PONTIFÍCIA UNIVERSIDADE CATÓLICA DE GOIÁS

Pró-Reitoria de Pós-Graduação e Pesquisa

Mestrado em Genética

A importância dos resultados do CMA no aconselhamento genético das famílias com probandos apresentando deficiência intelectual

IRENE PLAZA PINTO

Goiânia-GO

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"Só se pode alcançar um grande êxito quando nos mantemos fiéis a nós mesmos."

Friedrich Nietzsche

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SUMÁRIO

RESUMO

Deficiência intelectual é caracterizada por uma diminuição significativa em ambas funções cognitivas e adaptativas, afetando de 1 – 3 % da população em geral. É mundialmente um dos principais problemas sócio-econômicos, com uma etiologia altamente heterogênea e variável, podendo incluir fatores ambientais, desordens mendelianas e anormalidades cromossômicas, apresentados sozinhos ou combinados. Desordens de um único gene e cromossômicas são consideradas a causa de deficiência intelectual em 7-37% dos casos, enquanto que variação número de cópias submicroscópicas ocorrem em 5-15% dos casos, especialmente quando estão associadas com anormalidades congênitas múltiplas e/ou dismorfismos. Microarranjos genômicos têm sido extensivamente usados no estudo das causas genéticas da deficiência intelectual, sendo a análise cromossômica em microarranjos recomendada como teste diagnóstico de primeira escolha para pacientes com deficiência intelectual/atraso no desenvolvimento global, desordem do espectro do autista e/ou anormalidades congênitas múltiplas. Aconselhamento genético é um processo que lida com a ocorrência ou o risco de ocorrência de uma doença genética na família, ajudando-a a compreender a contribuição desta herança, envolvendo aspectos educacionais e reprodutivos. Sua prática visa a facilitação de escolhas autônomas informadas, num processo educacional, incluindo informações sobres as opções (disponibilidade, riscos e limitações destas opções), bem como o fornecimento de um suporte emocional. Promovendo, dessa forma, autonomia e adaptação ao diagnóstico, tendo como dogma central a não diretividade, exigindo do conselheiro geneticista uma posição neutra, apoiando e respeitando valores e decisões pessoais do paciente. Famílias de pessoas diagnosticadas com doenças genéticas precisam ser esclarecidas sobre a importância de passarem pelo processo de aconselhamento genético, e este deve ser feito de forma contínua e de acordo com a necessidade que os fatos indicarem, tendo o objetivo primário a promoção da saúde e a qualidade de vida.

Palavras-chave: deficiência intelectual; aconselhamento genético; CMA.

ABSTRACT

Intellectual disability is characterized by a significant decrease in both cognitive and adaptive functions, affecting 1-3% of the general population. Worldwide is a major socioeconomic problem, with a highly heterogeneous and variable etiology, which may include environmental factors, disorders Mendelian and chromosomal abnormalities, presented alone or in combination. Single gene and chromosomal disorders are considered the cause of intellectual deficiency in 7-37% of cases, while submicroscopic copy number variation occur in 5-15% of cases, especially when associated with multiple congenital abnormalities, and/or dysmorphisms. Genomic microarrays have been extensively used in the studying the genetic causes of intellectual disability, and the chromosomal microarray analysis is recommended as the first-tier cytogenetic diagnostic test for patients with intellectual disability/global developmental delay, autism spectrum disorders and/or multiple congenital anomalies. Genetic counseling is a process that deals with the occurrence or risk of occurrence of a genetic disorder in a family, helping them to understand the contribution of this heritage, involving education and reproductive aspects. The goals include facilitation of informed autonomous choices, education, which includes information on options (available, risks and limitations of those options), as well as provision of emotional support. Promoting, thus, autonomy and adaptation to the diagnosis, with the central tenet the nondirectiveness, requiring the genetic counselor to maintain a neutral stance, supporting and respecting the patient's personal values and decisions. Families of people diagnosed with genetic disorders need to be aware of the importance of going through the process of genetic counseling, and this should be done continuously and according to the need that the facts indicate, with the primary objective the promotion of health and the quality of life.

Keywords: Intellectual disability; genetic counseling; CMA.

LISTAS DE SIGLAS E ABREVIATURAS

- ADG: Atraso no desenvolvimento global.
- ASHG: Do inglês "*American Society of Human Genetics*", traduzido como Sociedade Americana de Genética Humana.
- ChAS: Do inglês "*Chromosome Analysis Suite*".
- CMA: Do inglês "*Chromosomal Microarray Analysis*", traduzido como análise cromossômica em microarranjos.
- CNV: Do inglês "*Copy Number Variation*", traduzido como variação no número de cópias.
- DI: Deficiência intelectual.
- DNA: Do inglês "*Deoxyribonucleic Acid*", traduzido como ácido desoxirribonucleico.
- DWM: Do inglês "*Dandy-Walker Malformation*", traduzido como Malformação de Dandy-Walker.
- FISH: Do inglês "*Fluorescence in situ Hybridization*", traduzido como hibridização *in situ* por fluorescência.
- LACEN: Laboratório de Saúde Publica Dr. Giovanni Cysneiros.
- LAGENE: Laboratório de Citogenética Humana e Genética Molecular.
- NSGC: Do inglês "*National Society of Genetic Counselors*", traduzido como Sociedade Nacional Americana de Conselheiros Geneticistas.
- OMIM: Do inglês "*Online Mendelian Inheritance in Man*", traduzido como On-line Herança Mendeliana no Homem.
- OMS: Organização Mundial de Saúde, do inglês "*World Health Organization – WHO*".
- PUC-GO: Pontifícia Universidade Católica de Goiás
- QI: Quociente de inteligência.
- REPLICON: Laboratório de Genética Molecular do Núcleo de Pesquisas REPLICON da PUC-GO.
- RPMI: Do inglês "*Roswell Park Memorial Institute*".
- SES-GO: Secretaria de Saúde do Estado de Goiás
- SNP: Do inglês "*Single Nucleotide Polymorphisms*" traduzido como polimorfismos de nucleotídeos únicos.

1. INTRODUÇÃO

A presente dissertação teve como objetivos avaliar microarranjos cromossômicos, usando a metodologia de Análise Cromossômica em Microarranjos (CMA) (do inglês, *Chromosomal Microarray Analysis* – CMA), em probandos que apresentavam o sinal clínico de deficiência intelectual (DI) e que foram encaminhados para os Laboratório de Genética Molecular do Núcleo de Pesquisas REPLICON-PUC-Goiás e LAGENE/LACEN–SES–GO ; desenvolver o aconselhamento genético não-diretivo e contínuo com as famílias dos probandos que realizaram o CMA, com o propósito de ajudar essas famílias a entenderem a condição e a tomarem decisões sobre a saúde de seus filhos.

A dissertação foi dividida em 7 capítulos, conforme descritos, resumidamente a seguir:

O primeiro capítulo trata de uma consideração sobre a deficiência intelectual e suas implicações como sinal prevalente em diversas doenças humanas.

O segundo capítulo faz uma abordagem sobre o aconselhamento genético e sua práxis para capacitar os consulentes à compreensão da condição que afeta suas famílias e se acomodarem à esta realidade.

O terceiro capítulo descreve as 3 metodologias usadas na investigação da causa genética da deficiência intelectual.

O quarto capítulo teve como objetivo relatar o primeiro caso de diagnóstico pós-natal no Brasil Central de uma criança com alterações citogenéticas envolvendo o cromossomo 13, usando 3 diferentes metodologias: cariótipo, FISH e CMA.

O quinto capítulo teve como objetivo fazer o relato do primeiro caso de uma criança com deficiência intelectual não sindrômica que apresentava microarranjos cromossômicos complexos identificados pelo CMA e que apresentava um cariótipo sem alteração visível ao microscópio.

O sexto capítulo teve como objetivo principal apresentar o artigo que identifica possíveis alterações genômicas submicroscópicas usando microarranjo cromossômico de alta densidade em um coorte de pacientes com deficiência intelectual ainda não diagnosticada, referidos por médicos ao sistema de saúde público do Brasil Central.

O sétimo capítulo faz uma discussão executiva abordando a inclusão do CMA e do aconselhamento genético como ferramentas de diagnóstico e suporte para as famílias de pacientes com DI.

2. CAPÍTULO I

2.1 Deficiência Intelectual e suas implicações

Deficiência intelectual (DI) é caracterizada por uma diminuição significativa em ambas funções cognitivas e adaptativas, originada antes dos 18 anos de idade. A DI pode se tornar evidente durante a primeira infância como atraso no desenvolvimento global (ADG), porém é melhor diagnosticada durante os anos escolares. De acordo com Quociente de Inteligência (QI), a DI é subgrupada em 5 graus de severidade: DI incerta (QI 70-85), DI leve (QI 55- 70), DI moderada (QI 40-55), DI severa (QI 25-40) e DI profunda (QI < 25) (Battaglia *et al.*, 2013; Bartnik *et al.*, 2014).

Atraso no desenvolvimento global é definido como um atraso significativo em dois ou mais domínios do desenvolvimento, incluindo atraso motor fino ou grosso, atraso na fala/linguagem, atraso cognitivo, atraso social/pessoal e atraso nas atividades da vida diária, acreditando-se que poderá ser preditivo para um futuro diagnóstico de DI (Moeschler, Shevell, 2014).

Estima-se que a DI afeta de 1 – 3 % da população em geral, sendo mundialmente um dos principais problemas sócio-econômicos, causando um grande impacto na vida dos pacientes e suas famílias. A etiologia da DI e ADG é altamente heterogênea e variável, podendo incluir fatores ambientais, desordens mendelianas e anormalidades cromossômicas, apresentados sozinhos ou combinados (Moeschler, 2008; Gijsbers *et al.*, 2009; Bartnik *et al.*, 2014; Musante, Ropers, 2014; Agha *et al.*, 2014).

Anormalidades cromossômicas e alterações de um único gene contribuem significativamente para todas as formas de DI. Defeitos no cromossomo X ocorrem em somente 10% dos casos de DI. Por outro lado, cerca de 45% da DI é causada por alterações nos cromossomos autossômicos e igualmente, 45% da DI é causada por fatores ambientais como exposição ambiental a certos teratógenos, radiação ionizante, infecções, saúde materna, privação social, injúrias durante o pré-natal e hipóxia neonatal (Kaufman *et al.*, 2010; Agha *et al.*, 2014).

Além disso, a DI pode ser subdividida na forma sindrômica, no qual pacientes apresentam uma ou múltiplas características clínicas ou co-morbidades em adição à DI, e na forma não-sindrômica, sendo definida pela presença da DI como uma única característica

clínica, apesar destas distinções não serem sempre clinicamente óbvias (Kaufman *et al.*, 2010; Schuurs-Hoeijmakers *et al.*, 2011). Embora seja clinicamente heterogênea, tanto a DI sindrômica quanto a DI não-sindrômica compartilham características neurológicas comuns, tais como o autismo, epilepsia, transtorno de déficit de atenção e hiperatividade e anormalidades comportamentais (Agha *et al.*, 2014).

Desordens de um único gene e cromossômicas são consideradas a causa de DI em 7-37% dos casos, enquanto que Variação no Número de Cópias (CNV) (do inglês, *Copy Number Variation* – CNV) submicroscópicas por perdas e ganhos de material genômico ocorrem em 5-15% dos casos de DI, especialmente, quando estão associadas com anormalidades congênitas múltiplas e/ou dismorfismos, demonstrando a importância das CNV na DI (Bernardini *et al.*, 2010; Qiao *et al.*, 2014; Gilissen *et al.*, 2014).

CNVs são segmentos de DNA, variando de kilobases a várias megabases, presentes em um número variado de cópias em diferentes indivíduos. As CNVs podem ser classificadas em ganhos (duplicação ou inserção), perdas (deleção) ou rearranjos complexos. Sua função ainda não está totalmente entendida, porém sua contribuição na variação genômica ganhou grande atenção por abranger mais nucleotídeos do genoma (Chung *et al.*, 2014). Além disso, as CNVs têm um importante papel na modulação do fenótipo em desordens de um único gene ou multigênicas (Bernardini *et al.*, 2010).

Coletivamente as CNVs tem um impacto considerável na saúde mental humana (Coe *et al.*, 2014) e estão implicadas na patogênese de várias desordens neuropsiquiátricas, aumentando o risco de desenvolver desordens neurocomportamentais de início precoce (Chong *et al.*, 2014). As CNVs foram identificadas pela primeira vez em pacientes com aspectos sindrômicos característicos, como na Síndrome de Williams-Beuren, Síndrome de Smith-Magenis, Síndrome de Sotos, Síndrome DiGeorge/Velocardiofacial (Kirov *et al.*, 2014).

Os microarranjos genômicos têm sido extensivamente usados no estudo das causas genéticas da DI e em desordens que são consideradas consequência clínica de CNVs (Vissers *et al.*, 2010**).** Rearranjos cromossômicos maiores que 5 – 10 Mb podem ser detectados pelo cariótipo convencional por bandeamento G. Entretanto, um número considerável de desordens clínicas é causado por rearranjos cromossômicos submicroscópicos, menores que 5 Mb (Vissers *et al.*, 2010**).** Neste ínterim, o cariótipo convencional por bandeamento G revela alterações cromossômicas de 3-5% em pacientes com DI idiopática e a análise citogenética molecular (FISH – Hibridização Fluorescente *in situ*) em regiões subteloméricas fornece um diagnóstico adicional em 3-6% dos casos (Bartnik *et al.*, 2014).

Análise cromossômica em microarranjos (CMA), usando marcadores de Polimorfismos de Nucleotídeos Únicos (SNP) (do inglês, *Single Nucleotide Polymorphisms* – SNP) e CNV, tem sido comumente aplicada como uma ferramenta de diagnóstico clínico em pacientes com deficiência intelectual/atraso no desenvolvimento global, desordens do espectro autista e anormalidades congênitas múltiplas (Chong *et al.*, 2014). O CMA examina todo o genoma humano em um único chip com uma resolução pelo menos 10 vezes maior que o cariótipo convencional por bandeamento G, sendo recomendado como teste diagnóstico de primeira escolha para pacientes com deficiência intelectual/atraso no desenvolvimento global, desordem do espectro do autismo e/ou anormalidades congênitas múltiplas (Miller *et al.*, 2010; Zilina *et al.*, 2014).

3. CAPÍTULO II

3.1 Aconselhamento genético

O aconselhamento genético tem a finalidade de ajudar pessoas a enfrentarem suas condições no campo da hereditariedade. Portanto, é um processo que lida com o risco de ocorrência ou de recorrência de uma doença genética na família, ajudando-a a compreender a contribuição desta herança, que podem envolver aspectos educacionais e reprodutivos (Guimarães, Coelho, 2010)

O conceito adotado pela Sociedade Americana de Genética Humana (ASHG) (do inglês, *American Society of Human Genetics* – ASHG), proposto na década de 70 e até hoje aceito, trata de um processo de comunicação que lida com problemas humanos associados com a ocorrência ou o risco de recorrência de uma doença genética em uma família. Este processo envolve a participação de uma ou mais pessoas treinadas para ajudar o indivíduo ou sua família à: 1) compreender os fatos médicos, incluindo o diagnóstico, provável curso da doença e as condutas disponíveis; 2) apreciar o modo como a hereditariedade contribui para a doença e o risco de recorrência para pacientes específicos; 3) entender as alternativas para lidar com o risco de recorrência; 4) escolher o curso de ação que pareça apropriado em virtude do seu risco, objetivos familiares, padrões éticos e religiosos, agindo de acordo com essa decisão; 5) ajustar-se da melhor maneira possível para a doença em um membro da família afetada e/ou para o risco de recorrência de tal doença (Epstein, 1975).

Atualmente, a Sociedade Nacional Americana de Conselheiros Geneticistas (NSGC) (do inglês, *National Society of Genetic Counselors* – NSGC) apresenta a definição de aconselhamento genético como um processo de comunicação que tem como objetivo auxiliar pais e familiares à compreenderem, entre outras coisas, o diagnóstico, etiologia, prognóstico, risco de ocorrência ou recorrência, possibilidades para o tratamento e os meios pelos quais irão se adaptar as circunstâncias impostas pela condição, com suporte psicológico e respeito positivo incondicional para a autonomia da família. Este processo integra a interpretação da família e dos históricos médicos para avaliar a possibilidade de ocorrência ou recorrência da doença, educação sobre a herança, testes, gestão, prevenção, recursos e pesquisa, bem como a promoção de escolhas informadas e adaptação ao risco ou condição (Resta *et al.*, 2006).

O aconselhamento genético visa a facilitação de escolhas autônomas informadas, um processo educacional, incluindo informações sobres as opções (disponibilidade, riscos e limitações destas opções), bem como o fornecimento de um suporte emocional (Hodgson, Spriggs, 2005). Portanto, o processo de comunicação no aconselhamento genético promove autonomia e adaptação ao diagnóstico, tendo como dogma central a não-diretividade, exigindo do conselheiro geneticista uma posição neutra, apoiando e respeitando valores e decisões pessoais do paciente (Sheets *et al.*, 2011).

O aconselhamento genético não-diretivo deve se basear em dois elementos básicos: 1) provisão da informação precisa, completa e sem vieses, para que os indivíduos possam tomar suas decisões; 2) estabelecer uma relação empática com alto grau de entendimento, para que as pessoas sejam efetivamente ajudadas a trabalharem para tomar suas decisões (Pina-Neto, 2008). O processo não diretivo descreve um estilo de aconselhamento em que o conselheiro geneticista não dá conselhos, não faz julgamentos ou emite opiniões, ele promove a autonomia do indivíduo, guiando-o para a tomada de decisão (Hodgson, Spriggs, 2005).

O anseio do serviço de aconselhamento genético é de que a decisão de utilizar este serviço seja inteiramente voluntária e igualmente e prontamente disponível para todos que necessitem e escolham usá-lo. Todo o processo de aconselhamento genético visa a compreensão ampla do indivíduo sobre a condição genética e suas implicações, o conhecimento das opções de terapêutica ou diminuição dos riscos de ocorrência ou recorrência da doença genética em questão e o fornecimento eventual de apoio psicoterapêutico (Uhlmann *et al.*, 2009; Bertollo *et al.*, 2013).

Os princípios éticos aplicados aos serviços de genética, recomendados pela Organização Mundial de Saúde (OMS), determinam: 1) respeito às pessoas e famílias, incluindo a verdade total, respeito pela decisão das pessoas e informação precisa e sem ser tendenciosa; 2) preservação da integridade da família; 3) revelação completa para os indivíduos e famílias de todas as informações relevantes para a saúde; 4) proteção da privacidade dos indivíduos e famílias de intrusões não justificadas por parte de empregadores, seguradoras e escolas; 5) informação aos indivíduos sobre a obrigação ética que eles se encontram de informar os parentes de que podem estar em risco genético; 5) informar aos indivíduos sobre a necessidade de que eles revelem o seu status de portadores a esposos/parceiros se uma criança está sendo desejada e as possibilidade de dano ao casamento das revelações; 7) informar as pessoas de suas obrigações morais de revelar o status genético que possam afetar a segurança pública; 8) apresentação das informações de forma menos tendenciosa possível; 9) uso de técnicas não-

diretivas, exceto nas questões de tratamento; 10) envolver as crianças e adolescentes o máximo possível nas decisões que lhes afetem; 11) obrigação dos serviços de seguimento dos afetados/famílias se apropriado e desejado (WHO, 1998).

No processo de aconselhamento genético, as ferramentas utilizadas são: 1) coleta de informação, aonde a parte integral da avaliação genética é a história familiar que é usualmente registrada na forma de um pedigree para esclarecer as relações e as características fenotípicas que podem ser relevantes para o diagnóstico; 2) estabelecimento ou verificação do diagnóstico, no qual testes genéticos ou genéticos molecular podem ser suficientes, não somente para diagnosticarem um indivíduo afetado ou portador, mas também para fornecerem pistas importantes para prognóstico ou severidade; 3) avaliação de risco; 4) fornecimento de informações, ajudando a família e o indivíduo afetado a entenderem o seu resultado, tendo certeza de que a visão anterior da família sobre a desordem é apropriada na luz do corrente entendimento de genética e tratamento; 5) aconselhamento psicológico, ajudar a família e o indivíduo afetado a serem ativos psicologicamente, a trabalharem com os sentimentos ambíguos, os conflitos interpessoais, as dificuldades da tomada de decisão e suas relações com os tipos de personalidade e assegurar que a informação está sendo assimilada (Uhlmann *et al.*, 2009).

Os conselheiros geneticistas devem atuar como facilitadores de um processo complexo de entendimento do que está ocorrendo com a família e agir para que os consulentes façam um processo de ajuste perante a nova situação a ser vivenciada pela família: a de ter um ou mais membros afetados por uma doença genética (Pina-Neto, 2008). Além do mais, quando um indivíduo (probando) recebe um diagnóstico de uma condição genética, esta informação genética geralmente tem implicações para outros membros da família que podem ter o risco de serem portadores da mesma alteração ou mutação (Hodgson *et al.*, 2014).

O aconselhamento genético é indicado para: 1. Avaliação de pessoas com deficiência intelectual ou atraso de desenvolvimento global; 2. Avaliação de pessoas com defeitos morfológicos congênitos únicos ou múltiplos; 3. Avaliação de pessoas com uma possível doença metabólica herdada; 4. Presença de possível doença monogênica; 5. Presença de doença cromossômica, incluindo rearranjos cromossômica equilibrados; 6. Pessoas com risco de uma condição genética, incluindo questões de diagnóstico pré sintomático ou risco de câncer; 7. Pessoa ou família com dúvidas sobre aspectos genéticos de qualquer condição médica; 8. Casais com históricos de abortos recorrentes; 9. Consanguinidade; 10. Doenças típicas de grupos

étnicos específicos; 11. Uso de teratógenos; 12. Aconselhamento pré-concepção e sobre fatores de risco, incluindo idade materna avançada e outras (Bertollo *et al.*, 2013).

No Brasil, a maioria dos pacientes e famílias acometidos de doenças puramente genéticas ou influenciados pelos genes, desconhece amplamente a condição médica que possui e não foram investigados de maneira adequada para evidenciar os fatores genéticos envolvidos (Brunoni, 2002), bem como a maioria desses serviços estão concentrados nas regiões Sul e Sudeste do país (Acosta *et al.*, 2013). Sendo assim, as famílias de pessoas diagnosticadas com doenças genéticas devem ser esclarecidas sobre a importância de passarem pelo processo de aconselhamento genético, e este deve ser feito de forma contínua e de acordo com a necessidade que os fatos indicarem, tendo o objetivo primário a promoção da saúde e a qualidade de vida (Bertollo *et al.*, 2013).

4. CAPÍTULO III

4.1 Metodologias usadas na investigação da causa genética da deficiência intelectual

4.1.1 Cariótipo convencional por bandeamento G

O sangue periférico é o mais utilizado para o diagnóstico citogenético pela facilidade na obtenção do material e pela simplicidade no cultivo das células, sendo a coleta para o cariótipo convencional realizada com o anticoagulante heparina. O meio de cultura ideal é o RPMI (*Roswell Park Memorial Institute*), desenvolvido para culturas *in vitro* de células humanas, que é enriquecido com fitoemaglutinina, L-glutamina e soro fetal bovino. Após 48 h, é adicionado colchicina, substância que tem o objetivo de impedir a formação do fuso acromático, estagnando as divisões celulares na metáfase, etapa em que os cromossomos estão mais individualizados e condensados, apropriada para análise do cariótipo. Após o uso da colchicina, inicia-se o processo de coleta e fixação das células com solução hipotônica e fixador Carnoy. Após o processo de coleta das células, faz-se a preparação das lâminas e a coloração por bandeamento GTG, usando a tripsina, que tem o objetivo de fazer a desproteinização dos cromossomos e preparar as células para a coloração com Giemsa. Este bandeamento produz bandas escuras e claras, permitindo a identificação de cada par cromossômico. A captura das imagens é feita pelo sistema de microscopia óptica motorizado, interligado com *software* para análise citogenética IKAROS® (*Metasystems, German*) (Maluf *et al.*, 2011; Verma, Babu, 1995).

A técnica por bandamento G permite a detecção de diversas alterações estruturais, como translocações, inversões, deleções e duplicações, além de alterações numéricas, atingindo uma resolução de aproximadamente 450-550 bandas por genoma, o que representa de 5 a 10 megabases (Mb) de DNA (Smeets, 2004; Trask, 2002).

4.1.2 Hibridização *in situ* por Fluorescência (FISH)

A partir da segunda metade da década de 1980 surgiu uma técnica locus-específica que permitiu verificar e detectar sequências de ácidos nucleicos específicas em células metafásicas e interfásicas, denominada Hibridização *in situ* por Fluorescência (FISH) (do inglês, *Flurorescence in situ Hybridization*) (Pinkel *et al.*, 1986; Lichter *et al.*, 1988; Maluf *et al.*, 2011).

Para esta técnica são utilizadas basicamente sondas de DNA contendo dois elementos, o inserto (DNA complementar à sequência-alvo no cromossomo) e o vetor (sequência de DNA não relacionada na qual o inserto será clonado, permitindo a replicação do DNA), consistindo na ligação de sequências de DNA marcadas com fluorocromos (sondas) aos genes ou cromossomos-alvo complementares. A marcação cromossomo-específica é visualizada como pontos florescentes de uma ou várias cores diferentes. (Maluf *et al.*, 2011). A resolução depende do tamanho da sonda (> 50 kb - 2 Mb), mas é cerca de 20 a 40 vezes superior à do cariótipo convencional (Wiegant *et al.*, 1992). A captura das imagens é feita pelo microscópio de epifluorescência (*Zeiss*) e análise é feita pelo *software* ISIS® (*Metasystems, German*).

4.1.3 Análise Cromossômica por Microarranjo (CMA)

A tecnologia alta densidade usada na plataforma do CMA (Affymetrix, Santa Clara, USA), concilia o alto poder de resolução para detectar CNVs com a sensibilidade de detecção de consanguinidade, dissomia uniparental e uma maior sensibilidade para detectar baixos níveis de aneuploidias em mosaico, pelos SNP-arrays (Gijsbers *et al.*, 2009; Koolen *et al.*, 2009; Miller *et al.*, 2010).

A amostra com 250 ng de DNA total é digerida com uma enzima de restrição – NspI, ligada a um adaptador, amplificada por PCR e purificada usando esferas magnéticas, fragmentada, marcada com biotina e hibridizada usando o GeneChipHD CytoScan Array (Affymetrix, Santa Clara, USA), de acordo com protocolo do fabricante. O array foi desenhado especificamente para diagnóstico citogenético, incluindo em torno de 2,7 milhões de marcadores de CNVs e 744.000 marcadores de SNP, e 1,9 milhões de sondas não-polimórficas cobrindo todo o genoma humano. Os arquivos CEL obtidos pelo escaneamento dos arrays são analisados usando o software *Chromosome Analysis Suite* (ChAS®) (Affymetrix, Santa Clara, USA) para determinar os genótipos. Perdas e ganhos genômicos que tinham o mínimo de 50 e 25 marcadores, respectivamente, e um tamanho de 100 kb foram inicialmente considerados para determinar deleções e duplicações relevantes.

Translocações aparentemente equilibradas não são detectáveis pela tecnologia do CMA, entretanto quase metade das translocações equilibradas são acompanhadas por um rearranjo detectável pela análise por microarranjo (Hochstenbach *et al.*, 2009; Miller *et al.*, 2010).

5. CAPÍTULO IV

GMR

Genetics and Molecular Research - Sistema para Gerenciamento de Publicações

Ribeirão Preto, 24 de Setembro de 2014

Prezados autores,

Informamos que o artigo "Postnatal diagnosis of constitutive ring chromosome 13 using both conventional and molecular cytogenetic approaches" GMR4981, de autoria L.B.Minasi,, I.P.Pinto,, J.G. de Almeida,, A.V. de Melo,, D.M. da C. e Cunha,, C.L.Ribeiro, G.P.Silva, M. das G. Brasil, D. de M. e Silva,, C.C. da Silva,,, and, A.D. da Cruz,,,, foi aceito para publicação na Genetics and Molecular Research (GMR).

Aproveitamos a oportunidade para informar que a GMR está indexada em 74 bases de dados, entre elas: Index Medicus, PubMed, Medline e ISI. E tem fator de impacto 1,184, segundo JCR - junho 2012.

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Postnatal diagnosis of constitutive ring chromosome 13 using both conventional and molecular cytogenetic approaches

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ABSTRACT

We describe the first postnatal diagnosis of a child from Central Brazil with *de novo* cytogenetic alterations in 13q showing malformations of the brain, eyes, distal limbs, and genitourinary tract, and severe intellectual disability. The karyotype was a constitutive 46,XX,r(13)[77]/45,XX,-13[17]/46,XX,idic r(13)[6]. Interphase and metaphase fluorescence *in situ* hybridization analyses also showed the absence of 13qter and the presence of 13q14.3 in the cells with r(13), and chromosome microarray analysis detected a 15.39Mb deletion in chromosome region 13q32.3-q34. This study is intended as the registry of a rare case of chromosomal rearrangement involving chromosome 13 in Central Brazil. Further studies are needed to define whether genetic haploinsufficiency is associated with each major 13q deletion anomaly.

Keywords: 13q deletion; Ring chromosome 13; Complex phenotype; Karyotype; Fluorescence *in situ* hybridization; Chromosomal microarray analysis

INTRODUCTION

Deletions of chromosome regions are generally the result of double-stranded chromosome breaks with loss of produced acentric fragments during the next cell division. Such deletions can lead to ring chromosomes. Patients with r(13) have various phenotypic abnormalities that correspond to specific breakpoints (Uwineza et al., 2013).

Lele et al. (1963) first described a partial deletion of 13q in retinoblastoma patients who also displayed intellectual disabilities and global developmental delays (Huang et al., 2012). The 13q deletion syndrome leads to phenotypes that include short stature, microcephaly, cerebral cortical malformations, Dandy-Walker malformation (DWM), corpus callosum agenesis, meningocele/encephalocele, neural tube defects, micro-/anophthalmia, cleft lip/palate, lung hypoplasia, heart defects, genital anomalies, and hand abnormalities (Kirchhoff et al., 2009; Chen et al., 2013; Valdes-Miranda et al., 2014). In this study, we used 3 laboratory methodologies to report the first postnatal diagnosis in Central Brazil of a child with cytogenetic abnormalities involving chromosome 13.

MATHERIAL AND METHODS

Sample preparation and cytogenetic characterization

The parents of the proband signed informed consent forms approved by the Ethics Committee on Human Research at the Pontifical Catholic University of Goiás, under protocol number 1721/2011. Cytogenetic studies were carried out with peripheral blood samples from the patient and her parents using G-banding techniques at a band resolution of > 550. Short-turn lymphocyte cultures were carried out for the proband and her progenitors following standard procedures (Verma and Babu, 1995). Chromosomal analyses were performed with the IKAROS® software (Metasystems Corporation, Jena, Germany).

Metaphase and Interphase fluorescence *in situ* hybridization (FISH) analyses were performed using 13q14.3 (spectrum red) and 13qter (spectrum green) specific probes according to standard FISH protocols. Chromosomes were counterstained with 4,6-diamino-2-phenyl-indole. Metaphases and interphase nuclei were captured using an epifluorescence microscope (Carl Zeiss, Jena, Germany) and the ISIS® software (Metasystems Corporation, Germany).

Genomic DNA was obtained from peripheral blood from the proband and her parents. Genomic DNA was isolated from whole blood using a QIAamp® DNA Mini kit (Qiagen, Limburg, Netherlands). The analyses were carried out on the proband and her biological parents to establish whether the DNA rearrangements were *de novo* or inherited. Total DNA (250ng) for each sample was digested with *NspI*, ligated, PCR amplified and purified, fragmented, biotin-labeled, and hybridized for use in a GeneChip™ HD CytoScan Array (Affymetrix, Santa Clara, CA, USA). The array was designed specifically for cytogenetic research, including ≈ 2,696,550 copy number variation markers, 743,304 single-nucleotide polymorphism markers, and > 1,953,246 non-polymorphic markers. CEL files obtained by scanning the arrays were analyzed using the Chromosome Analysis Suite software (Affymetrix). Gains and losses that affected a minimum of 50 and 25 markers, respectively, in a 100 kb length were initially considered.

Case presentation

A female child was born at 40 weeks gestation to a non-consanguineous Brazilian couple. Her birth weight was 2,430g and her crown-heel length was 45 cm. Delivery occurred via cesarean section. Malformations in the newborn were noted by the assistant medical staff at birth. At the age of 2 months, the proband underwent surgery for vesico-ureteral reflux. After surgery, the child developed kidney stones. At the age of 8 months, she was diagnosed with low tubular renal function in both kidneys. Her craniofacial dysmorphisms included a high and sloping forehead, hypotelorism, bilateral microphthalmia, deep set eyes with reduction and change in

shape of the eyeballs, atrophy of the optic nerves and chiasm, and epicanthal folds. Magnetic nuclear resonance revealed decreased cervical and axial tonus, reduced fontanelle, lack of visualization of the septum pellucidum with persistence of the cavum septum pellucidum and cavum vergae, hippocampal atrophy, absence of falx cerebri, and partial fusion of the thalami with an appearance suggestive of holoprosencephaly. She also had a broad and prominent nasal bridge, arched upper lip, hands with elongated fingers and disproportionate size, and heart problems.

At the age of 6 years, she weighed 16.9 kg, and was 1.06 m tall. Physical examination revealed severe delayed psychomotor development and intellectual disability. No history of birth defects in the family was reported.

RESULTS

GTG banding of 100 metaphases and corresponding FISH signals were analyzed at 8 months of age. After 5 years and 4 months, the research group contacted the parents of the child for follow-up testing using a chromosomal microarray analysis (CMA) approach that had become available in Central Brazil.

The karyotype was a constitutive 46,XX,r(13)[77]/45,XX,-13[17]/46,XX,idic r(13)[6] (Figure 1). Interphase and metaphase FISH analyses also showed the absence of 13qter (green spectrum) and the presence of 13q14.3 (red spectrum) in cells with ring chromosome 13 (Figure 2). An isodicentric chromosome was observed and confirmed by gray scale images on reverse 4,6-diamino-2-phenyl-indole staining, a tool included with the ISIS® software (Metasystems Corporation) to highlight centromeres. The ring chromosomes showed 2 red signals for the region 13q14.3 confirming the presence of chromosome 13 (see Figure 2). Chromosome microarray analysis detected 15.39Mb deletions at 13q32.3-q34 arr[hg19] 13q32.3q34(99,712,845-115,107,733)x1 (Figure 3). This deletion involved the following

genes: *DOCK9, UBAC2-AS1, UBAC2, MIR548AN, GPR18, GPR183, FKSG29, MIR623, TM9SF2, CLYBL, MIR4306, ZIC5, ZIC2, PCCA, PCCA-AS1, A2LD1, TMTC4, NALCN-AS1, NALCN, ITGBL1, FGF14, MIR2681, MIR4705, FGF14-IT1, LOC283481, TPP2, METTL21C, CCDC168, TEX30, KDELC1, BIVM, BIVM-ERCC5, ERCC5, METTL21CP1, SLC10A2, MIR548AS, DAOA-AS1, DAOA, LINC00343, LINC00460, EFNB2, ARGLU1, LINC00551, LINC00443, FAM155A, LIG4, ABHD13, TNFSF13B, MYO16, MYO16-AS1, IRS2, COL4A1, COL4A2, COL4A2-AS1, RAB20, CARKD, CARS2, ING1, LINC00346, ANKRD10, ARHGEF7, TEX29, SOX1, SPACA7, TUBGCP3, C13orf35, ATP11A, MCF2L-AS1, MCF2L, F7, F10, PROZ, PCID2, CUL4A, LAMP1, GRTP1, ADPRHL1, DCUN1D2, TMCO3, TFDP1, ATP4B, GRK1, LINC00552, FAM70B, GAS6-AS1, GAS6, LOC100506394, LINC00452, LINC00565, RASA3, CDC16, UPF3A* and, *CHAMP1*. The karyotypes and CMA analyses of both parents were unremarkable.

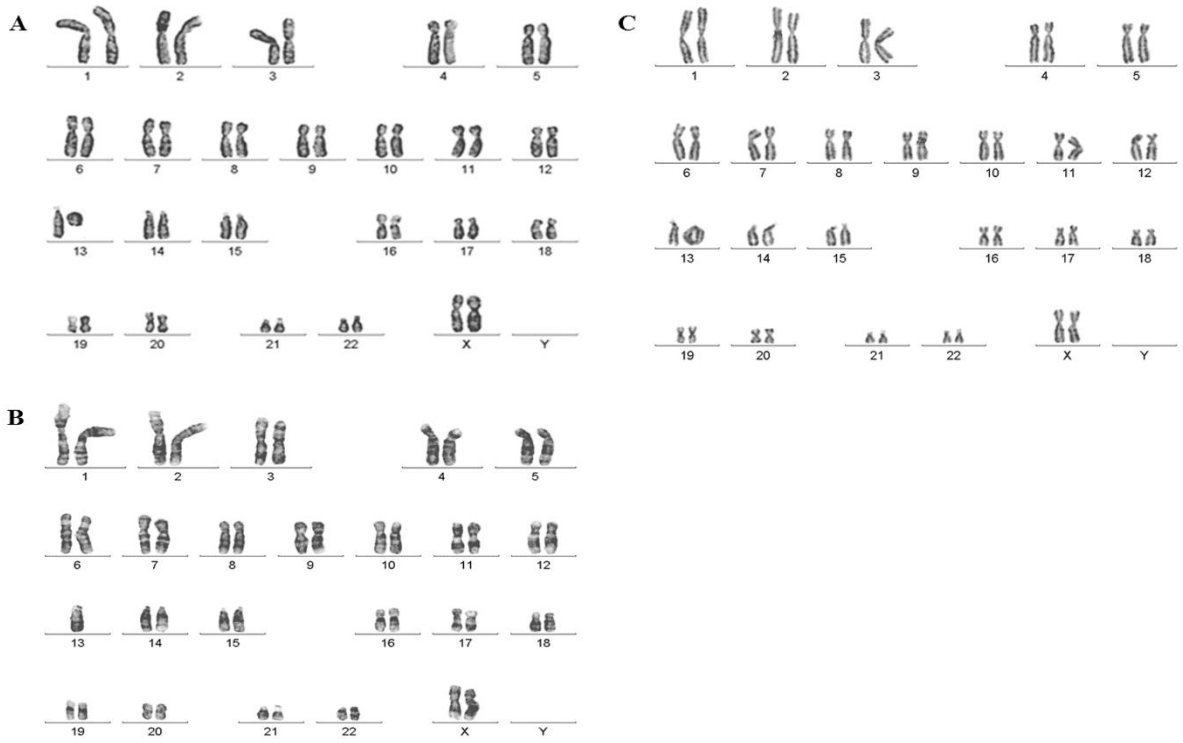

Figure 1. G-banded karyotype of the proband. (A) The Ring chromosome 13. **(B)** Monosomy of chromosome 13. **(C)** the Isodicentric ring chromosome 13.

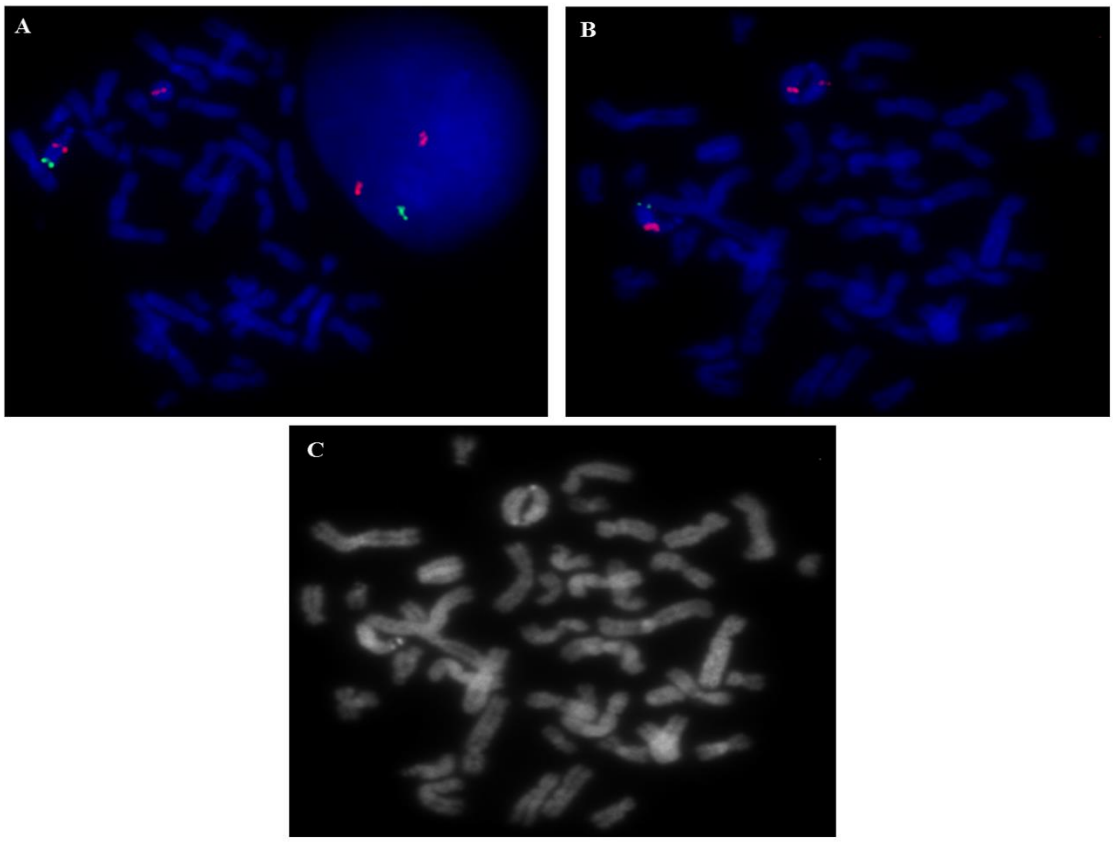

Figure 2. Fluorescence *in situ* hybridization analyses on peripheral lymphocytes. **(A)** Absence of the 13qter-specific probe signal (green spectrum) and presence of the 13q14.3-specific probe signal (red spectrum) on r(13) in metaphase and interphase cells. **(B)** Presence of the 13q14.3-specific probe signal (red spectrum) and absence of the 13qter-specific probe signal on idic r(13). **(C)** Isodicentric ring chromosome 13 defined by gray scale on reverse 4,6-diamino-2-phenyl-indole staining.

Figure 3. Chromosomal microarray analyses. Copy number variation is shown by 15.39 Mb deletions at chromosome bands 13q32.3-q34 (red dots).

DISCUSSION

This report presents the first case of a child from Central Brazil harboring alterations of chromosome 13. The GTG-banding karyotype showed constitutive r(13) in 77% of the cells, isodicentric 13 in 6% of the cells, and monosomy 13 in 17%. Both monosomy 13 and isodicentric 13 were confirmed with FISH, which was also useful in determining the deletion of the terminal end of the long arm of chromosome 13 in the ring formation. However, banding karyotyping and FISH were unable to define the precise breakpoint of the terminal deletion. It was evident that the ring chromosome led to a partial deletion of the long arm of chromosome 13. Thus, based on probe density, high-resolution CMA allowed refinement of the breakpoint region of the affected chromosome 13 and was useful in determining the gene content within the deleted region. However, due to the nature of the chromosomal rearrangements, CMA identified only the microdeletion that comprised the majority of the mosaicism in our proband.

Gain of isodicentric chromosome 13 and monosomy 13 were absent in the CMA due to its limitations for detecting low-level mosaicism.

Herein we report the first postnatal case of chromosome 13 with only r(13), isodicentric 13, and monosomy 13. Our findings are similar to those of a report on a prenatal product of a pregnancy in which the parents elected to terminate at 23 weeks of gestation (Chen et al., 2013). The proband had a deletion of 15.39 Mb involving 13q32, which is considered the critical band for the most severe phenotypes in 13q deletions syndrome.

Haploinsufficiency of the genes within the deleted region is the most probable cause of the proband phenotype. Brown et al. (1993) defined 13q32 as the critical region for the most severe phenotypes, showing malformations in the brain, eyes, distal limbs, and genitourinary and gastrointestinal tracts, severe mental retardation, and short stature. The previous description is in agreement with our findings. Thus, 13q32 deletions describe a complex phenotype related mainly to brain, eye, and urinary tract malformations and severe mental retardation. Ballarati et al. (2007) defined a minimal deletion interval associated with DWM to the 13q32.2-33.2 region and Gul et al. (2005) reported a 13q31.2/32.1-qter deletion in a fetus with DWM. In this context, the proband exhibited the traits of 13q32 deletion syndrome including DWM in accordance with previous reports.

Herein we contrast the phenotypic findings of our proband with Online Mendelian Inheritance in Man (OMIM) morbid genes. The present case was characterized by haploinsufficiency of *ZIC5* and *ZIC2*. Mutations in *ZIC2* have been associated with a causality mechanism of holoprosencephaly (HPE) and may contribute to the severe brain malformations of patients with del(13q) (Brown et al., 1998). Our data corroborate this finding as magnetic nuclear resonance image of the proband indicated HPE. Moreover, research has implicated *ZIC 2* deletions in 2% of HPE cases (Bendavid et al., 2006; 2007). Mutations of *ZIC2* are frequently *de novo* and 70%

of cases are due to deletions (Ribeiro et al., 2012), a finding that also support our results. Additional studies have indicated that *ZIC2* has a pleiotropic effect and variable penetrance that may be the result of a loss of contiguous genes that in turn may influence gene expression (Mademont-Soler et al., 2010).

The proband had severe eye malformations. In addition to this phenotypic observation, the haploinsufficiency of *EFNB2* has been proposed as a candidate gene for congenital eye malformations (Ballarati et al., 2007) and may be related to urorectal development and genital malformations in males (Walczak-Sztulpa et al., 2008). *EFNB2* encodes a member of the ephrin family with specificity for ephrin receptors, which, in animal models, participate in several aspects of visual system development (Williams et al., 2003). Controversies remain about which genes are involved in the malformation of the ocular system. A set of changes in the eyes is a phenotypic trait often observed in individuals with 13q32 deletion and other genes in this region may also participate in the development of the phenotype. Additional studies will be needed to corroborate or refute this hypothesis. The deletion presented in our report confirms the importance of investigating genes in the 13q32.3-q34 region to identify those that may be involved in eye malformation.

We also found reports implicating the haploinsufficiency of *SOX1* and *ARHGEF7* in the development of the brain and cerebral cortex. These genes may also be responsible for microcephaly and cortical development (Kirchhoff et al., 2009). Deletion of *COL4A1* has been associated with congenital anomalies of the eyes, brain, and kidneys in mice. Moreover, deletions of *COL4A1* were first shown to cause porencephaly, cerebral hemorrhage, and microangiopathy in humans (Rodahl et al., 2013). Heterozygous mutations of *COL4A2* have been associated with porencephaly 2 (OMIM 614483) (Yoneda et al., 2012) and increased nuchal translucency (Jeanne et al., 2012; Weng et al., 2012). Haploinsufficiency of *COL4A1*

and *COL4A2* may be associated with susceptibility to intracerebral hemorrhage (OMIM 614519) (Jeanne et al., 2012; Weng et al., 2012) and increased nuchal translucency (Chen et al., 2012).

Moreover, *F7* and *F10* have been associated with factor VII (Carew et al., 2000) and factor X deficiencies (Zhou et al., 2013), respectively. Coagulation deficiencies were not investigated in the proband. Furthermore, the haploinsufficiency of *ITGBL1* is associated with lung hypoplasia (Berg et al., 1999). No evidence of lung defects was found in our proband.

The current report of a girl with 13q32.3-q34 deletion associated with DWM and a complex phenotype is intended to help in the characterization of rare and variable phenotypes associated with chromosome 13 deletions. Karyotype–phenotype correlations will facilitate future investigations of the haploinsufficiency of specific genes. However, due to the rarity of cases, studies must be carried out as part of an international consortium. Thus, the current case report is intended to act as the registry of a rare case of chromosomal rearrangement involving chromosome 13 in Central Brazil. Further studies are needed to define whether genetic haploinsufficiency is associated with each major 13q deletion anomaly or whether one or more putative genes of the critical regions are contributing to these congenital malformations. Taken together, the data reported herein and those from the literature allow for an update of the genotype-phenotype map of the 13q32.3-q34 chromosome segment.

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6. CAPÍTULO V

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MOLECULAR CYTOGENETICS

CASE REPORT Open Access

A non-syndromic intellectual disability associated with a *de novo* microdeletion at 7q and 18p, microduplication at Xp, and 18q partial trisomy detected using chromosomal microarray analysis approach

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Abstract

Background: Chromosome abnormalities that segregate with a disease phenotype can facilitate the identification of disease loci and genes. The relationship between chromosome 18 anomalies with severe intellectual disability has attracted the attention of cytogeneticists worldwide. Duplications of the X chromosome can cause intellectual disability in females with variable phenotypic effects, due in part to variations in X inactivation patterns. Additionally, deletions of the 7qter region are associated with a range of phenotypes.

Results: We report the first case of *de novo* microdeletion at 7q and 18p, 18q partial trisomy, microduplication at Xp associated to intellectual disability in a Brazilian child, presenting a normal karyotype. Karyotyping showed any chromosome alteration. Chromosomal microarray analysis detected a *de novo* microdeletion at 18p11.32 and 18q partial trisomy, an inherited microdeletion at 7q31.1 and a *de novo* microduplication at Xp22.33p21.3.

Conclusions: Our report illustrates a case that presents complex genomic imbalances which may contribute to a severe clinical phenotypes. The rare and complex phenotypes have to be investigated to define the subsets and allow the phenotypes classification.

Keywords: Intellectual disability, CMA, 18q partial trisomy, Microdeletion, Microduplication, Mosaicism

Background

Chromosome abnormalities that segregate with a disease phenotype can facilitate the identification of disease loci and genes. The relationship between chromosome 18 anomalies with severe intellectual disability (ID) has attracted the attention of cytogeneticists worldwide [1]. Moreover, the partial trisomy of the chromosome 18 is a rare genetic chromosomal syndrome, corresponding to a variant and less severe form of Edwards's Syndrome (OMIM 300484). For this condition, the severity of the symptoms and the phenotype is highly variable depending on the extension of chromosome involvement and the level of compromised cells and tissues [2].

Eighty percent of the cases of Edwards's Syndrome present full trisomy, the other 20% present mosaic or partial 18 trisomy [1]. After birth, mosaic chromosomal abnormalities are essentially identified among individuals with phenotypic manifestation of recognizable aneuploidy (chromosomal) syndromes. Mosaic aneuploidy involving 18 chromosome was classified as a frequent mosaic autosomal aneuploidy [3].

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Several genomic disorders have been linked to X chromosome. Duplications of the X chromosome can cause ID in females with variable phenotypic effects, due in part to variations in X-inactivation patterns [4]. It has been estimated that up to 30% of genes on the short arm of the X chromosome could escape inactivation and undergo gene expression in cells and tissues [5]. Deletions of the 7qter region are associated with a range of phenotypes, and clinical findings comprise low birth weight, mental retardation, development delay, facial dysmorphisms and genitourinary malformations [6,7].

Here in we report the first case of *de novo* microdeletion at 7q and 18p, 18q partial trisomy, microduplication at Xp associated to a non-syndromic intellectual disability in a Brazilian child, presenting a normal karyotype.

Case presentation

A 4-years old female patient born to non-consanguineous parents, at 39 weeks gestation to a 33-year-old mother and 47-year-old father, and her birth weight was 2325 g. Child delivery was carried out through a caesarean section procedure. Physical examination of the proband reveled delayed psychomotor development, severe intellectual disability, and a height of 94,5 cm (<7rd), a weight of 9500 g (<7rd). Her craniofacial dysmorphisms included an oblong face with a prominent forehead/frontal bossing, a bulbous nasal tip on a small nose and abnormal teeth. She also had hypertelorism, lower ear implantation and limb weakness. The family history was unremarkable on both sides.

Results

Karyotyping at about 550 band resolution showed the proband with a female karyotype (46,XX), without any suggestion of chromosome alteration (Figure 1). Her parents also presented normal karyotype. CMA detected four genomic imbalances in the patient's genome, corresponding to a *de novo* 1.23 Mb microdeletion at 18p11.32 (136,226-1,369,804)x1 [NCBI 37.3/hg19] with 30% mosaicism, a 18q partial trisomy with 40% mosaicism (Figure 2), an inherited 386.73 kbp microdeletion at 7q31.1(110,923,434-111,310,159)x1 [NCBI 37.3/hg19] and a *de novo* 25.72 Mb microduplication at Xp22.33p21.3 (168,546-25,887,307)x3 [NCBI 37.3/hg19] (Figure 3). The progenitor's CMA confirmed *de novo* genomic imbalances in their child (Table 1).

Discussion

In our study, the use of microarray analyses allowed the identification of genomic rearrangements in a girl severely affected with intellectual disability, multiple congenital abnormalities and intense dysmorphology, despite her normal karyotype.

CMA showed 386.73 kbp microdeletion at 7q31.1, inherited from her mother, likely pathogenic because harbor *IMMP2L* gene that, according to authors, deletions involving this gene have been associated with attention deficit hyperactivity disorder (ADHD) [8], autism [9], and Tourette syndrome [10]. Moreover, the *IMMP2L* gene was also found in inherited rare CNV-associated gene set in ADHD patients [8].

Furthermore, we observed two genomic imbalances at 18 chromosome. A *de novo* mosaic microdeletion at 18p11.32, a genomic imbalance comprising 11 genes, and the gene only was *ADCYAP1*, also known as *PACAP*, related to intellectual disability [11]. Additionally, the monosomy of 18p chromosome refers to a chromosomal disorder, resulting

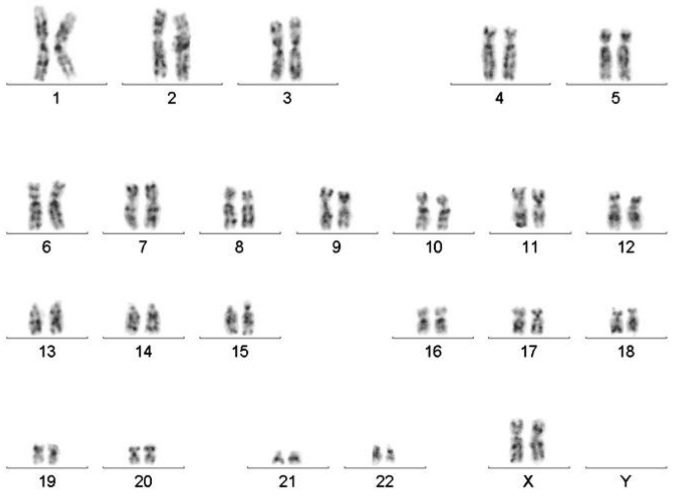

Figure 1 G-banded karyotype. Displaying no numerical or structural karyotype deviations (46,XX).

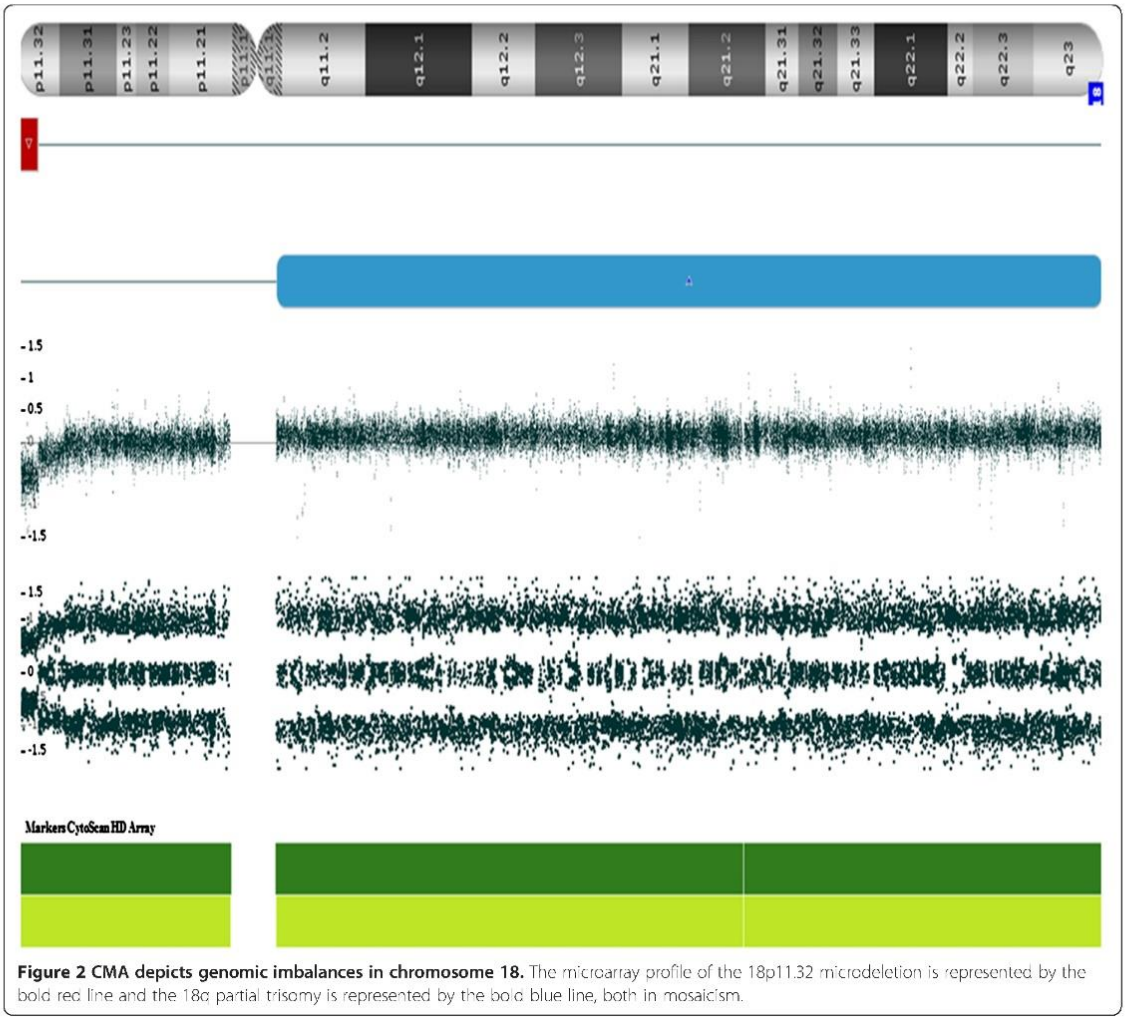

Figure 2 CMA depicts genomic imbalances in chromosome 18. The microarray profile of the 18p11.32 microdeletion is represented by the bold red line and the 18q partial trisomy is represented by the bold blue line, both in mosaicism.

from the absence of all or part of the short arm, and the clinical features frequently include mild to moderate mental retardation, short stature, and speech delay [12].

On the other hand, the most relevant alteration observed was a *de novo* 18q partial trisomy with 40% mosaicism. The degree of clinical manifestations in a patient who presents partial trisomy of chromosome 18 is variable, generally include a relatively mild phenotype with a high survival rate [1]. In the partial trisomy form, only a segment of the chromosome 18q is present in triplicate, often resulting from a balanced translocation or an inversion carried by one parent. This type of trisomy accounts for approximately 2% of cases presenting with the Edward's phenotype. The location and the extent of the triplicated segment and the possible associated deletion of genomic material due to unbalanced translocation can explain the variable phenotype associated with partial trisomies [13].

The clinical findings are highly variable in a phenotypic spectrum that spans from the absence of dysmorphic features with normal intelligence to the complete trisomy 18 syndrome. Thus, clinical and cytogenetic diagnostic of chromosome 18 partial trisomy in mosaic state is a challenge for both medical and laboratory personnel. The etiologic factors for mosaic trisomy 18 are unknown, but advanced maternal age is an important factor that increases the risk of chromosomal nondisjunction in offspring. Nevertheless, the association between paternal age (40 years of age or older) and chromosomal abnormalities may be investigated [14,15].

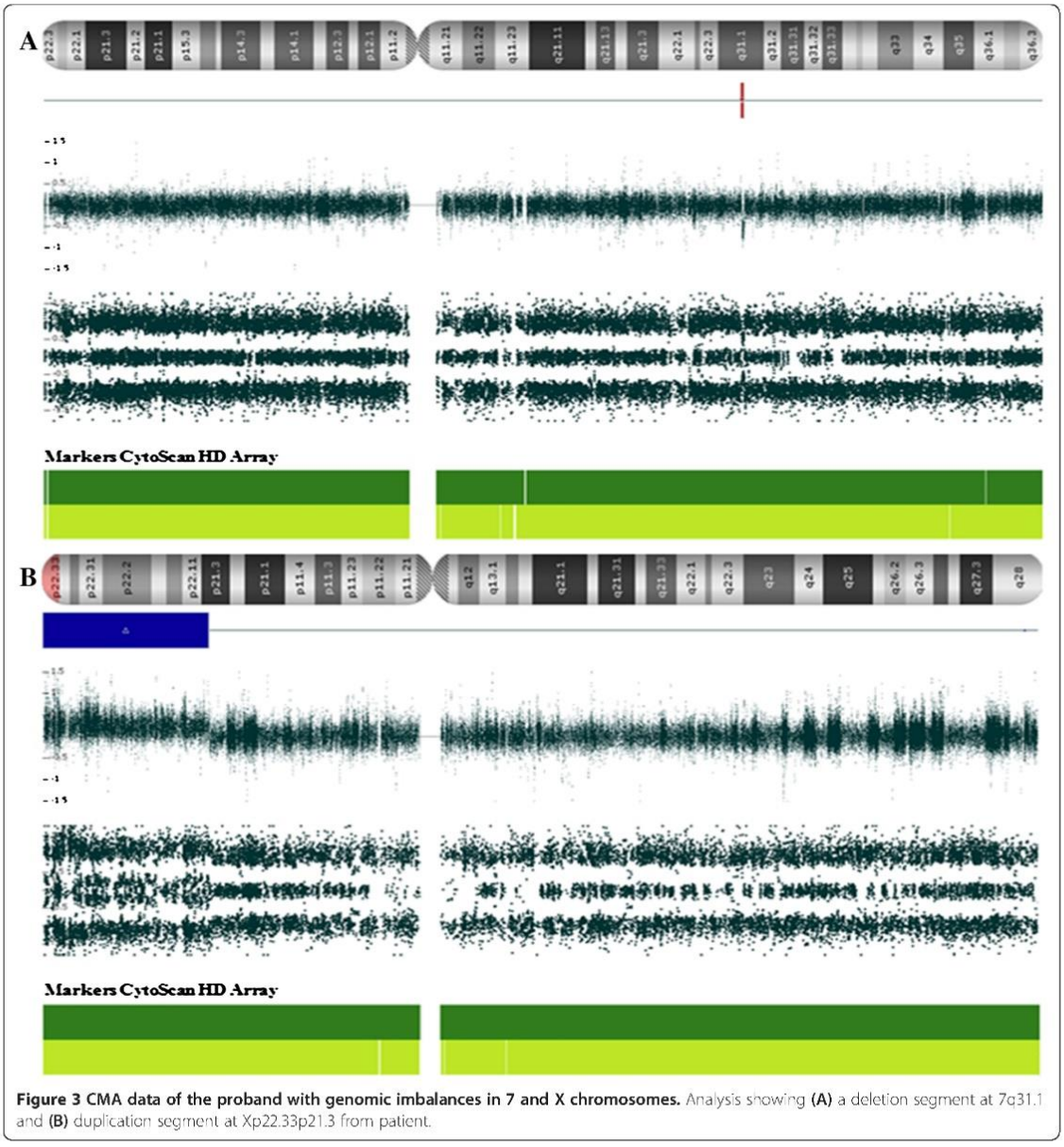

Figure 3 CMA data of the proband with genomic imbalances in 7 and X chromosomes. Analysis showing **(A)** a deletion segment at 7q31.1 and **(B)** duplication segment at Xp22.33p21.3 from patient.

We also found a *de novo* 25.72 Mb microduplication at Xp22.33p21.3, involving 109 genes. However, only 8 OMIM morbid genes have been described in association with intellectual disability, namely: *NLGN4X, AP1S2, NHS, CDKL5, RPS6KA3, MBTPS2, SMS, ARX*. Interstitial duplications of the short arm of the X chromosome have been rarely described. Female carriers of partial Xp duplication exhibit variable developmental defects, because of random or selective inactivation of X chromosome [16].

The encoded protein NLGN4X belongs to a family of neuronal cell surface proteins and mutations in this gene has been associated with autism and Asperger's Syndrome (OMIM 300497). Pathogenic mutations in the X-linked *NLGN4X* in autism spectrum disorders and/or mental retardation are rare [16].

Table 1 Clinical and molecular features of patient

Clinical features*	Age (yo)	Sex	CNV	Mosaic (%)	Cytoband	Size (Mb)	Marker count	Microarray nomenclature	Number of genes	Selected OMIN Morbid Genes**	Origin	Interpretation
			Loss		7q31.1	0.39	265	7q31.1 (110,923,434-111,310,159)x1	1	*IMMP2L*	Inherited mat	LP
			Loss	30	18p11.32	1.23	1400	18p11.32(136,226-1,369,804)x1	11	*ADCYAP1*	*de novo*	LP
GDD, MS, MCA	4	F	Gain	40	18q11.1		53197	18q11.1q23 (18,608,373-78,014,123)x2-3		*18q Partial Trissomy*	*de novo*	Pathogenic
			Gain		Xp22.33	25.72	31456	Xp22.33p21.3 (168,546-25,887,307)x3	147	*NLGN4X, AP1S2, NHS, CDKL5, RPS6KA3, MBTPS2, SMS, ARX*	*de novo*	Pathogenic

*GDD = Global Developmental Delay; MS = Multiple Stigmas, MCA = Multiple Congential Anomalies; **Genes related to ID/Autsm; yo = years old; LP = Likely Pathogenic.

Moreover, the encode protein AP1S2 is a member of the adaptin protein family, disruption of the AP1 complex via mutations in AP1S2 could disrupt normal neurotransmitter processing within the synapse [17].

The encoded protein NHS may function during the development of the eyes, teeth and brain. Mutations in the *NHS* gene are related to Nance-Horan's Syndrome (OMIM 302350), which is characterized by bilateral congenital cataracts, dental anomalies, craniofacial abnormalities and, in some cases, mental retardation. It is important in the limbic system, given the range of neuropsychological abnormalities, including mental retardation, autism, aggression, anxiety, stereotypical behavior and mood disturbance [18].

The *CDKL5* gene is a member of Ser/Thr kinase family and encodes a phosphorylated protein with a kinase activity. According to some researchers, female with *CDKL5* mutations experience no regression and the delay of psychomotor development is present since birth [19]. Alterations involving *CDKL5* have also been found in some patients with Hanefeld variants, a congenital form of Rett Syndrome, an X-linked dominant severe neurodevelopmental disorder that affect almost exclusively girls. Moreover, mutation or chromosomal translocations involving *CDKL5* have also been identified in patients with infantile spasms associated with mental retardation and in West's Syndrome (OMIM 308350) patients [20].

The *RPS6KA3* gene encodes a member of the RSK (ribosomal S6 kinase) family of Ser/Thr kinases. The activity of this protein has been implicated in controlling cell growth and differentiation. Mutations in the *RPS6KA3* gene were first reported in Coffin-Lowry Syndrome (OMIM 303600) [21], and this gene also affects nonsyndromic X-linked intellectual disability and nonsyndromic X-linked intellectual disability without bone abnormalities [22]. In addition, one case was reported in which a boy with mild ID and a maternally inherited microduplication at Xp22.12. The duplicated region included *RPS6KA3*, a key gene related to mental retardation or intellectual disability in humans [21].

The *MBTPS2* gene encodes a intramembrane zinc metalloprotease, which is essential in development, and mutation in this gene can cause the Ichthyosis Follicularis, Atrichia, and Photophobia Syndrome, with or without BRESHECK Syndrome (OMIM 308205), which is an X-linked multiple congenital anomaly disorder with variable severity, and some patients have additional features, including mental retardation [23].

Conclusion

This is the first case of a Brazilian child reported with complex genomic alterations involving chromosomes 7, 18 and X that went undetected by banding cytogenetics. However, the complex rearrangements were detected by chromosomal microarray analysis using clinical relevant probes. Synergistic effects from the rare genomic imbalances is likely responsible for the severe observed clinical phenotype in the proband. Further studies are required to define the role of the genes presented in this report to define the physiopathology of phenotypes similar to the one described here.

The rare and complex phenotypes need to be investigated to define the subsets and allow the phenotypes classification. Furthermore, this will allow adequate clinical management and a better follow up of the proband and the family.

Materials and methods

Following the Pontifícia Universidade Católica de Goiás ethics approval (CAAE 0051.0.168.000-11) an informed consent was signed and peripheral blood was obtained for cytogenetic studies including karyotyping and CMA. Conventional cell culture, harvesting, and GTG banding followed standardized procedures [24]. Chromosome analyses were done using the software IKAROS® (Metasystems Corporation, Germany).

After karyotyping, the array analysis were performed on both patient and her parents, in order to determine the origin of potential DNA imbalances, either *de novo* or inherited. Genomic DNA was isolated from whole blood using QIAamp® DNA Mini kit (Qiagen, Germany). Total DNA (250 ng) was amplified, labeled, and hybridized using GeneChip CytoScan™ HD array protocols (Affymetrix, USA) according to the manufacturer's instructions. The array was designed specifically for cytogenetic research, including ≈ 2,696,550 CNV markers, 743,304 SNP markers, and > 1,953,246 non-polymorphic markers. CEL files obtained by scanning the arrays were analyzed using the Chromosome Analysis Suite (ChAS) software (Affymetrix, USA). Gains and losses that affected a minimum of 50 and 25 markers, respectively, in a 100 kb length were initially considered.

CNVs were classified according to their nature, based on [25,26]. In summary, the CNVs found in each patient and their biological parents were compared with genomic variants in public databases, including Database of Genomic Variants (DGV), Database of Chromosomal Imbalance and Phenotype in Humans using Ensemble Resources (DECIPHER), and CytoScan™ HD Array Database. CNVs were classified as pathogenic, likely pathogenic, and of unknown clinical significance, according to [24,25]. CMA analysis followed the manufacter's instruction regarding mosaic, which follows the same principle of a complete CNV, a mosaic call was made by the software and included a clear result in SmoothSignal and Allele Peaks.

Consent

Written informed consent was obtained from the parents of the patients for publication of this report and the accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Abbreviations

CMA: Chromosomal microarray analysis; CNV: Copy number variation; ID: Intellectual disability; ChAS: Chromosome analysis suite; ASD: Autism spectrum disorders.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

IPP, LBM, ASC, DMS, CCS and ADC have made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; DMCC and CLR carried out the cytogenetics studies; IPP, AVM and RR carried out the chromosomal microarray analysis. All authors read and approved the final manuscript.

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7. CAPÍTULO VI

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Screening for Intellectual Disability Using High-Resolution CMA Technology in a Retrospective Cohort from Central Brazil

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Abstract

Intellectual disability is a complex, variable, and heterogeneous disorder, representing a disabling condition diagnosed worldwide, and the etiologies are multiple and highly heterogeneous. Microscopic chromosomal abnormalities and well-characterized genetic conditions are the most common causes of intellectual disability. Chromosomal Microarray Analysis analyses have made it possible to identify putatively pathogenic copy number variation that could explain the molecular etiology of intellectual disability. The aim of the current study was to identify possible submicroscopic genomic alterations using a high-density chromosomal microarray in a retrospective cohort of patients with otherwise undiagnosable intellectual disabilities referred by doctors from the public health system in Central Brazil. The CytoScan HD technology was used to detect changes in the genome copy number variation of patients who had intellectual disability and a normal karyotype. The analysis detected 18 CNVs in 60% of patients. Pathogenic CNVs represented about 22%, so it was possible to propose the etiology of intellectual disability for these patients. Likely pathogenic and unknown clinical significance CNVs represented 28% and 50%, respectively. Inherited and *de novo* CNVs were equally distributed. We report the nature of CNVs in patients from Central Brazil, representing a population not yet screened by microarray technologies.

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Introduction

The most widely accepted definition of mental retardation (MD) relays on impairment of both cognitive and social adaptive functions [1]. Recently, the terminology MD had been replaced with DD/ID referring to patients affected with developmental delay/intellectual disability. Epidemiological data suggests that DD/ID affect up to 3% of the worldwide population and it may concur with or without multiple congenital anomalies (MCA) and also can be found in patients with no phenotypic traits [2-4].

ID is a complex, variable, and heterogeneous disorder, representing a disabling condition diagnosed worldwide, most commonly associated with neuropsychiatric disorder in children and adolescents [1]. ID is a condition characterized by the impairment of cognitive and adaptive skills that begins before the age of 18 years [3,4]. The etiological diagnosis of ID remains a clinical challenge. For this particular trait, genetic and environmental factor may play an important role in its pathogenesis [1].

ID has a major impact on the life of the affected person, as well as his or her family and society. Thus, a specific diagnosis allows for a better understanding of the etiology of ID and patient clinical management, prognosis, and risk of recurrence [5]. Microscopic chromosomal abnormalities and well-characterized genetic conditions are the most common causes of ID, accounting for up to 40% of the observed cases. G-banding karyotyping is the standard genetic test for the laboratory investigation of ID and definitive diagnose is found in about 28% of ID cases following conventional karyotyping. Additionally, microscopic chromosome aberrations are found in association with up to 35% of the cases when using molecular cytogenetic tools, such as FISH, CGH, and MLPA that have contributed to increase the resolution of chromosomal rearrangement detection. However, about 50% of the molecular etiology of ID remains unknown. Therefore, at least half of children with ID remains without a diagnosis and poses a relevant clinical challenge [2,6,7].

Array-based chromosomal analyses have made it possible to identify putatively pathogenic copy number variation (CNVs) that

could explain the molecular etiology of ID. Current estimates have indicated that approximately 15% to 20% of ID cases are due to submicroscopic CNVs [1,8–10]. Recently, Chromosomal Microarray Analysis (CMA) using high density SNP probes increased genomic resolution and improved the detection of pathogenic microdeletions and microduplications. Moreover, the detection of cryptic genomic imbalances associated with apparently balanced chromosome rearrangements can also be detected when using CMA [11]. Nowadays, CMA has become the genetic test of choice to detect CNVs in patients with developmental disabilities and has increased the diagnostic yield for global developmental delay, intellectual disability, autism, and epilepsy [12,13].

From 2010 through 2012, several patients with initial diagnosis of ID were referred to our laboratory. Herein, we attempt to establish an explanation for the phenotype of 15 patients with ID using a high-density resolution SNP microarray to determine clinical relevant CNVs over the entire genome. We report that 10/15 patients showed deletions and/or duplications that could explain patients' phenotype. To our knowledge, this is the first report on genomic rearrangements and ID in a cohort from Central Brazil, representing a population not yet screened by microarray technology.

The aim of the current study was to identify possible submicroscopic genomic alterations using a high-density chromosomal microarray in a retrospective cohort of patients with otherwise undiagnosable intellectual disabilities referred by doctors from the public health system in Central Brazil.

Materials and Methods

Patients

All participants had ID without etiological diagnosis after undergoing a thorough clinical evaluation. Assistant physicians from the Goiás state public health system referred each patient to our genetic service at both the Laboratory of Human Cytogenetic and Molecular Genetics and the Biology Department at Pontifical Catholic University in Central Brazil. The study population was comprised of a retrospective cohort which included 305 probands with clinical diagnosis of ID with our without multiple congenital anomalies (MCA) assisted at the Laboratory from 2010 to 2012. From those, 182 patients had visible chromosome aberrations from which accurate and definite diagnoses were possible. Of the remaining 123 cases, we contacted every family in order to explain about CMA as a new available genetic test potentially useful to explain the ID of the proband and also to offer the test to the family. From the remaining cases, 3 children died, 39 children missed one or both biological parents, for 44 cases the follow up were not successful due to address/telephone changes and additional information unavailability to contact the families, and 22 families were invited to participate in study. Based on an autonomous voluntary decision, only 15 families joined the task and signed an ethical informed consent if the cases adhered to the following inclusion criteria: (1) Normal G-banding karyotyping; (2) PCR negative results for *FMR1* gene mutations; (3) No family history of DD/ID; (4) No medical history of hypoxia, intoxication, infection or cranial trauma; (5) No history of perinatal brain injury; (6) Probands born to intellectually normal non-consanguineous parents; (7) Availability of biological parents to participate in the study. Due to cognitive limitations and/or individual age, probands were not able to make the decision to enroll themselves in the study. Thus, their parents or guardians signed the informed consent forms approved by the Ethics Committee on Human Research at the Pontifical Catholic University of Goiás (CEP-PUC/GO), under the protocol number 1721/2011.

Biological Samples

For each proband and their biological parents, a total of 5 mL of peripheral blood was drawn using a standard vacuum extraction blood-collecting system containing EDTA. Genomic DNA was isolated from whole blood using QIAamp DNA Mini kit (Qiagen, Germany), following the manufacturer's instructions. Conventional cell cultures, harvesting, and G-banding at the level of 550 bands were performed for all patients following standardized procedures [14]. Chromosome observations were performed using a Zeiss Axioscope (Göttingen, Germany) and analyses using IKAROS (Metasystems Corporation, Altlussheim, Germany).

CMA: Chromosomal Microarray Analysis

The analyses were carried out on probands and their biological parents in order to establish the origin of DNA rearrangements if *de novo* or inherited. A total of 250 ng of isolated DNA for each sample was digested with NspI, ligated, PCR amplified and purified, fragmented, biotin-labeled, and hybridized to be used in a GeneChip HD CytoScan Array (Affymetrix, Santa Clara, USA), following strictly the manufacturer's protocol to identify potentially pathogenic CNVs. The array was designed specifically for cytogenetic diagnose, including ~2,7 million clinically relevant CNVs based on 743,304 SNP, and >1.9 million non-polymorphic probes covering the whole human genome. CEL files obtained by scanning the arrays were analyzed using the Chromosome Analysis Suite (ChAS) software (Affymetrix, Santa Clara, USA) in order to establish the genotypes. Genomic gains and losses that affected a minimum of 50 and 25 markers, respectively, in a 100 kb length were initially considered to determine the relevance of duplications and deletions. When using ChaS, CNVs boundaries were putatively inferred based on probe density. The two major quality control metrics for the GeneChip HD array were the Median Absolute Pairwise Difference (MAPD) and SNP-QC scores that apply to copy number and SNP probes, respectively. For our diagnostic setting, we applied the parameters ≤0.25 for MAPD and ≥15 for SNP-QC.

CNV classification

CNVs were classified according to their nature, based on [2,10,15]. In summary, the CNVs found in each patient and their biological parents were compared with genomic variants in public databases, including Database of Genomic Variants (DGV), Database of Chromosomal Imbalance and Phenotype in Humans using Ensemble Resources (DECIPHER), and CytoScan HD Array Database. CNVs were classified as pathogenic, likely pathogenic, and of unknown clinical significance, according to [10,15]. Benign CNVs were filtered out from subsequent analysis.

Results

Herein, we report the results for CMA of 15 families, including proband and their biological parents. The cohort was comprised of 6 males and 9 females with intellectual disability with age ranging from 2 to 25 years old, and their progenitors with age at conception ranging from 21 to 47 years old. For all probands, G-banding karyotypes showed no visible alterations (46 XX or 46XY). The clinical and molecular features of patients were included in Table 1.

We found a total of 18 CNVs that were identified in 9/15 (60%) patients. The CNV set was equally distributed, 9/18 (50%) and 9/18 (50%) were duplications and deletions, respectively. In six cases (40%), no chromosome rearrangement was observed. Moreover, molecular karyotyping of all 30 progenitors included in our cohort showed no evidence of chromosome rearrangements. Pathogenic

Table 1. Clinical and molecular features of 15 probands with intellectual disability screened with high-resolution CMA technology in Goás (Brazil).

Case	Clinical features*	Age (yo)	Sex	CNV	Mosaic (%)	Cytoband	Size (Mb)	Marker Count	Microarray nomenclature	Number of genes	Selected OMIN Morbid Genes**	Origin	Interpretation
001	GDD, SS	9	F	NAF									Negative***
002	GDD	11	M	Loss		1p13.3	0.13	54	1p13.3(108,726,456–108,853,796)x1	2	*NBPF4*	*de novo*	UCS
				Gain		1q44	0.53	600	1q44(246,174,090–246,702,392)x3	2	*SMYD3*	Inherited mat	UCS
				Gain		1q44	0.34	348	1q44(247,080,457–247,416,825)x3	8	*AHCTF1, ZNF124*	Inherited mat	UCS
				Gain	50	17p11.2	3.68	4151	17p11.2(16,769,800–20,446,820)x3	64	*COPS3, SMCR9, RAI1, SMCR5, TOM1L2, LRRC48, ATPAF2, DRG2, MYO15A, ALKBH5, FUI, SMCR8, SHMT1, USP32P2, CCDC144B, B9D1,MFAP4, RNF112*	*de novo*	Pathogenic
003	GDD	17	M	Gain		7q31.32	0.67	785	7q31.32(122,366,542–123,036,250)x3	3	*CADPS2*	Inherited pat	UCS
004	GDD, SS, MS	2	M	Gain		12q13.13.	0.52	396	12q13.13q13.2(54,462,464–54,980,062)x3	19	*SMUG1*	*de novo*	UCS
				Loss		14q11.2	0.21	298	14q11.2(22,732,618–22,941,375)x1	0		Inherited mat	UCS
005	GDD, MS	9	M	NAF									Negative
006	GDD	11	F	Loss		Xq27.3	4.18	10150	Xq27.3q28(144,580,614–148,757,072)x1	33	*CXorf1/TMEM257, FMR1, TMEM185A, IDS*	*de novo*	Pathogenic
007	GDD, MS, MCA	4	F	Loss		7q31.1	0.39	265	7q31.1(110,923,434–111,310,159)x1	1	*IMMP2L*	Inherited mat	LP
				Loss	30	18p11.32	1.23	1400	18p11.32(136,226–1,369,804)x1	11	*ADCYAP1*	*de novo*	LP
				Gain	40	18q11.1	5.80	2125	18q11.1q23(18,608,373–78,014,123)x2–3		*18q Partial Trissomy*	*de novo*	Pathogenic
				Gain		Xp22.33	25.72	31456	Xp22.33p21.3(168,546–25,887,307)x3	147	*NLGN4X, AP1S2, NHS, CDKL5, RPS6KA3, MBTPS2, SMS, ARX*	*de novo*	Pathogenic
008	GDD, SS	25	F	NAF									Negative
009	GDD, MS, ALS	10	F	NAF									Negative
010	GDD, MS	9	F	NAF									Negative
011	GDD, BD, MS	8	F	Loss		1p31.3	10.89	10080	1p31.3p31.1(68,693,129–79,580,916)x1	49	*AKS*	*de novo*	LP
012	GDD	6	M	Loss		14q11.2	0.34	580	14q11.2(22,599,355–22,943,573)x1	0		*de novo*	UCS
014	GDD, SS	14	M	Loss		14q11.2	0.14	188	14q11.2(22,799,790–22,944,507)x1	0		Inherited mat	UCS
				Gain		22q11.23	0.34	140	22q11.23q12.1(25,656,237–25,994,326)x3	4		Inherited mat	LP
015	GDD, MS	8	F	Loss		15q23	0.14	180	15q23(71,537,904–71,673,921)x1	1		Inherited mat	UCS
				Gain		Xq28	0.14	344	Xq28(152,720,466–152,860,955)x3	4	*ATP2B3, FAM58A*	Inherited mat	UCS
016	GDD, MS	5	F	NAF									Negative

* SS = Short Stature; GDD = Global Developmental Delay; MS = Multiple Stigmas, MCA = Multiple Congential Anomalies, ALS = Autism Like Symptoms; BD = Behavior Disorders; Disturbance of brain electrical activity; ** Genes related to ID/Autism; *** Negative means that no genomic rearrangements were found using CMA; yo = years old; NAF = No Alterations Found; UCS = Unknown Clinical Significance; LP = Likely Pathogenic.

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and likely pathogenic CNVs were classified based on their size, gene content, and previously reported cases of potential involvement with pathogenic mechanism in human and animal models. In our cohort, pathogenic CNVs represented about 22% (4/18) of all observed rearrangements and were associated with chromosomes 17, 18, and X (Figure 1 and Figure 2), involving genes that were related to the formation and/or maintenance of the central nervous system. Also, pathogenic CNVs included morbid genes from OMIM (Online Mendelian Inheritance in Man). Moreover, all pathogenic CNVs in this study had a *de novo* origin. Four out of 18 (22%) CNVs were classified as likely pathogenic and 10/18 (56%) CNVs were classified as of unknown clinical significance because they overlapped by more than 90% of the CNVs observed in the databases of normal control groups. Inherited and *de novo* CNVs were equally distributed. However, 8/18 (45%) of CNVs were maternally inherited.

Discussion

The correct diagnosis of a neurological disorder is crucial for predicting the probands' clinical follow up, to establish accurate prognostic, and to provide adequate genetic counseling. CMA technology is a relatively new strategy useful as an additional tool for genetic diagnosis. The method has been recommended as the first-tier diagnostic test for patients with global developmental delay, intellectual disability, autism spectrum disorders, and multiple congenital anomalies [13].

In the current study, high-resolution CMA was carried out on 15 patients with ID, and relevant CNVs were found in 9 patients. From those, it was possible to propose the etiology of intellectual disability for 3 patients (20%), which is consistent with other studies that have used this or similar technologies, and reported improving the diagnostic yield up to 10–25% [10,15–17].

No changes in copy number were observed in six (40%) patients. Thus, it was not possible to suggest a genetic cause for the ID and high-resolution CMA was not useful to diagnose these cases. The possibility of testing these individuals with more

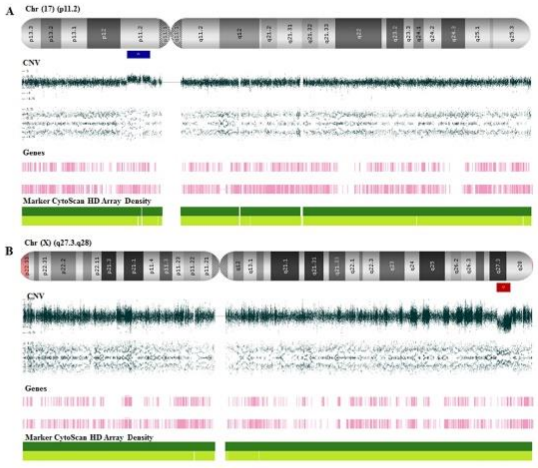

Figure 1. CNVs pathogenics from probands 002 and 006. (**A**) CMA from patient 002 showing a 3.677 Mb microduplication at 17p11.2 involving 64 genes. (**B**) CMA from patient 006 showing Xq27.3-q28 microduplication with 4.176 Mb that includes 4 genes related to intellectual disability.
doi:10.1371/journal.pone.0103117.g001

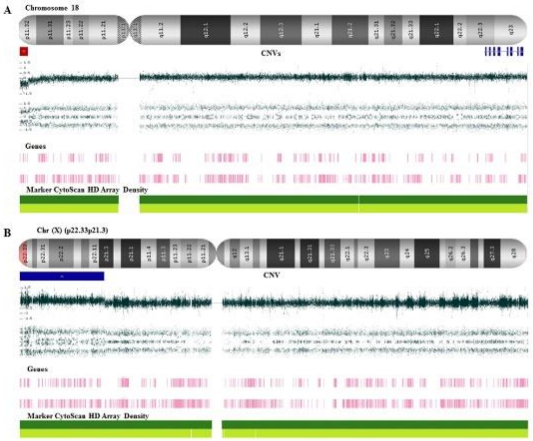

Figure 2. Genomic imbalances from proband 007. (**A**) 18p11.32 microdeletion (red line) and the 18q partial trisomy in mosaicismo (light blue line) (**B**) *de novo* microduplication at Xp22.33-Xp21.3 (blue line) that involves 147 genes.
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sensitive or specific genomic technologies, such as next-generation exome sequencing could be a fruitful diagnostic approach in order to identify gene mutations that may be causing the observed phenotype [18–20]. Moreover, ID is a polygenic complex and heterogeneous multifactorial trait, which remains a diagnostic challenge for human geneticists and heavily affected by environmental factors.

We identified 4 (22%) pathogenic CNVs, including chromosomal imbalances associated with 17p11.2, Xq27.3, 18q11.1 and Xp22.33. Case 2 was a boy who presented a *de novo* microduplication at 17p11.2. Interestingly, the log ratio probe intensity on this region was compatible with a mosaic duplication affecting about 50% of cells. The region has been implicated in the Potocki-Lupski Syndrome (MIM 610883) [21,22]. Additionally, microdeletion of this region has been associated with Smith-Magenis Syndrome (MIM 182290). Gain or loss of genomic material on chromosome band 17p11.2 inevitably leads to phenotypes that include ID as a relevant trait [23].

Patient 6 was a girl who showed a *de novo* microdeletion of about 4.2 Mb at Xq27.3, including the region of the Fragile X Syndrome (FXS). CGG trinucleotide repeats expansions are the most common cause of FXS. However, less frequently point mutations and partial or full deletions of the *FMR1* gene also cause the FXS. To date, only 10 female index patients with deletions harboring *FMR1* have been reported. Moreover, the severity of the phenotype for females with *FMR1* deletions correlates with their X-chromosome inactivation [23–25].

Case 7 was a girl severely affected with multiple congenital abnormalities and intense dysmorphology. This proband, presented a *de novo* 18q partial trisomy with 40% mosaicism identified by CMA. Furthermore, we analyzed 100 metaphase spreads and we observed the duplication of 18q in a mosaic state in 45% of the cells. We also found that the break point called by the CMAs was overestimated due to an array of small duplications upstream from the real duplication site, which created an artificially large partial trisomy of 18. Also we observed in CMA a small deletion in the terminal region stating at 18p11.32. The karyotyping reveled that in fact there was a duplication of a distal region of 18q starting at 18q22.3, resulting in a segment of 5.8 Mb. The chromosome

images revealed that the two breakpoints alongside the chromosome 18 created a rearranged chromosome, which included a pericentric inversion of that DNA segment. As a result, there was a loss of the terminal end of 18q, including its centromere. Although CMA was robust to identify the deletion and the duplication, it was not able to indicate the chromosomal inversion. Moreover, especial care must be devoted to the callings made CMA in order to avoid overestimation of the size of the segments involved in gain or loss of chromosome material.

Full trisomy 18 is known to cause Edward's Syndrome. However, partial trisomy of chromosome 18, especially involving 18q, has long been reported to cause peculiar phenotypes that show cognitive impairment, varying from a milder to a more severe form, with or without internal malformation. The extent of the partial trisomy and the level of the mosaic state affect the odds of patients' survival [26–31]. Interstitial duplications of the short arm of the X chromosome have been rarely described [32]. In Patient 7, we also observed a *de novo* microduplication at Xp22.33-Xp21.3 harboring 8 OMIM morbid genes (Table 1) that have been described in association with ID [32–36].

The CNVs designated as likely pathogenic comprise of 22% of our data. They were called likely pathogenic because they harbor genes having well-established association with abnormal phenotypes. Moreover, their genic content has been implicated in the process of neurological development (42), as mediators of neuroendocrine stress responses (51), to be expressed exclusively in the brain (64). However, none of the genes observed in the likely pathogenic CNVs was yet directly related to ID. We also observed a maternally inherited region which was involved with Chromosome 22q11.2 Duplication Syndrome (MIM 608363) [37]. This region is also found deleted in the DiGeorge Syndrome (MIM 188400) and Emanuel Syndrome (MIM 609029). Both syndromes are characterized by multiple congenital anomalies, significant developmental delay, and mental retardation [38].

At the case 15, despite the location of *ATP2B3* and *FAM58A* genes in Xq28, this region has not yet been implicated in ID *per se*. *ATP2B3* gene encodes a calcium-transporting ATPase predominantly expressed in the brain, and mutations in the gene have been associated with increased plasmatic concentrations of aldosterone and reduced plasmatic potassium [39]. Moreover, base substitution in *ATP2B3* identified by exome sequencing in a family with X-linked congenital ataxia (XCA) indicated the importance of calcium homeostasis in neurons. Nevertheless, the affected persons present neither mental retardation nor pyramidal tract involvement at their neurological examinations [40]. On the other hand, mutations in *FAM58A* cause an X-linked dominant disorder known as STAR Syndrome (MIM 300707). This syndrome presents facial dimorphism, toe syndactyly, telecanthus, anogenital and renal malformations [41]. Nevertheless, patients with STAR Syndrome do not show ID.

The proportion of CNVs classified as of unknown clinical significance was high (56%) in our study. According to researches, the ability to detect CNVs has far outpaced our ability to understand their role in a disease [16]. Inheritance studies are the primary strategy recommended to estimate the role of such CNVs in pathogenicity. Nevertheless, it is often imprudent to attribute clinical significance to a CNV based solely on its inheritance pattern as a growing number of CNVs show an incomplete penetrance and also because de novo CNVs may represent benign variants. The clinical and genetic interpretation of the data acquired by CMA technologies still remains a challenge and often require further specific investigations [42]. To confirm the pathogenicity of a CNV requires studies designed to understand the causative relation between the genomic imbalances to the investigated disease. The genetic interpretation and clinical relevance of the data acquired by CMA technologies remain a challenge. Primarily due to the deleterious effects caused by small genomic variations, such as point mutations, further influenced by reduced penetrance, variable expressivity, and gene dosage [43].

Despite the small size of the cohort screened in the current study, here we report the nature of CNVs in patients from Central Brazil, representing a population not yet screened by microarray technologies. Tests based on microarray technologies are relatively new and are likely to continue to evolve in the coming years. For both, patients and families, it is very important to establish a diagnosis for ID. Furthermore, the diagnosis has to be linked to genetic counseling to ensure that parent's views and preferences are taken into account, following a non-directive approach. The results of our study helped the families and their assistant physicians to reach an accurate diagnosis bringing closure to their post-natal search for an explanation regarding the ID observed in the family.

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Author Contributions

Conceived and designed the experiments: ADC CCS DMS. Performed the experiments: RRP IPP LBM CLR AVM DMCC ASC. Analyzed the data: IPP LBM DMS ASC ADC. Contributed reagents/materials/analysis tools: ADC DMS CCS. Wrote the paper: ADC RRP IPP LBM DMS.

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8. CAPÍTULO VII

8.1 Discussão Executiva

A deficiência intelectual é um problema de saúde pública e um grande problema sócio-econômico. Portanto, o diagnóstico preciso da DI é essencial para iniciar uma avaliação adequada nos serviços de referência, com apropriado e contínuo gerenciamento de comorbidades esperadas e o aconselhamento genético que atenda às necessidades das famílias (Srour, Shevell, 2014). Sendo, o diagnóstico correto em pacientes com DI/ADG, autismo e múltiplas anormalidades congênitas, essencial para prever a evolução clínica, estimar o risco de repetição na família ou simplesmente trazer alívio emocional para os pais (Zilina *et al.*, 2014).

Dessa maneira, a prática do aconselhamento genético se faz urgente nos serviços de saúde, voltado para as famílias que possuem indivíduos com DI, para casais cuja a prole está em risco de alguma doença ou anomalia devido à história familial positiva, consanguinidade, idade materna avançada, casais que tiveram filhos natimortos ou malformados ou duas ou mais perdas gestacionais sem causa aparente, casais sob tratamento de infertilidade ou membros de uma etnia onde alguma doença monogênica apresente maior incidência ou prevalência. Fornecendo, então, cuidados clínicos, educação e suporte emocional aos indivíduos e familiares que enfrentam doenças genéticas e herdadas (Bertollo *et al.*, 2013).

Novas técnicas de citogenética molecular com uma maior resolução, alta densidade e robustez são eficientes, capazes de identificarem em até 25% dos casos várias microdeleções e microduplicações associadas à DI (Rodrigues-Revenga *et al.*, 2013; Bartnik *et al.*, 2014). Provando serem importantes ferramentas para o estudo em pacientes com DI idiopática, essas novas técnicas trazem uma melhoria para qualidade de vida dos pacientes e suas famílias, proporcionando o entendimento da causa genética e a aceitação da condição já estabelecida.

Makela e colaboradores (2009) estudaram 20 famílias de crianças com DI com ou sem o diagnóstico etiológico e observaram que estas famílias tinham necessidades e sentimentos específicos em relação ao diagnóstico oferecido, mostrando que o diagnóstico estabelecido proporcionava nas famílias o poder de defender seus filhos, dava esperanças para tratamentos, ajudava na obtenção de serviços desejados e ajudava no acesso ao companheirismo emocional (ou “desafios semelhantes”) entre outras famílias.

A utilização da técnica de CMA nos pacientes com sinal clínico de DI, encaminhados ao serviço de genética dos Laboratórios REPLICON–PUC–Goiás e LAGENE/LACEN–SES–GO, mostrou-se eficiente e eficaz, conseguindo em até 25% dos casos fornecer às famílias um diagnóstico genético para a condição fenotípica de seus filhos/as. Além disso, trouxe para os médicos e profissionais que acompanham essas famílias informações adicionais que poderão propiciar um cuidado personalizado.

Além do mais, a realização do aconselhamento genético com estas famílias de probandos que apresentam DI, proporcionou o entendimento da condição genética estabelecida, suas características, possibilidades e implicações, e teve o intuito de capacitar essas famílias para tomada de decisão sobre a saúde de seus filhos, com o objetivo primordial na melhora da qualidade de vida dessas famílias e seus filhos/as. Esse processo de aconselhamento genético foi ainda muito tênue e breve, fazendo-se necessário a aplicação de um questionário antes e depois da realização do teste genético para que pudesse avaliar a evolução e eficácia de todo o processo de aconselhamento genético.

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ANEXO A

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título do Projeto: Investigação das Causas Genéticas e Genômicas do Retardo Mental Autossômico

Pesquisador Responsável: Dr. Aparecido Divino da Cruz

Nome (sujeito da pesquisa): _____,

RG/Certidão nascimento _____,

Naturalidade _____, Idade _____,

Endereço _____,

Neste ato representado por mim:

Nome dos representantes legais:

Pai _____,

Naturalidade _____, Idade _____,

Estado Civil _____, Profissão _____,

Endereço_____

Mãe _____

Naturalidade _____, Idade _____,

Estado Civil _____, Profissão _____,

Endereço_____

Está sendo convidado(a) a participar de um estudo intitulado: Investigação das Causas Genéticas e Genômicas do Retardo Mental Autossômico cujos objetivos e justificativas são: propor a investigação genética do paciente com indicação clínica de retardo mental, visto que o diagnóstico de um paciente com essa síndrome estabelece uma oportunidade valiosa de fazer estudos na família, identificando outros afetados e portadores, e de realizar um aconselhamento genético eficiente permitindo a prevenção de novos casos, possibilitando assim, um tratamento específico, diminuindo o impacto econômico, social e pessoal que esta síndrome acarreta.

Os critérios de inclusão são: pacientes encaminhados ao Núcleo de Pesquisas Replicon e LaGene com indicação clínica de retardo mental, acompanhados dos pais biológicos que concordarem em assinar o termo de consentimento livre e esclarecido (TCLE).

Os critérios de exclusão são: indivíduos maiores de 18 anos, com indicação clínica de retardo mental, que não concordarem em assinar o termo de consentimento livre e esclarecido,

ou menores, cujos pais biológicos não aceitarem a participação do(a) filho(a) neste estudo, ou que não assinarem o TCLE, ou ainda, pacientes que apresentarem outras doenças genéticas já diagnosticadas.

A minha participação no referido estudo será no sentido de permitir a doação voluntária de uma amostra de sangue de meu(minha) filho(a) biológico(a) para colaborar com o diagnóstico do retardo mental, juntamente com uma amostra, doada voluntariamente, de meu sangue para comparação com a finalidade de d
eterminar causas genéticas e sua hereditariedade.

Fui alertado de que, da pesquisa a se realizar, posso esperar alguns benefícios, tais como: a confirmação da causa do retardo mental (quando tiver uma etiologia genética). Estou ciente ainda, que a técnica proposta já foi realizada em outros estudos e é reprodutiva, evitando assim possíveis riscos.

Recebi, por outro lado, os esclarecimentos necessários sobre os possíveis desconfortos e riscos decorrentes do estudo, levando-se em conta que é uma pesquisa, e os resultados positivos ou negativos somente serão obtidos após a sua realização. Durante a coleta do sangue, você poderá sentir uma dor leve a moderado, em decorrência da aplicação da agulha. Podem, também, ocorrer a formação de hematomas que não são comuns, e caso isso ocorra, você será imediatamente encaminhado(a) ao Serviço Médico da Pontifícia Universidade Católica de Goiás (PUC-GO).

Estou ciente de que a minha privacidade será respeitada, ou seja, meu nome ou qualquer outro dado, ou elemento, que possa, de qualquer forma, me identificar, será mantido em sigilo. Também fui informado de que posso me recusar a participar do estudo, ou retirar meu consentimento a qualquer momento, sem precisar justificar, e de, por desejar sair da pesquisa, não sofrerei qualquer prejuízo à assistência que venho recebendo.

O pesquisador responsável, envolvido com o referido projeto é: Aparecido Divino da Cruz, e com ele poderei manter contato pelos telefones: (62)3946-1443/3946-1086.
É assegurada a minha assistência durante toda pesquisa, bem como me é garantido o livre acesso a todas as informações e esclarecimentos adicionais sobre o estudo e suas consequências, enfim, tudo o que eu queira saber antes, durante e depois da minha participação.

Tenho sido orientado ao teor de todo o aqui mencionado e compreendido a natureza e o objetivo do já referido estudo, autorizo a participação de (nome do sujeito da pesquisa) _____ na referida pesquisa, e

manifesto meu livre consentimento em participar, estando totalmente ciente de que não há nenhum valor econômico, a receber ou pagar.

No entanto, caso eu tenha qualquer despesa decorrente da participação na pesquisa, haverá ressarcimento na forma de dinheiro em espécie. De igual maneira, caso ocorra algum dano decorrente da minha participação no estudo, serei devidamente indenizado, conforme determina a lei.

Goiânia, ____ de_____de201__.

(nome e assinatura do Pai)

(nome e assinatura da Mãe)

Pesquisador Dr. Aparecido Divino da Cruz

ANEXO B

PROTOCOLO DE ATENDIMENTO

Número do protocolo: CMA _____-_____

Paciente:_____Sexo: ( )Mas ( )Fem

Data de nascimento: ____/____/_____ Idade_____ Etnia_____

Escolaridade:_____

Filiação:

Número protocolo: CMA _____ -_____

Mãe:_____Idade:_____

Número protocolo: CMA _____- _____

Pai:_____Idade:_____

Endereço:_____

_____

Bairro:_____ Cep:_____

Cidade:_____

Telefones: Resid: ( )_____Celular: ( )_____

Comercial: ( )_____ Recado: ( )_____

E-mail: _____

Faz uso de medicamentos? _____

_____

Encaminhado pelo médico:_____

Instituição:_____

Indicação:_____

Observações:_____

_____

_____

DADOS E MEDIDAS DO PACIENTE

Altura:_____ Peso:_____ Perímetro cefálico: _____Cintura:_____

Distância oculares:_____Narina:_____Mãos:_____Braços:_____

Pernas:_____Dedos:_____Olhos:_____Orelhas:_____

Tipo sanguíneo: _____ RH:_____ Implantação da orelha: _____Arcada dentária:_____

Palato_____ Pescoço:_____ Pés:_____ Genitália:_____

Tônus muscular:_____Ossos:_____Abdômen:_____

Umbigo:_____ Palma das mãos:_____ Pigmentação da pele:_____

Irritabilidade:_____ Convulsões:_____ Data início:___/___/____

Frequência_____ Movimentos anormais: ( )Mãos ( )Cabeça ( )Olhos.

Descrição do movimento_____

_____

Observação:_____

HABITOS

Pai:

Fuma? ( )Sim ( )Não. Se sim, há quanto tempo? _____

Consome bebida alcoólica? ( )Sim ( )Não. Se sim, há quanto tempo? _____

Já usou outras substâncias? ( )Sim ( )Não. Se sim, qual e há quanto tempo? _____

_____

Trabalha? ( )Sim ( )Não. Se sim, qual a profissão e há quanto tempo? _____

_____

Mãe:

Fuma? ( )Sim ( )Não. Se sim, há quanto tempo? _____

Se sim, fumou durante a gestação? _____

Consome bebida alcoólica? ( )Sim ( )Não. Se sim, há quanto tempo? _____

Se sim, bebeu durante a gestação? _____

Já usou outras substâncias? ( )Sim ( )Não. Se sim, qual e há quanto tempo? _____

_____

Se sim, usou durante a gestação? _____

Trabalha? ( )Sim ( )Não. Se sim, há quanto tempo e qual profissão? _____

_____

Observações: _____

DADOS FAMILIARES

Possui Irmãos? ( )Sim ( )Não.

Se sim, quantos e quais idades? _____

_____

_____

_____

Possui irmãos com alguma doença genética conhecida ou com transtornos físicos ou mentais?

_____

_____

_____

Possui algum parente próximo com alguma doença genética conhecida ou com transtornos físicos ou mentais? _____

_____

_____

_____

Observações: _____

_____

( )Laqueadura ( )Vasectomia

CONSANGUINIDADE

Irmãos do pai (Idade, Est. Civil, Filhos N e/ou A):_____

_____

_____

Irmãos da mãe (Idade, Est. Civil, Filhos N e/ou A):_____

_____

_____

Casos semelhantes na família? _____

_____

Observações:_____

_____

Possuem algum grau de parentesco? ( )Sim ( )Não

ANTECEDENTES GESTACIONAIS:

Duração da gravidez? _____

( )Febre – Período? _____( )Rubéola – Período? _____

( )Toxoplasmose – Período? _____( )VDRL – Período?_____

( )Hepatite – Período? _____( )HIV – Período? _____

Outras Infecções? _____ Período? _____

Outras Infecções? _____ Período? _____

Outras Infecções? _____ Período? _____

Radiografias: ( )Não ( )Sim Tipos: ( )Torácicas ( )Abdominais ( )Odontológicas

( )Com Proteção ( )Sem Proteção – Período? _____

Outras exposições a radiação? _____

( )Perdas Sanguíneas – Período? _____

( )Medicações: Tipo: _____ Motivo: _____Período: _____

( )Medicações: Tipo: _____ Motivo: _____ Período: _____

( )Medicações: Tipo: _____ Motivo: _____ Período: _____

( )Anticoncepcionais: Tipo: _____ Período: _____

( )Anticonvulsivantes : Tipo: _____ Período: _____

( )Sofre de gota: Remédio: _____ Período: _____

Outras Informações: _____

_____

_____

Parto:

( ) Hospitalar ( ) Domiciliar ( ) Médico ( ) Parteira

Hospital:_____

( )Normal – Tipo de apresentação?_____

( )Fórceps – Duração? _____

( )Cesariana – Motivo? _____

Condições do RN:

Peso: _____ Comprimento: _____ Pc:_____ Pt: _____

Choro: ( )Sim ( )Não Apgar _____/_____ Idade Gestacional: _____ semanas

Sucção: _____ ( )Cianose Perm. Matern.: _____

( )Ictericia: Época: _____( )Fototerapia ( )Exsanguíneo Transfusão

Bilirrubina Máxima: _____ (Dir) _____ (Ind) _____ (Tot) _____

Outras intercorrências: _____

_____

_____

Momento da notícia/Quem: _____

_____

Primeiro tratamento recebido no hospital:

( )Nenhum ( )Medicação ( )RX ( )Sem Informação

( )Outros:_____

_____

_____

_____

_____