



Infant zygosity can be assigned by parental report questionnaire data

Thomas S Price¹, Bernard Freeman¹, Ian Craig¹, Stephen A Petrill², Lorna Ebersole¹ and Robert Plomin¹

¹Social Genetic and Developmental Psychiatry Research Centre, Institute of Psychiatry, London, UK

²Wesleyan University, Middletown, CT, USA

A parental report questionnaire posted to a population sample of 18-month-old twins correctly assigned zygosity in 95% of cases when validated against zygosity determined by identity of polymorphic DNA markers. The questionnaire was as accurate when readministered at 3 years of age, with 96% of children being assigned the same zygosity on both occasions. The results validate the use of parental report questionnaire data to determine zygosity in infancy. *Twin Research* (2000) 3, 129–133.

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Introduction

A challenge for twin research is to find accurate means to determine the zygosity of twins. Using DNA, it is possible to establish with certainty whether twins share an identical genome and are therefore monozygotic (MZ), or differ genotypically and are therefore dizygotic (DZ). For large-scale studies of community samples, however, it is often difficult and expensive to obtain DNA. For this reason, twin researchers have developed indices of physical similarity of highly heritable traits in order to determine zygosity.^{1–6} Height, eye colour, hair colour and overall confusability are often used for this purpose. Diagnoses based on the questionnaire responses of adult twins typically misclassify 5% or fewer twin pairs as validated by blood group or DNA markers.^{1,3,6–9} Similar validity has been demonstrated for maternal reports of physical similarity,^{5,6,10,11} although there is a paucity of research on twins younger than 6 years old. Two investigations using twin samples of mixed ages up to 6 years old have correctly classified over 90% of twin pairs.^{12,13} To our knowledge only one study has documented the validity of zygosity diagnoses based on the questionnaire responses of parents of infant twins.¹⁴

The current study examined these issues in relation to a large population sample of infant twin pairs born in England and Wales. A parental report questionnaire of physical similarity was administered at the age of 18 months. The resulting

assessments of zygosity were compared in a subsample to DNA analysis using 8–10 highly polymorphic DNA markers in the genome, a technique which can assign zygosity with an extremely high degree of accuracy.^{15–19} A second subsample focused on infants whose zygosity was difficult to assign by questionnaire in order to investigate possible improvements to the design and scoring of the questionnaire using DNA-assigned zygosity as a criterion. The questionnaire was readministered at 3 years of age in order to assess the stability of the assignments of zygosity.

Method

Sample

The sampling frame for the present study, namely the Twins Early Development Study (TEDS), consisted of all twins born in England and Wales in 1994 and 1995. Parents of twins were identified from their children's birth records and initially contacted by the government agency responsible for birth registration, the Office for National Statistics (ONS). Participating families were sent questionnaire booklets when their twins were about 18 months old and further booklets just before the twins' third birthday. Currently information from the 3-year booklets is only available from twins born in 1994. In total, information was available from 6060 families of same-sex twins who returned the background booklet and 2028 families of same-sex twins who returned both booklets.

Two subsamples of twin pairs were assigned zygosity by genotyping. One sample contained 159 twin pairs who were unselected with respect to their responses to the zygosity questionnaire, having been

Correspondence: Thomas S Price, Social Genetic and Developmental Psychiatry Research Centre, Institute of Psychiatry, London, SE5 8AF, UK. Tel: +44 (0) 207 848 0961; Fax: +44 (0) 207 848 0866; E-mail: t.price@op.kcl.ac.uk
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recruited to participate in a DNA study using the TEDS sample. A further sample of 165 pairs was recruited from those children least likely to be assigned an accurate zygosity by the questionnaire described below. These included pairs in the median 10% of the bimodal distribution of physical similarity derived from responses to the questionnaire, and those who were not assigned a zygosity by the questionnaire.

The TEDS samples did not differ importantly from UK population averages on most demographic indicators, including the proportion of fathers in employment, fathers leaving school with no qualifications, and mothers achieving A levels (the UK equivalent to high school finishing qualifications). In comparison to the UK population, families returning the 18-month questionnaire contained somewhat fewer mothers who had left school without qualifications (10%, $n = 5841$, compared with 19%), fewer employed mothers (41%, $n = 5941$, compared with 49%), and fewer divorced parents (4%, $n = 5891$, compared with 7%). The TEDS samples differed very little from each other on any of these demographic indicators, with the sole exception of the unselected genotyped sample which contained only one non-white family (99% ethnically white, $n = 158$) compared with 92%, $n = 6043$, for families returning the 18-month questionnaire, and the UK population average of 92%. The UK population averages used in these comparisons were for comparable groups participating in the General Household Survey,²⁰ age-weighted where necessary to match the age profile of TEDS families.

DNA zygosity

In the current study, DNA for genotyping was collected from cheek cells using swabs. This method is more acceptable than taking blood, particularly in a population sample of young children, and can be easily and cheaply administered at home by parents and then returned by post.²¹ For each child, the allele lengths of 8–10 highly polymorphic simple sequence repeat (SSR) DNA markers were determined by electrophoresis on an agarose gel. These markers were TH, D3S1300, CYAR, FABP2, PLA2, DHFRP2, D22S264, D14S74, D1S255, and D17S798. Twins with only one divergent allele were genotyped a second time to limit the scope for genotyping error. Identity on all the markers can be used to assign monozygosity with greater than 99% accuracy. Replicated non-identity for any of the markers is a guarantee of dizygosity. Early somatic mutation at one of the SSR loci could theoretically lead to misclassification of MZ twins; however, mutation rates for SSRs are of the order of 10^{-4} and no

examples of twins reproducibly differing by only one marker were observed.

Zygosity questionnaire

Both the background and the 3-year booklets contained a zygosity questionnaire adapted from Goldsmith (1991),⁴ reproduced in the Appendix 1. Certain individual items were used as definite markers of zygosity. Twins described as looking as alike as 'two peas in a pod' were classified as MZ. This question alone has been shown to correctly classify a high proportion of twin pairs.⁷ Twin pairs described as 'not looking much alike at all' or as having clear differences in eye colour, hair colour or hair texture were classified as DZ, except where they were described elsewhere as being as alike as 'two peas in a pod' in which case they were left unclassified.

In all other instances, the items were scored numerically, with low scores given to responses indicating similarity between twins, and high scores given to responses indicating dissimilarity. These scores were summed and then divided by a maximum possible score on those questions that were answered in order to create a physical similarity quotient (PSQ) between 0, representing maximal physical similarity, and 1, representing maximum physical dissimilarity. Twins were not assigned zygosity where the maximum possible score of all the questions that were answered was equivalent to missing data on half or more of the questions. The physical similarity of same-sex twins is distributed bimodally, with MZ twins physically much more similar than DZ twins. Twin pairs whose PSQ scores fell in the overlap between the bimodal curves were not assigned zygosity unless the twins showed clear signs of zygosity as described above. Otherwise twin pairs with PSQ scores below the median were classified as MZ and twin pairs with PSQ scores above the median were classified as DZ.

Results

The distribution of scores from the questionnaire administered at 18 months of age indicates the expected bimodal distribution of physical similarity (see Figure 1). In total, 98.8%, $n = 6060$, of the entire sample supplied sufficient information to calculate a PSQ score. The median PSQ for the whole sample is 0.65. Unless the twins showed clear signs of one or other zygosity classification, the twin pairs in the overlap between the bimodal curves, the 5.6% who scored between 0.64 and 0.70, were not assigned a zygosity, whereas pairs with $PSQ > = 0.70$ were classified as DZ and pairs with $PSQ < = 0.64$ were classified as MZ. In all, 47.8% of the sample were

classified as MZ, 46.8% as DZ, and 5.5% were not classified.

PSQ scores from the unselected genotyped subsample also show a bimodal distribution, as can be seen from the histogram in Figure 2. The bars of the histogram are shaded according to the zygosity assigned by genotyping and by questionnaire: solid blocks of black and white correspond to concordant assignments of zygosity, cross-hatched blocks correspond to discrepancies in zygosity assignment.

This sample contains many more MZ pairs than DZ pairs despite the similar number of MZ and same-sex DZ pairs in the entire TEDS sample. Nevertheless, within the zygosity categories assigned by the questionnaire the physical similarity of twin pairs in this subsample was distributed similarly to twin pairs in the whole sample. This is

important since the questionnaire is more likely to assign the correct zygosity to twin pairs who are physically extremely similar or dissimilar. In the whole sample, pairs assigned MZ zygosity had a mean PSQ of 0.50 (SD 0.071) and pairs assigned DZ zygosity had a mean PSQ of 0.83 (SD 0.088). In the unselected genotyped subsample, pairs assigned MZ zygosity had a mean PSQ of 0.50 (SD 0.065) and pairs assigned DZ zygosity had a mean PSQ of 0.82 (SD 0.097).

From this sample, 153 twin pairs were assigned zygosity by the questionnaire from the background booklets. Of these, 145 (94.8%) were assigned the same zygosity using DNA markers. The proportion of twin pairs who were assigned MZ by DNA markers who were also assigned MZ by the questionnaire was 93.6%, $n = 110$. For twin pairs assigned DZ by DNA markers, the proportion assigned DZ by the questionnaire was 97.6%, $n = 43$. These proportions are not significantly different according to Fisher's exact χ^2 test, indicating that in our sample DZ pairs are no more likely than MZ pairs to be correctly assigned zygosity.

The results of genotyping the twin pairs of intermediate physical similarity or who could not be classified by the 18-month questionnaire validated the exclusion of the median 5.6% band of PSQ scores from assignments of zygosity. Most twin pairs within this band ($0.64 < \text{PSQ} < 0.70$) are not assigned a zygosity by the questionnaire, but those who are classified are done so on the basis of clear physical differences and hence tend to be assigned with reasonable accuracy (89.5%, $n = 38$). In the ranges of PSQ values immediately adjacent to these boundaries the zygosity classifications have lower levels of accuracy: 69.0%, $n = 42$, for $0.62 < \text{PSQ} < 0.64$ and 79.4%, $n = 34$, for $0.70 < \text{PSQ} < 0.72$. In mitigation, only 5.0% of twin pairs fall within these ranges. In the TEDS twin registry DNA has been sought from all families with $0.62 < \text{PSQ} < 0.72$.

The results from the questionnaire at age 3 indicate that questionnaire assignments of zygosity are stable as well as accurate in infancy. Using the same criteria for scoring the questionnaire as at age 18 months, 94.4% of twin pairs were assigned a zygosity of whom 96.1%, $n = 1856$, of twin pairs were assigned the same zygosity on both occasions. The questionnaire was at least as accurate at 3 years as at 18 months. From the unselected genotyped sample, 95.2%, $n = 147$, of the twin pairs assigned a zygosity by the questionnaire at age 3 years were assigned the same zygosity using DNA markers, including 95.4%, $n = 108$, of the twin pairs classified as MZ by DNA markers, and 94.9%, $n = 39$, of the twin pairs classified as DZ by DNA markers.

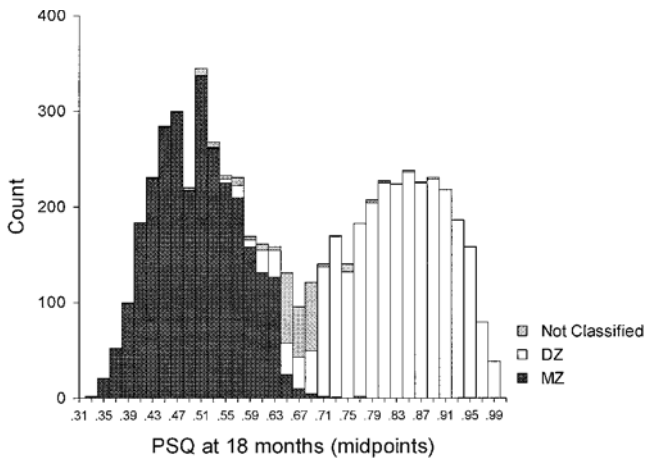


Figure 1 Assignments of zygosity by physical similarity at 18 months for the entire sample ($n = 5990$ same-sex pairs).

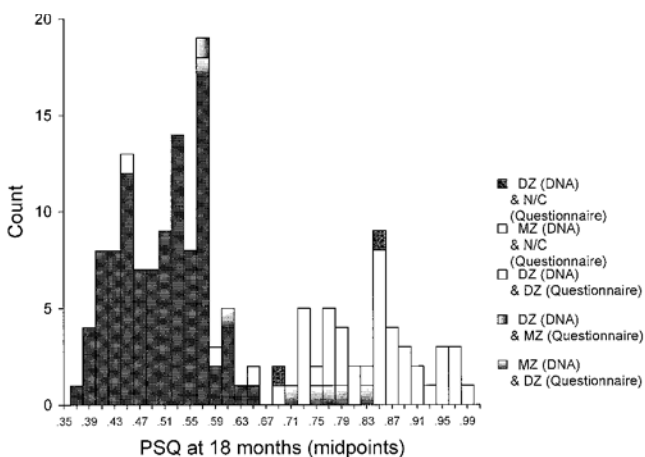


Figure 2 Assignments of zygosity by genotyping and by physical similarity at 18 months, for the unselected genotyped sample; $n = 153$ pairs; 110 MZ and 43 DZ on the basis of genotyping.

Discussion

Zygosity classifications using a physical similarity questionnaire rated by parents are stable in infancy and as accurate as similar methods used with adult populations. Validation against DNA assignments of zygosity indicated that using a parental report questionnaire, 94.4% of twins can be assigned a zygosity as early as 18 months of age with 94.8% accuracy. The questionnaire classifications were equally accurate at age 3, and 96.1% of twin pairs were assigned the same zygosity at both time points. Nevertheless, for even greater reliability, where absolute zygosity ascertainment is required, DNA extraction and testing are recommended. For large twin registries a combined approach genotyping only twin pairs of intermediate physical similarity can offer high levels of accuracy at low cost.

Although the number of MZ and same-sex DZ pairs in TEDS is similar, more parents of MZ pairs than DZ pairs responded to our zygosity study. Parents were told that they would be informed of their child's zygosity, so it could be that parents of MZ twins are more motivated to be certain of zygosity than parents of DZ twins.

Anecdotal evidence from non-participating parents suggests that a major reason for not sending back the cheek cell packs was the conviction that their children were not identical twins.

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Appendix 1

The parental report questionnaire has been adapted from Goldsmith (1991).⁴ The responses to questions 1–4 are scored: none (1), only slight difference (2), clear difference (3). The responses to questions 10 to 16 are scored: yes, easily (1); yes, sometimes (2); rarely or never (3). Otherwise the scores are indicated in parentheses after each possible response.

- Are there differences in the shade of your twins' hair?
- Are there differences in the texture of your twins' hair (fine or coarse, straight or curly etc.)?
- Are there differences in the colour of your twins' eyes?
- Are there differences in the shape of your twins' ear lobes?
- Did the twins' teeth begin to come through at about the same time? The twins had matching

- teeth on the same side or opposite sides come through within a few days of each other (1); The twins had different teeth come through within a few days of each other (2); the twins' first teeth did not come through within a few days of each other (3); the twins' teeth have not come through yet (missing).
- 6 Do you know your twins' ABO blood group and Rhesus (Rh) factors? If yes, and response categories for blood group and Rhesus factor have been ticked for both twins, score 0 each for same response for both twins, 1 for different responses.
- 7 As your twins have grown older, has the likeliness between them: remained the same (2); become less (3); become more (1)?
- 8 When looking at a new photograph of your twins, can you tell them apart (without looking at their clothes or using any other clues)? Yes, easily (1); Yes, but it is hard sometimes (2); No, I often confuse them in photographs (3).
- 9 Do any of the following people ever mistake the twins for each other?
- 10 Other parent of the twins.
- 11 Older brothers or sisters.
- 12 Other relatives.
- 13 Babysitter/day carer.
- 14 Parents' close friends.
- 15 Parents' casual friends.
- 16 People meeting the twins for the first time.
- 17 If the twins are ever mistaken for another, does this ever occur when they are together? Yes, often (1); Yes, sometimes (2); No, almost never (3); They are not mistaken for one another (4).
- 18 Would you say that your twins: are physically alike as 'two peas in a pod' (virtually the same) (1); are as physically alike as brothers and sisters are (2); do not look very much alike at all (3).