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Study on Possible Increase in Twinning Rate at a Small Village in South Brazil

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Abstract. A high frequency of twin births has been observed in Linha São Pedro, a small settlement which belongs to the city of Cândido Godói, located 524 km Northwest from Porto Alegre, Rio Grande do Sul, Brazil, in an ethnically homogeneous population of German descent restricted to a small geographic region. From 1990 to 1994, the proportion of twin births in Linha São Pedro was 10%, significantly higher than the 1.8% rate for the state of Rio Grande do Sul as a whole. Genealogical analysis showed a high recurrence of multiple births within families, as well as a high level of inbreeding in the community. Zygosity data indicated that 9 of the 17 pairs of twins studied (53%) were dizygotic. No external environmental factors were detected that could be influencing the appearance of this characteristic. This preliminary investigation confirmed the presumed existence of a high twinning rate in the community. The high familial recurrence and the high inbreeding rate suggests the presence of genetic twinning factors. Complementary studies of twins that have yet to be evaluated and the search for additional risk factors, as well as linkage studies, should contribute to a further understanding of the biological factors related to twin births in the human species.

Key words: Twinning rate, Twin births, Monozygotic twins, Dizygotic twins, Genetic factors

INTRODUCTION

No precise explanations exist for the twin phenomenon. Some hypotheses have tried to correlate fertility, maternal age, and hormone levels with the predisposition to twin births. Factors of predisposition for dizygotic twins include maternal age, parity, race and a family history of dizygotic twins [6]. In contrast, a predisposition for monozygotic twins has been considered to be independent of genetic factors [6, 18, 8, 2]. Its frequency

is the same among different racial groups, independent of maternal age and family history [6]. Other factors that could possibly affect its occurrence are not known.

It is difficult to differentiate single gene from multiple gene heredity in twinning. Ethnic differences in dizygotic twin rates are evidence that genetic factors are involved. The fact that the rate appears to be maternally inherited in interracial marriages also corroborates the maternal heredity hypothesis [12]. At the same time, other factors such as parity, maternal age, marital status and social class of the mother, cannot be disregarded [18, 7].

Linha São Pedro is a small village which belongs to the city of Cândido Godói, situated along an unsurfaced road in the Northwest region of the state of Rio Grande do Sul, Brazil, 35 km from Santa Rosa and 524 km from Porto Alegre. The population basically consists of German descendants. In 1993 its inhabitants conducted a survey of the number of pairs of twins among 84 families of the region, registering, within a radius of 4 km, 34 pairs of twins. Interested in knowing more about this, near the end of 1994 members of the community contacted the Medical Genetics Unit of the Hospital de Clinicas de Porto Alegre (HCPA).

In the literature there are few cases related to a high incidence of twins restricted to such a small area and among such an ethnically homogeneous population as exists in Linha São Pedro. As such, the study of this population could contribute to an explanation of the mechanisms involved in the etiology of twin births. Thus, the objectives of the present study were as follows:

1. to determine if the frequency of twinning in the population of Linha São Pedro, Cândido Godói municipality, RS, is significantly higher than expected;
2. to evaluate if the pairs of registered twins are monozygotic or dizygotic;
3. to identify the presence of possible risk factors for twinning in the population studied;
4. to propose mechanisms that could explain the phenomenon.

MATERIAL AND METHODS

All the pairs of twins residing in Linha São Pedro were interviewed, as well as those originally from Linha São Pedro but now living in nearby areas (Linha Santo Cristo and Linha São Miguel). In cases where the twins could not be located, or if they were children, immediate family members such as parents, grandparents, aunts and uncles were interviewed.

Data from the state of Rio Grande do Sul provided by the the Division of Health Information of the State Secretary of Health and Environment, were used as a reference guide concerning variables related to the incidence of twin births.

A detailed genealogy was obtained from each family, including the records of twin births, ancestral origin and kinship relations among the families who live in the community. In addition, data were obtained about existence of consanguinity or genetic diseases, as well as relevant data concerning the twins and their mothers, date of birth, development, maternal age and parity, the use of contraceptives or any fertility treatments.

A sample of 10 ml of blood in EDTA was taken from each pair of twins for DNA

analysis. Informed consent was obtained from each twin who participated in the study. Zygosity was determined by Southern Blotting with the Hae III restriction enzyme and hybridization with the single locus probes PLH1 – D5S110 [1]; CEB 42 [17]; p YNH24 – D2S44 [14]; pH 30 – D4S139 [13]; pTBQ7 – D10S28 [3]; EFD52 – D17S26 [15] and the multiple loci probes p 33.15 and p 33.6 [10].

Data related to the incidence of twin births were compared by the Chisquare test of homogeneity. Mean maternal age and number of children were compared between the mothers of mono – and dizygotic twins by the Wilcoxon – Mann-Whitney test.

RESULTS

The population of Linha São Pedro is composed of 349 habitants belonging to 84 families. Among these, 22 pairs of twins were found representing 11% of the population. Blood was collected from 17 of them for analysis of zygosity, as well as information about maternal age and number of siblings. It was not possible to collect blood from five pairs of twins. These data are presented in Table 1.

None of the mothers of twins interviewed (n = 22) reported the use of contraceptives or fertility treatments. The interviews produced no cases of genetic disease, or of disease of possible genetic etiology, among the twins. Only two cases revealed health problems affecting one of the members of the twin pair, one being a heart condition (the mother did not know the name of the condition) and the other a case of epilepsy. The interviews also generated data on deceased twins, on those no longer living in the region, and those who were not contacted during the first study completed by the community. Thus, these data include 48 pairs of twins related to the 84 families that live in Linha São Pedro. It was not possible to obtain data with respect to placental type, which would have served as a secondary indicator of zygosity, because the births occurred in regional hospitals and the mothers never came to know this information. In many cases it was not possible to find out the sex of twins who live in distant places because of imprecise information.

The mean maternal age for dizygotic twins mothers was 26.7 and for mozygotic twins 27.0. The mean number of siblings was 5.1 for dizygotic twins and 5.4 for monozygotic twins. These differences were not statistically significant ($p = 0.54$ and $p = 0.70$ respectively) both for maternal age, as for the number of children of mothers of mono – and dizygotic twins. One should consider the small sample size, especially in the case of monozygotic twins, that may have contributed to the lack of statistical significance.

Molecular analysis revealed that 9 (53%) of the 17 pairs studied are dizygotic (Table 1). The rest of the pairs are monozygotic (the probability of misdiagnosis using the previously mentioned probes is very low).

From 1990 to 1994, in accordance with the survey done by the community, there were 49 births in Linha São Pedro. Of these, 5 were twin births, which correspond to 10% of the births during this period. Data provided by the Information Division of the Secretary of Health and Environment of Rio Grande do Sul for the period from 1992 to 1994 show that the rate of twin births for this period in the state was 1.8%. Thus, it is confirmed that the incidence of twin births in Linha São Pedro is significantly higher in relation to the rest of the state ($p = 0.0189$).

Table 1 - Profile of the study sample with reference to twin pair age, maternal age, number of children and zygosity

Pair	Age	Maternal age at delivery	Number of children	Zygosity
1	30	27	7	MZ
2	40	–	7	DZ
3	29	37	13	MZ*
4	32	19	8	DZ
5	18	33	8	DZ
6	2	22	2	MZ*
7	30	22	4	DZ
8	15	20	4	MZ*
9	8	28	3	DZ
10	5	32	7	DZ
11	2	27	–	DZ
12	12	29	5	DZ
13	11	23	–	DZ
14	8	34	5	MZ*
15	4	30	4	MZ*
16	16	–	3	MZ*
17	2	19	2	MZ
18	13	28	4	DZ**
19	50	–	2	DZ**
20	33	18	9	not tested
21	2	26	3	DZ**
22	3	25	–	not tested

* very small probability of being dizygotic « 1/64

** not tested, dizygozity determined by difference in sex

DISCUSSION

It has been shown that the twinning rate in Linha São Pedro is quite high in relation to what is described in the literature. This fact stands out even more when one considers the ethnic origin of the individuals, especially since, according to several authors [2], dizygotic twinning rate is highest among Negroids, followed by Caucasoids. It is important to mention that the population in question, mostly of German origin, does not show any level of mixture with Black individuals.

The proportion of mono – and dizygotic pairs (47% and 53%) is slightly above the expected levels, i.e., approximately 30% monozygotic and 70% dizygotic. Monozygotic twinning is often considered to be sporadic and only modestly influenced by genetic background [16]. Our results seem to be in contradiction to this acceptation. The incidence of twin births for the period from 1990 to 1994 in Linha

São Pedro (10%) was much higher than reported by for the Brazilian population as a whole (0.8%). The incidence of twinning in the state of Rio Grande do Sul, in the same period, was 1.8%, which confirms the high rate of twin births in Linha São Pedro and reveals what seems to be a high twinning rate for this state. The population of African descent is considered less important in Rio Grande do Sul than in other Brazilian states. If we consider specifically the case of Linha São Pedro, as far as we know, no ancestry of African origin could have contributed to the rate of multiple birth. The Linha São Pedro case seems to be an isolated phenomenon, to which other etiological factors could be contributing.

The high familial recurrence demonstrated in the genealogies (Figure 1) suggests the presence of genetic twinning factors, which seem to predispose to both monozygotic and dizygotic twinning. A common mechanism for both types of twinning was already proposed by a group of researchers [6]. Considering the pattern of heredity, we think it is more likely a monogenic trait although the involvement of several genes cannot be ruled out. Many authors suggest that the genetic factors involved, although being few, may be pleiotropic [5], and that polygenic effects and genetic heterogeneity [4] may be involved. The high level of consanguinity and the absence of twins every other generation suggest a recessive trait, although this condition is obviously not fully penetrant. As for the penetrance, the twinning rate may be regarded as greatly underscored because it is well known that a twin pregnancy may end with a unique birth, by disappearance of what is called a "vanishing foetus". Some even estimate that up to 70% of twin conceptions do not come to term as twins [9].

The absence of environmental factors commonly associated with twinning, such as contraceptives and fertility treatments, makes the involvement of genetic twinning factors even more plausible. Thus, a concentration of genes that predispose to twinning appears to have occurred, genes probably present in the small gene pool that founded the population which has remained relatively isolated with a high level of inbreeding (drift). This situation, along with other conditions, such as the community being rural with large

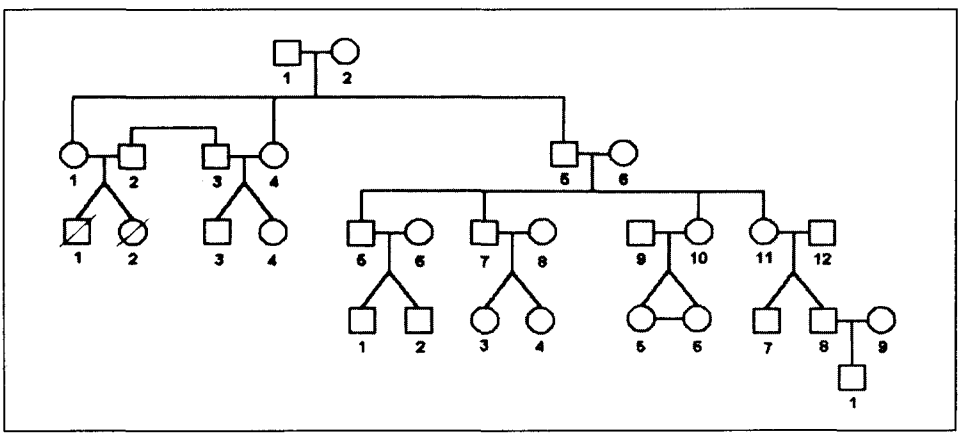

Fig. 1 - Example of one of the genealogies encountered in Linha São Pedro. Note the high familial recurrence and the lack of a clear pattern of heredity.

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numbers of children per family and a relatively long life expectancy, favors the high rate of twinning documented in the area.

The present study was a preliminary step, permitting the confirmation of the high rate of twinning in Linha São Pedro and attributing the principal cause to genetic factors. The mechanisms involved must be focused upon in further investigations, specially linkage studies through which genes with major influence on female fertility might be detected [11].

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