

Zygoty Misclassification of Recent Young Twins by Maternal Reports in Japan

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Abstract

The problem of zygoty misclassification of twins has been paid little attention in Japan. By analyzing maternal reports collected using an internet questionnaire, the author found that around 30% of monozygoty twins were misclassified as dizygoty twins, mainly by obstetricians. On the other hand, the percentage of dizygoty twin pairs misclassified as monozygoty twins was lower. Misclassification was more frequently observed in cases of spontaneous pregnancy compared to iatrogenic pregnancy. The percentage of monozygoty twins misclassified as dizygoty is in very good accordance with that of monozygoty twins having two placentas. The reported number of placentas was very strongly associated with the reported zygoty. Thus, many monozygoty twins in Japan were still misclassified as dizygoty by obstetricians, presumably based solely on the placental findings.

Keywords: Zygoty; Monozygoty twins; Dizygoty twins; Placenta; Questionnaire

Abbreviations MZ: Monozygoty; DZ: Dizygoty

Introduction

As is well known, there are two types of twins with different origins. Monozygoty (MZ) twins derive from the division of a single zygote, whereas dizygoty (DZ) twins derive from the independent release and subsequent fertilization of two ova [1,2]. Zygoty classification is the process of classifying whether same-sex twin pairs are MZ or DZ, since opposite-sex pairs are essentially all DZ [3].

In 1996, the Council of Multiple Birth Organizations adopted the Declaration of Rights and Statement of Needs of Twins and Higher Order Multiples. The declaration includes the statement that parents have a right to expect accurate recording of placentation and diagnosis of the zygoty of same-sex multiples at birth [4]. The three important reasons for determining zygoty at birth [5,6] are (1) medical [7], (2) scientific and (3) personal.

It has been recognized that zygoty misclassification occurs more frequently than medical professionals believe. In particular, many MZ twins are misclassified as being DZ because they have two placentas [8-16]. In Japan, this problem has been pointed out previously [10]. Zygoty is most accurately determined by DNA testing. Also, the usefulness of administering zygoty questionnaires to twins or twins' mothers has a long history and is well established in the field of twin studies, although the accuracy is lower compared to that of DNA testing [3,17].

The focus of the present study is to estimate the current situation of zygoty misclassification in Japan using maternal reports.

Subjects and Methods

Subjects

The subjects of the present study were all mothers of same-sex twin pairs who responded to an internet questionnaire concerning twins' zygoty. This internet service was originally offered by the present author to the three independent nationwide organizations that provide support for families with children of multiple births.

The internet questionnaire consisted of three parts. The first part requested basic information on the twins, i.e. the birth year and month, sex combination (male-male or female-female), gestational weeks, whether the pregnancy was conceived via spontaneous conception or fertility treatment, the reported number of placentas (one, two, or 'never told') and so on. The second part contained 11 questions that assist in zygoty classification (the so-called zygoty questionnaire) developed by us [3,18], which is commonly used in Japan. Zygoty was determined based on the single summed raw score, which ranged from 6 to 19, for six out of 11 question items (1-3 points for five question items and 1-4 points for one question item) in accordance with the given cutoff point. Strict criteria of zygoty classification were adopted to reduce the risk of misclassification as much as possible. A summed score of 6-10 was regarded as MZ, a score of 13-19 as DZ and a score of 11 or 12 as unclassified. If zygoty determination by DNA markers is regarded as the gold standard, the accuracy of the zygoty questionnaire was 98%, though about 10% of pairs were not classified by the questionnaire [3]. The third part of the questionnaire included parental beliefs about zygoty, namely what the reported zygoty was and where and by whom zygoty was reported.

All data were checked carefully to ensure there was no double-counting of subjects. If all basic information for a pair of twins was the same, they were regarded as the same twin pair and only one twin pair with lower summed raw score of six question items were analyzed. The maternal questionnaire is used mainly to obtain information from the mothers of young twins. In addition, the peak twinning rate in Japan occurred in 2005 [19]. Thus, the subjects analyzed in the present study

were limited to those twin pairs whose birth year was 2004 or later and who were under 12 years of age (i.e., 11 years old or younger) to analyze the recent data on zygosity misclassification. In total, the subjects included 408 mothers.

Method

The reported zygosity was classified as follows: 'told MZ', 'told DZ', 'told both MZ and DZ', told 'do not know' and 'never told.' The answer 'told MZ, told DZ, told both MZ and DZ' and told 'do not know' were regarded as talking about information on zygosity. The percentage of cases in which the parents were told the zygosity was calculated, accompanied by where and by whom it was reported.

The zygosity as classified by the questionnaire and that reported were compared and the percentage of zygosity misclassification was calculated by two methods. In the first estimation, being told both MZ and DZ was treated as an incorrect response and excluded from the number of misclassifications. The percentage of zygosity misclassification was calculated as the number of cases in which the parents were told DZ but the twins were classified as MZ divided by the total number of classified MZ pairs. Regarding misclassified DZ pairs, the inverse procedure was followed. Cases claiming that they were told both MZ and DZ were considered in the second estimation. The percentage of zygosity misclassification was calculated as the sum of the number told DZ plus half the number told both MZ and DZ and classified as MZ divided by the total number of pairs classified as MZ. Regarding the misclassified DZ pairs, the inverse procedure was used. The percentage of zygosity misclassification was also determined according to the method of conception (spontaneous vs. iatrogenic).

The relationship between the zygosity told to the parents and the number of placentas was analyzed using Fisher's exact test with a significance level of 0.05.

Ethical Consideration

Mothers of twins answered the questionnaire of their own will. Statistical analyses of the collected data are mentioned in the instruction for filling out the questionnaire.

Results

The basic data of the subjects are shown in Table 1. About 80% of subjects were mothers of twins aged 0-2 years. The percentage of female-female pairs was slightly higher than that of male-male pairs.

	N	%
Age of twins		
0 years of age	40	9.8
1 years of age	189	46.3
2 years of age	87	21.3
3 years of age	45	11
4-9 years of age	47	11.5
Sex combination		
Male-Male	192	47.1

	Female-Female	215	52.7
	Unknown	1	0.2
Method of pregnancy			
	Spontaneous pregnancy	347	85
	Fertility treatment	58	14.2
	No answer	3	0.7
Number of placentas			
	One	172	42.2
	Two	148	36.3
	Unknown	88	21.6

Table 1: Basic information on the subjects (n=408).

The percentage of mothers who were told that their twins' zygosity was MZ and/or DZ was calculated, along with information on where and by whom it was reported. The result is shown in Table 2. Ninety-one percent of mothers were told about their twins' zygosity, including being told 'do not know,' and 77.9% of mothers were told that their children's zygosity was MZ and/or DZ mainly by obstetricians.

	N (%)	
Told MZ and/or DZ	318 (77.9%)	
At obstetrics by obstetrician ^a		307
At obstetrics by midwife or nurse ^a		12
At pediatrics by pediatrician or nurse ^a		11
Others		13
Told 'do not know'	53 (13.0%)	
Never told	37 (9.1%)	

Table 2: Answer to the questions regarding told zygosity (if told any zygosity, where and by whom; Note: a: multiple answers permitted, n=408).

The relationship between the zygosity first reported and the classified zygosity was summarized according to the method of conception. The results are shown in Table 3. The percentage of MZ pairs who were classified as DZ was 26.5% (83/313) or 31.2% (97.5/313), whereas the percentage of DZ pairs who were classified as MZ was 8.7% (4/46) or 10.9% (5/46). The percentage of zygosity misclassifications was higher in the spontaneous pregnancy group compared to the fertility treatment group, irrespective of misclassified zygosity, i.e. being told DZ and classified as MZ or vice-versa. Regarding 83 mothers who were told DZ incorrectly, 79 (95.2%) were told by an obstetrician and three were told by a midwife (multiple answers permitted). No mother was told by a pediatrician. All of the four mothers who were incorrectly told as MZ were told by obstetricians.

Method of pregnancy		Told MZ	Told DZ	Told both MZ and DZ	Told 'do not know'	Never told	Total	Percentage of misclassification		MZ/DZ ratio
								1st estimation ^a	2nd estimation ^b	
Spontaneous	Classified as MZ	115	77	28	34	24	278	27.7%	32.7%	8.7
	Classified as DZ	4	19	2	5	2	32	12.5%	15.6%	
Iatrogenic	Classified as MZ	21	5	1	4	2	33	15.2%	16.7%	2.5
	Classified as DZ	0	10	0	0	3	13	0%	0%	
Total	Classified as MZ	137	83	29	38	26	313	26.5%	31.2%	6.8
	Classified as DZ	4	30	2	5	5	46	8.7%	10.9%	

Table 3: Zygosity classified by questionnaire and told zygosity according to the method of pregnancy (Note: MZ: monozygotic, DZ: dizygotic; Only those subjects who were told their children's zygosity and for whom zygosity determination was completed were analysed; a: The number of cases told they were DZ but classified as MZ (or the number told they were MZ but classified as DZ) was divided by the total number classified as MZ (or DZ); b: The sum of the number of cases told they were DZ and half the number told both MZ and DZ and classified as MZ (or the sum of the number of told MZ and half the number told both MZ and DZ and classified as DZ) was divided by the total number classified as MZ (or DZ)).

Told zygosity and the number of placenta showed a very strong association, with a significance level of $p < .0001$, as is shown in Table 4.

	Reported number of placentas	
	One	Two
Told MZ	135	2
Told DZ	6	88

Table 4: Relationship between told zygosity and number of placentas (Fisher's exact test $p < .0001$).

The mothers who used this internet zygosity questionnaire were asked why they chose to use it on the recently revised questionnaire ($n=74$). The main reason for using this zygosity questionnaire was the following; I would like to know as much about my children as possible ($n=54$), the internet questionnaire is easy to use ($n=46$) and they were often asked about their twins' zygosity by others ($n=36$) (multiple answers permitted).

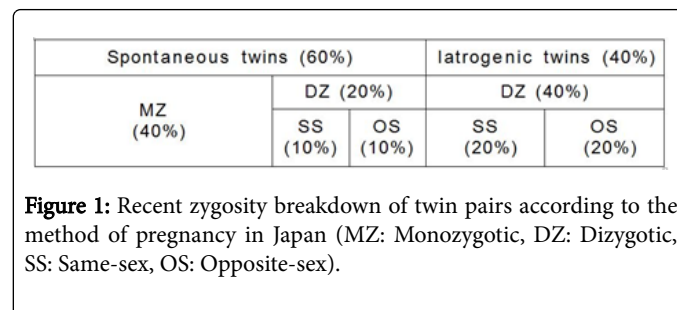
Discussion

The characteristics of the subjects

The recent zygosity breakdown of twin pairs according to the method of pregnancy in Japan is shown in Figure 1. About 60% of twin pregnancies are estimated to be spontaneous, while 40% are considered to be iatrogenic [19]. The proportion of spontaneous MZ twin pairs among all spontaneous twin pairs is about 2/3 [20]. Although about 1-2% of iatrogenic twins are estimated to be MZ pairs in Japan [21], most iatrogenic twins are DZ pairs. Among all twin pairs, including both spontaneous and iatrogenic pairs, MZ pairs make up 40% and same-sex DZ make up 30% as shown in Figure 1 and thus the MZ/same-sex DZ ratio is about 1.3 (40%/30%). The estimated MZ/same-sex DZ ratio of this study's subjects was 6.8, as shown in Table 3, meaning that there was an extreme tilt toward MZ pairs. This extreme imbalance toward parents of MZ pairs who are interested in knowing

their twins' zygosity is also observed by the study of Bamforth et al. [22].

The percentage of all same-sex twin pairs that result from fertility treatment is expected to be about 30% (20%/70%), as calculated using Figure 1. The percentage of iatrogenic pregnancies in this survey was 14.2% and this figure is about half the percentage that is to be expected.



Thus, among the subjects of the present study, mothers of spontaneous MZ pairs make up a high percentage, suggesting that they are interested in zygosity classification for a variety of reasons. Mothers who have taken fertility treatment might not be so interested in the zygosity diagnosis itself, since they believe their twins are DZ.

Told zygosity and classified zygosity

The results of the present study were nearly the same as those obtained in a previous study conducted in Japan [10]. The percentage of MZ pairs who were incorrectly believed to be DZ was 26.5% or 31.2% in this study and 25.6% in the previous study [10]. The percentage of DZ pairs who were incorrectly reported as MZ was 8.7% or 10.9% in this study and 10.8% in the previous study [10]. Since the birth years of the twins in the previous study were about 20 years earlier, the present results strongly suggested that the situation of zygosity misclassification in Japan has not improved over this period.

In general, the percentage of zygosity misclassification was not symmetric. More MZ twins were considered to be DZ than vice-versa [9]. This tendency was also supported by other recent studies [12,16]. The percentage of misclassification of MZ as DZ was around 30% throughout all of these studies. According to Jaarsveld et al. [12], the percentage of MZ pairs who were initially considered to be DZ was 27.5% (i.e. 179 out of 651), whereas the percentage of DZ pairs who were initially considered to be MZ was only 1.9% (i.e. 12 out of 621). According to the Herle et al. [16], the percentage of MZ pairs who were first reported to be DZ was 30.1% (220/731) at 8 months and 26.3% (119/453) at 29 months, much larger compared with the percentages of DZ pairs who were initially reported to be MZ, i.e. 0.8% (6/797) at 8 months and 0.4% (2/445) at 29 months.

The percentage of MZ pairs who were initially considered DZ was in very good accordance with the percentage of MZ twins having dizygous placentas. According to Machin [8], the percentage of MZ twins having a dizygous placenta is estimated to range from one-fourth to one-third (25-33%) of the total. Moreover, the zygosity that was initially reported was very strongly associated with the number of placentas, as shown in Table 4. Almost all mothers of MZ pairs who were told that they were DZ were told by an obstetrician. These results strongly suggest that even now, zygosity misclassification of MZ twins as being DZ occurs based on the easy placental findings of the obstetrician, not based on the communications of other health specialists such as midwives, in Japan.

The percentage of DZ pairs incorrectly told that they were MZ was about 10% in Japan. This figure is higher than that of other reports in Western countries [12,16]. Since the number of incorrect cases was only four out of 46 in the present study, no conclusive reason for these incorrect determinations could be found. In general, the misclassification of DZ as MZ occurred for several reasons, with fused placentation, in which two placentas seem to be one placenta at a glance, being one of them [15].

One novel finding of this study was that zygosity misclassification clearly occurred with a low frequency in iatrogenic pregnancies. This can be explained by the fact that most, but not all, twins born after single embryo transfer is MZ and most born after double or more embryo transfer are DZ. Thus, obstetricians could anticipate twins' zygosity irrespective of their confusion regarding chorionicity and zygosity. As far as the author knows, there are no studies of zygosity misclassification which consider the method of pregnancy.

The importance of proper knowledge about zygosity for medical professionals

The knowledge about zygosity possessed by twins' families and medical professionals is not always correct [12,14,22]. According to Cleary-Goldman et al. [23], the physicians of the American College of Obstetricians and Gynaecologists showed less knowledge regarding chorionicity and zygosity than anticipated. The knowledge gaps of obstetricians and gynecologists in twin prenatal development are suggested to be the cause of the misinformation [16]. Although there exists no such survey for obstetricians in Japan, the situation seems to not be very different from that in other Western countries according to the years of author's own experience as a supporter of families with multiples.

Some researchers, physicians and multiple births organization recommend that zygosity testing should be encouraged for all same-sex twins [5,6,13,24,25]. Apart from the pros and cons of standard

zygosity testing for same-sex twins at birth or earlier age, correct information should be offered by all medical/health professionals to the parents of twins and at the very least, incorrect information should not be offered.

Aside from its medical importance [5-7], zygosity knowledge can also invoke positive emotional responses in the parents [13,14]. For some twin pairs, to know that they are MZ have special meaning [11]. The main reason of mothers to answer present questionnaire was "to know as much about my children as possible", supporting the above point of view, although the questionnaire responders might have been biased toward those mothers who are very interested in their twins' zygosity. For all these reasons, possessing information on chorionicity and zygosity might be regarded as a birthright [4,6].

About 80% of mothers were told their children's zygosity at obstetrics. Medical professionals should be aware that zygosity classification has a great deal of meaning for some parents or twins themselves. Medical specialists who have much more knowledge than the mothers should not talk lightly about zygosity, the placenta and related information to mothers [10]. Yet, some medical/health professionals do not appear to recognize the importance of zygosity determination to the parents of twins [24].

Limitations

The results of the present study naturally reflect a highly self-selected population of mothers of twins, excluding those mothers who feel certain of their twins' zygosity or for whom zygosity is not an important issue.

The accuracy of zygosity determination using the questionnaire is not 100%; therefore zygosity misclassification by the zygosity questionnaire itself can occur. To decrease this possibility, strict criteria for zygosity determination were adopted in the present study, yielding about 10% unclassified pairs.

As the present data was obtained only from the twins' mothers and not from obstetricians, another possibility is that the mothers were mistaken regarding what they were told about the number of placentas or chorionicity for zygosity. This possibility cannot be denied.

Conclusion

The percentage of recent cases with zygosity misclassification of twins was not decreased compared to that of about 20 years ago in Japan. Around 30% of MZ pairs were still initially considered to be DZ, presumably based on the placental findings made by obstetricians. This tendency is more obvious in spontaneous pregnancies compared to iatrogenic pregnancies. Better education about the difference between chorionicity and zygosity for all health/medical professionals whose work is related to multiple births is strongly recommended. One possible way to achieve this is through support groups for families with multiples.

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