Verification of Self-Report of Zygosity Determined via DNA Testing in a Subset of the NAS-NRC Twin Registry 40 Years Later

Terry Reed,[1] Brenda L. Plassman,[2] Caroline M. Tanner,[3] Danielle M. Dick,[1]* Shannon A. Rinehart,[1] and William C. Nichols[4]

[1] Department of Medical & Molecular Genetics, Indiana University School of Medicine, Indianapolis, United States of America
[2] Department of Psychiatry & Behavioral Science, Duke University Medical Center, Durham North Carolina, United States of America
[3] The Parkinson's Institute, Sunnyvale California, United States of America
[4] Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Ohio, United States of America
* Now at Department of Psychiatry, Washington University School of Medicine, St Louis, Missouri, United States of America

The National Academy of Sciences — National Research Council (NAS-NRC) twin panel, created nearly 50 years ago, had twin zygosity determined primarily via a similarity questionnaire that has been estimated to correctly classify at least 95% of twins. In the course of a study on the genetics of healthy ageing in the NAS-NRC twins, DNA was collected for genome-wide scanning and zygosity confirmation was examined in 343 participating pairs. The sample was supplemented from two other studies using NAS-NRC twins where one or both co-twins were suspected to have Alzheimer disease or another dementia, or Parkinson's disease. Overall 578 twin pairs with DNA were analyzed. Zygosity assignment for 96.8% (519/536) was confirmed via questionnaire. Among 42 pairs whose questionnaire responses were inconclusive for assigning zygosity, 50% were found to be monozygous (MZ) and 50% were dizygous (DZ). There was some evidence for greater misclassification of presumed DZ pairs in the healthy ageing study where participation favored pairs who were similar in having a favorable health history and willingness to volunteer without any element of perceived risk for a specific disease influencing participation.

In 1955, the National Academy of Sciences — National Research Council (NAS-NRC) initiated the development of a veteran twin registry by matching birth certificate information, collected from 40 of the then 48 states (except for the city of New Orleans in Louisiana), to Veteran Administration records. The resulting registry of 15,924 Caucasian male twin pairs represented 93% of male twin births from 1917 through 1927 (Hrubec & Neel, 1978) and is close to a population-based, representative sample of male twin births for these years in the United States. A detailed description of the creation of the registry is published (Hrubec & Neel, 1978; Jablon et al., 1967) and was more recently summarized by Page (2002). In the NAS-NRC twin panel, approximately 80% of pairs assigned a zygosity were classified solely on their own assessment from a zygosity questionnaire first mailed in 1965 (Hrubec & Omenn, 1981). Two questions were utilized for establishing zygosity. One was based on similarity, 'As children were you and your twin alike as two peas in a pod or of only ordinary family resemblance?'; a second was based on confusion of identity, 'In childhood, did your parents, brothers and sisters, or teachers have trouble in telling you apart?'. The largest number of pairs classified as monozygotic (MZ) answered yes to both questions and the second largest number included one co-twin who answered yes to both questions, but his co-twin did not complete the questionnaire (Hrubec & Neel, 1978). Similarly, no–no responses to both questions by one or both co-twins led to classification as a dizygotic (DZ) pair. Magnus et al. (1983) showed that questionnaire responses from one co-twin are as highly accurate as responses from both members of a pair. Approximately 18% of the NAS-NRC pairs also had fingerprint and physical characteristics available. If similarity of these traits did not contradict the questionnaire assignment of zygosity, the pairs were classified based on questionnaire data. Otherwise, the twins were considered as unknown for zygosity along with pairs with incomplete or conflicting questionnaire responses (Hrubec & Neel, 1978). Jablon et al. (1967) found an average error of 4.3% in zygosity in a small subgroup of pairs with blood typing. It has been estimated that zygosity is correctly determined for at least 95% of twin pairs assigned a zygosity in the panel (Hrubec & Neel, 1978, 1981). Reitveld et al. (2000) reviewed zygosity determination in various twin studies and concluded that mailed questionnaires

to adult twins or parents of twin children are approximately 95% accurate when compared with serologic or serum protein marker typing. They also noted similar percentages have been reported for DNA typing in comparisons with questionnaires sent to parents of young twins.

In the process of performing a sib-pair linkage study using pairs from the NAS-NRC twin registry (Reed et al., 2004), we conducted a genome-wide microsatellite marker scan on 254 adult twin pairs that provided a blood sample. This report summarizes the rate of agreement for self-report information over 30 years ago and DNA confirmation for zygosity in these pairs plus an additional 89 pairs that provided a DNA sample but were not part of the linkage study. The sample was further augmented with 176 additional pairs from the NAS-NRC registry who took part in the Duke Twin Study of Memory in Ageing and provided a DNA sample that was used for zygosity confirmation. Pairs from the NAS-NRC twin registry who took part in a genetic study of Parkinson disease and provided a DNA sample (Tanner et al., 1999) are also included.

Materials and Methods

Zygosity confirmation testing was undertaken via DNA methods in 578 NAS-NRC twin pairs from three different studies.

Firstly, a health history questionnaire (Q8) was mailed in the fall of 1998 to all complete NAS-NRC twin pairs presumed to be alive. Excluding those who were deceased and those with invalid addresses, questionnaires were received from 6108 of 8848 (69%) individuals. In this group, 2059 were complete pairs with a mean age of 74.3 years. A definition of healthy physical ageing, termed *wellness*, was created from the Q8 responses (Reed & Dick, 2003). An individual met the wellness definition if he answered 'No' to all of the following questions: (1) Has a doctor ever told you that you had a heart attack?; (2) Have you ever had coronary bypass surgery or angioplasty?; (3) Have you ever been told by a doctor that you had a stroke?; (4) Has a doctor ever told you that you have diabetes?; (5) Have you ever been diagnosed with prostate cancer? These traits were selected to define an individual who has successfully aged into their 70s by eliminating most of the major health problems. To search for genes associated with healthy physical ageing, we first recruited and collected blood by mail for DNA extraction from DZ pairs concordant for the wellness definition. Because we were also interested in potential co-twin control comparisons we subsequently recruited, in order, DZ pairs discordant for the good health phenotype and finally MZ pairs where one or both co-twins met the wellness definition. Twin pairs recruited for the linkage study included a small number of pairs of unknown zygosity. Because of the interest in concordant DZ pairs for the linkage study, such pairs were recruited within the

DZ twin groups. Table 1 shows the number of pairs who were recruited in each of the four groups. A total of 711 samples were received; among these were 343 complete pairs. The lowest participation rate was in DZ pairs discordant for the wellness phenotype (19.4%). Approximately one third of the potential subjects participated from the other groups. Blood was received from both co-twins in 91.5% (343/375) of pairs agreeing to participate. Reasons for not returning a blood sample included death of a co-twin, illness in one or both co-twins, difficulty in finding a local health care facility willing to draw the blood, and unspecified reasons for no longer being interested in providing a blood sample for the study.

Secondly, as part of the Duke Twins Study of Memory in Ageing, members of the NAS-NRC Twin Registry were screened for dementia four times between 1990 and 2002 using a two-step telephone screening process. Individuals who screened positive for suspected dementia on both screening measures were then assessed in-person using a standardardized evaluation for dementia (Breitner et al., 1995). Blood or buccal DNA samples were collected at the time of the in-person assessment for those who completed this phase of the study. Buccal DNA was obtained via a mail DNA collection protocol for those who did not receive an in-person assessment (i.e., primarily cognitively intact co-twins).

Third, the sample was further supplemented by including 74 twin pairs who participated in a study of the genetics of Parkinson disease (Tanner et al., 1999). Of these pairs, 15 were already included in either the Duke or Indiana samples, leaving 59 additional unique pairs to be included.

The genotyping was done as follows. For the linkage study, a genome screen was completed using DNA samples sent to Cincinnati Children's Hospital Medical Center. Genotyping employed up to 400 dinucleotide markers from the ABI Prism Linkage Mapping Set (Applied Biosystems, Foster City, CA) with an average heterozygosity of 79% and an average intermarker spacing of 8.6 centimorgans (cM)

Table 1

Twin Subjects Who Agreed to Participate and Returned a Blood Sample by Zygosity and Concordance for the Healthy Ageing Phenotype

	Both co-twins agreed to participate	Provided a blood sample by mail (only one co-twin sent a blood sample)
DZ concordant	123	110 (12)
DZ discordant	68	59 (6)
MZ concordant	110	104 (4)
MZ discordant	74	_70_ (3)
		343 (25)

or map units as previously reported (Pankrantz et al., 2002; Reed et al., 2004).

We used the genome-wide microsatellite marker data to verify zygosity in the twin pairs using the computer program PREST (McPeek & Sun, 2000). PREST produces a variety of summary statistics to evaluate genetic relatedness. For each pair it lists the proportion of markers at which 0 alleles, 1 allele or 2 alleles are shared identical by descent (IBD). For full siblings, the expected proportions are .25, .50 and .25 respectively. For MZ twins, all alleles are expected to be shared IBD, so the expected proportions would be 0, 0 and 1 if there were no genotyping error. Pairs were classified as MZ or DZ according to whether their pattern of allele-sharing more closely resembled that expected for full siblings or for MZ twins. For MZ pairs, there was identity of both marker alleles (IBD-2) in greater than 97% of all loci tested. The reason that not every marker was completely identical in MZ pairs is that dinucleotide markers do have a small percentage of genotyping error. MZ twins are a good way to look for genotyping error. Allele nonidentity was 0.86% in our MZ pairs; this genotyping error rate is in line with the error rate reported in other studies (Bonin et al., 2004; Kirov et al., 2000).

For any pair that were listed as unknown or dizygotic in the NAS-NRC database, if the probability values were greater than $p = .05$ for full siblings or roughly approximated a proportion of a quarter of markers identical by descent (IBD = 2) at all loci, half of markers with one allele shared (IBD = 1) and a quarter of pairs not sharing any marker alleles at a locus (IDB = 0), the pairs were confirmed to be dizygotic. If there was a significantly higher sharing of alleles IBD-2 than would be expected for full siblings, the pairs were confirmed to be MZ twins.

After completion of the linkage study, blood samples collected were predominantly from presumed MZ groups. In Cincinnati, 10 microsatellite markers were genotyped on these pairs to establish zygosity. The chance a pair of DZ twins matched for all 10 of these markers was 4.4×10^{-5} (power = 99.996%) using the average heterozygosity (h) for each individual marker in the formula $(1 - [1/2 \times h])^2$ and multiplying the resulting probability for all 10 of the markers (Spitz et al., 1996). For these 87 pairs the program RELPAIR (Boehnke & Cox, 1997; Epstein et al., 2000) was used to determine if the IBD sharing for the markers was consistent with siblings (i.e., DZ twins). Nearly all MZ pairs matched on all or nearly all markers and had statistically significant increases in expected allele sharing IBD for siblings. Pairs who had a relatively high percentage of markers IBD-2 but did not quite reach statistical significance for greater allele sharing from siblings were retested in the Department of Medical & Molecular Genetics in Indianapolis. This laboratory used a commercially available system used for a variety of identity analyses (e.g., paternity testing) employing from 9 to all 13 polymerase chain reaction (PCR)–amplified Short Tandem Repeat (STR) loci (Budowle et al., 2001). The probability a DZ set will match on the first 9 markers is .0001 and for all 13 markers it is 2.5×10^{-5}. Two additional MZ pairs whose blood samples arrived well after the completion of the linkage study also had zygosity confirmed with the 13 STR. Similar methodology using a different panel of 13 STR loci was used for zygosity testing in all pairs with DNA typing in the Parkinson study (Hammond et al., 1994). Zygosity testing in the long-term Duke Alzheimer twin study has evolved with time. Initial blood samples were typed for zygosity using DNA fingerprinting (Jeffreys et al., 1985). With collection of buccal samples, 10 to 20 microsatellite markers were used in a few pairs for zygosity determination similar to those used for the 87 predominantly MZ pairs in the Indiana ageing twin study. A few pairs were tested using only three STR used for ruling out maternal contamination (Urquhart et al., 1995). Later samples used another commercially available zygosity-testing kit with eight STR markers (power = 99.95%). Except for the system using the three STR (power = 96%), the chance a pair of DZ twins match on all markers is well under 1%.

Table 2

Result of Zygosity Testing at Each Study Site and Zygosity Assignment for Pairs with Unknown Zygosity in the NAS-NRC Master File

	Healthy ageing [Indiana]	Memory [Duke]	Parkinsons	Total
Questionnaire diagnosis				
MZ zygosity confirmed	173 (99.4%)	81 (96.4%)	24 (88.9%)	278 (97.5%)
–MZ pairs actually DZ	8	2	0	10
–unknown confirmed as MZ	9	9	3	21
Total	190	92	27	309
DZ zygosity confirmed	143 (94.7%)	74 (97.4%)	24 (100%)	241 (96.0%)
–DZ pairs actually MZ	1	3	3	7
–unknown confirmed as DZ	9	7	5	21
Total	153	84	32	269

Results

Table 2 presents the results of the zygosity testing separately for both MZ and DZ pairs in each of the three NAS-NRC twin subsamples. Initially we suspected that the pairs of unknown zygosity might be predominantly DZ twins. As shown in the table 50% (21/42) were DZ and the proportions were consistent across the studies. Among the pairs in the full NAS-NRC twin panel classified as unknown, almost 90% were due to neither co-twin completing the zygosity questionnaire (Hrubec & Neel, 1978). It is not known how many of these subsequently participated in other surveys and whether part of the explanation for more MZ pairs in the unknown zygosity group than expected is due to both co-twins not answering the zygosity questionnaire. Table 2 shows that only one MZ pair was incorrectly classified (99.4% were correctly classified) in the healthy ageing study. In the DZ pairs, 143/151 (94.7%) were correctly classified.

The difference in the proportion of twin pairs correctly classified was significantly higher in the MZ pairs ($z = 2.45$, $p < .05$) in the Indiana subsample using a two-tailed test of proportions (Snedecor & Cochran, 1980). In the other two studies pairs were recruited for suspected specific diseases (Alzheimer or Parkinson's) in at least one co-twin. In the latter two samples, the proportion of misclassified DZ pairs was less than that for MZ pairs but not statistically significant.

A total of 54 pairs (28 DZ and 26 MZ) in the present sample were also members of the National Heart, Lung, and Blood Institute (NHLBI) twin study. As part of the NHLBI twin study pairs were assigned zygosity based on genotyping via serologic markers (22 antigens) and self-report information (Feinleib et al., 1977). When DNA samples were later collected on participants at the third examination of the NHLBI twins (Reed et al., 1993) zygosity was checked using DNA markers only for pairs with identical serology but classified as DZ based on questionnaire responses. Three of the 54 NHLBI pairs had a zygosity change from MZ in the master NAS-NRC file to DZ after DNA testing in the current study. Of these, two of the reclassified pairs previously were reclassified as DZ pairs in the NHLBI twin study via DNA testing of those with identical serology with DZ questionnaire responses.

Comment

Among the reports that used DNA genotyping to verify zygosity assignment, this is the first that involves comparison to questionnaire responses in adult twins. In our twins the questionnaires were completed over 30 years earlier. All of the other studies using DNA for verification involved comparison of questionnaire data completed by the parents of twin children (Charlemaine et al., 1997; Chen et al., 1999; Reitveld et al., 2000; Spitz et al., 1996) In the NAS-NRC twins, previous comparisons with serological markers indicated that there were more MZ pairs

who thought they were DZ (Jablon et al., 1967). With a sample size over threefold larger, Hrubec and Neel (1978) still found that 97.8% of DZ and 92.4% of MZ pairs were correctly classified via questionnaires in this cohort. Another United States twin adult twin cohort (King et al., 1980) also reported better classification of DZ pairs (97% vs. 83%). Much of the difference was attributed to the MZ pairs' parents being told their twins were DZ because of two placentas at birth. Torgersen (1979) found slightly better classification via questionnaire data based on similarity in adult DZ twin pairs, while Cederlof et al. (1961) reported better classification in MZ pairs. In children where twins are classified based on the responses of their parents to questionnaires, generally classification is better in MZ pairs. Nichols and Bilbro (1966) provide a good explanation for the latter observation 'in that as many as 10% of MZ pairs do not appear strikingly similar and are more easily misdiagnosed as DZ while fewer DZ pairs are similar enough to erroneously be called MZ'.

The twins in the ageing study had fewer errors in assignment of zygosity from MZ pairs in a cohort that overall had better classification of DZ twins. We believe it unlikely that this observation could reflect errors in handling of samples in the laboratory. The explanation could simply be a chance occurrence or the difference may reflect how the twins were ascertained for the ageing study. There is some evidence in the NAS-NRC twins and the NHLBI subset for greater mortality in DZ twins (D. Carmelli, personal communication). With ageing, a higher percentage of surviving pairs are more likely to be MZ. In this study we selected for pairs in which both co-twins had to survive into their 70s, both co-twins had to complete the Q8 questionnaire, at least one of the two co-twins had to meet the healthy physical ageing definition, and both had to agree to go to a local healthcare facility or provider to have blood samples drawn. In the other two studies included in this report pairs were recruited for Alzheimer or Parkinson's disease in at least one co-twin with likely interest for participation because of possible risk for the unaffected co-twin. In these combined groups, there was a trend towards better classification in DZ pairs more like estimates from the overall cohort. Twins of unknown zygosity via similarity questionnaires, are most likely very similar DZ pairs or dissimilar MZ pairs (Allen, 1976). Unknown zygosity pairs were spread rather evenly between MZ and DZ pairs across the three NAS-NRC subgroups.

Overall 96.8% of the pairs who provided a blood sample for genetic markers in the three studies were correctly classified in the NAS-NRC master database based on questionnaire responses in the 1960s. Our results suggest that using a conservative estimate of 95% of pairs correctly classified based on questionnaire data for the NAS-NRC twins and other twin cohorts regardless of whether serologic markers or

DNA genotyping is used (Hrubec & Neel, 1981; Magnus et al., 1983; Reitveld et al., 2000) is appropriate. The error rate has little effect on heritability estimates and if anything leads to more conservative figures. We also caution that with the use of highly polymorphic DNA markers, if a pair of twins differs in genotype on just one marker, the test probably should be repeated. For example, using the 10 microsatellite markers typed for pairs at the end of the ageing twin study, if a pair matches on the first nine markers the probability the pair is really DZ is .0001. If there is an allele difference on the 10th marker, then it may be as plausible to suspect a genotyping error as to go ahead and assume that the pair is now DZ. Repeating the test can resolve if the initial discordance is an error in genotyping; a difference in one allele might also be due to a rare postzygotic mutation. If DZ twins are used for studies to find genes associated with complex traits (Boomsma et al., 2002), then even small errors in zygosity assignment may affect power to detect true associations with limited sample sizes.

References

Allen, G. (1976). Scope and methodology of twin studies. *Acta Geneticae Medicae et Gemellologiae (Roma), 25,* 79–85.

Boehnke, M., & Cox, H. J. (1997). Accurate inference of relationships in sib-pair linkage studies. *American Journal of Human Genetics, 61,* 423–429.

Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C., & Taberlet, P. (2004). How to track and assess genotyping errors in population genetic studies. *Molecular Ecology, 13,* 3261–3273.

Boomsma, D., Busjahn, A., & Peltonen, L. (2002). Classical twin studies and beyond. *Nature Reviews*: *Genetics, 3,* 872–882.

Breitner, J. C. S., Welsh, K. A., Gau, B. A., McDonald, W. M., Steffens, D. C., Saunders, A. M., Magruder, K. M., Helms, M. J., Plassman, B. L., Folstein, M. F.,

Brandt, J., Robinette, C. D., & Page, W. F. (1995). Alzheimer's disease in the National Academy of Sciences-National Research Council Registry of ageing twin veterans. III: Detection of cases, longitudinal results, and observations on twin concordance. *Archives of Neurology, 52,* 763–771.

Budowle, B., Shea, B., Niezgoda, S., & Chakraborty, R. (2001). CODIS STR loci data from 41 sample populations. *Journal of Forensic Science, 46,* 453–489.

Cederlof, R., Friberg, L., Jonsson, E., & Kaij, L. (1961). Studies on similarity diagnosis in twins with the aid of mailed questionnaires. *Acta Genitica et Statistica Medica, 11,* 338–362.

Charlemaine, C., Duyme, M., Aubin, J.-T., Guis, F., Marquiset, N., de Pirieux, I., Strub, N., Brossard, Y., Jarry, G., Le Groupe Romulus, Frydman, R., & Pons, J. C. (1997). Twin zygosity diagnosis by mailed questionnaire below age twelve months. *Acta Geneticae Medicae et Gemellologiae (Roma), 46,* 147–156.

Chen, W. J., Chang, H.-W., Wu, M.-Z., Lin, C. C. H., Chang, C., Chiu, Y.-N., & Soong, W.-T. (1999). Diagnosis of zygosity by questionnaire and polymerase chain reaction in young twins. *Behavior Genetics, 29,* 115–124.

Epstein, M. P., Duren, W. L., & Boehnke, M. (2000). Improved inference of relationship for pairs of individuals. *American Journal of Human Genetics, 67,* 1219–1231.

Feinleib, M., Garrison, R. J., Fabsitz, R., Christian, J. C., Hrubec, Z., Borhani, N. O., Kannel, W. B., Rosenman, R., Schwartz, J. T., & Wagner, J. O. (1977). The NHLBI twin study of cardiovascular disease risk factors: Methodology and summary of results. *American Journal of Epidemiology, 106,* 284–295.

Hammond, H. A., Jin, L., Zhong, Y., Caskey, C. T., & Chakraborty, R. (1994). Evaluation of 13 short tandem repeat loci for use in personal identification applications. *American Journal of Human Genetics, 55,* 175–189.

Hrubec, Z., & Neel, J. V. (1978). The national academy of sciences-national research council twin registry: Ten years of operation. *Progress in Clinical & Biological Research, 24B,* 153–172.

Hrubec, Z., & Neel, J. V. (1981). Familial factors in early deaths: Twins followed 30 years to ages 51–61 in 1978. *Human Genetics, 59,* 39–46.

Hrubec, Z., & Omenn, G. S. (1981). Evidence of genetic predisposition to alcoholic cirrhosis and psychosis: Twin concordances for alcoholism and its biological end points by zygosity among male veterans. *Alcoholism, Clinical & Experimental Research, 5,* 207–215.

Jablon, S., Neel, J. V., Gershowitz, H., & Atkinson, G. F. (1967). The NAS-NRC twin panel: Methods of construction of the panel, zygosity diagnosis, and

proposed use. *American Journal of Human Genetics, 19,* 133–161.

Jeffreys, A. J., Wilson, V., & Thein, S. L. (1985). Individual-specific 'fingerprints' of human DNA. *Nature, 316,* 76–79.

King, M.-C., Friedman, G. D., Lattanzio, D., Rodgers, G., Lewis, A. M., Dupuy, M. E., & Williams, H. (1980). Diagnosis of twin zygosity by self-assessment and by genetic analysis. *Acta Geneticae Medicae et Gemellologiae (Roma), 29,* 121–126.

Kirov, G., Williams, N., Sham, P., Craddock, N., & Owen, M. J. (2000). Pooled genotyping of microsatellite markers in parent-offspring trios. *Genome Research, 10,* 105–115.

Magnus, P., Berg, K., & Nance, W. E. (1983). Predicting zygosity in Norwegian twin pairs born 1915–1960. *Clinical Genetics, 24,* 103–112.

Nichols, R. C., & Bilbro, W. C. (1966). The diagnosis of twin zygosity. *Acta Genitica et Statistica Medica, 16,* 265–275.

Page, W. F. (2002). The NAS-NRC twin registry of WWII military veteran twins. *Twin Research, 5,* 493–496.

Pankrantz, N., Nichols, W. C., Uniacke, S., Halter, C., Rudolph, A., Shults, C., Conneally, P. M., Foroud, T., & Parkinson Study Group. (2002). Genome screen to identify susceptibility genes for Parkinson disease in a sample without parkin mutations. *American Journal of Human Genetics, 71,* 124–135.

McPeek, M. S., & Sun, L. (2000). Statistical tests for detection of misspecified relationships by use of genome-screen data. *American Journal of Human Genetics, 66,* 1076–1094.

Reed, T., Carmelli, D., Christian, J. C., Selby, J. V., & Fabsitz, R. R. (1993). The NHLBI male veteran twin study data. *Genetic Epidemiology, 10,* 513–517.

Reed, T., & Dick, D. M. (2003). Heritability and validity of healthy physical ageing (wellness) in elderly male twins. *Twin Research, 6,* 227–234.

Reed, T., Dick, D. M., Uniacke, S. K., Foroud ,T., & Nichols, W. C. (2004). Genome-wide scan for a healthy ageing phenotype provides support for a locus near D4S1564 promoting healthy ageing. *Journal of Gerontology Biological Sciences, 59A,* 227–232.

Reitveld, M. J. H., van der Valk, J. C., Bongers, I. L., Stroet, T. M., Slagboom, P. E., & Boomsma, D. I. (2000). Zygosity diagnosis in young twins by parental report. *Twin Research, 3,* 134–141.

Snedecor, G. W., & Cochran, W. G. (1980). *Statistical methods* (7th ed.). Ames, IA: Iowa State University Press.

Spitz, E., Moutier, R., Reed, T., Bushnel, M. C., Marchaland, C., Roubertoux, P. L., & Carlier, M. (1996). Comparative diagnoses of twin zygosity by SSLP variant analysis, questionnaire, and dermatoglyphic analysis. *Behavior Genetics, 26,* 55–63.

Tanner, C. M., Ottman, R., Goldman, S. M., Ellenberg, J, Chan, P., Mayeux, R., & Lansgton, J. W. (1999). Parkinson disease in twins: An etiologic study. *Journal of the American Medical Association, 281,* 341–346.

Torgersen, S. (1979). The determination of twin zygosity by means of a mailed questionnaire. *Acta Geneticae Medicae et Gemellologiae (Roma), 28,* 225–236.

Urquhart, A., Oldroyd, N. J., Kimpton, C. P., & Gill, P. (1995). Highly discriminating heptaplex short tandem repeat PCR system for forensic identification. *Biotechniques, 18,* 116–121.