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Cytogenetics of genetic counseling patients in Pelotas, Rio Grande do Sul, Brazil

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ABSTRACT. From 1986 to 2002, we examined the chromosomal composition of 916 patients attended by two genetic counseling services in the city of Pelotas, in the Brazilian State of Rio Grande do Sul, to determine the genetic causes of their disturbances. Patterns of G-banding using trypsin and Giemsa (GTG) and C-banding using barium and Giemsa (CBG) were studied using phytohemagglutinin M-stimulated lymphocytes cultured from peripheral blood. Among the patients, 110 had Down's syndrome, 7 had Edward's syndrome, 4 had Patau's syndrome, 29 had Turner's syndrome, 5 had Klinefelter's syndrome, and 3 had "cri-du-chat" syndrome. Abnormal chromosomes were observed in 29.3% of the patients. Most of these (56.3%) were numerical abnormalities, with the remaining being structural variants.

Key words: Chromosomal disorders, Genetic counseling

INTRODUCTION

Chromosomal abnormalities affect at least 7.5% of all conceptions. Most of these abnormalities are spontaneously aborted and the frequency in live births is 0.6% (Connor and Ferguson-Smith, 1991). Three to four percent of all births are associated with a major congenital malformation, mental retardation, or genetic disorder, a rate that doubles by 7-8 years of age, with later-appearing or later-diagnosed genetic disorders (Milunsky, 1992).

Centers for genetic counseling are important sources of information about the frequencies of chromosomal disorders, with such data allowing a more detailed analysis of these disorders (Schinzel, 1984; Mitelman, 1985). In addition, these centers allow us to determine the types or profiles of individuals who seek genetic counseling (such profiles tend to vary among developed and developing countries), and also to establish the pattern and extent of chromosomal variability in distinct human populations (Geiger et al., 1987).

Although chromosomal disorders are included among the most important causes of childhood mortality in Latin American countries (OPS, 1984), in most Latin American countries, such disorders have not received much attention from state and federal governments, partly because the main health problems responsible for childhood morbidity and mortality have socioeconomic and environmental, rather than genetic, origins. As a result, few public health services perform cytogenetic studies, and most use antiquated techniques, due to funding restrictions.

We made a chromosomal analysis of patients attended by two Genetic Counseling Services in the city of Pelotas, in the southern Brazilian State of Rio Grande do Sul.

MATERIAL AND METHODS

One thousand seven hundred and four patients were attended by the Genetic Counseling Services in Pelotas from 1986 to 2002. Of these, 916 (53.8%) were analyzed cytogenetically. Blood samples were collected from the patients into heparinized test tubes. Cytogenetic analyses were done on cultured peripheral blood lymphocytes, stimulated with phytohemagglutinin M, using standard techniques (Moorhead et al., 1960). The karyotype of each patient was determined by G-banding using trypsin and Giemsa (GTG) (Caspersson et al., 1970) and C-banding using barium and Giemsa (CBG) (Salamanca and Armendares, 1974) when necessary. At least 30 cells were routinely analyzed; in cases of mosaicism, this number was increased to approximately 100 metaphases. The best metaphases were photographed to determine the karyotypes. Whenever translocations or unusual karyotypes were found, blood samples were collected from both parents and their chromosomes were also studied.

RESULTS

Of the 916 patients, 48.5% were females and 50.9% were males. Abnormal chromosomes were found in 29.3% of the cases, with 56.3% of these being numerical abnormalities; the remaining were structural variants. Cytogenetic analysis of the parents of individuals with structural abnormalities or of those with an unusual karyotype revealed normal chromosomes, except for one case of 46,XY, inv(12)(q23q24), in which the patient's mother had the same abnormality.

Among the abnormal karyotypes, 83.6% had autosomal alterations and the rest were sexual abnormalities (Table 1). Among the numerical abnormalities, 21.2% involved mosaicism and 78.5% had trisomy or monosomy. Among the autosomal chromosomal alterations, the most frequent were Down's syndrome (49.1%), Edward's syndrome (3.1%) and Patau's syndrome (1.8%). Turner's syndrome was the most frequent sexual abnormality (79.5%), followed by Klinefelter's syndrome (11.4%).

Table 1. Cytogenetic results for 916 patients examined by Genetic Services in Pelotas, RS, Brazil, from 1986 to 2002.

Chromosomal abnormalities	Karyotype	Number of cases	Percentage	Sex
Down's syndrome	47,XX,+21 or	45	4.9	F
	47,XY,+21	55	6.0	M
	46,XX/47,XX,+21	2	0.2	M
	46,XX,-14,+t(14q21q)	1	0.1	F
	46,XX,-21,+t(21q21q)	6	0.7	3M/3F
Edward's syndrome	47,XX,+18 or			
	47,XY,+18	6	0.7	3M/3F
	46,XX/47,XX,+18	1	0.1	M
Patau's syndrome	47,XY,+13	4	0.4	M
"Cri-du-chat" syndrome	46,XX,5p$^-$ or			
	46,XY,5p$^-$	3	0.3	2M/1F
Turner's syndrome	45,X	8	0.9	F
	45,X/46,XX	16	1.7	F
	46,XX, del(Xq)	3	0.3	F
	46,X, i(Xq)	2	0.2	F
Klinefelter's syndrome	47,XXY	5	0.5	M
Somatic mosaicism with 2	45,X/46,XX/47,XXY	1	0.1	M
or 3 cell lines	46,XX/46,XY	1	0.1	M
	46,XX/47,XXX	3	0.3	F
	45,X/46,XY	2	0.2	M
Other abnormalities		105	11.5	
Normal	46,XX or 46,XY	646	70.5	323M/323F
Cases with unsatisfactory cell growth		11	1.2	5M/6F
Total		916	100	

DISCUSSION

This karyotype information provides a foundation for a regional cytogenetic data library designed to help in the genetic counseling of families who require this service. Using this approach, a definitive diagnosis for a chromosomal disorder was reached in 29.3% of the patients. The frequency of chromosomal anomalies was higher than the 16.9% found by Miranda and Santos (1995), 5.5% by Brum (1999), 16% by SAG-EFES (Serviço de Aconselhamento Genético da Universidade Federal do Espirito Santo, 1998), and 3.8% by Kumar et al. (2001), while it was similar to the 28.6% found by Santos et al. (2000). The differences in the frequencies of the

chromosomal abnormalities among these studies could reflect variations in the criteria for inclusion of the patients and in the cytogenetic methods used.

Abnormalities involving the sex chromosomes

Among patients with Turner's syndrome, the most frequent sexual abnormality observed in this study, somatic mosaicism (45,X/46,XX) was more common (53.6%) than the classic karyotype (45,X) (28.6%) and structural abnormalities (17.9%). Klinefelter's syndrome was the second most frequent sexual abnormality, and only 20% of the cases showed the classic, well-defined phenotype, whereas the others had various types of sexual behavior problems.

Of the patients with sexual abnormalities, 3.5% were cases of somatic mosaicism with three cell lines (45,X/46,XX/47,XXX). Their phenotype included short stature and primary amenorrhea. An additional 3.5% were patients with 46,XX/47,XXX, and 7% had 45,X/46,XY. In the latter cases, the clinical findings were behavioral problems, and genital ambiguity resulting from overlap of the phenotypic characteristics produced by the 45,X and 46,XY cell lines.

Autosomal trisomies

Down's syndrome, the most common anomaly in the group of trisomies, was also the most frequent (47.1%) of all the chromosomal abnormalities. Nearly 60% of the Down's syndrome cases were children less than one year old, indicating that the diagnosis was precocious in most cases. Males accounted for 54.6% of the Down's cases. In a study with a larger number of Down's cases, Astete et al. (1991) observed a similar sex ratio (56.4% males).

Similar to previous studies (Jones, 1998), there was considerable karyotypic variability in individuals with Down's syndrome, including cases of free trisomy (51.8%) and partial free trisomy (1.8%) of chromosome 21, as well as Robertsonian translocations between chromosomes 14 and 21 (0.9%), and between the two chromosomes 21 (5.5%). These observations emphasize the importance of cytogenetic confirmation in cases of Down's syndrome. In addition to indicating the risks of recurrence of the syndrome, karyotyping can also be useful in the clinical follow-up of some disorders associated with Down's syndrome. As a result, it is possible to inform the patient's family about the susceptibility to acute leukemia, duodenal stenosis, and Alzheimer's disease, and to help them with the associated symptoms (Santos et al., 2000). Treatment can then be introduced at an early stage, thereby increasing the patient's life expectancy.

Of the six individuals diagnosed with Edward's syndrome, two males and three females had the classic karyotype (47,XY,+18 and 47,XX,+18), and one (female) showed somatic mosaicism (47,XX/47,XX,+18). According to Giaccardi et al. (1991), most fetuses with trisomy 18 are spontaneously aborted. When the pregnancy is brought to term, the post-natal lifetime is limited to one-two months in 80% of the cases, except when there is somatic mosaicism. Our patient with mosaicism died five months after the other individuals.

Only four females were identified as having Patau's syndrome. This disease is well known for its low life expectancy and the well-defined features that allow an early diagnosis in the first days of life, except in cases of mosaicism. Taylor (1968) reported the mean lifetime of children with trisomy 13 to be 89.2 days, although there can be exceptions. One of the patients, whose mother was 37 years old, with a classic karyotype had been accompanied until two years

and four months old. Somatic mosaicism was not observed in this individual. According to Fernandez et al. (1996), the detection of mosaicism depends mainly on the type and number of tissues analyzed, the number of cells studied, the sensitivity of the techniques applied, and the possible selection that may result in the disappearance of cell lines.

Structural chromosomal abnormalities

Three patients were diagnosed as carrying a deletion on chromosome 5 ($5p^-$), characteristic of "cri-du-chat" syndrome (Table 2). The breakage occurred in the 5p15.2 region, as reported in the literature. Gersh et al. (1995) stated that the critical 5p15.3 region contains a gene, which when it appears as a single copy, is responsible for the cat-like cry, whereas the genes responsible for the facial features and motor delay are located in the 5p15.2 region. The children in our study had the clinical characteristics of the syndrome. The children's parents were examined to determine whether one of them carried a reciprocal translocation involving 5p or had mosaicism with normal and $5p^-$ cells. No chromosomal alterations were found in the parents, thus greatly reducing the risk of recurrence in future siblings.

Table 2. Rare structural chromosomal abnormalities encountered in 916 patients examined by the Genetic Services in Pelotas, RS, Brazil, from 1986 to 2002.

Chromosomal abnormalities	Karyotype	Number of cases	Percentage (%)	Sex
Deletions	46,XY, del(1)(p36)	1	0.1	M
	46,XY, del(7)(q22;q36)	1	0.1	M
	46,XY, del(9)(p24)	1	0.1	M
	46,XY, del(9)(q13)	2	0.2	M
	47,XX, del(13)(q31;q34)+mar	1	0.1	F
	46,XX, del(14)(q12)	1	0.1	F
	46,XX, del(17)(p12;p13)	1	0.1	F
Duplications	46,XX, dup(3)(p22;p25)	2	0.2	F
	46,XX, dup(5)(q31;q32)	1	0.1	F
	46,XX, dup(8)(q12)	2	0.2	F
	46,XY, dup(10)(q25;q26)	1	0.1	M
	46,XX, dup(12)(p+)	1	0.1	F
	46,XY, dup(16)(q21)	1	0.1	M
Inversions	46,XY, inv(9)(q12;cent)	2	0.2	M
	46,XX, inv(12)(q23;q24) and 46,XX, inv(12)(q23;q24)	2	0.2	1M/1F

del = deletion, dup = duplication, inv = inversion.

CONCLUSION

Although cytogenetic techniques are sometimes tedious, they are very important for the correct identification of a variety of syndromes. The information obtained by such techniques provides a basis for determining the risks of recurrence and for deciding clinical treatment and

genetic counseling. However, the success of providing an accurate clinical diagnosis with cytogenetic techniques could be improved using fluorescence *in situ* hybridization and other complementary molecular approaches; however, these are not always available in small cities in developing countries. The replacement of cytogenetic diagnosis by direct DNA diagnosis is certainly of great importance, but cytogenetics still has an important role in Latin American public hospitals since molecular diagnosis is not yet a routine procedure in many of these institutions.

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