorionic membrane and nearly always only one placenta.

That the information given by the obstetrician does not influence the accuracy of the exact reporting of the “number of placentas” was not surprising, as some obstetricians still believe that two chorionic sacs or two separate placentas are a prove of dizygosity. The low frequency of correct reporting of the chorion type by the mothers is certainly partly due to the inability of the obstetricians to distinguish between the different types of placentas and, as a matter of fact, tell the mothers the wrong observation or interpretation.

If the questionnaire was used for the determination of chorion type, a total of 31 MZ twins (13%) should have been assigned as DC on the fact that there were two separate afterbirths at birth. Of these, 10 (32%) are MC and 12 (39%) reported falsely two afterbirths.

The accuracy on the question of the zygosity of the twins in the present study is high and of the same magnitude as reported earlier (Peeters et al., 1998). It is most probably that this accuracy is somewhat higher than in other studies (Jackson et al., 2001) because, as mentioned earlier, the obstetrician is informed about the placentation and the zygosity.

In conclusion, our findings suggest that, if biologically based chorion type classification is not done at or before birth, this simple questionnaire method is not reliable for the retrospective determination of the chorion type.

Endnote

- 1 Regarding the question asked to the mother “one after-birth” or “one placenta” means either a single placenta or two fused placentas. The term “afterbirth” was used in the questionnaire, because this term is most commonly known in the Dutch language

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