Zygosity Diagnosis in Young Twins by Questionnaire for Twins' Mothers and Twins' Self-reports

双生児母親用質問紙および双生児本人用質問紙による小児期双生児の卵性診断

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CONTENTS

Introduction

In our recent study (Ooki et al, 2003) it was revealed that about 30 population-based twin registers exist in the world. For example, in many European countries, such as Scandinavia, Germany, Belgium, the UK, Italy, The Netherlands, as well as many states of the United States and Australia, there exist historical twin registers. In Italy and several Asian countries, such as Sri Lanka, China, and Korea, a large nationwide register has also been under establishment. The aim of establishing twin register is mainly two reasons, namely to perform many twin studies in human genetics and to manage and control maternal and child health in multiple pregnancy. These findings supported that it is important to examine the establishment of twin register in Japan.

There are two types of twins, and they have different origins. Monozygotic (MZ) twins derive from the division of a single zygote, whereas dizygotic (DZ) twins derive from the independent release and subsequent fertilization of two ova. Zygosity diagnosis is the process of determining whether same-sex twin pairs are MZ or DZ, since different-sex pairs are always DZ. If twin pairs are discordant for certain genetic markers, for example blood typing, they are regarded as DZ, as MZ pairs are genetically identical, therefore all genotypes are concordant. If many genetic markers are concordant for twin pairs, the possibility of their being MZ is calculated according to the Bayes's theorem concerning conditional probability. Zygosity diagnosis is essential for genetic twin studies, as well as management of maternal and child health.

In most population-based twin registers, which aim at genetic epidemiologic twin studies, zygosity diagnosis is mainly performed by convenient questionnaires, not genetic markers, because of large sample size.

More than twenty studies have shown that the determination of zygosity in twins based on questionnaires can be done (Table 1) with considerable accuracy. The accuracy was established by comparing the zygosity diagnosed using questionnaire with that diagnosed using many genetic markers. These studies are summarized by a recent study of Rietveld et al. (2000), showing that the accuracy of the questionnaires employed is around 95%. The usefulness of parental reports has been gradually recognized. (Bønnelykke et al., 1989;

Chen et al., 1999; Cohen et al., 1973, 1975; Price et al., 2000; Rietveld et al., 2000).

We developed a zygosity questionnaire for twins themselves (Ooki et al., 1990) and for twins' mothers (Ooki et al., 1993), mainly for use in a genetic epidemiologic twin study. In Japan, multiple birth rates have been increasing since 1975, and the higher twinning rates since 1987 have been attributed to the higher proportion of mothers treated with ovulation-inducing hormones and partially attributed to in-vitro fertilization (Imaizumi & Nonaka, 1997). The need for an appropriate method of determining zygosity for use by twins' parents or nursing stuff has rapidly increased for many reasons (Derom et al., 2001) and is especially important when twins are too young to respond to questions.

The purpose of this study was to evaluate the accuracy of the zygosity questionnaire for twins' mothers already used in Japan, by adding questions to collect information about physical similarity, and then to compare the accuracy of the mothers' reports with that of self-reports using the same young twin pairs. Though the use of questionnaires for young twins has been increasing recently (Cohen et al., 1973, 1975; Bønnelykke et al., 1989), the comparison of mothers' reports with that of self-reports is relatively limited (Ooki et al., 1993; Chen et al., 1999).

Materials and Methods

Subjects

As there is no population-based twin registry in Japan, it is very difficult to collect blood samples of twins and zygosity questionnaires from their mothers. Original dataset was 74 pairs, consisting of 61 MZ and 13 same-sex DZ pairs (Ooki et al., 1993). In present study two pairs (one MZ and one DZ pair) were removed from original dataset because of insufficient data. Subsequent dataset was 152 pairs, consisting of 99 MZ and 53 DZ pairs. Therefore the total subjects of this study consisted of 159 MZ and 65 DZ twin pairs and their mothers.

The twins were all applicants and students of the secondary education school attached to the faculty of education of the University of Tokyo from 1985 to 2003. This school was established in 1948 and adopted a unique entrance system. About 50 pairs of twins, about 12 years of age and living in the Tokyo metropolitan area, take an examination every year, and about 15 pairs are admitted. The enrolled twins participate in the twin study of education and related projects. All of these twins' parents must hand in a Twins Protocol Questionnaire, which gathers information on family structure, obstetrical findings of mothers, physical, motor and mental development of twins from birth to 12 years of age, and zygosity. One of the parents of each applicant, usually the mother, participates in a medical interview by two or three interviewers (at least one of the authors being an interviewer in the periods of data collection), where this Twins Protocol Questionnaire is checked carefully to be sure there are no unanswered questions. Questions concerning knowledge of zygosity determination based on DNA/blood testing are also asked at the medical interview. It was confirmed that most subjects of the present study did not have much knowledge of their zygosity. First, we ascertained dizygotic twin pairs of applicants whose ABO blood type were discordant using information on obstetrical data described in the "Maternal and Child Health Handbook," presented by the Ministry of Health, Labour and Welfare for all pregnant women who are registered. This handbook was also used in the medical interview. A more accurate zygosity diagnosis using many genetic markers or DNA polymorphisms is performed for those twin pairs who are admitted to the school.

Zygosity questionnaire (Appendix 1 and Appendix 2)

Our zygosity questionnaire for twins themselves (Ooki et al., 1990) and for twins' mothers (Ooki et al., 1993) asked about "confusion of identity" between twins.

The questionnaire for twins is shown in Appendix 1. This questionnaire was established by analyzing 189 twin pairs, consisting of 165 MZ and 24 same-sex DZ, who entered the secondary education school attached to the faculty of education of the University of Tokyo from 1976 to 1989. Each twin answered the same questionnaire separately. The questionnaire included three questions regarding similarity and confusion: 'How are you alike?' 'How often are you mistaken for your twin?' and 'By whom are you mistaken for your twin?' According to the degree of similarity reported, 1-3, 1-3, and 1-4 points were allotted, respectively, for these three questions, and then the sum of the points for both twins was calculated. This sum could range from 6 (1,1,1 for each answer for each twin) to 20 (3,3,4 for each answer for each twin). Zygosity was determined according to the given cutoff point on the single summed raw score (Figure. 1). Torgersen (1979) developed this method originally, and a translated version was used in Japan for twins of a wide range of ages. Determination of the cutoff point can be flexible according to the particular use of the data.

The questionnaire for twins' mothers is shown in Appendix 2. This questionnaire was established by analyzing 74 twin pairs, consisting of 61 MZ and 13 same-sex DZ, who entered the secondary education school attached to the faculty of education of the University of Tokyo from 1985 to 1990. The questionnaires were divided into two parts. The first part contained 16 items regarding mainly physical similarities at about one year of age. According to the answers, the following similarity points were given: 1 (no difference), 2 (do not know), and 3 (clear difference). These items were not yet used for zygosity classification. The three questions of the second part asked mothers whether the twins were 'like two peas in a pod' and whether they were 'mistaken for one another and by whom' at about one year of age. The usefulness of these questions has already been reported (Ooki et al., 1993), and this questionnaire has been used mainly for childhood-age twins in Japan. According to the degree of similarity, 1 to 3 or 1 to 4 points were allotted, and then the points were totaled, with distribution from 3 (1,1,1 for each answer) to 10 (3,3,4 for each answer). Zygosity was determined according to a cutoff point on the single summed raw score. Torgersen (1979) used this method originally in a questionnaire for twin pairs, and our questionnaire for mothers was derived from Torgersen's questionnaire. If the summed score was 3-6, twin pairs were classified as monozygotic, and if the sum was 7-10, they were termed dizygotic. The determination of the cutoff point can be flexible according to the particular use of the data. This method has relatively high accuracy, around 90% (Figure. 2). The problem of this questionnaire is the narrow range of the summed score. The possibility of using information on physical features had been considered as a way to resolve this weak point. In this study, we also compared the accuracy of zygosity questionnaire for twins' mothers with that of twins' self-reports (Figure. 3). The total accuracy was nearly the same for both types of questionnaires.

Because the twins in this study were around 12 years of age, their similarity at about one year, which was explored in the questionnaire, had occurred ten or more years ago, so answers based on mothers' memories might not be accurate. If mothers could not remember their children's similarity, the interviewer recommended that they choose the answer 'do not know' or make an intuitive answer.

Zygosity testing

Zygosity determination of the subjects who are admitted to the school was performed using many genetic markers, which were slightly different according to the entrance year. The zygosity testing included ABO, CcDEe, MNSs, Haptoglobin, Acid phosphatase, Glutamate pyruvate transaminase, Estrase D, HLA-DR, DNA polymorphisms (for example, beta-globin gene cluster haplotype, Dopamine receptor gene: DRD4, Serotonine receptor gene: 5-HTT, and mtDNA 9bp deletion), and related tests by blood sample, and DNase2 by urea sample. As a rule twins and their parents were examined. If all markers are concordant for certain twin pairs, the probability of being MZ calculated using Bayes's theorem is more than 0.9999.

Statistical procedures

The authors accepted the results of zygosity testing by genetic markers as "gold standard" of zygosity. This zygosity was compared with the zygosity classified using questionnaire. The following statistical analyses were performed.

First, we analyzed the usefulness of physical features for determining zygosity. As shown in Appendix 3, three indexes were determined. Index A is the percentage of time that mothers of MZ twins answered 'no difference' to a certain item to which mothers of DZ twins answered 'clear difference'. Index B is the percentage of time that mothers of MZ twins answered 'clear difference' to a certain item to which mothers of DZ twins answered 'no difference'. Index C is the percentage of time that mothers of both MZ and DZ twins answered 'do not know' to a certain item. By definition, the sum of Index A, Index B, and Index C is equal to 100% for each item. Obviously items that have a high value for Index A and low values for Index B and Index C were very informative.

Next, stepwise logistic regression analyses were performed on the six patterns of selected question items to compare their accuracy. We used the PROC LOGISTIC program (SAS Institute, 1993) with a significance level of 0.10 for entry into or retention in the model. The total accuracy, sensitivity and specificity of the questionnaire at specific cutoff points were calculated by specifying 'CTABLE' options. Here total accuracy means all pairs classified correctly as DZ or MZ, sensitivity means DZ pairs classified correctly as DZ and specificity means MZ pairs classified correctly as MZ. In the analysis of twins' self-reports, twins were analyzed as part of a pair or as individuals. In the former case, summed points of both twins for each three questions were used as independent variables.

Finally, we analyzed the usefulness of summed score method for selected items. The accuracy of original dataset (Ooki et al., 1993) was also compared to that of subsequent dataset.

Results

Index A, Index B, and Index C of the sample are summarized in

Table 2. 'Shape of eyebrow,' 'shape of fingers,' and 'sleeping face' seemed to be useful, with Index A >75%, Index B <10%, and Index C <20%.

The results of logistic regression analysis are summarized in Table 3. As seen in Table 3, total accuracy was 91.5% when we used 16 items in the mothers' questionnaires dealing with physical similarity (first part of the mothers' questionnaire) (Pattern 1). The total accuracy was 91.5% when we used 3 items dealing with confusion of identity (second part of the mothers' questionnaire) (Pattern 2). The total accuracy was 95.1%, with a sensitivity of 92.3% and a specificity of 96.2%, when we used all 19 items (Pattern 3). In addition to 'the frequency of being mistaken for one another (IM_{18}),' 'shape of fingers (IM_{11}),' and 'shape of eyebrow (IM_4)' were selected with a significance level of 0.10. The total accuracy was 93.3% when using twins' self-reports (Pattern 4). This accuracy was nearly the same, even if self-reports of only one member of the twin pair were analyzed (comparison between Pattern 4' and Pattern 4"). The total accuracy was not changed very much if both mothers' reports and twins' self-reports were analyzed simultaneously (Pattern 5 or Pattern

6). Sensitivity was much lower than specificity when using the items dealing with only confusion of identity (Pattern 2, Pattern 4, or Pattern 5).

The distributions of summed score for selected items according to determined zygosity are shown in Table 4. The mothers' three items and the twins' three items were the same as items we previously reported (Ooki et al., 1990, 1993). The difference of distribution of summed scores between MZ and DZ pairs was clearly seen. With the mothers' three items, if the cutoff point was set between the score 6 and 7 according to our previous study (Ooki et al., 1993), total accuracy was 89.7% (201/224), with accuracy of MZ equal to 95.6% (152/159) and that of DZ equal to 75.4% (49/65) (Figure.4). With the twins' three items, if the cutoff point was set between the score 13 and 14 according to our previous study (Ooki et al., 1990), total accuracy was 90.2% (202/224), with accuracy of MZ equal to 89.3% (142/159) and that of DZ equal to 92.3% (60/65) (Figure. 5). These results were not satisfactory. With the mothers' five items, if the cutoff point was set between the score 9 and 10, total accuracy was 94.6% (212/224), with accuracy of MZ equal to 95.0% (151/159) and that of DZ equal to 93.8% (61/65) (Figure. 6).

The results of simple summed score method using original three items of mothers' questionnaire according to dataset were shown in Table 5. The deterioration in total accuracy was observed due to the change in composition of the sample, namely MZ/DZ ratio.

Discussion

One of the main focuses of this study was to evaluate the already reported zygosity questionnaire used in Japan for childhood-age twins and answered by their mothers (Ooki et al., 1993). In Japan it is said that even now zygosity misclassification based on inappropriate placental findings is very high. Therefore it is important to offer a simple and proper method to classify zygosity of childhood-age twins. The reason maternal reports were used is that in Japan mothers in general bring up their children, including multiples. Some reports suggest the usefulness of information from fathers (Chen et al., 1999; Rietveld et al., 2000), but cultural differences should be considered. The similarity of twin pairs at about one year of age was quite informative, which was in accordance with findings of a recent study

(Price et al., 2000).

The twin subjects used in this study were all Japanese infants. As a result, some items that were useful for zygosity determination in Caucasian samples, such as eye color, hair color, and facial color (Peeters et al., 1998; Spitz et al., 1996), were not useful because there was less variation. This study suggested that similarity of 'shape of fingers' and 'shape of eyebrow' during infancy were also useful items for zygosity determination, at least in this ethnic group. Recently, we confirmed this finding using two additional groups of twins' mothers (not published). We thought that mothers regard their children as similar or dissimilar intuitively and the reasons for their judgment are highly variable.

The zygosity questionnaire was developed along two dimensions, the similarity of physical characteristics and the confusion of identity (Rietveld et al., 2000). Our original questionnaire for twins' mothers and twins themselves asked only about the latter dimension.

According to a recent study (Chen et al., 1999) or our previous study (Ooki et al., 1993), either parental reports or self-reports can classify zygosity with nearly the same accuracy in adolescent twins.

As shown in Table 3, this tendency was also found in this study, if the similar question items, namely confusion of identity, were used to classify the zygosity. As to self-reports, the accuracy did not decrease even if the reports of only one member of a twin pair were used. The reason seemed to be the high concordance rate of the answers to each question.

Regarding the result of logistic regression analysis, sensitivity was much lower than specificity when we used only items dealing with confusion of identity (Pattern 3 or Pattern 4 in Table 3), which means that the percentage of DZ pairs classified correctly as DZ was much lower than MZ pairs classified correctly as MZ. This tendency has been observed in other studies (Chen et al., 1999; Rietveld et al., 2000). The lower accuracy for DZ pairs was partly because of the small sample size of DZ pairs. Another reason for this tendency seemed to be a lack of sensitivity to detecting fraternity in the questionnaire, which asked only about general similarity. Certainly some DZ pairs are so similar in appearance that questions concerning their general similarity cannot always detect their differences.

As shown in Table 4 and Table 5, simple summed score method using 3-item measure, which we reported previously (Ooki et al.,1993), was proved to be not so effective method, even if cutoff point was changed. The misclassification of MZ and DZ, namely specificity and sensitivity, was a trade-off according to the cutoff point.

It is important to know that the accuracy was inflated by not including unclassified twin pairs in the denominator for computing the percentage (Jackson et al., 2001). And total accuracy alone cannot explain the accuracy of 'MZ classified as MZ' and 'DZ classified as DZ,' because the ratio of MZ/DZ influences the total accuracy, as shown in Table 5. The total MZ/DZ ratio of this study was 2.45 (159/65). According to Imaizumi (1997), the recent Japanese same-sex MZ/DZ ratio ranges roughly from 2.00 to 3.66. The MZ/DZ ratio of this study fell within this range.

As shown in Table 3, the total accuracy of the mothers' questionnaire was certainly rose to 95.1% by adding questions dealing with physical similarity, even though the increase in accuracy is not statistically significant. Therefore we thought that 19 items questionnaire was more useful than 3 items questionnaire in practical situation. And in the parsimonious model obtained by logistic regression analysis (Pattern 3), both sensitivity and specificity reached levels greater than 90%, meaning that misclassification ratio is nearly the same between MZ and DZ twin pairs. This is suitable for practical use.

The total accuracy was not changed very much by considering the items of self-reports dealing with confusion of identity, in addition to mothers' reports (Pattern 6). But, it is relatively easy to obtain both mothers' reports and self-reports for young twins. Therefore, it is worthwhile to collect both types of information if possible. Moreover, the self-reported questionnaire could obtain higher accuracy if it included other questions on physical similarity, which has already been gathered in a variety of items for the present young subjects.

As shown in Table 4, the results of simple summed score methods of five items on the mothers' questionnaire showed nearly the same accuracy obtained from logistic regression analysis, suggesting the effectiveness of the simple summed score method in practical use, especially when we use this questionnaire for the purpose of offering information about twins' zygosity for twins' mothers easily. As

Bønnelykke et al. (1989) said, it seems of little use to construct more complex statistical methods, as the summed point of a few simple answers by mothers was sufficient for reliable zygosity classification. Moreover, as Jackson et al. (2001) pointed out, many of the complex formulae for zygosity classification are sample dependent, and accuracy in cross-validation is not always assured. The classification method should vary according to the use to which the questionnaire will be put. The use of a simple sum of checked scores for informative items is both practical and simple.

A limitation of this study is the retrospective methodology of the mothers' reports, which may be informed by factors other than the twins' resemblance as infants. In Japan, where zygosity testing using blood samples cannot be easily or widely performed, answers to the zygosity questionnaire provide very important information.

In the genetic epidemiologic study, zygosity questionnaire could be used for the first step of zygosity diagnosis, by setting strict criteria of accuracy. Zygosity testing using genetic markers may be performed only to the subjects who were unclassified zygosity by questionnaire.

In conclusion, twin zygosity can be estimated by the use of the questionnaire rated by mothers with sufficient accuracy even in very young twins about one year of age. There is no other established zygosity questionnaire in Japan. These questionnaires would be used as a useful tool for zygosity classification of twins, if population-based twin register would be established in Japan in the future.

We hope cross-cultural adaptation and validation study (Sumathipala et al., 2000) of this simple questionnaire will be performed in other Asian counties where a large twin register is now under construction.

Summary

The present study deals with the determination of zygosity in twins of childhood age by simple questionnaire. The subjects were 224 twin pairs and their mothers, consisting of 159 monozygotic and 65 same-sex dizygotic pairs, identified by genetic markers including DNA samples. Mothers of twins responded to 19 questionnaire items dealing with twin similarity in 16 items about physical features and 3 items about the degree of similarity and frequency of being mistaken

(confusion of identity) when twins were about one year of age. The twins themselves responded to 3 questionnaire items dealing with only confusion of identity items. The results of stepwise logistic regression analysis were as follows: The total accuracy of the mothers' questionnaire was 91.5% when using only the items dealing with confusion of identity, and this accuracy was slightly lower than that obtained by twins' self-reports dealing with nearly the same question items as to confusion of identity answered by both twins separately, with 93.3% accuracy. The total accuracy of mothers' questionnaire responses rose to 95.1% when we used all 19 items. In addition to 'the frequency of being mistaken,' two physical features, namely 'shape of fingers' and 'shape of eyebrow,' were very informative. In conclusion, twin zygosity can be estimated by the use of the mothers' simple questionnaire with sufficient accuracy even in very young twins about one year of age.

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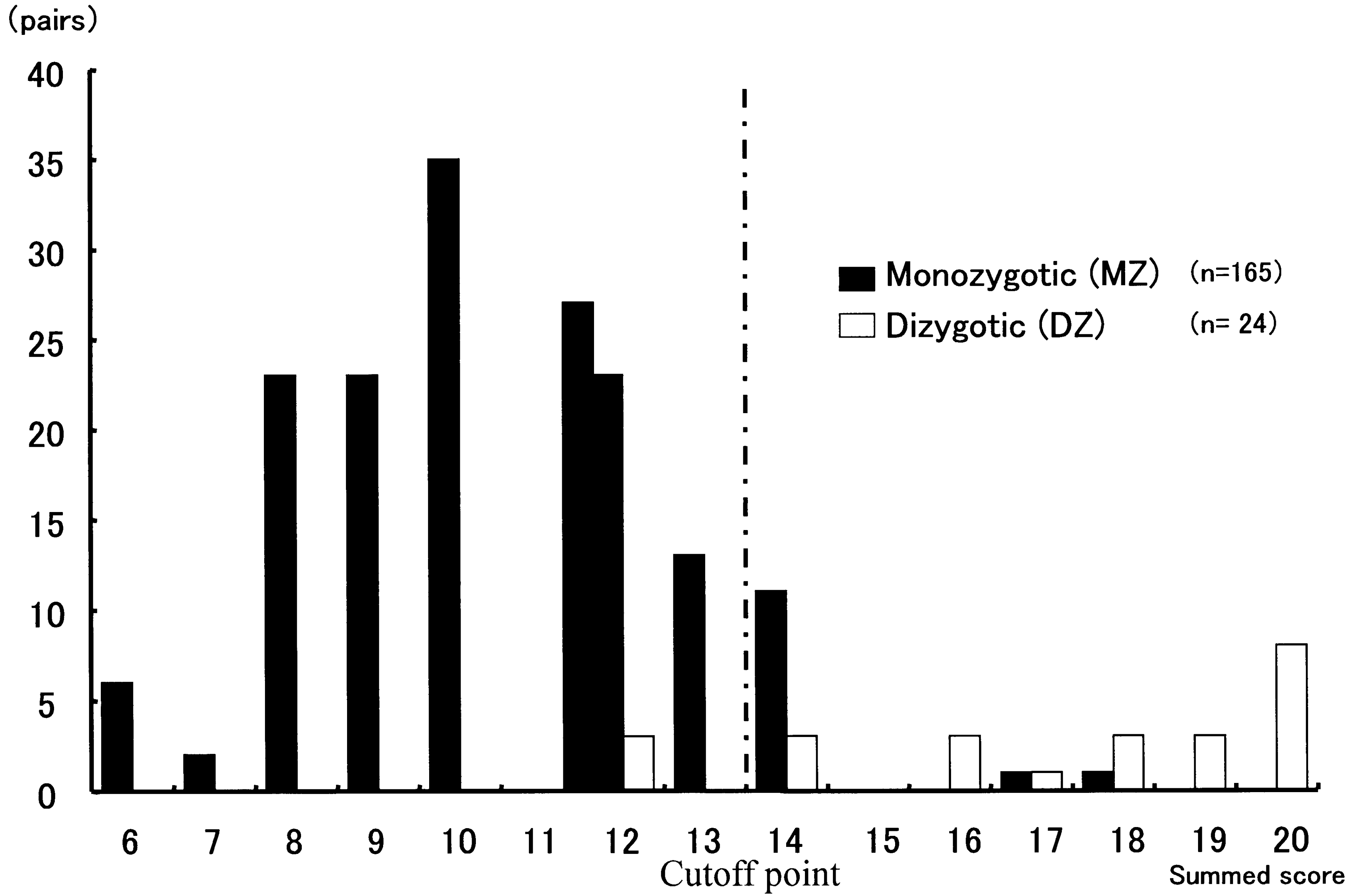

Figure.1 The distributions of simple summed scores of zygosity questionnaire for twins according to zygosity determined by genetic markers (Ooki et al, 1990)

(classified correctly as MZ or DZ;91.5%, classified correctly as MZ;92.1%, classified correctly as DZ;87.5%)

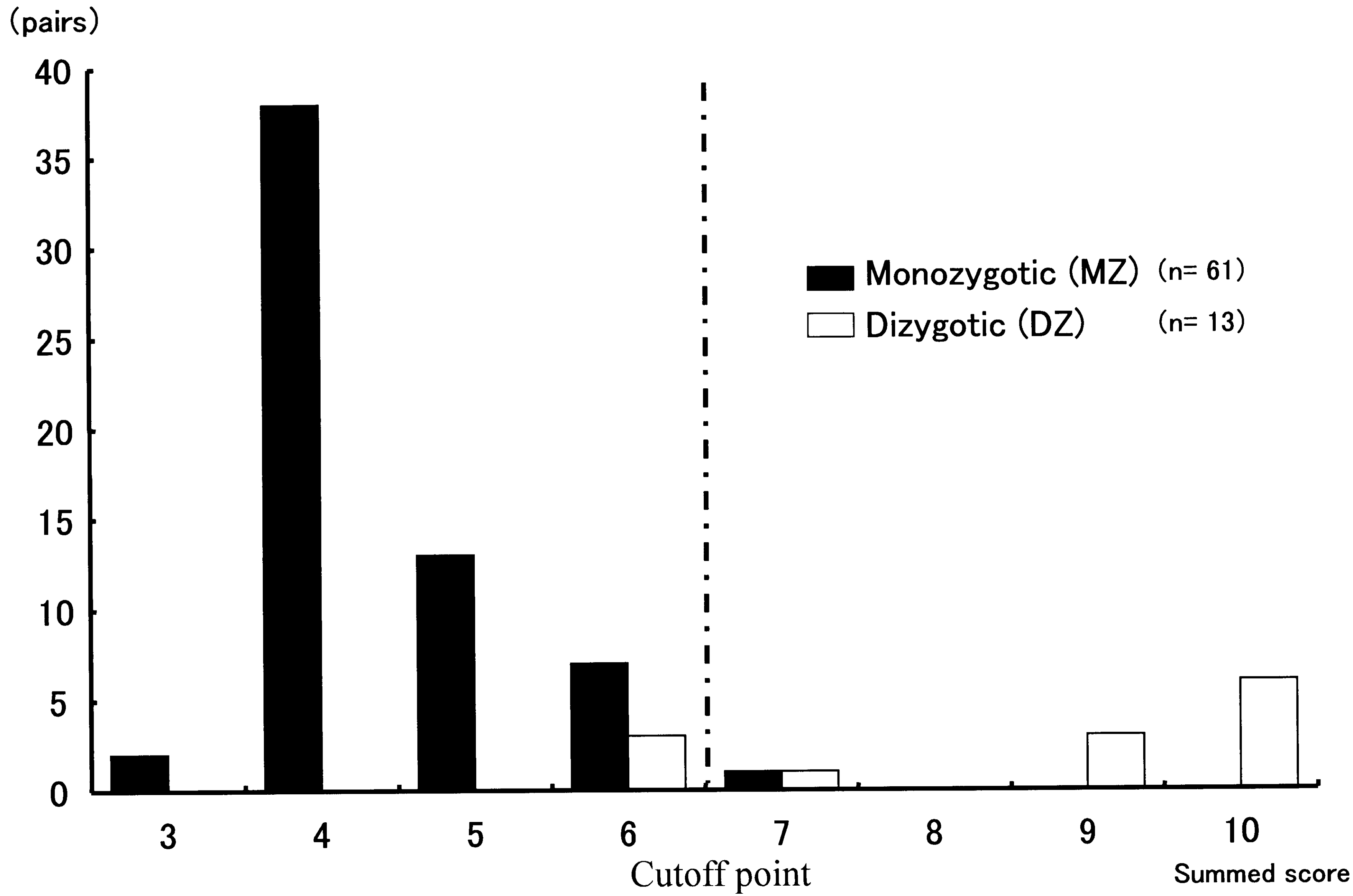

Figure.2 The distributions of simple summed scores of zygosity questionnaire for twins' mothers according to zygosity determined by genetic markers (Ooki et al, 1993)

(classified correctly as MZ or DZ;94.6%, classified correctly as MZ; 98.4%, classified correctly as DZ;76.9%)

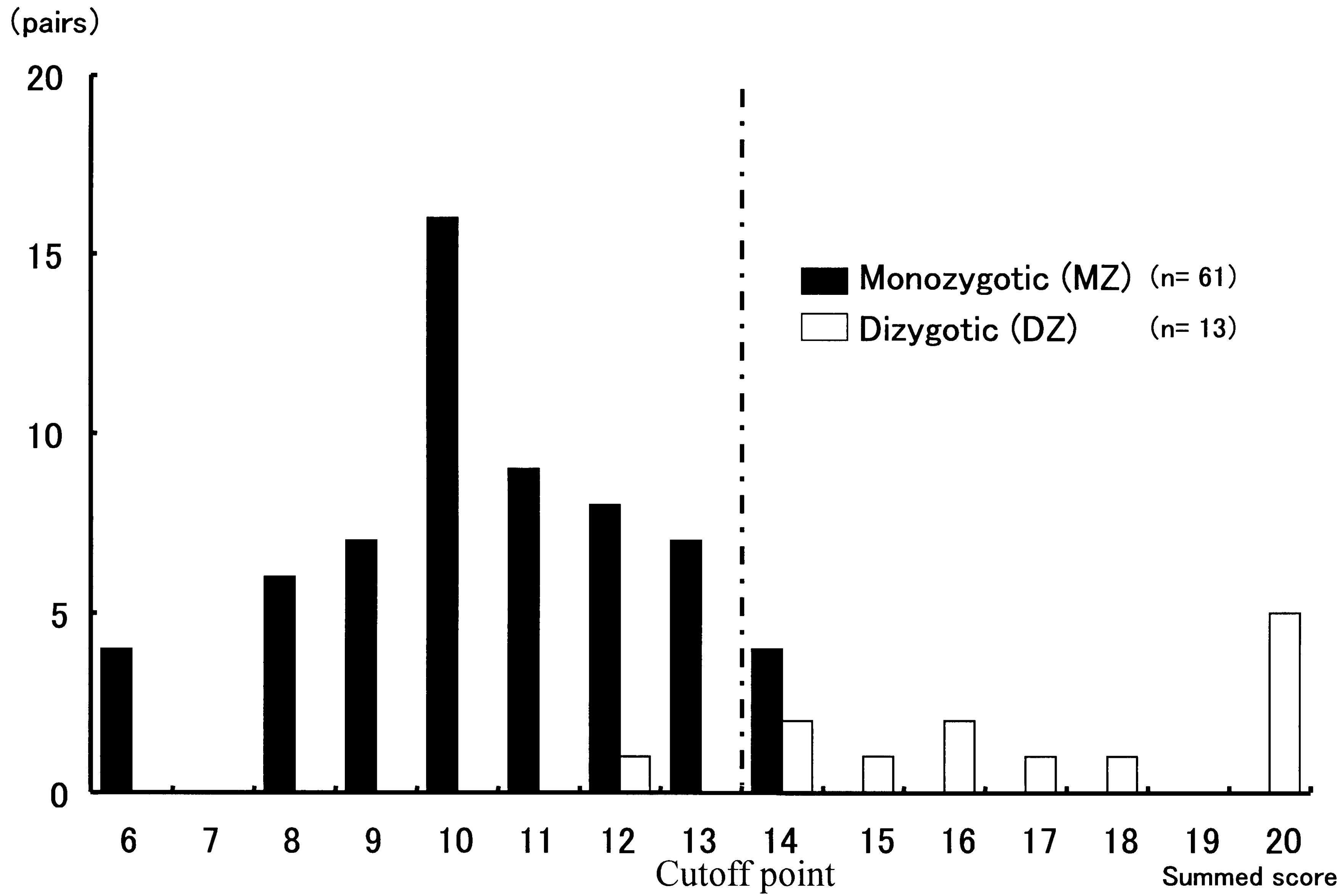

Figure.3 The distributions of simple summed scores of zygosity questionnaire for twins according to zygosity determined by genetic markers (Ooki et al, 1993)

(classified correctly as MZ or DZ;93.2%, classified correctly as MZ;93.4%, classified correctly as DZ;92.3%)

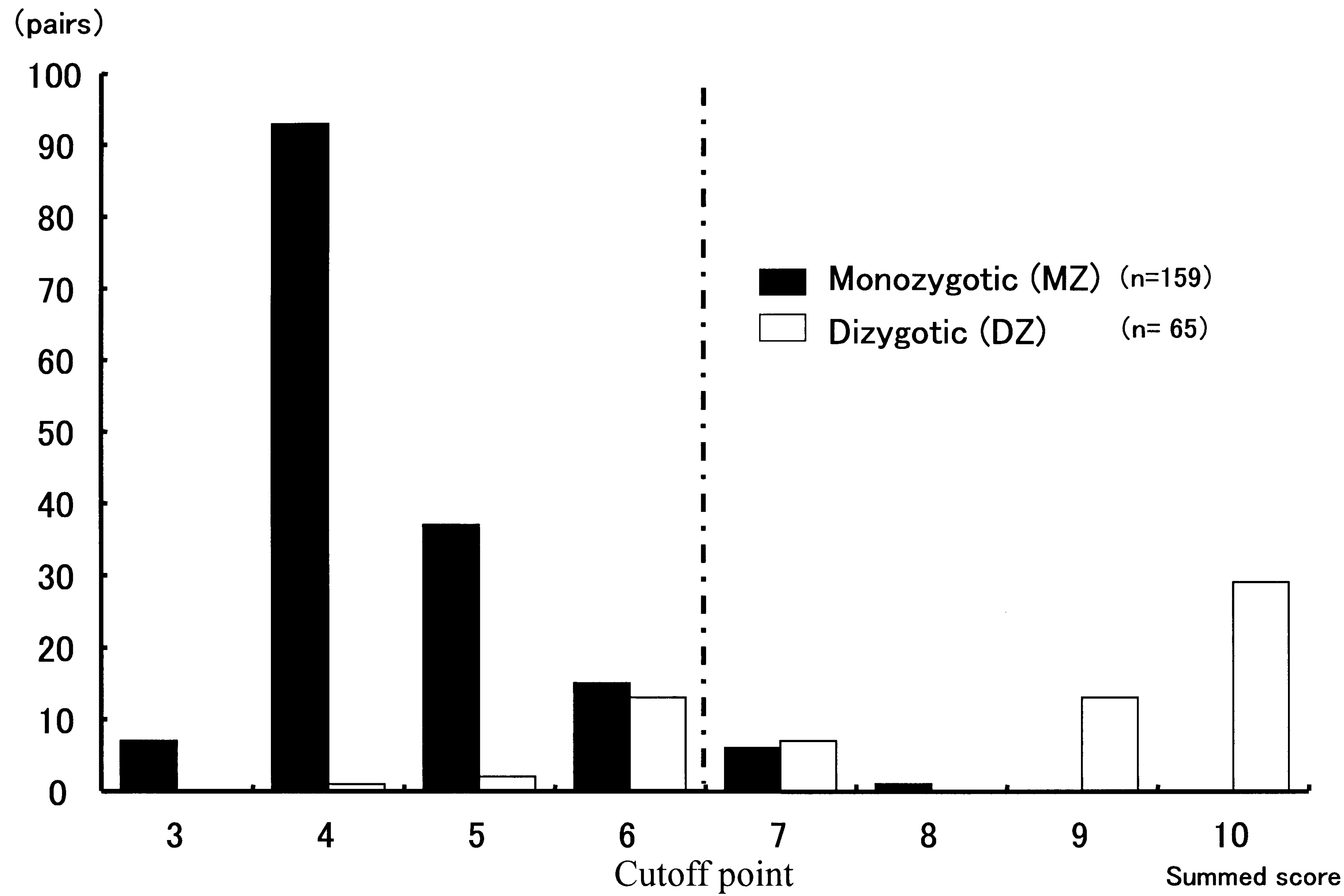

Figure.4 The distributions of simple summed scores of mothers' three questions according to zygosity determined by genetic markers

(classified correctly as MZ or DZ; 89.7%, classified correctly as MZ; 95.6%, classified correctly as DZ; 75.4%)

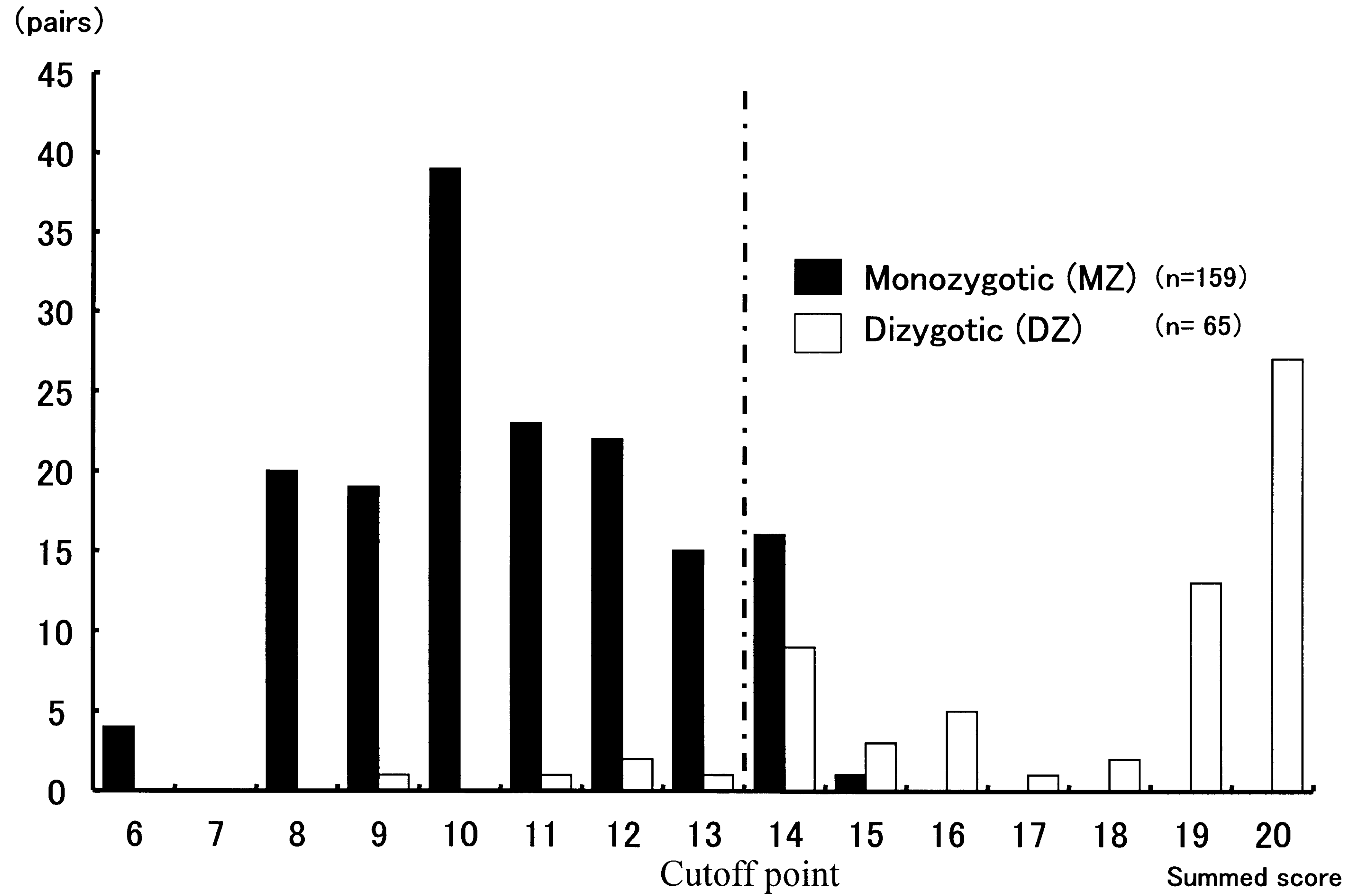

Figure.5 The distributions of simple summed scores of twins' three questions according to zygosity determined by genetic markers

(**classified correctly as MZ or DZ; 90.2%, classified correctly as MZ; 89.3%, classified correctly as DZ; 92.3%**)

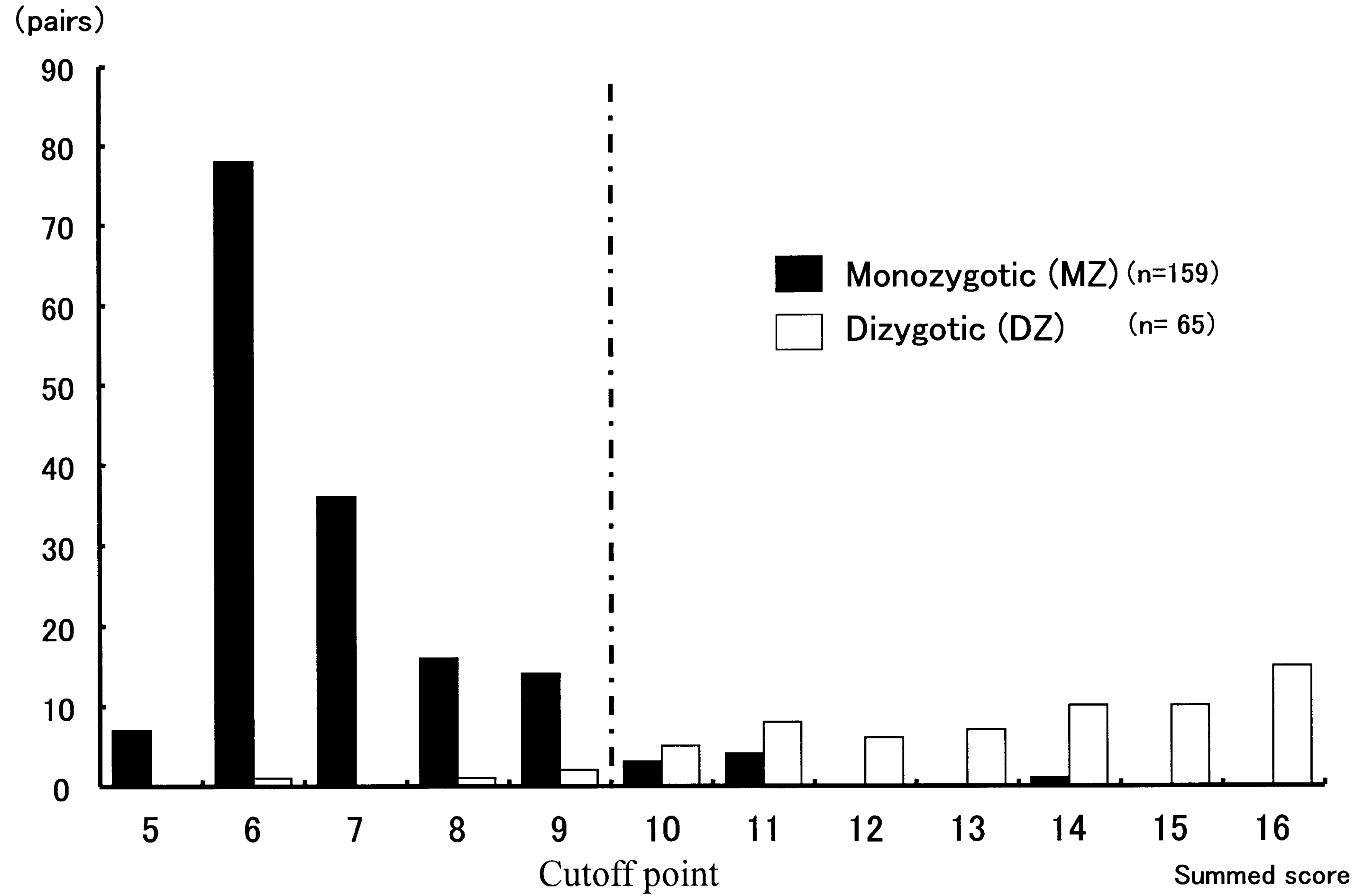

Figure.6 The distributions of simple summed scores of mothers' five questions according to zygosity determined by genetic markers

(classified correctly as MZ or DZ; 94.6%, classified correctly as MZ; 95.0%, classified correctly as DZ; 93.8%)

Table 1 Summary of studies on zygosity determination by written questionnaire

study	country	subjects (pairs)	age of twins	answerer
1 Cederlöf et al (1961)	Sweden	200	35-75years	both twins
2 Nichols et al (1966)	USA	123	school children	both twins
3 Jablon et al (1967)	USA	232	30-45years	one or both twins
4 Hauge et al (1968)	Denmark	335	adults	one or both twins
5 Schoenfeldt (1969)	USA	124	school children	both twins
6 Cohen et al (1973)	USA	120	mean 9.4years	mothers
		35	mean 4.2years	mothers
7 Cohen et al (1975)	USA	275	1-6years	mothers
8 Martin et al (1975)	Australia	47	15years	both parents
9 Kasriel et al (1976)	UK	178	adults	both twins
10 Sarna et al (1978)	Finland	104	20-69years	both twins
11 Torgersen et al (1979)	Norway	215	18-67years	both twins
12 King et al (1980)	USA	173	adults	both twins
13 Sarna et al (1980)	Finland	52	adults	both twins
		104	adults	both twins
14 Magnus et al (1983)	USA	207	33-61years	one or both twins
15 Bønnelykke et al (1989)	Denmark	125	0.5-6.5years	mothers
16 Eisen et al (1989)	USA	4774	adults	both twins
17 Ooki et al (1990)	Japan	189	12-16years	both twins
		93	52-77years	both twins
18 Ooki et al (1993)	Japan	74	school children	both twins and mothers
19 Spitz et al (1996)	France	79	8-12.5years	one parent
20 Charlemaine et al (1997)	Denmark	76	infants	one or both parents
21 Chen et al (1999)	Taiwan	105	12-16years	both twins and both parents
		47	2-12years	both parents
22 Rietveld et al (2000)	The Netherlands	618	6years	both parents
23 Sumathipala (2000)	Sri Lanka	25	infants	mothers
24 Price et al (2000)	the UK	153	about18months	one parent
25 Jackson et al (2001)	USA	110	11-25years	mothers
26 Ooki et al (present study)	Japan	224	11-12years	both twins and mothers

Table 2 The results of similarity of physical features reported by twins' mothers

	Index A	Index B	Index C
1. facial appearance	61.6%	8.5%	29.9%
2. the number of the hair whorl	59.4%	19.2%	21.4%
3. the position of the hair whorl	52.2%	17.9%	29.9%
4. shape of eyebrow	76.3%	7.6%	16.1%
5. shape of eyelid	61.2%	11.6%	27.2%
6. shape of eye	58.9%	12.5%	28.6%
7. shape of ear	62.9%	8.5%	28.6%
8. voice	52.7%	11.6%	35.7%
9. the number of the mole or spot	26.3%	26.8%	46.9%
10. the position of the mole or spot	28.1%	33.5%	38.4%
11. shape of fingers	75.4%	5.4%	19.2%
12. physique	71.9%	7.6%	20.5%
13. sleeping face	77.2%	9.4%	13.4%
14. sleeping posture	65.2%	9.8%	25.0%
15. liability to get illness	59.4%	12.9%	27.7%
16. tendency of concordance for illness	63.4%	18.3%	18.3%

Note: See text and Appendix 3 as to the explanation of each index.; Index A + Index B + Index C = 100%.; n=224 mothers of twin pairs, consisteing of 159 MZ and 65 DZ.

Table 3 The results of stepwise logistic regression analysis

	Samples and items used in analysis	Logistic model	Cutoff point	Diagnozed correctly	Diagnozed correctly as DZ	Diagnozed correctly as MZ
	Mothers' reports					
Pattern 1	IM_1-IM_{16}	$\log[p/(1-p)] = -8.5812 + 1.4715IM_4 + 1.7132IM_{11} + 1.5332IM_{13}$	0.40-0.48	91.5% (205/224)	86.2% (56/65)	93.7% (149/159)
Pattern 2	IM_{17}-IM_{19}	$\log[p/(1-p)] = -10.6472 + 2.1189IM_{17} + 3.1006IM_{18}$	0.46-0.94	91.5% (205/224)	72.3% (47/65)	99.4% (158/159)
Pattern 3	IM_1-IM_{19}	$\log[p/(1-p)] = -11.4420 + 1.0765IM_4 + 1.3922IM_{11} + 3.3479IM_{18}$	0.32-0.38	95.1% (213/224)	92.3% (60/65)	96.2% (153/159)
	Twins' self-reports					
Pattern 4	IT_1-IT_3 of twin pairs	$\log[p/(1-p)] = -15.1573 + 1.9486IT_1 + 1.3217IT_2$	0.34-0.42	93.3% (209/224)	83.1% (54/65)	97.5% (155/159)
Pattern 4'	IT_1-IT_3 of twin A	$\log[p/(1-p)] = -14.2945 + 2.8498IT_1 + 2.5700IT_2$	0.64-0.74	92.0% (206/224)	75.4% (49/65)	98.7% (157/159)
Pattern 4"	IT_1-IT_3 of twin B	$\log[p/(1-p)] = -10.9587 + 3.0191IT_1 + 1.9697IT_2$	0.66-0.72	92.0% (206/224)	73.8% (48/65)	99.4% (158/159)
	Both mothers' reports and twins' self-reports					
Pattern 5	IM_{17}-IM_{19} and IT_1-IT_3 of twin pairs	$\log[p/(1-p)] = -15.3675 + 2.1830IT_1 + 3.1656IM_{18}$	0.44-0.86	93.8% (210/224)	80.0% (52/65)	99.4% (158/159)
Pattern 6	IM_1-IM_{19} and IT_1-IT_3 of twin pairs	$\log[p/(1-p)] = -16.2354 + 1.9028IT_1 + 0.9873IM_4 + 0.8403IM_{11} + 2.4416IM_{18}$	0.32-0.38	96.0% (215/224)	96.9% (63/65)	95.6% (152/159)

Note: p=the probability of being DZ; IM_n=item n of the similarity questionnaire for mothers; IT_n=item n of the similarity questionnaire for twins. (See also Appendix 1 and 2)

Items used in pattern 2 are the same as items we previously reported (Ooki et al., 1993).

Items used in pattern 4 are the same as items we previously reported (Ooki et al., 1990).

Table 4 The distribution of summed score with cutoff point according to zygosity and selected items

summed score		Mothers' three items		Mothers' five items		Twins' three items	
		MZ (n=159)	DZ (n=65)	MZ (n=159)	DZ (n=65)	MZ (n=159)	DZ (n=65)
similar	3	7	0				
	4	93	1				
	5	37	2	7	0		
	6	15	13	78	1	4	0
	7	6	7	36	0	0	0
	8	1	0	16	1	20	0
	9	0	13	14	2	19	1
	10	0	29	3	5	39	0
	11			4	8	23	1
	12			0	6	22	2
	13			0	7	15	1
	14			1	10	16	9
	15			0	10	1	3
	16			0	15	0	5
	17					0	1
	18					0	2
	19					0	13
dissimilar	20					0	27

Note: Mothers' three items are the same as items we previously reported (Ooki et al., 1993).
Mothers' five items are the second part of questions (mothers' three items) , 'shape of fingers' and 'shape of eyebrow'.
Twins' three items are the same as items we previously reported (Ooki et al., 1990).

Table 5 The results of simple summed score method using mothers' three items according to dataset

	MZ/DZ ratio	Correct	DZ classified as DZ	MZ classified as MZ
original dataset (n=72)	5.00 (60/12)	94.4% (68/72)	75.0% (9/12)	98.3% (59/60)
subsequent dataset (n=152)	1.87 (99/53)	88.2% (134/152)	75.5% (40/53)	94.9% (94/99)

Note: If the summed score was 3-6, twin pairs were classified as MZ, and if the sum was 7-10, they were classified as DZ.(Ooki et al.,1993)

Appendix 1 Zygosity questionnaire for twins

Below you will find three questions about how alike you and your twin were in childhood. Please mark off the answers which are most fitting.

1 Were you and your twin "as alike as two peas in a pod?"
   1 As alike as two peas in a pod
   2 Usual sibling similarity
   3 Quite different

2 Were you and your twin mixed up as children?
   1 Yes, very often
   2 Now and then
   3 Never

3 In that case, by whom were you mixed up?
   1 Parents
   2 Teachers
   3 Others
   4 Nobody

Appendix 2 Zygosity questionnaire for twins' mothers

I Below you will find 16 questions on how alike your twin children were when "about one year of age". Please mark off one answer for each item

		no difference	do not know	clear difference
1	facial appearance	1	2	3
2	the number of the hair whorl	1	2	3
3	the position of the hair whorl	1	2	3
4	shape of eyebrow	1	2	3
5	shape of eyelid	1	2	3
6	shape of eye	1	2	3
7	shape of ear	1	2	3
8	voice	1	2	3
9	the number of the mole or spot	1	2	3
10	the position of the mole or spot	1	2	3
11	shape of fingers	1	2	3
12	physique	1	2	3
13	sleeping face	1	2	3
14	sleeping posture	1	2	3
15	liability to get illness	1	2	3
16	tendency of concordance for illness	1	2	3

II Below you will find three questions on how alike your twin children were when "about one year of age". Please mark off one answer for each item

17 Were your twin children "as alike as two peas in a pod?"

1 As alike as two peas in a pod
2 Usual sibling similarity
3 Quite different

18 Were they mixed up at that age?

1 Yes, very often
2 Now and then
3 Never

19 By whom were they mixed up?

1 Parents
2 Relatives or neighbors
3 Others
4 Nobody

Appendix 3 Definition of three similarity indexes

answer	mothers of MZ	mothers of DZ
no difference	a	b
do not now	c	d
clear difference	e	f

$a+b+c+d+e+f=t$.

$\text{IndexA}=(a+f)/t\times100$. $\text{IndexB}=(b+e)/t\times100$. $\text{IndexC}=(c+d)/t\times100$

$\text{IndexA}+\text{IndexB}+\text{IndexC}=100$.