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Diagnosis of Zygosity by Questionnaire and Polymarker Polymerase Chain Reaction in Young Twins

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We developed a zygosity questionnaire for use in young twins and assessed its validity using the results of DNA diagnosis. The participants were divided into two groups: 105 pairs of adolescent twins (12–16 years old), 47 pairs of child twins (2–12 years old), and their respective parents. The DNA diagnosis of zygosity was made with polymarker polymerase chain reaction (PCR) amplification of five loci, using the AmpliType PM PCR Amplification and Typing Kit; this method has an accuracy rate of 99.0%. A parsimonious model for each sample was established using stepwise logistic regression analysis of the 20 items of the questionnaire. The total accuracy rate of the model was satisfactory for both parental reports (three items) and self-reports (three items) of adolescent twins (97.4 and 95.6%, respectively), while that for parental reports on child twins (two items) was less satisfactory (92.5%). For adolescent twins, if DNA diagnostic workups were limited to those with discordant reports either from themselves or from their parents, the accuracy rate increased to 100% for parental reports and 98% for self-reports.

KEY WORDS: Polymarker PCR; twin zygosity; young twin; zygosity questionnaire.

INTRODUCTION

The diagnosis of zygosity in adult twins can be made easily with the use of self-report questionnaires, which have been validated by serum markers to have sufficient accuracy (93–98%) (Cederlof *et al.*, 1961; Nichols and Bilbro, 1966; Torgersen, 1979; Magnus *et al.*, 1983). Although molecular genetics techniques can establish the diagnosis with an accuracy rate of almost 100% (Hill and Jeffreys, 1985; Akane *et al.*, 1991; Erdmann *et al.*, 1993; Eufinger *et al.*, 1993) and noninvasive ways to obtain DNA have been developed (Lench *et al.*, 1988; Hayney *et al.*, 1996; Freeman *et al.*, 1997), the cost of these procedures has limited their use in large scale epidemiological studies. Thus, many well-established twin studies have relied solely on questionnaires for zygosity diagnosis and have applied more expensive confirmatory diagnostic procedures only in highly selective subsamples. There are relatively few zygosity questionnaires for young children (Cohen *et al.*, 1975; Goldsmith, 1991). A Danish study has found that even for twins aged 6 months to 6.5 years, zygosity questionnaires completed by parents have sufficient accuracy (91%) (Bønnelykke *et al.*, 1989). One study among Japanese junior high-school twins aged 12 to 16 years found that a self-reported questionnaire adapted from that of Torgersen had a predictive accuracy of 91.5% (Ooki *et al.*, 1990). Spitz *et al.* (1996) recently assessed a zygosity questionnaire adapted from that of Goldsmith using simple sequence repeat length polymorphism analysis in twins aged 8 to 12.5 years. They found that 92.4% of twins were correctly classified using only four questions.

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There has been no report on the validity of zygosity questionnaires in the Taiwanese Han (Chinese) population. Previous twin studies in Taiwan relied on dermatoglyphic analysis and serum markers for zygosity diagnosis (Lin *et al.*, 1969; Chen *et al.*, 1984). Furthermore, the sample size required for a twin study with sufficient statistical power is higher in Taiwan than in Western populations because of the low ratio of dizygotic (DZ)-to-monozygotic (MZ) twins (Chen *et al.*, 1987). This requirement makes it essential to have a well-validated twin zygosity questionnaire to facilitate twin research in such populations. In this study we designed a zygosity questionnaire for use in young twins and assessed its validity using polymarker polymerase chain reaction (PCR) amplification of five genetic loci (DNA diagnosis) (Budowle *et al.*, 1995). The reliability and predictive accuracy of the questionnaire were evaluated separately for adolescent and child twins.

METHOD

Participants

The participants in this study were divided into two groups. The first group consisted of adolescent twins who were junior high-school students in Taipei City. They were all participants in an ongoing twin study on sustained attention and schizotypy among adolescents. In that study, all of the twins in each junior high school were identified by school administrators and were invited to participate by researchers. In the present study, of the 170 pairs of same-sex twins identified from 13 schools in the initial stage (March to May 1997), 111 pairs agreed to participate (the participation rate was 65%). After informed written consent was obtained from both of the twins and their parents, twins were asked to provide a mouthwashing sample for DNA isolation and to complete a self-reported twin similarity questionnaire (20 items), together with other personality questionnaires. Both parents of the twins were also asked to fill out a parental twin similarity questionnaire (27 items). For each similarity questionnaire, items with missing values were completed via telephone interview by one of our researchers. Only twins with DNA zygosity data were included in the analysis ($n = 105$ pairs). Among them, five twins did not return the similarity questionnaire. Thus, a total of 205 questionnaires was available for analysis. The mean age and standard deviation of twins in this group were 14.4 ± 0.9 years (range: 12 to 16 years).

The second group of participants consisted of primary school children and preschoolers ($n = 47$ pairs). After informed written consent for participation in the study was obtained from their parents, child twins were asked to provide a mouthwashing sample and one of their parents was asked to fill out the parental twin similarity questionnaire. The majority of the twins (38 pairs) were recruited during an annual meeting of the Twin Association of Taipei City. The remaining twins (nine pairs) were recruited from another study on elective mutism. Venous blood was also collected in four pairs of this subsample. The mean age and standard deviation of twins in this group were 7.6 ± 3.1 years (range: 2 to 12 years).

Questionnaire

The twin similarity questionnaire was compiled from items of questionnaires developed by Cohen *et al.* (1975) and Goldsmith (1991) and also included five culture-specific items (items 9, 10, 11, 12, and 27). Separate questionnaires forms were designed for self-reporting and for parental reporting. The questionnaires were divided into three parts. The first part contained 13 items regarding physical similarities, in which participants were asked whether the twins were similar in terms of (1) eye color, (2) hair color, (3) facial appearance, (4) skin color, (5) weight, (6) height, (7) hair texture, (8) shape of ear lobes, (9) hair whorl, (10) thumb curvature, (11) palmar creases, and (12) eyebrows and (13) whether they are like two peas in a pod. There were three possible answers to the first 12 items: "no difference," "only a slight difference," and "clear difference"; the possible answers to item 13 were "definitely yes," "occasionally so," and "no."

The second part of the questionnaire contained seven items regarding confusion, in which participants were asked whether the twins were ever mistaken for one another by (14) parents, (15) other siblings, (16) teachers, (17) close friends, (18) casual friends, and (19) people meeting them for the first time. If the twins were sometimes mistaken for one another, participants were asked, (20) Do these occasions occur when the twins are together? The three possible answers to these items were "frequently," "occasionally," and "rarely or never." For participants who did not have other siblings, the answer for item 15 was considered to be "rarely or never" in the analysis. Similarly, for participants who reported that the twins had never been mistaken for one another (items 14–19) and hence did not answer item 20, the answer for item 20 was also considered to be "rarely or never" in the analysis.

The third part of the questionnaire was included only on the parental form. Parents were asked: (21) When

they are looking at a new photograph of their twins, can they correctly identify each twin? (22) Are their twins physically more similar than typical brothers and sisters? (23) Did the twins' first teeth begin to come in at about the same time? (24) Were they told at the time of birth whether the twins were identical or fraternal? (25) their pediatrician's opinion on whether the twins are identical or fraternal; (26) Do they consider their twins to be identical or fraternal? and (27) Did their twins have the same birthmarks? Because many parents did not answer items 23 to 26 and our analyses indicated that the remaining 3 questions contained information which was largely redundant of that obtained from the first 20 questions, this part of the questionnaire was excluded from subsequent analyses.

A test–retest reliability study of the questionnaire given 2 weeks apart was conducted among 36 junior high-school twins and 42 of their parents. Concordance in twin similarity reports between adolescent twins and their cotwins (n = 100 pairs) and between fathers and mothers (n = 73 pairs) was evaluated to determine the interrater reliability for the questionnaire.

DNA Genotyping

Buccal cavity cells were collected from each twin by mouthwashing for at least 10 s with a 10-ml solution of 4% sucrose (Lench *et al.*, 1988; Hayney *et al.*, 1996). After centrifugation in 10-ml test tubes at 2000*g* for 10 min, supernatants from the mouthwashing samples were centrifuged again in 1.5-ml Eppendorf vials at 8000*g* for 3 min. The sediment was immediately used for DNA extraction or stored at −70°C until used. DNA extraction was performed using a commercial kit GENOMIX (Talent, Italy). The yield of DNA was quantified spectrophotometrically in a subsample of 11 subjects. The average amount of extracted DNA for each individual was 0.7 μg (ranging from 0.05 to 3 μg). Six subjects whose DNA extraction for the first sample had failed were asked to return a second mouthwashing sucrose solution by mail in a bottle we sent to them. All of these samples were returned within 7 to 10 days and DNA was successfully extracted from all of them.

PCR was used to amplify the following five loci from the extracted DNA: low-density lipoprotein receptor (LDLR), glycophorin A (GYPA), hemoglobin G γ-globulin (HBGG), D7S8, and group-specific component (Gc). PCR was performed using the AmpliType PM PCR Amplification and Typing Kit (Perkin–Elmer Corp., Foster City, CA) according to the manufacturer's instructions. All five loci were typed simultaneously using reverse dot-blot analysis in which amplified DNA hybridizes to allele-specific oligonucleotide probes immobilized on nylon typing strips (Saiki *et al.*, 1989; Budowle *et al.*, 1995). Amplification was carried out in a DNA thermal cycler 480 (Perkin–Elmer Corp.). Twin pairs with differences in one or more loci were considered to be dizygotic; those identical for all five loci were considered monozygotic. The wet nylon strips were photographed and interpreted independently by two researchers (H.W.C. and C.C.H.L.). Strips with discordant interpretation (n = 11, 5.7%) were then remade and reinterpreted.

Statistical Analysis

The Hardy–Weinberg equilibrium was tested by χ^2 analysis with Yates' continuity correction (Weir, 1990) for each locus. The probability of monozygosity in twin pairs with identical genotypes at the five loci (denoted as T) was calculated using Bayes theorem:

$$\text{prob}(\text{MZ}|T) = \frac{1}{1 + QL}$$

where Q is the pretest odds (prevalence ratio of DZ to MZ in the population) and L is the likelihood ratio of prob(T|DZ) to prob(T|MZ). The DZ/MZ ratio in this study was estimated from the reported population twining rate in Taiwan (Chen *et al.*, 1987). The average DZ/MZ ratio for all twins was 2/4.92 for cohorts born from 1981 to 1984 and 2/4.90 for cohorts born from 1974 to 1984. Based on these data, Q was set to be 2/5 in this study. For a genetic system of two or three alleles, Smith and Penrose (1955) had derived the L for various genotypes in which the parental genotypes are not known. If more than one genetic system is employed, the L can be multiplied. For this study, we applied information from sex (only same sex twins were included) and the five polymarker loci. Thus $L = (1/2)\ L_1L_2L_3L_4L_5$.

The frequency of agreement between each item of the questionnaire and DNA diagnosis was evaluated for adolescent twins and child twins separately, because the ascertainment of the two groups was different. To construct a parsimonious model predicting twin zygosity for parental and self reports, respectively, we employed stepwise logistic regression with a significance level of 0.10 for entry into or staying in the model among three samples: parental reports on adolescent twins, adolescent self-reports, and parental reports on child twins.

Variables were entered into and removed from the model in such a way that each forward selection step was followed by one or more backward elimination steps. Stepwise logistic regression analyses were performed using the PROC LOGISTIC program (SAS Institute, 1989). The predictive accuracy, sensitivity, and specificity of the questionnaire at specific cutoff points were determined by specifying "CTABLE" in PROC LOGISTIC, which uses a jackknife approach to reduce the bias that results from classifying the same data used to derive the classification criterion.

RESULTS

DNA Diagnosis of Twin Zygosity

In estimating allele frequencies of the five polymarker loci, both adolescent and child twins were combined (Table I). None of the five loci deviated from Hardy–Weinberg equilibrium (all p's > 0.26). The allele frequencies were similar to those reported among Taiwanese adults (Lee *et al.*, 1995), with the exception of GYPA. Using allele frequencies from the twin samples and using our assumption that $Q = 2/5$, the probability of monozygosity for same-sex twins with the same genotype in all five loci, i.e., prob(MZ|T), was estimated for all possible genotype combinations ($n = 486$). On average, the prob (MZ|T) was 0.990 (range: 0.974 to 0.999). Among the four pairs of twins in whom both blood and buccal cell DNA was examined, the genotypes were completely identical for the two sources of DNA.

DNA diagnosis among the 105 pairs of same-sex adolescent twins (53 female and 52 male) revealed that 19 were DZ and 86 were MZ. Among the 47 same-sex child twins (21 female and 26 male), 8 were DZ and 39 were MZ.

Questionnaire Reliability

Many of the 20 items considered here had such little variation that it was not appropriate to calculate kappa statistics. Hence, only agreement probability is reported. When each item was scored on a 3-point scale, the agreement probability for the test–retest reliability of the questionnaire on adolescent twins (36 self-reports and 42 parental reports) was below 80% for 12 self-report items and for 17 parental report items. In contrast, if a 2-point scale was used for coding (0 = no difference or only a slight difference and 1 = clear difference; 0 = definitely yes or occasionally so and 1 = no; 0 = frequently or occasionally and 1 = rarely or never), only one self-report item and four parental report items had a test-retest agreement probability below 80% (Table II). Thus, a 2-point recoding scale was used for all analyses.

For adolescent twins, there were 205 self-reported twin similarity questionnaires and 157 parental reports of twin similarity (77 fathers and 80 mothers). Concordance in twin similarity reports between twins and their cotwins could be examined in 100 pairs of twins. The probability of agreement between their reports ranged from 70.4 to 99.0% (Table II). Concordance in twin sim-

Table I. Allele Frequencies of Five Polymarker Loci in Taiwan

		Young twins (present study) ($N = 179$)[a]		Unrelated adults (Lee *et al.*, 1995) ($N = 120$)		
Marker locus	Allele	N	(%)	N	(%)	χ^2 test (p value)
LDLR	A	88	(24.6)	51	(21.3)	0.40
	B	270	(75.4)	189	(78.8)	
GYPA	A	216	(60.3)	118	(49.2)	0.01
	B	142	(39.7)	122	(50.8)	
HBGG	A	79	(22.1)	56	(23.3)	0.78
	B	279	(77.9)	184	(76.7)	
D7S8	A	221	(61.7)	154	(64.2)	0.58
	B	137	(38.3)	86	(35.8)	
Gc	A	88	(24.6)	77	(32.1)	0.13
	B	157	(43.9)	93	(38.8)	
	C	113	(31.6)	70	(29.2)	

[a] The MZ twin was counted as one person and the DZ twin was counted as two persons; adolescent and child twins together.

Table II. Test–Retest and Interrater Reliability of the Twin Similarity Questionnaire

Item	Test–retest agreement (%)		Interrater agreement (%)	
	Self-report (n = 36)	Parental report (n = 42)	Twin–cotwin (n = 100)	Father–mother (n = 73)
1. Eye color	100.0	100.0	98.0	98.6
2. Hair color	100.0	97.6	99.0	98.6
3. Facial appearance	91.7	97.6	86.0	90.1
4. Skin color	100.0	90.2	93.0	95.7
5. Weight	86.1	85.7	85.0	86.3
6. Height	100.0	88.1	81.0	86.3
7. Hair texture	100.0	90.5	91.0	90.4
8. Ear lobe shape	100.0	95.1	95.0	93.0
9. Hair whorl	91.7	90.2	86.0	92.6
10. Thumb curvature	94.4	90.2	92.0	89.7
11. Palmar creases	91.7	73.7	75.8	84.1
12. Eyebrow	94.4	95.0	91.9	90.1
13. Like two peas in a pod	85.7	81.6	75.8	84.6
14. Mistaken by parents	88.6	80.5	73.7	77.8
15. Mistaken by other siblings	90.0	69.0	71.0	68.7
16. Mistaken by teachers	83.3	90.2	83.0	91.7
17. Mistaken by close friends	62.9	80.5	70.4	86.1
18. Mistaken by causal friends	91.7	92.7	82.8	88.9
19. Mistaken by strangers	97.1	97.6	91.6	87.5
20. Mistaken when together	88.6	81.6	84.4	84.6

ilarity reports between fathers and mothers could be examined for 73 pairs of twins. The probability of agreement between their reports ranged from 68.7 to 98.6%. In general, the probability of agreement between twins and between parents was similar. Only the following five items had an agreement probability below 80% for either test–retest or interrater reliability: items 11 (palmar creases), 13 (like two peas in a pod), 14 (mistaken by parents), 15 (mistaken by other siblings), and 17 (mistaken by close friends).

Questionnaire Prediction Accuracy

For each of the 20 items of the questionnaire, the frequency of response 0 or 1 was compared with the DNA diagnosis of zygosity (Table III). Three items (1, 2, and 4) had little discriminating power between monozygosity and dizygosity because very few persons reported any difference in these items. In general, parental reports on adolescent twins had higher concordance rates than the self-reports of adolescent twins, while parental reports on child twins had the lowest concordance rate. Among the five items that had sufficient concordance rates with the DNA diagnosis (defined as >80% for both MZ and DZ twins) for parental reports on adolescent twins, three dealt with twin confusion (items 16, 18, and 19) and two with physical similarity (items 3 and 13).

Stepwise logistic regression analysis among the 20 items was then employed to construct a parsimonious model for each sample. For the parental reports on adolescent twins, items 3, 13, and 19 were selected in the final model, whereas the final model for the self-reports of adolescent twins selected items 7, 13, and 18. For the parental reports on child twins, only items 2 and 6 were retained in the final model. Their regression coefficients and prediction accuracy indexes are listed in Table IV. If the significance level of entry into or staying in the model was changed from .10 to .05, the results remained the same except that no variable was retained in the final model for the parental reports on child twins. The total predictive accuracy of the models were satisfactory for both the parental reports and the self-reports of adolescent twins (97.4 and 95.6%, respectively), with a sensitivity of about 90% and a specificity of ≥97% in both models. However, the total predictive accuracy of the model for parental reports on child twins was less satisfactory (92.5%), with a sensitivity of only 57.1%.

To determine the score ranges on the three-item models where DZ and MZ adolescent twins overlapped, we calculated the distribution of MZ and DZ twins on

Table III. Concordance Between a Positive Score[a] on a 2-Point Scale of Individual Items of the Twin Similarity Questionnaire and the DNA Diagnosis

	Parental report on adolescent twins				Self-report by adolescent twins				Parental report on child twins			
	MZ (N = 124)		DZ (N = 33)		MZ (N = 167)		DZ (N = 38)		MZ (N = 39)		DZ (N = 8)	
Item	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
1. Eye color	1	(0.8)	0	(0.0)	0	(0.0)	2	(5.3)	0	(0.0)	0	(0.0)
2. Hair color	1	(0.8)	0	(0.0)	0	(0.0)	5	(13.2)	1	(2.6)	1	(12.5)
3. Facial appearance	11	(8.9)	29	(87.9)	17	(10.2)	31	(81.6)	2	(5.1)	3	(37.5)
4. Skin color	0	(0.0)[b]	5	(15.2)	1	(0.6)	7	(18.4)	0	(0.0)	1	(12.5)
5. Weight	18	(14.5)	25	(75.8)	39	(23.4)	22	(57.9)	6	(15.4)	4	(50.0)
6. Height	10	(8.1)	19	(57.6)	19	(11.4)	18	(47.4)	0	(0.0)	4	(50.0)
7. Hair texture	4	(3.2)	17	(51.5)	4	(2.4)	15	(39.5)	0	(0.0)	3	(37.5)
8. Ear lobe shape	1	(0.8)	9	(27.3)	5	(3.0)	4	(10.5)	0	(0.0)[c]	2	(25.0)
9. Hair whorl	14	(11.3)	11	(33.3)	14	(8.4)	9	(23.7)	5	(13.2)[b]	2	(25.0)
10. Thumb curvature	5	(4.0)	6	(18.2)	9	(5.4)	10	(26.3)	2	(5.4)[c]	1	(12.5)
11. Palmar creases	14	(11.3)	15	(45.5)	22	(13.2)	13	(34.2)	0	(0.0)	4	(50.0)
12. Eyebrow	2	(1.6)	12	(36.4)	5	(3.0)	18	(47.4)	1	(2.6)	2	(25.0)
13. Like two peas in a pod	13	(10.6)[b]	31	(93.9)	41	(24.6)	35	(92.1)	2	(5.1)	3	(37.5)
14. Mistaken by parents	45	(36.3)	29	(87.9)	104	(62.3)	36	(94.7)	8	(20.5)	4	(50.0)
15. Mistaken by other siblings	44	(35.5)	29	(87.9)	118	(70.7)	37	(97.4)	8	(20.5)	4	(50.0)
16. Mistaken by teachers	9	(7.3)	29	(87.9)	20	(12.0)	29	(76.3)	2	(5.4)[c]	2	(28.6)[b]
17. Mistaken by close friends	26	(21.0)	28	(84.9)	59	(35.3)	35	(92.1)	3	(7.9)[b]	2	(28.6)[b]
18. Mistaken by causal friends	5	(4.0)	28	(84.9)	18	(10.8)	33	(86.8)	0	(0.0)[b]	2	(25.0)
19. Mistaken by strangers	3	(2.4)	27	(81.8)	7	(4.2)	29	(76.3)	0	(0.0)[b]	1	(12.5)
20. Mistaken when together	33	(26.6)	30	(90.9)	22	(13.2)	33	(86.8)	1	(2.6)[b]	2	(25.0)

[a] Positive score = "clear difference" for items 1–12, "no" for item 13, and "rarely or never" for items 14–20.
[b] Data missing for one subject.
[c] Data missing for two subjects.

Table IV. Accuracy of the Parsimonious Logistic Model of Twin Similarity Questionnaire in Predicting DNA-Determined Zygosity Among Three Samples

	Number of			Prediction accuracy			
Sample	Reports	DZ twins	Logistic model	Cutoff point	Correct	Sensitivity	Specificity
Adolescent twins							
Parental report	155[a]	33	$\log[p/(1-p)] = -6.8252 + 4.2880I_3 + 4.2059I_{13} + 5.8429I_{19}$	0.38–0.80	97.4	90.9	99.2
Self-report	205	38	$\log[p/(1-p)] = -7.2517 + 4.1725I_7 + 4.7751I_{13} + 4.1098I_{18}$	0.08–0.64	95.6	89.5	97.0
Child twins							
Parental report	40[b]	7	$\log[p/(1-p)] = -2.8034 + 15.9915I_2 + 15.9915I_6$	0.06–0.98	92.5	57.1	100.0

Note: p = the probability of being DZ; I_n = item n of the similarity questionnaire.
[a] Two parental reports of MZ twins were deleted from the regression analysis because of missing one variable.
[b] Seven parental reports (six of MZ twins and one of DZ twins) were deleted from the regression analysis because of missing one or more variables.

all possible scores, and the corresponding predicted probability of being DZ (Table V). Among the parental reports on adolescent twins, only four misclassified twin zygosity (2.6% misclassification rate) if class 4 was chosen as the threshold to classify a twin pair as MZ (≤class 4) or DZ (>class 5). Among the four incorrect parental reports, two were from mothers and two were from fathers. All of their spouses' reports classified their twins' zygosity correctly. Among the self-reports of adolescent twins, nine misclassified twin zygosity (4.4% misclassification rate) if class 4 was chosen as the threshold to classify a twin pair as MZ (≤class 4) or DZ (>class 5). Among the nine self-reports that misclassified twin zygosity, two were from a pair of MZ twins and two from a pair of DZ twins. The remaining five twins had corresponding self-reports from their cotwins that classified zygosity correctly

DISCUSSION

Several DNA-based methods have been adopted for the diagnosis of twin zygosity, including those employing minisatellite (Hill and Jeffreys, 1985; Eufinger *et al.*, 1993) and microsatellite (Akane *et al.*, 1991; Erdmann *et al.*, 1993; Spitz *et al.*, 1996) probes. Common drawbacks of these methods are that they are time-consuming (each subject's DNA has to be amplified several times for different markers and gel electrophoresis is needed) and the resulting banding is sometimes difficult to read. In contrast, the method used in this study has several advantages including the ability to perform one-round PCR simultaneously for multiple markers (five in this study), the fact that neither gel electrophoresis nor isotope is needed, results can be obtained quickly (within 6 h in this study), and its high accuracy (as high as 99.0% in this study). Furthermore, we also demonstrated that DNA can be extracted from regularly mailed mouthwashing sucrose solutions within 7–10 days and is adequate for zygosity diagnosis. This feature is especially useful for epidemiological studies of twins.

On the basis of DNA diagnosis, the DZ-to-MZ ratio of the same-sex twins in this study was 1:4.53 for the adolescent twins and 1:4.88 for the child twins. These values are very close to the reported 1:4.92 or 1:4.90 population twinning rate of same-sex twins in Taiwan (Chen *et al.*, 1987). Thus, possible bias due to a higher participation rate of MZ twins is not likely to be an important confounding factor in this study.

The reliability analysis of the twin similarity questionnaire revealed several interesting features of the questionnaire that have seldom been discussed before. First, differentiation between "no difference" and "only a slight difference" or between "frequently mistaken" and "occasionally" was not reliable on the basis of test–retest reliability for adolescent twins themselves or their parents. Second, some items were not concordantly judged by twins themselves or their parents, including item 13 (like two peas in a pod) and several confusion questions. Because few studies have reported on the reliability of twin similarity questionnaires, we cannot determine whether this unreliability is specific to our study population.

In terms of predicting zygosity, many individual items were useless. It is not surprising that items useful in Western countries such as eye color and hair color in fact were useless in this study. Instead, parents and adolescent twins tended to base differentiation on general appearance (items 3 and 13). Individual items that had good predictive accuracy in this study were mainly items related to confusion. It is also interesting to note

Table V. Classes of Scores on the Three-Item Models of Similarity Questionnaire and Zygosity Status Among Adolescent Twins

	Parental reports on adolescent twins			Self-reports by adolescent twins		
Class of scores	I_3,I_{13},I_{19} (p)	MZ (n = 122)	DZ (n = 33)	I_7,I_{13},I_{18} (p)	MZ (n = 167)	DZ (n = 38)
1	0, 0, 0 (.001)	96	0	0, 0, 0 (.001)	109	0
2	0, 1, 0 (.068)	12	1	0, 0, 1 (.041)	14	0
3	1, 0, 0 (.073)	10	1	1, 0, 0 (.044)	3	0
4	0, 0, 1 (.272)	3	1	0, 1, 0 (.078)	36	4
5	1, 1, 0 (.841)	1	4	1, 0, 1 (.737)	0	3
6	0, 1, 1 (.962)	0	2	0, 1, 1 (.837)	4	19
7	1, 0, 1 (.965)	0	0	1, 1, 0 (.845)	1	1
8	1, 1, 1 (.999)	0	24	1, 1, 1 (.997)	0	11

Note: I_n = item n of the similarity questionnaire; p = predicted probability of being DZ.

that the accuracy of individual items of parental reports on child twins was very low.

In this study, a parsimonious model of more than one item for each sample was derived from logistic regression analysis that led to an improvement in predictive accuracy. The compositions of this model, however, were different for each of the three samples. For adolescent twins, the items for parental reports (items 3, 13 and 19) and self-reports (items 7, 13, and 18) are related to physical similarity and confusion among casual friends or strangers. These items are similar to the following items used in Japanese studies by Ooki *et al.* (1990, 1993): "How are you alike?" "How often are you mistaken?" and "By whom are you mistaken?" Our results indicate that either parental reports or self-reports can predict zygosity with sufficient accuracy in adolescent twins. This level of accuracy is comparable to that in previous studies of adults' self-reports (93–98%) (Cederlof *et al.*, 1961; Nichols and Bilbro, 1966; Torgersen, 1979; Magnus *et al.*, 1983) or adolescents' self-reports (91.5%) (Ooki *et al.*, 1990). This suggests that, when assessing zygosity in adolescent twins, parental questionnaires are not necessary, which could make data collection easier for this population.

In the three-item models for adolescent twins, if a single cutoff point was chosen, the sensitivity of identifying DZ (89.5–90.9%) was lower than the specificity (97.0–99.2%). This indicates that the main reason for the misclassification of zygosity by questionnaire was that some DZ twins were so similar in appearance that they and their parents could not see much difference between them. The overlap of score classes between DZ and MZ in our study was wider than that reported by Spitz *et al.* (1996), especially for self-reports of adolescent twins. One feasible way to improve the predictive accuracy rates is to conduct DNA diagnostic procedures on twins with discordant reports either from themselves or from their parents. In this report, this technique raised the correct rate from 97.4 to 100% for parental reports and from 95.6 to 98% for self-reports of adolescent twins.

In this study, for the parental reports on child twins, the parsimonious model consisted of only two items (similarity in hair texture and height). The predictive accuracy rate of the model is also comparable to that of previous studies in child twins (91–92.4%) (Bønnelykke *et al.*, 1989; Spitz *et al.*, 1996). However, the small number of DZ twins in the child sample render the results preliminary. Nevertheless, the concordance rates of individual items do indicate that parents had difficulty differentiating two young DZ twins prior to pubertal changes. Items other than those in our questionnaire may be needed to improve questionnaire accuracy in child twins.

It is of interest to note that adding other items to the preceding parsimonious models did not lead to an increased predictive accuracy rate. One reason for this is that twin similarity items tend to correlate with one another. Replication in further independent samples is warranted to establish the accuracy of the three-item models derived in this study.

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REFERENCES

Akane, A., Matsubara, K., Shiono, H., Yamada, M., and Nakagome, Y. (1991). Diagnosis of twin zygosity by hypervariable RFLP markers. *Am. J. Med. Genet.* **41**:96–98.

Bønnelykke, B., Hauge, M., Holm, N., Kristoffersen, K., and Gurtler, H. (1989). Evaluation of zygosity diagnosis in twin pairs below age seven by means of a mailed questionnaire. *Acta Genet. Med. Gemellol.* **38**:305–313.

Budowle, B., Lindsey, J. A., DeCou, J. A., Koons, B. W., Giusti, A. M., and Comey, C. T. (1995). Validation and population studies of the loci LDLR, GYPA, HBGG, D7S8, and Gc (PM loci), and HLA-DQa using a multiplex amplification and typing procedure. *J. Forens. Sci.* **40**:45–54.

Cederlof, R., Friberg, L., Jonsson, E., and Kaij, L. (1961). Studies on similarity diagnosis with the aid of mailed questionnaires. *Acta Genet.* **11**:338–362.

Chen, C. J., Cohen, B. H., Diamond, E. L., Lin, T. M., and Chen, J. S. (1984). Genetic variance and heritability of cardiovascular risk factors in Chinese adolescent twins. *Acta Genet. Med. Gemellol.* **33**:363–373.

Chen, C. J., Lin, T. M., Chang, C., and Cheng, Y.-J. (1987). Epidemiological characteristics of twinning rates in Taiwan. *Acta Genet. Med. Gemellol.* **36**:335–342.

Cohen, D. J., Dibble, E., Grawe, J. M., and Pollin, W. (1975). Reliably separating identical from fraternal twins. *Arch. Gen. Psychiatry* **32**:1371–1375.

Erdmann, J., Nöthen, M. M., Stratmann, M., Fimmers, R., Franzek, E., and Propping, P. (1993). The use of microsatellites in zygosity diagnosis of twins. *Acta Genet. Med. Gemellol.* **42**:45–51.

Eufinger, H., Rand, S., Scholz, W., and Machtens, E. (1993). Clefts of the lip and palate in twins: Use of DNA fingerprinting for zygosity determination. *Cleft Palate Craniofac. J.* **30**:564–568.

Freeman, B., Powell, J., Ball, D., Hill, L., Craig, I., and Plomin, R. (1997). DNA by mail: An inexpensive and noninvasive method

for collecting DNA samples from widely dispersed populations. *Behav. Genet.* **27**:251–257.

Goldsmith, H. H. (1991). A zygosity questionnaire for young twins: A research note. *Behav. Genet.* **21**:257–269.

Hayney, M. S., Poland, G. A., and Lipsky, J. J. (1996). A noninvasive 'swish and spit' method for collecting nucleated cells for HLA typing by PCR in population studies. *Hum. Hered.* **46**:108–111.

Hill, A. V., and Jeffreys, A. J. (1985). Use of minisatellite DNA probes for determination of twin zygosity at birth. *Lancet* **2**:1394–1395.

Lee, C.-I., Tsai, L.-C., and Liau, S.-C. (1995). Application of AmpliType PM Kit. *J. Police Sci.* **26**:256–268 (in Chinese)

Lench, N., Stanier, P., and Williamson, R. (1988). Simple non-invasive method to obtain DNA for gene analysis. *Lancet* **1**:1356–1358.

Lin, R. S., Chiu, F. Y., and Chen, K. P. (1969). A preliminary twin study in Taiwan. II. Dermatoglyphic aspect and zygosity-testing. *J. Formos. Med. Assoc.* **68**:212–219.

Magnus, P., Berg, K., and Nance, W. E. (1983). Predicting zygosity in Norwegian twin pairs born 1915–1960. *Clin. Genet.* **24**:103–112.

Nichols, R. C., and Bilbro, W. C. (1966). The diagnosis of twin zygosity. *Acta Genet. Basel* **16**:265–275.

Ooki, S., Yamada, K., Asaka, A., and Hayakawa, K. (1990). Zygosity diagnosis of twins by questionnaire. *Acta Genet. Med. Gemellol.* **39**:109–115.

Ooki, S., Yamada, K., and Asaka, A. (1993). Zygosity diagnosis of twin by questionnaire for twins' mothers. *Acta Genet. Med. Gemellol.* **42**:17–22.

Saiki, R. K., Walsh, P. S., Levenson, C. H., and Erlich, H. A. (1989). Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes. *Proc. Natl. Acad. Sci. USA* **86**:6230–6234.

SAS Institute, Inc. (1989). *SAS/STAT User's Guide, Version 6,* SAS Institute, Cary, NC.

Smith, S. M., and Penrose, L. S. (1955). Monozygotic and dizygostic twin diagnosis. *Ann. Hum. Genet.* **19**:273–289.

Spitz, E., Moutier, R., Reed, T., Busnel, M. C., Marchaland, C., Roubertoux, P. L., and Carlier, M. (1996). Comparative diagnoses of twin zygosity by SSLP variant analysis, questionnaire, and dermatoglyphic analysis. *Behav. Genet* **26**:55–63.

Torgersen, S. (1979). The determination of twin zygosity by means of a mailed questionnaire. *Acta Genet. Med. Gemellol.* **28**:225–236.

Weir, B. S. (1990). *Genetic Data Analysis,* Sinauer, Sunderland, MA.

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