UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INVESTIGAÇÃO ABRANGENTE SOBRE AS RAZÕES DA ALTA FREQUÊNCIA RELATIVA DA MUCOPOLISSACARIDOSE TIPO II NO BRASIL

Juliana Alves Josahkian

Tese submetida ao Programa de Pós-Graduação em Genética e Biologia Molecular da UFRGS como requisito parcial para obtenção do grau de Doutor em Ciências (Genética e Biologia Molecular).

Orientador: Prof. Dr. Roberto Giugliani

Coorientadora: Dra. Ana Carolina Brusius-Facchin

Porto Alegre, janeiro de 2022

Este trabalho foi desenvolvido no Laboratório de Genética Molecular do Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre e teve como instituições financiadoras: FIPE/HCPA (Projeto GPPG 2003-0066 e 2017-0664), INAGEMP (CNPq 465549/2014, CAPES 88887.136366/2017-00 e FAPERGS 17/2551.0000521-0) e a Fundação Médica do Rio Grande do Sul.

Aos meus pais, Márcia e Luiz.

SUMÁRIO

LISTA DE ABREVIATURAS

AH – ácido hialurônico

AR – autossômico recessivo

ARSB – gene arilsulfatase B

ARSK – gene arilsulfatase K

CS – sulfato de condroitina

C4S – sulfato de condroitina 4

C6S – sulfato de condroitina 6

DS – sulfato de dermatan

ELISA – ensaio de imunoabsorção enzimática

GAGs – glicosaminoglicanos

GALNS – gene galactosamina-6 sulfato sulfatase

GLB1 – gene beta-galactosidase-1

GNS – gene N-acetilglicosamina-6-sulfatase

GUSB – gene beta-glicuronidase

HGSNAT – gene heparan-alfa-glucosaminida-N-acetiltranferase

HS – sulfato de heparan

HYAL1 – gene hialuronoglicosaminidase 1

IDS – gene iduronato 2-sulfatase

IDUA – gene alfa-L-iduronidase

KS – sulfato de queratan

MPS – mucopolissacaridoses

NAGLU – gene N-acetil-alfa-glicosaminidase

SGSH – gene heparan sulfato sulfatase

SNC – sistema nervoso central

TCTH – transplante de células tronco hematopoiéticas

TRE – terapia de reposição enzimática

XR – ligado ao X recessivo

LISTA DE FIGURAS

LISTA DE TABELAS

RESUMO

Introdução: As mucopolissacaridoses são um grupo de 11 doenças lisossômicas causadas por defeitos enzimáticos específicos relacionados com o catabolismo dos glicosaminoglicanos, que se acumulam e podem ser identificados como biomarcadores dessas doenças. Apresentam quadro multissistêmico e progressivo, podendo haver envolvimento cognitivo. Todas elas têm padrão de herança autossômico recessivo, exceto a MPS tipo II, que é ligada ao X recessiva. O tratamento das MPS inclui medidas de suporte e reabilitação, além de tratamentos específicos disponíveis para alguns dos tipos, como o transplante de células tronco hematopoiéticas e a terapia de reposição enzimática intravenosa. A prevalência para cada uma delas varia conforme o país estudado, sendo que a MPS II, ao contrário do que acontece na maioria dos países da América do Norte e da Europa, é o tipo mais comum no Brasil. **Objetivos:** Investigar fatores que possam explicar a maior frequência relativa de MPS II no Brasil; verificar se a alta frequência relativa de MPS II tem maior predominância em alguma região brasileira; avaliar a variabilidade fenotípica intra-familiar em pacientes com MPS II; avaliar o tempo de atraso no diagnóstico dessa patologia no Brasil. **Métodos:** O estudo foi realizado com dados de pacientes diagnosticados com MPS pelo Serviço de Genética Médica do HCPA e pela Rede MPS Brasil entre 1982 e 2020. Nós calculamos a incidência das MPS no Brasil, por região e estados da federação a partir dos dados da nossa amostra e de informações do Sistema de Informações sobre Nascidos Vivos do sistema de saúde brasileiro. Para a MPS II, estudamos o perfil molecular dos pacientes e de suas mães. Também revisamos dados clínicos registrados em prontuários e relacionamos o fenótipo neuronopático e não-neuronopático da MPS II com as variantes encontradas nesses pacientes. **Resultados:** No Brasil, a prevalência da MPS ao nascimento (para cada 100.000 nascidos vivos) entre 1994 e 2018 foi de 1,57 e foi maior para a MPS II nas regiões centro-oeste, norte, sudeste e sul. Na região nordeste, a MPS VI foi o tipo mais comum. Estudando o perfil molecular de 280 pacientes de 206 famílias com MPS II, observamos que mutações de ponto foram encontradas em 70% dos casos, sendo as variantes patogênicas do tipo missense as mais comumente detectadas no gene *IDS*. Considerando as formas neuronopática e não-neuronopática da

MPS II, houve concordância do fenótipo entre os familiares afetados, exceto entre dois meio-irmãos. As mães dos pacientes com MPS II foram portadoras da variante considerada patogênica no gene *IDS* em 82% dos casos. Considerando os dados clínicos, a mediana do atraso ao diagnóstico para as MPS em geral foi de 40 (15,25 – 84) meses e não houve diferença na idade ao diagnóstico entre o primeiro caso e os demais casos de MPS diagnosticados em uma mesma família. Para pacientes com MPS II, a forma neuronopática foi diagnosticada mais cedo do que a forma não-neuronopática. Consanguinidade parental foi detectada em 4,1% dos casos de MPS II e em 35,1% dos casos com as formas autossômicas recessivas, sendo mais encontrada no Ceará, Distrito Federal e Paraíba e com a região nordeste mostrando uma tendência à maior consanguinidade. **Conclusão:** Na ausência de uma variante patogênica que contribua particularmente para a sua alta frequência, o maior número de afetados nas famílias com MPS II, associada à baixa taxa de consanguinidade na população em geral, podem ser fatores responsáveis pela maior proporção relativa de MPS II no Brasil, para a qual parece contribuir a falta de um aconselhamento genético eficiente.

Palavras-chave: Mucopolissacaridoses, Doenças Lisossômicas, Glicosaminoglicanos, Síndrome de Hunter, Terapia de Reposição Enzimática, Epidemiologia, Brasil.

ABSTRACT

Introduction: Mucopolysaccharidoses are a group of 11 lysosomal diseases caused by specif enzymatic defects related to the catabolism of glycosaminoglycans, which accumulate and can be indentified as biomarkers. They present as multisystemic and progressive diseases, and there may be cognitive involvement. All of them are inherited as an autosomal recessive trait, except for MPS type II, which is X-linked recessive. Treatment for MPS includes supportive and rehabilitative measures, as well as specific treatments availaible for some types, such as hematopoietic stem cell transplantation and intravenous enzyme replacement therapy. Their prevalence varies according to the country studied and, unlike what happens in most American and European countries, MPS II is the most common in Brazil. **Objectives:** To investigate factors that may explain the higher relative frequency of MPS II in Brazil; verify whether the high relative frequency of MPS II is related to any Brazilian region; to assess intra-familial phenotypic variability in patients with MPS II; to assess the delay in diagnosis for MPS in Brazil. **Methods:** The study was carried out with data from patients diagnosed with MPS from the Medical Genetics Service of Hospital de Clínicas de Porto Alegre and the MPS Brazil Network between 1982 and 2020. We calculated the incidence of MPS in Brazil, per region and federation states using data from our sample and from the Information System on Live Births of the Brazilian Health System. For MPS II, we studied the molecular profile of patients and their mothers. We also reviewed clinical data from medical records and related the neuronopathic and the non-neuronopathic MPS II phenotype with the variants found in the patients. **Results:** In Brazil, the birth prevalence of MPS (per 100,000 live births) between 1994 and 2018 was 1.57 and it was higher for MPS II on the Midle-West, North, Southeast, and South regions. In the Northeast region, the MPS VI was the most common type. Molecular profile of 280 patients from 206 families with MPS II showed that point mutations were found in 70% of the cases, with pathogenic missense variants being the most commonly detected in the *IDS* gene. Considering the neuronopathic and non-neuronopathic forms of MPS II, affected family members had concordant phenotype, except between two half-siblings. The mothers of patients with MPS II were carriers of the variant considered pathogenic in the *IDS* gene in 82% of the cases. Considering the clinical data, the median delay in diagnosis for MPS in

general was 40 (15.25 – 84) months and there was no difference in the age at diagnosis between the first case and further MPS cases diagnosed in the same family. For patients with MPS II, the neuronopathic form was diagnosed earlier than the non-neuronopathic form. Parental consanguinity was detected in 4.1% in MPS II cases and in 35.1% of the autossomal recessive ones, being more frequent in Ceará, Distrito Federal and Paraíba, and also the Northeast region showed a trend towards consanguinity. **Conclusion:** In the absence of a particular pathogenic variant that explains its high relative frequency, the higher number of affected members from MPS II families, associated with the low consanguinity of the general population may be factors responsible for the higher incidence of this MPS type in Brazil, for which the lack of adequate genetic counseling seems to contribute.

Keywords: Mucopolysaccharidoses, Lysosomal Diseases, Glycosaminoglycans, Hunter Syndrome, Enzyme Replacement Therapy, Epidemiology, Brazil.

CAPÍTULO 1 – INTRODUÇÃO

1.1 Mucopolissacaridoses

1.1.1 Definição e histórico

As mucopolissacaridoses (MPS) são um grupo de doenças lisossômicas causadas por defeitos enzimáticos específicos relacionados com o catabolismo dos glicosaminoglicanos (GAGs). Apresentam quadro multissistêmico e progressivo, podendo haver envolvimento cognitivo, e classificam-se de acordo com a enzima deficiente (Neufeld & Muenzer, 2014).

Foram reconhecidas pela primeira vez no início do século XX pelo Prof. John Thomson, em Edimburgo, mas os primeiros casos foram publicados em 1917, por Charles Hunter, que descreveu dois irmãos com dismorfias faciais, displasia óssea e protusão abdominal (Henderson, 1940; Hunter, 1917). Em 1919, a médica alemã Gertrud Hurler descreveu mais dois pacientes não aparentados com características clínicas semelhantes (Hurler, 1920) e em 1929, o pediatra uruguaio Louis Morquio descreveu uma família com quatro irmãos afetados por uma displasia óssea familiar, filhos de pais consanguíneos (Morquio, 1929). Todos esses casos somente foram reconhecidos como MPS a partir da identificação de acúmulo de GAGs em tecidos e urina de pessoas afetadas (Brante, 1952; Dorfman & Lorincz, 1957; Campbell & Fried, 1961).

Os GAGs são polissacarídeos de carga negativa e classificam-se em cinco grupos de acordo com sua subunidade repetida: sulfato de condroitina (CS), sulfato de dermatan (DS), sulfato de heparan (HS), sulfato de queratan (KS) e ácido hialurônico (HA) (Kubaski et al., 2017a). Estudos das enzimas específicas envolvidas em diferentes etapas de degradação dos GAGs dentro dos lisossomos e a identificação dos genes responsáveis levaram à classificação das MPS em 11 tipos/subtipos reconhecidos atualmente e à exclusão dos tipos V, identificado posteriormente como um fenótipo leve da MPS I, e VIII, cujos achados laboratoriais não se confirmaram (Giugliani, 2012). A tabela 1 mostra os 11 tipos/subtipos de MPS e as suas particularidades e a figura 1 ilustra as etapas da degradação dos GAGs HS, DS e KS.

Tabela 1: Classificação e particularidades dos 11 tipos/subtipos de MPS reconhecidos atualmente.

MPS	Epônimo	GAGs	Enzima	Padrão de herança	Gene	Localização cromossômica*	Nº de variantes descritas*
I	Hurler Hurler-Scheie Scheie	HS + DS	α-L-iduronidase	AR	*IDUA*	4p16.3	320
II	Hunter	HS + DS	Iduronato-2-sulfatase	XR	*IDS*	Xq28	739
IIIA	Sanfilippo A	HS	Heparan-N-sulfatase	AR	*SGSH*	17q25.3	163
IIIB	Sanfilippo B	HS	N-acetil-α-glicosaminidase	AR	*NAGLU*	17q21	256
IIIC	Sanfilippo C	HS	α-glicosamina-acetilCoA transferase	AR	*HGSNAT*	8p11.1	91
IIID	Sanfilippo D	HS	N-acetil-glicosamina 6-sulfatase	AR	*GNS*	12q14	28
IVA	Morquio A	KS + C6S	Galactosamina-6-sulfato sulfatase	AR	*GALNS*	16q24.3	378
IVB	Morquio B	KS	β-galactosidase ácida	AR	*GLB1*	3q21.33	265
VI	Maroteau-Lamy	DS + C4S	N-acetilgalactosamina-4-sulfatase (Arilsulfatase B)	AR	*ARSB*	5q11-q13	229
VII	Sly	HS + DS	β-glicuronidase	AR	*GUSB*	7q21.11	81
IX	Natowicz	AH	Hialuronidase 1	AR	*HYAL1*	7p21.3-21.2	7

Tabela modificada e traduzida de Giugliani, 2012; *HGMD professional 2021.2, consulta realizada em 04 de fevereiro de 2022.
AH: ácido hialurônico; C4S: sulfato de condroitina 4; C6S: sulfato de condroitina 6; DS: sulfato de dermatan; HS: sulfato de heparan; KS: sulfato de queratan; AR: autossômico recessivo; XR: ligado ao X recessivo.

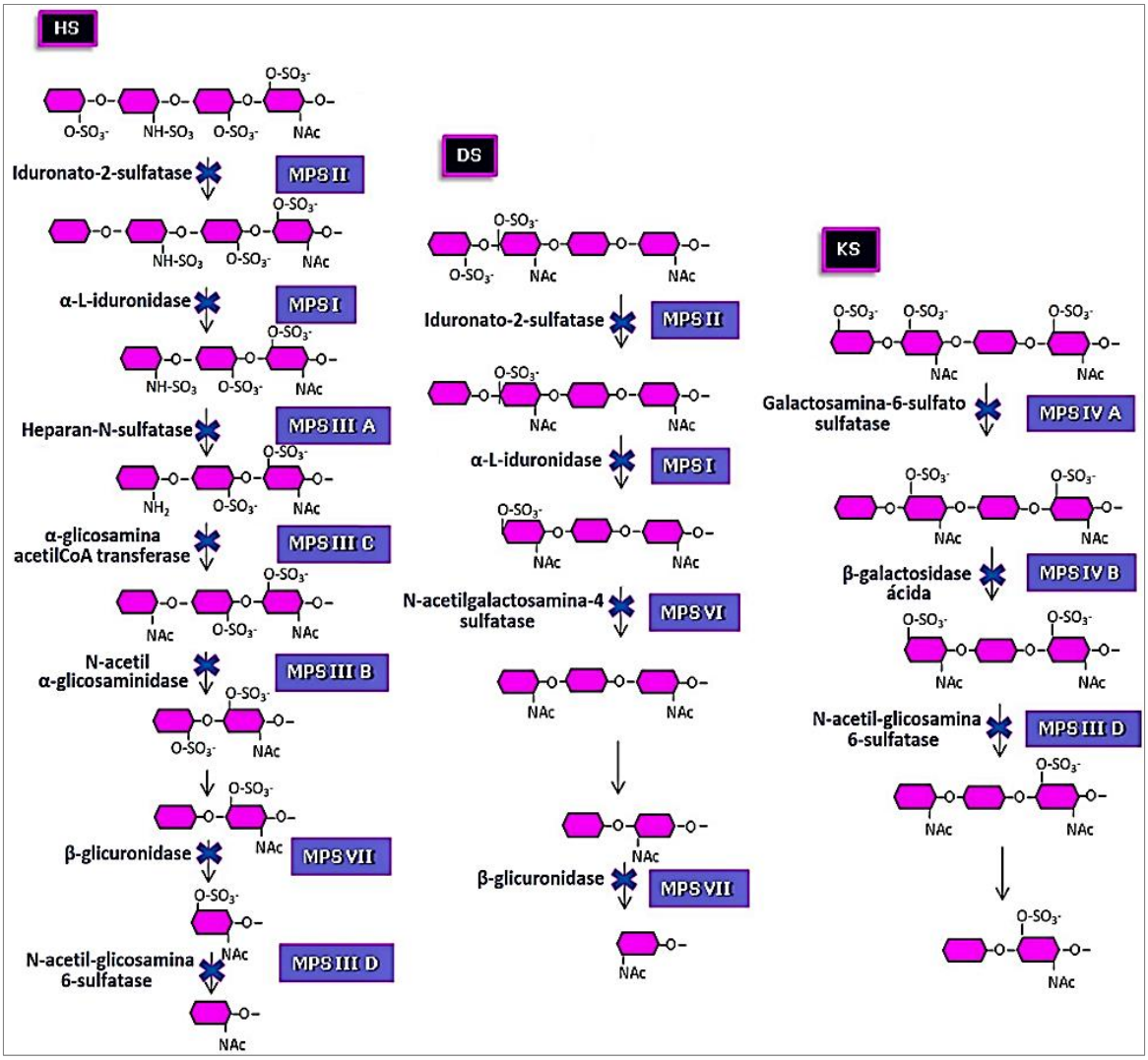

Figura 1. Etapas da degradação dos GAGs sulfato de heparan (HS), sulfato de dermatan (DS) e sulfato de queratan (KS), com a indicação da interrupção da atividade das enzimas envolvidas nas MPS tipos I a VII. Modificado e traduzido de Filocamo et al., 2018, sob os termos de Creative Commons Attribution 4.0 International (CC BY 4.0) Copyright © 2018 pelos autores.

O perfil de acúmulo de GAGs de cada tipo de MPS pode ser identificado no sangue e na urina dos pacientes afetados, servindo como um importante biomarcador, embora possam ser encontradas elevações secundárias de sulfato de queratan em pacientes com outros tipos de MPS que não a IV, cuja causa não pode ser explicada pelo envolvimento enzimático primário (Tomatsu et al., 2014).

Além dos tipos já bem estabelecidos de MPS, recentemente, em dezembro de 2021, foram descritos quatro indivíduos de duas famílias distintas, filhos de consanguíneos, com um possível novo tipo de MPS, de padrão de herança autossômico recessivo. Os autores sugerem que essa condição, que é decorrente da deficiência da enzima lisossômica

arilsulfatase K, em consequência de mutações bialélicas no gene *ARSK*, seja denominada MPS X (Verheyen et al., 2021).

1.1.2 Epidemiologia

As MPS são doenças raras e existe uma crescente preocupação em caracterizar o perfil epidemiológico dessas patologias, útil não somente para aconselhamento genético e seguimento das famílias afetadas, mas também para programação de políticas de saúde (Chen et al., 2016).

Estudos de incidência de MPS mostram grande variação conforme o país estudado, tendo sida descrita, para cada 100.000 nascidos vivos, de 4,8 em Portugal; 4,5 na Holanda; 4,46 na Austrália; 4,05 na Estônia; 4,0 na Irlanda do Norte; 3,72 na República Tcheca; 3,51 na Alemanha; 3,08 na Noruega; 2,27 na Tunísia; 2,04 em Taiwan; 1,94 na Columbia Britânica, Canadá; 1,8 na Polônia; 1,77 na Dinamarca; 1,75 na Suécia; 1,56 na Suiça; 1,53 no Japão; 1,35 na Coreia do Sul; 1,2 nos Estado Unidos e 1,04 no Brasil (Khan et al., 2017).

A prevalência estimada para cada tipo de MPS também variou conforme a região geográfica estudada. No Brasil, dentre os pacientes diagnosticados com MPS, o tipo II foi o mais comumente encontrado (Giugliani et al, 2017a; Federhen et al, 2020). A MPS II também foi a mais frequentemente observada na Estônia (incidência de 2,16/100.000 nascidos vivos); Taiwan (incidência de 1,07/100.000 nascidos vivos), Japão (incidência de 0,84/100.000 nascidos vivos), Coreia do Sul (incidência de 0,74/100.000 nascidos vivos), China e Suíça (incidência de 0,46/100.000 nascidos vivos) (Khan et al., 2017). Nos países asiáticos como Taiwan, Coreia do Sul, Japão e China, essa maior incidência de MPS II pode ser devido à alta frequência alélica da variante p.R468 no gene *IDS*, embora esta variante encontre-se em uma região pouco conservada e o códon para R468 seja considerado um *hotspot* (Rathmann et al., 1996; Khan et al, 2017). A genotipagem de um grupo de 103 pacientes da América Latina, sendo destes 91 brasileiros, mostrou grande heterogeneidade alélica, a maior parte alterações gênicas pequenas (<22 pb). Deste grupo da América Latina, somente seis pacientes apresentaram a variante p.R468 (Brusius-Facchin et al, 2014). Portanto, é pouco provável que a presença de variantes comuns seja a explicação para a alta frequência relativa de MPS II no Brasil.

No que diz respeito aos outros tipos de MPS, a MPS I foi a mais comumente encontrada na Columbia Britânica, Canadá (incidência de 0,58/100.000 nascidos vivos), Dinamarca (incidência de 0,54/100.000 nascidos vivos), Noruega (incidência de 1,85/100.000 nascidos vivos), Irlanda do Norte (incidência de 1,66/100.000 nascidos vivos), Portugal (incidência de 1,33/100.000 nascidos vivos); a MPS III foi a mais comumente encontrada na Tunísia (incidência de 0,7/100.000 nascidos vivos), Austrália (incidência de 1,51/100.000 nascidos vivos), República Tcheca (incidência de 0,91/100.000 nascidos vivos), Alemanha (incidência de 1,57/100.000 nascidos vivos), Holanda (incidência de 1,89/100.000 nascidos vivos), Polônia (incidência de 0,86/100.000 nascidos vivos) e Estados Unidos (incidência de 0,38/100.000 nascidos vivos); a MPS IV foi a mais comumente encontrada na Índia; a MPS VI foi a mais comumente encontrada na Arábia Saudita (Khan et al., 2017; Çelik et al, 2021).

A inclusão da pesquisa para esse grupo de doenças em testes de triagem neonatal, que já é uma realidade para alguns locais do mundo, tornará a informação sobre incidência mais precisa, já que muitos casos não são diagnosticados (Çelik et al, 2021).

1.1.3 Manifestações clínicas

As manifestações clínicas das MPS incluem dismorfias faciais, alterações ósseas, visceromegalias, cardiopatia e doença valvular cardíaca, problemas oculares, auditivos e do sistema respiratório, além de acometimento do sistema nervoso central e periférico (Coutinho, Lacerda e Alves, 2012; Zhou et al, 2020).

No que diz respeito ao acometimento do Sistema Nervoso Central (SNC), ele pode ser considerado primariamente comprometido nas MPS dos tipos I (forma Hurler), II (na forma neuronopática), III (todos os subtipos) e VII (na maioria dos casos), quando ocorre atraso do desenvolvimento neuropsicomotor, regressão neurocognitiva, crises convulsivas e/ou distúrbios comportamentais (Barone et al, 2018). Os exames de imagem do SNC de pacientes com MPS podem mostrar achados inespecíficos como alteração de sinal de substância branca e cinzenta, ventriculomegalia, hidrocefalia, atrofia cerebral principalmente cortical ou difusa, aumento do espaço perivascular e anomalias de fossa

posterior, entre outros achados menos comuns que já foram descritos (Zafeiriou & Batzios, 2013).

Os sinais e sintomas são progressivos e multissistêmicos, requerendo um esforço conjunto de várias especialidades no acompanhamento desses pacientes ao longo da vida (Stepien et al, 2020). A maioria dos pacientes é assintomática ao nascimento, com o acúmulo de GAGs e consequente prejuízo da função celular e na estrutura da matriz extracelular levando ao quadro clínico (Tomatsu et al., 2014). As características clínicas, gravidade e idade de aparecimento dos primeiros sintomas variam não só entre os diferentes tipos, mas também entre pacientes com o mesmo tipo de MPS (Suarez-Guerrero, et al., 2016).

1.1.4 Diagnóstico

Como método de rastreio para as MPS, pode ser feita a análise de GAGs totais na urina (Hopwood & Harrison, 1982). O diagnóstico deve ser confirmado a partir de ensaio enzimático e/ou estudo molecular (Coutinho, Lacerda e Alves, 2012).

A análise de GAGs pode ser quantitativa e/ou qualitativa. Métodos baseados em espectrofotometria com utilização de azul alcian ou azul de dimetilmetileno permitem a medida quantitativa dos GAGs totais na urina; métodos como a eletroforese, cromatografia em camada fina, cromatografia líquida acoplada à espectrometria de massas em tandem ou ensaio de imunoabsorção (ELISA) têm por finalidade identificar os tipos específicos de GAGs que estão aumentados, ajudando a direcionar o teste enzimático confirmatória a ser realizado (Khan et al., 2018). Nem sempre o paciente com MPS vai apresentar aumento de GAGs totais na urina e, por isso, embora essa análise seja geralmente o primeiro passo na investigação laboratorial, resultados normais nessa triagem não necessariamente afastam o diagnóstico (Lehman et al., 2011).

A análise da atividade enzimática é guiada pelo quadro clínico e pelo resultado da análise qualitativa de GAGs e permite definir o diagnóstico e especificar o tipo de MPS (Omar et al., 2019). A dosagem pode ser realizada em leucócitos, plasma, fibroblastos cultivados ou no sangue em papel filtro, sendo que alterações neste último tipo de amostra devem ser confirmadas, preferencialmente em leucócitos (Lehman et al., 2011).

A análise molecular, quando disponível, permite a identificação da(s) variante(s) patogênica(s) e serve para corroborar o diagnóstico enzimático, para o aconselhamento genético das famílias e, caso a variante já esteja bem caracterizada, para realizar a correlação genótipo-fenótipo e ter maiores informações sobre o prognóstico e eventualmente sobre o tipo de tratamento a ser proposto (Lehman et al., 2011; Omar et al., 2019).

1.1.5 Tratamento

O tratamento das MPS inclui medidas de suporte e reabilitação, visando a uma melhor qualidade de vida, além de tratamentos específicos que estão disponíveis para alguns dos tipos conhecidos.

O primeiro tratamento específico de sucesso descrito para a MPS foi o transplante de medula óssea em um paciente com MPS I-Hurler (Hobbs et al., 1982). Apesar de existirem relatos mostrando eficácia do transplante de células tronco hematopoiéticas para os tipos I, II, IV, VI e VII de MPS, essa opção é limitada devido à insuficiente evidência disponível, às complicações relacionadas ao procedimento, às dificuldades para conseguir um doador compatível, à carência de centros habilitados em vários países e ao diagnóstico tardio (Taylor et al., 2019; Zhou et al., 2020).

A terapia de reposição enzimática intravenosa (TRE) é outra opção de tratamento específico. Ela está disponível para as MPS I, II, IVA, VI e VII, mas tem limitações de resposta terapêutica devido à baixa biodisponibilidade em alguns tecidos e ao fato das enzimas convencionais não cruzarem a barreira hematoencefálica (Parini & Deodato, 2020).

Outras opções de tratamento são promissoras, especialmente no que diz respeito aos sintomas neurológicos centrais. Por exemplo, administração da TRE via intratecal ou intracerebro-ventricular, ou via intravenosa mas com modificação da molécula da enzima recombinante para que ela possa transpor a barreira hematoencefálica; terapia gênica in-vivo ou ex-vivo com o uso de vetores virais, ou edição genômica; e terapias de redução de substrato, visando reduzir o acúmulo de GAGs nos tecidos do paciente (Nan, Park e Maeng, 2020; Poswar, Baldo e Giugliani, 2017).

1.2 Mucopolissacaridose tipo II

1.2.1 Etiologia e genética

A mucopolissacaridose tipo II (MPS II), ou doença de Hunter, é o único tipo conhecido de MPS com padrão de herança ligado ao X (recessivo) e é causada por um defeito enzimático que leva ao prejuízo na degradação lisossômica dos GAGs sulfato de dermatan e sulfato de heparan (Bach et al., 1973). O gene envolvido nesta condição é o *IDS* (HGNC ID:5389; ENSG00000010404), responsável por codificar a enzima iduronato-2-sulfatase (EC 3.1.6.13) (D'avanzo et al. 2020). Ele é composto por 9 éxons e tem aproximadamente 24kb, estando localizado em Xq28 (Wilson et al., 1993).

A cerca de 20kb distal do gene *IDS* ativo foi identificado o pseudogene *IDS-2*, com estrutura semelhante ao gene *IDS*, cuja existência favorece eventos de recombinação que levam à disrupção do gene *IDS* (Timms et al, 1995). A figura 2 esquematiza a formação da inversão complexa envolvendo o *IDS* e o pseudogene *IDS-2*.

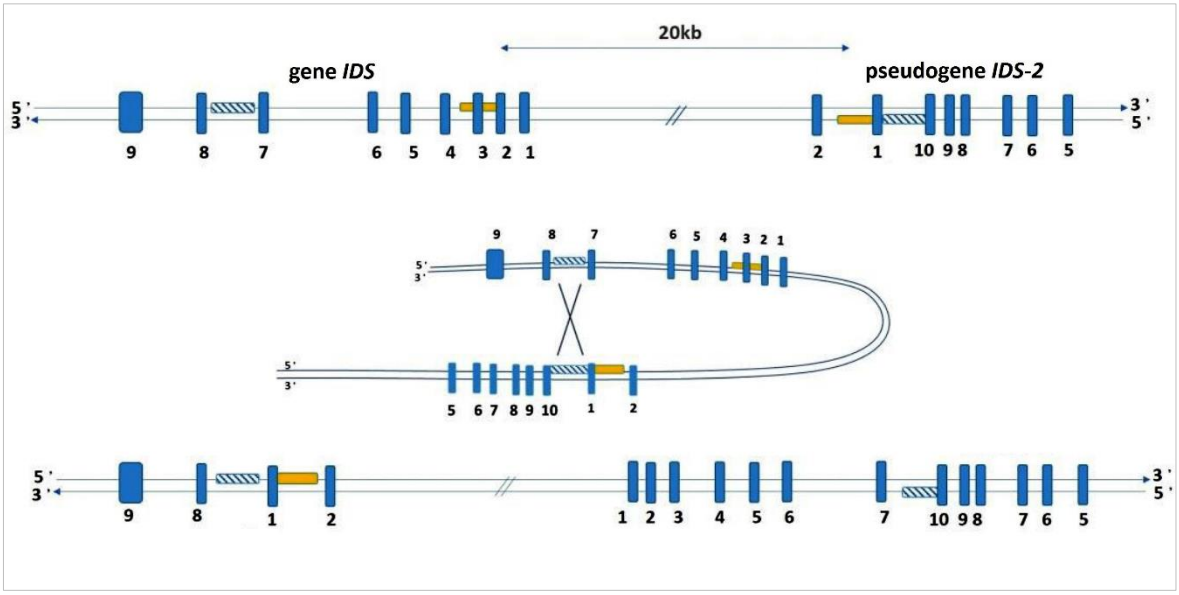

Figura 2. Esquema da formação da inversão complexa envolvendo o gene *IDS* e o pseudogene *IDS-2*. Os retângulos hachurados indicam as regiões homólogas que favorecem o rearranjo. Modificado e traduzido de Lin et al., 2019, sob os termos de Creative Commons Attribution 4.0 International (CC BY 4.0) Copyright © 2019 pelos autores.

Até o momento, 739 variantes diferentes já foram identificadas no gene *IDS* (HGMD, 2021).

1.2.2 Manifestações clínicas

Os pacientes com MPS II apresentam quadro clínico variável e multissistêmico, dentro de um amplo espectro fenotípico. As crianças afetadas são tipicamente normais ao nascimento (Guffon et al., 2015). Nos primeiros meses de vida já podem ser vistas algumas características da síndrome, como hérnias abdominais ou inguinais, hepatoesplenomegalia, episódios frequentes de diarreia aquosa, infecções respiratórias e otites recorrentes, mas elas geralmente são valorizadas somente após o surgimento de alterações mais evidentes e menos inespecíficas, levando a demora no diagnóstico (Burton & Giugliani, 2012; Galimberti et al, 2018). Características faciais típicas incluem macrocefalia, face infiltrada, ponte nasal baixa, lábios grossos e macroglossia (Anekar et al., 2015). Além das dismorfias faciais, pacientes com MPS II desenvolvem características dermatológicas que sugerem precocemente o diagnóstico, como hirsutismo, cabelos grossos e lisos, espessamento da pele (às vezes com o aspecto de “casca de laranja”), manchas mongólicas extensas e lesões cutâneas peroláceas do tipo pápulas na região superior das costas e braços (Galimberti et al., 2018; Giugliani et al., 2010).

A baixa estatura é uma característica consistente entre os afetados e evidente no final da primeira década de vida, embora já possa ser percebida uma queda na curva de crescimento em idades mais precoces (Jones et al., 2013). Ao nascimento, dados antropométricos mostram que pacientes com MPS II são maiores em comprimento em relação a população em geral, com queda na velocidade de crescimento observada em torno dos três anos de idade (Rozdzynska et al., 2010).

Os pacientes apresentam alterações ósseas conhecidas como “disostose múltipla”, que afeta todo o funcionamento do sistema músculo-esquelético, levando à restrição da mobilidade articular, mãos típicas em garra, marcha na ponta dos pés e deformidades de coluna (Martin et al., 2008). Nos exames radiológicos é possível encontrar espessamento da cortical óssea, ossificação epifisária irregular, costelas alargadas, platispondilia, aspecto de bico ou protusão na face anterior dos corpos vertebrais, podendo levar a problemas como a compressão medular (Galimberti et al., 2018). A síndrome do túnel do carpo é outra complicação relacionada com a MPS II, comprometendo ainda mais o uso funcional das mãos nesses pacientes (Norman-Taylor et al., 1995).

O sistema cardiovascular também está envolvido e inclui doença valvar, hipertrofia ventricular, hipertensão arterial e arritmias cardíacas (Scarpa et al., 2011).

Problemas respiratórios são frequentes e os pacientes apresentam obstrução progressiva de vias aéreas, apneia obstrutiva do sono e traqueobroncomalacea (Moreira et al., 2014). Pacientes com MPS II devem realizar periodicamente estudos do sono com polissonografia e avaliações para hipertrofia de tonsilas e deformidades em traqueia (Scarpa et al., 2011).

A doença pode ser dividida em duas formas de apresentação: a forma grave, caracterizada por acometimento primário do SNC com regressão neurológica e comprometimento cognitivo importante, denominada “neuronopática”; e a forma atenuada, sem neurodegeneração, denominada “não-neuronopática” (Scarpa et al., 2011; Schwartz et al., 2007; Parini et al, 2016). Na prática, às vezes pode ser difícil a distinção entre as duas formas, já que não há um intervalo claro entre elas (D’Avanzo et al., 2020).

As mulheres heterozigotas raramente apresentam sintomas, a menos que outro evento genético ocorra, como a inativação preferencial do X normal ou alguma alteração importante no cromossomo X que contém o alelo normal (Pinto et al., 2010).

A principal causa de morte em pacientes com MPS II é a insuficiência cardiorrespiratória (D’Avanzo et al., 2020). A crescente conscientização sobre a doença, aumento na identificação dos casos e manejo com avaliação em equipe multidisciplinar melhoram a expectativa e qualidade de vida dos pacientes, sendo que a forma neuronopática têm sobrevida menor em relação à forma não-neuronopática (Jones et al., 2009; Lin et al., 2016).

1.2.3 Diagnóstico laboratorial

A análise de GAGs na urina pelo método com azul de dimetilmetileno mostra um aumento desses metabólitos e a eletroforese ou espectrometria de massa em tandem identifica aumento preferencial dos tipos sulfato de heparan e sulfato de dermatan, embora tanto a análise qualitativa quanto a quantitativa possam dar falsos resultados (D’Avanzo et al., 2020; Giugliani et al., 2012).

Para diagnóstico, é necessária a documentação da deficiência da atividade da enzima iduronato-sulfatase em leucócitos plasmáticos ou em cultura de filbroblastos, que pode ser realizada após a triagem da atividade enzimática em amostras de sangue em papel filtro devido à facilidade de armazenamento e transporte nesta opção (Giugliani et al., 2016b).

A identificação da variante patogênica no gene *IDS* não é mandatória para o diagnóstico, mas pode ajudar a predizer o fenótipo e é geralmente necessária para identificar as heterozigotas, o que é fundamental para o aconselhamento genético, devendo ser realizada sempre que possível (Suarez-Guerrero et al., 2016). Por vezes, a identificação das heterozigotas pode ser feito apenas pela análise da história familiar.

1.2.4 Tratamento

A abordagem para tratamento dos pacientes com MPS II deve ser multidisciplinar, com envolvimento de especialidades médicas diversas e equipes de saúde com atendimento em fisioterapia, terapia ocupacional, psicologia, fonoterapia, que são essenciais para a promoção de saúde dessa população (Giugiliani et al., 2010).

Além do seguimento multidisciplinar para prevenção e manejo das complicações da doença, existem opções de tratamentos específicos aprovados ou em desenvolvimento para MPS II (Giugliani, 2010; Giugliani, 2016a).

Os esforços no desenvolvimento do tratamento com transplante de células tronco hematopoiéticas (TCTH) em pacientes com doenças lisossômicas de depósito têm por objetivo proporcionar ao paciente uma quantidade de células que produzam a enzima deficiente, melhorando o desenvolvimento cognitivo e prolongando a sobrevida, especialmente naqueles tratados precocemente (Peters et al, 1998). Para a MPS II, diversos estudos foram realizados para avaliar a recomendação desse tratamento devido às altas taxas de complicação observadas e à necessidade de realizar o transplante muito precocemente para se obter melhores resultados (Giugliani et al., 2010). Mais recentemente, estudos têm demonstrado resultados satisfatórios na utilização do TCTH para a MPS II e, especialmente com as melhorias nas técnicas de transplante, essa tem se

tornado uma opção terapêutica para pacientes em idade precoce e com boas condições clínicas (Barth & Horovitz, 2018; Kubaski et al., 2017b).

No Brasil, o TCTH alogênico é formalmente uma opção de tratamento para a MPS II forma neuronopática em idade precoce, de preferência até os três anos de idade, obedecendo rígidos critérios de seleção (BRASIL, 2018).

A terapia de reposição enzimática (TRE) intravenosa com idursulfase está disponível desde 2006 para tratamento da MPS II (Stapleton et al., 2017). Deve-se avaliar cuidadosamente cada caso antes da indicação de TRE, envolvendo quando possível a família do paciente afetado nas decisões, conversando sobre os benefícios esperados, limitações e critérios de suspensão do tratamento (Muenzer et al., 2012). No Brasil, está indicada para pacientes com diagnóstico confirmado de MPS II e que não apresentem regressão neurológica ou condição médica irreversível que leve a uma sobrevida provável inferior a 6 meses (BRASIL, 2018).

Outras possibilidades para tratamento estão sendo estudadas, como a TRE com administração intratecal ou intracerebro-ventricular de idursulfase, e terapias gênicas (Giugliani et al., 2016a).

CAPÍTULO 2 – JUSTIFICATIVA

As MPS são doenças raras, mas constituem importante problema de saúde para as famílias afetadas. O diagnóstico é muitas vezes difícil devido ao amplo espectro fenotípico e ao desconhecimento sobre o quadro clínico, inclusive por profissionais de saúde.

Ao contrário de muitos outros países, onde os tipos mais comuns são MPS I e MPS III, o tipo de MPS mais frequente no Brasil é a MPS II. A incidência de MPS II varia entre os países estudados e já foram até agora descritas 739 variantes diferentes no gene *IDS* (HGMD, 2021). Em algumas regiões, a frequência de variantes comuns no gene *IDS* parecem contribuir para a maior frequência deste tipo de MPS na população.

Há necessidade de se conhecer mais sobre a MPS II no Brasil em relação a fatores que poderiam explicar a alta frequência relativa da doença. Entre esses fatores se destacam o número de afetados por família e a epidemiologia genético-molecular. Paralelamente, a melhor compreensão sobre a localização geográfica e taxa de consanguinidade, sobre fatores que levam à demora no diagnóstico e à variabilidade da apresentação clínica, podem contribuir para um melhor entendimento sobre esse tipo de MPS em nosso país.

Esses dados serão importantes para o planejamento de políticas públicas relacionadas com manejo global, tratamento específico, prevenção primária e prevenção secundária das MPS em geral, e particularmente da MPS II, no Brasil.

CAPÍTULO 3 – OBJETIVOS

3.1. Objetivo geral

Investigar fatores que possam explicar a maior frequência relativa de MPS II no Brasil.

3.2. Objetivos específicos

a) Verificar se a alta frequência relativa de MPS II tem maior predominância em alguma região brasileira;
b) Verificar se o perfil genético-molecular dos pacientes com MPS II no Brasil poderia estar relacionado com a sua alta frequência relativa;
c) Avaliar a variabilidade fenotípica intra-familiar em pacientes brasileiros com MPS II;
d) Verificar se o número de afetados na família pode ser um fator relacionado com a alta frequência relativa de MPS II no Brasil;
e) Avaliar se a taxa de consanguinidade tem influência na frequência relativa das MPS em nosso país;
f) Avaliar a idade ao diagnóstico e comparar com a idade em que se manifestaram os primeiros sintomas, para assim identificar o tempo para diagnóstico dessa patologia em nosso país.

CAPÍTULO 4 – RESULTADOS

O trabalho desenvolvido nesta tese resultou em três artigos científicos, dois já publicados e um em fase final de preparação para ser submetido à publicação.

4.1. Artigo 1

Updated Birth Prevalence and Relative Frequency of Mucopolysaccharidoses Across Brazilian Regions

Artigo publicado na revista *Genetics and Molecular Biology* (2021), doi: 10.1590/1678-4685-GMB-2020-0138

Josahkian JA, Trapp FB, Burin MG, Michelin-Tirelli K, Magalhães APPS, Sebastião FM, Bender F, Mari JF, Brusius-Facchin AC, Leistner-Segal S, Málaga DR, Giugliani R (2021) Updated birth prevalence and relative frequency of mucopolysaccharidoses across Brazilian regions. Genet Mol Biol 27;44(1):e20200138.

Genetics and Molecular Biology, 44, 1, e2020138 (2021)

DOI: https://doi.org/10.1590/1678-4685-GMB-2020-0138

Research Article
Human and Medical Genetics

Updated birth prevalence and relative frequency of mucopolysaccharidoses across Brazilian regions

Juliana Alves Josahkian[1,2], Franciele Barbosa Trapp[3,4,5], Maira Graeff Burin[3,4,5], Kristiane Michelin-Tirelli[3,4,5], Ana Paula Pereira Scholz de Magalhães[3,4,5], Fernanda Medeiros Sebastião[3,4,5], Fernanda Bender[3,4,5], Jurema Fátima De Mari[3,4], Ana Carolina Brusius-Facchin[3,4,5], Sandra Leistner-Segal[3,4,5], Diana Rojas Málaga[6] and Roberto Giugliani[2,3,4,5,7,8,9]

[1]*Hospital Universitário de Santa Maria, Unidade de Clínica Médica, Santa Maria, RS, Brazil.*
[2]*Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Genética e Biologia Molecular, Porto Alegre, RS, Brazil.*
[3]*Rede MPS Brasil, Porto Alegre, RS, Brazil.*
[4]*Hospital de Clínicas de Porto Alegre, Serviço de Genética Médica, Porto Alegre, RS, Brazil.*
[5]*Hospital de Clínicas de Porto Alegre, Centro de Pesquisa Experimental, Grupo de Pesquisa BIODISCOVERY, Porto Alegre, RS, Brazil.*
[6]*Grupo Fleury, Pesquisa e Desenvolvimento - Biologia Molecular, São Paulo, SP, Brazil.*
[7]*Universidade Federal do Rio Grande do Sul, Departamento de Genética, Porto Alegre, RS, Brazil.*
[8]*Instituto Nacional de Genética Médica Populacional (INAGEMP), Porto Alegre, RS, Brazil.*
[9]*Hospital de Clínicas de Porto Alegre, Centro de Pesquisa Clínica, Grupo de Pesquisa DRBRASIL, Porto Alegre, RS, Brazil.*

Abstract

The mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders caused by 11 enzyme deficiencies, classified into seven types. Data on the birth prevalence of each MPS type are available for only a few countries, and the totality of cases may be underestimated. To determine the epidemiological profile of MPS in each Brazilian region, we analyzed data collected between 1982 and 2019 by a national reference laboratory and identified 1,652 patients. Using data between 1994 and 2018, the birth prevalence (by 100,000 live births) for MPS was 1.57. MPS II was the most common type of MPS in Brazil, and its birth prevalence was 0.48 (0.94 considering only male births). Regarding the number of cases per region, MPS II was the most frequent in the North and Center-West (followed by MPS VI), and also in the Southeast (followed by MPS I); MPS I and MPS II were the most common types in the South; and MPS VI was the most common in the Northeast (followed by MPS II). The differences observed in the relative frequencies of MPS types across Brazilian regions are likely linked to founder effect, endogamy, and consanguinity, but other factors may be present and need further investigation.

Keywords: Lysosomal storage diseases, metabolic diseases, mucopolysaccharidoses, epidemiology, Brazil.

Received: April 30, 2020; Accepted: December 04, 2020.

Introduction

Mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders caused by the deficiency of enzymes involved in the catabolism of glycosaminoglycans (GAGs). These conditions are multisystemic, progressive, and have variable clinical features (Neufeld and Muenzer, 2014), not only among the different types but also among patients with the same type of MPS. Severe cases are easier to diagnose, but attenuated cases are challenging to recognize and can be confounded with more common pathologies (Suarez-Guerrero *et al.*, 2015).

Studies of the specific enzymes involved in different steps of the GAG degradation pathway and the identification of which genes cause the disease allowed the classification of MPS in seven clinical types, which correspond to 11 enzyme deficiencies, currently recognized as MPS I, II, III (A, B, C, and D subtypes), IV (A and B subtypes), VI, VII, and IX. All MPS are autosomal recessive disorders, except MPS II, which is an X-linked recessive condition (Neufeld and Muenzer, 2014).

Epidemiological data about the MPS types are available for only a few countries and regions, and its birth prevalence may be underestimated as a consequence of the clinical heterogeneity of this group of diseases and the difficulties for its laboratory investigation (Giugliani, 2012). For this reason, a laboratory to provide diagnostic support for MPS was established at the Medical Genetics Service of Hospital de Clínicas de Porto Alegre (MGS/HCPA), Brazil. MGS/HCPA is a well-known reference center in the country, and it has received samples from patients with suspected MPS since 1982 (Giugliani *et al.*, 2017). In 2004, the demand for testing patients suspected of having MPS led to the creation of the MPS Brazil Network, with a specific investigation workflow (Giugliani *et al.*, 2016). In this manner, this study aimed to report the

Send correspondence to Juliana Alves Josahkian. Hospital Universitário de Santa Maria, Departamento de Clínica Médica, Av. Roraima, 1000, prédio 22, Camobi, 97105-900, Santa Maria, RS, Brazil. Email: julianajosahkian@gmail.com.

birth prevalence and relative frequency of the different MPS types in Brazil to determine the epidemiological profile of this condition per state, per region and in the country as a whole.

Material and Methods

We analyzed the records from the MGS/HCPA and the MPS Brazil Network of patients diagnosed with MPS between 1982 and 2019. MPS cases were diagnosed biochemically; the investigation frequently starts with a quantitative (colorimetric method with dimethylene blue) and qualitative (electrophoresis) analysis of urinary GAGs, followed by specific enzyme assays according to the first results and/or by identification of pathogenic variants by molecular genetic analysis. In this study, we calculated the relative frequency of each MPS type in Brazil, and also present data by region and state.

In this report we use the term *birth prevalence* to refer to the number of MPS cases diagnosed by the total number of live births in a specific period expressed as cases per 100,000 live births, as employed previously in the literature (Poupetová *et al.*, 2010; Khan *et al.*, 2017).

Data regarding live births from the Brazilian Health System database were available from 1994 to 2018, allowing us to calculate the birth prevalence in this period. Patients with MPS who were not born during this period were not included in the analyzes. The comparison between our findings and the estimations from other countries is also presented.

Results

From 1982 to 2019, 1,652 Brazilian patients were diagnosed with MPS at the Medical Genetics Service of Hospital de Clínicas de Porto Alegre. MPS II was the most commonly diagnosed condition (493 cases, 29.84%), followed by MPS VI (351 cases, 21.25%), MPS I (315 cases, 19.07%), MPS III – all subtypes (267 cases, 16.16%), MPS IV – both subtypes (205 cases, 12.41%) and MPS VII (21 cases, 1.27%). We did not observe any patients diagnosed with MPS IX.

Regarding MPS III, we identified the specific subtype (A, B, C, or D) for 95.50% of the cases. In this subset, the proportion of MPS IIIA was 26.67%; for IIIB, 45.49%; for IIIC, 27.45%; and there was just one case of IIID (0.39%).

The same approach was employed for MPS IV. In 96.09% of the cases, we were able to identify each specific subtype (A or B). In this subset, the proportion of MPS IVA was 96.45%, and the percentage of MPS IVB was 3.55%. By extrapolating these data to the total number of MPS III and MPS IV cases, we calculated the ratios presented in Table 1.

When considering the number of cases diagnosed from each Brazilian region, we found that MPS II was the most frequent in the North, Center-West, and Southeast regions; MPS I and MPS II were tied as the most common types in the South region; and MPS VI was the most frequent in the Northeast region. The number of cases diagnosed according to the Brazilian region and state of origin is shown in Table 1, and the distribution of these types of MPS in Brazil is shown in Figure 1.

Based on data provided by the Information System on Live Births (SINASC) of the Brazilian Health System database, between 1994 and 2018, a total of 74,215,086 live births occurred in Brazil – 37,977,308 being male babies (DATASUS, 2020). We are aware of 1,164 Brazilian patients diagnosed with MPS who were born in Brazil during this period. Among these patients, 217 were MPS I, 358 were MPS II, 199 were MPS III (54 IIIA, 84 IIIB, 50 IIIC, 1 IIID, and 10 were MPS III not specified), 117 were MPS IV (110 MPS IVA, 2 MPS IVB, and five with MPS IV not specified), 257 were MPS VI and 16 were MPS VII. For calculation purposes, the unspecified cases were distributed proportionally according to the frequency of MPS subtype. Thus, the numbers were adjusted to 57 for MPS IIIA, 88 for MPS IIIB, 53 for MPS IIIC, and 115 for MPS IVA.

The calculated incidence for MPS in Brazil, using the 1994 to 2018 data, was 1.57/100,000 live births. Regarding each MPS type, the birth prevalence by 100,000 live births was 0.29 for MPS I, 0.48 for MPS II (or 0.94, considering only male births), 0.08 for MPS IIIA, 0.12 for MPS IIIB, 0.07 for MPS IIIC, 0.001 for MPS IIID, 0.15 for MPS IVA, 0.003 for MPS IVB, 0.35 for MPS VI, 0.02 for MPS VII, and 0 for MPS IX.

The birth prevalence was also calculated for each Brazilian region. For the 358 patients (30.76%) without an informed place of birth, the region from where samples were obtained was set as "place of birth." Our results showed that MPS II had the highest score in all Brazilian regions, except in the Northeast, where MPS VI presented the highest birth prevalence rate. The number of cases diagnosed and the birth prevalence for MPS patients born from 1994 to 2018 in Brazil and each region of this country is detailed in Table 2.

This study was approved by the Ethics Committee of Universidade Federal do Rio Grande do Sul, Brazil (CAAE #82189417.5.0000.5347). This study was conducted in accordance with the ethical standards from the 1964 Declaration of Helsinki and its later amendments. Our manuscript does not contain data from any individual person.

Discussion

In this study, we explored the epidemiological data of MPS in Brazil. MPS II was the most common type of MPS in Brazil and the second most common lysosomal storage disease diagnosed in our laboratory in previously published studies conducted by our group (Giugliani *et al.*, 2017). Since Brazil has continental dimensions, analysis per region was critical for showing that MPS II is the most common in the North, Southeast, and Center-West. Indeed, MPS I and MPS II were tied as the most common types in the South, and MPS VI was the most frequent in the Northeast.

Birth prevalence rates calculated from 1994 to 2018 indicated that MPS II was the most frequent in all regions except the Northeast, where MPS VI has the highest rate. A founder effect that resulted in a high frequency of p.H178L pathogenic variant in the *ARSB* gene, responsible for MPS VI, may explain the high number of cases in Brazil's Northeast (Federhen *et al.*, 2020). This region also has areas of geographical isolation, endogamy, and a high number of consanguineous marriages that may lead to increased rates of MPS VI patients (Costa-Motta *et al.*, 2014; Vairo *et al.*, 2015). In addition, the birth prevalence of MPS II in the Northeast was similar to the one observed in other regions of Brazil, which suggests that the higher incidence of MPS VI

Table 1 – MPS types diagnosed in Brazil per region and by state[a] from 1982 to 2019.

MPS Type / Region/State	All	I	II	IIIA	IIIB	IIIC	IIID	IVA	IVB	VI	VII	IX
North	75	13	30	2	4	2	–	5	–	18	1	–
Acre	7	2	1	–	1	–	–	2	–	1	–	–
Amazonas	31	6	10	1	1	–	–	1	–	12	–	–
Amapá	–	–	–	–	–	–	–	–	–	–	–	–
Pará	28	3	13	1	2	2	–	1	–	5	1	–
Rondônia	7	1	5	–	–	–	–	1	–	–	–	–
Roraima	1	–	1	–	–	–	–	–	–	–	–	–
Tocantins	1	1	–	–	–	–	–	–	–	–	–	–
Center–West	103	20	33	6	8	3	–	6	–	24	3	–
Distrito Federal	49	12	9	4	4	2	–	4	–	13	1	–
Goiás	22	2	11	2	–	–	–	1	–	6	–	–
Mato Grosso	15	5	6	–	3	–	–	1	–	–	–	–
Mato Grosso do Sul	17	1	7	–	1	1	–	–	–	5	2	–
Southeast	679	140	207	33	56	37	–	69	6	124	7	–
Espírito Santo	22	–	11	1	4	2	–	–	–	3	1	–
Minas Gerais	135	33	22	7	15	5	–	15	–	36	2	–
São Paulo	388	86	126	21	23	24	–	36	6	62	4	–
Rio de Janeiro	134	21	48	4	14	6	–	18	–	23	–	–
Northeast	510	66	147	22	14	19	1	68	1	162	10	–
Alagoas	34	4	23	1	–	1	–	–	–	5	–	–
Bahia	111	15	34	6	7	1	1	9	1	31	6	–
Ceará	88	11	35	5	6	3	–	7	–	21	–	–
Maranhão	23	2	11	2	1	–	–	3	–	4	–	–
Paraíba	71	8	6	1	–	9	–	26	–	20	1	–
Pernambuco	118	15	20	2	–	5	–	17	–	58	1	–
Piauí	19	2	7	1	–	–	–	2	–	5	2	–
Rio Grande do Norte	31	6	4	2	–	–	–	4	–	15	–	–
Sergipe	15	3	7	2	–	–	–	–	–	3	–	–
South	285	76	76	8	40	12	–	50	–	23	–	–
Paraná	102	20	39	3	12	3	–	13	–	12	–	–
Rio Grande do Sul	142	42	32	3	21	6	–	28	–	10	–	–
Santa Catarina	41	14	5	2	7	3	–	9	–	1	–	–
Brazil, total	1652	315	493	71	122	73	1	198	7	351	21	–

[a] For MPS IIIA, IIIB, IIIC, and IIID, and for MPS IVA and IVB, the numbers represent an extrapolation.

is related to these factors, and not to a lower absolute number of births with MPS II. Similarly, as in Brazil, MPS II is the most common type found in Estonia, Taiwan, Japan, South Korea, China, and Switzerland (Krabbi *et al.*, 2012; Cho *et al.*, 2014; Chen *et al.*, 2016; Khan *et al.*, 2017). The higher birth prevalence of MPS II in East Asia was suggested to be a consequence of the p.R468 pathogenic variants in the *IDS* gene (Khan *et al.*, 2017). In South Korea, *IDS-IDS2* recombination mutations were the most frequently (Cho *et al.*, 2014). Molecular analysis of 103 unrelated South-Americans (including 91 Brazilian individuals) MPS II patients showed that small insertions, deletions, indels, and point mutations in the *IDS* gene were responsible for the disease in 81% of cases. Inversion/disruption or partial/total deletions of the *IDS* gene were found in 19% of the patients, and only eight pathogenic variants were found in more than one unrelated patient (Brusius-Facchin *et al.*, 2014). We do not have information about the rate of "de novo" mutation in the *IDS* gene in Brazil, but data from Latin America the literature estimate it as 10% (Amartino *et al.*, 2014).

A limitation of our study is that, although responsible for the vast majority of MPS diagnosis in Brazil, our laboratory is not the only to perform such tests, and some Brazilian cases may have been not included. Also, milder cases that are challenging to diagnose may be overlooked. Another estimate of the birth prevalence of MPS in Brazil, based on the frequency of heterozygotes for the most common pathogenic variant of the *IDUA* gene (p.Trp402Ter) in healthy blood donors and on the relative frequency of homozygosity for such variant in MPS I patients (Federhen *et al.*, 2020) was reported as

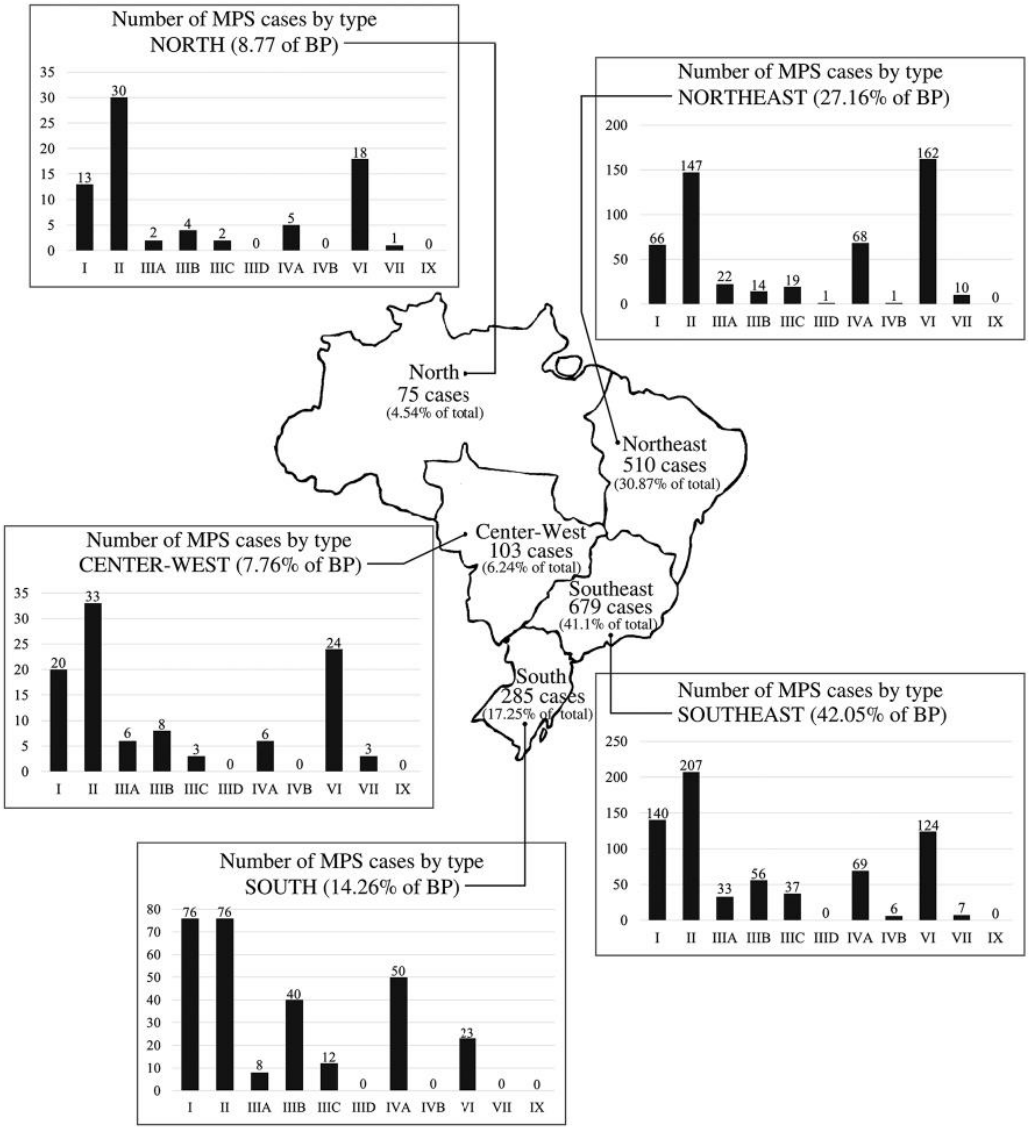

Figure 1 – Distribution according to Brazilian region of the Brazilian MPS cases diagnosed at the Medical Genetics Service of Hospital de Clínicas de Porto Alegre from 1982 to 2019 (BP: Brazilian population as estimated in 2019) (IBGE, 2020).

4.62/100,000 live births, nearly three times higher than the one found in this study (1.57). In this manner, although providing a comprehensive picture of the epidemiological profile of MPS in Brazil, the absolute numbers found in this study are possibly underestimated. A newborn screening (NBS) program would be more accurate to estimate the incidence of MPS. Although MPS testing is not included in the public NBS program in Brazil, pilot studies are being carried out in order to evaluate its feasibility for future incorporation (Camargo Neto *et al.*, 2018).

A previous estimation of the birth prevalence of MPS in Brazil was published with data from 1994 to 2015 (Federhen *et al.*, 2020). We have updated the birth prevalence across Brazilian regions up to 2018 and also demonstrated the distribution of MPS across each Brazilian State. We think this revision is important since the inclusion of only three years already demonstrated a change in the estimated birth prevalence of MPS by type in the South Region, where MPS I was previously the most common (Federhen *et al.*, 2020). Moreover, the knowledge of the distribution by state, which does not necessarily reflects the distribution by region, can help the design of targeted public policies. This report provides a comprehensive characterization of the epidemiological profile of the different MPS subtypes in Brazil and its variations across states and regions. The birth prevalence of MPS is variable across countries and regions and is likely linked to founder effect, endogamy, and consanguinity, but other factors that are still unclear may be present and may need further investigation. Our findings may help the assess of health needs in distinct populations and the delivery of medical care for these rare diseases.

Table 2 – Number of cases diagnosed and incidence (by 100,000 live births)[a] calculated for MPS patients born from 1994 to 2018 in Brazil and by region.

Region \ MPS	All	I	II	IIIA	IIIB	IIIC	IIID	IVA	IVB	VI	VII	IX
North	65 (0.88)	13 (0.18)	28 (0.38) (0.74)[b]	2 (0.03)	4 (0.05)	2 (0.03)	–	4 (0.05)	–	11 (0.15)	1 (0.01)	-
Northeast	379 (1.78)	52 (0.25)	109 (0.51) (1.00)[b]	18 (0.08)	13 (0.06)	14 (0.07)	1 (0.005)	40 (0.19)	–	123 (0.58)	9 (0.04)	-
Center-West	73 (1.26)	14 (0.24)	27 (0.47) (0.91)[b]	2 (0.03)	8 (0.14)	1 (0.02)	–	2 (0.03)	–	17 (0.29)	2 (0.03)	-
Southeast	476 (1.62)	98 (0.33)	143 (0.49) (0.95)[b]	27 (0.09)	41 (0.14)	27 (0.09)	–	43 (0.15)	2 (0.007)	91 (0.31)	4 (0.01)	-
South	171 (1.66)	40 (0.39)	51 (0.49) (0.97)[b]	8 (0.08)	22 (0.21)	9 (0.09)	–	26 (0.25)	–	15 (0.15)	–	-
Brazil, total	1164 (1.57)	217 (0.29)	358 (0.48) (0.94)[b]	57 (0.08)	88 (0.12)	53 (0.07)	1 (0.001)	115 (0.15)	2 (0.003)	257 (0.35)	16 (0.02)	-

[a] The incidence rate (shown inside parenthesis) was calculated using our data and the number of live births obtained from SINASC; [b] Considering only male live births.

Acknowledgments

The authors would like to thank the biologists, biochemists, biomedical, undergraduate, and graduate students who contributed to the work developed by the MPS BRASIL NETWORK. We would also acknowledge the health professionals who are members of this network and provided samples from suspected MPS patients. The authors acknowledge the support received from INAGEMP (CNPQ 465549/2014, CAPES 88887.136366/2017-00, FAPERGS 17/2551.0000521-0), from FIPE/HCPA (projects GPPG 2003-0066 and 2017-0664), and from Fundação Médica do Rio Grande do Sul. This work was performed in collaboration with the BIODISCOVERY (Experimental Research Centre, HCPA, Porto Alegre, Brazil) and DRBRASIL (Clinical Research Centre, HCPA, Porto Alegre Brazil) research groups.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

JAJ and RG conceived this study; JAJ performed formal analysis and wrote the manuscript; RG supervised the study and fully revised the document; RG, FBT, MGB, KMT, APPSM, FMS, FB, JFDM, ACBF, SLS and DRM contributed to the investigation, data collection, and creation of MPS BRAZIL NETWORK online platform. All authors read and approved the final version.

References

Amartino H, Ceci R, Masllorens F, Gal A, Arberas C, Bay L, Ilari R, Dipierri J, Specola N, Cabrera A, *et al.* (2014). Identification of 17 novel mutations in 40 Argentinian unrelated families with mucopolysaccharidosis type II (Hunter syndrome). Mol Genet Metab Rep 1:401-406.

Brusius-Facchin AC, Schwartz IV, Zimmer C, Ribeiro MG, Acosta AX, Horovitz D, Monlleó IL, Fontes MIB, Fett-Conte A, Oliveira Sobrinho RP, *et al.* (2014) Mucopolysaccharidosis type II: Identification of 30 novel mutations among Latin American patients. Mol Genet Metab 111:133–138.

Camargo Neto E, Schulte J, Pereira J, Bravo H, Sampaio-Filho C and Giugliani R (2018). Neonatal screening for four lysosomal storage diseases with a digital microfluidics platform: Initial results in Brazil. Genet Mol Biol 41:414–416.

Chen X, Qiu W, Ye J, Han L, Gu X and Zhang H (2016) Demographic characteristics and distribution of lysosomal storage disorder subtypes in Eastern China. J Hum Genet 61:345–349.

Cho SY, Sohn YB and Jin DK (2014) An overview of Korean patients with mucopolysaccharidosis and collaboration through the Asia Pacific MPS Network. Intractable Rare Dis Res 3:79–86.

Costa-Motta FM, Bender F, Acosta A, Abé-Sandes K, Machado T, Bomfim T, Boa Sorte T, Silva D, Bittles A, Giugliani R, *et al.* (2014) A Community-based study of Mucopolysaccharidosis Type VI in Brazil: The influence of founder effect, endogamy and consanguinity. Hum Hered 77:189–196.

Federhen A, Pasqualim G, de Freitas TF, Gonzalez EA, Trapp F, Matte U and Giugliani R (2020) Estimated birth prevalence of mucopolysaccharidosis in Brazil. Am J Med Genet A 182:469-483.

Giugliani R (2012) Mucopolysacccharidoses: from understanding to treatment, a century of discoveries. Genet Mol Biol 35 Suppl 4:924–931.

Giugliani R, Vairo FP, Riegel M, de Souza CF, Schwartz IV and Pena SD (2016) Rare disease landscape in Brazil: report of a successful experience in inborn errors of metabolism. Orphanet J Rare Dis 11:76.

Giugliani R, Federhen A, Michelin-Tirelli K, Riegel M and Burin M (2017) Relative frequency and estimated minimal frequency of lysosomal storage diseases in Brazil: Report from a reference laboratory. Genet Mol Biol 40:31–39.

Khan SA, Peracha H, Ballhausen D, Wiesbauer A, Rohrbach M, Gautschi M, Manson RW, Giugliani R, Suzuki Y, Orii KE, *et al.* (2017) Epidemiology of mucopolysaccharidoses. Mol Genet Metab 121:227–240.

Krabbi K, Joost K, Zordania R, Talvik I, Rein R, Huijmans JG, Verheijen FV and Ounap K (2012) The live-birth prevalence of mucopolysaccharidoses in Estonia. Genetic testing and molecular biomarkers 16:846–849.

Neufeld EF and Muenzer J. The Mucopolysaccharidoses (2014) In: Valle D, Beaud*et al.*, Vogelstein B, Kinzler KW, Antonarakis SE, Ballabio A, Gibson K and Mitchell G. (eds). The Online Metabolic and Molecular Bases of Inherited Disease. McGraw-Hill, New York, pp. 3421–3452.

Poupetová H, Ledvinová J, Berná L, Dvoráková L, Kozich V and Elleder M (2010) The birth prevalence of lysosomal storage disorders in the Czech Republic: comparison with data in different populations. J Inherit Metab Dis 33:387–396.

Suarez-Guerrero JL, Higuera PJIG, Flórez JSA and Contreras-Garcia GA (2015) Mucopolisacaridosis: características clínicas, diagnóstico y manejo. Rev Chil Pediatr 87:295–304.

Vairo F, Federhen A, Baldo G, Riegel M, Burin M, Leistner-Segal S and Giugliani R (2015) Diagnostic and treatment strategies in mucopolysaccharidosis VI. Appl Clin Genet 8:245–255.

Internet Resources

DATASUS. Tecnologia da informação a serviço do SUS, http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sinasc/cnv/nvuf.def (April 25, 2020)

IBGE. Instituto Brasileiro de Geografia e Estatística. Estimativas da população residente no brasil e unidades da federação com data de referência em 1º de julho de 2019, https://ftp.ibge.gov.br/Estimativas_de_Populacao/Estimativas_2019/POP2019_20201006.pdf (October 18, 2020)

Associate Editor: Angela Vianna-Morgante

4.2. Artigo 2

Genotype-phenotype studies in a large cohort of Brazilian patients with Hunter Syndrome.

Artigo publicado na revista *American Journal of Medical Genetics Part C: Seminars in Medical Genetics* (2021), doi: 10.1002/ajmg.c.31915.

Josahkian JA, Brusius-Facchin AC, Netto ABO, Leistner-Segal S, Málaga DR, Burin MG, Michelin-Tirelli K, Trapp FB, Cardoso-Dos-Santos AC, Ribeiro EM, Kim CA, de Siqueira ACM, Santos ML, do Valle DA, da Silva RTB, Horovitz DDG, de Medeiros PFV, de Souza CFM, Giuliani LR, Miguel DSCG, Santana-da-Silva LC, Galera MF, Giugliani R (2021) Genotype-phenotype studies in a large cohort of Brazilian patients with Hunter syndrome. Am J Med Genet C Semin Med Genet 187(3):349-356.

Received: 9 November 2020 | Revised: 25 February 2021 | Accepted: 18 April 2021
DOI: 10.1002/ajmg.c.31915

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics C Seminars in Medical Genetics WILEY

Genotype–phenotype studies in a large cohort of Brazilian patients with Hunter syndrome

Juliana Alves Josahkian[1,2] | Ana Carolina Brusius-Facchin[3] | Alice Brinckmann Oliveira Netto[3,4,5] | Sandra Leistner-Segal[3,4] | Diana Rojas Málaga[3,6] | Maira Graeff Burin[3] | Kristiane Michelin-Tirelli[3] | Franciele Barbosa Trapp[3] | Augusto César Cardoso-dos-Santos[2,4] | Erlane Marques Ribeiro[7] | Chong Ae Kim[8] | Ana Cecília Menezes de Siqueira[9] | Mara Lucia Santos[10] | Daniel Almeida do Valle[10] | Raquel Tavares Boy da Silva[11] | Dafne Dain Gandelman Horovitz[12] | Paula Frassinetti Vasconcelos de Medeiros[13] | Carolina Fischinger Moura de Souza[3] | Liane de Rosso Giuliani[14] | Diego Santana Chaves Geraldo Miguel[15] | Luiz Carlos Santana-da-Silva[16] | Marcial Francis Galera[17] | Roberto Giugliani[3,4,18]

[1]Department of Clinical Medicine, Hospital Universitário de Santa Maria (HUSM), Santa Maria, Rio Grande do Sul, Brazil

[2]Postgraduate Program in Genetics and Molecular Biology, UFRGS, Porto Alegre, Rio Grande do Sul, Brazil

[3]Medical Genetics Service, HCPA, Porto Alegre, Rio Grande do Sul, Brazil

[4]National Institute on Population Medical Genetics, INAGEMP, Porto Alegre, Rio Grande do Sul, Brazil

[5]Graduate in Biological Sciences, UFRGS, Porto Alegre, Rio Grande do Sul, Brazil

[6]Research and Development, Grupo Fleury, São Paulo, São Paulo, Brazil

[7]Serviço de Genética Médica, Hospital Infantil Albert Sabin, Fortaleza, Ceará, Brazil

[8]Genetic Unit, Pediatric Department, HC-FMUSP, São Paulo University, São Paulo, São Paulo, Brazil

[9]Instituto de Medicina Integral Professor Fernando Figueira (IMIP), Recife, Pernambuco, Brazil

[10]Neuropediatric Division, Hospital Pequeno Príncipe, Curitiba, Paraná, Brazil

[11]State University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil

[12]Medical Genetics Department, National Institute of Women, Children and Adolescents Health Fernandes Figueira—Fiocruz/Reference Center for Rare Diseases, Rio de Janeiro, Rio de Janeiro, Brazil

[13]Unidade Acadêmica de Medicina, Hospital Universitário Alcides Carneiro, Universidade Federal de Campina Grande, Campina Grande, Paraíba, Brazil

[14]Hospital Universitário Maria Aparecida Pedrossian (HUMAP), UFMS, Campo Grande, Mato Grosso do Sul, Brazil

[15]Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brazil

[16]Laboratory of Innate Errors of Metabolism, Institute of Biological Sciences, Federal University of Pará, Belém, Pará, Brazil

[17]Department of Pediatrics, Faculty of Medicine, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil

[18]Department of Genetics, UFRGS, Porto Alegre, Rio Grande do Sul, Brazil

Correspondence
Juliana Josahkian, Unidade de Clínica Médica, Hospital Universitário de Santa Maria. Avenida Roraima, 1000, prédio 22, Camobi, Santa Maria, RS, 97105-900, Brazil.
Email: julianajosahkian@gmail.com

Mucopolysaccharidosis type II (MPS II) is an X-linked inherited disease caused by pathogenic variants in the *IDS* gene, leading to deficiency of the lysosomal enzyme iduronate-2-sulfatase and consequent widespread storage of glycosaminoglycans, leading to several clinical consequences, with progressive manifestations which most

Funding information
FIPE/HCPA, Grant/Award Numbers: GPPG 2003-0066, GPPG 2017-0664; Instituto Nacional de Genética Médica Populacional, Grant/Award Numbers: CAPES 88887.136366/2017-00, CNPQ 465549/2014, FAPERGS 17/2551.0000521-0

times includes cognitive decline. MPS II has wide allelic and clinical heterogeneity and a complex genotype–phenotype correlation. We evaluated data from 501 Brazilian patients diagnosed with MPS II from 1982 to 2020. We genotyped 280 of these patients (55.9%), which were assigned to 206 different families. Point mutations were present in 70% of our patients, being missense variants the most frequent. We correlated the *IDS* pathogenic variants identified with the phenotype (neuronophatic or non-neuronopathic). Except for two half-brothers, there was no discordance in the genotype–phenotype correlation among family members, nor among MPS II patients from different families with the same single base-pair substitution variant. Mothers were carriers in 82.0% of the cases. This comprehensive study of the molecular profile of the MPS II cases in Brazil sheds light on the genotype–phenotype correlation and helps the better understanding of the disease and the prediction of its clinical course, enabling the provision of a more refined genetic counseling to the affected families.

KEYWORDS
Hunter syndrome, *IDS* gene, iduronate-2-sulfatase, lysosomal storage diseases, mucopolysaccharidosis type II

1 | INTRODUCTION

Mucopolysaccharidosis type II (MPS II, Hunter syndrome) is an X-linked inherited lysosomal storage disorder caused by a deficiency of iduronate-2-sulfatase activity (I2S, EC 3.1.6.13), that normally cleaves a sulfate group from the glycosaminoglycans (GAGs) heparan and dermatan sulfate (Pinto et al., 2004). Failure to hydrolyze the terminal iduronate-2-sulfate esters in these GAGs results in progressive accumulation of undegraded substrates within the lysosomes and in the clinical manifestations associated to MPS II (Demydchuk et al., 2017).

The *IDS* gene is located on the Xq28 boundary of the long arm of the X-chromosome, comprising nine exons spanning approximately 24 kb. A pseudogene (*IDS2*), containing sequences homologous to exon II, intron 2, exon III, and a chimerical intron 3 intron 7, is located approximately 20 kb far, telomeric to the active *IDS* gene, which makes this region prone to the occurrence of recombination events (Alkhzouz et al., 2017; Wraith et al., 2008).

According to Human Gene Mutation Database (HGMD—The Human Gene Mutation Database, www.uwcm.ac.uk/uwcm/mg/search2020), more than 660 mutations have been described, most of which being private point mutations (60.69%) and small deletions (18.48%; HGMD, 2020).

MPS II shows not only wide allelic heterogeneity, but also wide clinical variability. Clinical manifestations include neurological dysfunction, coarse facies, skeletal changes, recurrent infections, and visceral involvement (Alkhzouz et al., 2017). Patients are usually classified as having a "neuronopathic" form (with progressive neurological disease), or a "non-neuronopathic" form (with minimal or no neurologic involvement) (Whiteman & Kimura, 2017). Only a few female patients with MPS II have been published to date, most of them associated with a structural disorder in the chromosome X (Semyachkina et al., 2019).

Studies of the genotype–phenotype correlation are subject to various biases. As even simple Mendelian disorders are in fact complex traits, it is likely that genotype–phenotype correlation does not exist for several *IDS* gene mutations (Froissart, da Silva, Guffon, Bozon, & Maire, 2002). For instance, gross structural changes including recombination if *IDS-IDS2* and large deletion of *IDS* are always reported as being associated with severe phenotype, while small gene alterations are associated with a broad spectrum of disease severity (Kosuga et al., 2016; Zhang et al., 2011).

MPS II is the most frequent type of MPS in Brazil (Josahkian et al., 2021), the same occurring in some Asian countries such as Japan, South Korea, Taiwan, and China, as well as in a few European countries, as Switzerland and Estonia (Khan et al., 2017), and Israel (Schaap & Bach, 1980).

In the present study, we evaluated the genetic profile of patients diagnosed with MPS II by the Medical Genetics Service of Hospital de Clinicas de Porto Alegre (MGS/HCPA) and by the MPS Brazil Network (MBN), in order to reinforce the importance of molecular analysis to understand epidemiological data and for genetic counseling. Also, we classified the phenotypic forms in "neuronopathic" and "non-neuronopathic" and correlated the phenotype with the *IDS* pathogenic variants identified in our cohort.

2 | MATERIAL AND METHODS

2.1 | Editorial policies and ethical considerations

This study was approved by the Ethics Committee of Universidade Federal do Rio Grande do Sul (CAAE #82189417.5.0000.5347) and was conducted in accordance with the ethical standards from the Declaration of Helsinki and its later amendments. Patients or relatives

gave written informed consent for genetic testing through the MPS Brazil Network Project (project GPPG 2003-0066).

2.2 | Patients and laboratorial analysis

We evaluated the records of patients diagnosed with MPS II from January 1982 to June 2020 in the Medical Genetic Service of Hospital de Clínicas de Porto Alegre (MGS/HCPA), Brazil, a reference center for metabolic disorders diagnosis that receives samples from all over Brazil and from many Latin American countries. The dataset also includes patients diagnosed by the MPS Brazil Network (MBN), an association of several Brazilian services which headquarters is based at MGS/HCPA.

Patients were referred for diagnostic investigation according to clinical criteria and/or pedigree analysis. Diagnosis was based on abnormal urinary GAG excretion (increased total GAGS, with predominance of heparan sulfate and dermatan sulfatase), and deficient IDS activity in dried blood, plasma, leukocytes, or fibroblasts. In all cases, the possibility of multiple sulfatase deficiency was ruled out by the measurement of the activity of another sulfatase.

Whenever possible, genetic testing was performed in order to identify the mutation present in the patients, enabling carrier detection and appropriate genetic counseling to the families. For the molecular genetics analyses, genomic DNA was extracted from blood following standard procedures. Molecular analysis started with PCR/RFLP to check for the presence of inversion due to intrachromosomal recombination between the *IDS* gene and its homologous region on the *IDS2* pseudogene. When no inversion/disruption was present, next-generation sequencing (NGS) was performed using Ion S5 System platform (Thermo Scientific, Life Technologies Corporation, South San Francisco, CA). The raw data was processed with the Torrent Suite Software v5.10.5.0 (Thermo Fisher Scientific) using the standard pipeline parameters. After the variant identification in the DNA sample from the index case, patient's mothers, whenever possible, were tested to determine carrier status.

When we could not perform molecular tests for one patient but there is another person in the same family with molecular results, we inferred the patient's genotype from the pedigree analysis. Regarding the frequency of carrier mothers, we did not consider the family history to determine the carrier status, so even if the mother is an obligatory carrier, for calculation purposes we considered only the mothers who were actually genotyped, to avoid selection bias.

All cases studied have clinical and enzymatic diagnosis of MPS II. We classified the *IDS* variant types in our cohort according to The Human Gene Mutation Database in single base-pair substitutions, which comprises missense, nonsense, and splicing; and in small/large gene alterations, which comprises small deletions (≤20 bp), small insertions/duplications (≤20 bp), small indels (≤20 bp), gross deletions (>20 bp), gross insertions/duplications (>20 bp), complex rearrangements, and regulatory (Stenson et al., 2017). When two point mutations were found in one patient, we classified the variant as "neighbor" (Ricci et al., 2003; Schwartz et al., 2006). We also report one single base-pair substitution that lead to the incorporation of the same amino acid as a "synonymous" variant, since there is increasing evidence that those variants can be disease causing (Zhou et al., 2012).

To determine whether two or more MPS II patients were related, we considered the *IDS* variant detected, pedigree analysis and carrier status of the mother. When the same *IDS* variant was found but family history was not informative, we considered the family name to determine whether patients were related.

We evaluated the dispersion of *IDS* variants per Brazilian state and genotype data were used to investigate if the high prevalence of MPS II was due to a high frequency of a private variant.

The clinical characteristics were individually evaluated, being the MPS II patients classified in "neuronophatic" and "non-neuronophatic" forms as well as the findings were correlated with the *IDS* variants.

3 | RESULTS

From January 1982 to June 2020, 501 MPS II Brazilian patients were diagnosed at the MGS/HCPA and MBN. The age at diagnosis was established for 417 (83.2%) patients and ranged from 0 (pre-natal) to 621 months (or 51 years old), the median being 86.49 months (7.2 years). There was no female patient in our cohort.

Molecular testing was performed in 268 (53.5%) patients, and in 265, we were able to find a genetic variant that is already classified as pathogenic or that could be disease causing. In another 15 patients, it was possible infer the genotype from pedigree analysis, leaving 280 patients (55.9% of sample) with molecular results available (Table 1).

Out of those 280 MPS II patients with molecular results available, 206 were considered from distinct families (considering the *IDS* variant, pedigree charts, family name, and/or carrier status of the mother). Table 2 shows the distribution of variants types in these 206 different families.

Regarding the phenotype, we were able to classify 132 MPS II patients in neuronopathic or non-neuronopathic form, being the neuronopathic the most common, accounting for 68.9% (91/132) of cases, while the non-neuronopathic form corresponded to 31.1% (41/132) of cases. From those 132 patients, in 112 (77 "neuronopathic" and 35 "non-neuronopathic") we have molecular results available. Table 3 shows the type of *IDS* variant found in each phenotypic group.

Most of the *IDS* variants found in our cohort were detected in only one family and/or patient, but 21 single base-pair substitutions were found in at least more than one family. Figure 1 shows the distribution of the single base-pair variants that appear in more than one family in Brazil.

Analysis of other variants types showed that the missense "neighbor" variant c.467C>A/c.463T>C seems to appear in two different families (in three patients from the same family and in one patient from another family). We could not link those families after reviewing pedigree charts. Molecular results of their mothers were not available.

TABLE 1 Type of *IDS* variant in 280 MPS II patients

	Number of MPS II patients	
Variant type	***N* = 280**	**100%**
Single base-pair substitutions	196	70.0
Missense	127	45.36
Missense "neighbor"[a]	5	1.78
Nonsense	36	12.86
Splicing	19	6.79
Synonymous	9	3.21
Other lesions	84	30.0
Microdeletions	23	8.21
Microinsertions/duplications	12	4.29
Indels	3	1.07
Gross deletions	13	4.64
Gross insertions/duplications	0	0
Complex rearrangements	31	11.07
Regulatory	2	0.71

[a]There are five patients with missense "neighbor" variants in our cohort: four patients have a c.467C>A/c.463T>C; and one patient have a c.1466G>A/c.1454T>C.

TABLE 2 Type of *IDS* variant in 206 different families

	Number of MPS II families (%)	
Variant type	***N* = 206**	**100%**
Single base-pair substitutions	138	66.99
Missense	84	40.78
Missense "neighbor"	3	1.46
Nonsense	28	13.59
Splicing	15	7.28
Synonymous	8	3.88
Other lesions	68	33.01
Microdeletions	18	8.74
Microinsertions/duplications	8	3.88
Indels	2	0.97
Gross deletions	13	6.31
Gross insertions/duplications	0	0
Complex rearrangements	25	12.14
Regulatory	2	0.97

We also found a 178 bp deletion in the promoter region of the *IDS* gene (g.271996_272174del) in two unrelated boys. Family history was noncontributory and molecular analysis of these boy's mothers revealed that neither was a carrier.

Considering genotype–phenotype correlation in single base-pair substitution variants, Table 4 shows the distribution of 41 *IDS* variants according to neuronopathic and non-neuronopathic forms for 76 patients from 50 distinct families.

The two unrelated patients with a regulatory variant, a 178 bp deletion in a promoter region (g.271996_272174del), have the non-neuronopathic form of the disease.

Carrier testing for the *IDS* gene done on mothers of 89 unrelated MPS II patients showed that 73 (82.0%) were carriers.

4 | DISCUSSION

This report provides comprehensive information on genotype and phenotypic profile of Brazilian MPS II patients. Point mutations were present in 70% of our patients, the missense variants being the most frequent in our cohort, regardless the phenotype, which is in accordance to the literature (HGMD, 2020; Kosuga et al., 2016). In the three MPS II cases for whom we could not find any pathogenic variant, a complex rearrangement as conversion could be present (Lualdi et al., 2005). However, this type of alteration could not be detected with the molecular strategy used in this study.

The pathogenic effect of the c.1122C>T (p.Gly374sp) silent substitution is related to the activation of an upstream 5′ cryptic splice-site in exon 8 and results in the deletion of 60 nucleotides in the transcript. The normal transcript is produced as a minor product, with the major one being a truncated protein with 20 amino acids less than normal, due to abnormal mRNA processing (Uttarilli et al., 2016).

We chose to discriminate the missense "neighbor" variants to better characterize this molecular finding since it has already been reported like this in the literature (Schwartz et al., 2006). There are five patients with missense "neighbor" variants in our cohort. Four patients have a c.467C>A/c.463 T>C, and one patient have a c.1466G>A/c.1454 T>C. The "neighbor" mutations have been formerly described (Ricci et al., 2003; Schwartz et al., 2006; Timms, Huckett, Belmont, Shapira, & Gibbs, 1998) and are located in mutation-rich regions of the IDS gene (exons V, VIII, and IX) involving non-conserved codons.

Considering each phenotypic group, patients with the non-neuronopathic form only had single base-pair substitutions, except two unrelated patients that had a 178 bp deletion in the promoter region of the *IDS* gene, classified as regulatory. This regulatory variant was previous reported in the literature (Timms et al., 1998) and is speculated to interfere in RNA polymerase binding region creating an alternative translation start site (Brusius-Facchin et al., 2013).

For the neuronopathic form of the disease, single base-pair substitutions were detected in 55.8% of these patients. Complex rearrangements and microdeletions were responsible for 15.6 and 14.3%, respectively, followed by gross deletions (10.4%), microinsertions/duplications (2.6%) and indels (1.3%). The non-neuronopathic phenotype was mainly related to single base-pair substitutions (94.3%), except for the two mentioned patients with the regulatory variant. The finding that lesions other than single base-pair substitutions are related to the neuronopathic form has previously been reported (Kosuga et al., 2016).

TABLE 3 Type of *IDS* variant in 112 MPS II patients according to "neuronopathic" and "non-neuronopathic" phenotype

	Phenotype			
	Neuronopathic		Non-neuronopathic	
Variant type	*N* = 77	100%	*N* = 35	100%
Single base-pair substitutions	43	55.84	33	94.29
Missense	33	42.86	22	62.86
Missense "neighbor"	0	0	1	2.86
Nonsense	6	7.79	1	2.86
Splicing	4	5.19	6	17.14
Synonymous	0	0	3	8.57
Other lesions	34	44.16	2	5.71
Microdeletions	11	14.29	0	0
Microinsertions/duplications	2	2.60	0	0
Indels	1	1.30	0	0
Gross deletions	8	10.39	0	0
Gross insertions/duplications	0	0	0	0
Complex rearrangements	12	15.58	0	0
Regulatory	0	0	2	5.71

***IDS* variants identified in more than one family**

1. c.22C>T; p.Arg8Ter	8. c.309C>G; p.Tyr103Ter	15. c.1122C>T; p.Gly374Gly
2. c.187A>G; p.Asn63Asp	9. c.326G>A; p.Trp109Ter	16. c.1165C>T; p.Gln389Ter
3. c.241-2A>G	10. c.692C>T; p.Pro231Leu	17. c.1181-2A>G
4. c.253G>A; p.Ala85Thr	11. c.702C>A; p.Tyr234Ter	18. c.1327C>T; p.Arg443Ter
5. c.257C>T; p.Pro86Leu	12. c.913T>C; p.Ser305Pro	19. c.1400C>T; p.Pro467Leu
6. c.262C>T; p.Arg88Cys	13. c.998C>T; p.Ser333Leu	20. c.1402C>T; p.Arg468Trp
7. c.263G>A; p.Arg88His	14. c.1004A>G; p.His335Arg	21. c.1403G>A; p.Arg468Gln

FIGURE 1 Distribution of *IDS* single base-pair substitutions variants that appear in more than one family by Brazilian state (listed by their initials). When the same *IDS* variant appears in more than one family in the same state, it is indicated as follows: *Two different families in that state; **Three different families in that state. AL, Alagoas; AM, Amazonas; BA, Bahia; CE, Ceará; DF, Distrito Federal; ES, Espírito Santo; MA, Maranhão; MG, Minas Gerais; MS, Mato Grosso do Sul; MT, Mato Grosso; PB, Paraíba; PE, Pernambuco; PR, Paraná; RJ, Rio de Janeiro; RS, Rio Grande do Sul; SE, Sergipe; SP, São Paulo

In addition to MPS II being a rare disease, these is a large number of private variants in the *IDS* gene (D'Avanzo, Rigon, Zanetti, & Tomanin, 2020), making it even harder to clarify genotype–phenotype correlation for patients with single base pair substitutions. In our cohort, except for two half-brothers with the splicing variant c.1006 + 1G>A, there was no discordance in the genotype–phenotype correlation between family members nor among MPS II patients from different families that had the same single base-pair substitution variant. There may be other genetic and/or environmental factors to explain this lack of correlation in the half-brothers. For example, little is known about the influence of polygenic background in monogenic diseases, but it is plausible that it can impact the clinical presentation (Fahed et al., 2020).

There are some important limitations in our study. Although we could observe a correlation regarding neuronopathic and non-neuronopathic forms of MPS II presentation and the single base pair substitutions detected, the number of patients with the same variant is very limited to have a good generalizability. In vitro expression studies or animal model experiments would be important in trying to identify the mechanisms underlying the two different forms considered here, but it is outside the scope of our work. Also, it is pertinent to reflect that the categorization between these two forms is a subject of discussion since patients with a slow progression of neurological disease might be considered at first to have a non-neuronopathic form.

The rate for carrier mothers was 82.0%, more than expected assuming that one third of cases in X-linked recessive diseases are due to spontaneous mutations (Crow, 1997), but less than previously estimated for Latin American patients (Amartino et al., 2014).

TABLE 4 Summary of genotype–phenotype correlation for 41 single base-pair substitutions in the *IDS* gene in a Brazilian cohort of MPS II patients

Category	Variant	Consequence	Position	Patients (families)[a]	Phenotype
Missense	c.230C>A	p.Ala77Asp	E2	13 (1)	NN
	c.239A>G	p.Gln80Arg	E2	1 (1)	NN
	c.253G>A	p.Ala85Thr	E3	1 (1)	NN
	c.257C>T	p.Pro86Leu	E3	3 (2)	N
	c.262C>T	p.Arg88Cys	E3	4 (2)	N
	c.263G>A	p.Arg88His	E3	4 (2)	N
	c.285G>T	p.Arg95Ser	E3	1 (1)	N
	c.353C>T	p.Thr118Ile	E3	1 (1)	N
	c.412C>T	p.His138Tyr	E3	1 (1)	N
	c.418G>A	p.Gly140Arg	E3	1 (1)	NN
	c.455G >A	p.Ser152Asn	E4	2 (1)	NN
	c.479C>T	p.Pro160Leu	E4	1 (1)	N
	c.484 T>C	p.Ser162Pro	E4	1 (1)	NN
	c.560A>T	p.Asp187Val	E5	1 (1)	NN
	c.614C>A	p.Ala205Asp	E5	1 (1)	N
	c.668 T>A	p.Val223Asp	E5	1 (1)	N
	c.922G>C	p.Asp308His	E7	1 (1)	N
	c.998C>T	p.Ser333Leu	E7	2 (2)	N
	c.1001A>C	p.Asp334Ala	E7	2 (1)	N
	c.1001A>T	p.Asp334Val	E7	1 (1)	N
	c.1004A>G	p.His335Arg	E7	2 (1)	N
	c.1007G>T	p.Gly336Val	E8	2 (1)	N
	c.1030G>A	p.Glu344Lys	E8	1 (1)	N
	c.1047C>A	p.Ser349Arg	E8	1 (1)	N
	c.1265G>A	p.Cys422Tyr	E9	1 (1)	NN
	c.1400C>T	p.Pro467Leu	E9	1 (1)	N
	c.1402C>T	p.Arg468Trp	E9	2 (2)	N
	c.1403G>A	p.Arg468Gln	E9	1 (1)	N
	c.1406C>T	p.Pro469Leu	E9	1 (1)	N
	c.1433A>G	p.Asp478Gly	E9	1 (1)	NN
Missense "neighbor"	c.467C>A/c.463 T>C	p.Phe155Leu; Pro156Gln	E4	1(1)	NN
Nonsense	c.326G>A	p.Trp109Ter	E3	1 (1)	NN
	c.702C>A	p.Tyr234Ter	E5	2 (2)	N
	c.1165C>T	p.Gln389Ter	E8	3 (2)	N
Splicing	c.240 + 1G>C		IVS2	1 (1)	N
	c.709-1G>C		IVS6	1 (1)	N
	c.880+8G>A		IVS6	3 (1)	NN
	c.1006 + 1G>A		IVS7	2 (1)	N and NN
	c.1181-2A>G		IVS8	2 (1)	NN
	c.1181-15C>A		IVS8	1 (1)	N
Synonymous	c.1122C>T	p.Gly374Gly	E8	3 (3)	NN

Abbreviations: E, exon; IVS, intron; N, neuronopathic form; NN, non-neuronopathic form.

[a]The number of patients for whom we have information on the phenotypic form is indicated and the number of different families where the variant was found is inside the parenthesis.

5 | CONCLUSION

This report is a comprehensive study of the molecular profile of the MPS II cases in Brazil and sheds light on the genotype–phenotype correlation in this condition, which will help to better understand the disease and to provide a more refined genetic counseling for the families.

ACKNOWLEDGMENTS

The authors acknowledge the support given by the medical doctors, biologists, biochemists, biomedical, and graduate and undergraduate students from all over the country who contributed to the work developed by the MPS BRASIL NETWORK. We would also like to thank the health professionals who are members of this network and provided samples from suspected MPS patients. The authors acknowledge the support received from INAGEMP (CNPQ 465549/2014, CAPES 88887.136366/2017-00, FAPERGS 17/2551.0000521-0), from FIPE/HCPA (projects GPPG 2003-0066 and 2017-0664), and from Fundação Médica do Rio Grande do Sul. This work was performed in collaboration with the research groups BIODISCOVERY (Experimental Research Centre, HCPA, Porto Alegre, Brazil) and DR BRASIL (Clinical Research Centre, HCPA, Porto Alegre Brazil).

CONFLICT OF INTERESTS

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Juliana Alves Josahkian and Roberto Giugliani conceived and designed this study and took full responsibility for the article. Juliana Alves Josahkian wrote the article, and Roberto Giugliani supervised the study and fully revised the document. Ana Carolina Brusius-Facchin, Alice Brinckmann Oliveira Netto, Sandra Leistner-Segal, Diana Rojas Málaga, Maira Graeff Burin, Kristiane Michelin-Tirelli, Franciele Barbosa Trapp, Augusto César Cardoso-dos-Santos, Erlane Marques Ribeiro, Chong Ae Kim, Ana Cecília Menezes de Siqueira, Mara Lucia Santos, Daniel Almeida do Valle, Raquel Tavares Boy da Silva, Dafne Dain Gandelman Horovitz, Paula Frassinetti Vasconcelos de Medeiros, Carolina Fischinger Moura de Souza, Liane de Rosso Giuliani, Diego Santana Chaves Geraldo Miguel, Luiz Carlos Santana da Silva, Marcial Francis Galera: contributed to the collection, data analysis, and/or to the operation of the MPS BRASIL NETWORK. All authors have read and agreed to the final version of the article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Juliana Alves Josahkian https://orcid.org/0000-0002-7506-993X
Ana Carolina Brusius-Facchin https://orcid.org/0000-0003-1103-6589
Alice Brinckmann Oliveira Netto https://orcid.org/0000-0001-7888-8327
Sandra Leistner-Segal https://orcid.org/0000-0003-3792-6168
Diana Rojas Málaga https://orcid.org/0000-0001-8107-7621
Maira Graeff Burin https://orcid.org/0000-0002-1633-8941
Kristiane Michelin-Tirelli https://orcid.org/0000-0003-2907-5906
Franciele Barbosa Trapp https://orcid.org/0000-0001-5464-3763
Augusto César Cardoso-dos-Santos https://orcid.org/0000-0002-1499-9105
Erlane Marques Ribeiro https://orcid.org/0000-0002-7104-0128
Chong Ae Kim https://orcid.org/0000-0002-1754-1300
Ana Cecília Menezes de Siqueira https://orcid.org/0000-0002-1357-5263
Mara Lucia Santos https://orcid.org/0000-0002-5253-988X
Daniel Almeida do Valle https://orcid.org/0000-0001-9005-6726
Raquel Tavares Boy da Silva https://orcid.org/0000-0001-6432-4870
Dafne Dain Gandelman Horovitz https://orcid.org/0000-0001-7726-4301
Paula Frassinetti Vasconcelos de Medeiros https://orcid.org/0000-0003-1863-9358
Carolina Fischinger Moura de Souza https://orcid.org/0000-0002-7280-6260
Liane de Rosso Giuliani https://orcid.org/0000-0002-1505-623X
Luiz Carlos Santana-da-Silva https://orcid.org/0000-0003-1017-6221
Marcial Francis Galera https://orcid.org/0000-0002-1624-7572
Roberto Giugliani https://orcid.org/0000-0001-9655-3686

REFERENCES

Alkhzouz, C., Lazea, C., Bucerzan, S., Nascu, I., Kiss, E., Denes, C. L., & Grigorescu-Sido, P. (2017). Clinical and genetic characteristics of Romanian patients with mucopolysaccharidosis type II. *JIMD Reports*, *33*, 19–25. https://doi.org/10.1007/8904_2016_535

Amartino, H., Ceci, R., Masllorens, F., Gal, A., Arberas, C., Bay, L., … Rozenfeld, P. (2014). Identification of 17 novel mutations in 40 Argentinian unrelated families with mucopolysaccharidosis type II (Hunter syndrome). *Molecular Genetics and Metabolism Reports*, *1*, 401–406. https://doi.org/10.1016/j.ymgmr.2014.08.006

Brusius-Facchin, A. C., Abrahão, L., Schwartz, I. V. D., Lourenço, C. M., Santos, E. S., Zanetti, A., … Leistner-Segal, S. (2013). Extension of the molecular analysis to the promoter region of the iduronate 2-sulfatase gene reveals genomic alterations in mucopolysaccharidosis type II patients with normal coding sequence. *Gene*, *526*(2), 150–154. https://doi.org/10.1016/j.gene.2013.05.007

Crow, J. F. (1997). The high spontaneous mutation rate: Is it a health risk? *Proceedings of the National Academy of Sciences of the United States of America*, *94*(16), 8380–8386. https://doi.org/10.1073/pnas.94.16.8380

D'Avanzo, F., Rigon, L., Zanetti, A., & Tomanin, R. (2020). Mucopolysaccharidosis type II: One hundred years of research, diagnosis, and treatment. *International Journal of Molecular Sciences*, *21*(4), 1258. https://doi.org/10.3390/ijms21041258

Demydchuk, M., Hill, C. H., Zhou, A., Bunkóczi, G., Stein, P. E., Marchesan, D., … Read, R. J. (2017). Insights into Hunter syndrome from the structure of iduronate-2-sulfatase. *Nature Communications*, *8*, 15786. https://doi.org/10.1038/ncomms15786

Fahed, A. C., Wang, M., Homburger, J. R., Patel, A. P., Bick, A. G., Neben, C. L., … Khera, A. V. (2020). Polygenic background modifies penetrance of monogenic variants for tier 1 genomic conditions. *Nature*

Communications, *11*(1), 3635. https://doi.org/10.1038/s41467-020-17374-3

Froissart, R., da Silva, I. M., Guffon, N., Bozon, D., & Maire, I. (2002). Mucopolysaccharidosis type II—Genotype/phenotype aspects. *Acta Paediatrica*, *91*(11), 82–87. https://doi.org/10.1080/080352502762457996

HGMD. (2020). The human gene mutation database. Retrieved from http://www.hgmd.cf.ac.uk/ac/index.php

Josahkian, A. J., Trapp, F. B., Burin, M. G., Michelin-Tirelli, K., Magalhães, A. P. P. S., Sebastião, F. M., … Giugliani, R. (2021). Updated incidence and relative frequency of Mucopolysaccharidoses across Brazilian regions. *Genetics and Molecular Biology*, *44*(1), e20200138. https://doi.org/10.1590/1678-4685-gmb-2020-0138

Khan, S. A., Peracha, H., Ballhausen, D., Wiesbauer, A., Rohrbach, M., Gautschi, M., … Tomatsu, S. (2017). Epidemiology of mucopolysaccharidoses. *Molecular Genetics and Metabolism*, *121*(3), 227–240. https://doi.org/10.1016/j.ymgme.2017.05.016

Kosuga, M., Mashima, R., Hirakiyama, A., Fuji, N., Kumagai, T., Seo, J. H., … Okuyama, T. (2016). Molecular diagnosis of 65 families with mucopolysaccharidosis type II (Hunter syndrome) characterized by 16 novel mutations in the IDS gene: Genetic, pathological, and structural studies on iduronate-2-sulfatase. *Molecular Genetics and Metabolism*, *118*(3), 190–197. https://doi.org/10.1016/j.ymgme.2016.05.003

Lualdi, S., Regis, S., Di Rocco, M., Corsolini, F., Stroppiano, M., Antuzzi, D., & Filocamo, M. (2005). Characterization of iduronate-2-sulfatase gene-pseudogene recombinations in eight patients with Mucopolysaccharidosis type II revealed by a rapid PCR-based method. *Human Mutation*, *25*(5), 491–497. https://doi.org/10.1002/humu.20165

Pinto, R., Caseiro, C., Lemos, M., Lopes, L., Fontes, A., Ribeiro, H., … Sá Miranda, M. C. (2004). Prevalence of lysosomal storage diseases in Portugal. *European Journal of Human Genetics*, *12*(2), 87–92. https://doi.org/10.1038/sj.ejhg.5201044

Ricci, V., Filocamo, M., Regis, S., Corsolini, F., Stroppiano, M., Duca, M. D., & Gatti, R. (2003). Expression studies of two novel in CIS-mutations identified in an intermediate case of Hunter syndrome. *American Journal of Medical Genetics Part A*, *120A*(1), 84–87. https://doi.org/10.1002/ajmg.a.10215

Schaap, T., & Bach, G. (1980). Incidence of mucopolysaccharidoses in Israel: Is Hunter disease 345a "Jewish disease"? *Human Genetics*, *56*(2), 221–223. https://doi.org/10.1007/bf00295699

Schwartz, I. V., Lima, L. C., Tylee, K., Oliveira Sobrinho, R. P., Norato, D. Y. J., Duarte, A. R., Besley, G., Burin, M., Matte, U., Giugliani, R, & Leistner-Segal, S. (2006). Further cases of "neighbor" mutations in mucopolysaccharidosis type II. *American Journal of Medical Genetics. Part A*, *140*(15), 1684–1686. doi:https://doi.org/10.1002/ajmg.a.31317

Semyachkina, A. N., Voskoboeva, E. Y., Zakharova, E. Y., Nikolaeva, E. A., Kanivets, I. V., Kolotii, A. D., … Melnikova, N. V. (2019). Case report: A rare case of Hunter syndrome (type II mucopolysaccharidosis) in a girl. *BMC Medical Genetics*, *20*(1), 66. https://doi.org/10.1186/s12881-019-0807-x

Stenson, P. D., Mort, M., Ball, E. V., Evans, K., Hayden, M., Heywood, S., … Cooper, D. N. (2017). The human gene mutation database: Towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Human Genetics*, *136*(6), 665–677. https://doi.org/10.1007/s00439-017-1779-6

Timms, K. M., Huckett, L. E., Belmont, J. W., Shapira, S. K., & Gibbs, R. A. (1998). DNA deletion confined to the idorunate-2-sulfatase promoter abolishes IDS gene expression. *Human Mutation*, *11*(2), 121–126. https://doi.org/10.1002/(sici)1098-1004(1998)11:2<121::aid-humu4>3.0.co;2-m

Uttarilli, A., Ranganath, P., Matta, D., Md Nurul Jain, J., Prasad, K., Babu, A. S., … Dalal, A. (2016). Identification and characterization of 20 novel pathogenic variants in 60 unrelated Indian patients with mucopolysaccharidoses type I and type II. *Clinical Genetics*, *90*(6), 496–508. https://doi.org/10.1111/cge.12795

Whiteman, D., & Kimura, A. (2017). Development of idursulfase therapy for mucopolysaccharidosis type II (Hunter syndrome): The past, the present and the future. *Drug Design, Development and Therapy*, *11*, 2467–2480. https://doi.org/10.2147/dddt.s139601

Wraith, J. E., Scarpa, M., Beck, M., Bodamer, O. A., De Meirleir, L., Guffon, N., … Zeman, J. (2008). Mucopolysaccharidosis type II (Hunter syndrome): A clinical review and recommendations for treatment in the era of enzyme replacement therapy. *European Journal of Pediatrics*, *167*(3), 267–277. https://doi.org/10.1007/s00431-007-0635-4

Zhang, H., Li, J., Zhang, X., Wang, Y., Qiu, W., Ye, J., … Gu, X. (2011). Analysis of the IDS gene in 38 patients with Hunter syndrome: The c.879G>A (p.Gln293Gln) synonymous variation in a female create Exonic splicing. *PLoS ONE*, *6*(8), e22951. https://doi.org/10.1371/journal.pone.0022951

Zhou, T., Ko, E. A., Gu, W., Lim, I., Bang, H., & Ko, J. H. (2012). Non-silent story on synonymous sites in voltage-gated ion channel genes. *PLoS ONE*, *7*(10), e48541. doi.org/10.1371/journal.pone.0048541

How to cite this article: Josahkian, J. A., Brusius-Facchin, A. C., Netto, A. B. O., Leistner-Segal, S., Málaga, D. R., Burin, M. G., Michelin-Tirelli, K., Trapp, F. B., Cardoso-dos-Santos, A. C., Ribeiro, E. M., Kim, C. A., de Siqueira, A. C. M., Santos, M. L., do Valle, D. A., da Silva, R. T. B., Horovitz, D. D. G., de Medeiros, P. F. V., de Souza, C. F. M., Giuliani, L. d. R., Miguel, D. S. C. G., Santana-da-Silva, L. C., Galera, M. F., & Giugliani, R. (2021). Genotype–phenotype studies in a large cohort of Brazilian patients with Hunter syndrome. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics Part C*, 1–8. https://doi.org/10.1002/ajmg.c.31915

4.3 Artigo 3

Artigo em fase final de preparação para ser submetido à publicação.

A Contribution to the Understanding of the High Relative Frequency of Mucopolysaccharidosis type II in Brazil

Authors:
Juliana Alves Josahkian[1,2,*], Ana Carolina Brusius-Facchin[3,4], Maira Graeff Burin[3], Franciele Barbosa Trapp[3,4,5], Alice Brinckmann Oliveira Netto[3,6,7], Diana Rojas Málaga[8], Augusto César Cardoso-dos-Santos[2,6,7], Erlane Marques Ribeiro[9], Chong Ae Kim[10], Ana Cecília Menezes de Siqueira[11], Mara Lucia Santos[12], Daniel Almeida do Valle[12], Raquel Tavares Boy da Silva[13], Dafne Dain Gandelman Horovitz[14], Paula Frassinetti Vasconcelos de Medeiros[15], Carolina Fischinger Moura de Souza[3,5], Liane de Rosso Giuliani[16], and Roberto Giugliani[3,4,5,6,17,18]

Institutional affiliations:
1. Department of Clinical Medicine, Hospital Universitário de Santa Maria (HUSM), Santa Maria, Rio Grande do Sul, Brazil.
2. Postgraduate Program in Genetics and Molecular Biology, UFRGS, Porto Alegre, Rio Grande do Sul, Brazil.
3. Medical Genetics Service, HCPA, Porto Alegre, Rio Grande do Sul, Brazil
4. BioDiscovery Laboratory, Experimental Research Center, HCPA, Porto Alegre, Brazil
5. Casa dos Raros, Porto Alegre, Brazil
6. National Institute on Population Medical Genetics, INAGEMP, Porto Alegre, Rio Grande do Sul, Brazil
7. Graduate in Biological Sciences, UFRGS, Porto Alegre, Rio Grande do Sul, Brazil
8. Research and Development, Grupo Fleury, São Paulo, São Paulo, Brazil
9. Serviço de Genética Médica, Hospital Infantil Albert Sabin, Fortaleza, Ceará, Brazil.
10. Genetic Unit, Pediatric Department, HC-FMUSP, São Paulo University, São Paulo, São Paulo, Brazil.
11. Instituto de Medicina Integral Professor Fernando Figueira (IMIP), Recife, Pernambuco, Brazil.
12. Neuropediatric Division, Hospital Pequeno Príncipe, Curitiba, Paraná, Brazil.
13. State University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil.
14. Medical Genetics Department, National Institute of Women, Children and Adolescents Health Fernandes Figueira-Fiocruz/Reference Center for Rare Diseases, Rio de Janeiro, Rio de Janeiro, Brazil.
15. Unidade Acadêmica de Medicina, Hospital Universitário Alcides Carneiro, Universidade Federal de Campina Grande, Campina Grande, Paraíba, Brazil.
16. Hospital Universitário Maria Aparecida Pedrossian (HUMAP), UFMS, Campo Grande, Mato Grosso do Sul, Brazil.
17. Department of Genetics, UFRGS, Porto Alegre, RS, Brazil
18. DASA, São Paulo, Brazil

*** Corresponding Author:**
Juliana Alves Josahkian
ORCID ID: https://orcid.org/0000-0002-7506-993X

ABSTRACT

The Mucopolysaccharidoses (MPS) are a group of lysosomal disorders with storage of glycosaminoglycans leading to progressive multisystem involvement. The aim of this study was to explore the relative frequency of MPS diseases in Brazil, where MPS II is reported (unlike some other regions) as the most frequent type. We analyzed the records of 1,711 MPS patients diagnosed between 1982 and 2020 by the Medical Genetics Service of Hospital de Clínicas de Porto Alegre (MGS/HCPA) and by the MPS Brazil Network (MBN) and reviewed the medical records and the laboratory data of the patients, whenever possible. The median delay in diagnosis (time between onset of symptoms and diagnosis) was 40 (15.25 – 84) months. Unexpectedly, there were no differences in the age at diagnosis between the first and further cases diagnosed in the same family. For MPS II, patients with the neuronopathic form were diagnosed earlier than the ones with the non-neuronopathic form, which suggests that cases with central nervous system involvement are either more severe or lead to a higher perception of disease. As there was no predominant variant in the *IDS* gene identified in the MPS II families, we speculate that the occurrence of a higher number of cases of MPS II in the affected families, compared to the number of cases in the families with other MPS types, may be one of the factors that drive the higher relative frequency of MPS II in Brazil. Also, the low consanguinity rate in the general population may contribute to keep the proportion of autosomal recessive types relatively low. There are still many challenges for the diagnosis of rare diseases like MPS, and there is a need to improve awareness about these conditions and make diagnostic tests widely available. This becomes important as early diagnosis now enables prompt treatment for most cases, and also timely genetic counseling to the family members.

Keywords: Mucopolysaccharidoses, Lysosomal Diseases, Glycosaminoglycans, Hunter syndrome, Enzyme Replacement Therapy, Brazil**.**

RESUMO

Mucopolissacaridoses (MPS) são um grupo de doenças lisossômicas com acúmulo de glicosaminoglicanos que levam a comprometimento multissistêmico e progressivo. O objetivo deste estudo foi explorar a frequência relativa das MPS no Brasil, onde a MPS II é descrita (ao contrário do que ocorre em algumas outras regiões) como o tipo mais frequente. Nós analisamos os dados de 1.711 pacientes com MPS diagnosticados entre 1982 e 2020 pelo Serviço de Genética Médica do Hospital de Clínica de Porto Alegre (MGS/HCPA) e pela Rede MPS Brasil (MBN) e revisamos prontuários médicos e dados laboratoriais destes pacientes, quando possível. A mediana do atraso ao diagnóstico (período entre o início dos sintomas e o diagnóstico) foi de 40 (15,25 – 84) meses. Inesperadamente, não houve diferença na idade ao diagnóstico entre o primeiro caso e os demais casos diagnosticados em uma mesma família. Para pacientes com MPS II, a forma neuronopática foi diagnosticada mais cedo do que a forma não-neuronopática, o que sugere que sintomas relacionados ao sistema nervoso central sejam mais graves ou levem à uma maior percepção de doença. Como não houve predomínio de nenhuma variante patogênica específica no gene *IDS* identificada nas famílias com MPS II, nós especulamos que a ocorrência de um maior número de casos de MPS II nas famílias afetadas, comparado com o número de casos nas famílias com outros tipos de MPS, possa ser um dos fatores que influenciam a alta frequência relativa de MPS II no Brasil. Ainda, a baixa taxa de consanguinidade da população brasileira em geral pode contribuir para manter relativamente baixa a proporção dos tipos recessivos. Ainda existem muitos desafios no diagnóstico de doenças raras como as MPS e é preciso melhorar o conhecimento sobre essas condições e tornar os testes diagnósticos amplamente disponíveis. Isso se torna importante pois o diagnóstico precoce agora permite o tratamento imediato para a maioria dos casos e, além disso, o aconselhamento genético oportuno para a família.

Palavras-chave: Mucopolissacaridoses, Doenças Lisossômicas, Glicosaminoglicanos, Síndrome de Hunter, Terapia de Reposição Enzimática, Brasil.

1. INTRODUCTION

The mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders caused by deficiencies in one of the enzymes involved in the degradation pathway of glycosaminoglycans (GAGs), leading to progressive multisystem involvement (Muenzer, 2004). There are currently eleven enzyme deficiencies, each one related to pathogenic variants in a specific gene. All have autosomal recessive pattern of inheritance, with the exception of MPS II, which is X-linked recessive (Neufeld and Muenzer, 2001).

MPS patients present with a wide phenotypic spectrum, with the possibility of musculoskeletal, cardiovascular, respiratory, ophthalmological, gastrointestinal, and nervous system involvement. They are typically characterized by short stature, dysmorphias, hepatosplenomegaly, hernias, skin lesions, history of recurrent infections and ocular manifestations. Severe cases may also have cognitive impairment (Stapleton et al., 2018).

Unlike the autosomal recessive MPS types, patients with MPS II are almost always male, unless another genetic event occurs in the normal X chromosome of a female carrier (Semyachkina et al., 2019; Pinto et al., 2010). The MPS II patients are classically divided into neuronopathic and non-neuronopathic forms, according to the presence or not of neurocognitive regression (Scarpa et al., 2011; Suarez-Guerrero et al., 2016).

Epidemiological data are available for some countries and regions (Çelik et al., 2021; Khan et al., 2017). In Brazil, our group's previous work found that the birth prevalence for MPS was 1.57 in 100,000 live births, MPS II being the most common type (Josahkian et al., 2021a).

The MPS II is also the most common type observed in Asian countries such as Taiwan, South Korea, Japan, and China, possibly due to the high allele frequency of the p.R468 variant (Rathmann, 1996; Khan et al., 2017).

This study aimed to address factors that may help to explain the high relative frequency of MPS II in Brazil and to discuss some characteristics of the diagnostic trajectory of MPS patients.

2. MATERIAL AND METHODS

We analyzed the records of Brazilian MPS patients diagnosed between 1982 and 2020 by the Medical Genetics Service of Hospital de Clínicas de Porto Alegre (MGS/HCPA), and by the MPS Brazil Network (MBN). Based on these data, we sought to review the medical records and the laboratory data of patients diagnosed with MPS in order to obtain more specific data such as number of patients in each family, parental consanguinity, signs and symptoms which were first noted, age at first symptoms, age at diagnosis, among other details. In order to establish which patients belonged to the same family, we seek to obtain the pedigree in the families that had more than one case reported. We also compared the patient's surnames and/or their mother's full name and examined their molecular genetics results (data not shown), so patients with different pathogenic variants were considered from different families.

In addition, since MPS are rare diseases, we analyzed the variables age at diagnosis (considered the age at the confirmatory laboratorial test); age when the first signs or symptoms were reported (clinical onset, based on medical records); and the delay in diagnosis (difference between age at clinical onset and age at diagnosis) to better understand the diagnostic odyssey of these patients. The data are presented as median (first and third quartiles) or absolute frequency (percentage). Data distribution was analyzed using the Kolmogorov-Smirnov test. The comparisons between MPS types were performed using Kruskall-Wallis test followed by post hoc of Dunn. The Mann-Whitney test was used to check possible differences between those born before and after 2004, since this was the year when the MPS Brazil Network, aimed to promote knowledge and support the laboratorial diagnosis of MPS in all regions of Brazil was created (Vieira et al., 2019); we also checked if whether being the first case within a family made a difference in the time of diagnosis.

We examined the association between categorical variables using the chi-square test. Subsequently, the analysis of standardized residues was conducted to verify in which categories the proportions differed from the expected value. For this analysis, the null hypothesis was rejected when Z score was lower than -1.96 or higher than 1.96.

The association between age at diagnosis and age at clinical onset was analyzed using Spearman's rank correlation coefficient. Statistical significance was considered when $P<0.05$.

In the MPS II group we evaluated if presenting a neuronopathic or a non-neuronopathic form influenced the age at diagnosis. The label was assigned by each patient physician using the criteria "presence of cognitive involvement" for the neuronopathic form and "absence or minimal cognitive involvement, without regression", for the non-neuronopathic form. All patients who had a phenotype assigned were at least five years old, except one patient that were 3.5 years and clearly presented the neuronopathic form.

All analyzes were performed using SPSS version 20.0 (IBM Corp., N.Y., USA).

This study was approved by the Ethics Committee of Universidade Federal do Rio Grande do Sul (CAAE #82189417.5.0000.5347) and was conducted in accordance with the ethical standards from the Declaration of Helsinki and its later amendments. Patients or relatives gave written informed consent for genetic testing through the MPS Brazil Network Project (project GPPG 2003-0066).

3. RESULTS

From April 1982 to December 2020, 1,711 Brazilian MPS patients were diagnosed by the MGS/HCPA and by the MBN. The characteristics of the sample are shown in Table 1. MPS type II was the most frequent type diagnosed, with all cases male. Excluding the X-linked recessive MPS II, the gender proportion was 50.6% males/49.0% females/0.4% unknown for the autosomal recessive types combined. Regarding geographic distribution, 40.2% of the diagnosis were in patients from Southeastern Brazil. Due to the low number of patients diagnosed with MPS IIID and IVB these individuals were excluded from subsequent analyses.

Table 1 – Characteristics of the MPS patients diagnosed from 1982 to 2020 at the MGS/HCPA and MBN.

	Number of MPS patients	
Characteristic	N=1711	100%
Gender		
Male	1116	65.2
Female	590	34.5
Unkown	5	0.3
Gender (excluding MPS II)	N=1204	100%
Male	609	50.6
Female	590	49.0
Unkown	5	0.4
Brazilian region (% of Brazilian population)[a]		
Center-West (7.8%)	110	6.4
North (8.8%)	81	4.7
Northeast (27.1%)	537	31.4
South (14.3%)	294	17.2
Southeast (42.0%)	687	40.2
Unkown	2	0.1
MPS type		
I	325	19.0
II	507	29.6
IIIA	73	4.3
IIIB	119	7.0
IIIC	82	4.8
IIID	2	0.1
IVA	208	12.1
IVB	7	0.4
VI	366	21.4
VII	22	1.3

[a] Brazilian population as estimated in 01/07/2020 (IBGE, 2021).

Information about parental consanguinity was obtained for 775 patients and the presence of consanguinity of any degree was significantly associated with the type of MPS (χ^2 = 120.089, *P* < 0,001). The analysis of standardized residues indicated a higher proportion of consanguineous parents in types IIIC (46%), IVA (54.8%) and VII (61.5%). As expected, the consanguinity rate in MPS type II parents was relatively low (4.1%). This rate is statistically different than the one found for the aggregated data of all the autosomal recessive MPS types (35.1%); *P* < 0,001.

We observed that parental consanguinity was significantly associated with the states of Brazil (χ^2 = 49.738, *P* = 0,001) with a higher proportion of consanguineous parents in Ceará (50.0%), Distrito Federal (61.1%), and Paraíba (50.0%). We found a higher proportion of parental consanguinity in the Northeast region (38.6%), although our data showed only a tendency of association between the Brazilian regions and parental consanguinity (χ^2 = 8.432; *P* = 0,077).

Tables 2 and 3 show data analysis between parental consanguinity and MPS types and Brazilian regions.

Table 2 – Association between MPS types and parental consanguinity.

MPS type	Counsanguineous parents (%)	Standardized residues
I	39.2	1.90
II	4.1	-10.4*
IIIA	43.6	1.6
IIIB	41.7	1.7
IIIC	46.0	2.2*
IVA	54.8	5.1*
VI	37.4	1.7
VII	61.5	2.3*

* Z score < -1.96 or > 1.96.

Table 3 – Association between Brazilian regions and states and parental consanguinity.

Brazilian region/state	Consanguineous parents (%)	Standardized residues
Northeast	38.6	2.9*
AC	0.0	-1.2
AL	10.5	-2.0*
BA	46.4	1.7
CE	50.0	3.4*
MA	28.6	-0.2
PB	50.0	2.6*
PE	24.6	-1.2
PI	46.2	1.1
RN	30.8	-0.1
SE	30.8	-0.1
North	27.6	-0.5
AM	33.3	0.1
PA	31.2	-0.1
RO	0.0	-0.7
Center-West	30.4	-0.2
DF	61.1	2.7*
GO	8.3	-1.8
MS	11.1	-1.3
MT	14.3	-1.0
South	28.1	-1.0
PR	26.8	-0.9
SC	13.6	-1.9
RS	37.2	0.8
Southeast	28.3	-1.8
ES	20.0	-1.0
MG	35.1	0.5
RJ	25.4	-1.1
SP	27.7	-1.3

* Z score < -1.96 or > 1.96. AC: Acre; AL: Alagoas; AM: Amazonas; BA: Bahia; CE: Ceará; DF: Distrito Federal; ES: Espírito Santo; GO: Goiás; MA: Maranhão; MG: Minas Gerais; MS: Mato Grosso do Sul; MT: Mato Grosso; PA: Pará; PB: Paraíba; PE: Pernambuco; PI: Piauí; PR: Paraná; RJ: Rio de Janeiro; RN: Rio Grande do Norte; RO: Rondônia; RS: Rio Grande do Sul; SC: Santa Catarina; SE: Sergipe; SP: São Paulo.

We obtained the number of MPS patients in each family and compared between MPS II (205 different families, with 378 MPS II cases in total – 1.84 cases per family) and the autosomal recessive MPS forms (416 different families, with 623 autosomal recessive MPS cases in total – 1.49 cases per family) in order to access whether the number of patients per family could explain the higher incidence of MPS II in Brazil. Most families had only one

case (54.6% for MPS II and 60.6% for the other MPS types combined). Three MPS II families had the largest number of affected members within the same genealogy (11, 14 and 16 patients each). In addition, the families with autosomal recessive forms of MPS with five or more patients were all from the Northeast region; also, the four families with autosomal recessive MPS with four patients in each were also from the Northeast region, except one family that were from the Southeast region. Data is detailed in Table 4.

Table 4 – Number of patients per family for 205 MPS II families and 416 autosomal recessive MPS families:

	MPS II (N = 205)		MPS AR (N = 416)	
Number of patients in each family	N	%	N	%
1	112	54.6	252	60.6
2	60	29.3	141	33.9
3	21	10.2	15	3.6
4	6	2.9	4[a]	1.0
5	2	1.0	1[b]	0.2
6	-		1[b]	0.2
8	1	0.5	1[b]	0.2
9	-		1[b]	0.2
11	1	0.5	-	
14	1	0.5	-	
16	1	0.5	-	

N: number of families; [a] all families from the Northeast region, except one family from the Southeast region; [b] all families from the Northeast region.

To explore factors that could help understand the diagnostic odyssey for these patients we compared the age at diagnosis and the age of clinical onset. Considering all MPS types together, the median age at diagnosis was 56 (28 – 107.5) months, the median of clinical onset was 12 (6 – 24) months, and the median delay in diagnosis was 40 (15.25 – 84) months. Patients with MPS I had the earliest diagnosis, which was lower than the age

at diagnosis for MPS II, IIIA, IIIB, IIIC, and IVA. On the other hand, patients with MPS IIIC were identified later, with significant difference when compared to MPS II, IIIB, VI, and VII.

The age of clinical onset was accessed for 174 patients: 22 MPS I, 65 MPS II, five MPS IIIA, seven MPS IIIB, eight MPS IIIC, 21 MPS IVA, and 46 MPS VI. The clinical onset was later in MPS IIIC when compared to MPS I, II, IVA and VI. Patients with MPS I had a shorter diagnostic delay and it was larger for MPS IIIC compared to MPS I, II, and VI.

The comparison regarding age at diagnosis, age of clinical onset and the delay in diagnosis among the types of MPS is detailed in Table 5.

Table 5 – Age at diagnosis, clinical onset and delay at diagnosis for MPS types.

MPS type	Age at diagnosis	Age of clinical onset	Delay in diagnosis
I (N=267)	37 (17-82)	7.5 (4-12)[c]	14.5 (4.25-24.5)[c]
II (N=426)	68 (39-112)[a,c]	12 (6-28)[c]	40 (12.5-75.5)[c]
IIIA (N=71)	83 (56-112)[a,d]	24 (15-42)	109 (51-132.5)
IIIB (N=114)	75.5 (45-108.25)[a,c]	24 (15-36)	46 (10.75-67.5)
IIIC (N=80)	129 (84.75-175.75)[a]	36 (33-42)	112 (92-164.75)
IVA (N=193)	93 (51.5-220.5)[a,b,d]	12 (5-27)[c]	79 (39-123.5)[a]
VI (N=308)	52 (30-107.75)[c]	11 (4-15)[c]	34 (19-64)[c]
VII (N=20)	62.5 (25.75-95)[c]	-	-

Data are presented in months as median (first and third quartile). N: number of patients with known age at diagnosis; [a] $P < 0.05$ compared to MPS I; [b] $P < 0.05$ compared to MPS II; [c] $P < 0.05$ compared to MPS IIIC; [d] $P < 0.05$ compared to MPS VI.

The median age at diagnosis for patients born after 2004, the year of creation of MPS Brazil Network, was 39 (19 – 63), and compared to those born before that year, which was 97 (57 - 163) months, a difference which was statistically significant ($P < 0.001$).

There appears to be no major difference in the age at diagnosis for MPS patients that were the first or only case in a family compared to patients that had other cases previously diagnosed in their families, with a median of 61 (34 – 102.25) months for the first diagnosed MPS case in comparison to 73 (25 – 158) months for the further cases; $P = 0.077$. Also, there was no difference when the same analysis was performed considering only the MPS II patients, with a median of 66 (42 – 100) months for the first diagnosed case in comparison to 66.5 (23 – 149.25) months for further cases in the families, $P = 0.570$.

Furthermore, age at diagnosis was not different between Brazilian regions Center-West [65 (26.5 – 94.5) months], North [86 (50 – 121) months], Northeast [68 (36 – 127) months], South [64 (29.5 – 103.5) months], and Southeast [65 (34 – 126) months]. There was also no difference in the age at diagnosis among the Brazilian states, except between Espírito Santo [40 (30 – 79) months] and Paraíba [118 (54 – 265.5) months]; $P = 0.017$ (Figure 1).

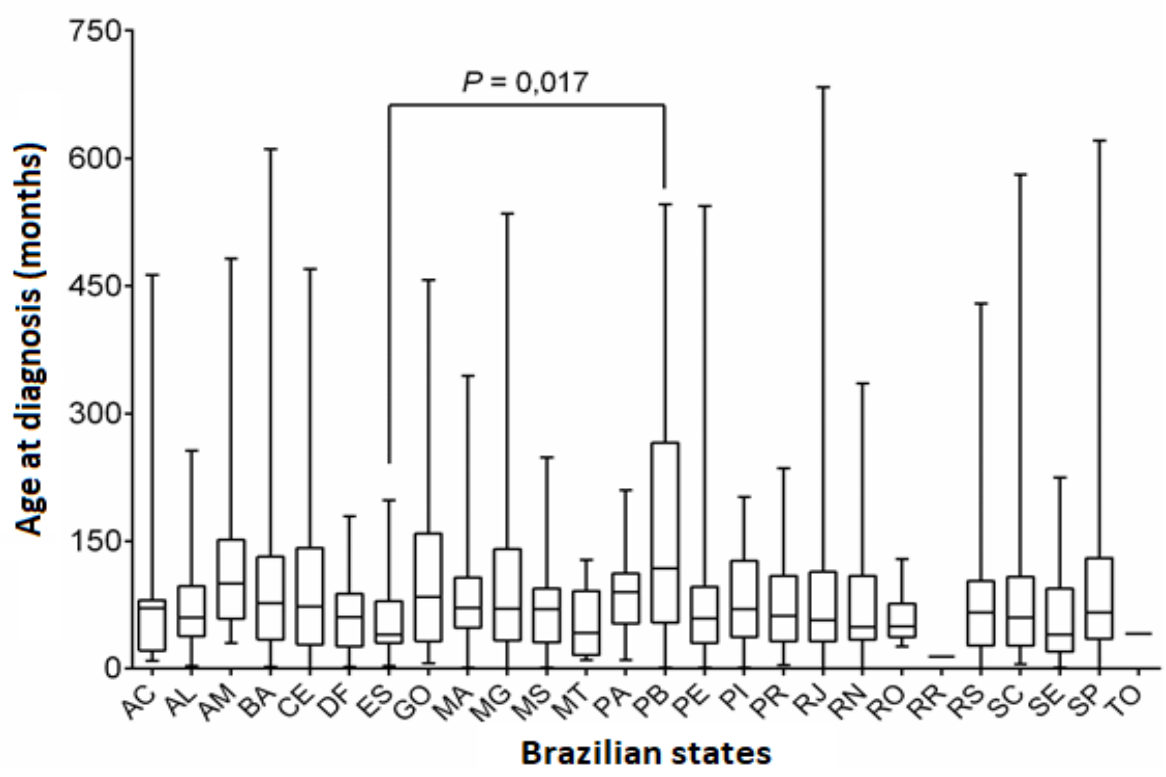

Figure 1 – Comparison of the age at diagnosis among Brazilian states.
AC: Acre; AL: Alagoas; AM: Amazonas; BA: Bahia; CE: Ceará; DF: Distrito Federal; ES: Espírito Santo; GO: Goiás; MA: Maranhão; MG: Minas Gerais; MS: Mato Grosso do Sul; MT: Mato Grosso; PA: Pará; PB: Paraíba; PE: Pernambuco; PI: Piauí; PR: Paraná; RJ: Rio de Janeiro; RN: Rio Grande do Norte; RO: Rondônia; RR: Roraima; RS: Rio Grande do Sul; SC: Santa Catarina; SE: Sergipe; SP: São Paulo; TO: Tocantins.

Regarding the age of clinical onset, there was no difference among the regions of the country ($P = 0.392$) nor the Brazilian states ($P = 0.187$).

The age at diagnosis was associated with the age of clinical onset (rho = 0.313; $P < 0.001$), with a faster diagnosis in patients in which the first signs or symptoms were noticed earlier (Figure 2).

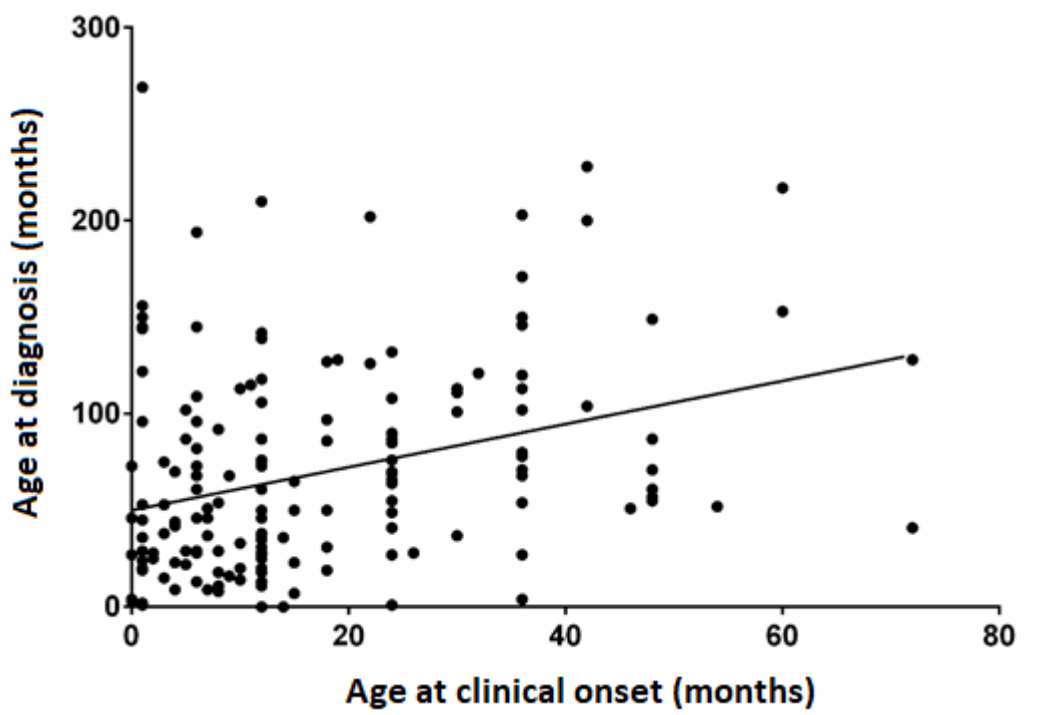

Figure 2 – Correlation between age at diagnosis and clinical onset of MPS.

In the MPS II group we evaluated if being a neuronopathic or a non-neuronopathic form influenced the age at diagnosis. We classified 128 MPS II patients as neuronopathic (N=85) or non-neuronopathic (N=43). For 64 MPS II patients, 42 neuronopathic and 22 non-neuronopathic form, we also could determine the age of clinical onset.

The neuronopathic MPS II patients differed from the non-neuronopathic ones in terms of age at diagnosis [neuronopathic: 56 (33 – 87.5) months and non-neuronopathic: 87 (42 – 236) months; P = 0.004], but there was no difference regarding the age of clinical onset [neuronopathic: 13.5 (6 – 31.5) months and non-neuronopathic: 12 (4.75 – 25.5) months; *P* = 0.655].

For 104 MPS patients we could identify which feature was perceived as the first sign or symptom of the disease, even if it was not the reason for seeking medical attention. Musculoskeletal changes were the most frequent feature, reported in 32 (30.8%) of the patients, and included joint restriction, claw hands, gibbus, pectus carinatum and/or genu valgum. Central nervous system symptoms were the second most common feature associated to MPS, in 26 (25%) patients, 18 of which had developmental delay, five had hyperactive behavior, two had seizures and one had hydrocephaly. Airway infections were reported as the first MPS sign for 18 (17.3%) patients. Umbilical or inguinal hernias were the first recognizable sign for 12 (11.5%) patients. Dysmorphic facial features were reported as the first sign for 10 (9.6%) patients. Four (3.9%) patients had visceromegaly and another two (1.9%) patients had diarrhea reported as the first recognizable feature of MPS.

4. DISCUSSION

Parental consanguinity was present in 35.1% of the cases of autosomal recessive MPS types, a much higher frequency compared to the MPS II cases (4.1%), as expected. We also found a higher proportion of parental consanguinity in Ceará, Distrito Federal, and Paraíba, with the same tendency for consanguinity in the Northeast region, historically the region with the highest inbreeding rate (Freire-Maia, 1957).

The Northeast is the only Brazilian region where MPS II is not the most frequent type. In Distrito Federal and Paraíba MPS II was not the predominant type, unlike what happens in other states and in Brazil as a whole (Josahkian, et al., 2021a).

High level of consanguinity is a known factor associated to recessive disorders, especially to rare diseases. Despite the heterogeneous genetic composition of the Brazilian population in general, the Northeast region of the country has been associated with endogamy, leading to a higher incidence of autosomal recessive diseases (Cardoso et al. 2019). This probably contributed to MPS II, the only MPS type with X-linked inheritance pattern, being not the most common MPS type in the Northeast region. In addition, the families with the autosomal recessive forms of MPS with the highest numbers of patients were from the Northeast region. We also think it is possible that the number of patients with MPS II per family contributes to the relatively high frequency of this MPS type in Brazil, since the number of affected patients is potentially higher (depending on whether there are female carriers in the family) than for the autosomal recessive forms of MPS, where affected members are expected to be limited to the sibship. The finding of a higher number of affected patients per MPS II family (1.84) than in families with autosomal recessive MPS (1.49) is in favor of this idea. The finding of over ten patients per family only in MPS II also supports this possibility.

Also endorsing this supposition, is the fact that the rate for carrier mothers is high, reported to be 82%, with some mothers that were initially found to be non-carriers reported to actually present pathogenic variants in mosaic (Josahkian et al., 2021b; Oliveira Netto et al., 2021).

When we look at all types of MPS combined, the median delay in diagnosis was 40 months, with a considerable percentage of the patients (25%) having over 84 months of

diagnostic delay. Patients with MPS I had the earliest diagnosis compared to types II, IIIA, IIIB, IIIC, and IVA. This may be related to the high proportion of the severe MPS I phenotype patients observed in this disease (Clarke et al, 2019), including in Brazil. In Brazil, in fact, almost 30 % of the MPS I cases are homozygous for one specific nonsense mutation associated to the severe phenotype (Federhen et al, 2020). The relatively late clinical onset of MPS IIIC that was observed in this study may be related to a specific mutation leading to this disease, which was more frequently found in an area of high consanguinity rate (Paraíba state) and most cases sharing similar genotypes (Martins et al, 2019).

It is undeniable that the knowledge of MPS has increased in the last years. The creation of the MPS Brazil Network, in 2004, seems to have significantly contributed to a faster, more efficient approach for MPS diagnosis in the country as the age at diagnosis for patients born after 2004 was lower when compared to those born before that year.

Unexpectedly, there appears to be no difference in the age at diagnosis if it was the first case diagnosed in a family or if there was already at least another MPS case in the same family. This is different from what was found in a multicenter study for MPS II, which showed that, despite the same median age of clinical onset, younger brothers were diagnosed earlier than their older brothers (Ficicioglu et al, 2018). We considered in our study every patient diagnosed within each family, even if there were no other case of MPS in that family. In the case of MPS II, the occurrence of an uncle or cousin with the same condition seems to not lead to an earlier diagnosis of the new cases. Potentially, psychological mechanisms may influence this delay in diagnosis in families who already had a first case. Anyhow, the large number of families with more than one affected patient exposes the need to improve access to effective genetic counseling throughout the country.

The nonspecific and slowly progressive symptoms are a challenge in the diagnosis of rare diseases, including MPS, as early signs and symptoms are often considered a personal characteristic or a minor feature and not indicative of a disease (Blöß et al., 2017). The fact that neuronopathic MPS II patients were diagnosed earlier than the non-neuronopathic ones suggests that the neuronopathic cases are more severe (with earlier manifestations) and/or the central nervous system dysfunction lead to a higher perception

of disease. Signs and symptoms like hernias, airway infections, and diarrhea are frequent in the pediatric population so, even if they could be retrospectively perceived as an early MPS feature, we cannot rely on them for diagnostic suspicion.

The pattern of age at diagnosis, clinical onset and delay in diagnosis seems to be the same across the country, with no regional particularities. On one way, it reinforces the reach of diagnostic support programs across the Brazilian territory, not limited to large reference centers physical localizations. However, it also emphasizes the need for increased awareness about this condition, since there is still a long gap between clinical onset and the diagnosis.

5. CONCLUSIONS

In this comprehensive analysis of a large sample of 1,711 Brazilian MPS patients diagnosed between 1982 and 2020, we confirmed previous findings about the higher relative proportion of MPS II compared to other MPS types. As there was no predominant variant detected in the *IDS* gene of MPS II families, we speculate that the higher average number of patients per family with this X-linked condition, compared to the average number observed in the autosomal recessive types, may be involved in this finding. The low consanguinity rate of the Brazilian population in general may contribute to the lower proportion of the autosomal recessive MPS types. Neuronopathic cases of MPS II have similar age of clinical disease onset as non-neuronophatic and, surprisingly, we found that there was no difference in the age at diagnosis of the first case in the family compared to the age at diagnosis of the additional cases. We also highlighted that there is still a significant delay in diagnosis, especially dramatic when the diagnostic odyssey faced by these patients leads to delayed or inappropriate treatments. This is concerning as there are specific treatments already approved, that lead to better outcomes when instituted early.

Acknowledgments: The authors acknowledge the support given by all members and staffs of the MPS BRASIL NETWORK. We would also like to acknowledge the support received from Fundação Médica do Rio Grande do Sul; INAGEMP (CNPQ 465549/2014, CAPES 88887.136366/2017-00, FAPERGS 17/2551.0000521-0) and FIPE/HCPA (projects GPPG 2003-0066 and 2017-0664). This work had the collaboration of BIODISCOVERY (Experimental Research Centre, HCPA, Porto Alegre, Brazil) and DR BRASIL (Clinical Research Centre, HCPA, Porto Alegre Brazil) research groups.

Conflicts of Interest: The authors declare no potential conflict of interest.

Author Contributions: JAJ and RG conceived this study. JAJ wrote the article, and RG supervised the study and fully revised the document. RG, ACBF, FBT, ABON and DRM contributed to data analysis, and/or to the operation of the MPS BRASIL NETWORK. JAJ, ACCS, EMR, CAK, ACMS, MLS, DAV, RTBS, DDGH, PFVM, CFMS, and LRG: contributed to the data collection and the review of medical records.

REFERENCES

Blöß S, Klemann C, Rother AK, Mehmecke S, Schumacher U, Mücke U, Mücke M, Stieber C, Klawonn F, Kortum X, et al (2017) Diagnostic needs for rare diseases and shared prediagnostic phenomena: Results of a German-wide expert Delphi survey. PLoS One 24;12(2):e0172532.

Cardoso GC, de Oliveira MZ, Paixão-Côrtes VR, Castilla EE, Schuler-Faccini L (2019) Clusters of genetic diseases in Brazil. J Community Genet 10(1):121-128.

Clarke LA, Giugliani R, Guffon N, Jones SA, Keenan HA, Munoz-Rojas MV, Okuyama T, Viskochil D, Whitley CB, Wijburg FA, Muenzer J (2019) Genotype-phenotype relationships in mucopolysaccharidosis type I (MPS I): Insights from the International MPS I Registry. Clin Genet 96(4):281-289.

Çelik B, Tomatsu SC, Tomatsu S, Khan SA (2021) Epidemiology of Mucopolysaccharidoses Update. Diagnostics (Basel) 10;11(2):273.

Federhen A, Pasqualim G, de Freitas TF, Gonzalez EA, Trapp F, Matte U, Giugliani R (2020) Estimated birth prevalence of mucopolysaccharidoses in Brazil. Am J Med Genet A 182(3):469-483.

Ficicioglu C, Giugliani R, Harmatz P, Mendelsohn NJ, Jego V, Parini R (2018) Intrafamilial variability in the clinical manifestations of mucopolysaccharidosis type II: Data from the Hunter Outcome Survey (HOS). Am J Med Genet A 176(2):301-310.

Freire-Maia N (1957) Inbreeding in Brazil. Am J Hum Genet Dec;9(4):284-98.

IBGE. Instituto Brasileiro de Geografia e Estatística. Estimativas da população residente no brasil e unidades da federação com data de referência em 1º de julho de 2020, https://www.ibge.gov.br/estatisticas/sociais/populacao/9103-estimativas-de populacao.html?=&t=downloads POP2020_20210331.pdf (December 07, 2021).

Josahkian JA, Trapp FB, Burin MG, Michelin-Tirelli K, Magalhães APPS, Sebastião FM, Bender F, Mari JF, Brusius-Facchin AC, Leistner-Segal S, et al. (2021a) Updated birth prevalence and relative frequency of mucopolysaccharidoses across Brazilian regions. Genet Mol Biol 27;44(1):e20200138.

Josahkian JA, Brusius-Facchin AC, Netto ABO, Leistner-Segal S, Málaga DR, Burin MG, Michelin-Tirelli K, Trapp FB, Cardoso-Dos-Santos AC, Ribeiro EM, et al. (2021b) Genotype-phenotype studies in a large cohort of Brazilian patients with Hunter syndrome. Am J Med Genet C Semin Med Genet 187(3):349-356.

Khan SA, Peracha H, Ballhausen D, Wiesbauer A, Rohrbach M, Gautschi M, Mason RW, Giugliani R, Suzuki Y, Orii KE, et al. (2017) Epidemiology of mucopolysaccharidoses. Mol Genet Metab 121(3):227-240.

Martins C, de Medeiros PFV, Leistner-Segal S, Dridi L, Elcioglu N, Wood J, Behnam M, Noyan B, Lacerda L, Geraghty MT, et al. (2019) Molecular characterization of a large group of Mucopolysaccharidosis type IIIC patients reveals the evolutionary history of the disease. Hum Mutat 40(8):1084-1100.

Muenzer J (2004) The mucopolysaccharidoses: a heterogeneous group of disorders with variable pediatric presentations. J Pediatr 144(5 Suppl):S27-34.

Neufeld EF, Muenzer J (2001) The mucopolysaccaridoses. In: Scriver C, Beaudet A, Sly W, Valle D, editors. The metabolic and molecular basis of inherited disease. New York: McGraw Hill pp. 3421–3452.

Oliveira Netto AB, Brusius-Facchin AC, Leistner-Segal S, Kubaski F, Josahkian J, Giugliani R (2021) Detection of Mosaic Variants in Mothers of MPS II Patients by Next Generation Sequencing. Front Mol Biosci 5;8:789350.

Pinto LL, Vieira TA, Giugliani R, Schwartz IV (2010) Expression of the disease on female carriers of X-linked lysosomal disorders: a brief review. Orphanet J Rare Dis 28;5:14.

Rathmann M, Bunge S, Beck M, Kresse H, Tylki-Szymanska A, Gal A (1996) Mucopolysaccharidosis type II (Hunter syndrome): mutation "hot spots" in the iduronate-2-sulfatase gene. Am J Hum Genet 59(6):1202-9.

Scarpa M, Almássy Z, Beck M, Bodamer O, Bruce IA, De Meirleir L, Guffon N, Guillén-Navarro E, Hensman P, Jones S, et al. (2011) Hunter Syndrome Europena Expert Council. Mucopolysaccharidosis type II: European recommendations for the diagnosis and multidisciplinary management of a rare disease. Orphanet J Rare Dis 7;6:72.

Semyachkina AN, Voskoboeva EY, Zakharova EY, Nikolaeva EA, Kanivets IV, Kolotii AD, Baydakova GV, Kharabadze MN, Kuramagomedova RG, Melnikova NV (2019) Case report: a rare case of Hunter syndrome (type II mucopolysaccharidosis) in a girl. BMC Med Genet 2;20(1):66.

Stapleton M, Arunkumar N, Kubaski F, Mason RW, Tadao O, Tomatsu S (2018) Clinical presentation and diagnosis of mucopolysaccharidoses. Mol Genet Metab 125(1-2):4-17.

Suarez-Guerrero JL, Gómez Higuera PJ, Arias Flórez JS, Contreras-García GA (2016) Mucopolisacaridosis: características clínicas, diagnóstico y de manejo [Mucopolysaccharidosis: clinical features, diagnosis and management]. Rev Chil Pediatr 87(4):295-304.

Vieira TA, Trapp FB, Souza CFM, Faccini LS, Jardim LB, Schwartz IVD, Riegel M, Vargas CR, Burin MG, Leistner-Segal S, et al. (2019) Information and Diagnosis Networks - tools to improve diagnosis and treatment for patients with rare genetic diseases. Genet Mol Biol 42(1 suppl 1):155-164.

CAPÍTULO 5 – CONSIDERAÇÕES FINAIS

Passaram-se mais de 100 anos desde a primeira descrição da MPS II, em 1917 (Hunter, 1917). Em 1973 foi demonstrada a correlação da síndrome com a deficiência de uma sulfatase (Bach et al., 1973); em 1993 o gene *IDS* foi sequenciado e dois anos depois foi descrito o pseudogene *IDS-2* (Wilson et al., 1993; Rathmann et al., 1996). Atualmente, 739 variantes envolvendo o gene *IDS* já foram relatadas (HGMD, 2021) e existem muitos estudos na tentativa de caracterizar a correlação genótipo-fenótipo (Fukurara et al., 2020).

O diagnóstico precoce é imprescindível devido à existência de tratamentos específicos e à possibilidade de recorrência familiar, com necessidade de aconselhamento genético a essas famílias. Por isso, a caracterização epidemiológica, clínica e molecular dos pacientes brasileiros é fundamental.

Nosso primeiro artigo é uma atualização sobre a prevalência ao nascimento das MPS no Brasil. Ele reforça a maior prevalência ao nascimento da MPS II no país como um todo, mas quando avaliada por região e por estado da federação, observa-se que nem sempre esse achado se mantém. Esse dado é fundamental, por exemplo, para direcionar políticas de saúde estaduais. Além disso, o perfil epidemiológico é dinâmico, com necessidade de constantes reavaliações, o que é especialmente importante nos casos de doenças raras como as MPS, em que o número de pacientes é limitado. A inclusão da MPS na triagem neonatal com certeza tornará os dados epidemiológicos mais precisos.

Considerando os fatores que poderiam explicar a maior frequência da MPS II no Brasil, a análise molecular dos pacientes mostrou, em nosso estudo (artigo 2), que não houve correlação do perfil genético com a sua alta frequência relativa entre os tipos de MPS, não havendo prevalência de uma determinada variante que pudesse explicar esse achado.

Com relação à correlação genótipo-fenótipo, sabe-se que na literatura ainda não há uma clara associação e uma mesma variante patogênica pode ser encontrada associada a apresentações clínicas diferentes. Entretanto, deleção de todo o gene *IDS* está invariavelmente associada à deficiência intelectual grave e algumas variantes parecem predizer o fenótipo, como as mutações neuronopáticas p.Pro86Leu, p.Ser117del e

p.Ser333Leu e as mutações não-neuronopáticas p.Asp269Val, p.Cys422Tyr e c.1122C>T (Vollebregt, 2017). Em nosso segundo artigo, não houve discordância entre os fenótipos neuronopático e não-neuronopático em pacientes com uma mesma variante no gene *IDS*, exceto em uma única família com dois meio-irmãos com a variante patogênica intrônica c.1006+1G>A.

Existem casos descritos com variabilidade fenotípica entre pacientes com MPS II de uma mesma família, mas, em geral, parece haver um alto grau de concordância intrafamiliar entre esses pacientes (Ficicioglu et al., 2018).

Variantes patogênicas missense têm consequências clínicas de difícil previsão. O modelo da estrutura da proteína IDS permite a inferência sobre as consequências fenotípicas, sendo que variantes patogênicas que afetam o sítio ativo da enzima, como as variantes p.R88H/C/P/L/G/S e p.K347I/Q/T/E, provavelmente levam a uma atividade catalítica ausente ou extremamente reduzida, com consequências graves e início mais precoce dos sintomas; outras variantes preveem consequências estruturais diferentes na enzima, conforme ilustrado na Figura 3, com gravidade variável (Demydchuk et al., 2017).

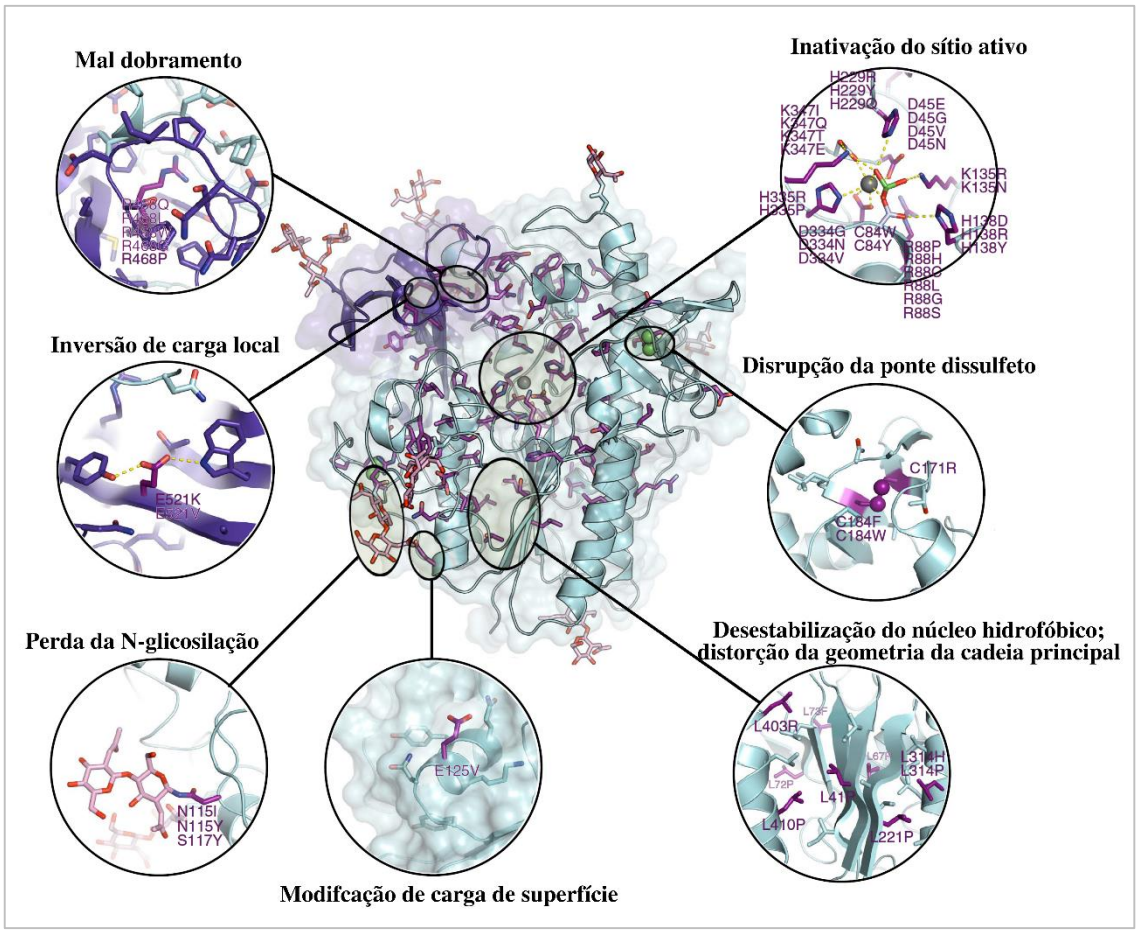

Figura 3. Variantes associadas a MPS II e suas consequências na estrutura da proteína. Modificado e traduzido de Demydchuk et al., 2017, sob os termos de Creative Commons Attribution 4.0 International (CC BY 4.0) Copyright © 2017 pelos autores.

Todos os nossos pacientes com MPS II com variantes que afetam o sítio ativo da enzima IDS apresentaram o fenótipo neuronopático, o que é compatível com o estudo de Demydchuk et al., 2017. Dos casos que investigamos cuja variante afeta o sítio ativo da enzima IDS, oito pacientes (de quatro famílias diferentes) têm variantes em p.R88; um tem uma variante em p.H138; três (de duas famílias diferentes) têm variantes em p.334; e dois pacientes (de uma mesma família) têm uma variante em p.335 (artigo 2 - Josahkian, et al., 2021).

Para as variantes missense que não afetam o sítio ativo da proteína, a utilização do modelo estrutural para prever o fenótipo é mais limitada. Por exemplo, as variantes em p.R468, representadas na figura 3 e que levam a alterações do dobramento enzimático, já foram descritas em pacientes com fenótipos variados. Em nosso estudo (artigo 2) há um paciente com fenótipo neuronopático causado pela variante patogênica c.1403G>A,

p.Arg468Gln; na literatura, há relatos de pacientes com uma forma grave, quando a substituição do aminoácido nessa posição é pela glutamina (como é o caso do nosso paciente) ou por leucina, e forma leve, quando a substituição é por triptofano (Christiakov et al., 2014; Sukegawa-Hayasaka et al., 2006; Whitley et al., 1993). Portanto, a correlação genótipo-fenótipo ainda é desafiadora para parte dos casos, especialmente quando não há outros casos na família.

Como o perfil genético-molecular dos pacientes com MPS II no Brasil não parece explicar a alta frequência relativa deste tipo de MPS no país, buscamos estudar outros fatores que poderiam justificar esse achado (artigo 3).

A baixa consanguinidade da população em geral e o maior número de afetados nas famílias com MPS II em relação às famílias com os outros tipos de MPS podem ser fatores responsáveis pela maior incidência da MPS II no Brasil. Por isso, nós sugerimos que o próprio padrão de herança poderia ser uma explicação para a alta frequência relativa de MPS II no país, só não sendo esta mais prevalente na região nordeste, onde fatores como consanguinidade e isolamento geográfico/ cultural têm importante influência modificadora. Entretanto, nossos dados de tamanho das famílias foram limitados para concluirmos essa hipótese.

Além disso, buscamos entender melhor o processo de diagnóstico. Nossos dados mostram que os pacientes poderiam ser diagnosticados mais cedo a partir de uma suspeita clínica precoce. Chama atenção o fato de que nas famílias em que já havia um paciente diagnosticado com MPS, os demais afetados não foram diagnosticados mais cedo em comparação ao primeiro caso. Isso aponta uma falha importante no aconselhamento genético.

Os fatores que influenciam a percepção sobre risco de recorrência de uma doença na família são complexos e o entendimento da capacidade de agir para prevenir doenças ou melhorar a qualidade de vida é visto como um dos principais promotores de comportamento preventivo (Acheson et al., 2010). Um estudo sobre percepção em saúde mostrou que reconhecer o histórico familiar de doença não equivale a assimilar que há maior risco e que somente 1/3 dos participantes ponderou vagamente o papel de genes na transmissão de alguma condição familiar, a maioria não considerando importante entender

mecanismos de herança genética para lidar com o risco de recorrência (Walter e Emery, 2005). Entendemos que o papel do aconselhamento genético oportuno e adequado é essencial na maneira como as famílias lidam com um determinado diagnóstico.

CAPÍTULO 6 – CONCLUSÕES

a) Verificar se a alta frequência relativa de MPS II tem maior predominância em alguma região brasileira

Nossos dados mostraram que somente na região Nordeste a MPS II não é a mais frequentemente encontrada ao nascimento, o que pode estar relacionado à maior taxa de consanguinidade e à maior proporção relativa das MPS autossômicas recessivas, em especial MPS VI.

b) Verificar se o perfil genético-molecular dos pacientes com MPS II no Brasil poderia estar relacionado com a sua alta frequência relativa

As variantes patogênicas encontradas nos pacientes/famílias com MPS II foram, em sua maioria, particulares, não tendo sido observado nos pacientes uma variante específica que pudesse contribuir para a alta frequência relativa deste tipo de MPS no país.

c) Avaliar variabilidade fenotípica intra-familiar em pacientes brasileiros com MPS II

Em geral, comparando os pacientes com MPS II em relação ao fenótipo neuronopático e não-neuronopático, houve concordância entre os pacientes de uma mesma família.

d) Verificar se o número de afetados por família pode ser um fator relacionado com a alta frequência relativa de MPS II no Brasil

Nossos dados sugerem que o número de afetados em uma mesma família foi maior nos casos de MPS II do que nas outras MPS, o que poderia, em parte, contribuir para a maior proporção relativa de MPS II.

e) Avaliar se a taxa de consanguinidade tem influência na frequência relativa das MPS em nosso país

A taxa de consanguinidade se mostrou mais elevada na região Nordeste, a única região onde a MPS II não é o tipo mais frequente de MPS, indicando que a baixa taxa de

consanguinidade da população brasileira em geral pode ser um fator que contribui para explicar a proporção relativa das MPS.

f) Avaliar a idade ao diagnóstico e comparar com a idade em que se manifestaram os primeiros sintomas, para assim identificar o tempo para diagnóstico dessa patologia em nosso país.

Identificamos uma demora no diagnóstico dos pacientes em todos os tipos de MPS, com a mediana de atraso no diagnóstico de 40 meses desde o relato dos primeiros sinais ou sintomas até o dianóstico laboratorial. Esse atraso diminuiu após a implantação da Rede MPS Brasil. Chama atenção o fato de que muitas famílias têm múltiplos casos, e que em famílias em que já há um paciente diagnosticado com MPS, os demais casos não são diagnosticados mais cedo, apontando falhas nos processos de diagnóstico e de aconselhamento genético.

CAPÍTULO 7 – PERSPECTIVAS

Este trabalho abordou alguns aspectos que poderiam estar relacionados com a alta frequência relativa da MPS II no Brasil, incluindo o papel do padrão de herança, consanguinidade, distribuição geográfica, variantes genéticas, número de casos na família, entre outros fatores.

As MPS são doenças de difícil reconhecimento precoce por sua raridade e por se apresentarem inicialmente com sinais e sintomas inespecíficos e comuns. A existência de tratamentos específicos e a possibilidade de recorrência familiar torna ainda mais urgente que se amplie o conhecimento geral sobre a doença para que o tempo entre o aparecimento dos primeiros sinais e/ou sintomas e o diagnóstico seja reduzido. Nosso estudo observou um atraso importante no diagnóstico e detectou falhas no processo de aconselhamento genético, especialmente crítico nas famílias onde já havia um paciente diagnosticado.

A caracterização epidemiológica, clínica e molecular dos pacientes brasileiros discutida neste trabalho pode trazer melhorias na qualidade de vida dos pacientes afetados e de suas famílias. Trazemos as seguintes reflexões como contribuição: (1) sugestão de disponibilizar mais amplamente o acesso aos testes diagnósticos; (2) sugestão de aumentar as atividades de educação continuada em MPS, especialmente para profissionais de saúde não especialistas; (3) sugestão de desenvolver ações para oferecer amplamente um aconselhamento genético adequado às famílias com MPS; (4) sugestão de ampliar a discussão sobre a inclusão da MPS nos testes de triagem neonatal, para reduzir o tempo até o diagnóstico.

Nosso grupo de pesquisa tem como perspectiva trabalhar na ampliação da divulgação do conhecimento sobre MPS, especialmente no que diz respeito a treinamento para aconselhamento genético adequado, bem como desenvolver novos biomarcadores que sejam facilmente acessíveis, para o diagnóstico mais rápido e eficiente deste grupo de doenças genéticas raras.

CAPÍTULO 8 – REFERÊNCIAS

Acheson LS, Wang C, Zyzanski SJ, Lynn A, Ruffin MT 4th, Gramling R, Rubinstein WS, O'Neill SM, Nease DEJr, Family Healthware Impact Trial (FHITr) Group (2010) Family history and perceptions about risk and prevention for chronic diseases in primary care: a report from the family healthware impact trial Genet Med 12(4):212-218.

Anekar J, Narayanan CD, Raj AC, Sandeepa NC, Nappalli D (2015) A rare case of mucopolysaccharidosis: hunter syndrome. J Clin Diagn Res. 9(4):ZD23-ZD26.

Bach G, Eisenberg F Jr, Cantz M, Neufeld EF (1973) The defect in the Hunter syndrome: deficiency of sulfoiduronate sulfatase. Proc Natl Acad Sci USA 70(7):2134-2138.

Barone R, Pellico A, Pittalà A, Gasperini S (2018) Neurobehavioral phenotypes of neuronopathic mucopolysaccharidoses. Ital J Pediatr 44(Suppl 2):121.

Barth AL, Horovitz DDG (2018) Hematopoietic Stem Cell Transplantation in Mucopolysaccharidosis Type II: A Literature Review and Critical Analysis. J. inborn errors metab. Screen 6:1-11.

Brante G (1952) Gargoylism – A mucopolysaccharidosis. Scand J Clin Lab Invest 4(1): 43–46.

BRASIL. Ministério da Saúde. Secretaria de Atenção à Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos. Portaria conjunta nº 16, de 24 de maio de 2018. Aprova o Protocolo Clínico e Diretrizes Terapêuticas da Mucopolissacaridose do tipo II. Disponível em: https://www.gov.br/saude/pt-br/assuntos/protocolos-clinicos-e-diretrizes-terapeuticas-pcdt/arquivos/2018/pcdt_mps-ii.pdf

Brusius-Facchin AC, Schwartz IV, Zimmer C, Ribeiro MG, Acosta AX, Horovitz D, Monlleó IL, Fontes MIB, Fett-Conte A, Oliveira Sobrinho RP, et al (2014) Mucopolysaccharidosis type II: Identification of 30 novel mutations among Latin American patients. Mol Genet Metab 111:133–138.

Burton BK, Giugliani R (2012) Diagnosing Hunter syndrome in pediatric practice: practical considerations and common pitfalls. Eur J Pediatr 171(4):631-639.

Campbell TN, Fried M (1961) Urinary Mucopolysaccharide Excretion in the Sex-Linked Form of the Hurler Syndrome. Proc Soc Exp Biol Med 108(2): 529-533.

Chen X, Qiu W, Ye J, Han L, Gu X, Zhang H (2016) Demographic characteristics and distribution of lysosomal storage disorder subtypes in Eastern China. J Hum Genet 61(4):345–349.

Chistiakov DA, Kuzenkova LM, Savost'anov KV, Gevorkyan AK, Pushkov AA, Nikitin AG, Vashakmadze ND, Zhurkova NV, Podkletnova TV, Namazova-Baranova LS, Baranov AA (2014) Genetic analysis of 17 children with Hunter syndrome: identification and functional characterization of four novel mutations in the iduronate-2-sulfatase gene. J Genet Genomics 20;41(4):197-203.

Çelik B, Tomatsu SC, Tomatsu S, Khan SA (2021) Epidemiology of Mucopolysaccharidoses Update. Diagnostics (Basel) 11(2):273.

Costa-Motta FM, Bender F, Acosta A, Abé-Sandes K, Machado T, Bomfim T, Boa Sorte T, Silva D, Bittles A, Giugliani R, et al. (2014) A Community-based study of Mucopolysaccharidosis Type VI in Brazil: The influence of founder effect, endogamy and consanguinity. Hum Hered 77:189–196.

Coutinho MF, Lacerda L, Alves S (2012) Glycosaminoglycan storage disorders: a review. Biochem Res Int 2012:471325.

D'Avanzo F, Rigon L, Zanetti A, Tomanin R (2020) Mucopolysaccharidosis Type II: One Hundred Years of Research, Diagnosis, and Treatment. Int J Mol Sci. 13;21(4):1258.

Demydchuk M, Hill CH, Zhou A, Bunkóczi G, Stein PE, Marchesan D, Deane JE, Read RJ (2017) Insights into Hunter syndrome from the structure of iduronate-2-sulfatase. Nat Commun 8:15786.

Dorfman A, Lorincz A (1957) Ocurrence of urinary acid mucopolysaccharides in the Hurler Syndrome. Proc Natl Acad Sci USA 43:443-446.

Federhen A, Pasqualim G, de Freitas TF, Gonzalez EA, Trapp F, Matte U, Giugliani R (2020) Estimated birth prevalence of mucopolysaccharidoses in Brazil. Am J Med Genet A 182(3):469-483.

Ficicioglu C, Giugliani R, Harmatz P, Mendelsohn NJ, Jego V, Parini R (2018) Intrafamilial variability in the clinical manifestations of mucopolysaccharidosis type II: Data from the Hunter Outcome Survey (HOS). Am J Med Genet A 176(2):301-310.

Filocamo M, Tomanin R, Bertola F, Morrone A (2018) Biochemical and molecular analysis in mucopolysaccharidoses: what a paediatrician must know. Ital J Pediatr 44(Suppl 2):129.

Fukuhara Y, Miura A, Yamazaki N, So T, Kosuga M, Yanagi K, Kaname T, Yamagata T, Sakuraba H, Okuyama T (2020) A cDNA analysis disclosed the discordance of genotype-phenotype correlation in a patient with attenuated MPS II and a 76-base deletion in the gene for iduronate-2-sulfatase. Mol Genet Metab Rep. 10;25:100692.

Galimberti C, Madeo A, Di Rocco M, Fiumara A (2018) Mucopolysaccharidoses: early diagnostic signs in infants and children. Ital J Pediatr 44(Suppl 2):133.

Giugliani R (2012) Mucopolysacccharidoses: from understanding to treatment, a century of discoveries. Genet Mol Biol 35(4 suppl):v924–v931.

Giugliani R, Federhen A, Rojas MVM, Vieira T, Artigalás O, Pinto LL, Azevedo AC, Acosta A, Bonfim C, Lourenço CM, et al (2010) Mucopolysaccharidosis I, II, and VI: Brief review and guidelines for treatment. Genet Mol Biol 33(4): 589-604.

Giugliani R, Federhen A, Vairo F, Vanzella C, Pasqualim G, da Silva LM, Giugliani L, De Boer AP, Souza CF, Matte U (2016a) Emerging drugs for the treatment of mucopolysaccharidoses. Expert Opin Emerg Drugs 21(1):9-26.

Giugliani R, Vairo FP, Riegel M, de Souza CF, Schwartz IV, Pena SD (2016b) Rare disease landscape in Brazil: report of a successful experience in inborn errors of metabolism. Orphanet J Rare Dis 10;11(1):76.

Guffon N, Heron B, Chabrol B, Feillet F, Montauban V, Valayannopoulos V (2015) Diagnosis, quality of life, and treatment of patients with Hunter syndrome in the French healthcare system: a retrospective observational study. Orphanet J Rare Dis.10:43.

Henderson JL (1940) Gargoylism: A review of the principal features with a report of five cases. Arch Dis Child 15:201-14.

HGMD (2022) The human gene mutation database, http://www.hgmd.cf.ac.uk/ac/index.php (acesso em 04 de fevereiro de 2022).

Hobbs JR, Barrett AJ, Chambers D, James DCO, Hugh-Jones K, Byrom N, Henry K, Lucas CF, Rogers TR, Benson PF, et al (1982) Reversal of clinical features of Hurler's disease and biochemical improvement after treatment by bone-marrow transplantation. J Inherit Metab Dis 5:59–60.

Hopwood JJ, Harrison JR (1982) High-resolution electrophoresis of urinary glycosaminoglycans: an improved screening test for the mucopolysaccharidoses. Anal. Biochem 119(1):120–127.

Hunter C (1917) A rare disease in two brothers. Proc R Soc Med 10:104-16.

Hurler G (1920) Über einen Typ multipler Abartungen, vorwiegend am Skelettsystem. Z Kinder-Heilk 24(5-6):220–234.

Jones SA, Parini R, Harmatz P, Giugliani R, Fang J, Mendelsohn NJ (2013) The effect of idursulfase on growth in patients with Hunter syndrome: Data from the Hunter Outcome Survey (HOS). Mol Genet Metab 109(1):41–48.

Jones SA, Almássy Z, Beck M, Burt K, Clarke JT, Giugliani R, Hendriksz C, Kroepfl T, Lavery L, Lin SP, et al. (2009) Mortality and cause of death in mucopolysaccharidosis type II-a historical review based on data from the Hunter Outcome Survey (HOS). J Inherit Metab Dis 32(4):534-43.

Khan SA, Peracha H, Ballhausen D, Wiesbauer A, Rohrbach M, Gautschi M, Mason RW, Giugliani R, Suzuki Y, Orii KE et al (2017) Epidemiology of mucopolysaccharidoses. Mol Genet Metab 121(3):227-240.

Khan SA, Mason RW, Giugliani R, Orii K, Fukao T, Suzuki Y, Yamaguchi S, Kobayashi H, Orii T, Tomatsu S (2018) Glycosaminoglycans analysis in blood and urine of patients with mucopolysaccharidosis. Mol Genet Metab 125(1-2):44-52.

Kubaski F, Osago H, Mason RW, Yamaguchi S, Kobayashi H, Tsuchiya M, Orii T, Tomatsu S (2017a) Glycosaminoglycans detection methods: Applications of mass spectrometry. Mol Genet Metab 120(1-2):67-77.

Kubaski F, Yabe H, Suzuki Y, Seto T, Hamazaki T, Mason RW, Xie L, Onsten TGH, Leistner-Segal S, Giugliani R, et al (2017b) Hematopoietic Stem Cell Transplantation for Patients with Mucopolysaccharidosis II. Biol Blood Marrow Transplant 23(10):1795-1803.

Lehman TJ, Miller N, Norquist B, Underhill L, Keutzer J (2011) Diagnosis of the mucopolysaccharidoses. *Rheumatology (Oxford)* 50 suppl5:v41–v48

Lin HY, Tu RY, Chern SR, Lo YT, Fran S, Wei FJ, Huang SF, Tsai SY, Chang YH, Lee CL, Lin SP, Chuang CK (2019) Identification and Functional Characterization of *IDS* Gene Mutations Underlying Taiwanese Hunter Syndrome (Mucopolysaccharidosis Type II). Int J Mol Sci 23;21(1):114.

Lin HY, Chuang CK, Huang YH, Tu RY, Lin FJ, Lin SJ, Chiu PC, Niu DM, Tsai FJ, Hwu WL, et al. (2016) Causes of death and clinical characteristics of 34 patients with Mucopolysaccharidosis II in Taiwan from 1995-2012. Orphanet J Rare Dis. 11(1):85.

Martin R, Beck M, Eng C, Giugliani R, Harmatz P, Munoz V, Muenzer J (2008) Recognition and Diagnosis of Mucopolysaccharidosis II (Hunter Syndrome). Pediatrics 121(2):e377–e386.

Moreira GA, Kyosen SO, Patti CL, Martins AM, Tufik S (2014) Prevalence of obstructive sleep apnea in patients with mucopolysaccharidosis types I, II, and VI in a reference center. *Sleep Breath* 18:791–797.

Morquio L (1929) Sur une forme de dystrophie osseuse familiale. Arch méd infants 32:129-135

Muenzer J. The mucopolysaccharidoses: a heterogeneous group of disorders with variable pediatric presentations (2004) J Pediatr 144 (5):S27-34.

Muenzer J, Bodamer O, Burton B, Clarke L, Frenking GS, Giugliani R, Jones S, Rojas MV, Scarpa M, Beck M, Harmatz P (2012) The role of enzyme replacement therapy in severe Hunter syndrome-an expert panel consensus. Eur J Pediatr 171(1):181-188.

Nan H, Park C, Maeng S (2020) Mucopolysaccharidoses I and II: Brief Review of Therapeutic Options and Supportive/Palliative Therapies. Biomed Res Int. 2020:2408402.

Neufeld EF, Muenzer J (2014) The Mucopolysaccharidoses. In: Valle D, Beaudet AL, Vogelstein B, Kinzler KW, Antonarakis SE, Ballabio A, Gibson K, Mitchell G. eds. The Online Metabolic and Molecular Bases of Inherited Disease. New York, NY: McGraw-Hill. Disponível em: http://ommbid.mhmedical.com/content.aspx?bookid=971&sectionid=62642135. Acesso em 28 de julho de 2017.

Norman-Taylor F, Fixsen JA, Sharrard WJ (1995) Hunter's Syndrome as a Cause of Childhood Carpal Tunnel Syndrome. J Pediatr Orthop B 4(1):106–109.

Omar A, Jalil JA, Shakrin NM, Ngu LH, Yunus ZM (2019) Selective screening for detection of mucopolysaccharidoses in Malaysia; A two-year study (2014-2016). Mol Genet Metab Rep 19:100469.

Parini R, Deodato F (2020) Intravenous Enzyme Replacement Therapy in Mucopolysaccharidoses: Clinical Effectiveness and Limitations. Int J Mol Sci 23;21(8):2975.

Parini R, Jones SA, Harmatz PR, Giugliani R, Mendelsohn NJ (2016) The natural history of growth in patients with Hunter syndrome: Data from the Hunter Outcome Survey (HOS). Mol Genet Metab 117(4):438-46.

Peters C, Shapiro EG, Anderson J, Henslee-Downey PJ, Klemperer MR, Cowan MJ, Saunders EF, deAlarcon PA, Twist C, Nachman JB, et al., (1998) Hurler syndrome: II. Outcome of HLA-genotypically identical sibling and HLA-haploidentical related donor bone marrow transplantation in fifty-four children. The Storage Disease Collaborative Study Group. Blood. 1;91(7):2601-8.

Pinto LL, Vieira TA, Giugliani R, Schwartz IV (2010) Expression of the disease on female carriers of X-linked lysosomal disorders: a brief review. Orphanet J Rare Dis 5(14):1-10.

Poswar F, Baldo G, Giugliani R (2017) Phase I and II clinical trials for the mucopolysaccharidoses. Expert Opin Investig Drugs 26(12):1331-1340.

Rathmann M, Bunge S, Beck M, Kresse H, Tylki-Szymanska A, Gal A (1996) Mucopolysaccharidosis type II (Hunter syndrome): mutation “hot spots” in the iduronate-2-sulfatase gene. Am. J. Hum. Genet 59(6):1202–1209.

Rozdzynska A, Tylki-Szymanska A, Jurecka A, Cieslik J (2010) Growth pattern and growth prediction of body height in children with mucopolysaccharidosis type II. Acta Paediatrica 100(3):456–460.

Scarpa M, Almássy Z, Beck M, Bodamer O, Bruce IA, De Meirleir L, Guffon N, Guillén-Navarro E, Hensman P, Jones S, et al. (2011) Mucopolysaccharidosis type II: European recommendations for the diagnosis and multidisciplinary management of a rare disease. Orphanet J Rare Dis 6(72):1-18.

Schwartz IV, Ribeiro MG, Mota JG, Toralles MB, Correia P, Horovitz D, Santos ES, Monlleo IL, Fett-Conte AC, Sobrinho RP (2007) A clinical study of 77 patients with mucopolysaccharidosis type II. Acta Paediatrica 96:63–70.

Stapleton M, Kubaski F, Mason RW, Yabe H, Suzuki Y, Orii KE, Orii T, Tomatsu S (2017) Presentation and Treatments for Mucopolysaccharidosis Type II (MPS II; Hunter Syndrome). *Expert Opin Orphan Drugs* 5(4):295-307.

Stepien KM, Gevorkyan AK, Hendriksz CJ, Lobzhanidze TV, Pérez-López J, Tol G, Del Toro Riera M, Vashakmadze ND, Lampe C (2020) Critical clinical situations in adult patients with Mucopolysaccharidoses (MPS). *Orphanet J Rare Dis* 15(1):114.

Suarez-Guerrero JL, Higuera, Pjig, Flórez JSA, Contreras-García GA (2016) Mucopolisacaridosis: características clínicas, diagnóstico y de manejo. Rev Chil Pediatr 87(4):295-304.

Sukegawa-Hayasaka K, Kato Z, Nakamura H, Tomatsu S, Fukao T, Kuwata K, Orii T, Kondo N (2006) Effect of Hunter disease (mucopolysaccharidosis type II) mutations on molecular phenotypes of iduronate-2-sulfatase: enzymatic activity, protein processing and structural analysis. J Inherit Metab Dis 29(6):755-61.

Taylor M, Khan S, Stapleton M, Wang J, Chen J, Wynn R, Yabe H, Chinen Y, Boelens JJ, Mason RW, et al. (2019) Hematopoietic Stem Cell Transplantation for Mucopolysaccharidoses: Past, Present, and Future. Biol Blood Marrow Transplant 25(7):e226-e246.

Timms KM, Lu F, Shen Y, Pierson CA, Muzny DM, Gu Y, Nelson DL, Gibbs RA (1995) 130 kb of DNA sequence reveals two new genes and a regional duplication distal to the human iduronate-2-sulfate sulfatase locus. Genome Res 5(1):71-8.

Tomatsu S, Shimada T, Mason RW, Montaño AM, Kelly J, LaMarr WA, Kubaski F, Giugliani R, Guha A, Yasuda E (2014) Establishment of glycosaminoglycan assays for mucopolysaccharidoses. Metabolites 11;4(3):655-79.

Vairo F, Federhen A, Baldo G, Riegel M, Burin M, Leistner-Segal S and Giugliani R (2015) Diagnostic and treatment strategies in mucopolysaccharidosis VI. Appl Clin Genet 8:245–255.

Verheyen S, Blatterer J, Speicher MR, Bhavani GS, Boons GJ, Ilse MB, Andrae D, Sproß J, Vaz FM, Kircher SG, et al. (2021). Novel subtype of mucopolysaccharidosis caused by arylsulfatase K (ARSK) deficiency. J Med Genet. Dec 16:jmedgenet-2021-108061. Epub ahead of print.

Vollebregt AAM, Hoogeveen-Westerveld M, Kroos MA, Oussoren E, Plug I, Ruijter GJ, Van Der Ploeg AT, Pijnappel WWMP (2017) Genotype–phenotype relationship in mucopolysaccharidosis II: predictive power of IDS variants for the neuronopathic phenotype. Dev Med Child Neurol 59(10):1063-1070.

Walter FM, Emery J (2005) 'Coming down the line'-- patients' understanding of their family history of common chronic disease *Ann Fam Med* 3(5):405-414.

Wilson PJ, Meaney CA, Hopwood JJ, Morris CP (1993) Sequence of the human iduronate 2-sulfatase (IDS) gene. Genomics 17(3):773-775.

Whitley CB, Anderson RA, Aronovich EL, Crotty PL, Anyane-Yeboa K, RussoD, Warburton D (1993) Caveat to genotype-phenotype correlation in mucopolysaccharidosis type II: Discordant clinical severity of R468W and R468Q mutations of the iduronate-2-sulfatase gene. Human Mutation 2(3):235–237.

Zafeiriou DI, Batzios SP (2013) Brain and spinal MR imaging findings in mucopolysaccharidoses: a review. AJNR Am J Neuroradiol. 34(1):5-13.

Zhou J, Lin J, Leung WT, Wang L (2020) A basic understanding of mucopolysaccharidosis: Incidence, clinical features, diagnosis, and management. *Intractable Rare Dis Res* 9(1):1-9.

ANEXO A – Parecer consubstanciado do CEP

UFRGS - PRÓ-REITORIA DE PESQUISA DA UNIVERSIDADE FEDERAL DO RIO GRANDE DO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: INVESTIGAÇÃO ABRANGENTE SOBRE AS RAZÕES DA ALTA FREQUÊNCIA RELATIVA DE MUCOPOLISSACARIDOSE TIPO II NO BRASIL

Pesquisador: ROBERTO GIUGLIANI

Área Temática:

Versão: 2

CAAE: 82189417.5.0000.5347

Instituição Proponente: Universidade Federal do Rio Grande do Sul

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.581.518

Apresentação do Projeto:

Trata-se da análise do retorno de diligência do projeto de tese intitulado "INVESTIGAÇÃO ABRANGENTE SOBRE AS RAZÕES DA ALTA FREQUÊNCIA RELATIVA DE MUCOPOLISSACARIDOSE TIPO II NO BRASIL", a ser desenvolvido no âmbito do Programa de Pós-Graduação em Genética e Biologia Molecular da Universidade Federal do Rio Grande do Sul.

As solicitações foram atendidas pelos pesquisadores.

Objetivo da Pesquisa:

Objetivo geral: Investigar fatores que possam explicar a maior frequência relativa de MPS II no Brasil.

Objetivos específicos:

- Verificar se o tamanho familiar pode ser um fator relacionado com a alta frequência relativa de MPS II;
- Verificar se o perfil genético-molecular dos pacientes com MPS II no Brasil poderia estar relacionado com a sua alta frequência relativa;
- Verificar se a alta frequência relativa de MPS II tem maior predominância em certas regiões brasileiras;
- Avaliar a variabilidade fenotípica intra-familiar nos pacientes brasileiros com MPS II

Endereço: Av. Paulo Gama, 110 - Sala 317 do Prédio Anexo 1 da Reitoria - Campus Centro
Bairro: Farroupilha **CEP:** 90.040-060
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3308-3738 **Fax:** (51)3308-4085 **E-mail:** etica@propesq.ufrgs.br

- Avaliar a idade ao diagnóstico e comparar com a idade em que se manifestaram os primeiros sintomas, para assim identificar a demora no diagnóstico dessa patologia em nosso país.

Avaliação dos Riscos e Benefícios:

Como mostrado no parecer anterior, o texto dos riscos e dos benefícios da pesquisa está adequado quanto aos aspectos éticos.

Comentários e Considerações sobre a Pesquisa:

Em atendimento à solicitação "Incluir, no projeto de pesquisa, informações referentes ao banco de dados", os pesquisadores informaram: "O banco de dados que será utilizado é de responsabilidade do Prof. Dr. Roberto Giugliani e encontra-se em uma plataforma online (RedCap), os dados são da Rede MPS Brasil e seus colaboradores que possuem consentimento informado. Foi construído utilizando dados da Rede MPS Brasil para o projeto nº CAAE 55935616.5.0000.5327, MUCOPOLISSACARIDOSES: UM ESTUDO ABRANGENTE SOBRE A EPIDEMIOLOGIA DA DOENÇA NO BRASIL".

Considerações sobre os Termos de apresentação obrigatória:

Em atendimento à solicitação, os pesquisadores anexaram carta de aprovação do projeto de pesquisa anterior no Comitê de ética do Hospital de Clínicas de Porto Alegre/RS.

Em atendimento à solicitação, os pesquisadores anexaram termo de concordância da Rede MPS, onde está claramente expressa a concordância com a utilização dos dados.

Conclusões ou Pendências e Lista de Inadequações:

Face ao exposto, o parecer é de aprovação do projeto de pesquisa quanto aos aspectos éticos.

Considerações Finais a critério do CEP:

Aprovado.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1053066.pdf	14/03/2018 15:41:31		Aceito
Outros	Termo_concordancia.pdf	14/03/2018 15:32:47	JULIANA ALVES JOSAHKIAN	Aceito
Projeto Detalhado / Brochura	ProjetoDetalhado2.pdf	14/03/2018 15:22:48	JULIANA ALVES JOSAHKIAN	Aceito

Investigador	ProjetoDetalhado2.pdf	14/03/2018 15:22:48	JULIANA ALVES JOSAHKIAN	Aceito
Outros	Carta_projeto_anterior.pdf	14/03/2018 15:21:10	JULIANA ALVES JOSAHKIAN	Aceito
Outros	CartaResposta.pdf	14/03/2018 15:16:48	JULIANA ALVES JOSAHKIAN	Aceito
Outros	2_Carta_CEP_Resposta_pend_documental.pdf	18/01/2018 12:44:02	ROBERTO GIUGLIANI	Aceito
Folha de Rosto	folhaDeRosto_atual.pdf	18/01/2018 12:42:15	ROBERTO GIUGLIANI	Aceito
Outros	1_Carta_CEP_Submissao.pdf	18/12/2017 13:35:07	ROBERTO GIUGLIANI	Aceito
Declaração de Pesquisadores	Solicitacao_Dispensa_TCLE_prof_Roberto.pdf	18/12/2017 13:21:21	ROBERTO GIUGLIANI	Aceito
Declaração de Pesquisadores	Solicitacao_Dispensa_TCLE_prof_Roberto.docx	18/12/2017 13:20:35	ROBERTO GIUGLIANI	Aceito
Outros	Solicitacao_Dispensa_TCLE_Juliana.docx	18/12/2017 13:20:14	ROBERTO GIUGLIANI	Aceito
Outros	Solicitacao_Dispensa_CLE_Juliana.pdf	18/12/2017 13:19:54	ROBERTO GIUGLIANI	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoDetalhado.pdf	15/12/2017 15:53:55	JULIANA ALVES JOSAHKIAN	Aceito
Orçamento	Orcamento.pdf	15/12/2017 13:53:54	JULIANA ALVES JOSAHKIAN	Aceito
Cronograma	Cronograma.pdf	15/12/2017 13:52:23	JULIANA ALVES JOSAHKIAN	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 05 de Abril de 2018

Assinado por:
MARIA DA GRAÇA CORSO DA MOTTA
(Coordenador)

ANEXO B – Coautoria em artigo publicado durante o período de doutorado

frontiers in Molecular Biosciences

ORIGINAL RESEARCH
published: 05 November 2021
doi: 10.3389/fmolb.2021.789350

Detection of Mosaic Variants in Mothers of MPS II Patients by Next Generation Sequencing

Alice Brinckmann Oliveira Netto[1,2], *Ana Carolina Brusius-Facchin*[1,3,4]*, *Sandra Leistner-Segal*[1], *Francyne Kubaski*[1,2,3,4], *Juliana Josahkian*[2,5] and *Roberto Giugliani*[1,3,4,6]

[1]Laboratory of Molecular Genetics, Medical Genetics Service, HCPA, Porto Alegre, Brazil, [2]Postgraduate Program in Genetics and Molecular Biology, UFRGS, Porto Alegre, Brazil, [3]National Institute on Population Medical Genetics, INAGEMP, Porto Alegre, Brazil, [4]BioDiscovery Laboratory, Experimental Research Center, HCPA, Porto Alegre, Brazil, [5]Department of Clinical Medicine, Hospital Universitario de Santa Maria (HUSM), Santa Maria, Brazil, [6]Department of Genetics, UFRGS, Porto Alegre, Brazil

OPEN ACCESS

Edited by:
Grzegorz Wegrzyn,
University of Gdansk, Poland

Reviewed by:
Ewelina Bukowska-Olech,
Poznan University of Medical Sciences, Poland
Patryk Lipiński,
Children's Memorial Health Institute (IPCZD), Poland

***Correspondence:**
Ana Carolina Brusius-Facchin
afacchin@hcpa.edu.br

Specialty section:
This article was submitted to Molecular Diagnostics and Therapeutics, a section of the journal Frontiers in Molecular Biosciences

Received: 04 October 2021
Accepted: 22 October 2021
Published: 05 November 2021

Citation:
Oliveira Netto AB, Brusius-Facchin AC, Leistner-Segal S, Kubaski F, Josahkian J and Giugliani R (2021) Detection of Mosaic Variants in Mothers of MPS II Patients by Next Generation Sequencing. Front. Mol. Biosci. 8:789350. doi: 10.3389/fmolb.2021.789350

Mucopolysaccharidosis type II is an X-linked lysosomal storage disorder caused by mutations in the *IDS* gene that encodes the iduronate-2-sulfatase enzyme. The *IDS* gene is located on the long arm of the X-chromosome, comprising 9 exons, spanning approximately 24 kb. The analysis of carriers, in addition to detecting mutations in patients, is essential for genetic counseling, since the risk of recurrence for male children is 50%. Mosaicism is a well-known phenomenon described in many genetic disorders caused by a variety of mechanisms that occur when a mutation arises in the early development of an embryo. Sanger sequencing is limited in detecting somatic mosaicism and sequence change levels of less than 20% may be missed. The Next Generation Sequencing (NGS) has been increasingly used in diagnosis. It is a sensitive and fast method for the detection of somatic mosaicism. Compared to Sanger sequencing, which represents a cumulative signal, NGS technology analyzes the sequence of each DNA read in a sample. NGS might therefore facilitate the detection of mosaicism in mothers of MPS II patients. The aim of this study was to reanalyze, by NGS, all MPS II mothers that showed to be non-carriers by Sanger analysis. Twelve non-carriers were selected for the reanalysis on the Ion PGM and Ion Torrent S5 platform, using a custom panel that includes the *IDS* gene. Results were visualized in the Integrative Genomics Viewer (IGV). We were able to detected the presence of the variant previously found in the index case in three of the mothers, with frequencies ranging between 13 and 49% of the reads. These results suggest the possibility of mosaicism in the mothers. The use of a more sensitive technology for detecting low-level mosaic mutations is essential for accurate recurrence-risk estimates. In our study, the NGS analysis showed to be an effective methodology to detect the mosaic event.

Keywords: mosaicism, mucopolysaccharidosis type II, hunter syndrome, next-generation sequencing, IDS gene, x-linked disease, carrier detection

1 INTRODUCTION

Mucopolysaccharidosis type II (MPS II), or Hunter syndrome (OMIM #309900) is an X-linked lysosomal storage disorder (LSD) caused by variants in the IDS gene, that encodes the iduronate-2-sulfatase enzyme. The deficiency of this enzyme lead to the accumulation of mainly two glycosaminoglycans (GAGs), dermatan sulfate and heparan sulfate, in the lysosomes, which are excreted in increased amounts in the urine (Neufeld and Muenzer, 2001). The accumulation of GAGs in multiple cells, tissues and organs, culminates in MPS II being a multisystemic disease. The most frequent clinical manifestations are skeletal abnormalities, heart disease, respiratory problems, visceromegaly, joint restriction, and, in severe cases, cognitive decline (Wraith et al., 2008). There is a broad spectrum for the phenotype that is classically divided into attenuated and severe forms, being this last one marked by progressive neurological impairment (Neufeld and Muenzer, 2001; Schwartz et al., 2007; Burton and Giugliani, 2012).

The IDS gene is located on the long arm of the X-chromosome (Xq28), comprising 9 exons and 8 introns, spanning approximately 24 kb. It was discovered by Bondenson et al. (1995) that the IDS gene has a pseudogene (IDS-2) situated approximately 20 kb from the telomeric side of the gene. The homology between the gene and the pseudogene corresponds to exons 2 and 3 and to introns 2, 3 and 7 of the IDS gene. The presence of IDS-2 makes it more susceptible for the occurrence of homologous recombination in the IDS gene (Bondenson et al., 1995b). According to the Human Gene Mutation Database v.2021.2, Public (HGMD) (Stenson et al., 2003), 626 different variants in the *IDS* gene have already been described, most of which being point mutations (49%) or small deletions (18%). As the disease has an X-linked recessive inheritance, most severely affected males do not generate offspring and homozygous females are predicted to be extremely rare (Neufeld and Muenzer, 2001).

The analysis of female carriers, in addition to detecting mutations in patients, is essential for genetic counseling, since the risk of recurrence, if the mother is heterozygous, is 50% for male children (Froissart et al., 1997). Presuming the absence of selection between carriers and non-carriers and considering that MPS II is X-linked, it is expected that approximately 1⁄3 of the patients' mothers are non-carriers and these cases are secondary to *de novo* variants (Haldane, 1935). In the work done by Chase et al. (1986), 23% of mothers of patients were identified as non-carriers, a value not that different of the expected (approximately 33%). More recent estimates are presented by Kondrashov (2003), that shows a rate of the loss-of-function mutation per locus per generation in *IDS* as 5×10^{-6}, and by Acuna-Hidalgo et al. (2016), which brings revised data on *de novo* mutations for various diseases using next-generation sequencing techniques.

When considering diagnosis and genetic counseling, somatic mosaicism demands great commitment and can cause serious consequences if not properly detected. Commonly, the sample used for DNA analysis comes from blood, and if the mosaicism extends to this tissue, erroneous diagnosis can be provided for the patient and for the family, when it is a case for counseling (Notini et al., 2008). The diagnosis of the mothers of patients to determine if they are carriers by biochemical assays is very limited (Schröder et al., 1993). So, for the detection of carriers, several molecular biology techniques are used and the ability of Sanger sequencing to detect somatic mosaicism is limited. So, sequence change levels of less than 20% may be missed (Gajecka, 2016). Next Generation Sequencing (NGS) has been increasingly used in diagnosis. It is a sensitive and fast method for the detection of somatic mosaicism (Gajecka, 2016; Lohmann and Klein, 2014; Thorpe et al., 2020). Compared to Sanger sequencing, which represents a cumulative signal, NGS technology analyzes the sequence of each DNA read in a sample (Metzker, 2010). The Targeted Next-Generation Sequencing (TNGS) approach enables the search in different associated genes, providing greater depth of coverage and increased sensitivity and specificity (Rehm et al., 2013). This approach has been used in the past years for the diagnosis of LSDs (Fernández-Marmiesse et al., 2014), and it has also been used by our laboratory, enabling molecular genetics characterization to countless patients (Brusius-Facchin et al., 2019; Josahkian et al., 2021). This study aimed to reanalyze, by Targeted Next Generation Sequencing, 12 mothers of patients with Mucopolysaccharidosis type II that showed to be non-carriers when investigated with Sanger sequencing analysis.

2 MATERIALS AND METHODS

2.1 Participants

12 mothers diagnosed as non-carriers after Sanger sequencing were selected for the reanalysis through TNGS. Genomic DNA was isolated from peripheral blood leukocytes and saliva and stored in the biorepository of the Molecular Genetics Laboratory of the Medical Genetics Service of Hospital de Clínicas de Porto Alegre (HCPA). All the samples are part of the project 13–0224, approved by the HCPA's Institutional Review Board (IRB0000921), which is recognized by the Office for Human Research. All participants signed the MPS Brazil Network informed consent form.

2.2 Sanger Sequencing

Mutational analyses were carried out for the specific region of the mutation present in the index case. Polymerase Chain Reaction (PCR)-amplified products were purified and subjected to direct sequencing using ABI 3500xl 96 capillary DNA analyzer (Applied Biosystems™) and the sequence was analyzed on BioEdit Sequence Alignment Editor. American College of Medical Genetics guidelines were followed for mutation nomenclature. For variant descriptions, reference sequences were NM_000,202.6 and NM_000,202.7.

2.3 Targeted-Next Generation Sequencing

The 12 mothers diagnosed as non-carriers after Sanger sequencing were reanalyzed through TNGS (second-tier test), using a customized panel that includes the *IDS* gene. The panel comprehends 26.75 kb, with 8 targets and 138 amplicons (Brusius-Facchin et al., 2019).

Eight of the samples were analyzed on Ion Torrent Personal Genome (PGM™) System (Thermo Fisher Scientific) and the

other four were analyzed on Ion GenStudio S5™ System (Thermo Fisher Scientific). The samples were prepared likewise, using Ion AmpliSeq™ Library kit (Thermo Fisher Scientific), following the manufacturer's recommendations (MAN0006943), and the quantification of the libraries was performed using Qubit® dsDNA HS kit (Thermo Fisher Scientific). The samples analyzed on the Ion PGM™ had the template preparation on the Ion OneTouch2 instrument (Thermo Fisher Scientific) using the Ion PGM Template OT2 200 kit (Thermo Fisher Scientific). Attune® Acoustic Focusing Flow Cytometer (Thermo Fisher Scientific) was used to define the percentage of positive Ion Sphere Particles (ISPs), following the protocol recommendations (Part. no. 4477181). The Ion OneTouch ES (Enrichment System) was used to enrich the positive ISPs and, subsequently, the samples were loaded onto Ion 314™ chip v2 (Thermo Fisher Scientific) according to the user guide (MAN0007273) and sequenced on the Ion PGM™ sequencer. The samples analyzed in the Ion S5™ had the template preparation on the Ion Chef™ instrument (Thermo Fisher Scientific), following the manufacturer's recommendations (MAN0016855), where the Ion 510™ chip (Thermo Fisher Scientific) was loaded. Afterward, the chip was transferred to Ion S5™ Sequencer and sequenced.

2.4 Bioinformatic Analysis

Raw data was processed and analyzed using Torrent Suite™ Software (Thermo Fisher Scientific), which imports into Ion Reporter™ Software (Thermo Fisher Scientific) a list of detected sequence variants, including SNPs and small insertions/deletions for analysis. The alignment of the sample sequence with the human genome reference 19 (Genome Reference Consortium GRCh37) was visualized and verified in the Integrative Genomics Viewer v2.3 (IGV) (Robinson et al., 2011; Thorvaldsdóttir et al., 2013). The position of the mutation present in the index case was analyzed to verify the allele frequency within this position, to conclude whether the mother had the mutation in mosaicism. Running metrics and coverage analyses were performed for the identification of technical deficiencies.

3 RESULTS

3.1 Sanger Sequencing

All samples had amplified PCR products and visualization in the chromatogram. However, no signal of allele alteration in the region of the variants present in the index case was visible or it was not possible to conclude if the variant was present. **Figure 1A** presents

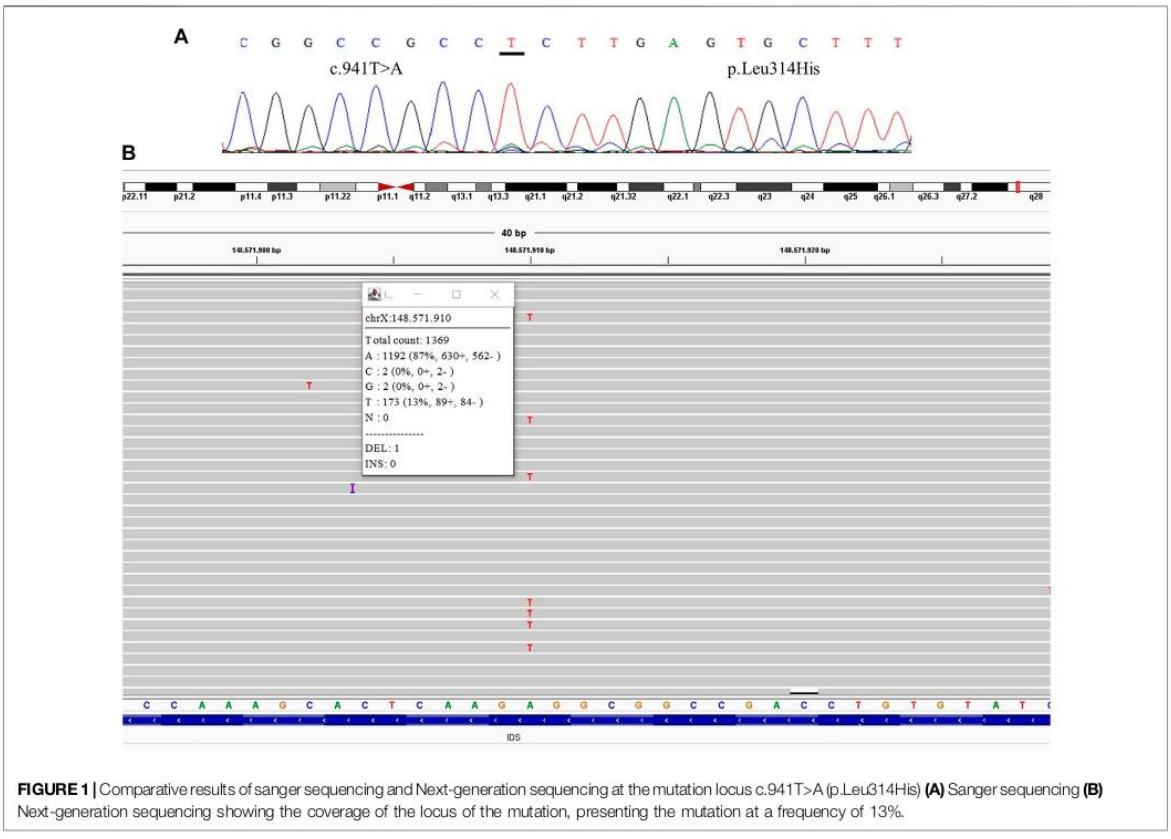

FIGURE 1 | Comparative results of sanger sequencing and Next-generation sequencing at the mutation locus c.941T>A (p.Leu314His) **(A)** Sanger sequencing **(B)** Next-generation sequencing showing the coverage of the locus of the mutation, presenting the mutation at a frequency of 13%.

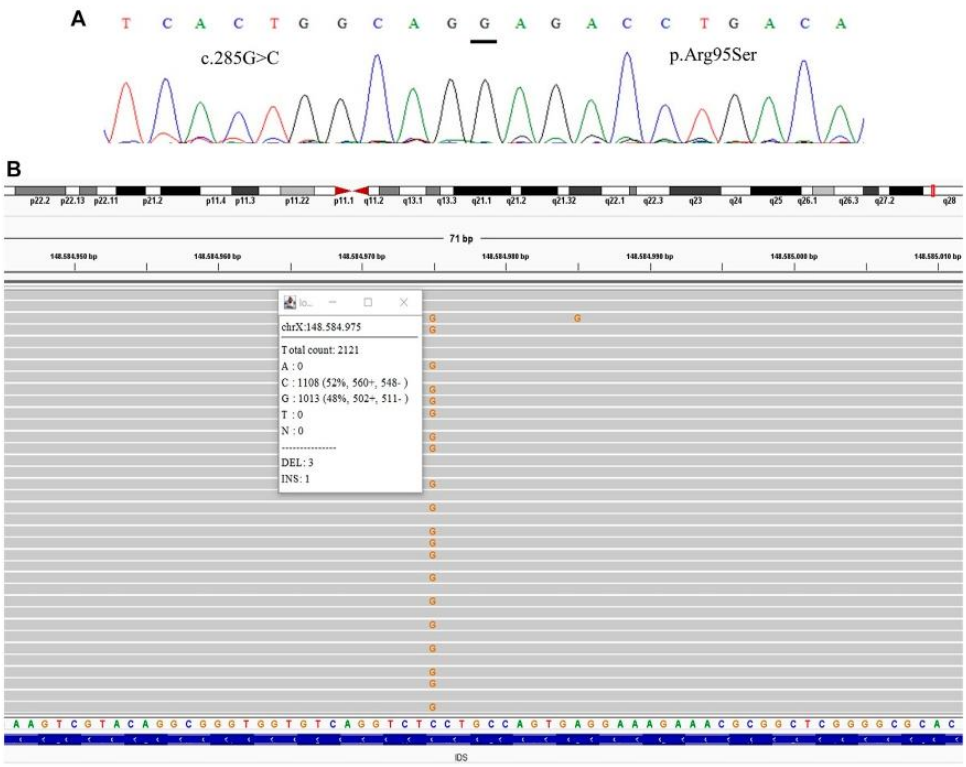

FIGURE 2 | Comparative results of sanger sequencing and Next-generation sequencing at the mutation locus c.285G > C (p.Arg95Ser) **(A)** Sanger sequencing **(B)** Next-generation sequencing showing the coverage of the locus of the mutation, presenting the mutation at a frequency of 48%.

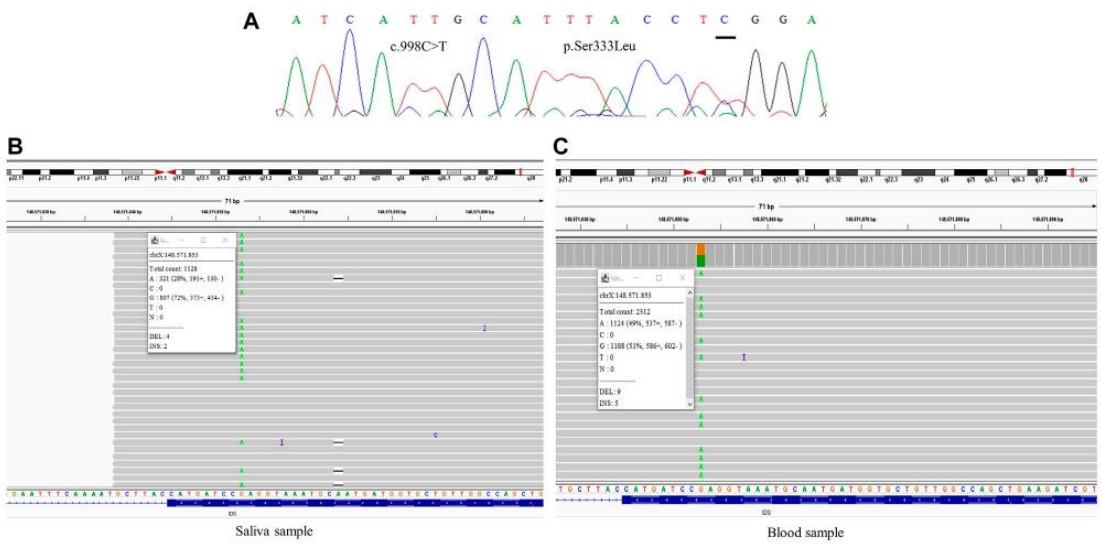

FIGURE 3 | Comparative results of sanger sequencing and Next-generation sequencing at the mutation locus c.998C>T (*p*.Ser333Leu) **(A)** Sanger sequencing **(B)** Next-generation sequencing showing the coverage of the locus of the mutation in saliva sample, at a frequency of 28% **(C)** Next-generation sequencing showing the coverage of the locus of the mutation in blood sample, at a frequency of 49%.

TABLE 1 | Coverage metrics of the customized gene panel, that includes the IDS gene.

Subject	Mapped reads	Reads on target	Depth of coverage	Uniformity of coverage
1	225,826	91.26%	1,462	95.23%
2	250,405	88.90%	1,571	95.47%
3	217,933	88.67%	1,361	95.79%
4	66,682	79.26%	618.9	94.05%
5	59,097	80.49%	561.3	92.74%
6	44,284	79.70%	418.2	90.49%
7	149,116	54.90%	996.3	94.81%
8	150,089	63.25%	1,148	95.53%
9	144,869	91.11%	1,622	95.74%
10	156,075	90.58%	1,650	93.91%
11	152,194	91.29%	1,700	95.05%
12	200.52	92.68%	1,296	95.46%
Mean	151,424	82.60%	1,215	94.51%

the sequencing of exon 7 showing the locus of the mutation c.941T>A p.(Leu314His) demonstrating no alteration. **Figure 2A** presents the sequencing of exon 3 showing the locus of the mutation c.285G > C p.(Arg95Ser), demonstrating no alteration. **Figure 3A** presents the sequencing of exon 7 showing the locus of the mutation c.998C>T *p*.(Ser333Leu) in a chromatogram with undefined peaks, precluding a reliable analysis.

3.2 Targeted Next-Generation Sequencing

We were able to detect the presence of the variant previously identified in the index case in three of the mothers after the TNGS. In one case the specific variant was present in a frequency of 13% of the reads, suggesting the possibility of mosaicism in the mother (**Figure 1**). In another case, we found a variant present in 48% of the reads, which was still not seen in the direct sequencing (**Figure 2**). As the variant is in exon 3 of the IDS gene, the frequency value might be related to readings of the IDS-2 pseudogene.

One of the mothers was analyzed with samples from two different sources, saliva and blood, showing mosaicism at different levels, with frequencies ranging between 28 and 49% of the reads (**Figure 3**).

The mutations found in the index cases were in a variety of exons (exon 3, 6, 7, 8, and 9). They were all classified as pathogenic and with different molecular consequences, including missense, nonsense, frameshift, and alternative splicing. The mutations we were able to find in mosaicism in the mothers were in exon 3 and 7, and all of them were missense.

Running metrics and coverage analysis have shown good sequencing data, achieving 151,424 of mapped reads and 170,39 mean coverage (value obtained using the coverage 100 x of the amplicons of the IDS gene). The average of reads on target, depth of coverage, and uniformity can be seen in **Table 1**.

There were other samples sequenced for the same gene panel beyond the mother's samples that served as control, demonstrating that the detection of mosaicism was not an error.

4 DISCUSSION

To the best of our knowledge, this is the first study to report mosaicism in Mucopolysaccharidosis type II through Targeted Next-Generation Sequencing. The use of a more sensitive technology for detecting low-level mosaic mutations, which may not be detectable by Sanger sequencing, is essential for accurate recurrence-risk estimates. In our study, the TNGS analysis showed to be an effective methodology to detect the event of mosaicism of Single-Nucleotide Variants (SNV), considering that we were able to identify three cases of mosaicism at different frequencies (between 13 and 49%) out of 12 that showed negative or inconclusive results in Sanger sequencing.

The fact that we did not find evidence of mosaicism in a greater number may have been because we performed the TNGS only with leukocyte DNA, which does not exclude the possibility of the occurrence of mosaicism in other tissues. In a single case, it was possible to perform the analysis with DNA extracted from saliva, as the Sanger sequencing of this case had shown undefined peaks and the mosaicism at a frequency of 49% in leukocyte DNA was an unexpected result, which motivated the analysis with a sample from a different source. As blood cells undergo several self-renewal processes in hematopoiesis, they are considered an unstable source of genetic material (Campbell et al., 2016). Furthermore, the possibility of finding somatic mosaicism should be strongly considered (Notini et al., 2008; Scarpa, 2018), by the fact that if there is no presence of mosaicism in other cell types, the *de novo* mutation rate we supposedly found (75%) would be very high compared to expected (33%) (Chase et al., 1986).

Mosaicism is a well-known phenomenon described in many genetic disorders, caused by a range of mechanisms that occur when a mutation arises in the early development of an embryo, from a unique fertilization event, producing cells with different genetic compositions (Notini et al., 2008). The mosaicism can be classified into three categories depending on the stage of development in which the mutation occurs: germline mosaicism (gonadal mosaicism), somatic mosaicism, and gonosomal mosaicism (combination of the two priors). However, it is known that a random inactivation of one of the chromosomes occurs, making only one of the chromosomes active, which is called dose compensation (Spolarics, 2007). Most of the genes present in the inactive chromosome remain inactive in all daughter cells, preserving the inactivation pattern. As a consequence of the random inactivation of the X chromosome, women are naturally

mosaics, having two cell populations relative to the X chromosome, each one being expressed in one of them, being expressed on each of them, in the way that, women are mosaics for various X-linked alleles (Migeon, 2008). Besides that, all individuals are mosaics, comprising variable genotypes acquired post-zygotically (Rodríguez-Nóvoa et al., 2020). Mosaicism arises in the post-zygotic phase and since there are countless mitotic cycles to generate an adult organism (~10^{14}), many mutations arise in this step of the development (Campbell et al., 2014). These mutations can turn out to be pathogenic, but to be clinically detected they need to be present in a considered level of cells, even though they can still be transmitted to the offspring when expressed at a low cellular level (Rodríguez-Nóvoa et al., 2020). In addition, there is another phenomenon occurring in women associated with the inactivation of the X chromosome and to variants present in this chromosome. A metabolic interaction occurs between the two cell types so that women who have one copy of a mutated allele are still able to produce enough gene products with just one normal allele (Migeon, 2006). This transference of gene product between cells ends up masking the genotype of the defective cell, possibly being one of the reasons why the detection of carrier mothers by measuring the enzyme activity is unreliable. This phenomenon has been reported in X-linked lysosomal diseases such as type II Mucopolysaccharidosis and Fabry disease (Migeon, 2006).

A study by Froissart et al. (1997) showed that there are cases of mosaicism in women with a single case of MPS II in the family. The mutation was present in different frequencies for the distinct tissues analyzed, meanwhile the state of heterozygosity was found in leukocytes. Therefore, when a mutation, whether recurrent or *de novo*, is identified in a family and the mother has a molecular diagnosis of non-carrier by conventional methods, the choice of a more sensitive technique for detection of mosaic mutations (at below level), is essential for recurrence risk estimates.

As has been mentioned, mosaicism in Mucopolysaccharidosis type II has already been described by Froissart et al. (2007), in which two cases of germline associated with somatic mosaicism was described, and by Alcántara-Ortigoza (2016), in which a case of germline and somatic mosaicism was described. Likewise, there are other X-linked diseases that have reported mosaicism: X-linked Alport syndrome (Fu et al., 2016; Yokota et al., 2017; Okamoto et al., 2019; Pinto et al., 2020), X-linked retinitis pigmentosa (Strubbe et al., 2021), X-linked acrogigantism syndrome (Daly et al., 2016), Fabry disease (Barriales-Villa et al., 2019; Pianese et al., 2019), Duchenne muscular dystrophy (Winerdal et al., 2020; Dinh et al., 2018), X-linked hypophosphatemic rickets (Saito et al., 2009; Lin et al., 2020; Goji et al., 2006) and Lesch–Nyhan syndrome (Willers, 2004). Still, some of the authors managed to diagnose mosaicism in SNV also using NGS (Fu et al., 2016; Yokota et al., 2017; Barriales-Villa et al., 2019; Okamoto et al., 2019; Pinto et al., 2020; Strubbe et al., 2021)

Recent studies indicate that the occurrence of mosaicism is much more common than it was expected and that the NGS technologies, which provide high sensitivity and high throughput, help to characterize the different levels of mosaicism, including low-level mosaicism (Gajecka, 2016). Additionally, the approach of NGS using specific gene panels (TNGS), like the one used in this article, is an option that allows for higher coverage and sensitivity, with a lower cost, that has already been reported as an appropriate method to identify somatic mosaicism (Baquero-Montoya et al., 2014). It is believed that NGS is the most adequate method for the discovery of mosaicism in SNV. However, for the detection of mosaicism in Copy-Number Variants (CNVs), other methods have already been reported as more indicated, with greater sensitivity and specificity (Liu et al., 2020).

Currently, the presence of variants in mosaicism has also been considered in genetic tests performed in pre-implantation of *in vitro* embryos, called Preimplantation Genetic Testing for Monogenic Disorders (PGT-M), for couples in which there was already some evidence of the presence of pathogenic variants (Hu et al., 2021). This reinforces the importance of NGS in the detection of mosaicism, enabling a more robust molecular diagnosis and the complete information needed for genetic counseling. Likewise, there is also a study in the literature that reports the case of a mother of a patient who could be a kidney donor that was re-evaluated and found to be a carrier of the index case mutation in mosaicism (Pinto et al., 2020), bringing another case in which the NGS enabled the complete diagnosis.

To this extent, we believe that the NGS analysis, using the TNGS approach, showed to be an effective methodology to detect the mosaic event, in different levels (13–49%), in the mothers of affected patients with Mucopolysaccharidosis type II. Since failure to identify low levels of mosaic mutations may lead to the misinterpretation of molecular results, especially for a carrier, the analysis with sensitive methods such as TNGS is very important for complete diagnostic and genetic counseling.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available due to ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hospital de Clinicas de Porto Alegre. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AB-F, and RG contributed to the conception and design of the study. AN, and AB-F contributed to the molecular analysis, data analysis and reporting of the work described. RG contributed to the data analysis and reporting of the work described. AN wrote the first draft of the manuscript. AB-F, SL-S, FK, and RG revised the final report. SL-S revised the molecular data. JJ revised the clinical data. All authors have read and agreed to the final version of the article.

FUNDING

This research was founded by INAGEMP (CNPq: Grant 465549/2014-4; CAPES: Grant 88887.136366/2017-00; FAPERGS: Grant 17/2551–0000521-0) and Fundação Médica do RS; ABON was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) with a master fellowship (Grant 130790/2021-4); FK was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) with a postdoctoral fellowship (Grant 88887.136,366/2017-00), and INAGEMP; RG has a research fellowship from CNPq (Grant 303219/2019-0).

ACKNOWLEDGMENTS

The authors acknowledge the support received by INAGEMP, CNPq, CAPES, FAPERGS and FUNDAÇÃO MÉDICA DO RS.

REFERENCES

Acuna-Hidalgo, R., Veltman, J. A., and Hoischen, A. (2016). New Insights into the Generation and Role of De Novo Mutations in Health and Disease. *Genome Biol.* 17 (1), 241. doi:10.1186/s13059-016-1110-1

Alcántara-Ortigoza, M. A., García-de Teresa, B., González-del Angel, A., Berumen, J., Guardado-Estrada, M., Fernández-Hernández, L., et al. (2016). Wide Allelic Heterogeneity with Predominance of largeIDSgene Complex Rearrangements in a Sample of Mexican Patients with Hunter Syndrome. *Clin. Genet.* 89 (5), 574–583. doi:10.1111/cge.12738

Baquero-Montoya, C., Gil-Rodríguez, M. C., Braunholz, D., Teresa-Rodrigo, M. E., Obieglo, C., Gener, B., et al. (2014). Somatic mosaicism in a Cornelia de Lange syndrome patient withNIPBLmutation identified by different next generation sequencing approaches. *Clin. Genet.* 86 (6), 595–597. doi:10.1111/cge.12333

Barriales-Villa, R., Ochoa, J. P., Santomé-Collazo, J. L., Mosquera-Reboredo, J., Cao-Vilariño, M., and Monserrat, L. (2019). First Reported Case of Fabry Disease Caused by a Somatic Mosaicism in the GLA Gene. *Revista Española de Cardiología (English Edition)* 72 (7), 585–587. doi:10.1016/j.rec.2018.06.013

Bondeson, M.-L., Dahl, N., Malmgren, H., Kleijer, W. J., Tönnesen, T., Carlberg, B.-M., et al. (1995). Inversion of the IDS Gene Resulting from Recombination with IDS-Related Sequences in a Common Cause of the Hunter Syndrome. *Hum. Mol. Genet.* 4 (4), 615–621. doi:10.1093/hmg/4.4.615

Brusius-Facchin, A. C., Siebert, M., Leão, D., Malaga, D. R., Pasqualim, G., Trapp, F., et al. (2019). Phenotype-oriented NGS Panels for Mucopolysaccharidoses: Validation and Potential Use in the Diagnostic Flowchart. *Genet. Mol. Biol.* 42 (1), 207–214. doi:10.1590/1678-4685-GMB-2018-0102

Burton, B. K., and Giugliani, R. (2012). Diagnosing Hunter Syndrome in Pediatric Practice: Practical Considerations and Common Pitfalls. *Eur. J. Pediatr.* 171 (4), 631–639. doi:10.1007/s00431-012-1703-y

Campbell, I. M., Shaw, C. A., Stankiewicz, P., and Lupski, J. R. (2016). Erratum to: Somatic Mosaicism: Implications for Disease and Transmission Genetics. *Trends Genet.* 32 (2), 138. doi:10.1016/j.tig.2015.07.004

Campbell, I. M., Yuan, B., Robberecht, C., Pfundt, R., Szafranski, P., McEntagart, M. E., et al. (2014). Parental Somatic Mosaicism Is Underrecognized and Influences Recurrence Risk of Genomic Disorders. *Am. J. Hum. Genet.* 95 (2), 173–182. doi:10.1016/j.ajhg.2014.07.003

Carlberg, B.-M., Pettesson, U., Bondeson, M.-L., Malmgren, H., and Dahl, N. (1995). Presence of an IDS-Related Locus (IDS2) in Xq28 Complicates the Mutational Analysis of Hunter Syndrome. *Eur. J. Hum. Genet.* 3 (4), 219–227. doi:10.1159/000472302

Chase, D. S., Morris, A. H., Ballabio, A., Pepper, S., Giannelli, F., and Adinolfi, M. (1986). Genetics of Hunter Syndrome: Carrier Detection, New Mutations, Segregation and Linkage Analysis. *Ann. Hum. Genet* 50 (4), 349–360. doi:10.1111/j.1469-1809.1986.tb01756.x

Daly, A. F., Yuan, B., Fina, F., Caberg, J.-H., Trivellin, G., Rostomyan, L., et al. (2016). Somatic Mosaicism Underlies X-Linked Acrogigantism Syndrome in Sporadic Male Subjects. *Endocr. Relat. Cancer* 23 (4), 221–233. doi:10.1530/ERC-16-0082

Dinh, L. T., Nguyen, D. H., Luong, L. H., Le, P. T., Le-Anh, T. P., Tran, D. Q., et al. (2018). Mosaicism in Carrier of Duchenne Muscular Dystrophy Mutation - Implication for Prenatal Diagnosis. *Taiwanese J. Obstet. Gynecol.* 57 (6), 878–880. doi:10.1016/j.tjog.2018.10.019

Fernández-Marmiesse, A., Morey, M., Pineda, M., Eiris, J., Couce, M., Castro-Gago, M., et al. (2014). Assessment of a Targeted Resequencing Assay as a Support Tool in the Diagnosis of Lysosomal Storage Disorders. *Orphanet J. Rare Dis.* 9, 59. doi:10.1186/1750-1172-9-59

Froissart, R., Da Silva, I. M., and Maire, I. (2007). Mucopolysaccharidosis Type II: an Update on Mutation Spectrum. *Acta Paediatr.* 96 (455), 71–77. doi:10.1111/j.1651-2227.2007.00213.x

Froissart, R., Maire, I., Bonnet, V., Levade, T., and Bozon, D. (1997). Germline and Somatic Mosaicism in a Female Carrier of Hunter Disease. *J. Med. Genet.* 34 (2), 137–140. doi:10.1136/jmg.34.2.137

Fu, X. J., Nozu, K., Kaito, H., Ninchoji, T., Morisada, N., Nakanishi, K., et al. (2016). Somatic Mosaicism and Variant Frequency Detected by Next-Generation Sequencing in X-Linked Alport Syndrome. *Eur. J. Hum. Genet.* 24 (3), 387–391. doi:10.1038/ejhg.2015.113

Gajecka, M. (2016). Unrevealed Mosaicism in the Next-Generation Sequencing Era. *Mol. Genet. Genomics* 291 (2), 513–530. doi:10.1007/s00438-015-1130-7

Goji, K., Ozaki, K., Sadewa, A. H., Nishio, H., and Matsuo, M. (2006). Somatic and Germline Mosaicism for a Mutation of the PHEX Gene Can lead to Genetic Transmission of X-Linked Hypophosphatemic Rickets that Mimics an Autosomal Dominant Trait. *J. Clin. Endocrinol. Metab.* 91 (2), 365–370. doi:10.1210/jc.2005-1776

Haldane, J. B. S. (2004). The Rate of Spontaneous Mutation of a Human Gene. 1935. *J. Genet.* 83 (3), 235–244. doi:10.1007/BF02717892

Hu, X., He, W. B., Zhang, S. P., Luo, K. L., Gong, F., Dai, J., et al. (2021). Next-generation Sequence-based Preimplantation Genetic Testing for Monogenic Disease Resulting from Maternal Mosaicism. *Mol. Genet. Genomic Med.* 9 (5), e1662. doi:10.1002/mgg3.1662

Josahkian, J. A., Brusius-Facchin, A. C., Netto, A. B. O., Leistner-Segal, S., Málaga, D. R., Burin, M. G., et al. (2021). Genotype-phenotype Studies in a Large Cohort of Brazilian Patients with Hunter Syndrome. *Am. J. Med. Genet.* 187, 349–356. doi:10.1002/ajmg.c.3191510.1002/ajmg.c.31915

Kondrashov, A. S. (2003). Direct Estimates of Human Per Nucleotide Mutation Rates at 20 Loci Causing Mendelian Diseases. *Hum. Mutat.* 21 (1), 12–27. doi:10.1002/humu.10147

Lin, Y., Cai, Y., Xu, J., Zeng, C., Sheng, H., Yu, Y., et al. (2020). 'Isolated' Germline Mosaicism in the Phenotypically normal Father of a Girl with X-Linked Hypophosphatemic Rickets. *Eur. J. Endocrinol.* 182 (1), K1–K6. doi:10.1530/EJE-19-0472

Liu, Q., Karolak, J. A., Grochowski, C. M., Wilson, T. A., Rosenfeld, J. A., Bacino, C. A., et al. (2020). Parental Somatic Mosaicism for CNV Deletions - A Need for More Sensitive and Precise Detection Methods in Clinical Diagnostics Settings. *Genomics* 112 (5), 2937–2941. doi:10.1016/j.ygeno.2020.05.003

Lohmann, K., and Klein, C. (2014). Next Generation Sequencing and the Future of Genetic Diagnosis. *Neurotherapeutics* 11 (4), 699–707. doi:10.1007/s13311-014-0288-8

Metzker, M. L. (2010). Sequencing Technologies - the Next Generation. *Nat. Rev. Genet.* 11 (1), 31–46. doi:10.1038/nrg2626

Migeon, B. R. (2006). The Role of X Inactivation and Cellular Mosaicism in Women's Health and Sex-specific Diseases. *JAMA* 295 (12), 1428–1433. doi:10.1001/jama.295.12.1428

Migeon, B. R. (2008). X Inactivation, Female Mosaicism, and Sex Differences in Renal Diseases. *Jasn* 19 (11), 2052–2059. doi:10.1681/ASN.2008020198

Neufeld, E. F., and Muenzer, J. (2001). "The Mucopolysaccharidosis," in *The Online Metabolic and Molecular Bases of Inherited Disease*. Editors D. L. Valle, S. Antonarakis, A. Ballabio, A. L. Beaudet, and G. A. Mitchell (New York, NY: MacGraw-Hill), 3421–3452.

Notini, A. J., Craig, J. M., and White, S. J. (2008). Copy Number Variation and Mosaicism. *Cytogenet. Genome Res.* 123 (1-4), 270–277. doi:10.1159/000184717

Okamoto, T., Nozu, K., Iijima, K., and Ariga, T. (2019). Germline Mosaicism Is a Pitfall in the Diagnosis of "sporadic" X-Linked Alport Syndrome. *J. Nephrol.* 32 (1), 155–159. doi:10.1007/s40620-018-0518-y

Pianese, L., Fortunato, A., Silvestri, S., Solano, F. G., Burlina, A., Burlina, A. P., et al. (2019). Maternal Germline Mosaicism in Fabry Disease. *Neurol. Sci.* 40 (6), 1279–1281. doi:10.1007/s10072-019-03754-1

Pinto, A. M., Daga, S., Fallerini, C., Bruttini, M., Baldassarri, M., Giliberti, A., et al. (2020). Detection of Cryptic Mosaicism in X-Linked Alport Syndrome Prompts to Re-evaluate Living-Donor Kidney Transplantation. *Transplantation* 104 (11), 2360–2364. doi:10.1097/TP.0000000000003104

Rehm, H. L., Bale, S. J., Bale, S. J., Bayrak-Toydemir, P., Berg, J. S., Brown, K. K., et al. (2013). ACMG Clinical Laboratory Standards for Next-Generation Sequencing. *Genet. Med.* 15 (9), 733–747. doi:10.1038/gim.2013.92

Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., et al. (2011). Integrative Genomics Viewer. *Nat. Biotechnol.* 29 (1), 24–26. doi:10.1038/nbt.1754

Rodríguez-Nóvoa, S., Rodríguez-Jiménez, C., Alonso, C., Rodriguez-Laguna, L., Gordo, G., Martinez-Glez, V., et al. (2020). Familial Hypercholesterolemia: A Single-Nucleotide Variant (SNV) in Mosaic at the Low Density Lipoprotein Receptor (LDLR). *Atherosclerosis* 311, 37–43. doi:10.1016/j.atherosclerosis.2020.08.002

Saito, T., Nishii, Y., Yasuda, T., Ito, N., Suzuki, H., Igarashi, T., et al. (2009). Familial Hypophosphatemic Rickets Caused by a Large Deletion in PHEX Gene. *Eur. J. Endocrinol.* 161 (4), 647–651. doi:10.1530/EJE-09-0261

Scarpa, M. (2018). "Mucopolysaccharidosis Type II," in *GeneReviews® [Internet]*. Editors M. P. Adam, H. H. Ardinger, and R. A. Pagon (Seattle (WA): University of Washington).

Schröder, W., Petruschka, L., Wehnert, M., Zschiesche, M., Seidlitz, G., Hopwood, J. J., et al. (1993). Carrier Detection of Hunter Syndrome (MPS II) by Biochemical and DNA Techniques in Families at Risk. *J. Med. Genet.* 30 (3), 210–213. doi:10.1136/jmg.30.3.210

Schwartz, I. V., Ribeiro, M. G., Mota, J. G., Toralles, M. B. P., Correia, P., Horovitz, D., et al. (2007). A Clinical Study of 77 Patients with Mucopolysaccharidosis Type II. *Acta Paediatr.* 96 (455), 63–70. doi:10.1111/j.1651-2227.2007.00212.x

Spolarics, Z. n. (2007). The X-Files of Inflammation. *Shock* 27 (6), 597–604. doi:10.1097/SHK.0b013e31802e40bd

Stenson, P. D., Ball, E. V., Mort, M., Phillips, A. D., Shiel, J. A., Thomas, N. S. T., et al. (2003). Human Gene Mutation Database (HGMD): 2003 Update. *Hum. Mutat.* 21 (6), 577–581. doi:10.1002/humu.10212

Strubbe, I., Van Cauwenbergh, C., De Zaeytijd, J., De Jaegere, S., De Bruyne, M., Rosseel, T., et al. (2021). Phenocopy of a Heterozygous Carrier of X-Linked Retinitis Pigmentosa Due to Mosaicism for a RHO Variant. *Sci. Rep.* 11 (1), 117. doi:10.1038/s41598-020-80400-3

Thorpe, J., Osei-Owusu, I. A., Avigdor, B. E., Tupler, R., and Pevsner, J. (2020). Mosaicism in Human Health and Disease. *Annu. Rev. Genet.* 54, 487–510. doi:10.1146/annurev-genet-041720-093403

Thorvaldsdóttir, H., Robinson, J. T., and Mesirov, J. P. (2013). Integrative Genomics Viewer (IGV): High-Performance Genomics Data Visualization and Exploration. *Brief. Bioinform.* 14 (2), 178–192. doi:10.1093/bib/bbs017

Willers, I. (2004). Germline Mosaicism Complicates Molecular Diagnosis of Lesch-Nyhan Syndrome. *Prenat. Diagn.* 24 (9), 737–740. doi:10.1002/pd.959

Winerdal, M., Westenius, E., Granfors, M., Pettersson, M., and Iwarsson, E. (2020). Confined Placental Mosaicism of Duchenne Muscular Dystrophy: a Case Report. *Mol. Cytogenet.* 13 (1), 51. doi:10.1186/s13039-020-00520-3

Wraith, J. E., Scarpa, M., Beck, M., Bodamer, O. A., De Meirleir, L., Guffon, N., et al. (2008). Mucopolysaccharidosis Type II (Hunter Syndrome): a Clinical Review and Recommendations for Treatment in the Era of Enzyme Replacement Therapy. *Eur. J. Pediatr.* 167 (3), 267–277. doi:10.1007/s00431-007-0635-4

Yokota, K., Nozu, K., Minamikawa, S., Yamamura, T., Nakanishi, K., Kaneda, H., et al. (2017). Female X-Linked Alport Syndrome with Somatic Mosaicism. *Clin. Exp. Nephrol.* 21 (5), 877–883. doi:10.1007/s10157-016-1352-y

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.