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Diagnosis of Twin Zygosity by Self-Assessment and by Genetic Analysis

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For 173 pairs of like-sex adult twins, self-assessment of zygosity was verified by laboratory diagnosis. Seventeen percent of twins who were very likely monozygous (MZ) believed themselves dizygous (DZ), frequently citing two placentas at their delivery as "evidence." We suggest that twins be asked what leads them to their assessment of their own zygosity. For 93% of Caucasian and 89% of American Black like-sex twins in our sample, DZ twins could be differentiated based on six polymorphic markers retrievable from frozen sera. MZ twins who believe themselves DZ can be considered "environmentally DZ, genetically MZ" twins, and might be used to study genetic and environmental influences on the treatment of twins and on twins' choices of social characteristics.

Key words: Zygosity determination, Genetic analysis, Genetic markers, Twin studies

INTRODUCTION

As part of an extensive investigation of health and disease in twins, we have been interested in the extent to which twins' own statements about their zygosity are confirmed by laboratory diagnosis based on analysis of polymorphic blood groups, red cell enzymes, and serum proteins. This question is of considerable practical importance in twin studies, since much effort and expense could be saved if twins' own assessment of their zygosity proved highly accurate.

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METHODS

Our study population is the Kaiser-Permanente Twin Registry, including approximately 8000 pairs of twins, the majority of whom live in the San Francisco Bay area. The entirely voluntary Registry includes slightly more female than male twins, and is heavily weighted toward children and young adults [7]. Each adult twin was asked to assess own zygosity by answering the question, "Do you and your twin look almost exactly alike?" The alternative responses were "Yes, almost exactly alike ('Like two peas in a pod'), that is, 'identical'"; or "No, that is, 'fraternal'."

We obtained blood samples from 173 adult like-sex twin pairs in the study population (Table 1). Questionnaire responses to the zygosity question were in disagreement for 7 of these pairs. However, at the time of appointment scheduling or blood sampling, 6 individuals told us they had misunderstood the question or had erred in their original answer. We decided to allow these corrections by the twins themselves prior to laboratory analysis. Therefore, the twins' own assessment of zygosity was: 87 pairs monozygous (MZ), 85 pairs dizygous (DZ), 1 pair in disagreement. Twenty independent genetic marker systems were used: ABO, Rh CcDEe, MNSs, Duffy A and B, Lewis A and B, Kell, acid phosphatase, adenylate kinase, adenosine deaminase, amylase 2, esterase D, glutamate-pyruvate transaminase, glyoxylase I, Gm immunoglobulins, haptoglobin, phosphoglucomutase 1, properdin factor B, protease inhibitor, third component of complement, and vitamin D-binding protein. The genetic analyses were undertaken "blind," ie, without knowledge of the twins' own assessment of their zygosity.

For each twin, 7 ml of blood was drawn into an EDTA-coated tube, and a 0.8-ml aliquot removed for bloodgroup typing. The remaining sample was separated by centrifugation at 150 *g* for 8 min. White cells and plasma were aspirated and centrifuged at 375 *g* for 10 min. Plasma was removed and stored, after flash freezing, at −80°C. Red cells were washed three times in 0.8% saline. An equal volume of glycerol-freezing solution (0.058M potassium citrate, 0.02M monobasic sodium phosphate, and 0.02M dibasic sodium phosphate in 40% glycerol in water) was added to each red cell sample, immediately mixed, then stored at −35°C. We have found the glycerol-freezing solution crucial for the long-term preservation of red cell enzyme activity.

Blood typing was performed using traditional procedures [19]. The red cell enzymes (AP, ADA, AK, Est D, GLO, GPT, and PGM) were analyzed by starch-gel electrophoresis [9]. Amylase 2 and haptoglobin were analyzed by acrylamide-gel electrophoresis, vitamin D binding protein by cellulose-acetate electrophoresis, properdin factor B and third complement component by agarose electrophoresis, and protease inhibitor by isoelectric focusing (references for these markers are indicated in Table 2).

RESULTS

Concordance of the twins' assessment and the laboratory diagnosis of zygosity are indicated in Table 3. Of the 87 twin pairs who indicated they were MZ, 85 (98%) were the same for

*TABLE 1. Demographic Characteristics of Twin Pairs Whose Zygosities Were Diagnosed by Self-Assessment and by Genetic Analysis**

	Male pairs			Female pairs			
Birth year	White	Black	Other†	White	Black	Other†	Total
1895–1909	3	0	0	3	0	0	6
1910–1919	4	0	1	8	2	0	15
1920–1929	9 (2)	0	0	13 (1)	4	1	27 (3)
1930–1939	6	0	0	8	0	0	14
1940–1949	9	1	1	35 (2)	6	1 (1)	53 (3)
1950–1962	23 (3)	0	1	31 (8)	0	3 (1)	58 (12)
Total	54	1	3	98	12	5	173 (18)

*The figures in parentheses indicate the number of twin pairs who believed themselves DZ or disagreed, but who were MZ by genetic analysis.

†Includes 3 Chinese, 1 Japanese, and 1 Creole pair, and 3 pairs of other, unspecified ethnicity.

*TABLE 2. Allelic Frequencies Among American Caucasians, Blacks, and Chinese, of Electrophoretic and Immunoglobulin Markers Used in the Diagnosis of Twin Zygosity**

		Allelic frequencies		
Locus	Allele	Caucasian	Black	Chinese
Acid phosphatase, AP	A	0.39	0.25	0.22
	B	0.55	0.72	0.78
	C	0.06	0.01	...
	R	...	0.02	...
Adenosine deaminase, ADA	1	0.95	0.98	0.98
	2	0.05	0.02	0.02
Adenylate kinase, AK	1	0.95	0.99	1.00
	2	0.05	0.01	<0.01
Amylase 2, AMY [16]	A	0.95	0.96	1.00
	B†	0.05	0.02	...
	C†	...	0.02	...
Esterase D, EST D [11]	1	0.90	0.90	0.86
	2	0.10	0.10	0.14**
Properdin factor B, Bf [1]	S	0.71	0.44	0.89
	F	0.28	0.51	0.11
	S′	0.01	...	...
	F′	...	0.05	...
Glyoxylase I, GLO [13]	1	0.40	0.31	0.12
	2	0.60	0.69	0.88**
Glutamate-pyruvate transaminase, GPT [4]	1	0.52	0.78	0.59
	2	0.48	0.22	0.41
Vitamin D-binding protein, Gc	1	0.72	0.89	0.77
	2	0.28	0.11	0.23
Haptoglobin, Hp	1	0.42	0.55	0.34
	2	0.58	0.45	0.66
Phosphoglucomutase 1, PGM_1	1	0.75	0.81	0.76
	2	0.25	0.19	0.22‡
Third component of complement, C3 [2]	S	0.77	0.95	0.99
	F	0.22	0.05	...
	Others	0.01	<0.01	0.01
Gm immunoglobulins [22, 23]	3, 5, 11	0.69	...	...
	1, 17, 21	0.20	...	0.23
	1, 2, 17, 21	0.10	...	0.09
	1, 5, 11, 17	<0.01	0.88#	...
	1, 11, 17	...	0.12#	...
	1, 11, 16, 17	<0.01	...	0.06
	1, 3, 5, 11	...	...	0.62
[a]Protease inhibitor, Pi [14]	M_1	0.64	0.90	0.66
	M_2	0.19	0.03	0.24
	M_3	0.11	0.05	0.09
	S	0.04	0.01	...
	Z	0.01	<0.01	...
	Others (E, F, I)	0.01	0.01	0.01**

*Allelic frequencies are from relevant citations by Mourant et al [17], unless otherwise indicated.
†BA and BB are indistinguishable on our system, as are CA and CC.
‡PGM_1^6 and PGM_1^7 also observed.
#Frequencies in African Blacks.

**Estimates based on 52 unrelated Chinese individuals tested in our laboratory.

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TABLE 3. Concordance of Laboratory Diagnosis of Zygosity and Twins' Own Assessment

	Laboratory diagnosis of zygosity		
Twins' assessment of zygosity	MZ	DZ	Total
MZ	85	2	87
DZ	17	68	85
Disagree	1	0	1
Total	103	70	173

all 20 marker systems. The two exceptions were a pair of twins who differed at both the haptoglobin and Gc loci, and a pair who differed at the ABO, acid phosphatase, and Gc loci. Duplicate tests on these twin pairs confirmed the laboratory results. Of the 85 twin pairs who indicated they were DZ, only 68 (80%) appeared DZ by our tests. The pair in which the twins disagreed appeared MZ on the basis of laboratory results.

For each of the 18 twin pairs who appeared MZ on the basis of laboratory results, but who believed themselves to be DZ or disagreed about their zygosity, we calculated the probability of dizygosity given the observed genotypes. We applied the method of Race and Sanger [19], using – for Caucasian twins – the probabilities calculated by Maynard Smith and Penrose [15] for the ABO, MNSs, Rhesus, Kell, Duffy, and Lewis systems. For the electrophoretic markers, we estimated relative chances of dizygosity from the figure presented by Gaines and Elston [8], based on the allele frequencies of Table 2. For each pair, the probability of dizygosity, given identity for all marker systems, was less than 0.001. It appears quite unlikely, therefore, than *any* of these 18 pairs are DZ. These 18 pairs were significantly younger than the total sample of twins ($P = 0.003$).

Each twin of these 18 pairs was asked what led him or her to believe their pair to be DZ. Several simply said they had always been told they were. Several other pairs said that their mothers had been told the twins were DZ "because there were two placentas." At the time these twin pairs were born it was known, but not universally recognized, that about 23% of like-sex twin pairs delivered with two placentas are MZ [5]. One other pair of male twins reported that they believed themselves DZ because the US Army had assigned them different ABO types (O and B). Both were clearly type O by our analysis.

Seventy pairs of twins were DZ by laboratory analysis. Both bloodgroup and electrophoretic markers were informative in detecting dizygosity. Bloodgroup analysis alone detected differences in 66 of the 70 pairs (94%). The 13 electrophoretic markers plus Gm detected differences in 69 of the 70 pairs (99%). Six of these markers are of particular practical interest because they can be analyzed accurately even in frozen sera stored for long periods. As a group, these six markers – AMY_2, Bf, Gc, Gm, Hp, and Pi – detected differences in 65 of the 70 twin pairs (93%).

DISCUSSION

Three quite different observations of practical importance in twin studies are indicated by this survey. First, among adult twins of like sex, self-assessment of zygosity may be in error in as many as 10% of cases. However, such errors are predominantly of one sort: 17% of twins who are almost certainly MZ believe they are DZ, while only 2% of DZ twins believe they are MZ. The error rate among laboratory-diagnosed MZ twins in this study is higher

than the comparable error rates from other studies [3, 6, 10, 12, 18, 20], especially considering that monozygosity was diagnosed by concordance at many more markers in the present study than previously. This difference may be due in part to the way in which the zygosity question was phrased. Both the Swedish and NHLBI questionnaires, for example, asked twins about their similarity of appearance as children, while our question used the present tense – "Do you and your twin look almost exactly alike?" However, there is a substantial percentage of twins in each study who believe themselves to be DZ, but for whom laboratory analysis indicates a very low probability of dizygosity (in this study $P < 0.001$ for each such pair). To resolve some such errors, we suggest as a simple addition to every twin questionnaire or interview the question: "What leads you to believe you are fraternal (or identical)?" We would tend to doubt responses of self-assessed DZ twins citing two placentas. We would consider responses such as "We've always looked quite different" to be much more reliable evidence of dizygosity. Based on our experience, we would tend to accept twins' own assessment of monozygosity, particularly if only a limited number of pairs can be verified by laboratory analysis. Since most research with twins relies on the genetic identity of MZ twins, it is important that self-assessment of monozygosity be accurate.

Second, our experience suggests that about 93% of like-sex DZ Caucasian twin pairs can be accurately diagnosed using six markers (AMY_2, Bf, Gc, Gm, Hp, and Pi) which can be analyzed from stored, frozen sera. About 89% of like-sex, DZ, American Black twin pairs can be accurately diagnosed using the same six markers. The discriminating power of these six markers is as high as that of the six bloodgroup systems (A_1A_2 BO, Rh DCcEe, MNSs, Duffy, Lewis, and Kell) commonly employed in zygosity diagnosis. We believe the serum markers may be particularly useful for field studies, for which twins may be interviewed away from laboratory facilities. It is quite easy to freeze, store, and ship sera, while field analysis of bloodgroups, or proper storage and shipping of whole blood or frozen red cell lysates, can be time-consuming and difficult.

Finally, twin pairs who believe they are DZ, but who are very probably MZ on the basis of laboratory analysis, form a most interesting group for further studies [21]. Since these pairs have been considered DZ by their families, they are, in effect, "environmentally DZ, genetically MZ" twins. Twin registries with a substantial number of such pairs might consider comparing the within-pair discordance for environmental or social characteristics (occupation, diet, smoking, etc) of these twins with the within-pair environmental discordance of other MZ twins and of DZ twins. The similarity of the within-pair environmental discordance for "environmentally DZ, genetically MZ" twins to the within-pair environmental discordance for other MZ twins, may reflect the extent of genetic influences on individual twins' selection of environmental characteristics, or the extent to which treatment of the twins is affected by their genetic similarity.

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