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Twin Zygosity Testing for Medical Purposes

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After being poisoned by eating the mushroom species *Cortinarius speciosissimus*, a twin developed interstitial nephritis with acute renal failure. He received a renal transplant from his living twin brother, who was presumed dizygotic on phenotypic grounds. Fifteen years later, the twins were zygosity tested by DNA "fingerprint analysis" and found to be monozygotic, despite important phenotypic discordances. The recipient has discontinued immunosuppression therapy and remains well after 9 months. We suggest that, for medical and other reasons, zygosity should be determined at birth on all like-sexed twins. Am. J. Med. Genet. 77:412–414, 1998.

KEY WORDS: twins; twinning; monozygotic; zygosity; transplantation

INTRODUCTION

Confusion is caused by the use of the words "identical" and "fraternal" with reference to monozygotic (MZ) and dizygotic (DZ) twin pairs. Few MZ twin pairs are absolutely "identical," and some pairs may show marked phenotypic and/or genotypic discordance. We report on a pair of male twins (D. M. S. and J. B. S., two of the authors of this article), who are phenotypically discordant and had therefore always considered themselves to be DZ. J. B. S. received a renal transplant from his twin brother, D. M. S; he was treated with immunosuppression therapy for 15 years, with significant complications. Immunosuppression therapy was discontinued when the correct diagnosis of MZ twinning was made. The recipient has maintained normal renal function. This case provides support for the position that unequivocal diagnosis of zygosity of like-sexed twins should be made in early life, the most convenient time being at birth.

In 1980, the renal transplant recipient, J.B.S., ingested a stew containing mushrooms subsequently identified as *Cortinarius speciosissimus* [Short et al., 1980, case 2]. Acute renal failure ensued after six days, and renal biopsy showed acute interstitial nephritis. He received intermittent hemodialysis followed by renal transplantation nine months after the incident. His living twin brother (D. M. S.) was the donor. On the assumption of dizygosity, the recipient received immunosuppression therapy, consisting of prednisolone and azathioprine, for 15 years. Complications included several bouts of pneumonia (one requiring hospitalization), viral warts of hands and feet that required regular outpatient treatment, and a marked Cushingoid change in facial appearance. The recipient also experienced stress and tension because of fears of transplant rejection.

From the earliest age, the twin brothers had presumed that they were DZ. Placental chorionicity was unknown. There were multiple sets of twins in the extended family, many of whom were DZ. The twins are discordant for handedness and laterality of occipital hair whorl, and the donor twin has heterochromia iridum. In other respects, they are phenotypically similar (Fig. 1). When he first met the twin brothers 15 years after the transplantation, one of the authors (G. A. M.) questioned the presumption of dizygosity for the following reasons: 1) MZ status is not excluded by a degree of phenotypic discordance [Machin, 1996], 2) mirror image–like effects are quite common in MZ and DZ pairs [Boklage, 1987] and, 3) these twins generally bore strong phenotypic resemblance (Fig. 1).

Because of the possibility of withdrawing immunosuppression therapy in the event that monozygosity could be proven, the twins agreed to undergo formal zygosity testing, which used DNA variable number tandem repeat sequences. These showed that the twins are apparently MZ. Following this result, the recipient has been weaned from immunosuppression therapy and feels well. His renal function remains normal, thus confirming the diagnosis of monozygosity.

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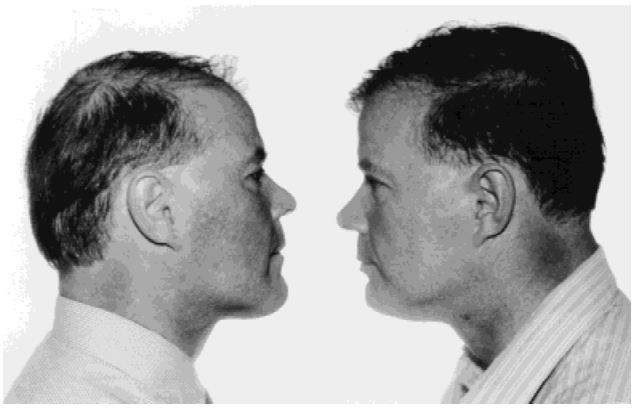

Fig. 1. Facial features of the twins, showing general similarity apart from opposite-sided hair parting and Cushingoid facies of recipient (left).

LABORATORY TESTS AND RESULTS

Zygosity was determined by peripheral leucocyte DNA restriction fragment length polymorphisms in variable number tandem repeats (VNTRs), analysed at low stringency [Hill and Jeffreys, 1985], and using the probes 3′-HVR [Higgs et al., 1986], NH24 [Nakamura et al., 1987], INS310 [Bell et al., 1981] and HMF1. In addition, a commercial polymerase chain reaction (PCR)–based dot-blot kit was used to test for allelic differences with probes for LDLR, GYPA, D7S8, and GC. No allelic differences were detected. The twins were diagnosed as MZ (Fig. 2).

DISCUSSION

MZ twins usually have closely similar phenotypes, but are seldom absolutely "identical." Parents can usually distinguish between their MZ twins, whereas friends and relatives may be unable to do so. Many twins and their parents have excessive expectations of the degree of "identity" required before a diagnosis of MZ status is readily accepted. The situation is further complicated by the fact that some MZ twin pairs show quite marked phenotypic discordances [Machin, 1996]; genetic mechanisms for these include: 1) discordance for chromosome constitution, 2) different patterns of X chromosome inactivation within female MZ twin pairs, 3) variable expression of imprintable genes (e.g., Wiedemann-Beckwith syndrome), and 4) discordance for major malformations (including holoprosencephaly, neural tube defect, symmelia, and Hirschsprung disease), and pigmentary skin dysplasias [Wulfsberg et al., 1991]. Degrees of "mirroring" phenomena, including opposite handedness, are also common in twins, both MZ and DZ [Boklage, 1987]. In addition, monochorionic (MC) MZ twins may show the results of discordant prenatal environments imposed by the vascular connections of their MC placentas [Machin et al., 1996]. Potential pitfalls in the use of twins for genetic research and zygosity determination have been summarized [Phillips, 1993].

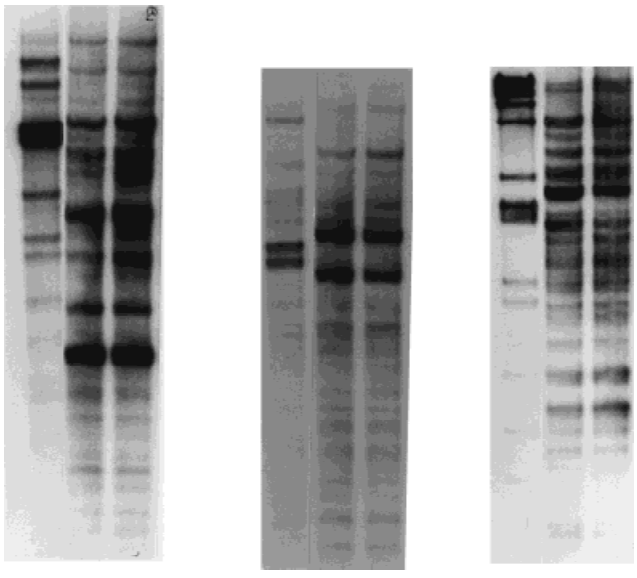

Fig. 2. DNA VNTR analysis. **Left lane** group, 3′-HVR; **middle lane** group, YNH24; **right lane** group, HMF1. In each group of three lanes, the control sample is on the left, with the twin pair center and right. Monozygosity is proven because no discordances are identified.

MZ twin pairs are good candidates for solid organ transplantation, including renal transplantation [Tilney, 1986]. However, bone marrow transplantation for leukemia has not been universally successful, presumably because the donor twin may already be in the preleukemic phase of the same disease, having exchanged, received, or donated a prenatal transfusion of hemopoietic stem cells via anastomosing fetal vessels of an MC placenta [Ford et al., 1993]. Immunosuppression is not required in MZ solid organ transplantation [Tilney, 1986]. Monozygosity should be excluded before immunosuppression therapy is used after organ transplantation from a like-sexed twin donor.

The present case illustrates that DZ status should not be assumed on the basis of significant phenotypic discordance within a twin pair. Such discordance may fall within the acceptable spectrum of MZ status. Heterochromia iridum presumably represents (postzygotic) mosaicism in neural crest-derived pigmentary cells of the irides of one of the twins. Discordant skin pigmentation has been described in a pair of female MZ twins, one of whom had diploid/triploid mosaicism in skin fibroblasts [Wulfsberg et al., 1991].

If zygosity had been known at the time of transplantation in this case, complications of immunosuppression and their treatment could have been avoided. The cost of immunosuppression and other therapy is estimated at US$40,000, while zygosity testing costs about US$100 per pair.

Eloquent pleas have been made that zygosity of like-sexed twins should be determined at the time of birth, based on the known MZ status of MC twin pairs, and DNA fingerprinting in like-sexed dichorionic pairs [Fisk and Bryan, 1993; Derom et al., 1991]. To our knowledge, this is only currently available in two centers (East Flanders, Belgium and Edmonton, Alberta, Canada). For medical, psychological, and educational reasons it is often essential that zygosity be known. It

can also be argued that twin pairs have the right to conclusive and objective data on their zygosity. Accurate zygosity diagnosis is also necessary for twin genetic research and publication, particularly concerning the biology and pathology of MZ twinning, and the degree of concordance for many chronic and degenerative diseases [Martin et al., 1997]. The cost of unnecessary immunosuppression therapy in the present case could instead have been used to fund zygosity tests for about 400 twin pairs.

If it were agreed that zygosity testing for twins is neither frivolous nor expensive, the optimal methodology is currently being discussed [Keith and Machin, 1997]. Large amounts of DNA can be collected from twin placentas at birth, so blood samples will not be required subsequently. VNTR methodology can be applied to these DNA samples. In later life, it is tempting to apply noninvasive collection methods (such as buccal mucosal samples), from which small amounts of DNA can be amplified by PCR for comparison of small numbers of oligonucleotide sequences, using commercial dot-blot kits. As with older methods of zygosity testing (such as multiple blood groups and other polymorphisms), statistical probability is increased if parental DNA is also available, so that informative loci can be identified.

However, there are two important drawbacks in the use of finite numbers of oligonucleotide sequences. First, the statistical power of the method may not be sufficient to detect dizygosity, because several loci might, by chance, be occupied by the same alleles in a twin DZ pair; hence, these twins might give results interpreted as monozygosity, with serious consequences for transplantation. There is at present no generally agreed minimal number of loci that will ensure the exclusion of dizygosity. Second, it is a counterintuitive paradox that postzygotic genetic events may cause MZ twin pairs to be genetically discordant; it would be easy to diagnose pseudodizygosity in such pairs, and exhaustive multilocus testing would probably falsely designate most truly MZ twin pairs as being DZ [Keith and Machin, 1997; Martin et al., 1997]. Again, there is as yet no uniformly agreed number of locus discordances that can be tolerated in zygosity testing before the diagnosis of dizygosity becomes irrevocable. For the present, it therefore seems prudent to use restriction fragment length polymorhism VNTR analysis at low stringency, wherein smaller postzygotic mutational differences within MZ pairs can be assessed against a background of predominantly similar alleles, scanned by two or three probes. Although no definite statistical probability of monozygosity can be scored by this method, "DNA fingerprinting" is now widely accepted in forensic medicine for the identification of individuals, and is equally applicable to twin zygosity testing.

This present case shows that the imprecise and misleading words "identical" and "fraternal" twins should be abandoned in favor of the correct terms "monozygotic" and "dizygotic." Zygosity testing at birth for all like-sexed dichorionic twins would provide those twins with essential information in the event of future medical problems.

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