

Diagnosing Zygosity in Infant Twins: Physical Similarity, Genotyping, and Chorionicity

Nadine Forget-Dubois¹, Daniel Pérusse¹, Gustavo Turecki², Alain Girard¹, Jean-Michel Billette¹, Guy Rouleau², Michel Boivin³, Jocelyn Malo¹, and Richard E. Tremblay¹

¹ Research Unit on Children's Psychosocial Maladjustment, University of Montreal, Montreal, Canada

² Douglas Hospital Research Center, McGill University, Montreal, Canada

³ Research Unit on Children's Psychosocial Maladjustment, Laval University, Quebec City, Canada

We compared the results of different methods for diagnosing zygosity in a sample of 237 same-sex pairs of twins assessed at 5 and 18 months of age. Despite the twins' very young age and early stage of development, physical similarity was concordant with genotyping in 91.9% of cases at 5 months and 93.8% of cases at 18 months, for a subsample of 123 and 113 pairs, respectively. This concordance rate was obtained following a case-by-case assessment of each pair's physical similarity using a shortened version of the Zygosity Questionnaire for Young Twins (Goldsmith, 1991). Taking into account the chorionicity data available from the twins' medical files, we were able to classify correctly 96% of the pairs, an accuracy rate comparable to previously reported rates obtained with older twins. Chorionicity data is especially useful since we found that monozygotic MZ twins are more difficult than dizygotic MZ twins to diagnose by physical similarity at these young ages. The relative cost-benefit of methods based on reported physical similarity and DNA analysis is discussed in light of these results.

Diagnosing zygosity accurately is a fundamental prerequisite of the twin design, which rests on the phenotypic comparison of monozygotic (MZ) and dizygotic (DZ) twins. Specifically, the misdiagnosis of zygosity (classifying MZs as DZs or the other way around) will result in an underestimation of heritability when genetic influences are present. Assessing the degree of physical similarity between twins using questionnaires is still a widely used method for diagnosing zygosity. Of course, diagnoses that are almost perfectly reliable can be obtained by genotyping; however, these analyses are expensive and may take a long time. Moreover, some participants may not agree to provide the biological specimens necessary for DNA extraction, in which case another method for assessing zygosity must be used. The twins themselves, their relatives, or spouses can report on physical resemblance. For young twins, this information is usually provided by the parents, who describe the physical differences between their twins and report on how often the twins are confused by other people.

A number of validation studies reported that zygosity can be reliably assessed in children by physical similarity, with accuracy rates ranging from 90% to 98% (Bonnelykke et al., 1989; Cohen et al., 1973, 1975; Nichols & Bilbro,

1966; Peeters et al., 1998; Saudino & Eaton, 1991). However, the participants in these studies displayed a large age range, with infants, school-age children and sometimes adults grouped in the same sample. To our knowledge, no validation study of zygosity by physical similarity has yet been conducted solely on infants and toddlers. Since some of the criteria used to classify older children may not be suitable for very young twins, the question remains about the possibility to correctly assess zygosity by physical similarity in twins younger than age two.

Goldsmith (1991) constructed a parent questionnaire specifically designed for the diagnosis of zygosity in twin children, the Zygosity Questionnaire for Young Twins (ZQYT). Several of the ZQYT items, however, may not be appropriate for infant twins whose physical development is still in a very early stage. For example, questions about hair color, shade and texture are not useful in cases where twins have not grown hair yet; differences in body weight can be misleading since MZ twins, particularly monozygotic twins, may not be concordant for weight following birth (Vlietinck et al., 1989), becoming more so progressively during childhood; height can be similarly uninformative as the two-inch difference suggested by Goldsmith for likely exclusion of MZ status may not be appropriate with infants.

A version of the ZQYT was validated by Spitz et al. (1996) using DNA analysis. The questionnaire provided a correct classification for 97.5% of the pairs. However, all subjects were aged 8 to 12.5 years at the time of the study, leaving unanswered the question of validity in the case of very young twins. In the present sample drawn from the Quebec Newborn Twin Registry (Pérusse, 1995), the twins were first evaluated at the age of 5 months. In this study, we assessed the accuracy of a shortened version of the ZQYT with twins aged 5 months and reassessed at 18 months, by comparing the diagnosis obtained from physi-

Address for correspondence: Daniel Pérusse, Research Unit on Children's Psychosocial Maladjustment-University of Montreal, Ste-Justine Hospital Research Center, Bio-psychosocial Unit, Block 5, floor A, 3175, Chemin de la Côte Ste-Catherine, Montreal, Canada, H3T 1C5. Email: Daniel.Perusse@umontreal.ca.

cal similarity with that resulting from genotyping. We also considered the information on placental analyses from medical records.

Materials and Methods

Sample

The Quebec Newborn Twin Registry (Pérusse, 1995) was ascertained from all twin births occurring in the Province of Quebec between 1 April 1995 and 31 December 1998. Names, addresses, and phone numbers of all mothers of newborn twins were collected every 14 days from the computerized birth records of the Quebec Bureau of Statistics. All parents living in the Greater Montreal Area were asked to enrol with their twins in the Quebec Newborn Twin Study (Forget-Dubois & Pérusse, 1997). Parents were contacted by letter and by phone, and laboratory appointments were scheduled for when the twins were aged 5 months, corrected for gestational duration. Several psychophysiological, cognitive, and behavioral measures were taken in the laboratory from the participating twins and their mothers (Dionne et al., 2003; Laplante et al., 2001). These assessments were followed within 2 weeks by a home visit to collect further psychosocial and demographic data on the twins and their families (*N* = 322 pairs). The sample was followed longitudinally using a similar protocol at 18 months. Of the 322 pairs, 237 were of same sex and had to be assessed for zygosity. A diagnosis of zygosity by genetic marker analysis was performed for 123 pairs, which we used to evaluate the accuracy of the diagnosis provided by the physical similarity questionnaire applied to 5-month-old and 18-month-old twins.

Because of the necessity to obtain the greatest number of reliable diagnoses in the shortest amount of time, and because of the cost of genotyping, the subsample of twins assessed by genetic marker analysis was not randomly selected. We gave priority to the pairs that presented no obvious physical differences according to the physical similarity questionnaire (see below). We consider that this procedure is conservative: if more twin pairs who had been straightforwardly diagnosed as DZ (on the basis of clear differences in eye and hair color) had been genotyped, it is likely that the correspondence rate between the two methods would have been higher. Therefore, it must be kept in mind that the accuracy rates of the physical similarity questionnaire reported in this paper are probably lower-bound estimates.

Zygosity Diagnosis

Physical Similarity

We constructed two shortened versions from Goldsmith's (1991) 31-item questionnaire. The first was designed to be answered by the mother or the father of the twins and included nine items of the ZQYT. Parents were asked about their children's hair color, shade and texture, eye color, and ear shape. Additional questions bore on general physical similarity, confusion by people meeting the twins for the first time, and mistaken identity. The second questionnaire included eight items and was to be filled by the experimenters who conducted the laboratory assessment. The assistants also reported on the twins' hair, eye color and ear

shape, and had to describe any other physical difference in an open-ended item. Two more questions assessed the difficulty for the assistants to correctly identify each twin and their overall degree of physical similarity.

For the 5-month assessment, the first 146 families of same-sex twins were reached by phone after the laboratory visit and one parent answered the questionnaire. The research assistant who had conducted the interview diagnosed zygosity immediately after completion of each call. In all cases where a diagnosis was made, a second assistant rated the interview to assess intercoder reliability. For the remaining 91 families, zygosity was assessed by two research assistants, in the presence of the children. Two assistants filled the questionnaire separately and provided a diagnosis. When no diagnosis was made or when the diagnoses were discordant, a panel of three raters reviewed videotapes of the twins taken in the laboratory for this purpose, and tried to reach a consensus. The videotapes showed the twins in front view and in profile. The 18-month zygosity assessments were made by two assistants in the presence of the twins and blind to the 5-month diagnosis. Again, disagreements were resolved by consensus by a three-rater panel, using videotapes of the twins. Figure 1 illustrates the entire coding process.

Guidelines and rules for coding were adapted from Goldsmith (1991) to reflect the abridged questionnaires used in the present study. The assessment for each pair consisted of a first-step exclusion of MZ status. When unsuccessful, this was followed by a second-step confirmation of MZ status. Hence, coders first considered that the twins were not MZ if they were "clearly different" on at least one physical feature. If all answers to the questionnaire

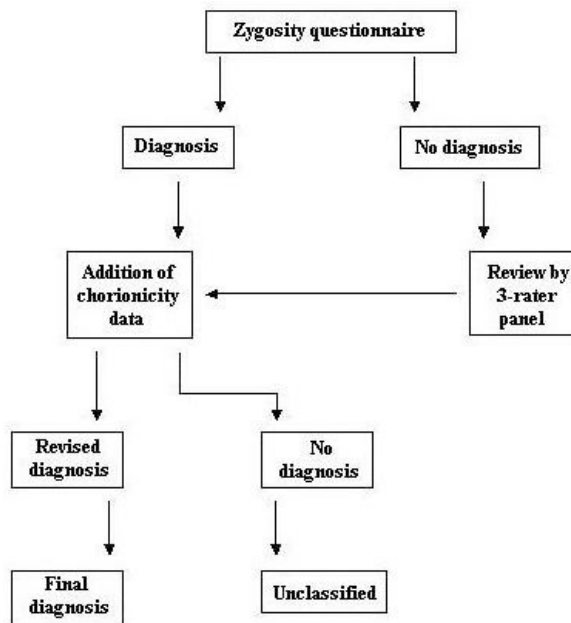


Figure 1
Zygosity diagnosis process.

indicated that there were no clear physical differences, and that the twins resembled one another “like two peas in a pod”, the pair was classified as MZ. Additional questions bearing on the twins’ confusion by strangers (or by research assistants) helped to resolve the cases falling between those two extremes, again following the guidelines provided by Goldsmith (1991). If a diagnosis was still impossible to make, the pair was provisionally left unclassified, to be resolved subsequently.

DNA Analysis

Extraction. Genomic DNA was extracted from oral epithelial cells obtained using mouth swabs (Freeman et al., 1997; Meulenbelt et al., 1995). A total of 20 mouth swabs were collected from the twins by their parents at their home through a period of three days, following a written protocol. Swabs were performed for approximately 30 seconds, using a different area of the mouth for each swab. No more than 10 consecutive swabs were taken and an interval of at least 4 hours was used before new swabs were collected from the same individual. Samples were stored up to 20 days at room temperature in a tube containing STE buffer (100 mM NaCl, 10 mM TrisHCl and 10 mM EDTA), proteinase K (0.2 mg/ml) and 0.5% SDS. DNA isolation was carried out by cell lysis and subsequent phenol/chloroform extraction using standard protocols. The average DNA yield was 70.60 ± 43.58 ug.

Markers. Eight (for 66.1% of the pairs) and then nine (for 33.9% of the pairs) microsatellite markers were used for genotyping. The markers were chosen because of high heterozygosity values, reliability, and chromosomal locations. In order to avoid studying loci that were not independently segregating, no syntenic markers were used.

PCR reactions. PCR was carried out in a total volume of 12.5 ul containing 40 ng of genomic DNA; 125 ng of primers; 200 uM each of dGTP, dCTP, and dTTP; 25 uM dATP; 1.5 uCi [35S] DATP; 0.5 units of Taq DNA polymerase (Bio/Can Scientific); and 2.0 ul of 10X buffer (Bio/Can Scientific) with MgCl₂ were included in the final concentration of 1.5 mM. Samples were over-laid with mineral oil and processed throughout 35 cycles of denaturation at 94°C, annealing at specific primer temperature, and elongation at 72°C, followed by a final elongation period of 72°C. PCR products were analyzed on a 6% denaturing polyacrylamide gel (38:2 acrylamide:bisacrylamide). Samples were run for a period of 2h in a vertical electrophoresis gel apparatus (Life Technologies). Gels were dried and exposed to X-ray films for 48 to 72h at room temperature. Autoradiographs were read and interpreted independently by two different readers. Readers were blind to any other source of information regarding zygosity.

Probability estimations. The probability that a DZ twin pair is concordant for the two alleles in a marker genotype was estimated using the method described by Smith and Penrose (1955) that uses the number of alleles and their frequencies in the general population. The probability was estimated as follows:

$$P(\text{concordant/DZ}) = \frac{\{[\sum_{i=1}^N p_i^2 + 1]^2 + \sum_{i \neq j}^N (p_i p_j)^2\}}{4}$$

Where p_i is the frequency of the i -th allele in a system containing N alleles. Because the markers used segregate independently, the probability that DZ twin pairs were concordant for more than one marker was estimated by the product of the probabilities to be concordant for each marker as well as to be same-sexed (probability assumed to be 0.5). The probability to be DZ and concordant for each specific marker varied between 0.2892 and 0.4322. The posterior probability to be MZ when concordant for all typed markers was computed for each remaining pair (Erdmann et al., 1993). In the calculation of this probability, we considered the prior probability that same-sex twins were MZ in our population (estimates were based on the following probabilities: same-sex: 0.5 and DZ/MZ: 2). However, we did not consider the prior probability of genotyping errors; although these errors do occur when single loci are used, the likelihood of having repeated errors leading to duplicate genotypes for several highly polymorphic markers was considered negligible.

Chorionicity

Chorionicity is routinely diagnosed in the case of twin births in hospitals in the Province of Quebec. The pathology unit reports a number of placental analyses: specimen, clinical information, clinical diagnosis, gross description, and chorionicity. Chorionicity was read directly from the files mailed in by the hospitals. We noticed that seven diagnoses of chorionicity were obviously erroneous (e.g., twins with different blood groups or of opposite sex were diagnosed as monochorionic), so we used other information available from the medical files to corroborate the pathologist’s evaluation. Such additional information included ultrasound report and operating report if the twins were delivered by cesarean section. All of the chorionicity diagnoses were thus double-checked by comparison with the available information on a case by case basis. After the completion of this process, chorionicity diagnoses were available for 87.6% of the whole sample (and for 93.5% of the pairs diagnosed by genotyping).

Differences in blood groups could also have been used to identify DZ twins. In this study, however, blood groups were used only to check the accuracy of the chorionicity diagnosis, because blood-group data were missing for 52% of the pairs. Moreover, physical differences that can have an impact on zygosity diagnosis are expected to be associated with chorion type for young twins (Vlietinck et al., 1989), so it seemed necessary to assess the impact of chorionicity data on the accuracy of physical similarity diagnosis independently of other factors.

Results

Interrater Agreement for Physical Similarity

For the 130 families reached by phone at the 5-month assessment, zygosity was coded by two assistants from the

same data and was used to assess interrater agreement. The first rater coded 50 pairs as MZ (38.5%), 60 pairs as DZ (46.1%) and 20 pairs as uncertain (15.4%). The second rater independently coded the 110 pairs for which a diagnosis had been made by the first rater. The two raters agreed on 103 pairs (93.6%), 94% for pairs diagnosed as MZ and 93.3% for pairs classified as DZ (difference not significant). Interrater agreement was high not only in terms of the diagnoses reached, but also as to whether a diagnosis could be made at all. Thus, only 2 of the 110 pairs (1.8%) diagnosed by the first rater could not be diagnosed by the second rater.

Validating Zygosity by DNA Analysis

The probability of being same-sex, concordant for all markers, and DZ approached zero when all the markers were typed: this probability was estimated at .0002 for the set of eight markers, and at .00006 for the set of nine markers.

DNA analysis was performed in 126 pairs. Three pairs were omitted because of an insufficient DNA yield. The number of markers typed for each pair ranged from three to nine. Given that error is very low for any specific marker and that all discordant pairs differed at least at two loci, the probability of being MZ was deemed to be zero for all pairs who differed for any marker. The pairs for which only three markers were typed were classified as DZ because they differed for two loci out of three.

The posterior probability to be MZ when concordant for all typed markers was computed for each remaining pair, according to the number of markers typed and the specific heterozygosity of each marker. For the pairs concordant for all markers typed, the number of successful markers ranged from four to nine. Accordingly, the probability to be MZ ranged from .9702 to .9998. Eighty pairs (65.0%) were thus classified as MZ and 43 pairs (35.0%) as DZ.

Accuracy of the Physical Similarity Assessment

5-month Assessment

Pairs for which a DNA diagnosis was obtained were used to determine the accuracy of the physical similarity assessment. Table 1 presents the comparison of zygosity obtained by the two methods for the 123 available pairs.

At 5 months, 98.4% of the pairs received a physical similarity diagnosis of zygosity. Excluding the two pairs left

unclassified, the accuracy rate was 91.7%. Eight out of the 10 mistaken pairs were MZ who had been erroneously classified as DZ, suggesting that the physical similarity assessment tended to overestimate DZ status. The greater proportion of mistaken MZ pairs could also be due to the larger proportion of MZ pairs in the sample, although MZs comprised 65% of the sample while they generated 80% of the misdiagnoses. The consideration of chorionicity data will help to clarify this issue (see below). Of the pairs that could not be classified, one turned out to be MZ and one DZ.

18-month Assessment

Nine pairs out of 123 were lost to attrition at the time of the 18-month laboratory visit and one was evaluated for zygosity in the home visit instead of the laboratory. The comparison between the diagnosis obtained by physical similarity and DNA analysis are shown in Table 1. At 18 months, 99.8% of the pairs received a physical similarity diagnosis. All the seven misclassified pairs were MZ mistaken for DZ. Excluding the unclassified pair, the diagnosis by physical similarity was accurate in 93.8% of the cases. The improvement of diagnosis accuracy between 5 and 18 months is small.

Continuity Between the 5-month and 18-month Assessment

Of the 113 pairs assessed at both ages, only two pairs who received a wrong diagnosis of zygosity based on physical similarity at 5 months were also misclassified at 18 months, suggesting that the causes of diagnosis error may not be all the same at the two ages. Excluding the unclassified pairs, the 5-month diagnosis corresponded to the 18-month diagnosis in 88.2% of cases. Seven pairs classified as MZ and six classified as DZ on the first assessment received an opposite diagnosis on the second assessment. Of the two pairs left unclassified at 5 months, one was diagnosed MZ and one DZ at 18 months. The only pair left unclassified at 18 months had been diagnosed MZ at 5 months.

Chorionicity and Zygosity

Of the 112 pairs in the complete sample diagnosed as MZ and for which chorionicity was available from the records, 64 (57.1%) pairs had been classified as monochorionic (MC) and 48 (42.9%) as dichorionic (DC). It is usually estimated that about two thirds of MZ twins are MC (Machin, 1996). MC status, if correctly assessed, indicates that the twins are MZ, while DC placenta does not allow a diagnosis of zygosity. We used this information to complement, and

Table 1
Accuracy of Zygosity Diagnosis

	Physical similarity		Physical similarity and chorionicity	
	5 months	18 months	5 months	18 months
% of sample correctly classified MZ	57.7%	55.8%	63.4%	61.1%
% of sample correctly classified DZ	32.5%	37.2%	30.9%	35.4%
% of sample misclassified	8.2%	6.2%	4.1%	3.5%
% of sample unclassified	1.6%	0.8%	1.6%	0.0%
Total number of pairs	123	113	123	113

Note: Comparison of physical similarity diagnoses and physical similarity supplemented with chorionicity diagnoses with DNA analysis, according to the age of the twins.

eventually correct, the zygosity diagnosis based on physical similarity alone. Thus, any MC pair was considered MZ regardless of the zygosity obtained by physical similarity. When the information provided by chorionicity was inconclusive (i.e., when the twins were DC) or unavailable, we relied solely on physical similarity. We then compared these revised diagnoses with those obtained by genotyping.

5-month Assessment (Revised)

The concordance between the zygosity diagnoses, based on physical similarity supplemented by chorionicity, with those obtained by genotyping are presented in Table 1. Of the 123 pairs, two could still not be classified. Leaving out these pairs, inclusion of the chorionicity data increased the correspondence between the physical similarity and genotyping diagnoses from 92% to 96%. The majority of the misdiagnosed pairs turned out to be MC-MZ erroneously classified as DZ, which explains why chorionicity improves the accuracy of the physical similarity diagnosis. It must be kept in mind that chorionicity is useful only in the absence of genotyping, since the latter is much more reliable. In the present case, two DZ pairs were erroneously classified MC, so chorionicity adds error along with information. The relevant question, therefore, is whether chorionicity improves the accuracy of the physical similarity diagnosis.

To estimate the significance of the improvement in the accuracy of the zygosity diagnosis, we compared a log-linear model where chorionicity was taken into account, with a model where it was not. We first built a saturated model that takes into account the chorionicity as well as the DNA and physical similarity data. In theory, these three sources of data define eight categories of twin pairs, as listed in Table 2. However, since none of the twins diagnosed as DZ by genotyping can be MC, the corresponding two categories were constrained to 0. The values predicted by the saturated model were very close to the observed values, although very small differences were generated by the estimation process ($-2LL$ of the saturated model = -162.677).

We then built a second model where chorionicity was included independently from genotyping and physical similarity, so that only the observed proportion of MC and DC twins in the sample determined the proportion of MC and DC twins falling into each category of pairs (i.e., there was no constraint). Thus, this model allows for the possibility that twins could be diagnosed as MC and DZ, because both chorionicity and physical similarity diagnoses are subject to error. Even if this procedure appears counter-intuitive, not constraining the MC-DZ cells to 0 takes this possibility of error into account, and two pairs actually fell in this category. The values predicted by this model are reported in Table 2. The fit of the model that does not take the information of chorionicity into account decreased significantly compared with the saturated model, which does. ($-2LL = -179.967$, likelihood ratio = 34.58 , $p = .000$, for 2 DF). This result shows that chorionicity adds a significant and specific contribution to the accuracy of the zygosity diagnosis by physical similarity.

To estimate the size of the improvement in the zygosity diagnosis provided by the addition of chorionicity, we com-

Table 2

Effect of Chorionicity on Zygosity Evaluation

Twin pairs			Observed number of pairs	Predicted number of pairs (without chorionicity effects)	Standardized residuals
G	P	C			
MZ	MZ	MC	35	25.9	1.8
MZ	MZ	DC	31	40.1	-1.4
MZ	DZ	MC	7	3.1	2.2
MZ	DZ	DC	1	4.9	-1.8
DZ	MZ	MC	0	0.8	-0.9
DZ	MZ	DC	2	1.2	0.7
DZ	DZ	MC	2	14.1	-3.2
DZ	DZ	DC	34	21.9	2.6

Note: Predicted values in zygosity categories when the effect of chorionicity is removed from the model (G = genotyping, P = physical similarity, C = chorionicity).

Table 3

Improvement in Diagnosis Using Chorionicity

	Odds to correctly classify MZ pairs	Odds to correctly classify DZ pairs	Odds-ratio to correctly classify all pairs
Classification by physical similarity only	33	4.5	148.5
Classification by physical similarity and chorionicity	15.5 ¹	34.0	527.0

Note: Comparison of the odds of classifying MZ and DZ pairs correctly, with and without considering chorionicity at 5 months. ¹DC-MZ pairs only.

pared the odds of being correctly classified by physical similarity alone with those of being correctly classified by physical similarity and chorionicity (Table 3). The full data for 5-month physical similarity, chorionicity, and genotyping were available for 112 pairs. Of the 68 pairs classified as MZ by physical similarity, 66 were correctly classified by physical similarity and two were misclassified, so the odds of being correctly classified as MZ by this method were 33 (66/2). Thirty-six of the 44 pairs classified as DZ by physical similarity were correctly identified (odds = $36/8 = 4.5$). Multiplying the independent odds of being successfully diagnosed as MZ and DZ yields an odds-ratio of 148.5 (i.e., the probability of being correctly classified is 148.5 times higher than the probability of being incorrectly classified using physical similarity alone).

When we used chorionicity in addition to physical similarity, all 44 MC pairs were classified as MZ, and only the 68 DC pairs remained to be diagnosed. Of the 33 pairs classified as MZ by physical similarity, 31 were correctly classified, so the odds of DC-MZ to be correctly diagnosed rather than misdiagnosed were 15.5 (31/2). Of the 35 pairs

classified as DZ by physical similarity, 34 were well classified (odds = 34/1). By multiplying these independent odds, we obtain an odds-ratio of 527.0 (i.e., the probability of being correctly classified for zygosity by physical similarity is 527 times higher than the probability of being incorrectly classified for the subsample of DC twins). Compared with the probability of classifying the twins correctly by physical similarity alone, the probability of classifying them correctly is thus 3.5 times (570/148.5) higher when the information contained in the chorionicity data is taken into account.

Since most of the wrongly classified pairs were MC-MZ, the probability of being correctly classified as DZ improves when chorionicity data are considered. This finding suggests that DC-MZ pairs are easier to diagnose by physical similarity than MC-MZ pairs at this young age. However, the imprecision of the chorionicity diagnosis may also introduce error; genotyping revealed that two of the 44 pairs diagnosed as MC were in fact DZ twins. Thus, the odds of being MZ when diagnosed as MC is $42/2 = 21$. This means that the probability of being MZ when reported to be MC is less than 1, though the probability of being MZ when diagnosed as MC is 21 times larger than the probability of being DZ.

18-month Assessment (Revised)

With the addition of chorionicity data to the 18-month physical similarity diagnosis, none of the 113 pairs for which we also had a diagnosis by genotyping were left unclassified. Four pairs were misclassified, giving an accuracy rate of 97.3%. Five out of seven erroneous physical similarity diagnoses were corrected by the addition of chorionicity data, but two more pairs were misclassified because of erroneous chorionicity.

Since no pairs classified as DZ by genotyping were misclassified as MZ by physical similarity, the odds of being correctly classified by physical similarity alone and by physical similarity supplemented by chorionicity could not be calculated, because this would have implied a division by 0. However, excluding the erroneous chorionicity diagnosis, five of the seven misclassified pairs were MC, one was DC, and chorionicity was not known for the remaining pair. This suggests that, in accordance with the 5-month diagnoses, the assessment of zygosity by physical similarity at 18 months is more difficult with MC-MZ than DC-MZ twins.

Continuity Between the 5-month and 18-month Revised Assessments

Since the chorionicity data were the same for the two times of assessment, the corrected diagnoses were expected to be more stable between the two ages than the diagnoses based on physical similarity alone. Indeed, for 111 pairs that could be diagnosed at the two ages, the 5-month zygosity diagnosis was the same at 18 months in 95.5% of the pairs. Four pairs diagnosed as MZ and one as DZ at 5 months received an opposite diagnosis at 18 months.

Discussion

We adapted the Zygosity Questionnaire for Young Twins (Goldsmith, 1991) to suit the needs of a sample of infant twins. We found that twins as young as 5 and 18 months

can be reliably assessed for zygosity according to physical similarity. The shortened version of the ZQYT can be administered to parents or directly filled by research assistants during the twins' visit to the laboratory. The physical similarity questionnaire alone yielded an accuracy rate of 91.7% at 5 months and 93.8% at 18 months when compared to zygosity diagnoses obtained by genotyping. These accuracy rates would have probably been higher if we had included in our sample more pairs easily classified as DZ on the basis of clear physical differences. As explained above, pairs more difficult to diagnose were given priority for the DNA analysis, in order to make the procedure more efficient. Few pairs were left unclassified at the completion of the process. The error generated by the physical similarity questionnaire was not random: at the two ages, the majority of mistaken diagnoses were MZ twins erroneously classified as DZ. This could possibly result from the higher proportion of MZ twins in the sample, but most of these misclassified pairs turned out to be monochorionic. This suggested that the diagnostic errors were linked to the chorionicity status of the pairs, so the addition of chorionicity data to physical similarity for diagnosing zygosity was especially useful. The odds of being correctly classified as MZ or DZ were 3.5 times larger in the subsample of dichorionic pairs than in the complete sample. This finding shows that the diagnosis by physical similarity alone is less reliable for monochorionic twins, which is probably explained by the fact that MC twins differ more than DC-MZ twins at birth on characteristics such as weight (Vlietinck et al., 1989). Indeed, the experienced prenatal environment of monochorionic twins may prove to be more different than that of dichorionic MZ twins, since sharing the same placenta can result in unequal distribution of blood flow between the two zygotes, resulting in phenotypic differences (Machin, 1996). In short, MC twins were more difficult to classify on the basis of physical similarity, but the chorionicity data could be used to correct the zygosity diagnosis.

Chorionicity is readily available from medical records. However, we found it necessary to verify the chorionicity data recorded in these files since we found that a few monochorionic diagnoses were obviously wrong. Thus, chorionicity complements physical similarity data but also introduces error. In spite of this problem, the simultaneous use of physical similarity and chorionicity in a case-by-case analysis allowed the correct classification of approximately 96% of pairs at both ages, an accuracy rate comparable to what Spitz and colleagues (1996) reported using a more extended version of the ZQYT with children aged 8 to 12.5 years, and with rates routinely found for adults (Kendler et al., 1993; Nichols & Bilbro, 1966; Riemann et al., 1997).

We conclude that zygosity can be reliably established for very young twins using a short physical similarity questionnaire supplemented by readily available data from medical records. Videotapes are necessary to resolve difficult cases, otherwise many pairs would remain unclassified in the absence of conclusive chorionicity data. This procedure represents an efficient and economic alternative to DNA analysis or blood typing. It is noninvasive and requires minimal effort from the twins' parents compared

with the mouth swab procedure necessary to collect a sufficient amount of DNA. Still, the collection of genetic material by mouth swabs performed by parents and sent by mail is very efficient and allows to check the accuracy of the physical similarity diagnosis and to proceed eventually to QTL analysis.

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