Chromosomal investigations in patients with mental retardation and/or congenital malformations

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Abstract

We investigated the chromosomal constitution of patients with mental retardation and/or congenital malformations in order to determine genetic causes for such disturbances. The GTG and CBG banding patterns were studied using phytohemagglutinin M-stimulated lymphocytes cultured from peripheral blood. Among 98 individuals with mental retardation and/or congenital malformations who were analyzed there were 12 cases of Down's syndrome, two of Edward's syndrome, one of Patau's syndrome, five of Turner's syndrome, two of Klinefelter's syndrome, one of "cri-du-chat" syndrome, one case of a balanced translocation between chromosomes 13 and 14, one case of a derivative chromosome and one of a marker chromosome. We found abnormal chromosomes in 26% of the patients, 82% of which were numerical abnormalities, with the remaining 18% being structural variants. We conclude that patients with mental retardation and/or congenital malformations should be routinely karyotyped.

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 percent to four percent of all births are associated with a major congenital malformation, mental retardation, or genetic disorder, a rate that doubles by seven to eight years of age, with later-appearing or later-diagnosed genetic disorders (Milunsky *et al*., 1992). A great number of chromosomal disorders are associated with mental retardation. According to the World Health Organization, the prevalence rate of mental retardation in industrialized countries comes close to 3% (Roeleveld *et al*., 1997). In developing countries, the prevalence of mental retardation is higher. In Brazil it has been estimated as 6.7 per 1000 live births (Félix *et al*., 1998).

In most Latin American countries, chromosomal disorders have not received much attention from state and federal governments, partly because the main health problems responsible for childhood morbidity and mortality are of socioeconomic and environmental origin. Chromosomal disorders are included among the most important causes of childhood mortality in Latin American countries (OPS, 1984); nevertheless, few public health services perform cytogenetic studies.

MATERIAL AND METHODS

We examined the frequency of chromosomal disorders in Rio de Janeiro (Brazil) over a two-year period. The patients were investigated by a public health genetic service and were initially screened at the Hospital Universitário Pedro Ernesto. The subjects consisted of patients with mental retardation and congenital malformations who were identified by a clinical geneticist as possibly carrying a chromosomal abnormality. Ninety-eight individuals were analyzed (45 males, 53 females; sex ratio = 0.85). The subjects ranged in age from two days old to 36 years old ($\overline{x} = 11.5 \pm 10.6$ years). There was no case of consanguinity. Most of the patients were from the pediatrics department, although a few others were from the neurology department, the main emergency room and adjacent hospitals. The analyzed individuals included 98 patients with congenital malformations, 85% of whom had both mental retardation and congenital malformations, while 15% had only congenital malformations. Blood samples were collected from all patients into heparinized-test tubes. Cytogenetic analyses were performed on cultured peripheral blood lymphocytes stimulated with phytohemagglutinin M, using standard techniques (Moorhead *et al.*, 1960). The karyotype was determined in all patients by GTG banding (Caspersson *et al.*, 1970) and, when necessary, CBG banding (Salamanca and Armendares, 1974). At least 30 cells were routinely analyzed, but in cases of mosaicism this number was increased to approximately 100 metaphases. The best metaphases were photographed to determine the karyotypes. In all cases in which translocations or unusual karyotypes were found, blood samples were collected from both parents and their chromosomes were studied.

RESULTS

Abnormal chromosomes were found in 26% of the patients, 82% of which were numerical abnormalities with

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the remaining (18%) being structural variants (Table I). The frequency of abnormal chromosomes in the individuals with congenital malformations and/or mental retardation was approximately 29%. Cytogenetic studies of the parents of individuals with translocations or of the other cases with an unusual karyotype showed normal chromosomes, except for one case of Down's syndrome (DS) with a translocation between chromosomes 14 and 21, where the patient's mother had a derived chromosome in some of her cells.

DISCUSSION

The results obtained provide the basis of a regional cytogenetic data library designed to help in the genetic counseling of families who require this service. With this approach, a definitive diagnosis for a chromosomal disorder was recorded in 28.6% of the patients with mental retardation and/or congenital malformations. The frequency of chromosomal anomalies was higher than that found by Fryns *et al.* (1986) (15.03%), Dereymaeker *et al.* (1988) (13.3%) and Fryns *et al.* (1990) (17.6%), but lower than that observed in two other studies (Gustavson *et al.*, 1977; Laxova *et al.*, 1977): 32 and 32.2%, respectively. The differences in the frequencies of chromosomal abnormalities among these studies are probably due to variations in the criteria for inclusion of patients and the cytogenetic methodology applied.

Abnormalities involving the sex chromosomes

Among Turner's syndrome patients, the most frequent sexual abnormality in our findings, somatic mosaicism (45,X/46,XX) was more common (four of five) than the classic karyotype (45,X). Klinefelter's syndrome was the second most frequent sexual abnormality and showed the classic, well-defined phenotype. Small testes were observed in both patients, whereas gynecomastia, which appears in only 1/3 of Klinefelter patients (Jorde *et al.*, 1996), occurred in one of the two patients.

There were two cases of somatic mosaicism with three cell lines involving the sex chromosomes. In the first case (45,X/46,XX/47,XXX), the phenotype included short stature and primary amenorrhea resulting from the 45,X cell line. The other abnormal cell line (47,XXX) had no well-defined expression because of X chromosome inactivation. In the second case (45,X/46,XY/47,XYY), the clinical findings were genital ambiguity, resulting from overlap of the phenotypic characteristics produced by the 45,X and 46,XY cell lines, and behavioral problems, such as offensiveness and psycho-social difficulties.

Even though fragile X syndrome is the most common form of inherited mental retardation and the second most frequent cause of mental retardation after DS, the diagnosis of this disorder based on cytogenetic detection of the fragile site Xq27.3 is laborious, since the fragile site has to be induced by specific chemical treatments, which could provoke misdiagnosis. In addition to the difficulties of identifying fragile X expression in females, there is considerable potential for false negatives due to males that are mosaic for the full mutation, and false positives due to closely linked fragile sites (FRAXE and FRAXF). Moreover, it does not identify the clinically normal carriers, limiting the cytogenetic value in genetic counseling (Pimentel, 1999). That is the reason why we did not include patients referred for fragile X analysis in this cytogenetic study.

Table I - Cytogenetic results for 98 mental retardation patients from the Hospital Universitário Pedro Ernesto and other adjacent hospitals, showing the frequency of abnormalities and the numbers of each sex involved.

Chromosomal abnormality	Karyotype	Number of cases	Percentage (%)	Sex
Down's syndrome	47,XX,+21 or 47,XY,+21	9	12.2%	1 M/11 F
	46,XX/47,XX,+21q-	1		
	46,XX,-14,+t(14q21q)	1		
	46,XX,-21,+t(21q21q)	1		
Edward's syndrome	47,XX,+18	1	2%	1 M/1 F
	46,XX/47,XX,+18	1		
Patau's syndrome	46,XX/47,XX,+13	1	1%	1 F
Turner's syndrome	45,X	1	5.1%	5 F
	45,X/46,XX	4		
Klinefelter's syndrome	47,XXY	2	2%	2 M
"Cri-du-chat" syndrome	46,XY,5p-	1	1%	1 M
Balanced translocation	45,XY,-13,-14,+t(13;14)	1	1%	1 M
Somatic mosaicism with 3 cell lines	45, X/46, XX/47,XXX	1	2%	1 M/1 F
	45,X/46,XY/47,XYY	1		
Derivative chromosome	46,XX/47,XX,+der(D)	1	1%	1 F
Marker chromosome	46,XY/47,XY,+mar (21)	1	1%	1 M
Normal	46,XX or 46,XY	57	58.2%	32 M/25 F
Cases with unsatisfactory cell growth	-	13	13.3%	5 M/7 F

M = male; F = female

Autosomal trisomies

DS, besides being the most common anomaly in the group of trisomies, was also the most frequent (11%) of all the chromosomal abnormalities. Eight of the 12 DS cases were children less than one year old, indicating that the diagnosis was precocious in most cases. The strong sex bias for DS (11 of 12 were female) observed in this cohort could be a consequence of the small sample size and are not suggestive of a higher incidence of females than males with DS in our region. Astete *et al.* (1991) described a cytogenetic study with a larger Down's sample size showing an almost equal sex ratio (56.4% of male gender) among the individuals with the syndrome.

In agreement with previous reports (Thompson *et al.*, 1993), there was considerable karyotype variability in individuals with DS, including cases of free trisomy (nine cases) and partial free trisomy (one case) of chromosome 21, as well as Robertsonian translocations between chromosomes 14 and 21 (one case) and between the two chromosomes 21 (one case). These observations emphasize the importance of cytogenetic confirmation in cases of DS. 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 DS. Certain diseases, such as duodenal stenosis, Alzheimer's disease and acute leukemia, seem to be more common in DS patients than in the general population (Fong and Brodeur, 1987). In the case of acute leukemia, for example, the incidence can be 14-30 times more frequent in DS patients (Iselius *et al.*, 1990). By identifying each karyotype associated with DS, it is possible to inform the patient's family about the susceptibility to acute leukemia, duodenal stenosis and Alzheimer's disease and help them notify the associated symptoms. Treatment can then be introduced early, thereby increasing the patient's life expectancy.

Of the two individuals diagnosed with Edward's syndrome, one had the classic karyotype (47,XX,+18) and the other showed somatic mosaicism (46,XX/47,XX,+18). According to Giaccardi *et al.* (1991), most fetuses with trisomy 18 are spontaneously aborted. However, when the pregnancy is brought to term, the post-natal lifetime is limited to 1-2 months in 80% of the cases, except when there is somatic mosaicism. In the latter situation, the individual lives longer because of the presence of normal cell lines, which attenuate the syndromic features. Our findings confirmed that a carrier of somatic mosaicism had a significantly longer lifetime than a carrier of the classic karyotype. The first patient had been clinically accompanied until four years old, while the latter died in the first month of life.

Only one individual was 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 may be exceptions (Zool *et al.*, 1993). Somatic mosaicism increased the lifetime of our patient and masked the clearcut expression of the syndrome because of the presence of a normal cell line. The latter cells delayed diagnosis, until when the girl was already five months old.

Somatic mosaicism

According to Fernandez *et al.* (1996), the detection of mosaicism depends mainly on four factors: a) the type and number of tissues analyzed, the number of cells studied (Hook, 1977), the sensitivity of the techniques applied and the possible selection that may result in the disappearance of cell lines (Procter *et al.*, 1984).

In addition to the cases of somatic mosaicism related to the syndromes described above, four patients had clinical indications of possible mosaicism, although they were not diagnosed as having any chromosomal abnormality. These cases may represent examples of "non-detected mosaicism" since mosaicism is usually expressed in only a few tissues of the organism and may be related to cell migration during embryonic development. If gonadal tissues are involved, the frequency of affected children will depend on the proportion of affected gametes (Thompson *et al.*, 1993). This is of great importance because genetic counseling should consider the possibility of an elevated risk of recurrence due to gonadal mosaicism in one parent. Cultures of other types of tissue ruled out the existence of gonadal mosaicism in the four patients reported here.

Structural chromosomal abnormalities

Deletions

One patient was diagnosed as carrying a deletion on chromosome 5 (5p-), characteristic of the "cri-du-chat" syndrome. 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 appearing 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 child observed here had the clinical characteristics of the syndrome, including motor delay, microcephaly, facial dysmorphism and the cat-like cry after birth. These findings agree with the observation that the breakage occurred in the 5p15.2 region. The child's parents were examined to verify 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.

Three other patients had the clinical signs of Prader-Willi syndrome, but there were no alterations in their chromosome 15. Seventy percent of individuals with this syndrome have a deletion in the q11q13 region of the chro-

mosome 15 (Lyons, 1996), which can be detected by cyto-
genetic techniques. In the remaining 30%, the condition
results from a maternal disomy (i.e., the individual has two
maternal copies and no paternal copy of 15q). This explains
why in such cases it is necessary to use DNA-specific tests
to detect this syndrome. The three patients in this study
were counseled to seek this type of test in order to con-
firm the syndrome.

Robertsonian translocations

Three individuals with Robertsonian translocations
were found. According to Opitz *et al.* (1979), Robertsonian
translocations generally involve a junction between the long
arms of the involved chromosomes, with loss of their short
arms. Since the short arms of acrocentric chromosomes
do not contain essential genetic material, their loss in bal-
anced translocations does not affect the carrier's pheno-
type. Nevertheless, the carrier's offspring can inherit an
extra long arm or may lack one of the acrocentric chromo-
somes involved in the parent's translocation and thus be
affected. The patient with a translocation between chro-
mosomes 13 and 14 showed mental retardation and some
dysmorphism, suggesting that in addition to the loss of the
short arms, he may have also lost some nearby regions.

Marker chromosomes

Only one case of a marker chromosome was found.
The marker chromosome appeared in 25% of this
individual's cells and was classified as a small acrocentric
chromosome, probably a segment of chromosome 21.

The high frequency of cases with unsatisfactory cell
growth (13%) was considered to be a consequence of the
heavy medication and X-rays to which the patients were
exposed.

In 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 determin-
ing the risks of recurrence and for deciding the necessary
clinical treatment and genetic counseling. However, the suc-
cess of providing a clinical diagnosis with cytogenetic tech-
niques could be improved using FISH and other comple-
mentary molecular studies. Certainly, the replacement of
cytogenetic diagnosis by direct DNA diagnosis has great
importance, but cytogenetic still has an important role to
play in Latin American public hospitals, since molecular
diagnosis is not yet a routine procedure in this region.

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RESUMO

Neste estudo investigamos a organização cromossômica de
pacientes com retardo mental e/ou malformações congênitas, vi-
sando a avaliação de causas genéticas associadas a estes distúr-
bios. Os padrões de bandas GTG e CBG foram estudados a partir
da cultura de linfócitos de sangue periférico, estimulados por
fitohemaglutinina M. Dentre os 98 indivíduos portadores de retardo
mental e/ou malformações congênitas analisados, diagnosticamos
as seguintes síndromes: 12 casos de Down, dois de Edwards, um
de Patau, cinco de Turner, dois de Klinefelter, um de "cri-du-chat",
e um caso de translocação balanceada entre os cromossomos 13
e 14, um caso de cromossomo derivado e um outro de cromos-
somo marcador. Encontramos anomalias cromossômicas em 26%
dos pacientes, das quais 82% eram alterações numéricas e o restante
(18%) representou rearranjos estruturais. Este percentual signifi-
cativo enfatiza o uso da cariotipagem de rotina em pacientes com
retardo mental e/ou malformações congênitas.

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