Retrospective karyotype study in mentally retarded patients

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Summary

Objective: To describe the chromosomal alterations in patients with mental retardation (MR) using G-banding karyotype analysis.

Method: A retrospective study of the results G-banding karyotype analysis of 369 patients investigated for MR was performed. Based on the structural rearrangements found, the authors searched all chromosomal regions related with breakpoints, and these were compared with the literature on MR and databases.

Results: 338 (91.6%) normal cases, and 31 (8.4%) with some type of chromosomal abnormality were identified. Among the altered cases, 21 patients (67.8%) were identified with structural chromosomal alterations, nine (29%) with numerical alterations, and one (3.2%) with numerical and structural alterations.

Conclusion: Structural chromosomal abnormalities were observed more frequently in this study. G-banding karyotyping contributes to the investigation of the causes of MR, showing that this technique can be useful for initial screening of patients. However, higher resolution techniques such as array based comparative genomic hybridization (aCGH) and multiplex ligation-dependent probe amplification (MPLA) can detect submicroscopic alterations commonly associated with MR.

Keywords: karyotype, intellectual disability, chromosome aberrations.

Introduction

According to the definition of classical cytogenetics, the term aneuploidy corresponds to changes in number of chromosomes, including the presence of an extra copy of a particular chromosome (trisomy) or the absence of one chromosome (monosomy) leading to abnormal karyotype. However, with the advent of new high-resolution molecular technologies, new aneuploidy syndromes have been identified, including deletions and duplications of chromosomal regions. Changes in gene dosage resulting from such deletions and duplications are commonly related to cases of mental retardation. It is estimated that over 15% of cases of severe mental retardation are due to microscopic cytogenetic abnormalities.

Mental retardation (MR), ethically accepted as intellectual retardation or cognitive delay, is characterized by significant limitations in intellectual functions and adaptive behavior of an individual, with onset of symptoms before the age of 18 years. Adaptive behavior refers to adaptive conceptual, social and practical skills, while intellectual functions are often measured using intelligence test instruments that generate an intelligence quotient (IQ).

In some cases, MR can be categorized as syndromic, i.e. the child has associated dysmorphic features identifying a genetic syndrome. However, intellectual disability is extremely heterogeneous, a consequence of the large number of different syndromes related to MR, which renders accurate diagnosis impossible based only on the clinical picture. Accurate diagnosis is essential to provide a specific treatment, to establish a clinical predictor of quality of life, to educate parents on the characteristics and progression of the syndrome, and finally, to define a reproductive prognosis for the family.

Many cases of MR resulting from duplication and recurrent deletions were initially identified by conventional microscopy techniques or banding. G-banding karyotype analysis is a technique used to identify individual human chromosomes in many laboratories. This technique, also known as GTG (G-bands by trypsin using Giemsa), is based on chromosomes digestion with proteolytic enzymes, which are then stained with Giemsa. After staining, each pair of chromosomes exhibits a characteristic pattern of light and dark bands (G bands), which can be individually differentiated under a microscope.
Knowing the importance of accurate diagnosis of MR, and being aware of the extensive clinical use of G-banding as an important method of diagnosis, this study aimed to carry out a retrospective analysis of all the results of G-banding karyotyping of patients with MR, performed in 2009 at Instituto Hermes Pardini, Sector of Human Cytogenetics, in Belo Horizonte, Minas Gerais, Brazil.

**METHOD**

The present study followed the guidelines of the Brazilian National Health Council – CNS Resolution 196/96, and the identity of all patients was kept confidential. Chromosome study was conducted at the Laboratory of Cytogenetics of Instituto Hermes Pardini using G-banding karyotype analysis.

For each patient, peripheral blood was collected in heparin and from these samples cell cultures were prepared. The cell cultures were treated with colchicine and subjected to hypotonic shock. Then, they were fixed on slides for subsequent staining of G-bands with trypsin and Giemsa.

Slides evaluation was performed using a microscope (NIKON®, model E 400) coupled to the karyotype analysis software (Applied Spectral Imaging®, version 6.0). Karyotypes were described according to the standards present in the 2013 International System for Human Cytogenetic Nomenclature (ISCN).5

A survey of karyotyping results was carried out, including types of rearrangements, chromosomal region, the possible genes involved, and MR syndromes associated. Based on the structural rearrangements found, we searched all chromosomal regions related with breakpoints, which were then compared with the literature on MR. For this comparison, renowned databases available for public consultation on the international computer network were used, such as the Mapviewer-National Center for Biotechnology Information.72 Then, all the specific chromosomal regions were researched in the scientific literature as to their association with MR.

**RESULTS**

During 2009, we evaluated the karyotyping results of 369 patients with MR, of which 143 (38.8%) were female, and 226 (61.2%) were male. For most of the patients, 338 (91.6%), karyotypes were compatible with normality, while 31 (8.4%) cases had some type of chromosomal abnormality. Of this total of 31 altered cases, 21 patients (67.8%) were identified with structural alterations, 9 (29%) with numerical abnormalities, and 1 (3.2%) with numerical and structural alterations (Figure 1).

Among the patients with identified numerical chromosomal abnormalities, five cases had aneuploidy of sex chromosomes: four cases of 47,XXY karyotype, and one case of X monosomy (45,X karyotype). Regarding autosomal chromosomes, three cases of free trisomy 21 (47,XX,+21 karyotype) were detected. The case of numerical and structural alteration corresponded to a 47,XXY,dup(18)(p12.2 p12.3) karyotype.

As for structural chromosomal abnormalities, reciprocal translocations, inversions, deletions, additional chromosomal segments, and insertions were observed. Based on the comparison of results of karyotypes with structural alterations and their specific chromosomal regions, we were able to identify breakpoints involving 1p22.3; 2p24; 2q22; 3p13; 3q26.2; 3q12; 5p15.1; 5p15.2; 5p15.3; 6q25.3; 7p14; 8p23.1; 8q11.2; 9p24; 10q26.3; 11q23.3; 11q21; 12q24.31; 15q25; 15q26; 17p11.2; 17q21; 18p11.1; 18p11.32; 20q11.2; 22q13. The respective rearrangements as well as
the correlation between the genes involved and syndromes related with MR are shown in Table 1. With the exception of the deletion on the short arm of chromosome 5 [46,XX,del(5)(p11.2) karyotype], found in three cases, all other changes occurred once in this study.

Considering the structural alterations, three cases revealed additional marker chromosomes of unknown origin, two of which were mosaic marker chromosomes. The cases of mosaic presented 47,XX,+mar[13]/46,XX[17] karyotype (mosaicism for one lineage with presence of an additional marker chromosome, and another lineage with normal complement) and 47,XY,+r[8]/46,XY[22] karyotype (mosaicism for one lineage with an additional marker chromosome ring, and another lineage with normal complement). In one case, the additional marker chromosome was seen in all cells analyzed (47,XX,+mar).

**Discussion**

This study presents a retrospective analysis of the results of G-banding karyotyping of individuals with MR assessed at Instituto Hermes Pardini in 2009, and the comparison of these results with literature data. Based on this evaluation, we were able to identify chromosomal alterations in 8.4% of patients only. Although this method is important either to explain or rule out causes of MR, it has limitations, especially in relation to the identification of submicroscopic alterations. Thus, other techniques such as fluorescent in situ hybridization (FISH),

comparative genomic hybridization (CGH), array based comparative genomic hybridization (aCGH),

and multiplex ligation-dependent probe amplification (MLPA),

are more suitable to detect submicroscopic rearrangements responsible for most cases of MR, especially in cases of idiopathic MR.

Among the numerical chromosomal abnormalities identified in this study, the 47,XYI karyotype was most frequent. This abnormality causes Klinefelter syndrome, the most common numerical chromosomal disorder among men. The estimated frequency of this disorder is between 1:500 and 1:1,000 live births. Men with Klinefelter syndrome usually have delayed auditory processing, language dysfunction and, in rare cases, severe intellectual retardation.2

Turner syndrome is less common than other sex chromosome aneuploidies, and its most important chromosome constitution is 45,X, found in one case in this study. Some patients, in addition to the unique physical characteristics of this syndrome, have a non-verbal IQ significantly lower than their verbal IQ; many require educational intervention, especially in mathematics.9,10

The only numerical alteration of autosomal chromosome found in this study was trisomy 21 (47,XX,+21). This chromosome constitution is observed in patients with Down syndrome, an example of neurogenetic disorder related to aneuploidy,1 and corresponds to the most common genetic cause of moderate mental retardation11,12 and intellectual disability.13

In this study we also identified three cases of additional marker chromosomes, two with mosaic karyotype (47,XX,+mar[13]/46,XX[17] and 47,XY,+r[8]/46,XY[22]). Supernumerary marker chromosomes are a heterogeneous group of structurally abnormal chromosomes derived from any of the 24 chromosomes, but they cannot be identified using conventional cytogenetic techniques.14 They are found in clinically normal individuals, but are often reported in patients with mental retardation and infertility.15 Mosaicism associated with marker chromosomes is a well-known finding, observed in approximately 50% of cases. Different types of mosaicism can be found, the most common being 47,XX,+mar/46,XX.14

The analysis of chromosomal abnormalities and comparison with literature data revealed that the alterations identified in this study occur in regions determined by breakpoints also described in previous studies with patients with MR. In the 46,XX.inv(2)(p24q22) inversion, for example, there is the q22 breakpoint located close to the ZEB2 gene. Deletions involving this gene are responsible for Mowat-Wilson syndrome, and MR is one of its main clinical manifestations.16 The 6q25.3 point of 46,XX,t(6;11)(q25.3;q21) translocation is associated with a deletion syndrome with moderate MR.17

The present study found three patients with terminal deletion in the short arm of chromosome 5 and p15.2 breakpoint [46,XX,del(5)(p15.2)]. Most deletions in the short arm of chromosome 5 are related to Cri-du-Chat syndrome, which is a genetic disease caused by deletion of variable size, with breakpoints ranging from p13 to p15.2.18 A distinctive cry (similar to that of a meowing kitten), dysmorphisms, psychomotor delay, and MR are some of the clinical manifestations of the syndrome. Studies confirm the critical region of the syndrome, mapped in Sp15, with genes related to Cri-du-Chat in p15.3, and dysmorphisms and MR in p15.2.18,19

With the aid of websites GenScript23 and ClinVar,74 we were able to identify genes within the chromosome regions involved with the identified structural alterations, and evaluate which of them have already been reported in relation with MR. We found reports on all of the regions identified in our study as containing genes that due to loss or gain in the number of copies resulted in devel-
<table>
<thead>
<tr>
<th>Karyotype</th>
<th>N° of cases</th>
<th>Chromosomal region</th>
<th>Rearrangement</th>
<th>Gene involved</th>
<th>MR syndrome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>46,XY,t(1;11) (p22.3;q23.3)</td>
<td>1</td>
<td>1p22</td>
<td>Duplication; deletion</td>
<td>-</td>
<td>Kabuki makeup syndrome Goldenhar syndrome</td>
<td>Lo et al. 1998;22 Carlier et al. 200823</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11q23.3-qter</td>
<td>Trisomy; deletion; monosomy</td>
<td>-</td>
<td>Dandy Walker variant</td>
<td>Weimer et al. 2006;24 Ounap et al. 200225</td>
</tr>
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<td>46,XX,inv(2) (p24q22)</td>
<td>1</td>
<td>2p24-pter; 2p24; 2p24.2-p25.1; 2p24.3-pter</td>
<td>Deletion</td>
<td>ZEB2</td>
<td>Mowat-Wilson syndrome</td>
<td>Balasubramanian et al. 2010;29 Ballarati et al. 2009;30 Saunders et al. 200931</td>
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<td>46,XY,inv(3) (p13q26.2)</td>
<td>1</td>
<td>3p13; 3p13-p12</td>
<td>Inversion</td>
<td>-</td>
<td>Bardet-Biedl syndrome</td>
<td>Héron et al. 2005;32 Ghdami et al. 200033</td>
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<td></td>
<td>3q26.2-q26.31; 3q25.1-26.2</td>
<td>Trisomy</td>
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<td>Chromosome 3q duplication syndrome</td>
<td>Abreu-González et al. 2013;34 Faas et al. 200235</td>
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<td>3q12-q23; 3q12-q21</td>
<td>Duplication; deletion</td>
<td>-</td>
<td>-</td>
<td>Gamerding et al. 2006;36 Okada et al. 198737</td>
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<tr>
<td></td>
<td></td>
<td>8q11.2; 8q11.2-q13</td>
<td>Duplication; deletion</td>
<td>CHD7</td>
<td>Charge syndrome</td>
<td>Amouroux et al. 2012;38 Verhoeven et al. 201239</td>
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<tr>
<td>46,XY,der(5)del(5) (p15.1)</td>
<td>1</td>
<td>5p15.1</td>
<td>Trisomy; duplication</td>
<td>-</td>
<td>Cri-du-Chat syndrome</td>
<td>Balaardio et al. 2003;40 Weeb et al. 198841</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5p15.2</td>
<td>Translocation and deletion</td>
<td>SRDSAT,POLS</td>
<td></td>
<td>Harvard et al. 2005;42 Wu et al. 200543</td>
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<tr>
<td></td>
<td></td>
<td>5p15.3</td>
<td>Deletion</td>
<td>-</td>
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<td>Moreira et al. 2008;44 Laczmanska et al. 200645</td>
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<td>46,XX,t(6;11) (q25.3;q21)</td>
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<td>TBP</td>
<td>Chromosome 6q deletion syndrome</td>
<td>Abu-Amero et al. 2010;47 Lukusa et al. 200148</td>
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<td></td>
<td>11q14.1-q23.2; 11q21</td>
<td>Deletion; translocation</td>
<td>FZD4</td>
<td>Trichorhinophalangeal syndrome</td>
<td>Li et al. 2006;49 Sanchez et al. 198550</td>
</tr>
<tr>
<td>46,XX,ins(7;?) (p14;?)</td>
<td>1</td>
<td>7p14.3; 7p14-p15; 7p14</td>
<td>Deletion</td>
<td>GLI3</td>
<td>Greig cephalopolysyndactyly syndrome</td>
<td>Debeer et al. 2007;46 Duno et al. 200449</td>
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<tr>
<td>46,XY,del(8) (p23.1)</td>
<td>1</td>
<td>8p23.1; 8p22-8p23.1; 8p23.1-ppter</td>
<td>Deletion; duplication</td>
<td>-</td>
<td>Kabuki syndrome</td>
<td>Wu et al. 2010;50 Sanlaville et al. 2005;51 Zafra et al. 200552</td>
</tr>
<tr>
<td>46,XY,add(9)(p24) t(10;15)</td>
<td>1</td>
<td>10q26-qter</td>
<td>Deletion</td>
<td>-</td>
<td>-</td>
<td>Lam et al. 200653</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15q25.3; 15q25.2; 15q25-qter</td>
<td>Trisomy; deletion</td>
<td>-</td>
<td>-</td>
<td>Kim et al. 2009;54 Wagenstaler et al. 200755</td>
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(Continue)
opmental delay; or significant speech delay; or growth delay; or intellectual delay.20,21

**Conclusão**

Genetics is one of the areas of expertise that has most contributed to the elucidation of the causes of MR, with various possibilities for diagnosis. This study illustrates the contribution of G-banding karyotype analysis for investigation of the causes of MR, since our results are in line with those reported in the literature. Structural chromosomal abnormalities were observed more frequently in this study. Knowing the critical regions on chromosomes is very important to correlate genotype and phenotype, and karyotype studies may help determine such regions. Therefore, although G-banding karyotype analysis may not be the most suitable test for syndromes related to mental retardation, since a large number of these syndromes are due to submicroscopic deletions and duplications, this technique proves to be useful for initial screening of affected individuals, and negative results should be evaluated using more accurate techniques such as aCGH and MLPA.

**Resumo**

Estudo retrospectivo de cariótipo em pacientes com retardo mental

**Objetivo:** descobrir as alterações cromossômicas em pacientes com retardo mental (RM) pela análise do cariótipo com bandas G.

**Método:** foi realizado um estudo retrospectivo dos resultados de cariótipo com bandas G de 369 pacientes em investigação de RM. A partir dos rearranjos estruturais encontrados, foram levantadas todas as regiões cromossômicas envolvidas nos pontos de quebra e elas foram comparadas com a literatura para RM e bancos de dados.

**Resultados:** foram identificados 338 (91,6%) casos normais e 31 (8,4%) com algum tipo de alteração cromossômica. Dentre os casos alterados, 21 pacientes (67,8%) foram identificados com alterações cromossômicas estruturais, 9 (29%) com alterações numéricas e 1 (3,2%) com alteração numérica e estrutural.

**Conclusão:** as alterações cromossômicas estruturais foram aquelas observadas com maior frequência. O cariótipo com bandas G contribui para a investigação das causas de RM, mostrando que essa técnica pode ser útil como uma primeira triagem dos pacientes. No entanto, técnicas mais resolutivas como o array based comparative genomic hybridization (aCGH) e o multiplex ligation dependent probe amplification (MLPA) permitem detectar alterações submicroscópicas comumente associadas ao RM.

**Palavras-chave:** cariótipo, deficiência intelectual, alterações cromossômicas.

<table>
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<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>46,XY,t(12;20)</td>
<td>1</td>
<td>12q24.31-qter</td>
<td>Translocation/microdeletion</td>
<td>-</td>
<td>-</td>
<td>Callier et al. 2007; Bao et al. 2005</td>
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<tr>
<td>(q24.31;q11.2)</td>
<td>20q11.2</td>
<td>Duplication</td>
<td>ASXL1</td>
<td>-</td>
<td>Ávila et al. 2013</td>
<td></td>
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<tr>
<td>(q26)</td>
<td>46,XX,inv(17)</td>
<td>17p11.2</td>
<td>Deletion</td>
<td>TNFRSF13B,FAM27L</td>
<td>Smith-Magenis syndrome</td>
<td>Boudreau et al. 2009; Partida-Pérez et al. 2012</td>
</tr>
<tr>
<td>(p11.2q21)</td>
<td>17q21.31; 17q21.32</td>
<td>Deletion; duplication</td>
<td>CRHR1,MAPT</td>
<td>17q21.31 microdeletion syndrome</td>
<td>Koole et al. 2006; Grisart et al. 2009; Partida-Pérez et al. 2012</td>
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<td>46,XY,inv(18)</td>
<td>1</td>
<td>18p11.1</td>
<td>Deletion</td>
<td>-</td>
<td>Chromosome 18p deletion syndrome</td>
<td>Portnoi et al. 2007; Wester et al. 2006</td>
</tr>
<tr>
<td>(p11.1p11.32)</td>
<td>18p11.2; 18p11.32</td>
<td>Deletion, trisomy</td>
<td>-</td>
<td>-</td>
<td>Portnoi et al. 2007; Moog et al. 1994</td>
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<tr>
<td>46,XX,t(22)</td>
<td>1</td>
<td>22q13; 22q13.3; 22q13.31; 22q13.1-q13.2</td>
<td>Deletion; translocation; duplication</td>
<td>SHANK3</td>
<td>22q13 deletion syndrome</td>
<td>Demirhan e Tunc, 2010; Koç et al. 2008</td>
</tr>
</tbody>
</table>

add: additional segment; del: deletion; der: derivative chromosome; ins: insertion; inv: inversion; r: ring chromosome; t: reciprocal translocation; p: short arm; q: long arm; ter: terminal.
References


