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Zygosity and Placental Structure in Monochorionic Twins

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Abstract. To test the assumption that monochorionic twins are monozygotic, cord bloods from 12 sets of monochorionic twins were tested for a total of 16 red cell antigens. Discrepancies were noted among the minor blood groups in 3 of the 12 sets. The result of this pilot study strengthens the contention of others who have previously questioned the general acceptance that monochorionicity and monozygosity are synonymous.

Key words: Monochorionic twins, Monozygotic twins, Placenta, Blood group antigens

INTRODUCTION

The sequence of events from conception to the postimplantation phase of blastocyst development leading to the formation of identical or monozygotic (MZ) twins are well described, and the derivation of the types of placental structure found in MZ twins has been elucidated [1,4,7,8,9]. It is also widely accepted that one type of placental structure, ie, the monochorionic placenta, is invariably the result of splitting of a single zygote during the blastocyst stage [4], so that the zygosity of individuals who share this type of placenta can be automatically inferred [1,4,7,8,9].

Several single case reports cast doubt on the validity of this concept of the scheme of postconceptional events. In particular, the comprehensive study of Bieber et al [2] on the cytogenetics and HLA profile of a monochorionic twin pair, one an acardius amorphous, and their parents, proved that this pair of monochorionic twins were genetically very different. Others have reported examples of MZ twins of unlike sex [3] or discordant for

particular malformations [6] or disease [5].

For many years at this center, zygosity testing of twins has been applied to those of like sex. Monochorionic twins have been assumed to be MZ. Accordingly, dichorionic like-sex twins only have received more detailed laboratory attention. Cord bloods from such twins have been drawn from their placentas and tested for a panel of 16 blood group antigens. Complete concordance for all such antigens has been taken as a strong indication of monozygosity; discrepancies have been interpreted as markers of dizygosity. For a period of one calendar year, the same approach has been applied to like-sex twins with monochorionic placentas. From a total of 12 such monochorionic twin sets, blood group discordance was noted in 3 (25%). The significance of this finding is discussed.

MATERIALS AND METHODS

Placentas were submitted for histopathological evaluation from 37 twin pairs in 1984. All specimens were delivered promptly, unfixed, to the laboratory. Information on the sex and weights of all such cases was provided with the laboratory request form. One of the cases was a twin abortion with features of the twin transfusion syndrome.

Following recording of general observations on size, shape and weight of placentas, the dividing septum was examined in single disc or fused specimens according to the method of Wigglesworth [9]. Gentle removal of the amniotic layer from either side of the placenta allowed easy assessment of the number of layers in the septum, and therefore chorionicity. A roll of the dividing septum was fixed in Bouin's fluid for routine light microscopic examination using haematoxylin and eosin staining, to confirm chorionicity. Additional blocks of the membranes, cords and placental parenchyma were taken for routine light microscopic study.

Subsequent to removing the membranes, the cords were clamped (if not already clamped by the labour ward staff) and coded A and B. The placentas were then thoroughly washed in tap water to remove any contaminating maternal blood. Then, the cord of all twins were unclamped, one at a time, and the blood removed from these into dry test tubes. Small volumes of blood were diluted with equal volumes of normal saline to increase their volume. The tubes were then numbered and coded for identification. Those from like-sex twins (mono- and dichorionic) were examined in the blood transfusion laboratory for the following major and minor blood groups, using standard blood transfusion methods: ABO, Rhesus, C^w, K, K-, Fy^a, Fy^b, Jk^a, S, $\bar{s}$, M, N, Pl and Lu^a. All results were recorded as positive or negative. The case of twin transfusion syndrome had its placenta and cords examined in the same way. This diagnosis was confirmed at necropsy.

Injection studies of the vasculature of single disc or fused placentas were completed at this stage.

RESULTS

A total of 37 twin sets were included in the period in question. Of these, 25 had separate placentas or dichorionic fused placentas. Of these 25 sets, 15 were of like sex and cord bloods were obtained from 13 of these for grouping; 10 were of unlike sex and were not

examined further. Of the 13 twin sets whose cord bloods were grouped, 8 were discordant (major and minor groups) and 5 were concordant for all 16 parameters, the latter adjudged to be MZ. Thus 38% of the separate or dichorionic like-sex twins were MZ.

Of the 37 twin sets, 12 were of monochorionic type. All 12 were of like sex. All 12 had cord blood group analysis performed. Complete concordance was found in 9, with discordances noted in 3 (25%). The discrepancies were always confined to single parameters among the minor blood group antigens, viz, K+ /K−, Pl+ /Pl−, and Jk^a+ /Jk^a−, the latter in the case of twin transfusion.

For the total of 35 twin sets studied according to sex, placental structure, and cord blood group antigen profiles, 40% were adjudged to be MZ, while 25% of those of like sex with a monochorionic placental structure appear to be genetically dissimilar.

DISCUSSION

The preliminary evidence presented from this pilot study of 12 monochorionic twin sets, based on detailed blood group antigen profile analysis, implies that one quarter of the pairs in this series are genetically dissimilar. This is a surprising observation and quite unanticipated, since monochorionic twins are generally regarded as MZ and therefore genetically identical. Several possible explanations require consideration.

Firstly, the possibility of a laboratory error must be entertained. This would be an extremely serious situation, as these analyses were performed in a regional reference laboratory for blood transfusion whose work would be seriously undermined by such a high frequency of error. These results were indeed checked and shown to be correct.

Secondly, mixing of fetal bloods could account for some errors. Indeed, all of the monochorionic twin placentas exhibited artery to artery and vein to vein anastomoses on their fetal surfaces when injected. However, mixing of bloods at this level, in MZ individuals, would not be expected to produce genetically determined differences and, as no chimaeric results were obtained, contamination by maternal blood is an unlikely explanation. The latter possibility should be circumvented by the care taken in extracting cord bloods. Thirdly, a genetic mutation in somatic cell division in one of any set could conceivably produce such minor blood group discordances in MZ twins, with one individual losing a particular phenotypic character retained by the other nonmutated twin. This seems an attractive proposition, although the frequency of such a development (25%) seems rather high. Others have previously argued along these lines for whole chromosomal gains or losses during somatic cell division [3,6]. The implication of this suggestion is similar to the final possible explanation ie, that monochorionic twins are genetically different.

Lastly, monochorionic twins, in at least some instances, may not be MZ. Bieber's dramatic case report provides strong evidence for this condition [2] and it is felt that this pilot study provides support for this idea. This is an extremely important question with widespread implications for all twin-based studies of epidemiology and genetics regardless of wheter the purported genetic differences between monochorionic twins arise by DZ conception or postconceptional somatic genetic mutation. The urgency of this question is under consideration in this laboratory, where this study is currently being repeated in a larger prospective series, with collaboration of the findings using in-situ hybridization and Southern blotting to analyse the DNA content of twin cord blood cells. Meanwhile, it

is important to avoid using the terms monochorionic and monozygotic interchangeably. In addition, it is stressed that these findings are preliminary and await confirmation.

CONCLUSION

Evidence of blood group discrepancies from a pilot study of 12 monochorionic like-sex twin sets implies that monochorionic twins are not always genetically identical, the differences probably arising either through genetic mutations in somatic cell division or via dizygous fertilization.

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