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Zygosity Testing: Laboratory and the Investigator's Judgment

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Abstract. The immediate global impressions of zygosity (based upon physical similarity) were recorded by the investigator at initial meetings with 105 pairs of twins. The accuracy of these ratings, as well as classifications provided by two objective procedures (physical resemblance questionnaire; dermatoglyphic analyses) and two subjective procedures (parental impressions; physician's impressions), were evaluated by comparison with results from bloodtyping for 53 pairs. The judgments of the investigator furnished the most accurate indication of zygosity (94-96% accuracy). Laboratory tests were repeated for five pairs when the results proved incompatible with the investigator's ratings. In all five cases, the investigator's judgments were confirmed, indicating that a laboratory error had occurred. It appears that the opinion of a skilled observer of twins can provide a convenient and highly effective alternative to bloodtyping.

Key words: Zygosity, Twins, Physical resemblance, Dermatoglyphics, Blood typing.

For many years, Mr. James Shields of the Genetics Unit at the Maudsley Hospital has been sending us samples of blood from the twins. We find that the blood groups practically never contradict the opinion of such a skilled observer of twins.

Race & Sanger 1975

INTRODUCTION

The availability of effective methods for zygosity diagnosis is critical in studies of twins, particularly when sample sizes are limited. This is because misclassification of twin pairs may yield spurious estimates of the relative magnitude of resemblance within sets assigned as DZ or MZ [8]. Determination of zygosity has typically relied on a comparative examination of 22 or more blood antigens [18] for the members of a twin pair; one or more discrepancies identify DZ twins, while complete concordance indicates MZ twins with a very high degree of reliability.

Bloodtyping is becoming an increasingly costly and time-consuming procedure, and is not without occasional discomfort to the subject. This has encouraged the development of alternative means for distinguishing among types of twin pairs, the most common being a physical resemblance questionnaire. Agreement between such methods and blood-group examination may be as high as 98% [16], yet there has been evidence of disappoint-

ing results. Scarr [12] reported that 17.4% of MZ pairs and 31.2% of DZ pairs were misclassified by mothers. More recently, only 60% of both adolescent MZ and DZ pairs were correct in their own perceptions of zygosity, as compared with blood-typing results [1]. Unsatisfactory results were also obtained in the study to be described.

The quotation provided here as an epigraph prompted a comparison between the accuracy of the investigator's judgments and other frequently used procedures. It was anticipated that an experienced examiner's opinion (1) would be as informative as objective standardized methods and (2) would prove more effective than the subjective impressions offered by parents and physicians.

MATERIALS AND METHODS

The 53 pairs of twins included in this analysis, selected due to the availability of bloodtyping information, were drawn from a larger sample of 105 pairs who participated in a twin study of cooperation, competition and altruism at the University of Chicago (Doctoral dissertion). The twins ranged in age from 5.04 to 13.28 years (mean 8.03, SD 1.51). One adult pair, 23.25 years of age, included in selected aspects of this study was excluded from the calculation of mean age. Recruitment was accomplished primarily through Mothers of Twins Clubs, schools, recreational centers and personal referrals, both in the Chicago and New York areas. Families of 111 pairs of twins were contacted personally and 105, or 94% (55 male and 50 female sets), consented to participate.

Zygosity determination required a major effort in this study. Two subsamples were selected and three objective and three subjective procedures were employed as described below.

OBJECTIVE PROCEDURES

1. **Serological Analyses.** Informations on bloodgroups was available for 53 pairs of twins, or half the sample. The following red bloodcell factors were compared between cotwins: ABO, Rh (D,C,c, E,e), Kell (K,k), Duffy (Fy^a,Fy^b), Kidd (Jk^a,Jk^b), Lewis (Le^a,Le^b), MNSs and P (P_1). Analyses were conducted at the Billings Hospital Blood Bank at the University of Chicago and at the New York Blood Center. Five of the pairs had been bloodtyped prior to participation in the study, either for medical reasons or involvement in previous twin research.
2. **Physical Resemblance Questionnaire.** Owing to the young age of the subjects, mothers completed items on a widely used physical resemblance questionnaire [7]. However, they were strongly encouraged to consult the twins for information concerning confusion by teachers and friends. Data on height were obtained by the investigator. This questionnaire was scored according to the rules established by its authors.
3. **Dermatoglyphic Analyses.** Fingerprints were obtained for 104 pairs of twins (one bloodtyped pair declined participation in this phase of the study) and evaluated for within-pair resemblance in pattern-type and ridge count. The probability that a pair was assigned as MZ or DZ was determined by (a) a discriminant function analysis (DFA) and (b) probability tables, based upon concordance for total ridge count (TRC) [15]. Dermatoglyphic data, collected for a sample of 360 twin pair (224 MZ sets and 136 DZ sets), independently ascertained by investigators at Indiana University [10,11], were used to calculate the discriminant function equation. In the present study, pairs were assigned as MZ if $p(DZ) \leqslant 0.50$, and DZ if $p(DZ) > 0.50$. Formulae for determining the probability of dizygosity, based upon total ridge count differences, are provided in Smith & Penrose [15].

SUBJECTIVE PROCEDURES

1. **Investigator's Impressions of Zygosity.** The investigator provided judgment of zygosity within the first two to three minutes of the initial meeting with each pair of twins as follows: "definitely" MZ, "somewhat" MZ, "definitely" DZ, "somewhat" DZ, uncertain. These judgments were based upon immediate, global impressions of physical similarity.
2. **Parental Impressions of Zygosity.** Mothers provided their beliefs as to zygosity of their twins as follows: MZ, DZ, "in-between" (one mother), uncertain, did not say.

3. **Physician's Impressions of Zygosity**. Parents were asked to provide the diagnoses given to them by their physician at the time of delivery. The response categories were the same as those used to assess their own impressions. (It is likely that, in some cases, parental judgments were guided by the physician's comments. However, in view of possible discrepancies, this analysis seemed worthwhile).

RESULTS

The bloodtyping procedure yielded 47 MZ and 6 DZ pairs. This striking imbalance can be explained as follows. Only pairs in which both cotwins achieved IQ scores of 100, or higher, and whose scores did not differ by more than 5 IQ points were to be selected from the initial pool of 105 pairs to participate in the cooperation and competition experiments mentioned above. Bloodtyping was first made available to the forty-seven pairs who fulfilled these criteria and then offered to pairs which appeared to be the most identical in appearance. Unfortunately, limited funding prevented the completion of blood analyses for more of the participants.

Information from the bloodtyping studies was used to evaluate the relative accuracy of the various methods employed. The table presents the percentages of correct classification of MZ and DZ pairs, which allows a rank-ordering of the efficacy of these procedures.

TABLE - Comparison of Methods Used for Zygosity Diagnosis

Method	Correct classification N [1]	MZ [2]	DZ [2]	Rank
Investigator's impressions	50/53[3] (94%)	45/45 (100%)	5/7 (71%)	1
	50/52[4] (96%)		5/6[4] (83%)	
Physical Resemblance Questionnaire	44/53 (83%)	41/44 (93%)	3/9 (33%)	3
Dermatoglyphics:				
DFA	40/52 (77%)	35/36 (97%)	5/16 (31%)	4
TRC	44/52 (85%)	39/40 (98%)	5/12 (43%)	2
Parental Impressions	39/53[5] (74%)	35/36 (97%)	4/12 (33%)	5
Physician's impressions	28/42 (67%)	26/28 (93%)	2/14 (14%)	6

[1] Proportion of all cases (MZ & DZ) classified correctly.
[2] Cases assigned as MZ or DZ by bloodtyping/cases assigned as MZ of DZ by the given method.
[3] One "uncertain" pair (DZ) counted as error in the overall analysis.
[4] Accuracy of investigator after reassignment of blood-concordant pair as DZ; this pair excluded from analysis.
[5] Five cases uncertain.

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The immediate impression of twin type provided by the investigator proved to be the most accurate measure of zygosity used in this study. Correct assignments were given in 94% (50/53) of the cases, with 100% accuracy for pairs assigned as MZ and 71% for pairs assigned as DZ. However, one blood-concordant pair (considered MZ for purposes of this experiment) was reassigned as DZ on account of differences in eye color and height. Excluding this pair from the analysis elevates the percentage of correct diagnoses to 83% for DZ twins, and 96% for the sample as a whole. However, the small sample of DZ twins restricts the meaningfulness and generalizability of outcome concerning DZ pairs. The single remaining misclassified pair proved to be MZ, but was given a judgment of "somewhat" DZ by the investigator. This pair was discordant for cleft palate, which may account, in part, for apparent differences in facial contours. (A comprehensive review of MZ twins discordant for this condition is provided by Cronin & Hunter [3]). An extensive examination of red bloodcell groups, enzymes and proteins for all family members (mother, father, older brother, and twins) yielded a probability of dizygosity of 0.000184. As anticipated, judgements of zygosity provided by parents and physicians were less accurate than those given by the examiner.

Dermatoglyphic analysis (total ridge count) was the most accurate objective measure of zygosity. Agreement with results from bloodtyping occurred in 85% of the cases. In contrast, dermatoglyphic analysis based on the discriminant function score agreed with only 77% of the bloodtyped cases. Despite a substantial heritable component, dermatoglyphic features are sensitive to environmental influences in utero [4]. As a result, the within-pair variation observed among MZ and DZ pairs may be enhanced or diminished, respectively, relative to one another, yielding group differences which are inconsistent with predictions based upon genetic relatedness. While twin studies of fingerprint variables may be of interest for possible evidence of asymmetry reversal or variation associated with placental membrane structure in MZ twins, they should not be used as primary indices of zygosity. (For example, a pair diagnosed as MZ by bloodgroup concordance, MZ-level 1 by the physical resemblance questionnaire, and "definitely" MZ by this investigator, displayed a ridge count difference of 32. A discrepancy of this size is associated with a p(DZ) = 1.29. In fact, a total ridge count difference greater than 12 has been observed among 38.46% of MZ pairs, in contrast with 85.15% of DZ pairs [15]). Much of the literature in this area does a serious disservice to the field by failing to sufficiently emphasize these issues.

Only 83% of the diagnoses provided by the physical resemblance questionnaire proved to be accurate. This instrument was slightly less accurate than the total ridge count difference, yet considerably less accurate than the judgments provided by the examiner. It was, however, more informative than the impressions given by parents and physicians. Finally, across all measures, assignments of twins as DZ proved to be less accurate than assignments of twins as MZ. In other words, the group of twins assigned as DZ included a larger percentage of misclassified sets (true MZ pairs) than the reverse. This would have the effect of reducing estimates of heritability, were such a sample to be used in genetic research.

DISCUSSION

Using serological data as the criterion, the greater validity of objective over subjective procedures for zygosity diagnosis was generally demonstrated. There were, however, two

notable exceptions. The first is the very high degree of accuracy (96%) of the examiner's impressions. Apparently there exist cues from the direct physical presence of the twins (eg, general expression or demeanor) which, in combination with facial similarity or dissimilarity, permit correct classification even after a very brief meeting. Shields [13] has commented that direct comparison of twins "enables one to make observations of similarities and differences in particulars which, though obvious to the eye are not easily recorded in words". In his studies of 74 bloodtyped MZ pairs, only one would have been misclassified in the absence of bloodgroup information, fingerprints and photographs; these cotwins had never been observed together and showed large differences in height and weight. He was, therefore, accurate in 98.6% of the MZ cases. In his assessment of 95 twin pairs discordant for one or more blood factors, only 3 would have been misclassified in the absence of this information. The percentage of accuracy which he achieved was 97% for the DZ twins, and 97.6% for the MZ and DZ samples combined. These figures are quite similar to those obtained in the present study overall (96%), and among the MZ sample (100%), (although the figure for the DZ twins (83%) was lower). Shields cites several previous studies which further demonstrate that the "practical value of this Gestalt of similarity is also seen in the fact that a history of a pair of twins having been so alike that they were frequently mistaken for one another weighs more heavily in favor of monozygosity than any other single item of evidence." Essen-Möller (cited in Torgersen [16]),made nearly the same claim over forty years ago. It seems that regard for the various methods used for zygosity diagnosis has come full circle. The observation presented in the epigraph to this paper [9] is clearly confirmed.

The second unusual finding is the limited ability of the physical resemblance questionnaire to predict zygosity. This problem deserves serious attention, given that assignments of twin-type in scientific research often rely solely upon such procedures. Separation of twins at school can minimize confusion by teachers and close friends; these items are important to level 1 and 2 decisions of zygosity in the physical resemblance questionnaire. In the future, questionnaires might attempt to control for some of these events, perhaps by giving greater weight to the opinion of a stranger or casual friend.

In the present study, there was a consistent bias toward misdiagnosing MZ twins as DZ twins. Another recent study by Matheny [6] reported a similar tendency among parents. In contrast, earlier studies have observed that parental ratings typically err in the direction of monozygosity [1,12,14]. It is possible that awareness and appreciation for the functioning of twins as individuals were less salient ten to twenty years ago than it is today. In support of this interpretation, the majority of families sampled, both in the present study and at Mothers of Twins Clubs meetings, report that school separation for twins is generally mandatory. Furthermore, a 1982 Mothers of Twins Club "Notebook" [19] indicates that a primary goal of this organization is to "... increase the awareness of the individuality of each child." A statement to this effect was not included in the statement of purpose in the May, 1974 edition. These events could explain, at least in part, the judgments made by parents in the more recent investigations.

The degree of error in zygosity diagnosis associated with bloodtyping has never been systematically evaluated, yet there is reason to suspect that it has been underestimated. Vogel & Motulsky [17] state that "... serology is not immune to errors. The investigator should therefore trust his eyes. Whenever possible, he should add (and document) a physiognomic comparison of the pair and should insist on repetition of serologic examinations whenever a twin pair is regarded as monozygotic in spite of dis-

cordance in a marker system."

Cooperation and collaboration between twin researchers and physical anthropologists are strongly encouraged, and arranging bloodtyping for cases in which physical comparisons do not yield clear-cut results is advised.

Such "difficult" cases are, unfortunately, not routinely reported in the literature. The few cases that have appeared are quite informative, yet describe the opposite dilemma: blood-concordant pairs which, in the opinion of the researchers, are DZ. Nichols & Bilbro [7] identified a pair for whom the blood-typing results (MZ) were most likely incorrect, given a four-inch height difference and "other discrepancies". Shields [13] classified two blood-concordant pairs as DZ, because of differences in eye color, hair texture and dermatoglyphic characteristics. Torgersen [16] reported reassignment of four blood-concordant pairs as DZ, due to differences in hair color, height and facial morphology. It may be recalled that this author reassigned one blood-concordant pair as DZ, because of a four-inch height difference and differences in eye color. However, in the present study, the problem of blood discordance among pairs judged to be MZ was also encountered. For five pairs, results from the bloodtyping laboratories (blood-discordant) did not agree with the impression of the examiner (MZ). However, upon replication (at the insistence of this investigator!), the results did prove compatibile. These events strongly underline the elevated risk of misclassification of twin pairs when the investigator does not maintain direct access to his or her sample or uses small numbers of twins.

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