Universidade Federal de Pernambuco
Centro de Biociências
Programa de Pós-Graduação em Biologia Aplicada à Saúde

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TRIAGEM GENÉTICA E CARACTERIZAÇÃO FENOTÍPICA DE PACIENTES COM CALCIFICAÇÕES CEREBRAIS NÃO LIGADAS A MUTAÇÕES NOS GENES SLC20A2, PDGFB, PDGFRB E XPR1

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Dissertação apresentada ao Curso de Pós-Graduação em Biologia Aplicada à Saúde da Universidade Federal de Pernambuco, como requisito à obtenção do título de mestre em Biologia Aplicada à Saúde.

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RESUMO

Calcificações cerebrais são um tipo de calcificação ectópica que pode estar associada a processos crônicos, agudos ou fisiológicos. A calcificação cerebral familiar primária (CCFP) é uma doença genética rara caracterizada por causar esse tipo de lesão, costumando ter um perfil de herança autossômico dominante. Quatro genes já foram relacionados a este tipo de herança (*SLC20A2*, *PDGFB*, *PDGFRB* e *XPR1*), mas esses achados explicam apenas cerca de 60% dos casos de CCFP. O fato de que muitos dos pacientes afetados não possuem mutações nesses quatro genes reforça a necessidade por mais estudos genéticos para identificação de novos genes candidatos. Além disso, é importante promover uma análise completa da apresentação clínica dos pacientes, permitindo que haja uma comparação em relação à severidade da doença, apresentação clínica, padrões de calcificação cerebral, idade de início da doença e outros fatores entre os dois grupos. Desse modo, esse estudo teve como objetivo realizar análise clínica aprofundada, associada a novas triagens genéticas, em pacientes com calcificação cerebral que possuem resultados negativos para os genes *SLC20A2*, *PDGFB*, *PDGFRB* e *XPR1* para garantir um correto diagnóstico diferencial. Análise de dados clínicos e radiológicos foram feitas, seguidas de triagens genéticas nos genes *PDGFC*, *PDGFD* e *PCDH12*. Através desse estudo foi possível diagnosticar um dos pacientes triados com Síndrome de Primrose, uma doença genética rara com menos de 30 casos descritos na literatura. Além disso, pequenas diferenças entre pacientes com e sem mutações nos genes ligado a CCFP foram identificadas, como na frequência de sintomas relatados e regiões cerebrais calcificadas. Por fim, nenhuma variação patológica foi encontrada nos genes *PDGFC*, *PDGFD* e *PCDH12*.

Palavras-chave: Calcificação Cerebral. Síndrome de Primrose. Neurodegeneração.

ABSTRACT

Brain calcifications are a type of ectopic calcification that can be associated to chronic, accute of physiological processes. Primary familial brain calcification (PFBC) is a rare genetic condition characterized by this type of lesion, usually displaying an autosomal dominant inheritance profile. Four genes have been reported to have this type of inheritance pattern in PFBC (*SLC20A2*, *PDGFB*, *PDGFRB* e *XPR1*), but these findings only account for 60% of all PFBC cases reported. The large number of affected patients without pathogenic variations to any of those genes reinforces the need for further genetic studies to identify new candidade genes. Besides that, it is importante to have a complete clinical analysis of these patients, allowing for a comparison of disease severity, clinical findings, brain calcification patterns, age at onset and other parameters between PFBC patients with and without identified mutations. This way, this study's aim was to promote a thorough clinical analysis, combined with genetic screening, in patients that have brain calcifications but show no mutations on *SLC20A2*, *PDGFB*, *PDGFRB* e *XPR1* in order to ensure a correct differential diagnosis. Analyses of both clinical and imaging data was perfomed, followed by genetic screening on *PDGFC*, *PDGFD* and *PCDH12*. By doing this we were able to diagnose one of the patients with Primrose Syndrome, a rare genetic condition with less than 30 cases reported in academic literature. Additionally, small differences were detected between patients with and without mutations in PFBC linked genes, such as different frequency of symptoms and brain areas most affected by calcifications. Finally, no pathogenic variation was found in *PDGFC*, *PDGFD* or *PCDH12*.

Key-words: Brain calcification. Primrose Syndrome. Neurodegeneration.

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LISTA DE ABREVIATURAS E SIGLAS

AGS	Síndrome de Aicardi-Goutières
CCFP	Calcificação cerebral familial primária
DGS	Síndrome DiGeorge
IBGC	*Idiopathic basal ganglia calcification*
MYORG	*Myogenesis Regulating Glycosidase*
PCDH12	*Protocadherin 12*
PCR	*Polymerase chain reaction*
PDGFB	*Platelet Derived Growth Factor subunit Beta*
PDGFC	*Platelet Derived Growth Factor C*
PDGFD	*Platelet Derived Growth Factor D*
PDGFRB	*Platelet Derived Growth Factor Receptor Beta*
PiT-2	T*ype III Sodium-dependent Inorganic Phosphate Transporter*
PRIMS	Síndrome de Primrose
PTH	Paratormônio
RMN	Ressonância Magnética
SLC20A2	*Solute Carrier Family 20 Member 2*
TC	Tomografia computadorizada
TORCH	Toxoplasmose, outros, rubéola, citomegalovirus, herpes simples
UFPE	Universidade Federal de Pernambuco
XPR1	*Xenotropic and Polytropic Retrovirus Receptor 1*
WES	*Whole exome sequencing*
WGS	*Whole genome sequencing*
ZIKV	Zika virus

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1 INTRODUÇÃO

1.1 PROBLEMATIZAÇÃO

A calcificação cerebral familiar primária (CCFP) é uma doença genética rara caracterizada por lesões cerebrais bilaterais detectadas por meio de tomografia computadorizada, normalmente em regiões subcorticais como os núcleos da base, tálamo e cerebelo (TAGLIA et al., 2014). A doença, que costuma ter um perfil de herança autossômico dominante, pode também ocorrer na ausência de histórico familiar (devido a mutações *de novo*) e já existem casos de CCFP causada de forma recessiva (RAMOS et al., 2017; YAO et al., 2018). Caracterizada por uma marcada hetereogeneidade clínica, a CCFP pode provocar uma ampla gama de sintomas, como demência, parksonismo, alterações de humor e convulsões, mas também existem casos de pacientes assintomáticos (LEMOS et al., 2015).

O diagnóstico da desordem, que também é conhecida como doença de Fahr, deve ser feito após exclusão de outros quadros onde calcificação cerebral ocorre secundariamente. Isso é possível pois, enquanto pacientes com CCFP apresentam níveis normal de cálcio sérico e hormônios paratireoideanos, assim como a ausência de causas metabólicas e infecciosas, pacientes com desordens como TORCH, pseudo-TORCH e pseudohipoparatireoidismo podem apresentar alterações em vários destes fatores (CASTRO; BORBA, 1999). Outro fator que merece destaque e se mostra importante na diferenciação dessas síndromes é o padrão de calcificação cerebral. Diferente de outros pacientes com calcificações secundárias que podem apresentar calcificação cerebral difusa, pacientes com CCFP sempre apresentam calcificações bilaterais e simétricas, normalmente restritas a regiões subcorticais (WESTENBERGER; KLEIN, 2014).

Estudos genéticos já foram capazes de identificar mutações relacionadas à doença, apesar de esses achados explicarem apenas cerca de 60% dos casos de CCFP. Os principais genes destacados como possíveis desencadeadores dessa desordem são *SLC20A2*, *XPR1*, *PDGFB* e *PDGFRB* (KELLER et al., 2013; LEGATI et al., 2015; NICOLAS et al., 2013a; WANG et al., 2012). O *SLC20A2*, gene que codifica um transportador de fosfato inorgânico (PiT2), aparenta ser o principal gene responsável por casos de CCFP e consequentemente é o mais estudado nessa doença (WANG et al., 2012). Já *PDGFB* e *PDGFRB*, genes que codificam o fator de crescimento derivado da plaqueta β e seu receptor, respectivamente, estão relacionados à integridade da barreira hemato-encefálica e foram relacionados à CCFP pela primeira vez em 2013 (KELLER et al., 2013; LEMOS et al., 2015; NICOLAS et al., 2013a). Mutações de perda de função em *XPR1*, por sua vez, foram descritas por Legati e colaboradores em 2015 e estão relacionadas a cerca de 8% dos casos negativos para mutações em *SLC20A2*, *PDGFB* e *PDGFRB*. Mais recentemente o gene *MYORG* também foi ligado a casos de CCFP, mas ainda não se sabe muito sobre sua função na etiopatogênese da doença (YAO et al., 2018).

1.2 JUSTIFICATIVA

Pacientes que possuem manifestações clínicas e imagens de tomografia computadorizada condizentes com CCFP, mas não possuem mutações em *SLC20A2*, *XPR1*, *PDGFB* e *PDGFRB* são ditos pacientes com CCFP não-confirmada geneticamente. Como foi previamente mencionado, cerca de 40% dos casos de calcificação cerebral familiar primária não foram relacionadas até o momento a nenhum dos 4 genes já ligados à doença (*SLC20A2*, *PDGFB*, *PDGFRB* e *XPR1*) (OLIVEIRA; OLIVEIRA, 2016). O fato de que muitos dos pacientes afetados não possuem mutações nesses quatro genes reforça a necessidade por mais estudos genéticos para identificação de novos genes candidatos (WESTENBERGER; KLEIN, 2014).

É importante promover uma análise completa da apresentação clínica dos pacientes com resultados negativos para os 4 genes já mencionados, assim como dos pacientes que possuam mutações em *SLC20A2*, *PDGFB*, *PDGFRB* e *XPR1*, permitindo que haja uma comparação em relação à severidade da doença, apresentação clínica, padrões de calcificação cerebral, idade de início da doença e outros fatores entre os dois grupos.

1.3 OBJETIVOS

1.3.1 **Objetivo Geral**

Realizar análise clínica aprofundada, associada à novas triagens genéticas, em pacientes com calcificação cerebral que possuem resultados negativos para os genes *SLC20A2*, *PDGFB*, *PDGFRB* e *XPR1* para garantir um correto diagnóstico diferencial.

1.3.2 Objetivos Específicos

a) Analisar sinais e sintomas exibidos por pacientes com CCFP não confirmada geneticamente;
b) Realizar pesquisa genética por novos genes candidatos em pacientes com resultados negativos para mutações nos genes *SLC20A2*, *PDGFB*, *PDGFRB* e *XPR1*;
c) Comparar informações clínicas, radiológicas e genéticas de pacientes com CCFP confirmada geneticamente com informações de pacientes com CCFP não confirmada geneticamente.

2 REFERENCIAL TEÓRICO

2.1 CALCIFICAÇÕES CEREBRAIS

Calcificações cerebrais são um tipo de calcificação ectópica caracterizadas por serem achados inespecíficos que podem estar associados a processos crônicos, agudos ou fisiológicos (LEMOS et al., 2013). São caracterizadas por depósitos de hidroxiapatita ($Ca_{10}[PO_4]_6[OH]_2$) em diversas regiões do encéfalo, como córtex, núcleos da base, plexo coróide e glândula pineal, podendo ainda conter ferro, zinco e magnésio (DENG; ZHENG; JANKOVIC, 2015).

2.1.1 Causas e padrões

As calcificações cerebrais podem ter diversas causas, o que repercute também nos seus aspectos clínicos e abordagem terapêutica (LIVINGSTON et al., 2013). Elas podem ser classificadas em calcificações fisiológicas, quando ocorrem como decorrência natural do envelhecimento, ou patológicas, podendo ser genéticas, infecciosas, endócrinas e metabólicas, vasculares ou neoplásicas (Quadro 1) (DENG; ZHENG; JANKOVIC, 2015; KIROĞLU et al., 2010).

Quadro 1 – Classificação dos diferentes tipos de calcificação cerebral.

Tipo de Calcificação Cerebral			Exemplos
Fisiológica e idade-dependente			Glândula Pineal, plexo coroide, dura mater.
Patológica	**Primária**	**Genética**	Calcificação Cerebral Familial Primária, Síndrome de Primrose, Síndrome de Aicardi-Goutieres, Esclerose Tuberosa.
	Secundária	**Infecciosa**	Infecções granulomatosas, encefalite viral crônica, Doenças tipo TORCH.
		Endócrina/ metabólica	Hipotireoidismo, hipoparatireoidismo, pseudohipoparatireoidismo.
		Vascular	Aterosclerose, aneurismas.
		Neoplásica	Oligodendroglioma, calcificação de metástases, menangioma.

Fonte: Adaptado de Kiroglu et al. (2010)

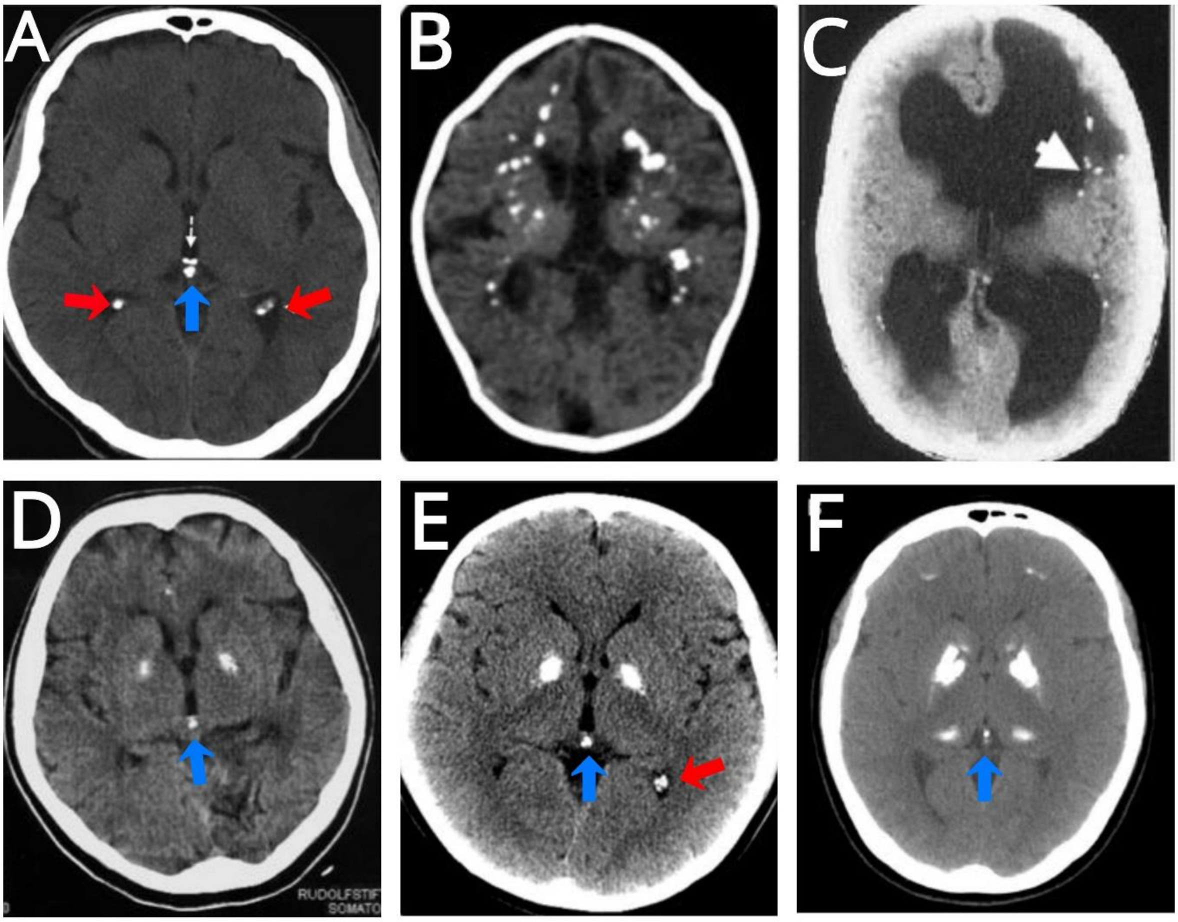
No contexto fisiológico, esse tipo de calcificação ectópica costuma acometer áreas como o plexo coroide e glândula pineal, podendo ser encontradas a partir da primeira década de vida, mas sendo detectadas com maior frequência a partir dos 50 anos (SEDGHIZADEH; NGUYEN; ENCISO, 2012; UDUMA; PIUS; MATHIEU, 2011; YALCIN et al., 2016). Essas calcificações costumam ser menores do que as patológicas e não são associadas a nenhum déficit clínico específico (KIROĞLU et al., 2010). Estudos estimam que este tipo de calcificação ectópica afete entre 17% e 70% da população, o que a torna um achado relativamente comum na rotina de exames de imagem do encéfalo (ALVES et al., 2013; OLIVEIRA; SILVA; OLIVEIRA, 2013; YALCIN et al., 2016).

As calcificações cerebrais patológicas, por sua vez, podem ter origem primária ou secundária. As calcificações cerebrais primárias são causadas por alterações genéticas, seja de caráter autossômico dominante ou recessivo, e os aspectos clínicos associados às calcificações dependem do gene afetado e, consequentemente, da doença causada por essa variação genética (QUINTÁNS; OLIVEIRA; SOBRIDO, 2018). Exemplos de doenças genéticas onde calcificações cerebrais já foram descritas como parte dos sinais encontrados incluem Calcificações Cerebrais Familiais Primárias (CCFP), Síndrome de Primrose (PRIMS) e Síndrome de Aicardi-Goutières, todas com quadros clínicos bastante distintos (CORDEDDU et al., 2014; RAMOS et al., 2017). A CCFP e PRIMS serão descritas em mais detalhes nos tópicos 2.2 e 4.2, respectivamente.

As calcificações cerebrais patológicas secundárias são causadas por causas não genéticas como traumas físicos, desbalançeamentos metabólicos, infecções ou neoplasias (Quadro 1) (BEKIESINSKA-FIGATOWSKA; MIERZEWSKA; JURKIEWICZ, 2013). Elas têm apresentações clínicas diferentes de acordo com a causa e podem ocorrer em diversos momentos da vida, como desde a vida fetal em casos de infecção por vírus *in utero*, ou depois dos 45 anos em casos de hipoparatireoidismo secundário a cirurgias (ABATE; CLARKE, 2017; CHEERAN; LOKENSGARD; SCHLEISS, 2009; VASCO ARAGÃO et al., 2016).

Os padrões de calcificações cerebrais são diversos e podem ser indicativos da etiologia da lesão (LIVINGSTON et al., 2013). Como exemplos, as calcificações fisiológicas costumam estar restritas a certas estruturas, como glândula pineal e plexo coroide (YALCIN et al., 2016). Similarmente, muitos quadros patológicos primários constam com esse tipo de calcificação ectópica em regiões bem definidas, como os núcleos da base nos casos de CCFP, PRIMS e Síndrome de Cockayne (CORDEDDU et al., 2014; LIVINGSTON et al., 2013; RAMOS et al., 2017). As calcificações podem ainda ser encontradas de maneira puntiforme no encéfalo, na região cortical, cerebelo, hipocampo, zonas periventriculares entre outras (DE BROUWER et al., 2018; DENG; ZHENG; JANKOVIC, 2015). Por fim, as calcificações podem ainda ser classificadas em simétricas, atingindo as mesmas estruturas em ambos os hemisférios cerebrais, ou difusas, não obedecendo a nenhum eixo de simetria. (Figura 1)

Figura 1 – Alguns dos diferentes tipos de padrões de calcificações cerebrais em imagens de tomografia computadorizada. (A) Calcificações fisiológicas demarcadas por setas (KIROGLU et al., 2010); (B) Calcificações puntiformes e periventriculares em quadro de síndrome de Aircardi-Goutières (LA PIANA et al., 2016); (C) Calcificações puntiformes e periventriculares em quadro de infecção congênita por CMV (CHEERAN et al., 2009); Calcificações bilaterais e simétricas em núcleos da base em quadros de (D) mitocondriopatia (FINSTERER et al., 2005), (E) pseudohipoparatireoidismo (KOBAYASHI et al., 2018) e (F) CCFP (FERREIRA et al., 2014). Calcificações na glândula pineal (seta azul). Calcificações no plexo coroide (seta vermelha).



Fonte: Adaptado de (CHEERAN; LOKENSGARD; SCHLEISS, 2009; FERREIRA et al., 2014; FINSTERER; KOPSA, 2005; KIROĞLU et al., 2010; KOBAYASHI; TSUYUZAKI; KOIZUMI, 2018; LA PIANA et al., 2016)

2.1.2 Métodos de detecção

Calcificações cerebrais podem ser detectadas por meio de exames de imagem, mas nem todos são capazes de detectar a lesão devido à sua localização anatômica. Desse modo, os métodos mais indicados para a detecção e avaliação de calcificações cerebrais são ressonâncias magnéticas e tomografias computadorizadas (LIVINGSTON et al., 2013).

A tomografia computadorizada (TC) é o método mais eficaz na detecção de depósitos de cálcio no encéfalo, sendo assim a melhor opção no estudo desse tipo de lesão por gerar imagens nítidas (RAMOS et al., 2017). A ressonância magnética (RMN), por sua vez, não é tão sensível como a TC na detecção de calcificações cerebrais, apesar de promover uma melhor definição das estruturas cerebrais (QUINTÁNS; OLIVEIRA; SOBRIDO, 2018). Desse modo, a execução dos dois exames quando disponível pode ser de interesse médico para garantir tanto a detecção de todas as lesões quanto a identificação precisa das estruturas afetadas (LIVINGSTON et al., 2013).

2.2 CALCIFICAÇÃO CEREBRAL FAMILIAR PRIMÁRIA

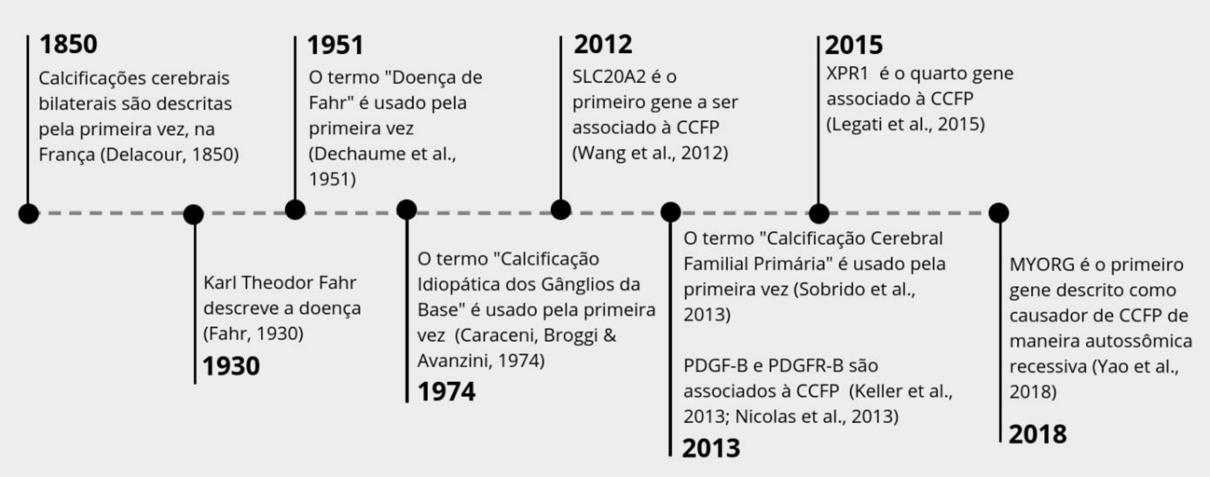
A calcificação cerebral familiar primária (CCFP) é uma doença neurodegenerativa rara caracterizada por depósitos de hidroxiapatita bilaterais e simétricos no encéfalo na ausência de causas metabólicas, infecciosas ou traumáticas (QUINTÁNS; OLIVEIRA; SOBRIDO, 2018). Apesar de ser causada em sua maioria por variações genéticas de caráter autossômico dominante, já foi relatado na literatura que a doença também pode ser causada por variações de caráter autossômico recessivo ou mutações *de novo* (FERREIRA et al., 2014; NICOLAS et al., 2015; YAO et al., 2018).

Nos tópicos a seguir serão descritos o histórico da doença, as diversas nomenclaturas usadas para descrevê-la ao longo dos anos, e seus sinais e sintomas, assim como prevalência, métodos diagnósticos e tratamento.

2.2.1 Histórico e nomenclatura

Doença conhecida há pelo menos 80 anos, mais de 35 termos já foram usados para fazer referência a CCFP (MANYAM, 2005). O nome “doença de Fahr” foi usado pela primeira vez em 1951 por Dechaume e colaboradores, fazendo referência à Karl Theodore Fahr, um médico alemão que descreveu a doença em 1930 (Figura 2) (DECHAUME et al., 1951). Desde então, diversos relatos da doença foram feitos em vários países, e com eles vieram novos termos ou variações de termos previamente utilizados. Em 1974, o termo “calcificação idiopática dos gânglios da base” (*idiopathic basal ganglia calcification*, IBGC) foi utilizado pela primeira vez, se tornando um dos termos mais usados para fazer referência à doença (CARACENI; BROGGI; AVANZINI, 1974). O termo CCFP, por sua vez, foi utilizado pela primeira vez em 2013 após causas genéticas para a doença serem identificadas (SOBRIDO et al., 2013).

Figura 2 – Linha do tempo destacando os principais acontecimentos relacionados às Calcificações Cerebrais Famliais Primárias, desde sua primeira descrição.



1850
Calcificações cerebrais bilaterais são descritas pela primeira vez, na França (Delacour, 1850)

Karl Theodor Fahr descreve a doença (Fahr, 1930)
1930

1951
O termo "Doença de Fahr" é usado pela primeira vez (Dechaume et al., 1951)

O termo "Calcificação Idiopática dos Gânglios da Base" é usado pela primeira vez (Caraceni, Broggi & Avanzini, 1974)
1974

2012
SLC20A2 é o primeiro gene a ser associado à CCFP (Wang et al., 2012)

O termo "Calcificação Cerebral Familial Primária" é usado pela primeira vez (Sobrido et al., 2013)

PDGF-B e PDGFR-B são associados à CCFP (Keller et al., 2013; Nicolas et al., 2013)
2013

2015
XPR1 é o quarto gene associado à CCFP (Legati et al., 2015)

MYORG é o primeiro gene descrito como causador de CCFP de maneira autossômica recessiva (Yao et al., 2018)
2018

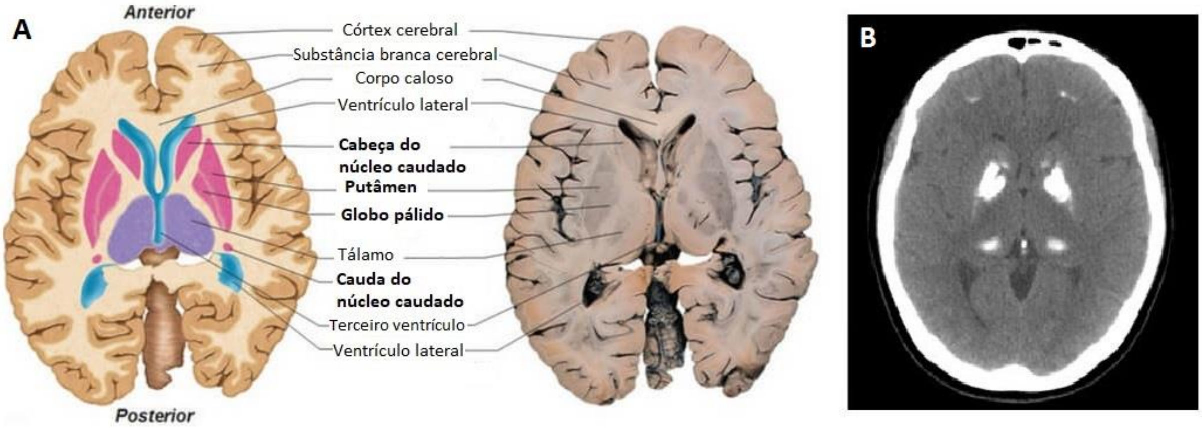
Fonte: Adaptado de (FERREIRA; MENDES DE OLIVEIRA, 2018).

Atualmente, refletindo uma preferência da comunidade médica como um todo, o uso dos epônimos vem sendo substituído por nomes que refletem melhor a origem genética da doença, assim como seus principais sintomas (WOYWODT; MATTESON, 2007). Desse modo, calcificações cerebrais familiais primárias (CCFP) é o nome mais usado pela comunidade científica nos últimos anos por refletir diretamente a causa da doença (primária), assim como sua lesão característica (calcificações cerebrais, não necessariamente restritas aos núcleos da base) (FERREIRA; MENDES DE OLIVEIRA, 2018).

2.2.2 Sinais e sintomas

O principal sinal da CCFP é a presença de calcificações cerebrais bilaterais e simétricas, que podem atingir os núcleos da base (putamen, globo pálido e núcleo caudado), tálamos, núcleo denteado, ponte, substância branca subcortical entre outros (Figura 3) (RAMOS et al., 2017). Os núcleos da base são as estruturas mais comumente afetadas, demonstrando uma elevada vulnerabilidade frente a processos patológicos como a CCFP devido à sua alta atividade metabólica e, consequentemente, elevada dependência no abastecimento sanguíneo (BEKIESINSKA-FIGATOWSKA; MIERZEWSKA; JURKIEWICZ, 2013; NICOLAS et al., 2015; TADIC et al., 2015). Essas calcificações costumam ser detectadas por RMNs ou TCs realizados de maneira rotineira ou como método de investigação de lesões neurológicas (RAMOS et al., 2017).

Figura 3 – Imagens de plano horizontal do cérebro destacando os núcleos da base. (A) Núcleos da base em desenho anatômico, em rosa, e em corte cerebral (ANTRANIK, 2018); (B) Imagem de tomografia computadorizada onde os núcleos da base estão demarcados por calcificações, em branco (FERREIRA et al., 2014).


A
Anterior
Córtex cerebral
Substância branca cerebral
Corpo caloso
Ventrículo lateral
Cabeça do núcleo caudado
Putâmen
Globo pálido
Tálamo
Cauda do núcleo caudado
Terceiro ventrículo
Ventrículo lateral
Posterior
B

Fonte: Adaptado de ANTRANIK (2018) e (FERREIRA et al., 2014)

Além das calcificações, a CCFP não possui nenhum outro sinal típico da doença que possa ser detectado por exames de imagem ou laboratoriais (RAMOS et al., 2017). Dessa maneira, pacientes com CCFP possuem exames hematológicos e bioquímicos dentro dos valores da normalidade, os diferenciando de pessoas com alterações da paratireoide, metabolismo do cálcio ou deficiência de vitamina D (RAMOS et al., 2018).

Já em relação aos sintomas relatados por pacientes com CCFP, é importante ressaltar a existência de uma grande variação fenotípica entre os pacientes diagnosticados com esta condição. A penetrância dos sintomas é incompleta, de modo que enquanto alguns pacientes são assintomáticos, outros possuem diversos sintomas cognitivos, psiquiátricos e distúrbios do movimento (LEMOS et al., 2013).

Estima-se que casos sintomáticos de CCFP correspondem a cerca de 60% do total de pacientes afetados por calcificações. Esse valor, entretanto, pode variar amplamente de estudo para estudo devido à adoção de diferentes critérios de inclusão (TADIC et al., 2015). De forma geral, estudos indicam que o aparecimento dos sintomas ocorre principalmente entre os 30 e 50 anos, podendo variar desde a infância até depois dos 80 anos (NICOLAS et al., 2015; RAMOS et al., 2018).

Por afetarem principalmente os núcleos da base, regiões cerebrais conhecidas por possuírem função primordialmente motora, é comum que pacientes com CCFP relatem a presença de distúrbios de movimento (KOPELL et al., 2006). Um estudo recente, realizado com pacientes geneticamente diagnosticados com CCFP em quatro países, indicou que distúrbios de movimento são os sintomas mais comumente relatados por esses pacientes, em especial parksonismo (RAMOS et al., 2018). Outros sintomas comumente relatados são tremores das extremidades, coreia, distonia e ataxia (NICOLAS et al., 2015; TADIC et al., 2015).

Os sintomas psiquiátricos, presentes em grande parte dos pacientes diagnosticados com CCFP, incluem transtornos de humor (como depressão e transtorno bipolar), sintomas psicóticos e episódios agressivos. Sintomas cognitivos, por sua vez, podem variar desde demência a relatos de dificuldade com memória (NICOLAS et al., 2015). Por fim, sintomas como dor de cabeça e enxaqueca, episódios convulsivos, problemas na fala e equilíbrio também já foram relatados por pacientes portadores de CCFP (NICOLAS et al., 2013b).

2.2.3 Prevalência

A CCFP sempre foi considerada uma doença rara (KELLER et al., 2013; WANG et al., 2012). Apesar disso, um estudo de 2018 indica que a prevalência dessa doença pode ser mais alta do que se imaginava. Nicolas e colaboradores

(2018) estimaram, através de um estudo usando informações de bancos de dados genômicos, que a real prevalência de CCFP na população mundial está entre 3,4/10.000 e 1,9/1.000. Esse estudo sugere, então, que uma grande parcela de pacientes portadores de variações genéticas causadoras de CCFP não é diagnosticada com a doença, provavelmente por não desenvolverem sintomas que os levariam a procurar serviços médicos (NICOLAS et al., 2018). Outros fatores que podem contribuir para a baixa taxa de diagnóstico de CCFP na população é a sobreposição clínica com outros quadros patológicos, o desconhecimento sobre a condição e a baixa disponibilidade de testes genéticos que são capazes de definir o diagnóstico.

2.2.4 Diagnóstico

Como mencionado anteriormente, o único sinal clássico da CCFP é a presença de calcificações cerebrais, sendo estas bilaterais e simétricas, detectadas por meio de RMNs ou TCs e tendo penetrância de 100%, de modo que o diagnóstico de CCFP depende obrigatoriamente da presença dessas lesões (TADIC et al., 2015). É importante, entretanto, garantir que as lesões não são causadas por distúrbios metabólicos, infecções ou traumas, garantindo um diagnóstico diferencial completo e acurado, como será visto no tópico 2.2.4.2.

A presença de sintomas, principalmente motores, psiquiátricos ou cognitivos, apesar de indicativos da doença, não é necessária para confirmação do diagnóstico. Estudos indicam que cerca de 40% de pacientes com CCFP são assintomáticos, o que pode contribuir para a baixa detecção das calcificações, notificação dos achados e, consequentemente, prevalência da doença (NICOLAS et al., 2018; TADIC et al., 2015).

Por fim, o diagnóstico de CCFP pode ser confirmado através de estudos genéticos quando se identifica uma variação em um dos cinco genes já ligados à doença, que serão detalhados no tópico a seguir.

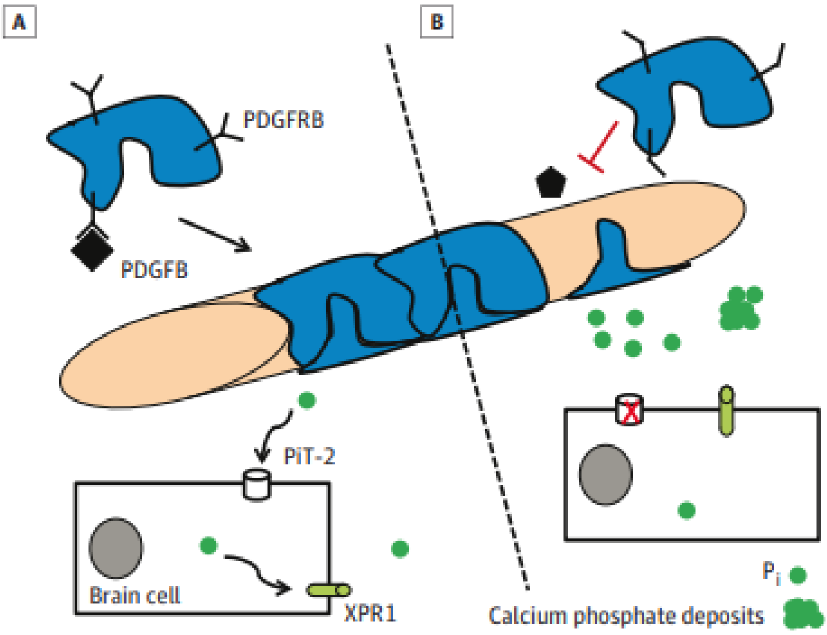
2.2.4.1 Genética

O primeiro gene identificado como causador da CCFP é, coincidentemente, o responsável pela maior parte dos casos da doença (DENG; ZHENG; JANKOVIC, 2015; WANG et al., 2012). O *SLC20A2* (*Solute Carrier Family 20 Member 2*) codifica uma proteína transmembrana (PiT-2, *type III sodium-dependent inorganic phosphate transporter*) relacionada à manutenção da homeostase do fosfato, mineral importante para diversos processos celulares (GIOVANNINI et al., 2013; WANG et al., 2012). Até o momento, o *SLC20A2* foi relacionado a 40% dos casos com causa genética identificada e existem tanto casos relacionados à herança autossômica dominante quanto casos de mutações *de novo* (FERREIRA et al., 2014; RAMOS et al., 2017; WANG et al., 2012).

Estudos em modelo animal mostram que a deleção desse gene causa calcificação bilateral dos núcleos da base em camundongos, confirmando o que é descrito em pacientes com CCFP (JENSEN et al., 2013). Inicialmente acreditava-se que perda da função do PiT-2 causava o acúmulo extracelular de fosfato e, consequentemente, a formação e deposição de fosfato de cálcio (

Figura 4) (TADIC et al., 2015). Estudos recentes mostram, entretanto, que a calcificação inicia de maneira intracelular, principalmente em pericitos e astrócitos (JENSEN et al., 2018).

Figura 4 – Função dos principais genes relacionados à CCFP.



Fonte: Tadic et al. 2015

Após o *SLC20A2*, os genes *PDGFB* (*Platelet Derived Growth Factor subunit Beta*) e *PDGFRB* (*Platelet Derived Growth Factor Receptor Beta*) foram relacionados à doença, também com padrão de herança autossômica dominante (KELLER et al., 2013; NICOLAS et al., 2013a). Juntos, estima-se que esses dois genes sejam responsáveis por cerca de 13% dos casos de CCFP (RAMOS et al., 2017). Estes genes, que codificam respectivamente o fator de crescimento derivado de plaqueta beta e seu receptor, aparentam atuar sobre a integridade e permeabilidade da barreira hemato-encefálica (

Figura 4) (MOURA; LEMOS; OLIVEIRA, 2017).

Em 2015, o *XPR1 (Xenotropic and Polytropic Retrovirus Receptor 1)* foi o quarto gene a ser relacionado à CCFP, afetando cerca de 2% dos pacientes (LEGATI et al., 2015; RAMOS et al., 2017). Através de modelos celulares a função do XPR1 foi esclarecida, de modo que a proteína atua na exportação celular de fosfato inorgânico, de maneira oposta ao PiT-2, contribuindo para a manutenção da homeostase do fosfato (

Figura 4) (ANHEIM et al., 2016; GIOVANNINI et al., 2013). Além disso, estudos avaliando a expressão de mRNA do *XPR1* em modelos animais mostraram maior expressão desta proteína em áreas cerebrais comumente afetadas na CCFP, assim como interação desta proteína com o PDGFRB (YAO et al., 2017).

Por fim, em 2018 o primeiro gene responsável por causar CCFP de maneira aussômica recessiva foi identificado. O *MYORG* (*Myogenesis Regulating Glycosidase*), previamente conhecido como *KIAA1161* ou *NET37*, foi identificado em uma família chinesa no início de 2018 e não se sabe muito sobre a sua função (YAO et al., 2018). Estudos iniciais mostram, entretanto, que animais *knockout* para esse gene desenvolvem calcificações condizentes com o que é visto na CCFP e que o *MYORG* é expresso mais fortemente em astrócitos (YAO et al., 2018).

Desse modo exames genéticos são, até o momento, a única maneira de confirmar o diagnóstico de CCFP – mas, por outro lado, não são capazes de excluir tal hipótese diagnóstica. Pacientes portadores de variações patogênicas nos genes mencionados acima podem ser ditos como portadores de CCFP confirmada geneticamente, mas existe uma grande parcela (cerca de 40%) de pacientes que são considerados como casos não confirmados geneticamente (NICOLAS et al., 2015; OLIVEIRA; OLIVEIRA, 2016). Esse grupo de pacientes é caracterizado por possuir calcificações cerebrais típicas de CCFP na ausência de causas secundárias, além de não possuírem variações nos genes *SLC20A2*, *PDGFB*, *PDGFRB* e *XPR1*. É importante ressaltar, entretanto, que devido à recente identificação do papel do

MYORG na CCFP muitos pacientes ainda não foram triados para variações neste gene.

O grande número de pacientes portadores de CCFP não confirmada geneticamente indica que outros genes estão ligados à doença, de modo que triagens para novos genes candidatos são feitas em tentativa de explicar uma maior parte dos casos (WESTENBERGER; KLEIN, 2014). Nesses casos, podem ser feitos dois tipos de abordagens: primeiramente, pesquisas genéticas focadas em genes específicos são indicadas principalmente quando se tem alguma nova hipótese ou gene candidato em mente. Uma segunda opção seria a realização de sequenciamentos de nova geração como *whole exome sequencing* (WES) e *whole genome sequencing* (WGS), que analisa o exoma ou genoma completos de cada paciente, detectando todos os SNPs presentes nas amostras e destacando aqueles que tem algum potencial patogênico (RAMOS et al., 2017).

2.2.4.2 Diagnóstico diferencial

O diagnóstico diferencial da CCFP é de extrema importância não só para garantir o diagnóstico correto, mas também para garantir a escolha terapêutica mais adequada para cada paciente. Exemplos de condições capazes de provocar lesões semelhantes às típicas da CCFP e que devem, portanto, fazer parte do diagnóstico diferencial incluem principalmente distúrbios do metabolismo do cálcio, quadros infecciosos, distúrbios mitocondriais e outras desordens genéticas.

Os principais distúrbios do metabolismo do cálcio que devem ser considerados durante o diagnóstico diferencial da CCFP são o hipoparatireoidismo e o pseudohipoparatireoidismo. O primeiro quadro, que pode acontecer de maneira idiopática ou pós-cirúrgica, causa hipocalcemia devido à baixos níveis ou ausência do hormônio paratireoidiano (PTH), enquanto o segundo acontece por resistência ao mesmo. A exclusão dessas hipóteses diagnósticas pode ser feita através da realização de exames bioquímicos para análise dos níveis séricos de cálcio, fósforo e paratormônio (CASTRO; BORBA, 1999). No Quadro 2 é possível ver como cada um desses parâmetros se apresenta nos diferentes quadros patológicos.

Quadro 2 – Perfil bioquímico de desordens que causam calficações cerebrais. CCFP – Calcificações Cerebrais Familiais Primárias. HP – Hipoparatireoidismo. PHP – Pseudohipoparatireoidismo. PTH – Paratormônio.

DOENÇA	CÁLCIO SÉRICO	FÓSFORO	PTH
CCFP	Normal	Normal	Normal
HP	Diminuído	Elevado	Diminuído
PHP	Diminuído	Elevado	Elevado

Fonte: Adaptado de (CASTRO; BORBA, 1999; COSTA et al., 2013)

Exemplos de quadros infecciosos capazes de causar calcificações cerebrais incluem infecções do tipo TORCH (toxoplasmose, outros, rubéola, citomegalovírus, herpes simples) e zika vírus (ZIKV) (HUSSAIN et al., 2018; LEEPER; LUTZKANIN, 2018). Fetos são especialmente sensíveis a tais quadros infecciosos e, nesses casos, é comum a detecção de outras anormalidades estruturais no cérebro como ventriculomegalia, polimicrogiria e microcefalia (CHEERAN; LOKENSGARD; SCHLEISS, 2009). Associadas ao histórico médico de cada paciente, o conjunto de anormalidades estruturais do cérebro podem ser usadas como indicativo de uma causa infecciosa (HUSSAIN et al., 2018). A pesquisa de marcadores indicativos de infecção, como anticorpos, proteínas virais e/ou bacterianas podem, por fim, esclarecer o diagnóstico (VASCO ARAGÃO et al., 2016).

Desordens mitocondriais são aquelas causadas por variações no DNA mitocondrial. Por serem organelas relacionadas à síntese energética, disfunções mitocondriais afetam diversos tipos celulares, de modo que neurônios são bastante a mudanças no metabolismo energético. Variantes genéticas no DNA mitocondrial podem causar uma grande variedade de desordens neurológicas, incluindo transtornos psiquiátricos, do desenvolvimento e desordens neurodegenerativas, como a MELAS (*Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes*) (CRUZ et al., 2018). Um dos achados radiológicos de MELAS são calcificações cerebrais nos núcleos da base, justificando a importância da sua consideração no diagnóstico de CCFP (KHANDWALA; AHMED; SHEIKH, 2018). A diferenciação das duas desordens pode ser feita de maneira genética ou através de análise clínica, já que pacientes com MELAS costumam apresentar fraqueza e dor muscular acompanhadas de perda de apetite e vômitos, quadros não relatados com frequência por pacientes com CCFP.

Uma série de doenças genéticas devem ser consideradas durante o diagnóstico diferencial da CCFP. A síndrome DiGeorge (DGS), por exemplo, é uma doença causada por deleções no cromossomo 22 onde calcificações bilaterais e simétricas já foram descritas. Apesar disso, DGS difere de CCFP por ser caracterizada por quadros de hipocalcemia, fendas palatinas e uma série de dismorfismos que podem ser usados para diferenciar os dois quadros clinicamente (DENG; ZHENG; JANKOVIC, 2015). Já a síndrome de Aicardi-Goutieres (AGS), apesar de também causar calcificações intracranianas, tem padrão clínico mais semelhante a casos de infecções virais congênitas. Como exemplos, pacientes com AGS tem início da doença ainda durante a infância e apresentam atrasos no desenvolvimento, microcefalia, atrofia cerebral e sorologia negativa para infecções virais. Todos estes fatores, mesmo na ausência de testes genéticos, já podem ser utilizados para diferenciar casos de CCFP e AGS (DENG; ZHENG; JANKOVIC, 2015).

Por fim, a síndrome de Primrose (PRIMS) é outra síndrome genética que deve ser considerada no diagnóstico diferencial da CCFP. Causada por mutações *de novo* no gene *ZBTB20*, PRIMS é caracterizada por pacientes com atrasos no desenvolvimento, muitas vezes não andando ou falando, além de causar características faciais típicas, perda auditiva, e problemas musculoesqueléticos (CORDEDDU et al., 2014). Pacientes portadores dessa síndrome desenvolvem dois tipos de calcificação ectópica, normalmente entre a segunda e terceira década de vida, um deles sendo nos núcleos da base (CARVALHO; SPECK-MARTINS, 2011; DALAL et al., 2010). Desse modo, pacientes investigados para CCFP que possuam transtornos do desenvolvimento e características sindrômicas devem ser investigados também para a síndrome de Primrose.

2.2.5 Tratamento

Até o momento, o tratamento de pacientes diagnosticados com CCFP faz referência direta aos sintomas relatados por cada um, de modo que não existe nenhuma abordagem terapêutica específica para a doença devido à elevada heterogeneidade clínica relatada por pacientes. Medicamentos utilizados em casos de CCFP incluem antipsicóticos e antidepressivos para tratar sintomas psiquiátricos, e levo-dopa e fisioterapia, quando em casos marcados por distúrbios do movimento. Outras opções farmacológicas incluem ainda anticonvulsivantes e analgésicos a depender do quadro clínico de cada paciente.

O uso de bifosfonatos já foi investigado como mecanismo capaz de prevenir a expansão das lesões de calcificação. Bifosfonatos são drogas análogas ao pirofosfato inorgânico, sendo comumente usadas no tratamento de osteoporose por diminuirem a reabsorção óssea através da ativação da apopotose de osteoclastos (ZAMEER et al., 2018). O uso de bifosfonatos no tratamento de calcificações cerebrais vem sendo estudado ao longo dos anos, de modo que o alendronato, um tipo de bifosfonato, aparenta ser a melhor opção por ser capaz de atravessar a barreira hemato-encefálica. Estudos avaliando o efeito do alendronato na progressão de sintomas e das lesões de calcificação chegaram a resultados inconclusivos, com pacientes que relataram uma estabilização na doença enquanto outros observaram uma piora progressiva (OLIVEIRA; OLIVEIRA, 2016). As lesões nos núcleos da base, entretanto, se mantiveram inalteradas.

3 MÉTODOS

3.1 LOCAL DE ESTUDO E ASPECTOS ÉTICOS

O projeto foi desenvolvido no Laboratório de Neuroimunogenética do Laboratório de Imunopatologia Keizo Asami, localizado na Universidade Federal de Pernambuco (LIKA-UFPE). As amostras e dados obtidos se deram dentro de projetos aprovados pelo comitê de ética do CCS-UFPE (CAAE-0296.0.172.000-08 e 09475912.8.0000.5208).

3.2 CRITÉRIOS DE INCLUSÃO

O critério de inclusão deste estudo foi a presença de calcificações cerebrais simétricas e bilaterais, detectadas por meio de RMN e/ou TC, na ausência de apresentações clínicas características das desordens secundárias mais comumente confundidas com CCFP, como hipoparatireoidismo e pseudohipoparatireoidismo (RAMOS et al., 2018). Exemplos desses sintomas são parestesias, mialgia, cãibras e fraqueza muscular (COSTA et al., 2013).

A possibilidade de triar genes ligados à CCFP em pacientes com calcificações de causa desconhecida, ou até com diagnóstico suspeito secundário, se deu devido a projetos paralelos financiados pelo CNPq, no âmbito de triar os genes ligados à CCFP em paciente com endocrinopatias e outras doenças, como esclerose tuberosa. Com isso tentamos incluir mais pacientes, mesmo aqueles que não preenchessem perfeitamente os critérios de laboratoriais CCFP, desde que não tivessem clínica típicas de outras condições secundárias. Esta escolha favoreceu a inclusão de mais pacientes que pudessem não estarem ligados a CCFP, o que diminui a especificidade, mas aumenta a sensibilidade do estudo.

3.3 COLETA DE DADOS CLÍNICOS

Dados clínicos foram coletados no primeiro contato com o paciente, e em um segundo momento quando necessário obter informações mais detalhadas sobre histórico clínico (sinais e sintomas relacionados à problemas motores, cognitivos, psiquiátricos, entre outros). Essa coleta foi feita por meio de contato pessoal e/ou através do uso de questionários disponibilizados *on-line* (Apêndice F).

Quando possível e disponível, informações referentes à exames laboratoriais, imagens de ressonância magnética e/ou tomografia computadorizada, assim como seus respectivos laudos, foram coletados. As áreas das imagens de RM e TC a serem analisadas incluem núcleo caudado, tálamo, núcleos lenticulares, substância branca subcortical, córtex cerebral, cerebelo, ponte, mesencéfalo e medula.

Todas as informações obtidas foram encaminhadas ao Laboratório de Neuroimunogenética do LIKA-UFPE, centro de referência em diagnóstico de CCFP no Brasil.

O diagnóstico de provável CCFP foi feito baseado nas informações descritas no tópico 2.2.4, seguindo o padrão adotado nos últimos anos em estudos com propostas semelhantes (NICOLAS et al., 2015; RAMOS et al., 2018). Os pacientes foram diagnosticados com CCFP confirmada geneticamente ou não-confirmada geneticamente baseado no resultado de estudos genéticos para mutações nos genes *SLC20A2*, *PDGFB*, *PDGFRB* e *XPR1*, como detalhado no tópico a seguir.

3.4 ESTUDO GENÉTICO

Os pacientes diagnosticados com CCFP confirmada geneticamente foram apenas aqueles que obtiveram positividade para mutações em éxons codificadores de proteína e regiões de fronteira entre éxons e íntrons dos genes *SLC20A2*, *PDGFB*, *PDGFRB* e *XPR1* (informações obtidas após sequenciamento das amostras pelo método de Sanger) (NICOLAS et al., 2015).

Os pacientes que não tiveram o diagnóstico de CCFP confirmado pela detecção de variações nos 4 genes já mencionados foram selecionados para passar por uma nova triagem genética em genes candidatos. Esses genes foram *PDGFC* e *PDGFD*, homólogos ao gene *PDGFB*, e *PCDH12*, gene previamente relacionado a outros tipos de calcificações cerebrais (NICOLAS et al., 2017).

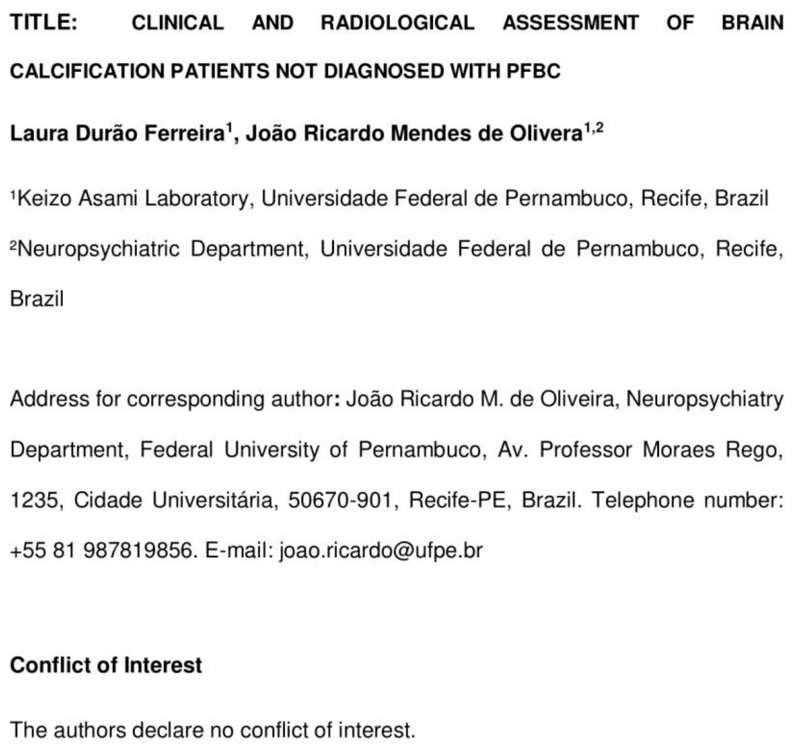
A técnica utilizada para triagem genética em genes candidatos foi o sequenciamento automático de Sanger. Primers utilizados para tais experimentos estão descritos no Apêndice F. Os produtos de PCR foram sequenciados em um sequenciador ABI 3700 no departamento de genética da Universidade Federal de Pernambuco (UFPE). Houve também o uso eventual do sequenciador do tipo MegaBace 1000 (Sunnyvale, CA), que se encontra instalado nas dependências do Laboratório de Imunopatologia Keizo Asami (LIKA), também na UFPE.

3.5 ANÁLISE ESTATÍSTICA

As análises estatísticas estão descritas em mais detalhes no capítulo 4.1. De maneira geral, o software estatítico RStudio (Versão 1.1.456) foi utilizado para realizar as análises. Gráficos foram feitos no Microsoft Excel, e significância estatística foi definida como $p<0.05$.

4 RESULTADOS

4.1 ARTIGO 1 - CLINICAL AND RADIOLOGICAL ASSESSMENT OF BRAIN CALCIFICATION PATIENTS NOT DIAGNOSED WITH PFBC



TITLE: CLINICAL AND RADIOLOGICAL ASSESSMENT OF BRAIN CALCIFICATION PATIENTS NOT DIAGNOSED WITH PFBC

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Conflict of Interest

The authors declare no conflict of interest.

Introduction

Primary Familial Brain Calcification (PFBC) is a rare genetic disorder characterized by bilateral brain calcification, affecting primarily the basal nuclei, and mostly caused by mutations inherited in an autosomal dominant manner. This disorder can also be inherited in an autosomal recessive manner or be caused by *de novo* mutations, but a large part of patients clinically diagnosed with PFBC does not have a determined genetic cause.

PFBC diagnosis depends not only on the detection of bilateral brain calcifications, but most importantly on the coupling of this finding to the absence of any metabolic, traumatic or infectious causes.

Several studies analyzing the clinical presentations of PFBC patients have been performed, but focusing only on patients that have had the PFBC diagnosis genetically confirmed by the detection of mutations on known genes (*SLC20A2*, *PDGFB*, *PDGFRB* or *XPR1*). These studies have provided useful information to the medical community regarding how each type of patient is more likely to present, how much the age of onset varies between groups, and even how they respond to treatment. As an example, it is now known that PFBC patients with mutations on *SLC20A2* tend to have higher total calcification scores when compared to other groups, and that symptoms tend to vary widely both between and within families. (NICOLAS et al., 2015; RAMOS et al., 2018)

However, although several studies analyzing PFBC's main signs and symptoms have been performed with genetically confirmed PFBC patients, those who do not have pathogenic variations on the 5 genes linked to the disease are mostly excluded from the analyses. Due to 40% of PFBC's total patients being classified as not genetically confirmed cases, studies that focus on these cases are necessary to hopefully obtain more information about their main clinical presentations, progression and how they differ to the group of genetically confirmed PFBC patients. (OLIVEIRA; OLIVEIRA, 2016) Additionally, further genetic screening is important to look for genetic causes to the cases that are still considered as not genetically confirmed. (WESTENBERGER; KLEIN, 2014)

This way, the aim of this study was to evaluate the clinical, radiological and biochemical profiles of individuals with brain calcifications that do not have any variations on PFBC related genes. Additionally, the phenotypes of genetically confirmed and not genetically confirmed PFBC patients was compared to look for similarities and, most importantly, disparities between both groups.

Methods

Patients

Medical records were obtained from patients referred to the PFBC study group at the Federal University of Pernambuco (Recife, Brazil), who were diagnosed with bilateral brain calcifications, detected either by CT scans or MRI. Exclusion of patients based on biochemical assessment was not performed at this point as a way to increase sample size, while allowing for the screening of patients with different underlying conditions. Therefore, even though the study became less specific for PFBC, it allowed for the identification of comorbidities affecting those who have brain calcification. Selected patients signed written informed consent forms, and the project was approved under protocols CAAE-0296.0.172.000-08 and 09475912.8.0000.5208.

Genetic Screening

DNA obtained from blood samples was used for Sanger sequencing, primarily of *SLC20A2*, *PDGFB*, *PDGFRB* and *XPR1*, four genes previously involved with PFBC. (KELLER et al., 2013; LEGATI et al., 2015; NICOLAS et al., 2013; WANG et al., 2012) Diagnosis of genetically confirmed PFBC was done when pathogenic variations were found in any of these genes, as previously described (GC-PFBC patients). (NICOLAS et al., 2015) Those who did not have any pathogenic variations to the four PFBC linked genes were included in this study as not-genetically confirmed PFBC (NGC-PFBC patients). Further genetic screening in the included patients was performed for *PDGFC* and *PDGFD*, two genes similar in structure and function to *PDGFB*, and *PCDH12*, a gene that has been previously linked to different types of inherited brain calcifications. (LEWANDOWSKI et al., 2016; NICOLAS et al., 2017)

Clinical Assessment

Each patient’s medical records were obtained from different Brazilian hospitals, causing the files to vary in its components due to a lack of a standardized system in the country. Most of those, however, were comprised of imaging and biochemical exams, as well as some level of clinical examination notes.

For the clinical analysis, information such as sex, types of signs and symptoms reported, comorbidities, age at onset, age at last CT scan available and neuroimaging results were collected from the medical records. Signs and symptoms classification were loosely based on the list provided by Manyam, Walters and Narla in 2001, and further used by (NICOLAS et al., 2015), and depended largely on the information available in each patient's records. Similar to those studies, patients were considered to be symptomatic even if diagnosed with only one of the list's signs. Analysis of hematological and biochemical parameters was performed in those who had the files available. Parameters such as serum calcium, parathormone, phosphorus and vitamin D were looked at to possibly differentiate any cases of hypoparathyroidism, pseudohypoparathyroidism, vitamin D deficiency and calcium metabolism abnormalities. (CASTRO; BORBA, 1999)

Statistical Analysis

Continuous data are expressed as mean and percentages of the total, and t-tests were used to determine differences between groups. Categorical data, such as frequency of symptoms reported and brain regions affected by calcification, are expressed as percentages, and the chi-square test for equality of proportions was used to assess differences between groups. Logistic regression and Spearman's correlation coefficient were used to examine the existence of associations between age at last CT scan and number of brain regions affected by calcification. Statistical significance was defined as $p<0.05$. All statistical tests were performed of RStudio software, version 1.1.456. Graphs shown here were plotted on Microsoft Excel.

Results and Discussion

A total of 38 patients with brain calcifications were included in this study. Of these, twelve (31.6%) had been previously diagnosed with GC-PFBC, carrying mutations on either *SLC20A2* or *PDGFB*. (FERREIRA et al., 2014; KELLER et al., 2013; RAMOS et al., 2018; WANG et al., 2012) The remaining patients (26, 68.4%) did not carry any pathogenic variations in any of the PFBC related genes.

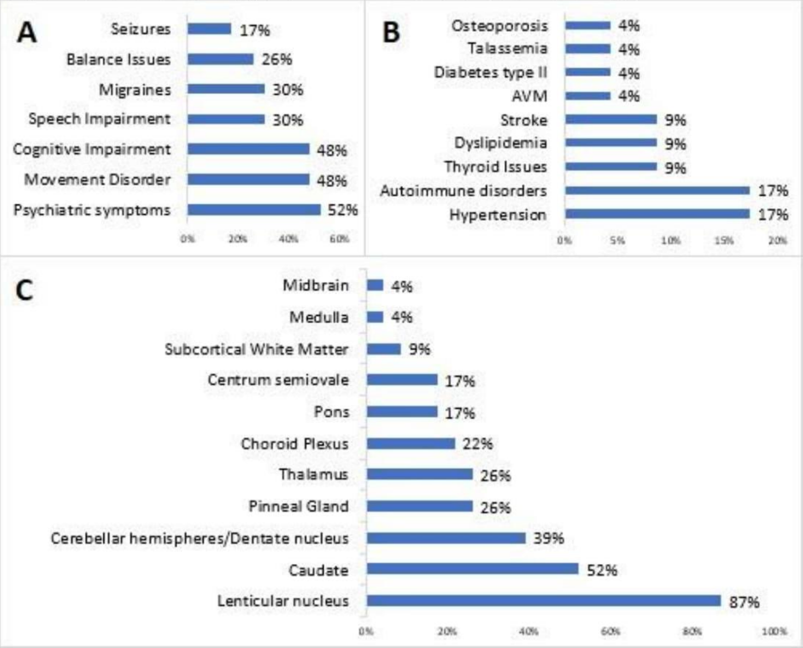
Analysis of biochemical parameters was performed next. Parameters available for evaluation varied between patients, but those who are directly involved with calcium metabolism were prioritized, such as serum calcium, serum phosphate, 25-OH vitamin D, alkaline phosphatase and parathormone (PTH). Although most patients had regular levels of the parameters, two presented high levels of PTH (1318 pg/mL and 358.8 pg/mL) accompanied by low levels of serum calcium (5.5 mg/dL and 5.3 mg/dL). Combined with the clinical information of both patients (who had a history of chorea, athetosis and dystonia, and repetitive seizures, respectively), both were diagnosed with pseudohypoparathyroidism. (CASTRO; BORBA, 1999) Additionally, one patient's clinical profile was consistent with the phenotype described in Primrose Syndrome. Therefore, another diagnostic approach was used for this case, resulting in the detection of a missense mutation in the ZBTB20 gene (c.1873A>G, p.Met625Val) and confirmation of the Primrose Syndrome diagnosis (see topic 4.2). The remaining 23 (60.5%) patients were labeled as NGC-PFBC.

NGC-PFBC Characterization and Genetic Testing

Out of the NGC-PFBC patients, seventeen (73.9%) were female and six were male (26.1%). All patients were symptomatic, and the overall age at onset ranged from 4 to 88 years of age (mean = 32.11), with a 37 mean for females and 23 for males (not significant - NS).

Psychiatric symptoms were the category of symptoms most frequently reported in the NGC-PFBC group (12/23), directly followed by movement disorders and cognitive impairment (11/23 each). (Figure 1A) The most commonly reported psychiatric symptoms were depression (2/12) and hallucinations (3/12); within movement disorders we have included gait difficulties (8/11), tremors (5/11), myoclonus (2/11) and others; finally, cognitive impairment is a broad category that includes symptoms ranging from memory deficits to dementia. Other signs and symptoms categories that were also reported by patients were speech impairment (7/23), self-proclaimed migraines (7/23), dizziness/balance issues (6/23), and seizures (4/23).

Figure 1 – Clinical characterization of not genetically confirmed PFBC patients. (A) Signs and symptoms. (B) Comorbidities reported by patients. (C) Frequency of calcifications reported in different brain regions. AVM – Arteriovenous malformation.



Regarding comorbidities, 10 out of the 23 patients included reported suffering from one or more of the conditions shown in Figure 1B. Hypertension and, interestingly, auto-immune disorders were the two types of comorbidities most frequently reported by patients (4/23 each). Among the auto-immune conditions, lupus was reported twice.

Analysis of patients CT scans, MRIs and medical records revealed that the area of the brain most commonly affected by ectopic calcification was the lenticular nucleus (20/23), followed by caudate (12/23) and cerebellar hemispheres/dentate nucleus (9/23). The maximum amount of brain regions calcified in a patient was 8, with a median of 3 and mean of 3.5.

A positive, although weak correlation was seen between the age at the last CT scan and the number of brain regions calcified (r=0.32, r^2=0.1), suggesting that older patients tend to have more calcifications than younger patients.

Finally, genetic screening for pathogenic variants in *PCDH12*, *PDGFC* and *PDGFD* was performed in 11 of the 23 NGC-PFBC patients and no damaging variations were found.

GC-PFBC Characterization

Genetically confirmed patients were equally divided in males and females; 3 (25%) of these patients, who are members of the same family carry, *PDGFB* mutations, while the remaining patients (75%) all carry *SLC20A2* mutations. All patients here included as GC-PFBC have been part of previous studies. (FERREIRA et al., 2014; KELLER et al., 2013; RAMOS et al., 2018; WANG et al., 2012) All patients were symptomatic, and the mean age of onset was 36 (10-80 years of age). No significant difference was seen in the age at onset or in the amount of brain areas calcified between genders.

Symptoms most frequently reported were balance issues (7/12, 58.3%), movement disorder (6/12, 50%) and psychiatric symptoms (4/12, 33.3%). Brain areas affected by calcifications included lenticular nucleus and caudate (7/12, 58.3% each), cerebellar hemispheres/dentate nucleus and subcortical while matter (6/12, 50% each), and thalamus (5/12, 41.6%), followed by pineal gland, choroid plexus, pons and centrum semiovale. Finally, and similar to what was seen in the NGC-PFBC group, a weak and positive correlation was seen between the age at the last CT scan and the number of brain regions calcified ($r=0.37$, $r^2=0.14$).

Comparison between GC-PFBC and NGC-PFBC groups

There were no statistically significant differences between ages at onset between groups, nor in the amount of brain areas calcified ($p>0.05$). Although clinically it seems like both groups present very similarly, statistical differences were detected in cognitive impairment, where 8% of GC-PFBC and 48% NGC-PFBC were affected ($p=0.019$), and in balance issues, reported in 58% of GC-PFBC and 26% of NGC-PFBC ($p=0.032$). Overall, however, both groups descriptions regarding mean and range of age at onset, and most common signs and symptoms reported were very similar to those described in previous studies. (NICOLAS et al., 2015; RAMOS et al., 2018)

Regarding imaging exams, the brain areas most commonly affected by ectopic calcifications were the same in both groups, suggesting that PFBC affects the brain in similar manners regardless of its genetic etiology. It is important to point out that this analysis did not evaluate how affected each of the brain areas was, as this was intended to be a qualitative study and no score was attributed to the each of lesions. Taking this into consideration, subcortical white matter was more commonly affected in GC-PFBC patients than in NGC-PFBC patients of our study sample (50% and 8%, respectively; $p=0.005$).

Conclusion

To our understanding, this is the first study to phenotypically characterize PFBC patients that do not carry mutations on *SLC20A2*, *PDGFB*, *PDGFRB* or *XPR1*. Although no major statistically significant differences were observed between our study groups, we believe it is important to look at NGC-PFBC patients as they are usually excluded from studies that evaluate PFBC's clinical profiles. Therefore, we believe studies such as this are essential not only to contribute to a comprehensive understanding of PFBC's clinical presentations, but also to provide information on a large parcel of patients affected by this condition. Finally, due to the small sample of this study, replications of this analysis in larger samples are encouraged to ensure correct conclusions about how PFBC patients without genetic confirmation present clinically and radiologically.

Acknowledgements

We would like to thank the patients for their participation in the study. L.D.F. received a fellowship from Fundação de Amparo à Ciência e Tecnologia de Pernambuco (FACEPE). J.R.M.O received funding from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Process Number 310150/2016-7). This study makes use of clinical data from patients that have been previously diagnosed with PFBC, and whose case reports have been previously published. Citations are included in the text to appropriately reference all original work. This way, we would like to thank R. Gitirana, L. Pimentel, J. Ferreira, E. Santos-Júnior and R.L. Borges-Medeiros for having previously contributed to the genetic triage of these patients.

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4.2 ARTIGO 2 – A ZBTB20 HETEROZYGOUS VARIANT LINKED TO A CASE OF PRIMROSE SYNDROME WITH NOVEL CLINICAL FINDINGS

TITLE: A *ZBTB20* HETEROZYGOUS VARIANT LINKED TO A CASE OF PRIMROSE SYNDROME WITH NOVEL CLINICAL FINDINGS

Small title: A *ZBTB20* gene variation causes Primrose syndrome

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Conflict of Interest

The authors declare no conflict of interest.

ABSTRACT

Primrose syndrome (PRIMS), a rare genetic disorder with several clinical findings including intellectual disability, macrocephaly, typical facial features, and progressive muscle wasting, is caused by *de novo* variations in the *ZBTB20* gene. We report a 28-year-old Brazilian male, who has a heterozygous missense variation in *ZBTB20* (c.1873A>G, p.Met625Val) and presents clinical features consistent with PRIMS. Additionally, the patient has Morton's toe, persistence of the cavum septi pellucidi and carvum vergae, and diminished body height and weight, features yet unreported in PRIMS.

Keywords: Primrose syndrome; ZBTB20; calcified pinnae; developmental delay; brain calcification; intellectual disability.

INTRODUCTION

Primrose syndrome (PRIMS; OMIM #259050) is a very rare autosomal dominant disorder described for the first time in 1982 [1]. This syndrome has a variable phenotype characterized by muscle wasting, cognitive deficiency, hearing loss, post-natal overgrowth, and characteristic facial features. Calcified external ears is often present [2-5].

In 2014, Cordeddu and colleagues described heterozygous variants in *ZBTB20* (most confirmed as *de novo*) in eight patients previously diagnosed with PRIMS. Additional screenings from other groups reinforced that, and until now there are a total of 20 *ZBTB20* variants linked to new patients [2–4, 6-9].

ZBTB20, located on 3q13.31, encodes a zinc finger protein that contains a highly conserved BTB domain at N-terminus and five C2H2 zinc fingers domains (ZnF) at C-terminus [10]. It plays an essential role as transcriptional repressor interacting with genomic DNA by its ZnF domains, but has also been implicated in regulating glucose homeostasis, immune responses, and development [10, 11].

We report here a 28-year-old male with PRIMS diagnosis harbouring variation in *ZBTB20* gene and different clinical features than those already described, including Morton's toe, persistence of the cavum septi pellucidi and carvum vergae, and diminished body height and weight. This is the second reported case of PRIMS in Brazil.

SUBJECTS AND METHODS

Studies with the patient were approved by Institutional Review Board at the registry CAAE-0296.0.172.000-08. The patient's mother provided written informed consent for genetic testing, publication of clinical pictures and authorized the processing of personal data. Genetic analysis of *ZBTB20* was performed by PCR amplification of coding exons and intron-coding exons boundaries, using primers previously reported [2], followed by direct Sanger sequencing.

RESULTS

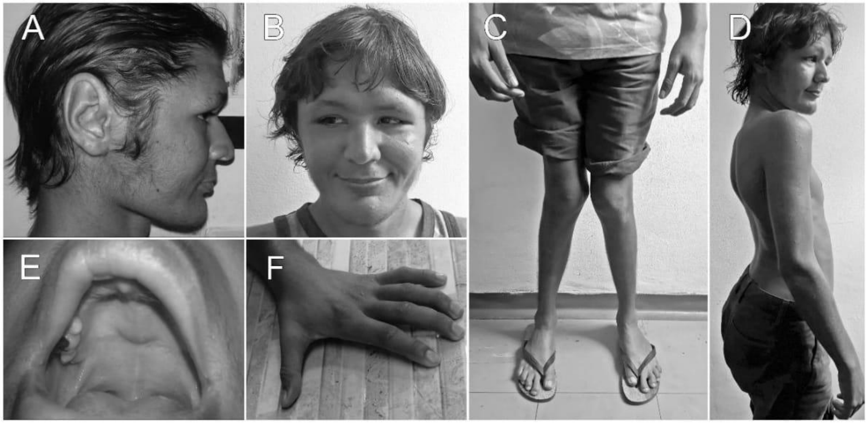
Clinical Report

Born at term from a caesarean birth after an uneventful pregnancy, the patient is the fourth child (with healthy siblings) from his unrelated mother and father, who were 41 and 39, respectively, at the time of the birth. Even though no complications happened during the procedure, it is reported that he did not cry when born (mother refers 'cyanosis'), at 3.250 kg and 48 cm.

At age 18 months doctors detected strabismus. Although no specific timeline can be established, it is known that developmental and cognitive delays were noticed by the family in the following decade. Relatives report that the patient was able to sit unassisted at 6 months, although he never crawled and walked late. Additionally, the patient was diagnosed with moderate hearing loss; he was prescribed hearing aids, but they were not worn continuously.

The patient has a series of skeletal abnormalities and behavioural issues that were noticed during his life. At age 18 the mother first noticed the distal muscle wasting, affecting his knees, shoulders, distal and proximal phalanges (Figure 1C and 1F). The condition progressively worsened and caused difficulty walking. At age 21 he presented aggressive behaviour, was diagnosed with autism spectrum disorder and treated with risperidone. impaired hearing persisted, and a systolic murmur was detected. The skeletal abnormalities previously noted by relatives during his childhood were diagnosed at this moment as lumbar hyperlordosis and genu valgum (Figure 1C and 1D), prompting his referral to physical therapy.

Figure 1. Pictures of the patient displaying brachycephalic head shape, large calcified ears and jaw (A); Deep set eyes, downslanted palpebral fissures, ptosis, wide forehead, and prominent lower lip (B); Genu valgum, thin limbs and Morton's toe (C); Hyperlordosis (D); Torus palatinus (E); and shortening of muscles, causing ulnar claw (F).



A neuroimaging study (CT and MRI) at age 21 detected diminished gyri in the frontal lobe, persistence of the *cavum septi pellucidi* (CSP) and *cavum vergae* (CV) cyst, and bilateral basal ganglia calcifications. Six years later, calcified pinnae were noticed during a routine consultation.

Regarding growth, the patient did not grow as expected for a child his age. At age 1, he weighed 10kg (65th percentile) and measured 83 cm (100th percentile); At age 3, he weighed 13 kg (21st percentile) and measured 90 cm (5th percentile); Finally, at age 6, the patient weighed 19kg (21th percentile) and measured 98 cm (0.2nd percentile). At the age of 27 he weighed 53 kg and his height was 1,65 m, with normal skull circumference (58 cm). On physical exam, the patient has brachycephaly, wide forehead, ptosis, deep set eyes, torus palatinus and sparse body hair (Figure 1A, 1B, 1D, 1E). Though unrelated, Morton's toe was also seen (Figure 1C). He also showed mutism, developing communication by mimicry.

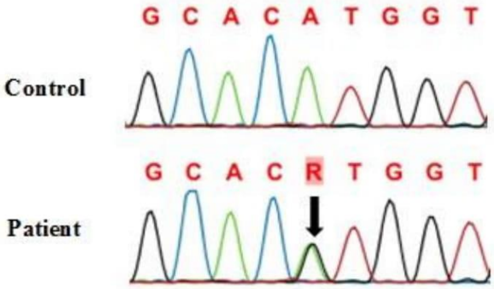
Biochemical analysis such as parathormone, calcium, phosphorus, thyroid-stimulating hormone, free T3 and T4 was normal, but he showed a slight increase in fasting blood glucose (Addison's disease was excluded), and the patient had positive IgG for cytomegalovirus and toxoplasmosis. Electroencephalogram and electroneuromyography were normal.

Considering all clinical features, Primrose Syndrome was considered a probable diagnosis. Sanger sequencing identified a heterozygous missense variation c.1873A>G in the coding exon 4 of the *ZBTB20* (NM_001164342.2). This variation predicts a methionine to valine substitution in position 625 of the protein (p.Met625Val, NP_001157814.1), at the second zinc finger domain. *In silico* assessment by PolyPhen-2, SIFT and MutationTaster suggested the p.Met625Val to be likely pathogenic, damaging and probably deleterious, respectively. This variation was not found in ExAC Browser, 1000 Genome Project, gnomAD, or ABraOM. Curiously, that variant was previously deposited in NCBI's ClinVar and dbSNP database (rs1064795382), even though the clinical data was not publicly available.

Molecular findings

We identified a heterozygous missense variation c.1873A>G in the coding exon 4 of the *ZBTB20* (NM_001164342.2) (Figure 2). This variation predicts a methionine to valine substitution in position 625 of the protein (p.Met625Val, NP_001157814.1), at the second zinc finger domain. *In silico* assessment by PolyPhen-2, SIFT and MutationTaster suggested the p.Met625Val to be likely pathogenic, damaging and probably deleterious, respectively.

Figure 2. *ZBTB20* gene variation in genomic DNA of the patient and control subject. Electropherograms showing a heterozygous missense c.1873A>G variation in *ZBTB20* (arrow) (NM_001164342.2) (below), and absent in wild-type control (above).



This variation was not found in ExAC Browser, 1000 Genome Project, gnomAD, or ABraOM. Curiously, that variant was previously deposited in NCBI's ClinVar and dbSNP database (rs1064795382), even though the clinical data was not publicly available.

DISCUSSION

PRIMS is a very rare genetic condition with several clinical variable presentations, including intellectual disability, progressive muscle wasting, brain calcification, calcified pinnae and various dysmorphic features, some of them only detectable more clearly at the second decade of life [2, 5, 6]. Over 60% of variants reported are localized in ZnF domains of the *ZBTB20*, and predicted to alter their binding affinity to DNA [2–4, 6, 7, 9]. These evidences might show that variations that alter the wild type ZnF domains in *ZBTB20* are more likely to affect protein structure and function in a more pathogenic manner than the variations in other sites, as BTB domain.

The patient here reported has a heterozygous missense variation c.1873A>G (p.Met625Val) in the last coding exon of the *ZBTB20*. The same variation was previously detected and deposited to NCBI's ClinVar and dbSNP databases (rs1064795382). There is no available clinical information about the patient that can be associated to that variation. However, it is extremely interesting that the same missense *ZBTB20* variation would happen in two unrelated individuals, as this would represent only the second time this happens in the context of *ZBTB20* variations associated to PRIMS [2, 12].

Similarly, independent occurrences of four variations in two different codons of *ZBTB20* have been previously reported affecting four unrelated patients with PRIMS [2, 6, 7, 9]. It is also important to highlight that nine novel pathogenic mutations in ZBTB20 were recently reported in DECIPHER database, however, the patients remain officially undiagnosed [13]. Many of the clinical features available are associated with PRISM, which shows the potential of reverse phenotyping in the recent scenario of large scale sequencing in patients with neurodevelopmental disorders of unknown etiology. Additionally, the possibility of pleiotropism should be considered and *ZBTB20* could be involved in conditions other than PRISM.

So far, increased postnatal growth of affected individuals has been widely described as one of the hallmarks of this disorder [4]. Interestingly, the patient described here does not have increased weight or height. In fact, data gathered from his childhood shows he was under the 3rd percentile for growth at age 6 while only reaching the 25th percentile for his weight. At the age of 27 he weighed 53 kg and his height was 1,65 m, a clear difference when compared to previously reported PRIMS patients [3-5].

Additionally, there was visualization of CSP and CV cyst on both brain magnetic resonance image and CT scans, as well as Morton's toe upon clinical evaluation. Although these structures might be considered to be anatomical variants, this is the first report of such findings in a patient with PRIMS and could be related to the disorder [9, 15]. Furthermore, the patient presents with elevated fasting blood glucose, in agreement with findings in other PRIMS patients [2].

This case of PRIMS highlights the importance to go beyond metabolic and infectious causes when diagnosing developmental disorders. Additionally, the patient did not meet all the clinical criteria previously described as essential for PRIMS diagnosis, but sequencing confirmed the presence of a variation on *ZBTB20*. Therefore, additional studies including more patients are necessary not only to ensure correct clinical descriptions of the syndrome, but also to help determine its true frequency.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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5 CONSIDERAÇÕES FINAIS

Dado o exposto, conclui-se que é possível que pacientes com CCFP confirmada geneticamente podem diferir clinicamente e radiologicamente de pacientes com CCFP não confirmada geneticamente. É necessário, entretanto, que estudos futuros incluam um maior número de pacientes já que, devido à hetereogeneidade clínica da CCFP, diferentes grupos de pacientes apresentam diferentes frequências de sintomas. Desse modo, estudos com mais pacientes podem garantir uma melhor representação da população afetada com CCFP como um todo, permitindo a adoção de abordagens terapêuticas que beneficiem o máximo de pacientes possível.

Além disso, o presente trabalho demonstra a importância do diagnóstico diferencial para casos suspeitos de CCPF. A detecção das lesões de calcificação cerebral não deve ser considerada como aspecto único para definição de diagnósticos, de modo que a prática clínica deve focar em uma avaliação completa que envolva a obtenção de informações clínicas, bioquímicas, hematológicas, radiológicas e genéticas quando possível. Apesar disso, e considerando as limitações financeiras e estruturais do sistema público de saúde brasileiro, o conhecimento dos principais quadros a serem considerados durante o diagnóstico diferencial da CCFP já é suficiente para auxiliar o direcionamento clínico, garantindo assim as melhores abordagens terapêuticas para cada caso.

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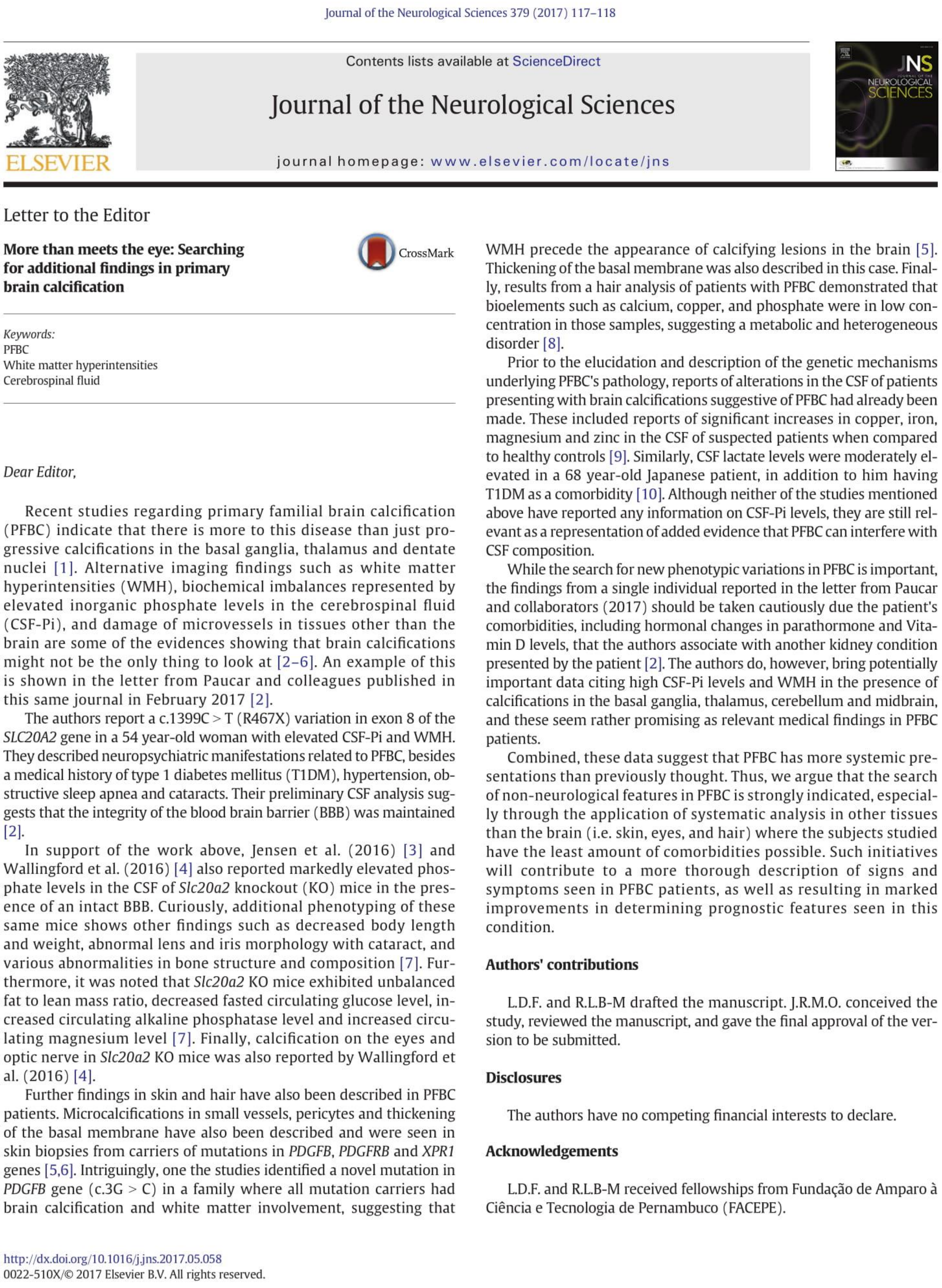
APÊNDICE A – CARTA PUBLICADA NO JOURNAL OF THE NEUROLOGICAL SCIENCES



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Letter to the Editor

More than meets the eye: Searching for additional findings in primary brain calcification

Keywords:
PFBC
White matter hyperintensities
Cerebrospinal fluid

Dear Editor,

Recent studies regarding primary familial brain calcification (PFBC) indicate that there is more to this disease than just progressive calcifications in the basal ganglia, thalamus and dentate nuclei [1]. Alternative imaging findings such as white matter hyperintensities (WMH), biochemical imbalances represented by elevated inorganic phosphate levels in the cerebrospinal fluid (CSF-Pi), and damage of microvessels in tissues other than the brain are some of the evidences showing that brain calcifications might not be the only thing to look at [2–6]. An example of this is shown in the letter from Paucar and colleagues published in this same journal in February 2017 [2].

The authors report a c.1399C > T (R467X) variation in exon 8 of the *SLC20A2* gene in a 54 year-old woman with elevated CSF-Pi and WMH. They described neuropsychiatric manifestations related to PFBC, besides a medical history of type 1 diabetes mellitus (T1DM), hypertension, obstructive sleep apnea and cataracts. Their preliminary CSF analysis suggests that the integrity of the blood brain barrier (BBB) was maintained [2].

In support of the work above, Jensen et al. (2016) [3] and Wallingford et al. (2016) [4] also reported markedly elevated phosphate levels in the CSF of *Slc20a2* knockout (KO) mice in the presence of an intact BBB. Curiously, additional phenotyping of these same mice shows other findings such as decreased body length and weight, abnormal lens and iris morphology with cataract, and various abnormalities in bone structure and composition [7]. Furthermore, it was noted that *Slc20a2* KO mice exhibited unbalanced fat to lean mass ratio, decreased fasted circulating glucose level, increased circulating alkaline phosphatase level and increased circulating magnesium level [7]. Finally, calcification on the eyes and optic nerve in *Slc20a2* KO mice was also reported by Wallingford et al. (2016) [4].

Further findings in skin and hair have also been described in PFBC patients. Microcalcifications in small vessels, pericytes and thickening of the basal membrane have also been described and were seen in skin biopsies from carriers of mutations in *PDGFB*, *PDGFRB* and *XPR1* genes [5,6]. Intriguingly, one the studies identified a novel mutation in *PDGFB* gene (c.3G > C) in a family where all mutation carriers had brain calcification and white matter involvement, suggesting that WMH precede the appearance of calcifying lesions in the brain [5]. Thickening of the basal membrane was also described in this case. Finally, results from a hair analysis of patients with PFBC demonstrated that bioelements such as calcium, copper, and phosphate were in low concentration in those samples, suggesting a metabolic and heterogeneous disorder [8].

Prior to the elucidation and description of the genetic mechanisms underlying PFBC's pathology, reports of alterations in the CSF of patients presenting with brain calcifications suggestive of PFBC had already been made. These included reports of significant increases in copper, iron, magnesium and zinc in the CSF of suspected patients when compared to healthy controls [9]. Similarly, CSF lactate levels were moderately elevated in a 68 year-old Japanese patient, in addition to him having T1DM as a comorbidity [10]. Although neither of the studies mentioned above have reported any information on CSF-Pi levels, they are still relevant as a representation of added evidence that PFBC can interfere with CSF composition.

While the search for new phenotypic variations in PFBC is important, the findings from a single individual reported in the letter from Paucar and collaborators (2017) should be taken cautiously due the patient's comorbidities, including hormonal changes in parathormone and Vitamin D levels, that the authors associate with another kidney condition presented by the patient [2]. The authors do, however, bring potentially important data citing high CSF-Pi levels and WMH in the presence of calcifications in the basal ganglia, thalamus, cerebellum and midbrain, and these seem rather promising as relevant medical findings in PFBC patients.

Combined, these data suggest that PFBC has more systemic presentations than previously thought. Thus, we argue that the search of non-neurological features in PFBC is strongly indicated, especially through the application of systematic analysis in other tissues than the brain (i.e. skin, eyes, and hair) where the subjects studied have the least amount of comorbidities possible. Such initiatives will contribute to a more thorough description of signs and symptoms seen in PFBC patients, as well as resulting in marked improvements in determining prognostic features seen in this condition.

Authors' contributions

L.D.F. and R.L.B-M drafted the manuscript. J.R.M.O. conceived the study, reviewed the manuscript, and gave the final approval of the version to be submitted.

Disclosures

The authors have no competing financial interests to declare.

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APÊNDICE B – ARTIGO PUBLICADO NO THE JOURNAL OF NEUROPSYCHIATRY AND CLINICAL NEUROSCIENCE

OPINION

The Need for Consensus on Primary Familial Brain Calcification Nomenclature

Laura Durão Ferreira, B.Sc., João Ricardo Mendes de Oliveira, M.D., Ph.D.

Primary familial brain calcification is characterized by the presence of symmetrical and bilateral brain calcifications, mainly on the basal ganglia but also on other brain structures, such as the thalamus, cerebellum, and cerebral white matter.[1] Over the years, a variety of names have been associated with this disease, and the commonly used eponym, "Fahr's disease," has slowly been substituted by other terms, following a tendency seen in medicine as a whole.[2,3]

Although Fahr's disease was first described in 1850 in France, its name refers to Karl Theodor Fahr, the German physician who described the disorder 80 years later.[4,5] (Figure 1) The eponym was used for the first time in 1951,[6] and variations of it are seen, ranging from possessive to nonpossessive forms, as well as from syndrome to disease. This results in four different variations of the original eponym: Fahr's disease, Fahr disease, Fahr's syndrome, and Fahr syndrome. Furthermore, since 1952 a large selection of terms has been used in the literature when referring to this disorder, and more recently there has been a tendency to move toward biologically based names.[3,7]

The diminished use of eponyms is not exclusive to Fahr's disease. Although common in the past, it has fallen in popularity among researchers and physicians around the world for several reasons. The main reason is that in recent years the scientific community has mostly agreed that disease names should focus primarily on conveying the underlying cause of the disease, not on the physician who first described it.[3] Some disadvantageous aspects related to the use of eponyms include the possibility of several names being attributed to a single disorder, which can generate confusion, as well as the use of a single eponym to describe different disorders.[2] In addition, some eponyms are being avoided due to the connection between the physicians honored and unethical practices.[8] On the other hand, it can be argued that the use of eponyms makes referring to a disease easier—especially for patients—saves physicians time, and prevents misunderstanding.[9,10] Some well-known examples of commonly used eponyms include Alzheimer's, Parkinson's, and Huntington's disease, and there is no indication that these terms will be discontinued any time soon. Regardless, the use of the correct term when referring to a disease is important not only to ensure that no confusion is generated between health care providers and researchers but also in order to represent the disease in its entirety.[11]

With regard to Fahr's disease, the names used from 1951 until now include, but are not limited to, the four Fahr eponyms, idiopathic basal ganglia calcification,[12] and primary familial brain calcification,[13] resulting in a total of more than 35 different names.[14] The terms mentioned above are the most commonly used, and there appears to be no exact criteria to distinguish them or to define when one is used over the others. Use of the correct nomenclature is especially important for this disease, because the term used has the capacity to identify whether the patient's calcifications developed primarily, when they can be attributed to genetics, or whether they developed secondarily in response to traumatic, metabolic, or infectious stimuli.[1]

Taking this into consideration, here we aimed to compare the use of different terms used when referring to primary familial brain calcification throughout the years and, possibly, to identify a tendency from the research community to abolish or adopt a specific term.

METHODS

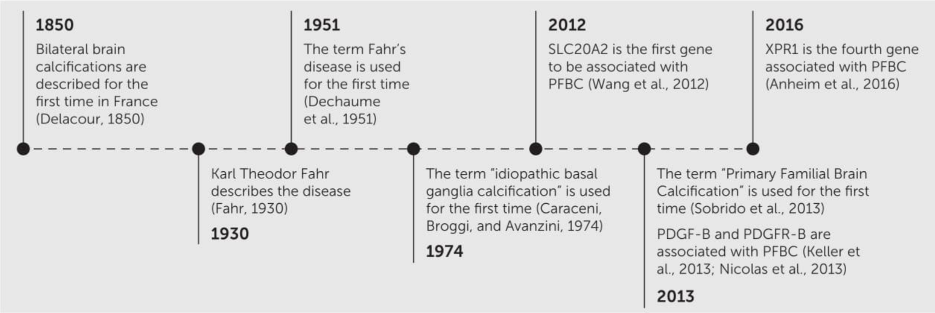
In January 2018, we searched PubMed for Fahr eponyms (Fahr's disease and syndrome, possessive and nonpossessive forms), as well as for the terms "primary familial brain calcification" and "idiopathic basal ganglia calcification." To ensure that the data analyzed reflect the scientific production of an entire year, studies dated to 2018 were not included in the analysis. Papers in all languages available on the platform were included, while abstracts of work presented at conferences, symposiums, and similar events were not included. Graphs were plotted by using Microsoft Excel.

RESULTS

Overall, our results showed that Fahr eponyms and the term idiopathic basal ganglia calcification have been used more than the term primary familial brain calcification (Figure 2). This was expected, because the term primary familial brain calcification was introduced in 2013,[13] while Fahr eponyms were first used in 1951[6] and idiopathic basal ganglia calcification in 1974,[12] as shown in the timeline displayed in Figure 1.

Since the introduction of the term primary familial brain calcification in 2013, use of the term in scientific papers has

FIGURE 1. Timeline of the Most Important Events Since the First Description of Primary Familial Brain Calcification (1850–2016).



1850
Bilateral brain calcifications are described for the first time in France (Delacour, 1850)

1930
Karl Theodor Fahr describes the disease (Fahr, 1930)

1951
The term Fahr's disease is used for the first time (Dechaume et al., 1951)

1974
The term "idiopathic basal ganglia calcification" is used for the first time (Caraceni, Broggi, and Avanzini, 1974)

2012
SLC20A2 is the first gene to be associated with PFBC (Wang et al., 2012)

2013
The term "Primary Familial Brain Calcification" is used for the first time (Sobrido et al., 2013)
PDGF-B and PDGFR-B are associated with PFBC (Keller et al., 2013; Nicolas et al., 2013)

2016
XPR1 is the fourth gene associated with PFBC (Anheim et al., 2016)

increased, indicating the acceptance of the term by researchers and physicians. This is simultaneous to decline in the use of Fahr eponyms. However, to date, these eponyms are used more than any of the alternative terms (Figure 3). Moreover, until 2017, the term idiopathic basal ganglia calcification was used more than the term primary familial brain calcification and has been used in studies in which mutations are identified, although this contradicts use of the term "idiopathic." Nevertheless, a decline in the use of the term idiopathic basal ganglia calcification can be observed, which is a positive sign and indicates that more thought is being put into choosing the correct terminology (Figure 3).

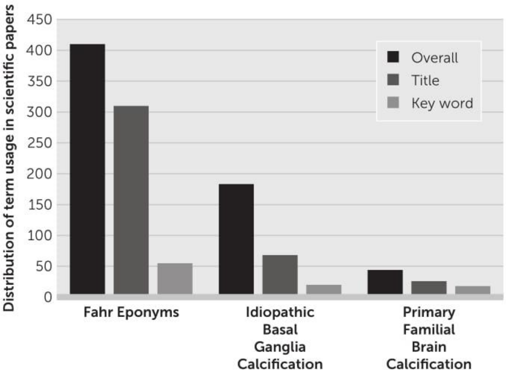
CONCLUSIONS

It is our position that there is no foreseeable end to the discussion regarding which term—primary familial brain calcification or idiopathic basal ganglia calcification—is the most appropriate. Considering that there is currently no exact definition for either term, it is easy to understand why they are used interchangeably. Until a neurology or psychiatry institution formally approves one or more terms, the speculation will continue, and each specialty group will use the term they judge best. We preferentially use the term primary familial brain calcification, because we believe it to be the most appropriate to designate brain calcifications of genetic origin that occur in the absence of any metabolic, infectious, or traumatic stress. Regardless, it is important to conduct a critical analysis, especially regarding the use of terms such as "idiopathic," "primary," and "familial," to ensure that the disease is not misconstrued on the basis of the name alone.

Additionally, we would like to propose a variation of the term primary familial brain calcification, since it currently excludes cases of de novo mutations. We believe that a term such as "primary bilateral brain calcification" can be used for both inherited and sporadic cases, which in turn facilitates differentiation between primary and secondary cases as a whole. Moreover, the term highlights the disease's main finding (calcification in both cerebral hemispheres) and does not erroneously indicate that the lesions are restricted to a specific group of structures, as does idiopathic basal ganglia calcification. The proposed term can encompass the variations—"familial primary bilateral brain calcification," "sporadic primary bilateral brain calcification," and "idiopathic primary bilateral brain calcification "—when it is necessary to further define whether a genetic mutation has been found. Although the first two terms would refer to familial and de novo cases, respectively, the latter should only be used in cases in which all appropriate genetic screening has been performed and no pathologic variants are detected.

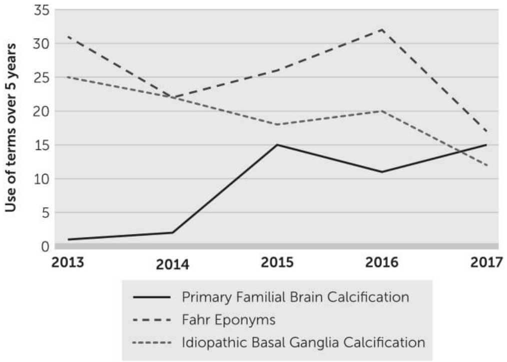
Thus, we believe that primary bilateral brain calcification and its subcategories are good alternatives to the current denominations of primary familial brain calcification. Taken together, these terms represent the disease as a whole while clearly differentiating patients with known mutations from those whose genetic base for the disease is unidentified. Only in time will it be possible to determine whether the scientific community elects these terms as the most suitable, whether older terms such as primary familial brain calcification

FIGURE 2. Distribution of Term Usage for Primary Familial Brain Calcification, Idiopathic Basal Ganglia Calcification, and Fahr Eponyms in Scientific Articles[a]



[a] Search of articles was conducted using PubMed.

FIGURE 3. Use of Various Terms Over a 5-Year Period (2013–2017)[a]



[a] Use of the terms primary familial brain calcification and idiopathic basal ganglia calcification, as well as Fahr eponyms; search of articles was conducted using PubMed.

remain in use, or whether new options are proposed. Either way, it is important to focus on choosing clear and straightforward terms that will facilitate communication and improve understanding in the field.

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APÊNDICE C – CO-AUTORIA DE PÔSTER APREENTADO EM SIMPÓSIO (VIII SINATER)

TITLE: SEARCHING FOR NEW GENES LINKED TO PRIMARY BRAIN CALCIFICATIONS

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INTRODUCTION: Primary brain calcification (PBC) is a rare neuropsychiatric condition that might be inherited in an autosomal dominant pattern or even caused by *de novo* mutations. Symmetrical and bilateral calcifications located mainly in basal ganglia, thalamus and cerebellum are detected through neuroimaging screening. The symptoms are unspecific and might include parkinsonism, psychosis, depression, and ataxia. Recent studies found mutations in four genes (*SLC20A2*, *PDGFRB*, *PDGFB* and *XPR1*), however they represent only 60% of all familial cases, suggesting that additional genes remain to be elucidated **OBJECTIVE:** We report our progress in screening new candidate genes (*SLC20A1*, *PDGFA*, *PDGFC*, *PDGFD*, *PDGFRA* and *PCDH12*), variants and endophenotypes in 24 patients without mutations at the 4 genes linked to PBC. **METHODOLOGY:** The exons and intron-exons boundaries sequencing of intended genes was performed using a 3500 Genetic Analyzer and CLC Main Workbench software for the assembly **RESULTS:** Only benign variants were found in the genetic results available thus far. In total, eleven patients were screened for *PDGFA* and *PDGFC*, twelve for *PDGFD*, one for *PDGFRA*, seven for *PCDH12,* and five for *SLC20A1*. Clinically, four patients presented psychiatric symptoms, such as hallucinations and repetitive-obsessive behavior. The same amount of patients suffered with movement disorder, and three have cognitive impairment. Four out of the 24 patients report suffering from convulsions in their lifetime. Two people reported hand tremors, and one declares suffering from recurrent migraines. Interestingly, when analyzing for comorbidities, two patients report having Lupus, one reports having diabetes, and one has a likely diagnosis of Hashimoto's disease. **CONCLUSION:** Even though no pathogenic mutations have been detected in any of the patients, it is important to address that the sample size does not allow a final conclusion yet. Samples are constantly undergoing further analyses, in the hopes of increasing sample size and obtaining more results.

KEYWORDS: primary brain calcification; mutations

FINANCIAL SUPPORT: Fundação de Amparo à Ciência e Tecnologia de Pernambuco (FACEPE).

APÊNDICE D – APRESENTAÇÃO ORAL DE TRABALHO EM SIMPÓSIO (IX SINATER)

TITLE: PRIMROSE SYNDROME: A CASE REPORT OF A BRAZILIAN PATIENT WITH A RARE DISEASE CAUSED BY HETEROZYGOUS VARIANTS TO *ZBTB20*

Laura Durão Ferreira[1], Rayssa Leal Borges-Medeiros[1], João Ricardo Mendes de Oliveira[1,2]

1- Laboratory of Immunopathology Keizo Asami, Federal University of Pernambuco, Recife, Brazil.
2- Neuropsychiatry Department, Federal University of Pernambuco, Recife, Brazil.

INTRODUCTION: Primrose syndrome (PRIMS), a rare genetic disorder with several clinical findings including intellectual disability, macrocephaly, typical facial features, and progressive muscle wasting, is caused by *de novo* variations in the *ZBTB20* gene. It has less than 30 cases reported worldwide, and due to its low frequency, it is not widely known by medical professionals. We discuss here the case of a 28-year-old Brazilian male who has a medical history marked by intellectual disability, developmental delays and presents several other clinical features consistent with PRIMS. **OBJECTIVE**: To determine if the patient has a pathogenic variation to the *ZBTB20* gene, therefore confirming a PRIMS diagnosis. **METHODOLOGY:** The patient's mother provided written informed consent for genetic testing, use of clinical pictures and authorized the processing of personal data. Genetic analysis of *ZBTB20* was performed by PCR amplification of coding exons and intron-coding exons boundaries, followed by direct Sanger sequencing. **RESULTS**: Analysis of the patient's medical files showed evidence of a progressive disorder affecting global development. Other signs consistent with PRIMS phenotype included calcified pinnae, basal ganglia calcification and characteristic facial features, besides skeletal and muscle abnormalities. We identified a heterozygous missense variation in the coding exon 4 of *ZBTB20* (c.1873A>G, p.Met625Val). *In silico* assessment suggested the p.Met625Val to be likely pathogenic. **CONCLUSION**: This case of PRIMS highlights the importance of a thorough clinical assessment combined with genetic testing when diagnosing developmental disorders. Additionally, it shows how such disorders are progressive, and how many of its characteristic signs do not appear until later in life. Therefore, additional studies including more patients with Primrose Syndrome are necessary not only to ensure correct clinical descriptions of the syndrome, but also to help determine its true frequency.

KEYWORDS: Primrose syndrome; ZBTB20; calcified pinnae; developmental delay; brain calcification.

FINANCIAL SUPPORT: L.D.F. and R.L.B-M received fellowships from Fundação de Amparo à Ciência e Tecnologia de Pernambuco (FACEPE). J.R.M.O received funding from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Process Number 310150/2016-7).

APÊNDICE E – RELATÓRIO SOBRE CALCIFICAÇÃO CEREBRAL FAMILIAR PRIMÁRIA PARA A NATIONAL ORGANIZATION FOR RARE DISORDERS

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Primary Familial Brain Calcification

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Synonyms of Primary Familial Brain Calcification

- Fahr disease, formerly
- familial idiopathic basal ganglia calcification
- striopallidodentate calcinosis
- PFBC

General Discussion

Summary

Primary familial brain calcification (PFBC) is a rare neurodegenerative disorder characterized by the presence of abnormal calcium/hydroxyapatite deposits (calcifications) in the brain. The clinical presentations generally attributed to these brain calcifications are highly variable, ranging from asymptomatic patients, to severely affected patients with progressive neuropsychiatric features. To date, pathogenic mutations in five genes have been associated with PFBC: *SLC20A2*, *PDGFB*, *PDGFRB*, *XPR1* and, just recently, *MYORG*.

Introduction

Primary familial brain calcification (PFBC) has recently become the preferred name for this condition because mutations in specific genes are now known to cause the disease. Previously, familial idiopathic basal ganglia calcification was the preferred name, and Fahr's disease is often used for either familial or sporadic brain calcification. It is unknown if these are the same or different diseases.

Signs & Symptoms

PFBC is characterized by symmetric and bilateral brain calcifications mainly in the basal nuclei, but also seen in the cerebellum (dentate nucleus), thalami and/or cerebral white matter. These calcium deposits are commonly found in the fourth to fifth decade of life, while neuropsychiatric symptoms (when present) usually begin in the third to fifth decade of life. Additionally, some individuals with PFBC may be clinically asymptomatic for several decades.

Early symptoms may include clumsiness, fatigue, slow or slurred speech and difficulty swallowing (dysphagia). Progressive deterioration of mental/cognitive abilities (dementia) and loss of previous motor development are accompanied by spastic paralysis and in some patients, twisting movements of the hands and feet (athetosis). Features of Parkinson disease found in this disorder may include tremors and rigidity (Parkinsonism), a masklike facial expression, shuffling walk, and a pill rolling motion of the fingers. Muscle cramping (dystonia), uncontrollable spasmodic irregular movements (chorea), and seizures can also occur. Occasional symptoms include sensory changes, headaches and urinary incontinence.

Associated symptoms include loss of contact with reality (psychosis), mood swings and loss of acquired motor skills. As the condition progresses, paralysis may develop that is associated with increased muscle stiffness (rigidity) and restricted movements (spastic paralysis). Additional abnormalities may include relatively slow, involuntary, continual writhing movements (athetosis) or chorea, a related condition characterized by irregular, rapid, jerky movements.

A recent study indicated that Parkinsonism was the most frequent symptom in a group of 44 PFBC patients, followed by cognitive impairment, psychiatric symptoms and cerebellar signs (Ramos et al., 2018). Other analyses have also suggested that males are more severely affected than females, especially those who have *SLC20A2* gene mutations, followed by those with *PDGFB* and *PDGFRB* gene mutations (Nicolas et al., 2015).

Causes

PFBC is caused by mutations in several different genes, and it can either be inherited or develop spontaneously. In inherited cases, the vast majority follow autosomal dominant inheritance, but autosomal recessive inheritance and new gene mutations in an affected person have also been reported.

Autosomal dominant genetic disorders occur when a single copy of an abnormal gene is necessary to cause a disease. The abnormal gene can be inherited from either parent, and the risk of passing the abnormal gene from affected parent to offspring is 50% for each pregnancy, and is the same for males and females. Four different genes have been described to cause PFBC in an autosomal dominant fashion: *SLC20A2*, *PDGFB*, *PDGFRB* and *XPR1*.

Wang and colleagues (2012) reported 7 families with PFBC from China, Spain and Brazil, with different mutations in the *SLC20A2* gene on chromosome 8, encoding for a phosphate inorganic transporter (PiT-2). In the following years, more than 50 mutations in this gene have been identified, and mutations in this gene are the most common cause of PFBC (Lemos et al., 2015).

Since then, three other genes have been linked to autosomal dominant forms of PFBC: the beta subunit of platelet-derived growth factor (*PDGFB*) and its receptor (*PDGFRB*), which are involved in the blood brain barrier integrity (Nicolas et al., 2013; Keller et al., 2013); and more recently, the xenotropic and polytropic retrovirus receptor 1 (*XPR1*) gene, which is involved in intracellular phosphate homeostasis (Legati et al., 2015).

Autosomal recessive genetic disorders occur when two copies of an abnormal gene are necessary to cause a disease. The abnormal gene then has to be inherited from both parents in order to cause the disease in the offspring. So far, the only gene reported to be involved with an autosomal recessive form of PFBC was the *MYORG* gene (previously known as *KIAA1161* or *NET37*) on chromosome 9, affecting 6 unrelated Chinese families (Yao et al., 2018).

Lastly, in some individuals the disorder is due to spontaneous (*de novo*) genetic mutations that may occur in the egg or sperm cell. It is not known how many people have PFBC as a result of new gene mutations. In such situations, the disorder is not inherited from the parents, but can still be passed on to the offspring (with a 50% chance).

Affected Populations

The prevalence of PFBC is unknown, but more people are being diagnosed probably due to the growing availability of neuroimaging screening and genetic testing.

A recent study indicates that the prevalence of PFBC may be higher than what was initially thought. Through a population-based genomic analysis, the authors estimated that the prevalence of the condition ranges from 4.5/10,000 to 2.1/1,000 (Nicolas et al., 2018).

Related Disorders

Calcification of the basal nuclei in the brain is found in many medical conditions and can be caused by infections as well as metabolic and other genetic syndromes. It is also not uncommon to observe calcium deposits of the basal ganglia in individuals over 60 years of age and this finding is not usually associated with diseases. Symptoms of the following disorders may be similar to those of PFBC. Comparisons may be useful for a differential diagnosis:

Parkinson disease is a slowly progressive neurologic condition characterized by involuntary trembling (tremor), muscular stiffness or inflexibility (rigidity), slowness of movement and difficulty carrying out voluntary movements. Degenerative changes occur in areas deep within the brain (substantia nigra and other pigmented regions of the brain), causing a decrease in dopamine levels in the brain. Dopamine is a neurotransmitter, which is a chemical that sends a signal in the brain.

Hypoparathyroidism is a condition characterized by insufficient production of parathyroid hormones by the parathyroid glands, the small, oval glands located near the thyroid gland in the neck. Parathyroid hormones (along with vitamin D and the hormone calcitonin, which is produced by the thyroid gland) play a role in regulating levels of calcium in the blood. Due to deficiency of parathyroid hormones, affected individuals exhibit abnormally low levels of calcium in the blood (hypocalcemia). Symptoms and findings associated with hypoparathyroidism may include weakness, muscle cramps, excessive nervousness, headaches, and/or increased excitability (hyperexcitability) of nerves resulting in uncontrollable twitching and cramping spasms of certain muscles such as those of the hands, feet, arms, and/or face (tetany). Hypoparathyroidism may result from absence of the parathyroid glands, removal or damage to the parathyroid glands or from several different underlying disorders. In rare cases, hypoparathyroidism may be inherited in an autosomal recessive pattern. (For more information on this disorder, choose “hypoparathyroidism” as your search term in the Rare Disease Database.)

Pseudohypoparathyroidism is a hereditary disorder characterized by an inadequate response to the parathyroid hormone, although the hormone is present in normal amounts. This inadequate response affects bone growth and affected individuals may also experience headaches, unusual sensations, weakness, easy fatigue, reduced energy, blurred vision, and/or hypersensitivity to light. Additional symptoms and findings may include stiffness or cramps in the arms and/or legs, palpitations, and/or abdominal pain. In addition, individuals with pseudohypoparathyroidism may have an abnormally round face, thick short stature, unusually short fourth fingers, and intellectual disability. Hormonal and calcium replacement therapy is often helpful, but the lack of growth may persist. (For more information on this disorder, choose “pseudohypoparathyroidism” as your search term in the Rare Disease Database.)

Diagnosis

Neuroimaging techniques such as computed tomography (CT) of the brain (the most sensitive technique) and magnetic resonance imaging (MRI) are used to diagnose the calcium deposits in the brain. Although not necessary, the combination of these findings with a progressive movement disorder, neuropsychiatric problems beginning in the 40’s or 50’s, and a lack of biochemical abnormalities or other known causes (infection, toxic exposure, trauma) makes the diagnosis very likely.

Additionally, genetic screening for mutations on PFBC-causative genes *SLC20A2*, *PDGFB*, *PDGFRB*, *XPR1* and *MYORG* should be performed, as it is currently the best way to determine with certainty if an individual has PFBC or not. This screening requires molecular diagnostic tests on DNA and can usually be performed by either private diagnostic laboratories or PFBC research groups. The molecular assays may be a simple DNA sequencing or any high-throughput DNA sequencing technologies, such as whole exome sequencing (WES) or whole genome sequencing (WGS).

Genetic counseling is recommended for affected individuals and their relatives, especially for those with mutations in PFBC-associated genes.

Standard Therapies

To date, no specific treatment for PFBC is known. Medications can be used to treat symptoms associated with this condition, such as movement disorders, seizures, anxiety, depression, psychosis and urinary incontinence. Off label prescription of biphosphanates have been reported in few patients (Loeb et al., 1998, 2006, Oliveira & Oliveira, 2017).

Investigational Therapies

Speech and gait were improved in one patient treated with disodium etidronate, but other neurologic symptoms and calcification were unchanged.

Levodopa therapy was found to be effective in treating parkinsonian features in one individual who had PFBC and Parkinson disease.

The anticonvulsant oxcarbazepine was effective in treating a Turkish patient with basal ganglia calcification and dyskinesia.

Information on current clinical trials is posted on the Internet at www.clinicaltrials.gov (http://www.clinicaltrials.gov). All studies receiving U.S. Government funding, and some supported by private industry, are posted on this government website.

For information about clinical trials being conducted at the NIH Clinical Center in Bethesda, MD, contact the NIH Patient Recruitment Office:

Tollfree: (800) 411-1222
TTY: (866) 411-1010
Email: prpl@cc.nih.gov (mailto:prpl@cc.nih.gov)

Some current clinical trials also are posted on the following page on the NORD website: https://rarediseases.org/for-patients-and-families/information-resources/info-clinical-trials-and-research-studies/ (https://rarediseases.org/for-patients-and-families/information-resources/info-clinical-trials-and-research-studies/)

For information about clinical trials sponsored by private sources, contact: www.centerwatch.com (http://www.centerwatch.com)

For information about clinical trials conducted in Europe, contact: https://www.clinicaltrialsregister.eu/ (https://www.clinicaltrialsregister.eu/)

Contact for additional information about Primary Familial Brain Calcification:

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joao.ricardo@ufpe.br (mailto:joao.ricardo@ufpe.br)

Supporting Organizations

Genetic and Rare Diseases (GARD) Information Center (https://rarediseases.org/organizations/genetic-and-rare-diseases-gard-information-center/)

PO Box 8126
Gaithersburg, MD 20898-8126
Phone: (301) 251-4925
Toll-free: (888) 205-2311
Website: http://rarediseases.info.nih.gov/GARD/ (http://rarediseases.info.nih.gov/GARD/)

NIH/National Institute of Neurological Disorders and Stroke (https://rarediseases.org/organizations/nihnational-institute-of-neurological-disorders-and-stroke/)

P.O. Box 5801
Bethesda, MD 20824
Phone: (301) 496-5751
Toll-free: (800) 352-9424
Website: http://www.ninds.nih.gov/ (http://www.ninds.nih.gov/)

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Years Published

1989, 1996, 1999, 2000, 2009, 2012, 2015, 2018

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Standard Therapies
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Supporting Organizations
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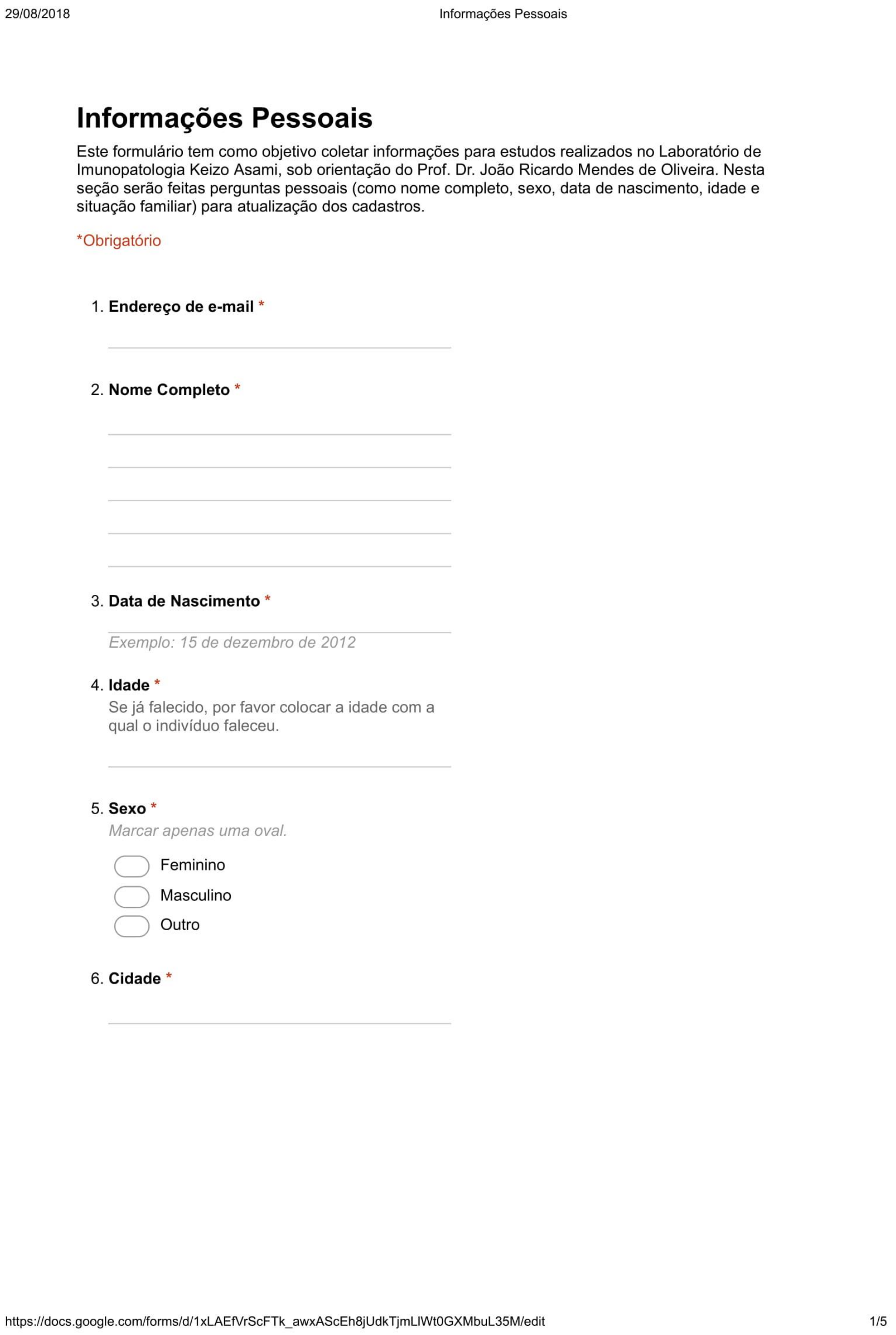
Search Rare Diseases

Enter a disease name or synonym to search NORD's database of reports.

NORD's Rare Disease Database provides brief introductions for patients and their families to more than 1,200 rare diseases. This is not a comprehensive database since there are nearly 7,000 diseases considered rare in the U.S. We add new topics as we are able to do so, with the help of rare disease medical experts.

If you are seeking information about a rare disease that is not in this database, we would suggest contacting the Genetic and Rare Diseases Information Center (GARD) (http://rarediseases.info.nih.gov/GARD/Default.aspx) at the National Institutes of Health. NIH has the most complete database of rare diseases in the U.S.

APÊNDICE F – QUESTIONÁRIO ONLINE USADO PARA A COLETA DE DADOS CLÍNICOS



29/08/2018 Informações Pessoais

Informações Pessoais

Este formulário tem como objetivo coletar informações para estudos realizados no Laboratório de Imunopatologia Keizo Asami, sob orientação do Prof. Dr. João Ricardo Mendes de Oliveira. Nesta seção serão feitas perguntas pessoais (como nome completo, sexo, data de nascimento, idade e situação familiar) para atualização dos cadastros.

*Obrigatório

1. **Endereço de e-mail** *

2. **Nome Completo** *

3. **Data de Nascimento** *

   Exemplo: 15 de dezembro de 2012

4. **Idade** *

   Se já falecido, por favor colocar a idade com a qual o indivíduo faleceu.

5. **Sexo** *

   Marcar apenas uma oval.

   ○ Feminino
   ○ Masculino
   ○ Outro

6. **Cidade** *

https://docs.google.com/forms/d/1xLAEfVrScFTk_awxAScEh8jUdkTjmLIWt0GXMbuL35M/edit 1/5

7. **Estado** *

Marcar apenas uma oval.

○ Acre - AC
○ Alagoas - AL
○ Amapá - AP
○ Amazonas - AM
○ Bahia - BA
○ Ceará - CE
○ Distrito Federal - DF
○ Espírito Santo - ES
○ Goiás - GO
○ Maranhão - MA
○ Minas Gerais - MG
○ Mato Grosso - MT
○ Mato Grosso do Sul - MS
○ Pará - PA
○ Paraíba - PB
○ Pernambuco - PE
○ Piauí - PI
○ Paraná - PR
○ Rio de Janeiro - RJ
○ Rio Grande do Norte - RN
○ Rio Grande do Sul - RS
○ Rondônia - RO
○ Roraima - RR
○ Santa Catarina - SC
○ Sergipe - SE
○ São Paulo - SP
○ Tocantins - TO

8. **Pai/Mãe Vivos?** *

Marcar apenas uma oval.

○ Ambos
○ Só mãe
○ Só pai
○ Não

9. **Tem irmãos?** *

Marcar apenas uma oval.

○ Sim
○ Não

10. **Tem filhos?** *

Marcar apenas uma oval.

○ Sim
○ Não

11. **Falecido?** *

Marcar apenas uma oval.

○ Sim
○ Não

Informações Clínicas

Nesta seção serão feitas perguntas referentes à sinais e sintomas que cada um venha a apresentar. Gostaríamos que as perguntas sejam respondidas da maneira mais detalhada possível para que cada caso seja entendido em sua totalidade.

12. **Faz uso de medicações?** *

Se sim, favor listá-los em "Outros"

Marque todas que se aplicam.

☐ Sim
☐ Não
☐ Outro: ____________________

13. **Já apresentou/foi diagnosticado com algum sintoma/transtorno psiquiátrico?** *

Como depressão, ansiedade, alucinações, alterações bruscas de humor, comportamento agressivo etc. Se sim, por favor os descreva em "outros".

Marque todas que se aplicam.

☐ Sim
☐ Não
☐ Não sei
☐ Outro: ____________________

14. **Já apresentou algum dos problemas motores abaixo?** *

Marque todas que se aplicam.

☐ Dificuldade ao caminhar
☐ Problemas de equilíbrio
☐ Problemas de coordenação motora
☐ Tremores em mãos/pés
☐ Rigidez de membros inferiores/superiores
☐ Perda de força muscular
☐ Não sei
☐ Outro: ____________________

15. **Já teve episódios de convulsão?** *

Marcar apenas uma oval.

○ Sim, mais de uma vez
○ Sim, uma única vez
○ Não
○ Não sei

16. **Já apresentou problemas na fala?** *

Se sim, por favor os descreva em "Outros".
Marque todas que se aplicam.

☐ Sim
☐ Não
☐ Não sei
☐ Outro: ____________________

17. **Já apresentou problemas de aprendizado ou de memória?** *

Marcar apenas uma oval.

○ Sim, ambos
○ Sim, apenas de aprendizado
○ Sim, apenas de memória
○ Não
○ Não sei

18. **Sofre de enxaquecas?** *

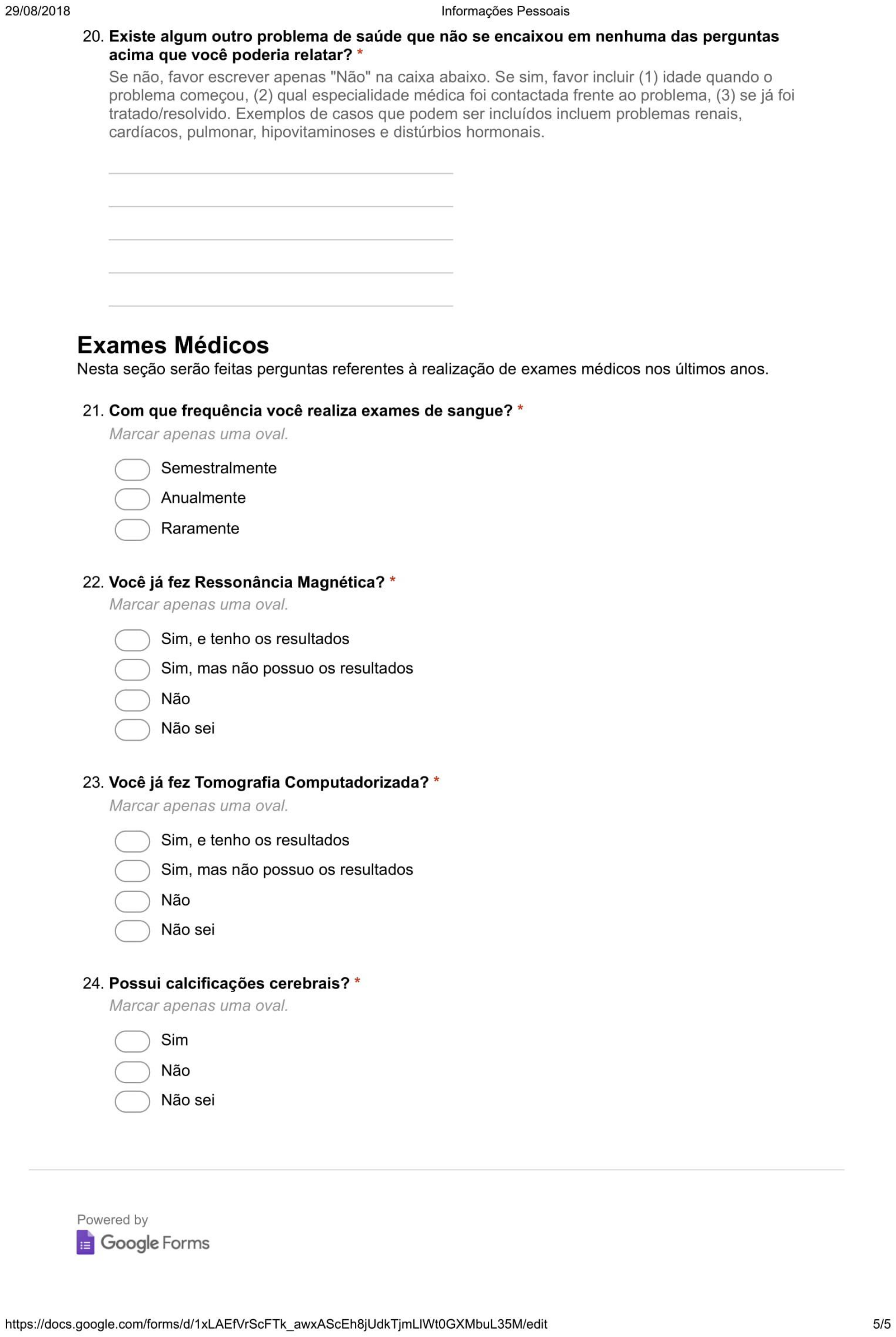
Marcar apenas uma oval.

○ Sim, com frequência
○ Sim, às vezes
○ Sim, raramente
○ Não
○ Não sei

19. **Tem algum dos quadros descritos abaixo?** *

Marque todas que se aplicam.

☐ Hipertensão
☐ Diabetes tipo I (insulino-dependente)
☐ Diabetes tipo II
☐ Dislipidemia (Colesterol Total e/ou Triglicerídeos elevado)
☐ Lúpus
☐ Artrite Reumatóide
☐ Problemas na Tireóide
☐ Osteoporose
☐ Nenhuma das opções acima



20. **Existe algum outro problema de saúde que não se encaixou em nenhuma das perguntas acima que você poderia relatar?** *

Se não, favor escrever apenas "Não" na caixa abaixo. Se sim, favor incluir (1) idade quando o problema começou, (2) qual especialidade médica foi contactada frente ao problema, (3) se já foi tratado/resolvido. Exemplos de casos que podem ser incluídos incluem problemas renais, cardíacos, pulmonar, hipovitaminoses e distúrbios hormonais.

Exames Médicos

Nesta seção serão feitas perguntas referentes à realização de exames médicos nos últimos anos.

21. **Com que frequência você realiza exames de sangue?** *

Marcar apenas uma oval.

○ Semestralmente
○ Anualmente
○ Raramente

22. **Você já fez Ressonância Magnética?** *

Marcar apenas uma oval.

○ Sim, e tenho os resultados
○ Sim, mas não possuo os resultados
○ Não
○ Não sei

23. **Você já fez Tomografia Computadorizada?** *

Marcar apenas uma oval.

○ Sim, e tenho os resultados
○ Sim, mas não possuo os resultados
○ Não
○ Não sei

24. **Possui calcificações cerebrais?** *

Marcar apenas uma oval.

○ Sim
○ Não
○ Não sei

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APÊNDICE G – PRIMERS UTILIZADOS PARA TRIAGEM GENÉTICA

GENE	NOME DO PRIMER	SEQUÊNCIA 5'-3'	REFERÊNCIA
PDGFC	PDGFC E1 Fw	CTGGCGGTGGTGAAAGAGA	Laboratório de Neuroimunogenética – LIKA/UFPE
	PDGFC E1 Rv	AATGAGAGACCAAAGATAC	
	PDGFC E2 Fw	TTTTCTGGATCAGTTCTGGA	
	PDGFC E2 Rv	AAAAGATGTCATTTAAAATC	
	PDGFC E3 Fw	ATTATATGTTCCCGGTTAAA	
	PDGFC E3 Rv	CATAAAAATGAAATCCCAGC	
	PDGFC E4 Fw	ATTTCTTGCATATGTCCAGA	
	PDGFC E4 Rv	ATAAGAAAATGTATTGAATA	
	PDGFC E5 Fw	TAATGAAATACATTCAGGC	
	PDGFC E5 Rv	CATGAATACTACGTCTTTT	
	PDGFC E6 Fw	AAAAGCCAGTCATAGACATT	
	PDGFC E6 Rv	CCTCCTCTCAAAAGAGCTGT	
PDGFD	PDGFD E1 Fw	AAAGTTCAGCATGCAGGAAG	Laboratório de Neuroimunogenética – LIKA/UFPE
	PDGFD E1 Rv	GAAAGGGGAAAAACATAGAA	
	PDGFD E2 Fw	GAAGTATGATGTGTTGTAAA	

	PDGFD E2 Rv	AGCGACACATGTTGGGTCCA	
	PDGFD E3 Fw	TTTCATTTCAGATTGTGGT	
	PDGFD E3 Rv	TCACTCACCCTCACTGAAT	
	PDGFD E4 Fw	TCACCAGTACTTACAAGAAA	
	PDGFD E4 Rv	CTGGCAGTTCCTGGAAACAT	
	PDGFD E5 Fw	AAACGTATGATGTCTTATAC	
	PDGFD E5 Rv	GGTTTCAAATACTTTGGATA	
	PDGFD E6 Fw	AAAGTTGTCCTCACAGAATC	
	PDGFD E6 Rv	CAAACCTAGTGTGGGTAGAA	
	PDGFD E7 Fw	AGCAAGCCCATAGCTTCTGT	
	PDGFD E7 Rv	AGCAACCACTTGTGTTCATT	
PCDH12	PCDH12 E1.1 Fw	TCTTGATCGGCGTTTGGACA	Laboratório de Neuroimunogenética – LIKA/UFPE
	PCDH12 E1.1 Rv	TCATCAGGGCCCACAATGAC	
	PCDH12 E1.2 Fw	AAATCTCTGAGAGCGCCTCT	
	PCDH12 E1.2 Rv	TTTGGGCCACTGCTCTCTGT	
	PCDH12 E1.3 Fw	GATTCAGGACACAATGGTTT	
	PCDH12 E1.3 Rv	TGCCACAATGGTTGTCAAAA	

	PCDH12 E1.4 Fw	AAAAGCCAGCCTCTCCGTGC
	PCDH12 E1.4 Rv	TTTGTGGGACTGCCCGACTT
	PCDH12 E1.5 Fw	GGCTGTACTGTTGGGCATCT
	PCDH12 E1.5 Rv	TCAGAAAGTGCCAGGTGAGC
	PCDH12 E2 Fw	GGTGACATGACAGAGCAGGG
	PCDH12 E2 Rv	CCTCACGACCAAAACTCCCA
	PCDH12 E3 Fw	CTCTAGAGGCCAGGCTGTGTA
	PCDH12 E3 Rv	GTCAAGGTAACTCCAGGGGG
	PCDH12 E4 Fw	GCTGCTTCCCCACTTGGTTC
	PCDH12 E4 Rv	ATCTCAGCCACAAACCGTCC