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The European Multiple Birth Study (EMBS)

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Abstract. The more that twin and other multiple pregnancies are investigated, the more it becomes mandatory that collaborative studies are set up in order to attain the critical number of cases needed to achieve meaningful and reliable results. The European Multiple Birth Study (EMBS) aims to study two aspects of twin and multiple pregnancy: 1) management of pregnancy and labour, with emphasis on the prevention of preterm delivery; 2) accurate determination of zygosity, a prerequisite for the proper use of the twin method in a variety of fields, eg, congenital malformations, genetics, clinical investigations, etc. The rationale, the methods and the organisation of the study are described and discussed.

Key words: Twin register, Twin pregnancy, Neonatal care, Zygosity, Birthweight, Congenital malformations

INTRODUCTION

Recent twin research has shown that the study of twins is very attractive to clinical epidemiologists and geneticists because of its ability to unravel difficult issues of causality [3]. Furthermore, the twin method has been shown to be the most powerful method for studying the fascinating question of nurture vs nature [8]. It allows the detection not only of genetic effects but also two particular types of environmental sources of variations: the common environment and the specific environment effects [4]. On the other hand, a large number of cases are required for the proper use of the twin method in a variety of

fields. For this, we are establishing a European registry of twins (EMBS, European Multiple Birth Study) with accurate determination of zygosity at the time of birth, which will be the largest of its kind. Its establishment is a major objective in itself and will eventually constitute a valid sample of MZ and DZ twins for genetic and clinical studies.

METHODS AND RESULTS

Accurate Determination of Zygosity

Several methods are used for the diagnosis of zygosity at birth in our survey:

1) Determination of the sex of the infants;
2) Accurate recording of the placental structure including histology of the dividing membrane;
3) Determination of a series of blood groups: ABO, Rhesus, MNSs, Duffy and Kell;
4) Placental alkaline phosphatase [10];
5) Determination of Restriction Fragment Length Polymorphisms (RFLPs) on placental tissue [2].

Only in same-sex dichorionic pairs, approximately 25% of all twins, is there a need to resort to the study of genetic markers [2,4,10]. Using the first four classical methods of zygosity determination, an overall correct diagnosis ($P > 0.95$) of zygosity can be made in 92% of the twin pairs. Only in the remaining 8%, will DNA genotyping be necessary and relevant. The pairs with at least two different genetic markers are classified as DZ. Determination of the blood groups will be done in each collaborating teaching hospital.

A sample of placental tissue corresponding to each infant will be sent to the Department of Obstetrics, University of Gent, at regular intervals. There placental alkaline phosphatase determinations are done routinely and the RFLP DNA genotyping will be elaborated further.

Collection and Processing of the Data

Depending on their interests, each collaborating centre will fill out one of the two EMBS files (available on request from senior author) and send it, together with a piece of placental tissue, to the coordinating centre in Leuven. The collaborating centre will cover the cost of the gross and microscopic examination of the placenta and the umbilical-cord blood typing. The determination of placental alkaline phosphatase and the placental DNA genotyping will be performed free of charge at the Department of Obstetrics, University of Gent. Costs of equipment, mailing and storing placental samples will be covered by a Belgian grant.

The basic data sheets of each twin pair will be fed into the IBM 3033 and IBM 4331 computers of the centre in Leuven. Results of the investigation of placental tissue (ie, alkaline phosphatase and DNA genotyping) will be added in the same way to the twins' charts. The final results of the zygosity determination of each twin pair will be sent to the different collaborating centres.

DISCUSSION

The authors already have experience in the collection and analysis of twin data with regard to the menagement of pregnancy and labour [9], the investigation of the placenta and the determination of genetic markers. They have developed appropriate forms and guidelines, computer programmes for storing and analysing the data and a large data bank of twin literature. Their registry already numbers more than 2900 twin pairs with known zygosity and placentation. Their facilities could be easily expanded in order to accommodate a large number of additional cases.

The specific aim of EMBS is to gather a sufficient number of cases in order to achieve meaningful and reliable results. In a first instance, EMBS will study the following two aspects:

1) Management of twin pregnancy and labor. Twin pregnancies involve a much higher preterm delivery rate than single ones and low-birthweight babies are much more frequent [1,5,12]. The very-low-birthweight rate is used as the principal predictor of neonatal mortality in industrialized countries [7]. A preterm labour prevention policy must be applied to lower neonatal mortality rates.

2) Etiology of congenital malformations in twins with known zygosity. Until now, the study of malformations in twins has not been used to its full extent for various reasons:

- Most series are too small to include a sufficient number of malformed children.
- In most large studies the diagnosis of zygosity is not accurate and has been considered only indirectly by classifying twin pairs as of like sex or unlike sex [6,11,13]. In a number of instances the investigations do not begin at the optimal time, ie, the time of birth.
- Finally, in the group of paramount importance, the MZ twins, insufficient attention is paid to the type of placentation, the influence of which has yet to be studied, on a larger scale.

Several centres have already agreed to participate and it is planned to invite others so that meaningful numbers will be obtained as rapidly as possible.

Questionnaires, forms and guidelines are available from the first author upon request.

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