

Age- and Sex-differences in the Validity of Questionnaire-based Zygosity in Twins

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Questionnaire-based zygosity assessment in twins has generally been found to be valid. In this report we evaluate sex- and age-differences in the validity of such questionnaire-based classification when using the four questions that have been the basis of zygosity assessment in The Danish Twin Registry for half a century. Three hundred and forty-two male and 531 female twin pairs were zygosity diagnosed using genetic markers and the results compared with the original questionnaire based classification. We found significant differences in the accuracy of questionnaire based zygosity diagnosis when stratifying the data for sex as well as age: males and monozygotic having the highest misclassification. However, even in the group with the highest misclassification rate the frequency was less than 8%. The overall misclassification rate was only 4%, with a clear tendency towards a higher proportion of misclassified monozygotic than dizygotic twins. The results demonstrate that questionnaire based zygosity diagnosis can still be regarded as a valid and valuable classification method for most purposes.

Twin studies provide a powerful tool for assessing the relative contribution of genetic and environmental factors for diseases and other phenotypes. Obviously, successful use of twins in this kind of studies requires an accurate classification of the twins as either monozygotic (MZ) or dizygotic (DZ). For reasonably small twin studies the reliable biological assessment of zygosity using genetic markers will often be the method of choice. However, for practical and economical reasons questionnaire-based zygosity assessment is more feasible, when dealing with large sample populations, for example when zygosity diagnosis of entire twin registries are required.

In 1954 the Danish Twin Registry was initiated as the first nation-wide twin register in the world. Since then, succeeding birth cohorts of twins have been added to the register, which now comprises more than 65,000 twin pairs born from 1870 to 1996 (Skytthe et al., 2002). The zygosity classification of all same-sexed twins in the Danish Twin Registry has been based on answers to four questions on the degree of similarity between twin partners (Table 1). This diagnosis of zygosity based on similarity in appearance is generally regarded as highly reliable, with a frequency of misclassification below 5% when comparing with blood group determinants (Hauge, 1981).

In this paper we re-evaluate the validity of the questionnaire based zygosity classification of 873 Danish twin pairs

aged 17–96 years by comparison with the results obtained using genetic marker analysis, which is considered to be the current “golden standard”.

Methods and Materials

A total of 873 twin pairs (342 male pairs and 531 female pairs) from the Danish Twin Registry have previously been selected for participation in three recent projects, in which the zygosity was accurately determined using genetic markers. This large sample of twins constitutes a subsample of the entire Danish Twin Register, with 256 pairs being selected from the birth cohorts 1900–1930, 143 pairs selected from the 1931–1952 birth cohorts, and 474 twin pairs selected from the 1953–1982 birth cohorts. In 199 of the twin pairs zygosity was classified using five highly polymorphic microsatellite markers, where identity in all markers had greater than .998 probability of monozygosity, as described previously (Christensen et al., 2000). 617 twin pairs were classified with 9 polymorphic microsatellite or RFLP markers using Applied Biosystems AmpFISTR Profiler Plus Amp Kit (Schousboe et al., 2003). Finally, 57 pairs were classified with 15 different microsatellite markers using Applied Biosystems AmpFLSTR Identifier PCR Amplification Kit (unpublished results). Using these two marker sets the probability of monozygosity determined by identity in all markers is estimated to be at least .9998 and .9999, respectively.

The original questionnaire based zygosity diagnosis was performed using the four questions in Table 1. Although minor variations in classification of unusual answer patterns may have occurred during the half century of existence of the Danish Twin Register, generally the twin pairs were classified as MZ if both of them described themselves as strikingly similar and/or stated that people who knew them well had difficulties distinguishing them from each other. Furthermore, a positive answer on the question of same hair- and eye-color in childhood was a requirement for an MZ diagnosis. Twin pairs were classified as DZ if both twins described themselves as no more alike than

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Table 1
The Four Questions Used for Zygosity Assessment in the Danish Twin Registry

1. Do you and your twin look ... — like two ordinary siblings?
— like two peas in a pod?*
- not very much alike?
2. In school, is/was it difficult for your teachers and friends to tell you apart?
3. Is/was it difficult for your family and friends to tell you apart?
4. In childhood, did you and your twin have both the same eye color and the same hair color?

Note: * The equivalent Danish phrase is “like two drops of water”.

ordinary sibs, and had never experienced that people had difficulties telling them apart. The zygosity was classified as unknown (UZ) in cases of disagreement between the twin partners and if twins thought of themselves as strikingly similar but had never been mistaken for each other. Only complete twin pairs were used in this study, that is only pairs where answers from both twins were available.

The questionnaire-based zygosity diagnosis was compared with zygosity determined by genetic marker analysis in total as well as when stratified for sex and age, and differences were evaluated using the Chi-square test.

Results

For the combined data (Table 2) the overall frequency of misclassification was 4%, whereas the frequency of unclassifiable (UZ) twin pairs using questionnaires was less than 1% (5 DZ and 1 MZ pairs). There were significantly more of the MZ twin pairs who had been misclassified as DZ twins (6%) than DZ twins misclassified as MZ twins (3%), when questionnaire based zygosity classification is judged against DNA analysis ($p = .02$).

The overall accuracy of questionnaire based zygosity determination did not differ between men and women (Table 3). However, whereas an equal percentage of female twins were correctly diagnosed as either MZ (10 misclassified out of 239) or DZ (11 misclassified out of 286), the

misclassified male pairs were almost exclusively MZ pairs diagnosed as DZ using questionnaires (14 out of 169), as only one out of 173 DZ male pairs by DNA analysis were classified wrongly as MZ using questionnaires. The difference in the distribution of misclassified MZ and DZ pairs in men and women was statistically significant ($p = .009$).

When grouping the data according to birth cohorts (Table 4) an age-associated difference in zygosity assessment was found, as the total accuracy of zygosity diagnosis in the birth cohorts 1931–1952 (98%) and 1953–1982 (97%) were both significantly higher than for twins in the birth cohorts 1900–1930 (93%) ($p < .04$).

Discussion

The validity of questionnaire based zygosity determination using the answers of either the twins themselves or their parents compared to biological assessment have been investigated in several studies, with a reported accuracy of zygosity diagnosis ranging between 91% and 98% (e.g., Bonnelykke et al., 1989; Charlemaine et al., 1997; Chen et al., 1999; Jackson et al., 2001; Ooki et al., 1990; Peeters et al., 1998; Spitz et al., 1996; Torgersen et al., 1979). The evaluation of the questionnaire based zygosity classification of a large subsample of twins from the Danish Twin Register presented here, demonstrated that the overall accuracy of zygosity

Table 2
Comparison of Zygosity Determination Using Questionnaires and DNA Marker Analysis Among 867 Twin Pairs from the Danish Twin Registry

Zygosity from DNA	Zygosity from questionnaires		Total
	MZ	DZ	
MZ	384 (94%)	24 (6%)	408
DZ	12 (3%)	447 (97%)	459

Note: MZ = monozygotic, DZ = dizygotic. The 6 pairs with unknown zygosity are not included.

Table 3
Accuracy of Zygosity Diagnosis by Means of Questionnaires for Men and Women

	MZ accuracy	DZ accuracy	Total accuracy
Men ($N = 342$)	92% (155/169)	99% (172/173)	96% (327/342)
Women ($N = 525$)	96% (229/239)	96% (275/286)	96% (504/525)

Note: MZ accuracy = percentage of MZ pairs by DNA analysis that are accurately diagnosed using questionnaires. DZ accuracy = percentage of DZ pairs by DNA analysis that are accurately diagnosed using questionnaires. The 6 pairs with unknown zygosity are not included.

Table 4

Accuracy of Zygosity Diagnosis by Means of Questionnaires in Different Age Groups

Birth cohorts	MZ accuracy	DZ accuracy	Total accuracy
1900–1931 (<i>N</i> = 250)	94% (94/100)	93% (139/150)	93% (233/250)
1932–1952 (<i>N</i> = 143)	97% (72/74)	99% (68/69)	98% (140/143)
1953–1982 (<i>N</i> = 474)	93% (218/234)	100% (240/240)	97% (458/474)
All cohorts (<i>N</i> = 867)	94% (384/408)	97% (447/459)	96% (831/867)

Note: MZ accuracy = percentage of MZ pairs by DNA analysis that are accurately diagnosed using questionnaires. DZ accuracy = percentage of DZ pairs by DNA analysis that are accurately diagnosed using questionnaires. Pairs with unknown zygosity are not included.

diagnosis using the questionnaires applied in the Danish Twin Register is among the highest obtained.

Furthermore, we found that the overall percentage of misclassified MZ twins is twice as high as the percentage of misclassified DZ twins. This indicates that the twins tend to focus on small differences in appearance and implies a tendency towards underestimation of the heritability, when questionnaire based zygosity diagnosis is used in classical twin studies.

Comparing the accuracy of zygosity diagnosis in men and women reveals that this tendency is mainly owing to more MZ male twin pairs being misdiagnosed as DZ twins than the opposite, perhaps suggesting that men have a more profound need to be seen as separate individuals than women. There was no difference in the misclassification rate of MZ and DZ female twin pairs.

Comparing the accuracy of zygosity diagnosis according to age indicated that the zygosity diagnosis based on similarity in appearance was superior in the younger birth cohorts. The ascertainment of the earliest of the birth cohorts 1900–1930 was initiated in the 1950s and 1960s but was not completed until the 1990s. This means that a large proportion of the participating twins from these cohorts were about 60 years of age or older at the time of inclusion. Contrary to this, the twins from the birth cohorts 1931–1952 and 1953–1982 were ascertained in the 1990s, making the participants between 17 and 65 years. The difference in misclassification rate between the oldest and the younger birth cohorts may thus reflect the fact that environmentally induced differences in appearance become more pronounced with age, making it more difficult to decide on “original” resemblance. In addition, as a consequence of the advanced age of most of the participating twins in the oldest cohorts, the higher misclassification rate may relate to a simple lack in recalling for instance if they had the same hair and eye color in childhood. In support of these arguments, all the unclassified twins included in this study were from the 1900–1931 birth cohorts. However, as the study in which 617 of the included twin pairs were zygosity diagnosed by DNA marker analysis, used a sampling strategy excluding unclassified twin pairs, the frequency of unclassified cases should be interpreted with caution.

The primary objective of this investigation was to re-evaluate the validity of the zygosity classification relying on the essentially comparable questionnaires conventionally used, and the confirmation of a high reliability of zygosity diagnosis thus justifies the use of these traditional questionnaires.

However, recently Heath et al. (2003) introduced a latent class analysis providing an even more valid zygosity classification as well as a mean to quantify the certainty of the classification. Implementation of this method in future twin zygosity assessments may prove beneficial, especially as it can be used to identify problems with DNA based zygosity diagnosis in some cases. Unfortunately, as The Danish Twin Registry has not computerized the single items of the zygosity questionnaire for all cohorts, we can not do this kind of analysis.

In summary, we have evaluated zygosity diagnosis based on questionnaires in a large sample of same-sexed twins from the Danish Twin Register. We find significant differences in the accuracy of zygosity diagnosis when comparing men and women as well as different birth cohorts. However, the results demonstrate that the overall reliability of zygosity determination using only four simple questions on similarity in appearance is very high, with a total misclassification frequency of only 4%, and even in the group with the highest proportion of misclassification (MZ males) the frequency was no greater than 8%. The presented results thus signifies that questionnaire based zygosity assessment still constitutes a valid classification method for most purposes.

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