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Can Birthweight Discordancy Within Monozygotic Twin Pairs Be Used as an Indicator of Chorionicity?

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Both zygosity and chorionicity provide important information in twin research. The East Flanders Prospective Twin Survey (EFPTS) determines zygosity and chorionicity at birth and therefore provides a gold standard for the testing of diagnostic parameters that can be used to determine chorionicity. The aim of the present study was to investigate whether birthweight discordancy can be used as an indicator of chorionicity. The study sample consisted of 4,060 live-born twin pairs from the EFPTS. We studied MZ twins, using univariate and multivariate logistic regression analyses to calculate odds ratios (OR) and 95% confidence intervals (CI) of being MC in relation to discordancy level. Diagnostic parameters, including sensitivity and specificity, were calculated. A two-fold cross-validation was carried out and a bootstrap distribution with 10,000 samples was created to estimate the standard deviations. For discordancy levels of below 10%, 10–15%, 15–20%, 20–25% and above 25%, the ORs (95% CI) were 1.16 (0.91–1.47), 1.38 (1.05–1.80), 2.13 (1.51–3.01), 2.73 (1.73–4.29) and 2.81 (2.81–4.35) respectively. There were no gender differences. Sensitivity was 42.2% (SD 5.6%), specificity was 72.8% (SD 6.3%), positive predictive value was 72.8% (1.5%) and the negative predictive value was 39.2% (0.7%). In conclusion, although a higher discordancy level resulted in higher ORs of being an MC twin, birthweight discordancy level can only be used to some weak extent as a proxy for chorionicity, highlighting the need to assess and record chorionicity data in obstetrical units.

Keywords: birthweight discordancy, chorionicity, zygosity, diagnostic test, sensitivity, specificity

The basic assumption in twin studies is that monozygotic (MZ) and dizygotic (DZ) twins experience the same prenatal and postnatal environment. This assumption has been questioned, as the prenatal environment of MZ monochorionic (MC) twins differs from that of MZ dichorionic (DC) twins and DZ

(always DC) twins (Cheung et al., 2000; Corey et al., 1979; Davis et al., 1995; Gardiner et al., 2003). For example, in MC twins with twin-to-twin transfusion syndrome vascular programming is evident (Cheung et al., 2000; Gardiner et al., 2003). Therefore, chorionicity as well as zygosity provide essential information in twin studies.

The East Flanders Prospective Twin Survey (EFPTS, Belgium) is the only large twin register that collects information about chorionicity at birth. Chorionicity is determined by examination of the placentas and fetal membranes within 48 hours after delivery according to a standardized protocol (Derom et al., 1995). This method of chorionicity determination can be used as a gold standard for the testing of diagnostic parameters. For example, a simple questionnaire for the retrospective determination of chorionicity has been used by the EFPTS (Derom et al., 2003). The questionnaire was based on the fact that 50% of both the MZDC and DZ twins have separate placentas (and are therefore DC) and that 80% of the MZ twins with one placental mass are MC. Therefore, if the number of separate placentas can be correctly assigned, the chorionicity of half of the MZ twins (those with separate placentas) can be correctly determined retrospectively and of the remaining twins 80% will be MC. However, the numbers of placentas was not accurately reported by the mothers and the questionnaire proved to be unreliable for this purpose (Derom et al., 2003).

An alternative diagnostic parameter for retrospective chorionicity determination could be the use of birthweight discordancy. This parameter is based on the fact that MC twins have lower and more discordant

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birthweights than DC twins (Blickstein & Keith, 2004; Fick et al., 2006; Gielen et al., 2008; Gonzalez-Quintero et al., 2003). One would expect MZ DC twins to be the least discordant, because they are genetically identical, have their own placenta and, therefore, do not have to compete for nutrients. DZ DC twins and MZ MC twins would be expected to be more discordant because DZ DC twins share on average half of the segregating genes and MZ MC twins are confronted with unequal placenta sharing.

The gender of the twin pair could influence the birthweight discordancy, since a birthweight difference exists between female–female (FF) and male–male (MM) pairs, with females having lower weights (Loos et al., 2001; Min et al., 2000). Furthermore, the difference in fetal growth between males and females appears to be rather pronounced before the third trimester, suggesting that males show a faster growth and at an earlier stage (de Zegher et al., 1999). As a result, female embryos could be somewhat less mature at the time of splitting (Derom et al., 2007). In addition skewed X-inactivation could also cause more birthweight discordancy in FF twin pairs (Martin et al., 1997). At present, it is unknown whether the level of discordancy of MM pairs is similar to that of FF pairs in MZ twin pairs and thus whether gender should be taken into account for retrospective chorionicity estimation.

The aim of the present study was to investigate whether birthweight discordancy can be used as an indicator of chorionicity. We hypothesized that a higher intra-pair birthweight difference results in a higher odds of being an MC twin pair, that intra-pair birthweight differences can be used to retrospectively predict chorionicity and that these results are similar for MZ MM pairs and MZ FF pairs.

Methods

The study sample consisted of twin pairs selected from the population based East Flanders Prospective Twin Survey (EFPTS), Belgium (Derom et al., 2006). A vast majority (> 99%) of the population of the province of East Flanders is of Caucasian origin (Gielen et al., 2008). Between July 1964 and the end of December 2006, the EFPTS registered 7218 twin pairs who met the World Health Organization criteria for live-born infants (birthweight \geq 500g or gestational age \geq 22 weeks, if birthweight unknown).

Obstetric and perinatal data were recorded. Placentas and fetal membranes were examined within 48 hours after delivery and chorionicity was assessed macroscopically following a standardized protocol (Derom et al., 1995). Gestational age was reported by the obstetrician, based on the last menstruation or a first trimester ultrasound investigation, and was calculated as the number of completed weeks of pregnancy. Zygosity was determined by sequential analysis based on sex, chorionicity, umbilical cord blood groups, placental alkaline phosphatase, and, since 1982, DNA

fingerprints (Loos et al., 1998; Vlietinck, 1986). Zygosity and chorionicity were determined with an accuracy of 0.999 (Vlietinck, 1986).

Only live-born twin pairs of the same sex, with no major congenital malformations, were included in this study ($n = 4,720$ pairs). Opposite sex pairs were excluded, because they are per definition DC. Pairs of whom one or two twins were stillborn or suffered from a major congenital malformation were excluded. Twin pairs with missing or inconsistent data (birthweight ($n = 34$ pairs), gestational age ($n = 428$), chorionicity and zygosity ($n = 126$), parity ($n = 31$) and maternal age ($n = 41$)) were excluded. In total, 4,060 twin pairs were analyzed.

Statistical Analysis

Discordancy level was calculated as the relative birthweight difference ($[\text{highest birthweight} - \text{lowest birthweight}] / \text{highest birthweight}$) and was analyzed as a continuous variable and as a discrete variable. Six groups of discordancy level were defined: 0–5%, 5–10%, 10–15%, 15–20%, 20–25%, and > 25%. To compare the discordancy levels of the DZ, MZ DC and MZ MC twins, analysis of variance was used for discordancy as a continuous variable, and a t test was used for comparison of males and females within a group. χ^2 -tests were used for comparisons of discordancy level as a discrete variable of the DZ, MZ DC and MZ MC twins.

Both univariate and multivariate logistic regression analyses were applied to estimate the odds of being an MC twin pair in relation to discordancy level, and odds ratios (OR) and 95% confidence intervals (CI) were calculated. Gestational age, the gender of the twin pair, parity (primiparity vs. multiparity), and maternal age were regarded as potential confounders. Because obstetrical procedures have changed over the years, we also controlled for birth year. In addition, interactions between birthweight difference and the potential confounders were tested by stratified analyses. A trend test was performed by entering the discrete discordancy level as a continuous variable into the logistic regression analysis. Because DZ twins are always DC and the discordancy levels of the DZ twins were equal to those of MZ MC twins (see results section), the logistic regression analyses were only performed for MZ twins.

Next, diagnostic parameters including sensitivity, specificity, positive predictive value and negative predictive value were calculated. As we concluded that sensitivity and specificity were of equal importance for this study, the highest area under the ROC curve (AUC) was chosen to present the best combination of sensitivity and specificity. The cut-off values varied from .50 to .80, with steps of .05. A two-fold cross-validation was carried out, in which a randomly chosen half of the sample was used as the test set and the other half as the training set. A bootstrap distribution with 10,000 samples was created to estimate the standard deviations of the diagnostic parameters.

Table 1

Relative Birth Weight Differences According to Zygosity and Chorionicity of the 4,060 Twin Pairs

	DZDC <i>n</i> = 2083 (51%) pairs		MZDC <i>n</i> = 686 (17%) pairs		MZMC <i>n</i> = 1291 (32%) pairs	
Continuous	mean (SD)		mean (SD)		mean (SD)	
BW difference (g)	308 (271)		225* (212)		297 (254)	
Males/Females	315 (275)/ 300 (266)		220 (204)/ 232 (219)		291 (252)/ 301 (255)	
Relative BW difference (%)	11.5 (9.8)		8.8* (7.5)		11.6 (9.6)	
Males/Females	11.6 (9.7)/ 11.4 (9.8)		8.5 (7.4)/ 9.0 (7.6)		11.2 (9.4)/ 11.9 (9.6)	
Relative BW difference	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
0–5%	603	29	260**	38	364	28
5–10%	505	24	193	28	312	24
10–15%	375	18	123	18	237	18
15–20%	252	12	54**	8	161	12
20–25%	164	8	27**	4	103	8
> 25%	184	9	29**	4	114	9

Note: BW= birth weight, DZ= dizygotic, MZ= monozygotic, DC= dichorionic, MC=monochorionic

*Proc ANOVA $p < 0.0001$ Overall contingency χ^2 -test: $p < .0001$, ** Expected cell χ^2 -test > 3.84 ($p < .05$)

The analyses were conducted with the SAS version 9.1 software package. The reported p values are two-sided and were considered statistically significant when $p \leq .05$.

Results

Sixty-five per cent of the MZ twins were MC. In other words, without any further information, the prior chance for an MZ twin to be MC is 65% (Table 1). While the mean birthweight difference for an MZ twin was 191g/7.7%, the MZMC twins were significantly more discordant for birthweight than the MZDC twins, whereas the discordancy level was the same for DZDC and MZMC twins (160g/6.5% [MZDC] vs. 211g /8.2% [DZDC] and 210g/8.5% [MZMC]; Table 1). There were no differences between early and late gestation, nor between males and females, except that the relative birthweight difference for MZMC females was greater than for MZMC males.

The univariate analyses showed that the higher the level of discordancy, the higher the chance of being an MC twin (Table 2) became. For a discordancy level up to 10% there was no higher chance of being MC. However, the posterior chances of being MC increased even between 10 and 15% with an OR (95% CI) of 1.38 (1.05–1.80). A discordancy level between 15 and 20% resulted in an OR of 2.1 (1.51–3.01), a discordancy level between 20 and 25% resulted in an OR of 2.73 (1.73–4.29) and a discordancy level above 25% resulted in an OR of 2.8 (2.81–4.35) (Table 2). This trend was significant ($p \leq .008$). The OR per unit (%) increase was 1.04 (1.03–1.05). Additional analyses adjusted for gestational age, the gender of the twin pair, parity, maternal age, and birth year did not change the results substantially (Table 2).

Figure 1 shows the estimated unadjusted probabilities of being MC for MZ twins, when modeled using discordancy level as a continuous variable in logistic

Table 2

Odds Ratios (OR) for Being a MC Twin Pair*

Relative birth weight difference	OR	Unadjusted		OR	Adjusted**	
		Lower 95% CI	Upper 95% CI		Lower 95% CI	Upper 95% CI
0–5%	Ref.			Ref.		
5–10%	1.155	0.908	1.468	1.156	0.909	1.470
10–15%	1.376	1.051	1.802	1.386	1.057	1.816
15–20%	2.130	1.505	3.013	2.151	1.519	3.045
20–25%	2.725	1.733	4.285	2.778	1.764	4.374
> 25%	2.808	1.813	4.349	2.886	1.860	4.477
Test for trend: $p = .008$						
Per unit (%) increase	1.041	1.029	1.053	1.042	1.030	1.055

Note: * Analysis of 1977 MZ twin pairs

**Adjusted for gestational age, the gender of the twin pair, parity, maternal age, and birth year

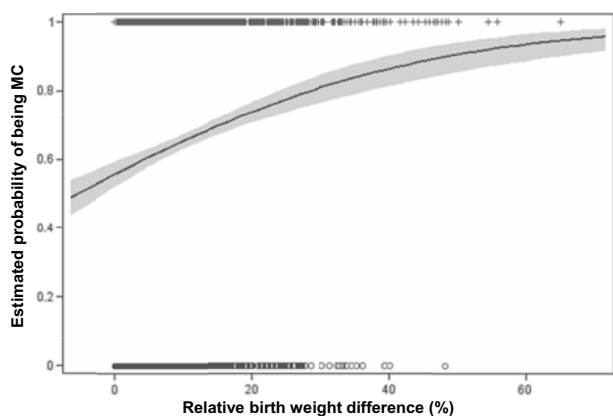


Figure 1
 Estimated probability (unadjusted) of being monozygotic.
 Note: + monozygotic, o dizygotic, Estimated, 95% prediction limits

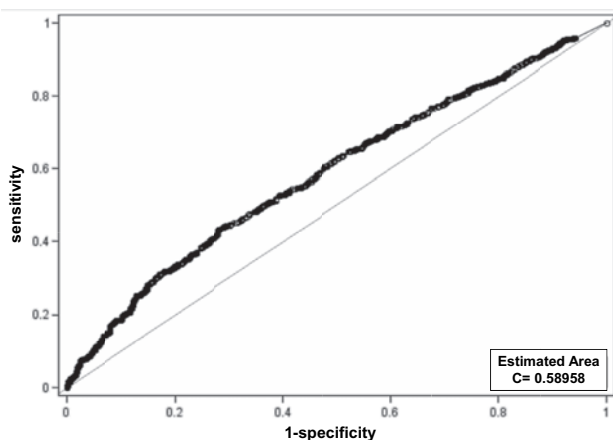


Figure 2
 ROC curve for relative birthweight difference.

regression analyses. A discordancy level of 0% corresponded to a probability of 56–57% of being an MC twin. A discordancy level of 9.8% corresponded with a probability of 65% of being an MC twin, whereas, for example, a discordancy level of 25% corresponded with a probability of being an MC twin of 77%.

Sensitivity, specificity, positive predictive value and negative predictive value did not differ substantially for the adjusted and unadjusted analyses. The bootstrap distribution was therefore only created for the unadjusted analyses. The highest AUC was found

when a cut-off value of .65 was used to predict monozygosity (Figure 2). The corresponding sensitivity, specificity, positive predictive value and negative predictive value for this cut-off value, are presented in Table 3. Sensitivity and specificity were comparable for the discrete and continuous discordancy levels. Sensitivity was lower than the specificity and both were below 71%. The negative predictive value was lower than the positive predictive value and both were below 73%.

Discussion

Birthweight discordancy level is not a reliable indicator of chorionicity in MZ twins. Although MZDC twins were less discordant at birth than MZMC twins and higher intra-pair birthweight differences resulted in higher ORs of being an MC twin pair, with an OR (95% CI) of 2.81 (1.81–4.35) when the birthweight difference exceeded 25%, intra-pair birthweight differences cannot be used to retrospectively predict chorionicity, as is reflected in the low sensitivity and specificity. These results are similar for MZ MM pairs and MZ FF pairs.

None of the covariates considered contributed to the model. For example, gestational age did not influence the results. Although the birthweight difference increased during gestation, as the weights increase during gestation, the increase was of the same magnitude for MC and for DC twins. Another explanation could be the fact that we analyzed live-born twin pairs. Therefore, we do not have information about discordancy levels of twins born before 25 weeks of gestation. MC twins suffering from severe twin-to-twin transfusion (and high discordancy levels) are not included in this study, because of the high mortality rates before 25 weeks of gestation. Furthermore, we found no differences between discordancy levels of males and females after 25 weeks of gestation. However, we cannot exclude the possibility of early differences in growth between males and females (de Zegher et al., 1999), nor an influence of the moment of splitting in MZ twins (Derom et al., 2007) or skewed X-inactivation in female MZ twins (Martin et al., 1997). But if such differences result in differences of discordancy levels, they must disappear after the first trimester.

While the mean birthweight difference for an MZ twin is 10.6% and the prior chance for being MC is 65%, the logistic regression analyses showed that the

Table 3
 Validity of the Test of Discordancy to Estimate Chorionicity

Relative birth weight difference	Sensitivity (SD)	Specificity (SD)	PPV (SD)	NPV (SD)
Discrete*	42.15% (5.60)	70.05% (6.30)	72.79% (1.50)	39.17% (0.65)
Per % increase	49.24% (0.90)	63.71% (1.15)	71.86% (0.46)	40.01% (0.34)

Note: PPV = positive predictive value, NPV = negative predictive value
 *six groups of discordancy levels were defined: 0–5%, 5–10%, 10–15%, 15–20%, 20–25%, and > 25%

posterior chances ranges from 56% for extremely concordant twin pairs to above 90% for extremely discordant pairs (Figure 1). Interestingly, a posterior chance of 65% corresponds to a discordancy level of 9.8%, which is slightly less than the mean birthweight difference. This also indicates that discordancy level cannot be a valid diagnostic test to determine chorionicity and results in a low sensitivity and specificity.

An alternative solution for twin registers with unknown chorionicity, could be the use of chorionicity assigned by ultrasound (Lee et al., 2006). At present, antenatal prediction of chorionicity is accurate, especially in the first trimester (Lee et al., 2006). However, inclusion of this information in twin studies is only possible on condition that (1) twin registers have access to the electronic patient records, (2) the twins have to be traceable by a unique identification number, and (3) that the information about chorionicity has to be included in these electronic medical records. Unfortunately, this is never the case at present.

The present study tested whether birthweight discordancy levels can be used as a diagnostic test for retrospective determination of chorionicity among MZ twins. However, although a higher discordancy level resulted in higher ORs of being an MC twin pair, discordancy level can only be used to some weak extent as a proxy for chorionicity, highlighting the need to assess and record chorionicity data in obstetrical units.

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