UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Caracterização clínica com ênfase em aspectos neuropsiquiátricos, cardiológicos e qualidade de vida de uma coorte de pacientes do Sul do Brasil com diagnóstico tardio de Homocistinúria Clássica.

Karina Carvalho Donis

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

Orientadora: Profa. Dra. Ida Vanessa Doederlein Schwartz

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Este trabalho foi desenvolvido no Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre e no Centro de Pesquisa Clínica do Hospital de Clínicas de Porto Alegre. Os recursos para seu desenvolvimento foram provenientes do FIPE-HCPA.

SUMÁRIO

Lista de Abreviaturas

BD - Escala de Depressão de Beck

BPRS - Brief Psychiatric Rating Scale

CACP - Camptodactyly-Arthropathy-Coxa-Vara-Pericarditis

CBS-Cistationina β-sintase

CDC - Centro para Controle e Prevenção de Doenças

FS IQ - Full Scale IQ Test

hcy - homocisteína

HCU - Homocistinúria Clássica

MeCbl - Metilcobalamina

NMDA -N-metil-D-aspartato

SAA - Aminoácidos sulfurados

SAH - S-Adenosilhomocisteína

SAM - S-adenosilmetionina

tHcy - homocisteína total

THF - Tetrahidrofolato

TDAH - Transtorno do Déficit de Atenção com Hiperatividade

TOD - Transtorno Opositivo Desafiador

QI – Quociente de Inteligência

WASI - Escala Wechsler Abreviada de Inteligência

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RESUMO

Introdução: A Homocistinúria Clássica ou Deficiência de Cistationina β-sintase é uma doença genética, autossômica recessiva, multissistêmica de curso lento e progressivo que ocorre pela alteração no metabolismo dos aminoácidos sulfurados, com aumento dos níveis de homocisteína, metionina, S adenosil homocisteína e redução de cistationina e cisteína. A Homocistinúria Clássica pode ser classificada em três formas: responsiva à piridoxina (vitamina B6), não-responsiva à piridoxina e com resposta intermediária à piridoxina. Sem o diagnóstico e tratamento precoces, os pacientes apresentam um quadro clínico caracterizado por anormalidades oftalmológicas, ósseas, vasculares e no sistema nervoso central. Todos os pacientes devem receber tratamento com suplementação de ácido fólico e vitamina B12 se houver deficiência. O tratamento recomendado para pacientes responsivos é o uso de piridoxina 10 mg/Kg/dia evitando doses acima de 500 mg/dia por risco de neuropatia periférica. O uso do tratamento dietético e betaína deve ser considerado para os pacientes que não atingiram níveis ideais de homocisteína total apenas com a suplementação de piridoxina. **Objetivos**: avaliar a qualidade de vida, detectar prevalência de deficiência intelectual, descrever quadro psiquiátrico e caracterizar alterações em Sistema Nervoso Central através de volumetria cerebral em pacientes com Homocistinúria Clássica acompanhados no ambulatório de Genética do Hospital de Clínicas de Porto Alegre. **Métodos**: o estudo foi realizado em três etapas. Os pacientes incluídos são acompanhados em um ambulatório especializado no Hospital de Clínicas de Porto Alegre. A amostra foi por conveniência e os pacientes tinham que ter diagnóstico confirmado de Homocistinúria Clássica. Na primeira etapa, realizou-se um estudo retrospectivo avaliando qualidade de vida através da aplicação do questionário WHOQOL-BREF em 11 pacientes. Além disso, foi avaliado o QI através do questionário WASI de 8 pacientes. Na segunda etapa, para avaliar quadro psiquiátrico, foi realizado um estudo transversal, prospectivo através da aplicação das escalas *Brief Psychiatric Rating Scale* (BPRS), Escala de Ansiedade de Beck, Hamilton e Escala de Depressão de Beck (BD) em 8 pacientes. Após, um estudo transversal de volumetria cerebral foi realizado nestes 8 pacientes. Na terceira etapa, foi realizada uma análise retrospectiva de 14

pacientes para avaliar alterações cardiológicas através da análise de eletrocardiogramas e ecocardiogramas. **Resultados**: na primeira etapa foi verificado que os pacientes em tratamento para Homocistinúria Clássica apresentaram escores mais elevados no domínio psicológico quando comparados aos pacientes não tratados. A segunda etapa mostrou que o volume total do tálamo controlado pelo volume craniano e idade teve correlações negativas com a Escala de Depressão de Beck, Escala de Hamilton, BPRS e com dois subitens do BPRS, preocupações somáticas e maneirismos. Pacientes com Homocistinúria Clássica, quando comparados a indivíduos saudáveis, pareados por idade e sexo, apresentaram mais locais de hipointensidade de substância branca. Na terceira etapa foi verificada uma alta prevalência de valvulopatias. **Conclusão**: Nossos dados sugerem que o tratamento de pacientes com Homocistinúria Clássica associa-se a uma melhora na qualidade de vida no aspecto psicológico, que as alterações no QI são prevalentes e que os pacientes apresentam quadro compatível com depressão, ansiedade e esquizofrenia. Há uma tendência no envolvimento do tálamo como uma das estruturas relacionadas às condições psiquiátricas nos pacientes com Homocistinúria Clássica e valvulopatias são frequentemente encontradas em pacientes com Homocistinúria Clássica não responsivos à piridoxina. Apenas um paciente responsivo à piridoxina apresentou ectasia de raiz da aorta.

Palavras - chave: Homocistinúria Clássica, Deficiência de Cistationina β-sintase, qualidade de vida, volumetria cerebral, valvulopatia.

ABSTRACT

Introduction: Classic Homocystinuria or Cystathionine β-synthase Deficiency, is a genetic autosomal recessive and multisystemic disease. It is characterized by a slow and progressive course and impaired metabolism of sulfur amino acids, leading to increased levels of homocysteine, methionine, S adenosyl homocysteine and reduced cystathionine and cysteine. Classical Homocystinuria can be classified into three forms: responsive to pyridoxine (vitamin B6), unresponsive to pyridoxine and intermediate response to pyridoxine. Ophthalmological, bone, vascular and central nervous system abnormalities can occur if no early diagnosis or treatment are provided. All patients should be treated with folic acid and vitamin B12 supplementation, if they are deficient. Recommended pyridoxine dose for responsive patients is 10 mg/kg/day, up to 500 mg/day, due to the risk of peripheral neuropathy caused by higher doses. Additionally, dietary treatment and betaine should be considered on those who did not reach ideal levels of total homocysteine with pyridoxine supplementation. There is little data about cardiac, neuropsychiatric problems and quality of life in Classic Homocystinuria patients and, to this date, there is no data about brain volume. **Objectives**: to assess quality of life, detect prevalence of intellectual disability, describe psychiatric conditions and characterize alterations in the Central Nervous System through cerebral volumetry in HCU patients followed at Genetics Service of Hospital de Clínicas de Porto Alegre. **Method**: the study was performed in three steps. Included patients are monitored at a specialized clinic at the Hospital de Clinicas de Porto Alegre. The sample was by convenience and the patients have a confirmed diagnosis of Classical Homocystinuria. First, a retrospective study assessed quality of life in 11 patients, through WHOQOL-BREF questionnaire. In addition to that, the IQ was assessed using the WASI questionnaire in eight patients. In the second step, psychiatric conditions were evaluated in eight patients through a cross-sectional, prospective study, using Brief Psychiatric Rating Scale (BPRS), Beck Anxiety Scale, Hamilton and Beck Depression Scale (BD). Afterwards, a cross-sectional study of cerebral volumetry was carried out in these eight patients. Finally, the third step was a retrospective analysis of 14 patients, in order to assess cardiac alterations via electrocardiogram and echocardiogram. **Results**: in the first stage, it was found that

patients undergoing treatment had higher scores in the psychological domain when compared to untreated patients. The second step showed that total thalamus volume, adjusted by cranial volume and age, had negative correlations with the Beck Depression Scale, Hamilton Scale, BPRS and with two BPRS sub-items: somatic concerns and mannerisms. Patients with Classical Homocystinuria, when compared to healthy individuals, matched for age and sex, had more sites of white matter hypointensity. In the third step, a high prevalence of valvulopathies was verified. **Conclusion**: Our data suggest that the treatment of patients with Classic Homocystinuria is associated with an improvement in quality of life in the psychological aspect, that changes in IQ are prevalent and that patients present depression, anxiety and schizophrenia. There is a trend towards involvement of the thalamus as one of the structures related to psychiatric conditions in Classic Homocystinuria patients and valvulopathies are frequently found in unresponsive pyridoxine Classic Homocystinuria patients. Only one pyridoxine-responsive patient had aortic root ectasia.

Keywords: Classic Homocystinuria, Cystathionine β-synthase Deficiency, quality of life, cerebral volumetry, valvulopathy.

1.1.Homocistinúria Clássica – Base Histórica

O primeiro relato de Homocistinúria Clássica (HCU) ou Deficiência de Cistationina β-sintase (CBS) foi em 1962, na Irlanda do Norte, em um estudo sobre anormalidades metabólicas detectadas em urina de crianças com deficiência intelectual. Nina Carson e Desmond Neill verificaram que duas irmãs, uma de 5 anos e outra de 7 anos, eliminavam grande quantidade de homocistina na urina. As irmãs apresentavam deficiência intelectual, atraso do desenvolvimento neuropsicomotor, alterações esqueléticas, alterações em pele, cabelos e subluxação do cristalino (1). Em 1964, foi descoberto o defeito enzimático responsável pela doença (2) e em 1967 foi sugerida a primeira estratégia de tratamento com restrição dietética de metionina e uso de piridoxina. (3,4,5). O gene *CBS* foi mapeado somente em 1998 (6).

1.2 Epidemiologia

A prevalência de HCU varia entre 1:1800 a 1:900,000 com base na incidência de nascimento de pacientes detectados por triagem neonatal e / ou estimativas de pacientes clinicamente verificados. (7,8,9). No Catar, a prevalência estimada é de 1:1800, sendo a mais alta no mundo (9). A incidência estimada no sudeste do Brasil é de aproximadamente 9.7:100,000 indivíduos (10).

1.3 Fisiopatogenia

A HCU resulta na alteração no metabolismo dos aminoácidos sulfurados (SAA), com elevação em fluidos corporais dos níveis de homocisteína (hcy), metionina, S adenosil homocisteína e redução de cistationina e cisteína (11).

A CBS é expressa predominantemente no fígado, pâncreas, rins e cérebro. A metionina é convertida em hcy liberando um grupo metila que é usado em diversas reações de metilação e pode ser convertida novamente em metionina. O doador de grupo metila pode ser 5-metiltetra-hidrofolato, catalisado pela metionina

sintase com metilcobalamina como cofactor, ou betaína, especialmente em pacientes tratados com este medicamento. Alternativamente, a hcy é irreversivelmente metabolizada em cisteína pela via de transulfuração, através da condensação de hcy e serina para formar cistationina, catalisada pela enzima CBS. Após, a cistationina é clivada pela cistationina γ-liase formando cisteína e 2-oxobutirato (12).

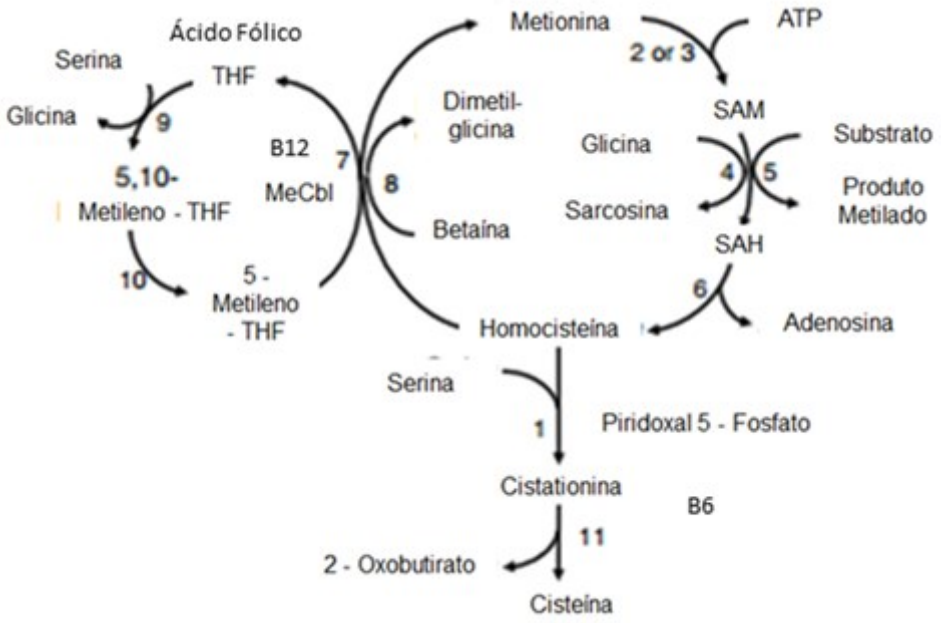

Figura 1: Homocistinúria Clássica e rota do metabolismo da metionina e da homocisteína (adaptada de Morris *et al.*, 2016).

Na Homocistinúria Clássica, ocorre atividade deficiente da enzima representada, na Figura, pelo número “1”. SAM, S-adenosilmetionina; SAH, S-adenosilhomocisteína; THF, Tetrahidrofolato; MeCbl, Metilcobalamina. 1, Cistationina beta- sintase ou CBS; 2, metionina adenosiltransferase I/III; 3, metionina adenosiltransferase II; 4, glicina N - metiltransferase; 5, numerosas metiltransferases; 6, S - adenosilhomocisteína hidrolase; 7, metionina sintase; 8, betaína homocisteína metilltransferase; 9, Serina hidroximetiltransferase; 10, metilenetetrahidrofolato redutase; 11, cistationina gamma-liase.

1.4 Base Genética e o Gene *CBS*

A HCU é uma doença genética de herança autossômica recessiva causada por variantes patogênicas bialélicas no gene *CBS*, localizado no cromossomo 21q22.3 (12). O gene tem 23 éxons e 25-30 kb. Até o momento, mais de 200 variantes patogênicas foram identificadas no gene *CBS*, sendo que a maioria delas são raras e privadas (10). Entretanto, quatro variantes, p.Ile278Thr, p.Thr191Met, p.Gly307Ser e p.Arg336Cys, são as mais prevalentes e responsáveis por mais da metade de todos os alelos HCU em todo o mundo (13).

1.5 Manifestações Clínicas

A HCU se apresenta com um amplo espectro de gravidade, desde indivíduos assintomáticos até indivíduos com grave acometimento multissistêmico (12) e pode ser classificada em três formas baseadas na resposta à suplementação de piridoxina (vitamina B6) (14).

A forma responsiva à piridoxina é caracterizada pela resposta dos pacientes ao uso desta vitamina que têm seus níveis plasmáticos de tHcy reduzidos abaixo de 50 umol/L. Na triagem neonatal, representa cerca de 12,7% dos casos identificados e 47% dos casos diagnosticados tardiamente (15).

Na forma não-responsiva à piridoxina, os indivíduos apresentam níveis plasmáticos de tHcy acima de 80% dos níveis baseline após teste de responsividade à piridoxina. Em triagem neonatal, representa cerca de 78,2% dos casos identificados (15).

A terceira forma é a resposta parcial à piridoxina na qual os pacientes que estão em uso de piridoxina têm seus níveis plasmáticos de tHcy reduzidos, mas não de forma substancial, não atingindo o alvo-terapêutico. Os níveis plasmáticos de tHcy se mantêm abaixo de 80% dos níveis baseline após teste de responsividade a vitamina B6. Em triagem neonatal, representa cerca de 7% dos casos identificados e 12,7% dos casos diagnosticados tardiamente (15).

Deve-se realizar o teste de suplementação com piridoxina para determinação do grau de responsividade à piridoxina ao diagnóstico (12). Estão descritas diferentes maneiras para a realização desse teste na literatura, com

variações em relação à dose utilizada de piridoxina, à posologia e ao intervalo de tempo para verificação da responsividade (redução de tHcy pós-teste).

Os pacientes com HCU apresentam um quadro clínico clássico caracterizado por anormalidades graves em pelo menos quatro sistemas sem a instituição de diagnóstico e tratamento precoces (12):

1. Sistema oftalmológico: miopia, deslocamento de cristalino, glaucoma e descolamento de retina.

2. Sistema ósseo: habitus marfanoide, osteoporose, vértebras bicôncavas, escoliose, pés cavos, palato arqueado, espículas metafisárias e aracnodactilia.

3. Sistema vascular: tromboembolismo, livedo *reticularis* e *flush* malar.

4. Sistema nervoso central: acidente vascular cerebral, manifestações psiquiátricas, deficiência intelectual, sinais extrapiramidais e crise convulsiva.

A HCU é uma doença multissistêmica de curso lento e progressivo, cujas primeiras manifestações podem surgir nos lactentes com sintomas inespecíficos, como déficit de crescimento e atraso do desenvolvimento neuropsicomotor (16).

1.5.1 Quadro psiquiátrico

Sabe-se que mais de 50% dos pacientes com HCU apresentam pelo menos um sintoma psiquiátrico, incluindo transtorno de comportamento (17%), depressão (10%), transtorno obsessivo-compulsivo (7,6%) e / ou transtornos de personalidade (19%). Há relatos de casos descrevendo pacientes HCU com episódios psicóticos (17,18,19).

A experiência em Manchester no Reino Unido com 30 anos no atendimento de 31 pacientes com HCU mostrou que destes 31 pacientes, 23 foram classificados como não responsivos à piridoxina, sendo 12 destes diagnosticados por métodos de triagem neonatal. O paciente mais velho desse grupo tinha 25 anos no momento da publicação. Nos 23 pacientes tratados com restrição de proteína e suplementação com fórmula, a mediana de consumo de metionina foi de 230 mg/dia (variando entre 160-900 mg/dia), sendo particularmente mais difícil a adesão em crianças mais velhas e adolescentes. Nenhum dos 11 pacientes com diagnóstico precoce (triagem neonatal), e início do tratamento com restrição dietética e suplementação com fórmula isenta de metionina, desenvolveu sintomas

característicos de HCU como hábito marfanóide, aracnodactilia e *ectopia lentis*. A mediana do QI nesses indivíduos foi de 100 (variando entre 84-117), significativamente superior aos dos indivíduos com tratamento tardio (pacientes não-responsivos à piridoxina com tratamento tardio, mediana de QI 58, variando 20-86, p<0,0001; pacientes responsivos à piridoxina com tratamento tardio, mediana de QI 82, variando 57-101, p=0,02) (20).

Almuqbil *et al.,*2019 avaliou 25 pacientes (20 não responsivos à piridoxina e 5 responsivos à piridoxina). Destes, a triagem neonatal diagnosticou 14 casos. Vinte e quatro pacientes estavam em tratamento combinado. Dezesseis pacientes (64%) apresentavam algum sintoma psiquiátrico como apresentado na tabela 1 (21).

Adicionalmente, foi realizado teste de QI com a escala Full Scale IQ Test (FS IQ) e em 60% dos casos, o FS IQ foi um desvio padrão ou mais abaixo da média populacional e em 26% dos casos apresentaram > 2 desvios padrão abaixo da média (FS IQ <70) considerado deficiência intelectual (21). A presença de sintomas psiquiátricos foi associada ao diagnóstico tardio (r = 9,07, p =0,065) e às pontuações mais baixas em medidas de memória (r = -0,650, p = 0,058). Entre os pacientes com déficit cognitivo, 91% foram relatados como tendo sintomas psiquiátricos, enquanto 38% dos pacientes com QI dentro ou acima de 1 desvio padrão da média normativa (QI> 85) tinham algum sintoma psiquiátrico (21).

A literatura mostra um aumento na prevalência de sintomas como depressão e ansiedade nos pacientes HCU. Segundo o Centro para Controle e Prevenção de Doenças (CDC) dos Estados Unidos, a prevalência de depressão na população geral acima de 18 anos é 4.7% e de ansiedade na população geral acima de 18 anos é 11.2% (22). Isso suporta a hipótese que as elevações de metionina e tHcy podem estar envolvidas com o fenótipo psiquiátrico. (23) A presença de sintomas psiquiátricos em pacientes com aumento de metionina já foi associada a déficits cognitivos e diagnóstico tardio. A tHcy age no receptor N-metil-D-aspartato (NMDA), que está implicado em déficits cognitivos e esquizofrenia (21).

Tabela 1 - Sintomas psiquiátricos associados a Homocistinúria Clássica

Sintoma	Diagnóstico precoce N=14 (56%)	Diagnóstico tardio N=11 (44%) (8-40 anos)	Indivíduos afetados (%)
Ansiedade	4	4	8 (33%)
Depressão	3	5	8 (33%)
TDAH	2	1	3 (12%)
Agressividade	1	1	2 (8%)
Suicídio	2	0	2 (8%)
Abuso de drogas	1	1	2 (8%)
Isolamento social	0	1	2 (8%)
Comportamento	0	1	1 (4%)
Alucinações	1	0	1 (4%)
Alterações de humor	1	0	1 (4%)
TOD	0	1	1 (4%)
Paranoia	0	1	1 (33%)
Transtorno invasivo do desenvolvimento	1	0	1 (33%)

Traduzida e adaptada de Almuqbil *et al.*, 2019

TDAH: Transtorno do Déficit de Atenção com Hiperatividade; TOD: Transtorno Opositivo Desafiador.

1.5.2 Qualidade de vida

Há poucos dados sobre qualidade de vida nos pacientes com HCU. Pesquisado no Pubmed “quality of life” and “CBS deficiency” e “Classic Homocystinuria” e não foi encontrado nenhum dado específico sobre qualidade de

vida nestes pacientes. Não há dados sobre a relação entre níveis de tHcy, gênero, fenótipo e qualidade de vida (12).

Cazzorla *et al.*, 2012 publicou um estudo sobre qualidade de vida em pacientes adultos com doenças metabólicas usando a escala WHOQOL - 100. Nesta amostra de 82 pacientes, apenas 8 tinham diagnóstico de HCU (6 pacientes com a forma não responsiva à piridoxina). Os resultados obtidos mostraram uma diferença estatística entre os pacientes com tratamento dietético e aqueles com tratamento farmacológico. Todo o grupo de pacientes com doenças tratadas com dieta especial tiveram melhor qualidade de vida geral. Os pacientes com HCU não foram avaliados separadamente devido ao número reduzido da amostra (24).

1.5.3 Alterações em exames de imagem do Sistema Nervoso Central

Mudd *et al.*,1985 publicou uma coorte de 629 pacientes com diagnóstico de HCU e relatou eventos tromboembólicos em 25% dos pacientes. De 253 eventos isquêmicos, 51% foram trombose venosa periférica (com um quarto resultando em embolia pulmonar), 32% foram acidentes vasculares cerebrais, 11% eram oclusões arteriais periféricas, 4% eram infartos do miocárdio e 2% eram eventos isquêmicos em outras áreas. Nesta coorte, o risco de ter um evento vascular foi de 25% antes dos 16 anos e 50% aos 29 anos (15). O número de eventos tromboembólicos observados durante a terapia com piridoxina em pacientes HCU que respondem a piridoxina foi muito menor do que o número esperado se a terapia não tivesse sido iniciada.

O tromboembolismo é a causa mais comum de morte na homocistinúria clássica, e diferentes mecanismos têm sido propostos. Modelos animais e várias investigações observacionais em humanos mostraram que um nível elevado de tHcy sérica é um fator de risco para aterosclerose precoce. (25). A hiper-homocisteinemia causa disfunção endotelial devido à diminuição da biodisponibilidade do endógeno vasodilatador óxido nítrico e através do estresse oxidativo. Além disso, um estado hipercoagulável subjacente foi sugerido com base na trombose aumentada e na ativação plaquetária. Essas alterações podem alterar a estabilidade da parede arterial e explicar a ocorrência de trombose intra-arterial,

dissecção arterial e arteriopatia simulando displasia fibromuscular em jovens com HCU (26).

Além de achados relacionados a eventos tromboembólicos, os achados descritos nas ressonâncias de crânio dos pacientes com HCU incluem infartos neurovasculares, restrição na difusão nas sequências T1 e T2 em globo pálido bilateral e tálamo (27), edema cerebral e anormalidades na substância branca foram reportadas em alguns pacientes que foram tratados com betaína, associados com altas concentrações plamáticas de metionina (12).

A betaína oral pode contribuir para o edema cerebral, pois age como um osmólito intracelular (28). Embora a restrição à difusão seja mais comumente causada por falha energética celular levando à diminuição da atividade Na + / K + ATPase e subsequente edema das células citotóxicas, um osmólito intracelular também pode produzir mudanças semelhantes nas medições de difusão (27).

Outro mecanismo que poderia explicar a restrição à difusão da substância branca é a mielinopatia vacuolante, caracterizada pelo acúmulo de água em vacúolos intramielínicos extracelulares (29). Este mecanismo foi associado à HCU apenas por meio de exame patológico, sem correlação radiológica (30, 31).

A metionina, e não a tHcy, é geralmente aceita como o agente causador dos achados de imagem observados. Em diversos relatos de caso, as anormalidades da substância branca coincidiram com níveis de metionina plasmática significativamente elevados entre 904 e 2823 µmol/L (27) e em alguns deles, se resolveram após a redução da metionina plasmática, embora isso tenha sido acompanhado pela redução ou interrupção da terapia com betaína, um fator de confusão potencial (22, 32, 33).

Não há dados sobre volumetria em Sistema Nervoso Central nos pacientes com HCU, nem sobre a relação entre níveis de tHcy, metionina, gênero, fenótipo e quadro psiquiátrico.

Foram descritas algumas associações entre estruturas do sistema nervoso central e distúrbios psiquiátricos. A redução do volume hipocampal, núcleo caudado, amígdala, córtex frontal, temporal, parietal, tálamo e ínsula estiveram relacionados com depressão (34, 35, 36, 37, 38). Redução dos volumes do hipocampo, volume da substância cinzenta na região temporal superior posterior

esquerda, ínsula, sub-região do córtex cingulado anterior, hipocampo, tálamo e amígdala na esquizofrenia (39, 40, 41, 42, 43, 44, 45, 46, 47). O transtorno de ansiedade social foi associado a anormalidades do córtex parietal e pré-motor (48). Esses achados ainda não foram descritos na HCU.

1.5.4 Alterações cardiológicas

Existem poucas informações na literatura sobre danos cardiológicos na HCU. A fibrilina-1 desempenha um papel complexo e não completamente compreendido, interagindo com as células do músculo liso vascular e regulando a atividade do fator de crescimento, particularmente do TGF-β1 que pode levar à expressão de metaloproteinase da matriz e, portanto, degradação da matriz e inflamação. Embora não muito bem compreendida, a elevação crônica da tHys poderia levar a danos no tecido conjuntivo através da redução das ligações dissulfeto de fibrilina-1 e, portanto, ao aumento da atividade de TGF-β1 levando a mudanças na estrutura e função cardíaca (49, 50)

A maior parte da literatura é baseada em relatos de casos, para situações como taquicardia postural ortostática (51), ou calcificações arteriais (52), decorrente da disfunção endotelial já documentada no HCU. Pacientes com HCU também apresentam maior incidência de dilatação da raiz da aorta em sua avaliação ecocardiográfica (53). Lorenzini *et al.*, avaliou 34 pacientes com HCU (29% responsivo à piridoxina). Oito pacientes (24%) tinham história de hipertensão. Sete pacientes (21%) apresentaram dilatação da raiz aórtica, leve em dois casos (6%), moderada em quatro (12%) e grave em um (3%). Nenhum apresentou dilatação da aorta ascendente. Regurgitação aórtica significativa, secundária à dilatação moderada da raiz da aorta, foi documentada em dois pacientes. Um único paciente apresentou insuficiência mitral importante devido ao prolapso de ambos os folhetos valvares, além de dilatação leve da raiz da aorta (53).

1.6. Diagnóstico

O diagnóstico definitivo da HCU exige a confirmação laboratorial, que é realizada através de três formas distintas: dosagem de metabólitos, dosagem da atividade enzimática e/ou análise de DNA.

O teste do cianeto nitroprussiato pode detectar altos níveis de tHcy na urina, mas tem baixa sensibilidade, principalmente quando a urina está diluída (níveis baixos de tHcy), nunca devendo ser usado isoladamente para exclusão ou confirmação do diagnóstico (54). O teste mais comumente usado para o diagnóstico de HCU é a dosagem de tHcy que é realizada por métodos cromatográficos, como a cromatografia líquida de alta performance, juntamente com a dosagem de metionina presente na dosagem por HPLC (55). A dosagem de hcy livre somente é detectável quando as concentrações de tHcy são aproximadamente 50-60 µmol/L. Esta medida não é recomendada por ter baixa sensibilidade. Em pacientes não tratados, a tHcy está geralmente acima de 100 µmol/L, mas pode ser mais baixa. A metionina pode estar elevada ou borderline no plasma, a concentração de cistationina é baixa no plasma e há um aumento da relação metionina/cistationina (12). De forma geral, uma dosagem elevada de tHcy em plasma, associada à elevação plasmática nos níveis de metionina e à exclusão de acidúrias orgânicas, confirma o diagnóstico de HCU.

A medida da atividade da enzima CBS pode ser realizada em fibroblastos cultivados da pele ou em tecido hepático, sendo seu resultado sensível e específico. Contudo não é o exame de primeira linha para o diagnóstico de HCU, pela necessidade de procedimento invasivo (biópsia), alto custo e a pouca disponibilidade de laboratórios para realizar este teste (56). A análise de DNA também pode ser usada no diagnóstico da doença. Quando um paciente é diagnosticado, deve ser oferecido triagem de outros membros da família com medida de tHcy e metionina e análise molecular ou enzimática (12).

A classificação da HCU em responsiva à piridoxina, parcialmente responsiva à piridoxina ou não responsiva à piridoxina pode ser realizada conforme a figura 2 (12).

O modo de testagem mais utilizado consiste na dosagem dos níveis basais de tHcy durante uma dieta normal (sem restrições) suplementação de ácido fólico e de deficiência de vitamina B12 se necessário, e administração de piridoxina 10mg/Kg/dia (mínimo de 100 mg/dia e máximo de 500 mg/dia) e dosagem de tHcy após 1 semana (12).

Caso a tHcy esteja abaixo de 50 umol/L, o ideal é repetir a tHcy em 2 semanas, se este valor se mantiver, considerar o indivíduo afetado como responsivo à piridoxina. Se a tHcy estiver acima de 50 umol/L, repetir o teste em 6 semanas. O paciente é considerado parcialmente responsivo se a tHcy estiver menor que 80% do valor inicial e não responsivo se a tHcy estiver maior que 80% do valor inicial.

Se após 1 semana estiver acima de 50 umol/L, deve-se repetir a tHcy em 2 semanas. Se a tHcy estiver abaixo de 50 umol/L considerar o indivíduo afetado responsivo à piridoxina. Se a tHcy estiver acima de 50 umol/L repetir em 6 semanas. Após, caso a tHcy esteja menor que 80% do valor inicial, é considerado parcialmente responsivo e se a tHcy estiver maior que 80% do valor inicial, o indivíduo é considerado não responsivo à piridoxina. (12)

Figura 2: Esquema para definição de responsividade à piridoxina (adaptada de Morris *et al.*, 2016).

1.7 Diagnóstico Diferencial

O diagnóstico diferencial da HCU compreende principalmente a Síndrome de Marfan. Síndrome de Weil Marchesani, Ehlers Danlos e Deficiência de Sulfito Oxidase também devem ser pesquisadas caso seja excluída HCU. No caso de um paciente apresentar tromboembolismo, problemas psiquiátricos e deficiência intelectual, HCU deve ser considerada no diagnóstico diferencial, mesmo se o paciente não apresentar outras características de HCU (12). A hiperhomocisteinemia está presente em insuficiência renal, deficiência nutricional de vitamina B12, defeito no metabolismo celular da cobalamina e folato, doenças genéticas relacionadas com a absorção da vitamina B12 ou defeitos na remetilação da hcy (12).

1.8 Associação Genótipo-Fenótipo

Em algumas populações são encontradas variantes patogênicas específicas como c.919G>A (p.Gly307Ser) em irlandeses, c.572C>T (p.Thr191Met) em portugueses, espanhóis e sul americanos, c.1006C>T (p.Arg336Cys) no Qatari (todas não responsivas a piridoxina). Uma variante comum detectada em populações europeias, c.833 T>C (p.Ile278Thr), causa quando em homozigose, HCU responsiva à piridoxina. (10,12).

Foram avaliadas 35 pacientes (30 famílias) no Brasil com diagnóstico de HCU e observou–se os seguintes genótipos: p.Ile278Thr (18.2%), p.Trp323Ter (11.3%), p.Thr191Met (11.3%) e c.828+1G>A (11.3%). Oito novas variantes foram encontradas [c.2T>C, c.209+1delG, c.284T>C, c.329A>T, c.444delG, c.864_868delGAG c.989_991delAGG, and c.1223+5G>T]. A variante p.Ile278Thr foi encontrada em homozigose em três pacientes responsivos à piridoxina. Pacientes homozigotos para variante p.Trp323Ter, eram não responsivos à piridoxina. Pacientes portadores da variante p.Thr191Met geralmente não respondem à piridoxina, mas uma grande variabilidade de gravidade e sintomas clínicos pode ser observada. A variante c.828 + 1G> A foi relacionada ao início precoce dos sintomas (≤5 anos) e todos os pacientes não responderam à piridoxina, mesmo ao composto heterozigoto (57).

1.9 Tratamento

O tratamento recomendado inclui a suplementação de ácido fólico e vitamina B12 caso os pacientes apresentem alguma deficiência. Pacientes responsivos à piridoxina devem usar piridoxina 10 mg/Kg/dia evitando dose acima de 500 mg/dia por risco de neuropatia.

O uso do tratamento dietético deve ser considerado para os indivíduos afetados que não atingiram níveis ideais de tHcy com a suplementação de piridoxina. A dieta, juntamente com a fórmula metabólica, pode ser usada como único tratamento ou como terapia adjuvante juntamente com a piridoxina e/ou betaína. A maioria dos pacientes não responsivos à piridoxina necessitam de uma dieta com restrição de proteínas naturais e de uma fórmula metabólica isenta de metionina (12).

A Betaína deve ser considerada nos casos que não conseguem alcançar os níveis alvo de tHcy com os outros tratamentos. A dose inicial para crianças é 50 mg/kg duas vezes ao dia e para adultos 3 gramas duas vezes ao dia e deve ser ajustada de acordo com a resposta (12).

Além das terapias tradicionais, novas terapias estão em estudo como a terapia de reposição enzimática e suplementação de N-acetilcisteína. A descrição das terapias está na tabela 2 (58, 59).

Tabela 2 - Terapias atuais e em estudo para Homocistinúria Clássica

Terapia	Estágio	Mecanismo de ação
Piridoxina	Em uso atualmente	Co-fator da CBS reduzindo os níveis de tHcy.
Fórmula Metabólica livre de metionina	Em uso atualmente	Reduz o acúmulo de tHcy, limitando o consumo da metionina.
Dieta restrita em metionina	Em uso atualmente	Reduz o acúmulo de tHcy, limitando o consumo da metionina.
Betaína	Em uso atualmente	Atua na transformação da tHcy em metionina.
OT-58 (terapia de reposição enzimática)	Fase I/II	Converte Hcy livre em cistationina resultando na diminuição da tHcy no plasma e tecidos.
AEB4104 (terapia de reposição enzimática)	Fase pré –clínica	Atua tanto no dissulfeto de Hcy livre quanto no dissulfeto de Hcy-Hcy no plasma.
Erymethionase (terapia de reposição enzimática)	Fase pré –clínica	Degrada metionina e Hcy livre que entra em hemácias.
RTX-CBS (terapia de reposição enzimática)	Fase pré –clínica	Atua na Hcy livre que entra nas hemácias.

Traduzida e adaptada de Bublil *et al.,* 2019

1.10 Monitorização do Tratamento

O objetivo do tratamento é reduzir as concentrações plasmáticas de tHcy para um nível seguro, mantendo nutrição adequada e concentrações normais de metionina e outros aminoácidos essenciais. A dosagem de tHcy, nos pacientes responsivos à piridoxina, deve ser mantida abaixo de <50 µmol/L. Os pacientes que não respondem à piridoxina, a tHcy deve ser mantida abaixo de 100 µmol/L. O diagnóstico precoce, e consequentemente, tratamento precoce, previne as complicações da HCU, já no caso do diagnóstico tardio, o objetivo é prevenir futuras complicações, como por exemplo, o tromboembolismo. (12).

No Serviço de Genética do Hospital de Clínicas de Porto Alegre, a avaliação clínica, bioquímica (tHcy e metionina) e avaliação nutricional com nutricionista especializada ocorre a cada 4 meses. Os exames gerais, para acompanhamento nutricional, são realizados anualmente, incluindo hemograma com plaquetas, vitamina B12, cálcio, magnésio, fosfatase alcalina, paratormônio e vitamina D. A Densitometria Óssea é realizada a cada dois anos. Se houver mudança no exame neurológico ou se necessário por alguma queixa específica, é realizada ressonância magnética de crânio.

1.11 Triagem Neonatal

No Brasil, não é realizada a triagem neonatal para HCU. A triagem neonatal para HCU em geral é realizada pela presença de metionina em papel filtro, já que a dosagem de tHcy é mais difícil. Entretanto, em até 50% dos casos, pode-se encontrar falso-negativos, pois as formas mais leves de HCU, que são as responsivas à piridoxina, podem não apresentar aumento da metionina nos primeiros dias de vida (60, 61, 62). Além disso, outras doenças também podem ocasionar hipermetioninemia.

A triagem neonatal também pode ser realizada pesquisando a tHcy em papel filtro ou pesquisando variantes comuns em populações de alto risco. Ambos os métodos têm custo mais elevado e exigem maior capacidade técnica. (62, 63).

Capítulo II - Objetivos

2.1 Objetivos Gerais

Caracterizar uma coorte de pacientes com Homocistinúria Clássica do Sul do Brasil em relação aos aspectos neuropsiquiátricos, qualidade de vida e cardiológicos.

2.2 Objetivos Específicos

a) Avaliar a qualidade de vida em pacientes com HCU acompanhados no ambulatório de genética do Hospital de Clínicas de Porto Alegre através da aplicação do questionário WHOQOL-BREF.
b) Detectar a prevalência de deficiência intelectual nos pacientes HCU por meio da Escala Wechsler Abreviada de Inteligência (WASI).
c) Descrever quadro psiquiátrico neste grupo de pacientes e verificar prevalência de sintomas como depressão, ansiedade e esquizofrenia.
d) Caracterizar alterações em Sistema Nervoso Central através de volumetria cerebral.
e) Caracterizar as alterações cardiológicas através de eletrocardiogramas e ecocardiogramas.

Capítulo III – Artigo 1

Quality of Life in Patients with Classic Homocystinuria

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Abstract

Introduction: Classic Homocystinuria (HCU; OMIM 236200) is an autosomal recessive inborn error of metabolism characterized by increased total plasmatic homocysteine (tHcy) and methionine, which may present with a wide spectrum of severity and age of onset of symptoms. **Aims:** To evaluate the QoL of late-diagnosed individuals with HCU through the WHOQOL - BREF Questionnaire. **Methods:** retrospective study, sampled by convenience. Eleven Brazilian patients were included (male= 6; mean age at diagnosis= 11.2 ± 8.9 years; mean age at inclusion= 25.2 ±7.9 years; non-responsive to pyridoxine= 9). All patients answered the WHOQOL-BREF questionnaire (pre-treatment= 2/11; on treatment= 9/11) and the Wechsler Abbreviated Scale of Intelligence (WASI) test was applied to 8/11 patients. **Results:** We did not find a difference in QoL between pyridoxine-unresponsive and pyridoxine-responsive patients on treatment. Treated patients had a higher psychological domain score than untreated patients. **Conclusions:** Treatment of HCU appears to be associated with a better quality of life.

Keywords: Classic Homocystinuria, Cystathionine β-Synthase Deficiency, Quality of Life, WHOQOL-BREF.

Introduction

Cystathionine β-synthase deficiency (CBS deficiency) or Classic Homocystinuria (HCU; OMIM 236200) is an autosomal recessive inborn error of metabolism, characterized by increased total plasmatic homocysteine (tHcy) and methionine. Patients with HCU may present with a wide spectrum of severity and age of onset of symptoms. Major clinical features include optic lens dislocation, osteoporosis, 'marfanoid' habitus, learning difficulties and predisposition to thromboembolism. HCU is typically classified into three forms based on responsiveness to pyridoxine (vitamin B6) supplementation. Individuals who are pyridoxine-responsive usually present with attenuated symptoms and may be treated with pyridoxine and folic acid. Pyridoxine-unresponsive patients may be treated with higher doses of pyridoxine and folic acid, a protein-restricted diet with

methionine-free metabolic formula supplementation and betaine. A third patient classification is the partial pyridoxine-responsive form, which is treated with methionine-free metabolic formula supplementation and a protein-restricted diet. (Morris et al, 2017 and Sacharow et al, 2004).

In individuals with HCU, intelligence quotient (IQ) ranges from 10 to 138. Pyridoxine responsive patients, when compared to pyridoxine-unresponsive, are more likely to be cognitively intact or only mildly affected. For instance, the mean IQ of untreated individuals is 79 for the former group versus 57 for the latter (Yap et al., 2001b).

Considerable progress in the diagnosis and therapeutics for patients with inborn errors of metabolism and, consequently, HCU, has resulted in long- term survival; however, it is necessary to take into account their quality of life (QoL) and their well-being (Lee, 2002 and Zeltneret al., 2014).

According to the World Health Organization (WHO), QoL accounts for individuals' perception of their position in life based on culture, values, goals, expectations and concerns. QoL is a complex concept influenced by the person's physical health, psychological state, personal beliefs, social relationships and their relationship to salient features of their environment. There are many instruments to assess QoL as WHOQOL-100 and WHOQOL-BREF, which enable comparison between responses to items in both versions. WHOQOL-100 has six broad domains of QoL, summing up 100 assessment items. However, this instrument is very comprehensive and time-consuming, so WHO Quality of Life Group developed an abbreviated version: WHOQOL-BREF, which contains two items from Overall Quality of Life and General Health, and one item from each of the 24 facets included in the complete version, spreading out four domains: physical, psychological, social relationships, and environment. (Fleck et al., 2000)

HCU is a multisystemic disease that requires lifelong follow-up and treatment, which greatly interferes in the QoL. However, as HCU is an ultra-rare disease, scarce data on the QoL of these patients is available. (Morris et al., 2017). Anyhow, there are QoL reports in adult patients with inherited metabolic diseases. They measured 6 patients with B6-non-responsive HCU, but they weren’t analyzed separately (Cazzorla et al., 2012). Herein, we used the WHOQOL-BREF

Questionnaire to evaluate the QoL of patients with CBS deficiency, who have been followed up in a Brazilian reference center for diagnosis and treatment of IEM.

Methods

It was a retrospective study, based on review of medical records of eleven affected individuals with HCU, seen at the Medical Genetics Service of the Hospital de Clínicas de Porto Alegre, Brazil. We used WHOQOL-BREF questionnaire during patients' routine follow-up visits from August 2012 to December 2017. Questionnaires were read by researchers, due to decreased visual acuity presented by affected individuals. We compared our patients QoL, measured by WHOQOL - BREF, to the best available data in the literature: six adults, all pyridoxine-unresponsive HCU patients, described by Cazzorla *et al*. (2012), who were evaluated with WHOQOL - 100 Questionnaire. Above mentioned patients had a late diagnosis, no intellectual deficiency (IQ>70), mean age of 32.2 yo and were on protein-free diet at time of questionnaire application. To compare with a Italian cohort, we used a method for converting raw scores to transformed scores (minimum 4 and maximum 20). Patients with partial pyridoxine-responsive form were analyzed in the same group as pyridoxine-unresponsive patients. Additional data included: CBS deficiency form, current age, age at diagnosis, type of treatment and plasmatic tHcy. The effect size was calculated using the Cohen D Test to compare the resuts about our pyridoxine-unresponsive and pyridoxine-resonsive patients. Mann – Whitney Test and $p<0.05$ U were used to compared our patients QoL with italian patients QoL.

Eight patients agreed upon having their cognitive ability, for this the IQ was evaluated, using the Wechsler Abbreviated Scale of Intelligence II (WASI). This test is a tool that comprises two domains: Verbal IQ, that reflects semantic knowledge, memory, formation of verbal concepts, verbal and abstract logical reasoning; and Execution IQ, reflecting visuospatial information processing, attention to details and visual-motor coordination. The test score was classified as superior (≥130), very high (120-129), bright normal (110 - 119), average (90-109), low average (80-89), borderline (70-79) and extremely low (≤69).

Aims of this study were:1) to apply the WHOQOL - BREF Questionnaire in adults patients with HCU; 2) compare the domains between treated and non-treated patients, pyridoxine-unresponsive and pyridoxine-responsive patients; 3) compared WHOQOL - BREF Questionnaire data with 6 adult pyridoxine-unresponsive patient from literature and 4) discuss the impact of treatment with pyridoxine, folic acid, metabolic formula, diet or betaine in QoL.

Results

Eleven patients, who answered 35 questionnaires, were evaluated; it included pyridoxine-responsive and pyridoxine-unresponsive, as well as in treatment (9 patients) or without treatment (2 patients). Two patients were pyridoxine-responsive, receiving pyridoxine and folic acid. In pyridoxine-unresponsive patients, all of them were taking pyridoxine and folic acid at the time of questionnaire application. In addition to that, one patient was on a restricted protein diet, six patients on metabolic formula, plus protein-restricted diet and six individuals were taking betaine.

Patients' characteristics are summarized in Table 1. Two patients answered the WHOQOL-BREF before and nine patients after starting HCU-specific treatment. Eight patients performed IQ tests, and their results were compared based on schooling and Pyridoxine responsiveness (table 1). Some patients had specific considerations about IQ test application. Patient 1 desired to cooperate with the IQ test, however she tended to relate the items to personal reports, leading to long answers, who were often disconnected from the task required. This way, her scores were below average in both verbal and execution tasks. Patient 2 presented average score results for verbal skills, however her execution score was well below average. Although she had an understanding of test instructions, motivation, cooperation and was committed to perform each item of the execution tasks, her performance pointed out difficulties in this skill; this way, her total IQ score was below average. Patient 3 missed the appointment follow up and IQ test couldn't be performed. Patient 4 understood the questions, was motivated, cooperative, concentrated and committed to issue his best response, taking a long time in each subtest item. In addition to that, he could recognize when his answer was not

adequate. Regarding Patient 4 results, there was a considerable difference between verbal and execution skills; his scores were average for the former and below average for the latter, pushing his total IQ score towards "Extremely low" range. Unfortunately, patient 6 and patient 8 weren't cooperative and had significant visual impairment, making it implausible to apply the test.

A comparison among patient's groups is shown in Table 2. Patients' mean age (±SD) at inclusion was 25.2 (±7.9) years; nine patients were classified as pyridoxine-unresponsive, including one with partial responsiveness, (mean age 24.8 ± 6.3 years) and two were pyridoxine-responsive (ages 18.8 and 35.1 years). At diagnosis patients were (mean ±SD) 11.2 ± 8.9 years old. Nine patients were on HCU-specific treatment, i.e, pyridoxine, folic acid, methionine-free metabolic formula, specific diet or betaine; two patients did not start the treatment.

Two sisters (Patients 1 and 2), both pyridoxine-unresponsive subjects, answered two questionnaires each: one before treatment, when they were 32.5 and 20 years old, respectively and another, after one year on treatment. Treatment prescribed was pyridoxine, folic acid and protein-free diet, however, they did not follow the diet as recommended. The comparison between their QoL (WHOQOL - BREF) on diagnosis and one year after, is shown in Figure 1.

When compared our patients QoL with italian patients QoL were no statistically significant differences in most domains, except for "Environment"was statistically different between both groups, Brazilians had higher median punctuation than Italians ($p<0.05$ U de Mann – Whitney Test).

Discussion

QoL involves a complex process of mediation and determination that makes several variables possible to be associated with the patient's perception. QoL has two relevant aspects: subjectivity and multidimensionality, the last one explores the individual's perception of how their personal situation can be affected when many dimensions are considered. In addition to that, QoL can only be evaluated by the individual perspective and not by the view of scientists or health professionals (Gomes et al., 2014).

To our knowledge, this is the first study to evaluate QoL exclusively in HCU patients. Literature-wise, there are some studies assessing QoL in phenylketonuria, galactosemia and other metabolic diseases requiring dietary treatment in adulthood (Simon et al., 2008 and Morris et al., 2017).

HCU is a multisystemic disease with a wide symptom presentation. Besides that, it requires adherence to complex treatment that can impact on daily life. Since there is no specific questionnaire that addresses QoL in HCU patients, we used the WHOQOL - BREF because it is not time consuming, easy to be applied and evaluates main domains necessary to comprehend QoL.

Although both Pyridoxine-responsive and Pyridoxine-unresponsive patients are treated with oral pyridoxine and folinic acid supplementation, there are significant differences on their therapeutics. In the first group, there is no dietary restriction, but it also involves betaine, methionine-free metabolic formula. On the other hand, the second group requires a protein-restricted diet, free methionine metabolic formula and, in certain cases, betaine. Despite differences in treatment, interestingly overall QoL was similar, as expressed by WHOQOL score and its domains.

Two patients have had their questionnaires answered before and after treatment. They present a higher score in most domains after starting treatment, being followed-up and receiving support for their medical condition.

When comparing untreated patients (n= 9) with treated patients (n= 2), those receiving some kind of treatment had higher scores in the psychological domain, which means a better QoL in this aspect. Based on our clinical experience, we believe that starting a therapy which in patients' perception leads to an improvement in their QoL, allows an improvement in their self-esteem and positive feelings, reflected both on psychological domain. We did not perform statistical analyses due to small sample size (n=2).

Regarding the Environment domain, the perception of Italian and Brazilian patients is different. This domain assesses safety and physical environment, home environment, financial resources, health and social care, opportunities for acquiring new information and skills, leisure and transportation. Interestingly, it was higher in

Brazilian patients compared to the Italians, meaning that, at the time of the interview and questionnaire application, patients were satisfied with their environment.

A common reported problem, addressed by most patients, was the difficulty of buying adequate food for their diet, either because they could not find it in regular shops or because variety was not available. Moreover, three patients, employed by national companies, reported problems concerning meals offered at work; they didn't have access to food meeting their dietary requirements. Another challenge, faced by Brazilian patients, is the acquisition of methionine-free metabolic formula, because is very expensive. So, these situations can lead to poor adherence to the prescribed diet, affecting overall QoL.

Because HCU is an ultra-rare disease and our sample was by convenience, we did not exclude individuals with IQ<70. Eight patients had their IQ tested, but only two patients presented IQ> 70. As public-funded universal newborn screening in Brazil does not include HCU, most patients are diagnosed later in their life, already presenting symptoms and some intellectual deficiency. The individuals pyridoxine-unresponsive were identified on newborn screening, received early treatment and had good compliance, their mean IQ was 105 (Yap, et al., 2001B). Another study evaluated 63 HCU patients with psychiatric disturbances, concluding that IQ<70 was two-thirds more common among pyridoxine-unresponsive patients compared to those pyridoxine-responsive (Abbott et al., 1987).

Conclusion

The QoL has a close relation with various human life facets, needing to be contextualized in each environment, culture, expectations and concerns. We did not find a difference in QoL between pyridoxine-unresponsive and pyridoxine-responsive patients, but treated patients had a higher psychological score than untreated ones. Patients in Brazil and Italy present similar scores in almost all domains, except "environmental", probably due to existing cultural differences.

In chronic metabolic diseases with lifelong treatment, such as HCU, it is necessary to develop strategies to improve QoL and to understand the impact of these diseases on the patient's life. One way to evaluate these items would be to develop a specific instrument for QoL evaluation in patients with HCU.

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Figures

Figure 1 - Pre vs post-treatment WHOQOL - BREF in two Brazilian patients with Classic Homocystinuria

Table -1 Clinical summary of Classical Homocystinuria patients included in the study (n=11)

Patient	Age at First Questionnaire (years)	Gender	Pyridoxine responsiveness	tHcy at diagnosis (µmol/L)*	tHcy at inclusion (µmol/L)	Genotype	Visual Acuity**	Age at IQ test (years)	Education (years)	Verbal IQ***	Execution IQ***	Total IQ***
1	32.5	F	N	189.4	228.7	c.[828+1G>A]; c.[828+1G>A]	NA	33	11	49	49	43
2	20.0	F	N	228	152.8	c.[828+1G>A]; c.[828+1G>A]	NA	21	12	83	60	69
3	18.0	F	N	89.4	158.2	c.[572C>T]; c.[572C>T]	OD 1.0	20	NA	NA	NA	NA
						p.[Thr191Met]; p.[Thr191Met]	OS 1.0					
4	22.2	M	N	-*	247.1	c.[444delG]; c.[444delG]	OD 0.8	27	11	82	47	62
						p.[Asn149fs]; p.[Asn149fs]	OS 0.8					
5	23.7	F	N	-*	205.8	c.[828+1G>A]; c.[1126G>A]	OD 0.1	28	8	56	60	54
						p.? p.[Asp376Asn]	OS 1.0					
6	30.5	F	N	-*	159.6	c.[253G>A]; c.[253G>A]	OD 0.1	32	Zero	NA	NA	NA
						p.[Gly85Arg]; p.[Gly85Arg]	OS 0.1					
7	18.0	M	P	-*	52.3	c.[284T>C]; c.[284T>C]	OD 0.1	23	8	55	62	55
						p.[Ile95Thr]; p.[Ile95Thr]	OS 0.1					
8	39.0	M	N	-*	176.	c.[253G>A]; c.[253G>A]	OD blind	41	Zero	NA	NA	NA
						p.[Gly85Arg]; p.[Gly85Arg]	OS 0.02					
9	16.2	M	N	348	131.5	c.[572C>T]; c.[209+1delG]	OD 1.0	20	11	93	97	94
						p.[Thr191Met]; p.?	OS 1.0					
10	18.8	M	Y	-*	67.6	c.[46C>T]; c.[1058C>T]	OD 0.2	25	12	67	55	58
						p.[Pro49Leu]; p.[Thr353Met]	OS 0.2					
11	35.1	M	Y	431.2	24.4	c.[833T>C]; c.[833T>C]	OD 1.0	39	11	80	86	80
						p.[Ile278Thr]; p.[Ile278Thr]	OS 1.0					

*positive nitroprusside cyanide test; **visual acuity with best correction provided; ***WASI Score: superior: >130 points, very high: 120-129, bright normal: 110-119, average: 90-109, low average: 80-89, borderline: 70-79, extremely low: <69; F= female; M= male; N= no; NA= not available; OD=right eye, OS=left eye; P = partial; tHcy, tHcy: total plasmatic homocysteine, reference range: 5-15 µmol/L; Y= yes.

Table 2 - WHOQOL – BREF findings: Pyridoxine-responsive vs Pyridoxine-unresponsive patients and Pre vs post-treatment

Variables	Pyridoxine-responsive (n=2)	Pyridoxine-unresponsive (n=9)	Variables	No treatment (n=2)	Any treatment (n=9)
Mean age (SD) years	26.9 (±11.5)	24.8 (±7.8)	**Mean age (SD)**	26.3 (±8.9)	25.0 (±8.3)
Gender (F:M)	0:2	5:4	**Gender (F:M)**	2:0	3:6
Treatment			**Responsiveness to Pyridoxine**		
Domains (median; IQ 25-75)			**Domains (median; IQ 25-75)**		
-physical health	13.0 (11.0 - 13.5)	13.0 (10.5 – 15.0)	**-physical health**	12.0 (11.0 – 12.5)	13.0 (10.5 – 16.0)
-psychological	14.0 (13.2 - 14.7)	13.0 (12.0 – 15.0)	**-psychological**	12.0 (12.0 – 12.0)	15.0 (13.0 – 15.0)
-social relationships	14.0 (13.0 - 14.2)	12.0 (12.0 - 17.0)	**-social relationships**	14.0 (13.2 - 15.7)	13.0 (12.0 - 17.0)
-environmental	13.0 (13.2 – 13.7)	14.0 (12.0 – 14.0)	**-environmental**	13.0 (12.5 – 13.5)	14.0 (13.0 – 14.0)
-Total	14.0 (13.0 – 15.0)	13.0 (12.0 – 14.0)	**-Total**	13.0 (13.0 – 13.0)	14.0 (12.0 – 15.0)

F: female; M: male; IQ: intelligence quociente; SD: standard deviation.

Capítulo IV – Artigo 2

Brain Volume MRI and Psychiatric Symptoms in late-diagnosed Classic Homocystinuria patients

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Abstract: Classic Homocystinuria (HCU; OMIM 236200) is an autosomal recessive inborn error of metabolism that presents as multisystem disease, including psychiatric conditions and structural brain changes. This study aims to characterize the brain MRI findings and the association between brain volumetry and psychiatric symptoms, such as depression, anxiety, and psychosis in HCU patients. Methodology: This was a cross-sectional study. The sampling was by convenience. Patients followed at the Medical Genetics Service at Hospital de Clínicas de Porto Alegre, Brazil could be recruited if they had the genetic diagnosis of HCU. Brief Psychiatric Rating Scale (BPRS) scale, Beck 's Anxiety Scale, Hamilton's Depression Scale, Beck Depression Inventory (BDI) scale and WASI test were applied. Volumetric findings on brain MRI were evaluated using high-resolution volumetric T1-weighted data on a 1.5T simultaneously with the scales to evaluate psychiatric symptoms. Results: We included eight late diagnosed and unresponsive to pyridoxine HCU patients (four male) with a mean age of 26.8 years (SD±6.5). All were late diagnosed (partial responsive to B6= 1; non-responsive= 7). Five patients were classified as having a minimum, two as mild, and one as severe depression. Minimum anxiety symptoms were found in five patients, mild symptoms in one, and moderate in two. Moreover, psychotic symptoms were noticed in five patients. Cerebral white matter changes were found in 4 patients' MRI. Correlation was inverse between thalamus volume and Beck's Depression Scale (R=-0.813; p=0.049), Hamilton's Depression Scale (R=-0.819; p=0.046), BPRS (R=-0.834; p=0.039) and two BPRS sub-items: somatic concerns (R=-0.838; p=0.037) and mannerisms (R=-0.838; p=0.037). When comparing HCU patients with 15 healthy individuals showed a significant difference in white matter hypodensities (p=0.003). Conclusion: This study showed a high prevalence of depression, anxiety and schizophrenia symptoms in pyridoxine-unresponsive HCU patients. Also, it was seen a tendency towards thalamus involvement, as one of the structures related to the psychiatric condition in HCU pyridoxine-unresponsive subjects. When compared to controls, a statistical significant difference in white matter was observed.

Introduction

Classic Homocystinuria (HCU; OMIM 236200) is a rare autosomal recessive inborn error of metabolism due to Cystathionine β-Synthase deficiency (CBS) that results in alteration of sulfur amino acids metabolism (SAA), increased levels of homocysteine (Hcy), methionine, S adenosyl homocysteine, and reduction of cystathionine and cysteine (1). HCU can be classified into three forms according to response to pyridoxine treatment: responsive, non-responsive, or intermediate (1,2). It is a slow-progressive multisystem disease. Patients with a late diagnosis who are on treatment have a clinical condition characterized by ophthalmological, skeletal, vascular, and central nervous system abnormalities (1,2). However, there are few studies published in the literature regarding psychiatric conditions in HCU patients, and - to our knowledge - there is no data available about structural changes in brain volume.

Developmental delay is often the first sign in children with HCU. It is also observed an Intelligence Quotient (IQ) ranging from 10 to 138 in HCU patients. Pyridoxine-responsive patients are more likely than pyridoxine-unresponsive to be cognitively intact or only mildly affected; the mean IQ of untreated responsive individuals is 79 versus 57 for those not responsive (3). Moreover, affected individuals with HCU may present several psychiatric abnormalities such as personality disorders, schizophrenia, anxiety, depression, obsessive-compulsive behavior, and psychotic episodes. Psychiatric symptoms can be the first sign of the disease in some individuals (4,5,6). A recent study showed that psychiatric symptoms were found in 64% of patients, including anxiety in 33% and depression in 33% (7).

Brain MRI findings in HCU patients include arterial and venous neurovascular infarctions (8), cerebral edema, and cerebral white matter abnormalities, which have been reported in few patients treated with betaine, associated with high plasmatic methionine (9). Besides that, one case had basal ganglia nuclei involvement: this patient presented bilateral and symmetrical T2 prolongation and water diffusion restriction in bilateral globe pallidus and thalamus (8). Interestingly, plasmatic methionine and not tHcy is usually accepted as a causative agent of image findings. In several case reports, white matter abnormalities coincided with elevated plasma methionine levels, between 904 and 2823 μmol/L (8). Another theory is that abnormal homocysteine, methionine, and S-adenosylmethionine storage in the CSF, seen due

to secondary decreased CBS activity, can be the cause of cerebral white matter abnormalities (10).

It has been described an association of changes in central nervous system structures and psychiatric disorders, such as depression and volumetric reduction of hippocampus, left and right caudate nucleus, amygdala, thalamus, insula, frontal, temporal, and parietal cortex (11-15). Moreover, it has been described, reduction of hippocampal volume, grey matter volume in left postero-superior temporal region, insula, anterior cingulate cortex, thalamus, and amygdala in schizophrenia (16-24). Social anxiety disorder was associated with parietal and premotor cortex abnormalities (25). Curiously, these findings were not described yet in HCU.

Therefore, this study aims to characterize structural brain MRI findings and the associations between brain volume and psychiatric conditions such as depression, anxiety, and psychosis in HCU patients.

Methods

This study was approved by the local independent ethics committee, and all data collection and procedures were in accordance with the ethical standards.

This was a cross-sectional study. The sampling was by convenience. Patients with genetic diagnosis of HCU followed at the Medical Genetics Service at Hospital de Clínicas de Porto Alegre, Brazil were recruited. Brief Psychiatric Rating Scale (BPRS) scale, Beck 's Anxiety Scale, Hamilton's Depression Scale, and Beck Depression Inventory (BDI) scale were applied to evaluate depression, anxiety, and psychotic symptoms by trained research. The Wechsler Abbreviated Scale of Intelligence (WASI) test was also applied by specialized psychologists to assess cognition (via Intelligence Quotient, IQ) in these patients. We analysed tHcy and methionine values when the patients did the scales.

Volumetric findings on brain MRI were evaluated using high-resolution volumetric T1-weighted data on a 1.5T simultaneously with the scales to evaluate psychiatric symptoms. All scans were inspected for motion artifacts, and an experienced physician confirmed the presence or absence of pathological findings. Volumetric segmentation was performed using Free Surfer image analysis suite software v.5.1.0 (http://surfer.nmr.mgh.harvard.edu/). All images were processed and verified by the same researcher.

It was first described the psychiatric symptoms and after it was made correlations (rho) between these symptoms and MRI. The structures analyzed were total thalamus, total accumbens area, total hippocampus, white matter, cortex volume, subcortical volume, corpus callosum, total caudate, total putamen, total pallidum and total amygdala. Results were compared with 15 healthy individuals paired by gender and age, though Mann Whitney test.

Statistical analysis was performed using the Statistical Package for Social Sciences Program (version 18.0 SPSS Inc., Chicago, IL), and the level of significance was 5%.

Results

Eight HCU patients were evaluated, seven pyridoxine-unresponsive and one partial-responsive. Among them, four were male (mean age= 26.8 years ±6.5), and four patients were adherent to prescribed treatment (defined as plasmatic tHcy <100 µmol/L at the time of the evaluation). The summary of characteristics of the patients and main findings of the study are summarized in Table 1.

Regarding depressive symptoms, five pacientes had minimum, two patients had mild, and one had severe symptoms, as classified by Hamilton's Depression Scale and Beck Depression Inventory. Anxiety symptoms were minimum in five, mild in one, and moderate in two patients, as assessed by Beck 's Anxiety Scale. Moreover, BPRS scale suggested presence of psychotic symptoms in five patients.

Structural changes in white matter were seen in four patients (Figure 1). Patient 1b and 2a presented high plasmatic methionine levels three months prior to MRI brain evaluated by this study (734.1 and 930.4 µmol/L, respectively. Reference range: 13-37). Patient 2a had a Superior sagittal and transverse sinus thrombosis and patient 3 had a stroke.

When compared to healthy individuals paired by age and gender, HCU patients had more sites of white matter hypointensities (p =0.003). No significant differences were seen in grey matter volume, total subcortical volume or total ventricular volume, between cases and controls.

In HCU patients, the analyses of volumetria showed that the total thalamus, controlled by intracranial volume and age, had negative correlations with Beck's Depression Scale (R=-0.813; p=0.049), Hamilton's Scale (R=-0.819; p=0.046), BPRS

(R=-0.834; p=0.039) and with two BPRS sub-items, somatic concerns (R=-0.838; p=0.037) and mannerisms (R=-0.838; p=0.037). There was no statistically significant correlation between thcy and methionine levels and brain structures.

Discussion

This is the first study to correlate brain volume variation with depression, anxiety and schizophrenia in HCU patients. This study is in accordance with published literature, as we also identified the presence of psychiatric symptoms such as depression, anxiety and psychosis in HCU patients (7). Besides characterizing psychiatric alterations, we compared them with brain volumetry. Our findings showed a negative correlation between thalamus volume and the presence of psychiatric diseases in individuals with HCU. It is known that reductions in thalamic volume when compared with healthy controls are related to psychiatric disorders, mainly schizophrenia (27). In HCU, there are many factors involved in the pathophysiology of psychiatric illness. Some theories associate the psychiatric condition with the action of tHcy as an antagonist of NMDA receptors, central to the glutamatergic role in psychiatric illness (26).

Brain Volumetry is assessed using the T1 volumetric sequence. For better global and white matter assessment, we performed brain MRI: T2 WI, Flair WI and DTI. White matter changes were detected in 50% of the patients' sample. Most accepted theory, for explaining white matter lesions, is an increase in methionine levels; as expected, only one of those 4 patients with white matter changes had methionine <700 µmol/L in the three months before. However, other theories speculate that abnormal accumulation of homocysteine, methionine, and S-adenosylmethionine in the CSF secondary to decreased CBS activity causes white matter diffusion restriction via inhibition of Na + / K + ATPase and resultant cytotoxic edema (8). Therefore, more than one mechanism could explain the presence of lesions in white matter.

As in many rare diseases studies, the main limitation of our study is the small sample size. Besides that, only pyridoxine-unresponsive patients were evaluated, hence it is not possible to compare HCU patients regarding pyridoxine response. As an exploratory study, we could infer some relations, however, we suggest further studies to validate our findings. A strength of our study is the confirmation of said psychiatric findings, through the application of well-known instruments.

Conclusion

This study demonstrated high prevalence of psychiatric symptoms in pyridoxine-unresponsive HCU patients, a tendency of thalamus involvement, as one of the structures related to the psychiatric condition in patients with HCU. Except for changes in white matter, there were no other differences in brain volumetry between HCU patients and controls.

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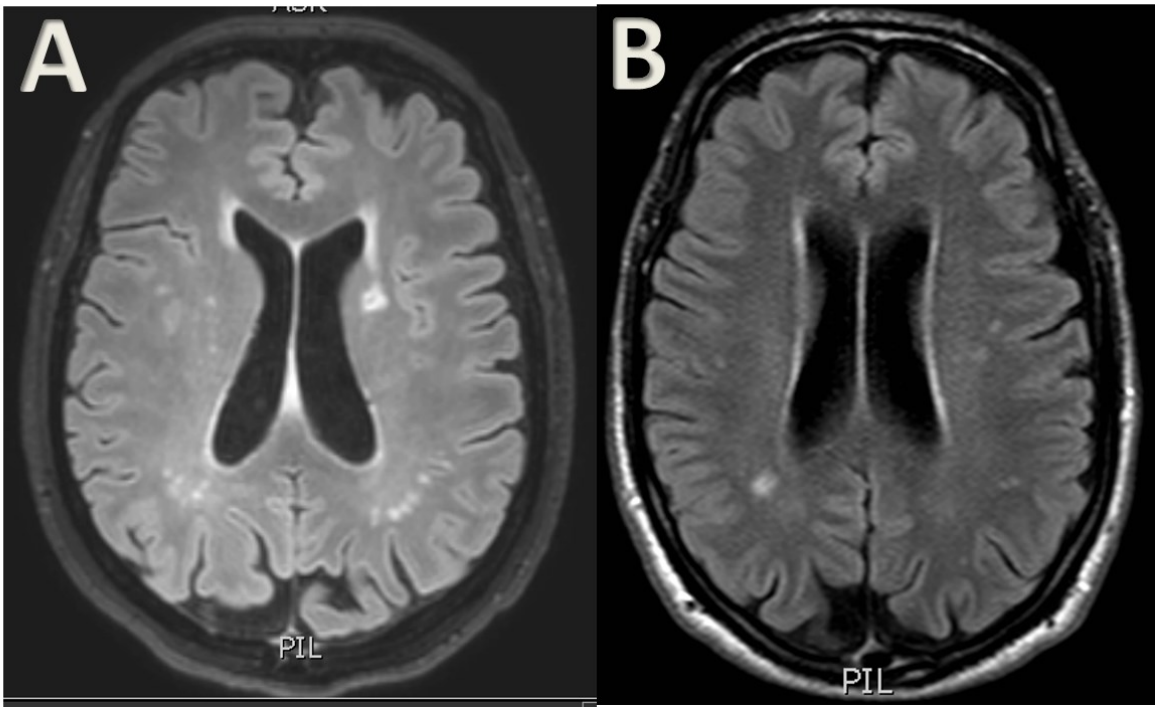

Figure 1 - White matter changes in MRI Flair sequence: deep, subcortical and periventricular hyperintensities. 1A- Patient 2a, 32 years old and 1B- Patient 2b, 41 years old.

Table 1: Patient's characteristics and main findings of this study.

Patient	Gender	Age (y)	Age at diagnosis (y)	Pyridoxine-responsive	tHcy at diagnosis (µmol/L)	tHcy at evaluation	Methionine at evaluation (µmol/L) (Ref 13-37)	Genotype #	BPRS*	Beck 's Anxiety Scale **	Hamilton' s Depression Scale ***	BDI#	Brain MR findingsI	HCU treatment	Total IQ& (verbal, execution)
1a	F	33	32	No	189.4	199.6	473.2	c.[828+1G>A]; c.[828+1G>A] p.[828ins104,737del92]	33	12	8	6	Slight prominence of the subarachnoid sulcus	Pyridoxine, folic acid, irregular restrict protein diet	43 (49,49)
1b	F	20	20	No	228	261.2	448.0	c.[828+1G>A]; c.[828+1G>A] p.[828ins104,737del92]	18	4	2	2	Subcortical WM Hyperintensities in T2	Pyridoxine, folic acid, irregular restrict protein diet	69 (83,60)
2a	F	32	5	No	-	329.0	691.0	c.[253G>A]; c.[253G>A] p.[Gly85Arg]; p.[Gly85Arg]	48	21	19	23	Deep, subcortical and periventricular WM hyperintensities (T2), seen in cerebral hemispheres, likely previous microangiopathic changes. Slight reduction in encephalic volume. Transverse and sigmoid sinuses thrombosis.	Pyridoxine, folic acid, irregular protein-restricted diet, betaine	-
2b	M	41	14	No	-	254.9	744.9	c.[253G>A]; c.[253G>A] p.[Gly85Arg]; p.[Gly85Arg]	24	4	0	0	Deep, subcortical and periventricular WM hyperintensities (T2) in cerebral hemispheres compatible with previous microangiopathic changes Slight reduction in diffuse encephalic volume.	Pyridoxine, folic acid, irregular protein-restricted diet, irregular methionine-free metabolic formula, betaine	-
3	M	22	2	Partial	-	36.2	43.5	c.[284T>C]; c.[284T>C] p.[Ile95Thr]; p.[Ile95Thr]	18	2	1	0	Periventricular WM hyperintensity in T2	Pyridoxine, folic acid, protein-restricted diet, methionine-free metabolic formula, betaine	55 (55,62)
4	M	27	6	No	-	70.0	336.0	c.[444delG]; c.[444delG] p.[Asn149fs]; p.[Asn149fs]	18	0	0	0	Prominence of telencephalic cortical sulcus	Pyridoxine, folic acid, protein-restricted diet, betaine	62 (82,47)
5	F	28	8	No	-	60.2	60.5	c.[828+1G>A]; c.[1126G>A] p.[828ins104,737del92]; p.[Asp376Asn]	37	27	11	11	Slight reduction in posterior parietal volume.	Pyridoxine, folic acid, irregular protein-restricted diet	54 (56,60)
6	M	20	13	No	348.0	92.1	453.6	c.[828+1G>A; c.[209+1delG p.[828ins104,737del92]; p?	23	0	6	3	Normal	Pyridoxine, folic acid, irregular protein-restricted diet, methionine-free metabolic formula, betaine	94 (93,97)

tHcy, total plasmatic homocysteine - reference range: 5-15 µmol/L; y, years; WM, White Matter; *Abnormal BPRS > 18 points; ** Beck 's Anxiety Scale, minimum: 0–10, mild: 11-19, moderate: 20-30, severe: 31-63; *** Hamilton' s Depression Scale: minimum: 0-7, mild: 8-13, moderate: 14-18, severe: 19-22, very severe: >23; #BDI: minimum: 0-13; mild: 14-19; moderate: 20-28; severe: 29-63; & IQ, measured by WASI scale, superior: ≥130, very high: 120-129, bight normal; 110-119, average: 90-109, low average: 80-89, borderline: 70-79, extremely low: <69 #See reference 28 to genotype of patients 1–6

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Cardiovascular findings in classic homocystinuria

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ABSTRACT

Objective: describe cardiovascular findings from echocardiograms and electrocardiograms in patients with Classic Homocystinuria
Methods: this retrospective exploratory study evaluated fourteen subjects with Classic Homocystinuria (median age − 27.3 years; male n − 8, B6-non-responsive n − 9 patients), recruited by convenience sampling from patients seen Hospital de Clínicas de Porto Alegre (Brazil), between January 1997 and July 2020. Data on clinical findings, echocardiogram and electrocardiogram were retrieved from medical records.
Results: Eight patients presented some abnormalities on echocardiogram (n − 6) or electrocardiogram (n − 5). The most frequent finding was mild tricuspid regurgitation (n − 3), followed by mitral valve prolapse, mild mitral regurgitation, enlarged left atrium and aortic valve sclerosis (n − 2 patients each). Aortic root ectasia was found in one patient. Venous thrombosis was reported in six patients: deep vein thrombosis of lower limbs (n − 3), ischaemic stroke (n − 1), cerebral venous sinus thrombosis (n − 1) and pulmonary vein thrombosis (n − 1).
Conclusion: mild valvulopathies seen to be common in patients with Classic Homocystinuria, but more studies regarding echocardiogram and electrocardiogram in this population are needed to draw absolute conclusions.

1. Introduction

Classic Homocystinuria (HCU) or Cystathionine β-Synthase Deficiency (CBS) is a rare autosomal recessive inborn error of metabolism (OMIM 236200), characterized by markedly increased concentrations of plasma total homocysteine (tHcy) and methionine [1]. The incidence of HCU is estimated to be at least 0.38:100,000, varying from −0.72:100,000 in non-Finnish Europeans, −0.45:100,000 to the lower rates reported among Africans (−0.20:100,000) and Asians (−0.02:100,000) [2]. HCU can be classified according to responsiveness to pyridoxine (vitamin B6), as responsive and non-responsive, but it is also known that some patients will have an intermediate metabolism [1]. Treatment with pyridoxine is prescribed for all patients; a combination of methionine-restricted diet, methionine-free metabolic formula, vitamin B12, betaine and folate is used in pyridoxine non-responsive individuals [1].

Clinical manifestations observed in responsive patients usually are milder and develop later in life [3]. Thromboembolic events are common, due to the well-known association between elevated plasma homocysteine and intraluminal venous thrombi formation [4]. Besides cardiovascular events, systemic manifestations are also seen, such as ectopia lentis, marfanoid habitus, osteoporosis, intellectual disability and psychiatric illness [3,5].

There is a paucity of information regarding heart disease in HCU patients. Although not fully understood, it is known that damage to connective tissue can happen [6] and chronically elevated plasma tHcy could reduce in fibrillin-1 disulfide bonds, leading to changes in both cardiac structure and function [7]. Mainly findings were described in

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Table 1
– Clinical summary of the Classic Homocystinuria patients included in the study (n = 14).

Patient	Gender	Pyridoxine Responsiveness	tHcy at Diagnosis (μmol/L) (Ref 5–15)	tHcy Mean (SD) (μmol/L) (Ref 5–15)	Age at Diagnosis (years)	Arterial Hypertension	Genotype*	Thromboembolic Events (age)	HCU Treatment
1a	F	No	273.0	–	7	No	c.[253G > A]; c.[253G > A] p.[Gly85Arg]; p.[Gly85Arg]	Ischaemic stroke (22 years)	Restricteded protein diet
1b	F	No	–	266.5 (±79.6)	5	Yes	c.[253G > A]; c.[253G > A] p.[Gly85Arg]; p.[Gly85Arg]	Cerebral venous sinus thrombosis (30 years)	Pyridoxine, folic acid, irregular restricteded protein diet, betaine
1c	M	No	–	156.7 (±62.2)	14	No	c.[253G > A]; c.[253G > A] p.[Gly85Arg]; p.[Gly85Arg]	No	Pyridoxine, folic acid, irregular restricteded protein diet, irregular methionine-free metabolic formula, betaine
2	M	No	348.0	141.7 (±40.6)	13	No	c.[828 + 1G > A; c.[209 + 1delG p.[828ins104,737del92]; p?	No	Pyridoxine, folic acid, irregular restricteded protein diet, methionine-free metabolic formula, betaine
3a	F	No	228.0	205.2 (±61.7)	20	No	c.[828 + 1G > A]; c.[828 + 1G > A] p.[828ins104,737del92]	Left leg and pulmonary venous thrombosis (15 years)	Pyridoxine, folic acid, irregular restricteded protein diet,
3b	F	No	189.4	205.3 (±97.6)	32	No	c.[828 + 1G > A]; c.[828 + 1G > A] p.[828ins104,737del92]	Right leg venous thrombosis (19 years)	Pyridoxine, folic acid, irregular restricteded protein diet,
4	F	No	–	88.9 (±15.1)	8	No	c.[828 + 1G > A]; c.[1126G > A] p.[828ins104,737del92]; p.[Asp376Asn]	No	Pyridoxine, folic acid, irregular restricteded protein diet
5	F	No	89.4	123.4 (±29.8)	18	No	c.[572C > T]; c.[572C > T] p.[Thr191Met]; p.[Thr191Met]	No	Pyridoxine, folic acid, betaine
6	M	Yes	431.2	17.1 (±3.6)	34	Yes	c.[833 T > C]; c.[833 T > C] p.[Ile278Thr]; p.[Ile278Thr]	No	Pyridoxine, folic acid
7	M	Yes	–	12.4 (±2.7)	4	Yes	c.[146C > T]; c.[1058C > T] p.[Pro49Leu]; p.[Thr353Met]	No	Pyridoxine, folic acid
8	M	Partial	–	63.4 (±33.1)	2	No	c.[284 T > C]; c.[284 T > C] p.[Ile95Thr]; p.[Ile95Thr]	Ischaemic stroke (8 months)	Pyridoxine, folic acid, restricteded protein diet, methionine-free metabolic formula, betaine
9	M	No	–	116.2 (±41.3)	6	No	c.[444delG]; c.[444delG] p.[Asn149fs]; p.[Asn149fs]	Right leg venous thrombosis (16 years)	Pyridoxine, folic acid, restricteded protein diet, betaine
10	M	Partial	184.6	98.4 (±74.0.1)	4	No	c.[526G > A]; c.[1598 T > G] p. [Glu176Lys]/p.[Val533Gly]	No	Pyridoxine, folic acid, restricteded protein diet, methionine-free metabolic formula
11	M	Yes	150.3	189.8 (±55.9)	55	Yes	c.[833 T > C]; c.[833 T > C] p.[Ile278Thr]; p.[Ile278Thr]	No	No treatment

F/M: female/male; tHcy: total homocysteine. *See reference 19 to genotype of patients 1–9.

case reports, like orthostatic postural tachycardia [8] or calcified atrial mass [9], the later a result from endothelial dysfunction - already documented in HCU. In addition to that, patients with classic phenotype also have higher incidence of aortic root ectasia, seen by echocardiography [10].

2. Materials and methods

We performed a retrospective, exploratory study in a reference center for metabolic diseases at Hospital de Clínicas de Porto Alegre, Brazil. All procedures and data collection were in accordance with the

Table 2
– Electrocardiogram Findings in HCU patients ($n = 11$).

Patient	ECG 1 (age)	ECG 2 (age)	ECG 3 (age)	ECG 4 (age)	ECG 5 (age)	ECG 6 (age)
1a	Normal (24.0 yo)	Normal (27.4 yo)	NP	NP	NP	NP
1b	Left atrial overload (16.7 yo)	Normal (19.4 yo)	Normal (22.8 yo)	Normal (27.0 yo)	Normal (32.7 yo)	NP
1c	Early ventricular repolarization (25.2 yo)	Normal (27.9 yo)	Left ventricular overload Early ventricular repolarization (30.1 yo)	Left ventricular overload (39.5 yo)	Left ventricular overload Early ventricular repolarization (40.3 yo)	Left ventricular overload (41.8 yo)
2	Normal (16.6 yo)	Normal (19.0 yo)	Normal (20.8 yo)	Normal (22 yo)	NP	NP
3a	Normal (21.5 yo)	NP	NP	NP	NP	NP
3b	Incomplete right bundle branch block (32.5 yo)	Normal (34.0 yo)	NP	NP	NP	NP
6	Normal (39.0 yo)	Normal (40.0 yo)	Normal (41.0 yo)	NP	NP	NP
8	Normal (8.6 yo)	Left ventricular overload (17.8 yo)	Left ventricular overload (21.2 yo)	Normal (22.4 yo)	Left ventricular overload (23.6 yo)	NP
9	Left bundle branch block (25.3 yo)	Left bundle branch block (26.9 yo)	NP	NP	NP	NP
10	Normal (4.3 yo)	Normal (5.3 yo)	NP	NP	NP	NP
11	Normal (55.3 yo)	Normal (55.4 yo)	NP	NP	NP	NP

NP – Not performed.

ethical standards of the local research committee and Helsinki Declaration. A convenience sample of 14 patients were included, whose information (electrocardiogram, echocardiogram and clinical data), from January 1997 to July 2020, was retrieved from medical records.

Pyridoxine responsiveness was defined as tHcy reduction in plasma to less than 50 μmol/L after treatment with pyridoxine; partial responsiveness was considered when more than 20% decrease in tHcy levels happened, but remained above 50 μmol/L and non-responsive when tHcy fell less than 20% after pyridoxine [1]. Arterial hypertension was defined according to AHA/ACC guidelines [11]. Aortic root measurement in the echocardiogram, was from leading edge to leading edge and a standardized z score was determined using the method of Devereaux for adults (>18 years old) and the method of Gautier for children and adolescents [12,13]. A z score < 2.0 was considered normal. The minimal regurgitation was not considered in the analysis, because usually it represents a physiological finding [14].

Statistical analyses were carried out in IBM SPSS Statistics, Version 21.0 (SPSS Inc., Chicago, IL) and the level of significance was considered 5%. Binary regression model was used to verify the effect of tHcy and pyridoxine responsiveness in hypertension, left ventricular overload, mild mitral regurgitation, mild tricuspid regurgitation, aortic valve sclerosis, mitral valve prolapse and enlarged left atrium.

3. Results

Fourteen patients from 11 families, 4.3 to 55.42 years old (median age – 27.3; IQ – 22.5–33.8) were included. Other main characteristics are summarized in Table 1. Patients 1a, 1b and 1c are siblings, same as 3a and 3b. Thirteen patients were receiving specific HCU treatment at the time of the study. Arterial hypertension was diagnosed in four patients, all of them taking at least one antihypertensive medication and all, except one, pyridoxine-responsive. Episodes of thrombosis were reported in six patients: two suffered ischaemic stroke, three had deep vein thrombosis in lower limbs and one patient presented cerebral venous sinus thrombosis.

A total of 34 electrocardiograms results were available for 11 patients and 46 complete echocardiograms for all patients. Eight patients presented at least one abnormality on echocardiogram ($n = 6$) or electrocardiogram ($n = 5$). Detailed electrocardiogram results and the age they were performed, are shown in Table 2; most patients had at least one, to a maximum of six different exams, however only incomplete reports were available for patients 4, 5 and 7. Regarding echocardiographic heart studies, all echocardiograms were transthoracic, being an exception patient 1a's second test, that was transesophageal. All patients left ventricular ejection fraction were greater than 50%, $n = 4/14$ patients (28.6%) had at least a valvar change at some point, all of them mild and not related to clinical manifestations. The most frequent finding on echocardiogram was mild tricuspid regurgitation ($n = 3$), followed by mitral valve prolapse, left atrial enlargement, aortic valve sclerosis and mild mitral regurgitation, described in two patients each (Table 3). Aortic root ectasia was found in one patient. No correlation was found between tHcy and hypertension, left ventricular overload, mild mitral regurgitation, mild tricuspid regurgitation, aortic valve sclerosis, mitral valve prolapse and enlarged left atrium; likewise, these variables did not have any statistically significant correlation with pyridoxine responsiveness.

4. Discussion

There is a lack of knowledge regarding heart disease in HCU patients, only some case series describe findings on electro and echocardiogram exams. Valve changes in HCU patients such as mitral prolapse, mitral and tricuspid regurgitation are rarely described [15,16]. Among the study subjects, we found a high prevalence of those, such as mitral prolapse, mitral and tricuspid regurgitation and aortic valve sclerosis. These results might be seen as exploratory, since such findings were not extensively described in HCU and our cohort was not matched with healthy subjects. We hypothesize that this correlation can be valid, as connective tissue - the main heart valve component - can be impaired in HCU patients [6,7]. The prevalence of findings, such as tricuspid regurgitation and mitral regurgitation, in pyridoxine-responsive ($n = 3$) and partially responsive ($n = 2$) patients seem to be lower than in non-responsive patients ($n = 9$). On the other hand, aortic valve abnormalities were more common in pyridoxine-responsive patients, possibly

Table 3
– Echocardiogram Findings in 14 HCU patients.

Patient	Echo 1	Echo 2	Echo 3	Echo 4	Echo 5	Echo 6	Echo 7	Aortic Root
1a	Normal (22.0 yo)	Normal (22.0 yo)	Normal (27.4 yo)	NP	NP	NP	NP	Normal (Z < 2)
1b	Mild mitral regurgitation (22.7 yo)	Mild Diastolic deficit (27.0 yo)	Mild mitral and tricuspid regurgitation (30.7 yo)	Mild mitral and tricuspid regurgitation (32.4)	Normal (Minimal mitral and tricuspid regurgitation) (32.7 yo)	Normal (Minimal mitral and tricuspid regurgitation) (33.3 yo)	Normal (Minimal mitral and tricuspid regurgitation) (34.0 yo)	Normal (Z < 2)
1c	Normal (30.0 yo)	Normal (31.2 yo)	Mild mitral regurgitation. (38.3 yo)	Normal (40.3 yo)	Mild mitral regurgitation (40.9 yo)	Mitral valve prolapse and mild regurgitation. Mild tricuspid regurgitation (41.8 yo)	Mitral valve prolapse and mild regurgitation. (42.0 yo)	Normal (Z < 2)
2	Normal (17.5 yo)	Normal (19.0 yo)	Normal (20.0 yo)	Normal (22.0 yo)	NP	NP	NP	Normal (Z < 2)
3a	Normal (21.5 yo)	NP	NP	NP	NP	NP	NP	Normal (Z < 2)
3b	Normal (32.5 yo)	Normal (34.0 yo)	NP	NP	NP	NP	NP	Normal (Z < 2)
4	Mild tricuspid regurgitation Mild left atrial enlargement (25.8 yo)	NP	NP	NP	NP	NP	NP	Normal (Z < 2)
5	Normal (17.3 yo)	NP	NP	NP	NP	NP	NP	Normal (Z < 2)
6	Aortic valve sclerosis and mild regurgitation (37.1 yo)	Mild aortic regurgitation (39.0 yo)	Mild aortic regurgitation (40.0 yo)	Aortic valve sclerosis and mild regurgitation (41.0 yo)	NP	NP	NP	Normal (Z < 2)
7	Normal (21.3 yo)	Normal (23.8 yo)	NP	NP	NP	NP	NP	Normal (Z < 2)
8	Small interventricular communication (6.9 yo)	Small interventricular communication (8.6 yo)	Small interventricular communication (11.6 yo)	Mitral valve prolapse. (17.8 yo)	Mitral valve prolapse. (21.2 yo)	Mitral valve prolapse. (22.4 yo)	Mitral valve prolapse. (23.6 yo)	Normal (Z < 2)
9	Normal (19.0 yo)	Normal (26.1 yo)	Normal (27.1 yo)					Normal (Z < 2)
10	Normal (4.3 yo)	Normal (5.33 yo)	Normal (6.0 yo)	NP	NP	NP	NP	Normal (Z < 2)
11	Aortic valve sclerosis Aortic root ectasia. Mild diastolic dysfunction. Slight enlargement of left atrium (55.4 yo)	NP	NP	NP	NP	NP	NP	Aortic root ectasia (Z ≥ 2)

NP – Not performed.

because this group was older.

Regarding electrocardiogram features, the main alteration was left ventricular overload, likely due to mitral regurgitation and mitral prolapse present in these patients. One female, 32 years old, normal blood pressure, had an incomplete right bundle branch block (RBBB), a common encounter at all ages, more prevalent in male and associated with hypertension, but not to other cardiovascular risk factors [17]. Moreover, partial RBBB is not associated with cardiovascular mortality, but some patients can evolve later with complete RBBB [17]. Another male from our cohort, 25 years old, normotensive, presented a left bundle branch block (LBBB) in one electrocardiogram, but his echocardiograms were all normal from age 19 to 27 years. Interestingly, LBBB is associated with arterial hypertension, age, coronary artery disease, left ventricular hypertrophy, ST-T abnormalities and an increased cardiothoracic ratio, none of them present in this subject [18]. Early ventricular repolarization was detected in one patient, who also had left ventricular overload. The former can be found in up to 13% of healthy individuals, although it is identified as a potential cause of sudden death [18]. It is possible that these combined findings are correlated.

We found a prevalence of 30% of arterial hypertension, similar to described in the literature for HCU [10]. Aortic root ectasia was present in one patient of our cohort, also described previously among HCU individuals [10]. He was known to suffer from arterial hypertension, but his blood pressure was controlled by pharmacological therapy. The relationship between arterial hypertension and aortic root ectasia is controversial, despite the large number of studies that tried to establish correlation [10]. Furthermore, no patients presented myocardial infarction (acute or previous), what is dissonant from 4% prevalence, described in the literature [1]. Ultimately, no familial pattern was observed for either echocardiograms or electrocardiograms findings.

5. Conclusion

This study described 10-year cardiological follow-up in a cohort of fourteen patients with HCU, showing high prevalence of mild valvulopathies. Our main limitations were the lack of a control group and its

retrospective and exploratory design. According to our results, we suggest considering an echocardiogram and electrocardiogram for each patient, at least in their first visit and monitor periodically their arterial blood pressure. That is justified by the fact that previous studies suggest an increased risk of ischaemic heart disease in HCU patients. Additionally, we believe that our report is not the definitive answer regarding echocardiogram and electrocardiogram in this population, but the opening for more discussion and new questions.

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Authors' roles

1- Conception and design of study: A. Conception, B. Organization, C. Execution;
2- Acquisition and analysis of data: A. Acquisition; B. Analysis of data.
3- Manuscript: A.Writing of the first draft; B. Review and Critique.
1- Marco Antônio Baptista Kalil: 1A, 1B, 1C, 2A, 2B, 3A (Nothing to disclose).
2- Karina Carvalho Donis: 1A, 1B, 1C, 2A, 2B, 3B (Nothing to disclose).
3- Fabiano de Oliveira Poswar: 2A, 2B, 3B (Nothing to disclose).
4- Bruna Bento dos Santos: 1C, 2A, 2B, 3B (Nothing to disclose).
5- Ângela Barreto Santiago Santos: 2B, 3B (Nothing to disclose).
6- Ida Vanessa Doederlein Schwartz: 1A, 1B, 1C, 2B, 3B (Nothing to disclose).

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Capítulo VI – Artigo 4

An adult with cystathionine beta-synthase deficiency, idiopathic rheumatoid arthritis, and deafness: A case report

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Keywords: Classic Homocystinuria, Camptodactyly-Arthropathy-Coxa Vara-Pericarditis Syndrome, Deafness

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Situation: submited

Abstract

Cystathionine beta-synthase (CBS) deficiency or Classic Homocystinuria (HCU) is an autosomal recessive disorder characterized by increased plasmatic homocysteine and methionine with variable expressivity and wide range of clinical manifestations. Affected individuals are typically classified as pyridoxine-responsive, partial pyridoxine-responsive, and pyridoxine-unresponsive. We report on an adult with *ectopia lentis,* idiopathic rheumatoid arthritis and deafness who was ultimately diagnosed with pyridoxine-responsive HCU after genetics evaluation. Because he presented symptoms not usually associated with HCU, exome sequencing was performed, confirming the diagnosis of three monogenic diseases: HCU, Camptodactyly-Arthropathy-Coxa Vara-Pericarditis Syndrome (CACP), and autosomal recessive nonsyndromic deafness, allowing improvement of patient's treatment.

Introduction

Cystathionine beta-synthase (CBS) deficiency or classic homocystinuria (HCU; OMIM 236200) is a rare autosomal recessive inborn error of metabolism, due to impaired conversion of homocysteine (Hcy) to cystathionine leading to accumulation of the former. There is a wide range of clinical manifestations, varying from severe childhood-onset multisystem disease to asymptomatic individuals until adulthood. The main findings are dislocation of the optic lenses, osteoporosis, 'marfanoid' habitus, learning difficulties, and predisposition to thromboembolism [1].

HCU is typically classified into three phenotypes: pyridoxine-responsive, partial pyridoxine-responsive, and pyridoxine-unresponsive. Individuals with the responsive form usually have milder phenotypes and are treated with pyridoxine (vitamin B6) and folic acid. On the other hand, unresponsive patients may require, in addition to pyridoxine and folic acid, restricted diet with methionine-free metabolic formula supplementation and betaine. Most of the individuals with atypical or attenuated phenotypes remain undiagnosed for several years [1].

Here we report on an unprecedented case of an adult patient with *ectopia lentis*, idiopathic rheumatoid arthritis, deafness, and psychiatric disorder who was

referred to Genetic evaluation and ultimately was diagnosed with three monogenic diseases.

Case report

A 33-year-old male patient was sent to genetic evaluation due to multi-system symptoms. He was the first-born to a consanguineous couple, with no family history of genetic diseases (Figure 1).

At three months of age, he presented with hand contractures and underwent surgical correction when he was one year old. At age of two years, he had edema on the knees and at six years of age, he was evaluated by a rheumatologist and was diagnosed with juvenile idiopathic arthritis (JIA). At that time, inflammatory markers were normal and treatment with methotrexate and corticosteroids was initiated. His psychomotor development was normal and he developed bilateral hearing loss at age of 9. He had bilateral total hip arthroplasty at age of 13, surgery for *ectopia lentis* at 23 years of age and a single seizure at 26 years of age. After a few years, he started having auditory hallucinations but no definitive psychiatric diagnosis was made at that time. Lesions suggesting osteonecrosis of the femur head were seen on a hip X-ray performed at age 33, likely due to long-term use of corticosteroids. No acetabular cyst was detected (Figure 2). Hand X-ray was done at 37 years of age and showed periarticular osteopenia, deformity with subluxation of proximal interphalangeal joints bilaterally, bone proliferations along the ulnar radio articulations bilaterally, pseudocysts in the styloid processes, and tenuous images with calcic attenuation projected in the region of the cartilage fibrous triangular laterally (Figure 3).

A comprehensive biochemical investigation was done and showed tHcy 431 umol/L (Reference range: 5-15) and methionine: 42 umol/L (Ref: 13-37). Due to a presumable HCU diagnosis, treatment with pyridoxine 500 mg/day was initiated and the homocysteine level decreased to 31 umol/L.

Targeted genetic analysis confirmed a homozygous pathogenic variant in *CBS* [NM_000071.2] c.833T>C (p.Ile278Thr), which has been associated with pyridoxine responsiveness (Figure 4). However, HCU alone did not explain the JIA diagnosis; hand and elbow contractures; and hearing loss. Hence, exome

sequencing was performed and, in addition to the pathogenic variant in *CBS*, it revealed a homozygous pathogenic variant in *TMPRSS3* [NM_001256317.1] c.413C>A (p.Ala138Glu) which has been associated with autosomal recessive nonsyndromic deafness, as well as a homozygous likely pathogenic variant in *PRG4* [NM_ 001127708.3] c.3755_3756insA (p.(Lys1253*) related to Camptodactyly-Arthropathy-Coxa Vara-Pericarditis (CACP) Syndrome (Figure 5a, 5b, 5c). At the time all diagnoses were achieved, his medication regimen was: pyridoxine 500 mg/day, folic acid 5 mg/day, valproic acid 750 mg 3 times/day, olanzapine 15 mg/day, phenobarbital 100 mg/day, escitalopram 10 mg/day, and methotrexate 5 mg twice a month. Once the JIA diagnosis was dismissed, methotrexate was stopped.

All his siblings were evaluated and were found to have normal levels of tHcy and methionine, normal hearing, and normal musculoskeletal exam.

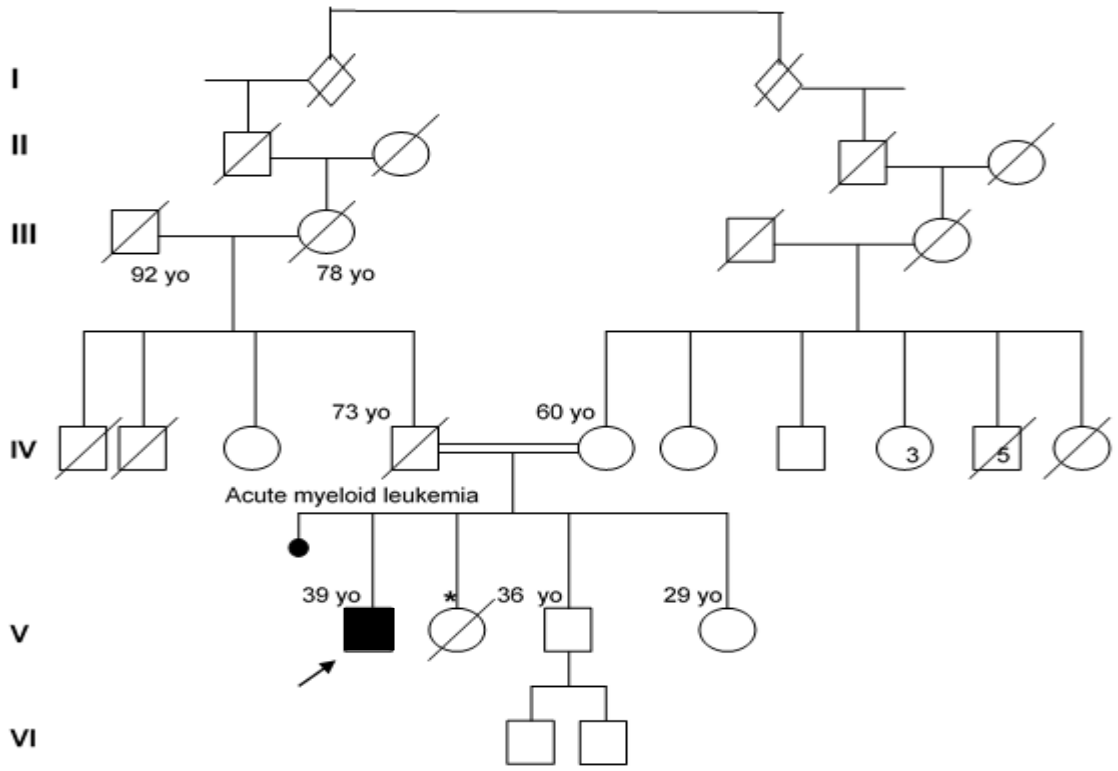

Figure 1: Family History.

* Died at 5-month-old (skin lesions/meningitis/hospitalized for 3 months)

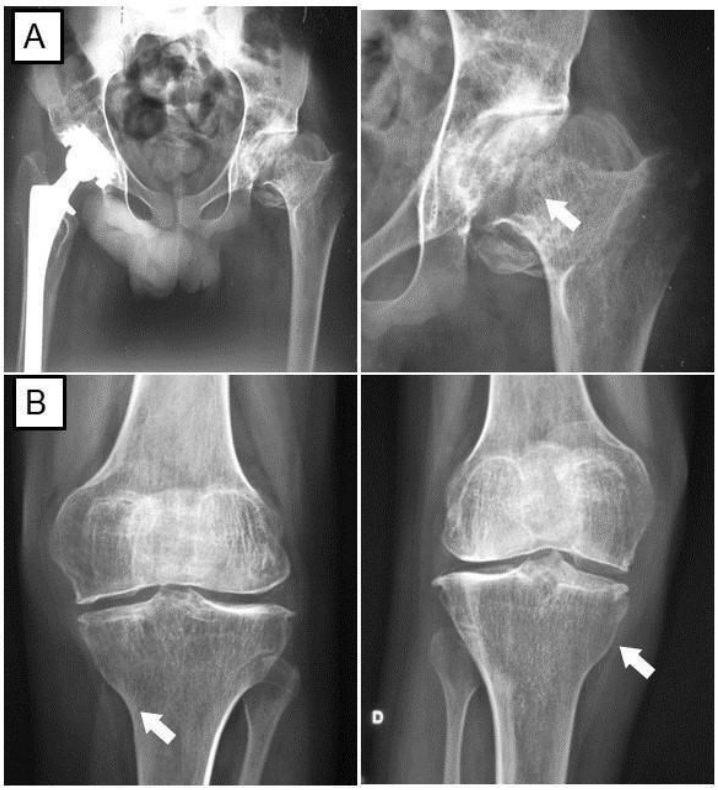

Figure 2: Bilateral hip and knee X- ray showing osteonecrosis of the femur head and right prosthesis (A) and osteopenia (B).

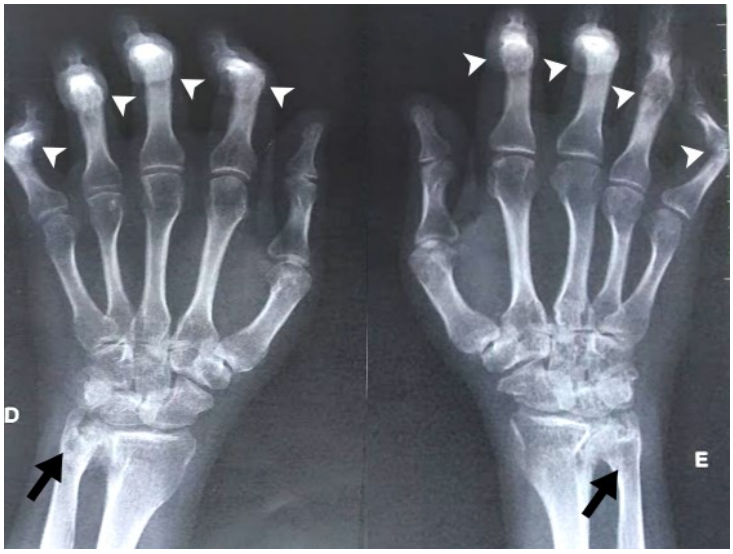

Figure 3- Bilateral hand X- ray. Pseudocysts are seen in the styloid processes (arrows) and bilateral deformity with subluxation of proximal interphalangeal joints (arrowheads)

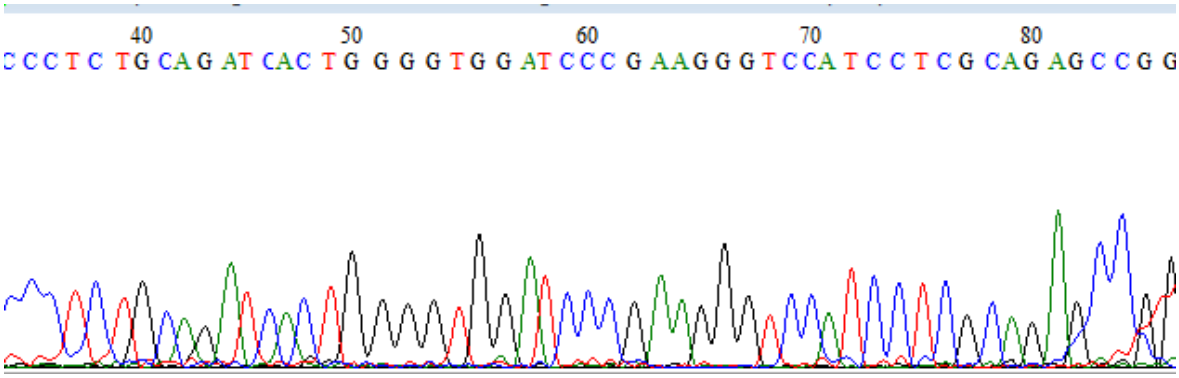

Figure 4: Homozygous pathogenic variant c.833T>C (p.Ile278Thr) in *CBS* gene

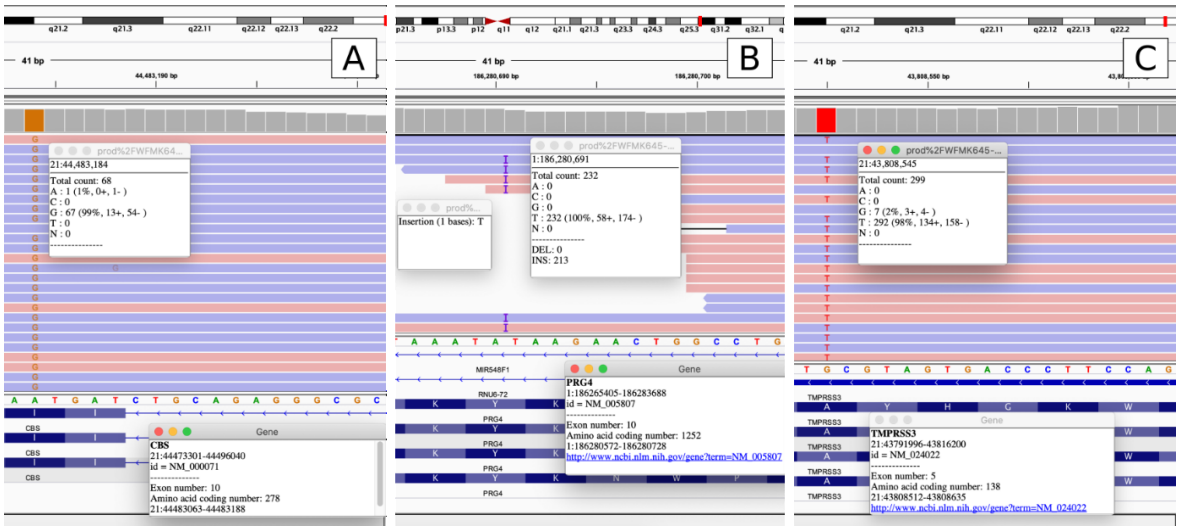

Figure 5: Homozygous pathogenic variants identified through exome sequencing in *CBS* (A), *PRG4* (B), and *TMPRSS3* (C).

Discussion

The exome sequencing may be used if, despite the consistent phenotypic features, a single genetic locus did can not explain the full phenotypic spectrum of a condition [2]. Since this patient had multi-system symptoms that were not usually seen in individuals with HCU, exome sequencing was performed and was able to unreavel three monogenic diseases. Data suggests exome sequencing results in diagnoses of 30-50% of patients tested and around 4.6-7% of patients are diagnosed with two independent monogenic conditions [3].

First clinical hypothesis and diagnosis in this patient was HCU, with a pyridoxine-responsive phenotype [4] however, he presented some atypical findings such as hearing loss and JIA, that could not be explained by HCU. After performing exome sequencing to clarify the phenotype, he was diagnosed with CACP syndrome and a recessive nonsyndromic deafness. Furthermore, the variant in the *TMPRSS3* gene is just 675 Kb of that variant present in *CBS* gene, in chromosome 21, so both are segregated in conjunction.

Pathogenic variants in *TMPRSS3* have been implicated in prelingual and postlingual hearing impairment. There is a genotype-phenotype correlation where loss-of-function variants cause the most severe phenotype whereas missense variants are associated with a milder form of hearing impairment. [5]. Our patient's variant has been reported as associated with postlingual hearing impairment such as what our patient presented [6].

Proteoglycan 4 (PRG4) is expressed in chondrocytes of the superficial zone and is involved in lubricating and protecting cells in the surfaces of joints and tendons as well as in non-skeletal tissue including liver and pericardium[7]. Pathogenic variants in *PRG4* identified on chromosome 1q25-q31 cause CACP syndrome which is characterized by congenital or early-onset camptodactyly, childhood-onset non-inflammatory arthropathy of large joints - such as elbows, hips, knees, and ankles - synovial hyperplasia, progressive coxa vara deformity and non-inflammatory pericardial or pleural effusion [7,8]. Due to overlap of symptoms, CACP may be misdiagnosed as JIA which is treated with immunomodulators. Importantly, the specific musculoskeletal features of CACP syndrome do not respond to this type of medication so the affected individuals should be treated with pain medications and with physical therapy [7]. Some clinical features that may help to differentiate CACP from JIA is the presence of coxa vara, positive family history, normal inflammatory markers, absence of inflammation in synovial aspirates or synovial biopsies and typical imaging findings including the presence of hip effusion with acetabular cysts[9,10].

Providing the correct diagnosis to our patient allowed us to withdraw the methotrexate which is known to cause long-term and severe adverse effects such as predisposition to infections, lung and skin problems, dizziness, and

gastrointestinal issues as well as tailor the physical therapy and other medications to treat his diseases.

Conclusion

Here we report on the success of exome sequencing in diagnosing an individual with three autosomal recessive disorders. This case highlights the importance of additional investigations when patient‘s symptoms are not explained by a single disease. An accurate diagnosis of coexistence of multiple conditions, may allow for the cessation of ineffective and potentially harmful treatments.

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Capítulo VII – Discussão

Nesta seção, serão discutidos em maiores detalhes alguns tópicos não abordados extensivamente nos artigos.

Em relação aos aspectos de qualidade de vida, acrescentamos que o questionário WHOQOL - BREF foi escolhido por ser um instrumento prático e de rápida avaliação, já que anualmente os pacientes são monitorados em relação à qualidade de vida na consulta assistencial. Como os pacientes com HCU podem apresentar dificuldade visual e deficiência intelectual, o ideal é que o médico ou uma pessoa treinada aplique o questionário para evitar erros de preenchimento.

A HCU é uma doença multissistêmica com um tratamento complexo. Estes dois fatores podem interferir na qualidade de vida destes pacientes. É importante entendermos quais são os aspectos mais prejudicados para podermos melhorar o manejo. Como a amostra foi por conveniência, tivemos poucos pacientes responsivos à piridoxina e poucos pacientes não tratados, mas observamos que parece haver uma melhora da qualidade de vida no aspecto psicológico após o diagnóstico e início do manejo dos pacientes.

A avaliação do QI dos pacientes foi realizada através do instrumento WASI por duas psicólogas treinadas para a aplicação da escala. A WASI é um instrumento de avaliação aplicável a crianças de 6 anos a idosos de 89 anos de idade. Fornece informações sobre os QIs Total, de Execução e Verbal a partir de quatro subtestes (Vocabulário, Cubos, Semelhanças e Raciocínio Matricial). A abrangência da idade e a facilidade na aplicação foi decisiva na escolha da escala. Apenas dois pacientes apresentam QI>70. Para avaliação de qualidade de vida, não excluímos os pacientes com QI<70, pois a HCU é uma doença rara e a amostra é por conveniência. Como não temos triagem neonatal para HCU no Brasil, a maioria dos casos apresentam diagnóstico tardio e alguma deficiência intelectual.

Alterações psiquiátricas como transtorno de personalidade, esquizofrenia, ansiedade, depressão, comportamento obsessivo compulsivo e episódios psicóticos são achados encontrados em pacientes com HCU. Neste estudo verificamos a frequência dos sintomas relacionados à depressão, ansiedade e

esquizofrenia através de escalas específicas e observamos uma alta prevalência de principalmente de sintomas de esquizofrenia. Após caracterizar os sintomas psiquiátricos neste grupo de pacientes, foi realizado exame de imagem para realização de volumetria das estruturas cerebrais e comparação com os achados psiquiátricos.

A volumetria encefálica foi realizada em apenas 8 pacientes também por amostra de conveniência. A coorte foi comparada com um grupo de indivíduos saudáveis já avaliados pela equipe da psiquiatria. Como a maior parte dos pacientes com HCU apresentam algum sintoma psiquiátrico, pensava-se que apareceriam redução de uma ou mais estruturas encefálicas que, como já relatado, estão associadas com depressão, ansiedade e esquizofrenia. Não foi encontrada esta redução. Um dos motivos para este resultado pode ser o número reduzido de pacientes que participaram do estudo. Entretanto encontramos achados interessantes em relação ao tálamo, que é uma estrutura subcortical que integra informações sensoriais e corticais, o que é importante para coerência, cognição e percepção. A disfunção talâmica é encontrada na esquizofrenia. Há estudos mostrando evidências de redução do volume do tálamo em pessoas em risco e também com diagnóstico de esquizofrenia. O volume do tálamo apresentou uma correlação negativa com as escalas de depressão, ansiedade e esquizofrenia e diferença nas hipointensidades da substância branca, achado que pode ser encontrado no HCU.

Além da parte neuropsiquiátrica, foi estudado o sistema cardiológico, mais especificamente a parte estrutural cardíaca para entender a extensão do acometimento nos indivíduos afetados com HCU. Através de uma análise retrospectiva de ecocardiogramas e eletrocardiogramas, encontramos apenas um caso de ectasia de aorta e achados leves nestes exames.

Por último, houve o relato de um caso de HCU piridoxina responsivo, filho de pais consanguíneos que apresentava sintomas que não pareciam estar relacionados ao quadro da HCU como deficiência auditiva e alterações em articulações diagnosticada como Artrite Reumatoide Juvenil. Este caso foi um desafio para o diagnóstico e que demorou mais de 30 anos para ter seu quadro completamente esclarecido. Realizado sequenciamento de exoma e detectado que

além de HCU, paciente apresenta surdez não sindrômica autossômica recessiva relacionada ao gene *TMPRSS3* e CACP (Camptodactyly-Arthropathy-Coxa-Vara-Pericarditis) relacionada ao gene *PRG4.* Este diagnóstico teve impacto no tratamento do paciente que estava em uso de Metotrexato e após o diagnóstico pode parar com a medicação que não é indicada nesta situação. Também foi realizado aconselhamento genético para a família e testagem dos familiares em risco.

Capítulo VIII – Conclusão

a) Avaliar a qualidade de vida em pacientes com HCU acompanhados no ambulatório de genética do Hospital de Clínicas de Porto Alegre através da aplicação do questionário WHOQOL BREF.

Nossos dados sugerem que o tratamento de pacientes com HCU associa-se a uma melhora na qualidade de vida no aspecto psicológico após o diagnóstico e início do manejo dos pacientes com HCU.

b) Realizar teste WASI em pacientes com HCU para detectar prevalência de deficiência intelectual neste grupo de pacientes.

A análise dos dados sugere que redução no QI é um achado prevalente nos pacientes com HCU com diagnótico tardio. Um QI Total extremamente baixo foi encontrado em 75% dos pacientes.

c) Descrever quadro psiquiátrico neste grupo de pacientes e verificar prevalência de sintomas como depressão, ansiedade e esquizofrenia.

O estudo sugere que pacientes com HCU em nossa população apresentam quadro compatível com depressão, ansiedade e esquizofrenia. Assim devemos pesquisar estas patologias na avaliação clínica destes pacientes.

d) Caracterizar alterações em Sistema Nervoso Central através de volumetria cerebral.

Há uma tendência no envolvimento do tálamo como uma das estruturas relacionadas às condições psiquiátricas nos pacientes com HCU. Além disso, não há diferença na volumetria cerebral quando comparamos pacientes HCU e controles, entretanto há diferença nas alterações de substância branca.

e) Caracterizar as alterações cardiológicas através de eletrocardiogramas e ecocardiogramas.

Valvulopatias são frequentemente encontradas em pacientes com HCU não responsivos à piridoxina. Apenas um paciente responsivo à piridoxina apresentou ectasia de raiz da aorta.

Capítulo IX – Perspectivas

Este trabalho abordou três questões centrais na HCU: quadro neuropsiquiátrico, qualidade de vida e aspectos cardiológicos.

Este estudo traz como contribuição para os pacientes com HCU, para os profissionais da saúde vinculados ao tratamento destes pacientes e à comunidade científica: (1) sugestão de avaliação neuropsiquiátrica objetiva através de aplicação de escalas para verificar qualidade de vida, suspeita de depressão, ansiedade e esquizofrenia; (2) sugestão de pelo menos uma aplicação de teste de QI para melhor entendimento e acompanhamento destes pacientes; (3) sugestão de realização de ressonância magnética de crânio para detectar alterações de substância branca e microvasculatura; e (4) avaliação cardiológica para prevenção de complicações relacionadas a HCU.

Nosso grupo de pesquisa tem como perspectiva aprimorar a avaliação neuropsiquiátrica nos pacientes com HCU aumentando o número de pacientes através da aplicação de instrumentos anuais e exames de imagem. Também pretendemos desenvolver uma escala de qualidade de vida específica para pacientes com HCU.

Capítulo X – Referências Bibliográficas

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Capítulo XI – Anexos

11.1 Carta de Aprovação do Projeto (GPPG – nº 2014-0596)

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO CIENTÍFICA

A Comissão Científica do Hospital de Clínicas de Porto Alegre analisou o projeto:

Projeto: 140596
Data da Versão do Projeto: 23/10/2014

Pesquisadores:
IDA VANESSA DOEDERLEIN SCHWARTZ
LILIA FATIRET REFOSCO
KAMILA CASTRO GROKOSKI
ANA PAULA VANZ
TATIELE NALIN
BORAIA PÓLONI
CAHOLINA FISCHINGER MOURA DE SOUZA
TACIANE ALEGRA

Título: Rede e registro Europeu de homocistinúrias e defeitos de metilação (E-HOD)

Este projeto foi APROVADO em seus aspectos éticos, metodológicos, logísticos e financeiros para ser realizado no Hospital de Clínicas de Porto Alegre.
Esta aprovação está baseada nos pareceres dos respectivos Comitês de Ética e do Serviço de Gestão em Pesquisa.

- Os pesquisadores vinculados ao projeto não participaram de qualquer etapa do processo de avaliação de seus projetos.

- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao Grupo de Pesquisa e Pós-Graduação (GPPG)

Porto Alegre, 26 de agosto de 2015.

Prof. José Roberto Goldim
Coordenador CEP/HCPA

11.2 Carta de Aprovação do Projeto (GPPG – nº2020/0648)

HOSPITAL DE CLÍNICAS DE PORTO ALEGRE

Grupo de Pesquisa e Pós Graduação

Carta de Aprovação

Projeto

2020/0648

Pesquisadores:

IDA VANESSA DOEDERLEIN SCHWARTZ

MARCO ANTONIO BAPTISTA KALIL	SORAIA POLONI	KARINA CARVALHO DONIS
CHARLES LUBIANCA KOHEM	FABIANO DE OLIVEIRA POSWAR	FILIPPO PINTO VAIRO

Número de Participantes: 1

Título: Relato de caso: An adult with cystathionine beta-synthase deficiency, idiopathic rheumatoid arthritis, and deafness: A case report

Este projeto foi APROVADO em seus aspectos éticos, metodológicos, logísticos e financeiros para ser realizado no Hospital de Clínicas de Porto Alegre.
Esta aprovação está baseada nos pareceres dos respectivos Comitês de Ética e do Serviço de Gestão em Pesquisa.

- Os pesquisadores vinculados ao projeto não participaram de qualquer etapa do processo de avalição de seus projetos.

- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao Grupo de Pesquisa e Pós-Graduação (GPPG).

13/01/2021

Impresso do sistema AGHUse-Pesquisa por ANDERSON ZANARDO MACHADO em 13/01/2021 09:30:21

11.3 Carta de Aprovação do Projeto (GPPG – nº100348)

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

A Comissão Científica e a Comissão de Pesquisa e Ética em Saúde, que é reconhecida pela Comissão Nacional de Ética em Pesquisa (CONEP/MS como Comitê de Ética em Pesquisa do HCPA e pelo Office for Human Research Protections (OHRP/USDHHS, como Institutional Review Board (IRB00000921) analisaram o projeto:

Projeto: 100348 **Versão do Projeto:** 04/10/2010 **Versão do TCLE:** 04/10/2010

Pesquisadores:
FLAVIO KAPCZINSKI
RAFFAEL MASSUDA
PAULO SILVA BELMONTE DE ABREU
CLARISSA SEVERINO GAMA

Título: ESTUDO DA COGNIÇÃO E MARCADORES DE TOXICIDADE SISTÊMICA EM POPULAÇÃO DE ALTO RISCO PARA DESENVOLVER ESQUIZOFRENIA

Este projeto foi Aprovado em seus aspectos éticos e metodológicos de acordo com as Diretrizes e Normas Internacionais e Nacionais especialmente as Resoluções 196/96 e complementares do Conselho Nacional de Saúde. Os membros do CEP/HCPA não participaram do processo de avaliação dos projetos onde constam como pesquisadores. Toda e qualquer alteração do Projeto deverá ser comunicada imediatamente ao CEP/HCPA.

Porto Alegre 05 de outubro de 2010

Profª Nadine Clausell
Coordenadora GPPG e CEP/HCPA

11.4 BPRS - Brief Psychiatric Rating Scale

BPRS- ESCALA BREVE DE AVALIAÇÃO PSIQUIÁTRICA DE ESQUIZOFRENIA

Nome:
Idade: **Sexo:**

Preencher a escala quando da 1ª solicitação de tratamento e em cada reavaliação comprovando acompanhamento clínico e psiquiátrico.

(1) Ausente (3) Discreto (5) Moderadamente grave (7) Extremamente grave
(2) Muito discreto (4) Grave (6) Grave

	Tempo 0	**Tempo após tratamento .....(meses)** **Medicamento .....**
1 - Preocupações somáticas		
2 - Ansiedade		
3 - Retraimento afetivo		
4 - Desorganização conceitual		
5 - Sentimento de culpa		
6 - Tensão		
7 - Maneirismo e Atitude		
8 - Megalomania		
9 - Humor depressivo		
10 - Hostilidade		
11 - Desconfiança		
12 - Comportamento alucinatório 13 - Retardamento motor		
14 - Não cooperação		
15 - Pensamentos não habituais		
16 - Embotamento afetivo		
17 - Excitação 18 - Desorientação		
Escore Total		

Considerado melhora clínica quando da redução de pelo menos 30% dos escores prévios da escala BPRS-A. (menor que 30% pode ser considerado falha terapêutica).

Local ..... Data ..... Assinatura e Carimbo com CRM do Médico Assistente.

11.5 Escala de Depressão de Beck

Nome:_____ **Idade:**_____ **Data:** _____/_____/_____

Este questionário consiste em 21 grupos de afirmações. Depois de ler cuidadosamente cada grupo, faça um círculo em torno do número (0, 1, 2 ou 3) próximo à afirmação, em cada grupo, que descreve **melhor** a maneira que você tem se sentido na **última semana, incluindo hoje.** Se várias afirmações num grupo parecerem se aplicar igualmente bem, faça um círculo em cada uma. **Tome cuidado de ler todas as afirmações, em cada grupo, antes de fazer sua escolha.**

1	0 Não me sinto triste 1 Eu me sinto triste 2 Estou sempre triste e não consigo sair disto 3 Estou tão triste ou infeliz que não consigo suportar	**7**	0 Não me sinto decepcionado comigo mesmo 1 Estou decepcionado comigo mesmo 2 Estou enojado de mim 3 Eu me odeio
2	0 Não estou especialmente desanimado quanto ao futuro 1 Eu me sinto desanimado quanto ao futuro 2 Acho que nada tenho a esperar 3 Acho o futuro sem esperanças e tenho a impressão de que as coisas não podem melhorar	**8**	0 Não me sinto de qualquer modo pior que os outros 1 Sou crítico em relação a mim por minhas fraquezas ou erros 2 Eu me culpo sempre por minhas falhas 3 Eu me culpo por tudo de mal que acontece
3	0 Não me sinto um fracasso 1 Acho que fracassei mais do que uma pessoa comum 2 Quando olho pra trás, na minha vida, tudo o que posso ver é um monte de fracassos 3 Acho que, como pessoa, sou um completo fracasso	**9**	0 Não tenho quaisquer idéias de me matar 1 Tenho idéias de me matar, mas não as executaria 2 Gostaria de me matar 3 Eu me mataria se tivesse oportunidade
4	0 Tenho tanto prazer em tudo como antes 1 Não sinto mais prazer nas coisas como antes 2 Não encontro um prazer real em mais nada 3 Estou insatisfeito ou aborrecido com tudo	**10**	0 Não choro mais que o habitual 1 Choro mais agora do que costumava 2 Agora, choro o tempo todo 3 Costumava ser capaz de chorar, mas agora não consigo, mesmo que o queria
5	0 Não me sinto especialmente culpado 1 Eu me sinto culpado grande parte do tempo 2 Eu me sinto culpado na maior parte do tempo 3 Eu me sinto sempre culpado	**11**	0 Não sou mais irritado agora do que já fui 1 Fico aborrecido ou irritado mais facilmente do que costumava 2 Agora, eu me sinto irritado o tempo todo 3 Não me irrito mais com coisas que costumavam me irritar

6	0 Não acho que esteja sendo punido 1 Acho que posso ser punido 2 Creio que vou ser punido 3 Acho que estou sendo punido	**12**	0 Não perdi o interesse pelas outras pessoas 1 Estou menos interessado pelas outras pessoas do que costumava estar 2 Perdi a maior parte do meu interesse pelas outras pessoas 3 Perdi todo o interesse pelas outras pessoas

13	0 Tomo decisões tão bem quanto antes 1 Adio as tomadas de decisões mais do que costumava 2 Tenho mais dificuldades de tomar decisões do que antes 3 Absolutamente não consigo mais tomar decisões	**18**	0 O meu apetite não está pior do que o habitual 1 Meu apetite não é tão bom como costumava ser 2 Meu apetite é muito pior agora 3 Absolutamente não tenho mais apetite
14	0 Não acho que de qualquer modo pareço pior do que antes 1 Estou preocupado em estar parecendo velho ou sem atrativo 2 Acho que há mudanças permanentes na minha aparência, que me fazem parecer sem atrativo 3 Acredito que pareço feio	**19**	0 Não tenho perdido muito peso se é que perdi algum recentemente 1 Perdi mais do que 2 quilos e meio 2 Perdi mais do que 5 quilos 3 Perdi mais do que 7 quilos Estou tentando perder peso de propósito, comendo menos: Sim _____ Não _____
15	0 Posso trabalhar tão bem quanto antes 1 É preciso algum esforço extra para fazer alguma coisa 2 Tenho que me esforçar muito para fazer alguma coisa 3 Não consigo mais fazer qualquer trabalho	**20**	0 Não estou mais preocupado com a minha saúde do que o habitual 1 Estou preocupado com problemas físicos, tais como dores, indisposição do estômago ou constipação 2 Estou muito preocupado com problemas físicos e é difícil pensar em outra coisa 3 Estou tão preocupado com meus problemas físicos que não consigo pensar em qualquer outra coisa
16	0 Consigo dormir tão bem como o habitual 1 Não durmo tão bem como costumava 2 Acordo 1 a 2 horas mais cedo do que habitualmente e acho difícil voltar a dormir 3 Acordo várias horas mais cedo do que costumava e não consigo voltar a dormir	**21**	0 Não notei qualquer mudança recente no meu interesse por sexo 1 Estou menos interessado por sexo do que costumava 2 Estou muito menos interessado por sexo agora 3 Perdi completamente o interesse por sexo

17	0 Não fico mais cansado do que o habitual 1 Fico cansado mais facilmente do que costumava 2 Fico cansado em fazer qualquer coisa 3 Estou cansado demais para fazer qualquer coisa		

11.6 Escala de Ansiedade de Beck

Nome:_____ **Idade:**_____ **Data:** _____/_____/_____

Abaixo está uma lista de sintomas comuns de ansiedade. Por favor, leia cuidadosamente cada item da lista. Identifique o quanto você tem sido incomodado por cada sintoma durante a **última semana, incluindo hoje**, colocando um "x" no espaço correspondente, na mesma linha de cada sintoma.

	Absolutamente não	**Levemente** Não me incomodou muito	**Moderadamente** Foi muito desagradável mas pude suportar	**Gravemente** Dificilmente pude suportar
1. Dormência ou formigamento				
2. Sensação de calor				
3. Tremores nas pernas				
4. Incapaz de relaxar				
5. Medo que aconteça o pior				
6. Atordoado ou tonto				
7. Palpitação ou aceleração do coração				
8. Sem equilíbrio				
9. Aterrorizado				
10. Nervoso				
11. Sensação de sufocação				
12. Tremores nas mãos				
13. Trêmulo				
14. Medo de perder o controle				
15. Dificuldade de respirar				
16. Medo de morrer				
17. Assustado				
18. Indigestão ou desconforto no abdômen				
19. Sensação de desmaio				
20. Rosto afogueado				
21. Suor (não devido ao calor)				

11.7 Escala de Hamilton

ESCALA DE HAMILTON
AVALIAÇÃO DA DEPRESSÃO (HAM-D 21 itens)

1	**HUMOR DEPRIMIDO** 0. Ausente 1. Sentimentos relatados apenas ao ser perguntado 2. Sentimentos relatados espontaneamente, com palavras 3. Comunica os sentimentos com expressão facial, postura, voz e tendência ao choro 4. Sentimentos deduzidos da comunicação verbal e não verbal do paciente	**ESCORE**
2	**SENTIMENTOS DE CULPA** 0. Ausentes 1. Auto-recriminação; sente que decepcionou os outros 2. Idéias de culpa ou ruminação sobre erros passados ou más Acões 3. A doença atual é um castigo. Delírio de culpa 4. Ouve vozes de acusação ou denúncia e/ou tem alucinações visuais ameaçadoras	
3	**SUICIDIO** 0. Ausente 1. Sente que a vida não vale a pena 2. Desejaria estar morto; pensa na possibilidade de sua morte 3. Idéias ou gestos suicidas 4. Tentativa de suicídio (qualquer tentativa séria)	
4	**INSÔNIA INICIAL** 0. Sem dificuldade 1. Tem alguma dificuldade ocasional, isto é, mais de meia hora 2. Queixa de dificuldade para conciliar todas as noites	
5	**INSÔNIA INTERMEDIÁRIA** 0. Sem dificuldade 1. Queixa-se de inquietude e perturbação durante a noite 2. Acorda à noite; qualquer saída da cama (exceto para urinar)	
6	**INSONIA TARDIA** 0. Sem dificuldade 1. Acorda de madrugada, mas volta a dormir 2. Incapaz de voltar a conciliar o sono ao deixar a cama	
7	**TRABALHOS E ATIVIDADES** 0. Sem dificuldade 1. Pensamento/sentimento de incapacidade, fadiga, fraqueza relacionada às atividades; trabalho ou passatempos 2. Perda de interesse por atividades (passatempos, trabalho) – quer diretamente relatada pelo paciente, ou indiretamente, por desatenção, indecisão e vacilação (sente que precisa se esforçar para o trabalho ou atividades). 3. Diminuição do tempo gasto em atividades ou queda da produtividade. No hospital, marcar 3 se o paciente passa menos de 3h em atividades externas (passatempos ou trabalho hospitalar) 4. Parou de trabalhar devido à doença atual. No hospital, marcar 4 se o paciente não se ocupar de outras atividades além de pequenas tarefas do leito, ou for incapaz de realizá-las sem auxílio	
8	**RETARDO** 0. Pensamento e fala normais	

	1. Leve retardo durante a entrevista 2. Retardo óbvio à entrevista 3. Estupor completo	
9	**AGITAÇÃO** 0. Nenhuma 1. Brinca com as mãos ou com os cabelos, etc 2. Troce as mãos, rói as unhas, puxa os cabelos, morde os lábios	
10	**ANSIEDADE PSÍQUICA** 0. Sem ansiedade 1. Tensão e irritabilidade subjetivas 2. Preocupação com trivialidades 3. Atitude apreensiva aparente no rosto ou fala 4. Medos expressos sem serem inquiridos	
11	**ANSIEDADE SOMÁTICA** (sintomas fisiológicos de ansiedade: boca seca, flatulência, indigestão, diarréia, cólicas, eructações; palpitações, cefaléia, hiperventilação, suspiros, sudorese, freqüência urinária) 0. Ausente 1. Leve 2. Moderada 3. Grave 4. Incapacitante	
12	**SINTOMAS SOMÁTICOS GASTROINTESTINAIS** 0. Nenhum 1. Perda do apetite, mas alimenta-se voluntariamente; sensações de peso no abdome 2. Dificuldade de comer se não insistirem. Solicita ou exige laxativos ou medicações para os intestinos ou para sintomas digestivos	
13	**SINTOMAS SOMÁTICOS EM GERAL** 0. Nenhum 1. Peso nos membros, costas ou cabeça. Dores nas costas, cefaléia, mialgia. Perda de energia e cansaço 2. Qualquer sintoma bem caracterizado e nítido, marcar 2	
14	**SINTOMAS GENITAIS** (perda da libido, sintomas menstruais) 0. Ausentes 1. Leves distúrbios menstruais 2. Intensos	
15	**HIPOCONDRIA** 0. Ausente 1. Auto-observação aumentada (com relação ao corpo) 2. Preocupação com a saúde 3. Queixas freqüentes, pedidos de ajuda, etc 4. Idéias delirantes hipocondríacas	
16	**PERDA DE PESO** (Marcar A ou B; A – pela história; B – pela avaliação semanal do psiquiatra responsável) A. 0. Sem perda de peso 1. Provável perda de peso da doença atual 2. Perda de peso definida B. 0. Menos de 0,5kg de perda por semana 1. Mais de 0,5kg de perda por semana 2. Mais de 1kg de perda por semana	

17	**CONSCIENCIA DA DOENÇA** 0. Reconhece que está deprimido e doente 1. Reconhece a doença mas atribui-lhe a causa à má alimentação, ao clima, ao excesso de trabalho, a vírus, necessidade de repouso 2. Nega estar doente	
18	**VARIAÇÃO DIURNA** (se há variação dos sintomas pela manhã ou à noite; caso não haja variação, marcar 0) 0. Ausentes 1. Leve 2. Grave	
19	**DESPERSONALIZAÇÃO E DESREALIZAÇÃO** (Idéias niilistas, sensações de irrealidade) 0. Ausentes 1. Leves 2. Moderadas 3. Graves 4. Incapacitantes	
20	**SINTOMAS PARANOIDES** 0. Nenhum 1. Desconfiança 2. Idéias de referência 3. Delírio de referência e perseguição	
21	**SINTOMAS OBSESSIVOS E COMPULSIVOS** 0. Nenhum 1. Leves 2. Graves	

ESCORE TOTAL = _____ PONTOS

11.8 WHOQOL - Abreviado

WHOQOL - ABREVIADO

Versão em Português

PROGRAMA DE SAÚDE MENTAL
ORGANIZAÇÃO MUNDIAL DA SAÚDE
GENEBRA

Coordenação do GRUPO WHOQOL no Brasil

Dr. Marcelo Pio de Almeida Fleck
Professor Adjunto
Departamento de Psiquiatria e Medicina Legal
Universidade Federal do Rio Grande do Sul
Porto Alegre – RS - Brasil

1

Instruções

Este questionário é sobre como você se sente a respeito de sua qualidade de vida, saúde e outras áreas de sua vida. **Por favor, responda a todas as questões** . Se você não tem certeza sobre que resposta dar em uma questão, por favor, escolha entre as alternativas a que lhe parece mais apropriada. Esta, muitas vezes, poderá ser sua primeira escolha.

Por favor, tenha em mente seus valores, aspirações, prazeres e preocupações. Nós estamos perguntando o que você acha de sua vida, tomando como referência as **duas últimas semanas** . Por exemplo, pensando nas últimas duas semanas, uma questão poderia ser:

	nada	muito pouco	médio	muito	completamente
Você recebe dos outros o apoio de que necessita?	1	2	3	4	5

Você deve circular o número que melhor corresponde ao quanto você recebe dos outros o apoio de que necessita nestas últimas duas semanas. Portanto, você deve circular o número 4 se você recebeu "muito" apoio como abaixo.

	nada	muito pouco	médio	muito	completamente
Você recebe dos outros o apoio de que necessita?	1	2	3	4	5

Você deve circular o número 1 se você não recebeu "nada" de apoio.

Por favor, leia cada questão, veja o que você acha e circule no número e lhe parece a melhor resposta.

		muito ruim	ruim	nem ruim nem boa	boa	muito boa
1	Como você avaliaria sua qualidade de vida?	1	2	3	4	5

		muito insatisfeito	insatisfeito	nem satisfeito nem insatisfeito	satisfeito	muito satisfeito
2	Quão satisfeito(a) você está com a sua saúde?	1	2	3	4	5

As questões seguintes são sobre **o quanto** você tem sentido algumas coisas nas últimas duas semanas.

		nada	muito pouco	mais ou menos	bastante	extremamente
3	Em que medida você acha que sua dor (física) impede você de fazer o que você precisa?	1	2	3	4	5
4	O quanto você precisa de algum tratamento médico para levar sua vida diária?	1	2	3	4	5
5	O quanto você aproveita a vida?	1	2	3	4	5
6	Em que medida você acha que a sua vida tem sentido?	1	2	3	4	5
7	O quanto você consegue se concentrar?	1	2	3	4	5
8	Quão seguro(a) você se sente em sua vida diária?	1	2	3	4	5
9	Quão saudável é o seu ambiente físico (clima, barulho, poluição, atrativos)?	1	2	3	4	5

As questões seguintes perguntam sobre **quão completamente** você tem sentido ou é capaz de fazer certas coisas nestas últimas duas semanas.

		nada	muito pouco	médio	muito	completamente
10	Você tem energia suficiente para seu dia-a-dia?	1	2	3	4	5
11	Você é capaz de aceitar sua aparência física?	1	2	3	4	5
12	Você tem dinheiro suficiente para satisfazer suas necessidades?	1	2	3	4	5
13	Quão disponíveis para você estão as informações que precisa no seu dia-a-dia?	1	2	3	4	5
14	Em que medida você tem oportunidades de atividade de lazer?	1	2	3	4	5

As questões seguintes perguntam sobre **quão bem ou satisfeito** você se sentiu a respeito de vários aspectos de sua vida nas últimas duas semanas.

		muito ruim	ruim	nem ruim nem bom	bom	muito bom
15	Quão bem você é capaz de se locomover?	1	2	3	4	5

		muito insatisfeito	insatisfeito	nem satisfeito nem insatisfeito	satisfeito	muito satisfeito
16	Quão satisfeito(a) você está com o seu sono?	1	2	3	4	5
17	Quão satisfeito(a) você está com sua capacidade de desempenhar as atividades do seu dia-a-dia?	1	2	3	4	5
18	Quão satisfeito(a) você está com sua capacidade para o trabalho?	1	2	3	4	5
19	Quão satisfeito(a) você está consigo mesmo?	1	2	3	4	5
20	Quão satisfeito(a) você está com suas relações pessoais (amigos, parentes, conhecidos, colegas)?	1	2	3	4	5
21	Quão satisfeito(a) você está com sua vida sexual?	1	2	3	4	5
22	Quão satisfeito(a) você está com o apoio que você recebe de seus amigos?	1	2	3	4	5
23	Quão satisfeito(a) você está com as condições do local onde mora?	1	2	3	4	5
24	Quão satisfeito(a) você está com o seu acesso aos serviços de saúde?	1	2	3	4	5
25	Quão satisfeito(a) você está com o seu meio de transporte?	1	2	3	4	5

As questões seguintes referem-se a **com que freqüência** você sentiu ou experimentou certas coisas nas últimas duas semanas.

		nunca	algumas vezes	frequentemente	muito frequentemente	sempre
26	Com que freqüência você tem sentimentos negativos tais como mau humor, desespero, ansiedade, depressão?	1	2	3	4	5

Alguém lhe ajudou a preencher este questionário?.....

Quanto tempo você levou para preencher este questionário?.....

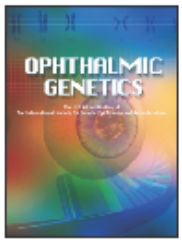
Você tem algum comentário sobre o questionário?

OBRIGADO PELA SUA COLABORAÇÃO

4

Capítulo XII – Apêndices

12.1 Artigo publicado durante o período do doutorado “Ocular manifestations in classic homocystinuria.”



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Ocular manifestations in classic homocystinuria

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CASE REPORT

Ocular manifestations in classic homocystinuria

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ABSTRACT

Background: Classic homocystinuria (HCU), or cystathionine beta-synthase (CBS) deficiency, is a rare inborn error of methionine metabolism. Main clinical features may include skeletal and vascular manifestations, developmental delay, intellectual disability and eye disorders.

Material and methods: This is an observational and retrospective study aiming at describing eye abnormalities presented by a cohort of late-diagnosed HCU patients. Data regarding ophthalmological evaluation included visual acuity, refraction, biomicroscopy, Perkins tonometry, fundus examination, retinography, biometry, ocular ultrasound, optical coherence tomography, anterior segment photography and topography.

Results: Ten patients with HCU (20 eyes) were included. The most frequent findings were *ectopia lentis* (n = 20) and myopia (n = 9). Biometry, ultrasound, OCT and topography findings were available for four patients. One patient had keratoconus; one had abnormal retinal pigmentation; and two had lens surgery scars with irregular astigmatism.

Conclusions: Eye abnormalities are very frequent in late-diagnosed HCU patients. The presence of *ectopia lentis* should always raise the diagnostic hypothesis of HCU.

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KEYWORDS
Classic homocystinuria; CBS deficiency; eye abnormalities; *ectopialentis*

Introduction

Classic homocystinuria (HCU) is a rare inborn error of methionine metabolism caused by cystathionine beta-synthase deficiency (CBS deficiency, OMIM 236200), an enzyme that catalyses the conversion of homocysteine to cystathionine. The minimum worldwide prevalence of HCU was estimated to be ~0.38:100,000 (1) and the pathophysiology of CBS deficiency is not fully understood. In addition to homocysteine (Hcy) accumulation, the defect leads to increased concentrations of S-adenosylhomocysteine (SAH), enhanced remethylation to methionine, and depletion of cystathionine and cysteine (2). High levels of Hcy concentrations modify sulfhydryl groups on proteins and interfere with the cross-linking of sulfhydryl groups in proteins such as elastin, which modify intracellular signaling and cause endoplasmic reticulum stress with vascular endothelial dysfunction (3).

Alterations of structural proteins, apoptosis and oxidative stress are associated with the increased SAH, impairs methylation reactions and decrease cystathionine and cysteine, which is thought to contribute to connective tissue abnormalities and lens dislocation (2). Patients with HCU present with hyperhomocysteinemia, hypermethioninaemia and low levels of cysteine (3–5). Clinical features may include skeletal abnormalities, developmental delay, intellectual disability and lethal thromboembolic phenomena, besides ocular complications.

HCU patients are treated according to the responsiveness to pyridoxine. Patients who are responsive are able to maintain the homocysteine levels (tHcy) within the target range while taking the vitamin; the non-responsive ones need to be treated with a low natural protein diet, methionine-free metabolic formula and, if necessary, betaine (2). Early initiation of treatment, during the first month of life, reduces the incidence of *ectopia lentis* and thromboembolic events, so HCU is one of the diseases amenable to neonatal screening (6).

This report concerns the first in-depth ocular examination of a series of HCU patients from Brazil.

Materials and methods

This is an observational and retrospective study, approved by the local IRB. Patients were recruited from the Inborn Errors of Metabolism Clinics at the Medical Genetics Division, Hospital de Clínicas de Porto Alegre-south of Brazil (IEM Clinics-MGS, HCPA). They were classified into responsive or non-responsive to pyridoxine according to the standard recommendations (2). After treatment, responsive patients maintained tHcy<50 micromoL/L. The majority of non-responsive patients were non-adherent and their level of tHcy was>100 micromoL/L, although some were able to keep it <100micromol/L (the target level).

As a routine, HCU patients have an appointment with the Ocular Genetics Section of Ophthalmology Division-HCPA. The eye examination comprises visual acuity, non-dilated and cycloplegic refraction, Perkins tonometry, non-dilated and dilated slit lamp biomicroscopy and dilated retinal exam. Retinography, biometry, ocular ultrasound, macular optical

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coherence tomography (OCT), anterior segment photography and corneal topography are not routine exams, but performed when different from expected features appear during ocular evaluation.

All patient had their diagnosis confirmed both biochemically (by demonstration of hyperhomocysteinaemia and hypermethioninaemia) and genetically (by detection of biallelic pathogenic variants in the *CBS* gene). Some of these patients have been included in the paper by Poloni et al. (7), which described the diagnosis and treatment findings of 72 late-diagnosed HCU patients from all over Brazil, without thorough ophthalmological description (ocular complains were the earliest detected symptom in 53% of cases; the main reason for diagnostic suspicion in 63% of cases and the most prevalent manifestation at diagnosis in 67% of cases). Their genotypes were described by Poloni et al.. Patient 10 has also been described as a case report and published by Gus et al. (8).

Results

Ten patients (20 eyes), aged 16 to 40 years old (mean age 26.3 ± 7.5), were included. The findings are summarized in Table 1 and Figures 1 and 2. The most frequent findings were *ectopia lentis* (n = 20) and myopia (n = 9). All patients presented with eye abnormalities, including the patient diagnosed at 2yo and the responsive patients.

Five patients had previously undergone lensectomy at other facilities before being examined by the authors. In our series, four of the seven eyes in which the lens could be evaluated, had a superior dislocation (57% of cases). Biometry, ultrasound, macular OCT, and topography findings were available for only four patients (Patients 2, 6, 8 and 10; Table 1). One patient had keratoconus (Patient 10, Table 1; Figure 2); two had abnormal lens surgery scars (one thinned and the other hypertrophic), both of whom had irregular astigmatism at topography; and one had altered retinal pigmentation (peripheral cluster of hyperpigmentation in one eye, not characteristic of retinal degeneration). Macular OCT abnormalities were not detected. Glaucoma was found in 15% of the cases, all of them in aphakic eyes.

Discussion

We described herein a comprehensive ophthalmologic evaluation of 10 Brazilian HCU patients (20 eyes). Neonatal screening (NBS) for HCU is not available in the public health system in Brazil, so all patients were late diagnosed. They were diagnosed and treated at the Medical Genetics Division of HCPA and posteriorly referred to the Ophthalmology Division.

Ocular problems can seriously compromise the quality of life of HCU patients. The most striking ocular problem is *ectopia lentis*. Other ophthalmic findings include high myopia, microcystoid peripheral retinal degeneration, secondary pupillary block glaucoma, retinal detachment, microphthalmia, optic atrophy, retinal arterial occlusions and band keratopathy (1). The lens dislocation has been described in patients as young as 3yo.

Biochemical abnormalities of HCU are supposed to cause the clinical features of this disorder, but underlying mechanisms are not fully understood. Ocular diseases are possibly related to the reduced level of cysteine, since the lens zonules have high cysteine content (2,4,5). Raised tHcy concentrations modify sulfhydryl groups on proteins and also interfere with the cross-linking of sulfhydryl groups in proteins such as elastin (2). High myopia might potentially occur due to alterations of the scleral connective tissue, as much as irregular scars, but the latest can also be a result of inadequate suture after lens removal. Keratoconus can be related to abnormal collagen fibers in corneal stroma. Those abnormalities may hypothetically cause the connective tissue structures of the eye less resistant to intra-ocular pressure.

It is well known that early diagnosis can prevent the ocular manifestations of HCU, but the absence of newborn screening for this condition in Brazil means that all patients were diagnosed late. Weak zonules of Zinn and sclera can lead to lens instability, increased refractive index, iridodonesis and globe enlargement, all of which contribute to progressive myopia at a young age. Increasing myopia is useful as a sign of treatment refractoriness or poor adherence, with poor biochemical control, and the presence of high myopia in HCU patients is around 45% (9–12).

Since the lens is significantly mobile in HCU patients, it can move completely from the original position, causing either anterior or posterior luxation – with or without angle-closure glaucoma (10). All glaucoma patients of these series presented high intra-ocular pressure levels after lens removal without intra-ocular lens insertion, and aphakia is a risk factor for glaucoma *perse* (aphakic glaucoma).

The prevalence of *ectopia lentis* in patients with HCU is around 70% until age 7, rising to 95% by the fifth decade and is classically inferior or inferonasal (13,14,15). In this series, however, 57% of the patients had a superior dislocation – usually described in Marfan patients. Since the lens may dislocate to any direction, its position in a given patient cannot be considered pathognomonic of HCU nor Marfan, because other heritable disorders may be associated with *ectopia lentis*: Ehlers–Danlos syndromes, Weill–Marchesani syndrome, hyperlysinemia, sulfite oxidase deficiency and Treacher Collins syndrome (16). It is always suspicious of a genetic disease, indeed, in the absence of eye trauma history.

Image taking exams of anterior and posterior eye were performed in only four patients, which allowed better evaluation. The authors strongly recommend that different imaging devices are used for all genetic syndrome patients in routine examination, since unexpected abnormalities can be diagnosed. After this outcomes, routine ophthalmological examination of the Ocular Genetic Section of HCPA started to include all of them.

Conclusion

Several ocular abnormalities were found in this group of late-diagnosed HCU patients. Some of them have been previously described in literature, while others are novel observations. Although *ectopia lentis* in classic homocystinuria is rarely

Table 1. Summary of clinical and ocular findings of patients with Classic Homocystinuria (n = 10).

Patient	Gender	Age (years)	Age at diagnosis (years)	Pyridoxine-responsive-ness	Eye	Surgery	Time after surgery (years)	Visual acuity	Ectopia lentis	Myopia	Cataract	Glaucoma	Other
1.	M	34	33	Yes	R	Cataract	6	20/20	Yes, AC IOL	-	-	-	Pseudophakia
					L	Cataract	6	20/20	Yes, AC IOL	-	-	-	Pseudophakia
2.	M	21	2	No	R	NP	-	20/200	Yes, supero-nasal	-	Yes	-	
					L	NP	-	20/200	Yes, supero-nasal	-	Yes	-	
3.	F	25	8	No	R	NP	-	CF 1,5 m	Yes, supero-nasal	-	Yes	-	
					L	Cataract	4	20/20	Yes, IOL	-	-	-	Pseudophakia
4.	M	37	14	No	R	Cataract	33	CF 3 m	Yes	-	-	Yes (TREC)	Surgical aphakia, Vitreo CA
					L	Cataract	33	NLP	Yes	-	-	-	Phtisis
5.	F	28	7	No	R	Cataract	25	20/200	Yes	High myopia	Yes	Yes	Surgical aphakia
					L	Cataract	25	20/200	Yes	High myopia	Yes	Yes	Surgical aphakia
6.	M	16	13	No	R	Cataract	9	20/20	Yes, IOL	-	-	-	Pseudophakia
					L	Cataract	9	20/20	Yes, IOL	-	-	-	Pseudophakia
7.	M	22	4	Yes	R	NP	-	20/200	Yes, posterior	High myopia	-	-	-
					L	NP	-	20/200	Yes, posterior	High myopia	-	-	-
8.	M	25	6	No	R	Cataract	17	20/40	Yes	Moderate myopia	-	-	Surgical aphakia, Strabismus
					L	Cataract	17	20/30	Yes	Moderate myopia	-	-	Surgical aphakia, Strabismus
9.	F	18	18	No	R	NP	-	20/20	Yes, infero-temporal	Moderate myopia	-	-	-
					L	NP	-	20/20	Yes, supero-temporal	Moderate myopia	-	-	-
10.	M	37	10	Yes	R	Lensectomy and Pams plana vitrectomy	11	20/50	Yes	Moderate myopia	-	-	Surgical aphakia, Keratoconus
					L	Lensectomy	21	20/40	Yes	-	-	-	Surgical aphakia, Keratoconus

L: left eye. R: right eye. AC: anterior chamber. CF: counting fingers. IOL: Intraocular lens. NP: not performed. NLP: no light perception. NRP: retinal pigment epithelium. TREC: trabeculectomy. Myopia defined as: high (> 6 diopters), moderate (3–6 diopters). Pseudophakia: defined as placement of an artificial lens after the natural lens has been removed.

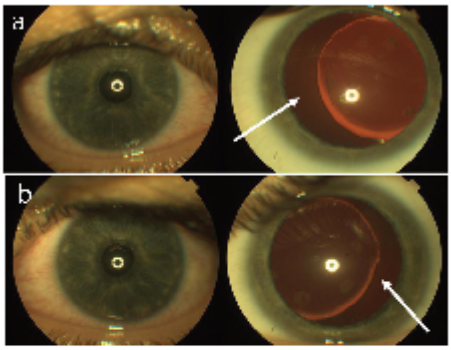

Figure 1. Bilateral *ectopia lentis*, manifesting as nasal displacement of the lens, in a patient with pyridoxine unresponsive HCU.

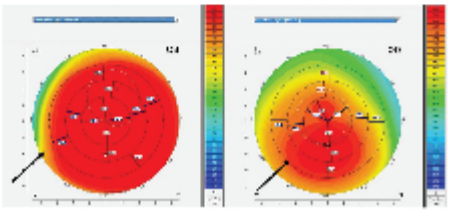

Figure 2. Bilateral keratoconus in a patient with pyridoxine-responsive HCU at age 37yo.

superior, the direction of displacement was highly variable in this series, a remarkable finding.

When *ectopia lentis* is found incidentally on eye examination, the ophthalmologist or optometrist should include HCU in the differential diagnosis. All eye examinations of children should include dilated slit lamp evaluation, and if *ectopia lentis* is diagnosed, the patient should be immediately referred to a genetical evaluation.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Research paper

Is the gut microbiota dysbiotic in patients with classical homocystinuria?

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ABSTRACT

Classical homocystinuria (HCU) is characterized by increased plasma levels of total homocysteine (tHcy) and methionine (Met). Treatment may involve supplementation of B vitamins and essential amino acids, as well as restricted Met intake. Dysbiosis has been described in some inborn errors of metabolism, but has not been investigated in HCU. The aim of this study was to investigate the gut microbiota of HCU patients on treatment. Six unrelated HCU patients (males = 5, median age = 25.5 years) and six age-and-sex-matched healthy controls (males = 5, median age = 24.5 years) had their fecal microbiota characterized through partial 16S rRNA gene sequencing. Fecal pH, a 3-day dietary record, medical history, and current medications were recorded for both groups. All patients were nonresponsive to pyridoxine and were on a Met-restricted diet and presented with high tHcy. Oral supplementation of folate (n = 6) and pyridoxine (n = 5), oral intake of betaine (n = 4), and IM vitamin B12 supplementation (n = 4), were reported only in the HCU group. Patients had decreased daily intake of fat, cholesterol, vitamin D, and selenium compared to controls ($p < 0.05$). There was no difference in alpha and beta diversity between the groups. HCU patients had overrepresentation of the *Eubacterium coprostanoligenes group* and underrepresentation of the *Alistipes*, *Family XIII UCG-001*, and *Parabacteroidetes* genera. HCU patients and controls had similar gut microbiota diversity, despite differential abundance of some bacterial genera. Diet, betaine, vitamin B supplementation, and host genetics may contribute to these differences in microbial ecology.

1. Introduction

Classical homocystinuria (HCU; OMIM 236200), or cystathionine beta-synthase (CBS; EC 4.2.1.22) deficiency, is an inborn error of metabolism (IEM) which predominantly affects the transsulfuration pathway. CBS is a key enzyme for the transsulfuration pathway, because it irreversibly catalyzes the conversion of homocysteine (Hcy) into cystathionine, using pyridoxal phosphate (an active form of pyridoxine) as a cofactor. CBS is expressed mainly in the liver, but also in the pancreas, kidneys, and brain [1–3].

The pathophysiology of HCU is still not fully understood. The spectrum of clinical manifestations is broad, ranging from paucisymptomatic patients to a very severe multisystem disease. The main organ systems affected are the eyes (*ectopia lentis* and/or severe myopia), skeleton (abnormally high stature, long limbs, osteoporosis, bone deformities), central nervous system (CNS) (developmental delay/intellectual disability, seizures, psychiatric and behavioral problems, extrapyramidal signs), and vascular system (thromboembolic events). The HCU phenotype is closely related to pyridoxine responsiveness; usually, pyridoxine-responsive patients have a milder phenotype and a later onset

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than nonresponsive patients [3]. Pyridoxine responsiveness is commonly defined according to plasma total homocysteine (tHcy) concentration achieved after a challenging test with pyridoxine, with tHcy decreasing to below 50 μmol/L [3,4].

The aim of HCU treatment is to reduce tHcy to a safe level; nevertheless, normal concentrations are usually unachievable, especially in nonresponsive patients. Treatment consists of pyridoxine supplementation (for responsive patients) and a Methione(Met)-restricted diet and/or betaine (for nonresponsive patients). As a consequence of the Met-restricted diet, nutritional supplementation with a metabolic formula (a Met-free mixture of L-amino acids and micronutrients) is necessary; in addition, folate and vitamin B12 levels should be monitored and supplemented if necessary. Treatment must be lifelong [3].

The gut microbiota is composed of trillions of microorganisms [5]. Recent studies have shown an impact of these microorganisms on physiological processes, and changes in microbiota have been linked to a range of diseases [6,7], including CNS [8,9] and vascular disorders [10–12]. Additionally, health-illness continuum in humans is associated with differences in microbial communities and their functions [13].

Diet and genetics are known to influence the gut microbiome [14,15]. The restrictive diet, use of a synthetic L-amino acids formula and vitamin supplementation used in HCU treatment may affect the gut microbiota [16]. Within this context, the present study aims to investigate possible relationships between HCU treatment and the gut microbiota.

2. Methods

2.1. Experimental design

This observational, cross-sectional study used a convenience sampling strategy, which included six HCU patients and six age- and-sex-matched healthy controls. The study protocol was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (HCPA) (project number 2015-0218). All participants and/or their legal guardians provided written informed consent. Patients were recruited from outpatient clinics of the Medical Genetics Service (MGS), HCPA, Rio Grande do Sul, Brazil. Control subjects were recruited from the Santa Cecília Basic Health Unit, Porto Alegre, Brazil, which is located nearby to HCPA; subjects who performed routine medical appointments at this Basic Health Unit were invited to participate in the study. The inclusion criteria for patients were: a) having a genetic diagnosis of HCU (e.g., presence of biallelic pathogenic mutations in the *CBS* gene); b) being on treatment; c) having no other chronic disease; and d) no current use of antibiotics. The inclusion criteria for controls were a) no relation to HCU patients, b) no chronic or acute diseases, and c) no current use of antibiotics.

Clinical information, such as treatment strategies and tHcy and Met levels, was obtained by reviewing medical records. Besides that, a specific questionnaire was applied to every participant, which included questions on the use of medicines (antibiotics, laxatives), and of probiotics and fermented milk, and fibers supplementation in the last 6 months. Additionally, the use of dietary fiber supplements was also recorded. The criteria used to define metabolic control was based on the 1-year median of tHcy measurements before the stool collection. If the median tHcy value was within target, the patient was considered to have good metabolic control. The target level of tHcy is 50 μmol/L for pyridoxine-responsive patients, and <100 μmol/L for nonresponsive patients [17].

All subjects received a kit which consisted of a Styrofoam box containing a sterile container in which to store the stool sample, a gel ice pack, and a sterile spatula to collect the sample. The participants also received printed instructions for stool collection, storage, and transport. They were instructed to collect stool in their own homes on the day before their medical appointments at HCPA, store the specimen in a household freezer (−20 °C), and deliver it the next day, on ice, during the scheduled appointment. All participants were also provided printed instructions and a sheet to record 3 days of dietary information. The participants were instructed to write down everything they had eaten over the 3-day period, as well as the amount and manner of preparation of all foods and beverages. Stool samples were preferably to be collected on the third day of the food record. Upon returning to the clinic, each participant answered a questionnaire about personal features, including dietary and bowel habits and current medications.

2.2. Nutritional assessment

Patients and controls were instructed to complete a 3-day dietary record (day 3 – the day when the stool sample was collected; days 1 and 2 – before the collection of the stool sample). Macro- and micronutrient intake were analyzed using Nutribase™ software (NB16Cloud, Cybersoft Inc. Phoenix, AZ, USA). As there is no Brazilian table of the Met content on foods, daily Met intake could not be estimated; hence, only total protein intake was calculated. Daily nutrient intake was computed considering the average of the three days of food records. Vitamin supplements and other medications were not included in the nutritional analysis.

2.3. pH measurement, bacterial DNA extraction, 16S rRNA gene amplification and sequencing

Frozen stool samples of participants were thawed and aliquoted at room temperature (20 °C) for pH measurement [18]. To measure the fecal pH, samples were diluted 1:10 (w/v) in distilled water. The dilute was homogenized and incubated for 5 min at room temperature, and fecal pH was measured with an electronic pH meter (K39–1014B, KASVI, PR, Brazil) after complete immersion of the electrode for 3 min.

Bacterial DNA was isolated from 300 mg of frozen stool sample using the QIAmp Fast DNA Stool Mini Kit (Qiagen, Valencia, CA, USA), following manufacturer instructions. DNA quality was determined by spectrophotometry in a NanoVue™ system (GE Healthcare, Chicago, IL, USA). The sequencing library was prepared following the procedures described by Barboza et al. [19]. The gut microbial community was determined by amplification of the V4 region of 16S rRNA with the barcode bacterial/archeal primers 515F and 806R [20]. PCR reactions were carried out with 2U of Platinum Taq DNA High Fidelity Polymerase (Invitrogen, Carlsbad, CA, USA), 4 μL 10X High Fidelity PCR Buffer, 2 mM $MgSO_4$, 0.2 mM dNTPs, 0.1 μM of each barcoded primer, 25 μg of Ultrapure BSA (Invitrogen, Carlsbad, CA, USA) and approximately 50 ng of DNA template in a final volume of 25 μL. PCR conditions were 95 °C for 5 min, 30 cycles at 94 °C for 45 s, 56 °C for 45 s, and 72 °C for 1 min, followed by a final extension step of 10 min at 72 °C. After visualization on a 1.5% agarose gel, the PCR products were purified with Agencourt AMPure XP Reagent (Beckman Coulter, Brea, CA, USA). The final concentration of the PCR product was quantified with a Qubit Fluorometer kit (Invitrogen, Carlsbad, CA, USA), following manufacturer instructions, and combined in equimolar ratios to create a mixture composed of amplified 16S gene fragments of each sample. Ultimately, this composite was used for library preparation in the Ion One-Touch 2 System, using Ion PGM Template OT2 400 Kit (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was performed with Ion PGM Sequencing 400 on the Ion PGM System, using 318 Chip kit v2.

Table 1
Comparison between the HCU and control groups.

Variable	HCU[a] (n = 6)	Control[a] (n = 6)	p-value
Sex (male:female)	5:1	5:1	1.000
Age (years)	25.5 (15.2–31.2)	24.5 (17.2–32.0)	0.810
Weight (kg)	63.0 (52.5–74.8)	68.9 (50.0–84.5)	0.631
Height (cm)	173.9 (154.6–182.3)	177.0 (153.2–183.5)	0.873
BMI (kg/m^2)	22.0 (19.7–24.0)	22.1 (19.7–24.8)	1.000
Fecal pH	7.2 (6.74–7.6)	7.3 (7.0–7.6)	0.873
Antibiotics[b] (yes:no)	2:4	1:5	1.000
Laxatives[b] (yes: no)	0:6	0:6	1.000
Probiotics[b] (yes: no)	0:6	1:5	1.000
Fibers supplementation[b] (yes:no)	0:6	0:6	1.000
Vitamin supplementation[b] (yes:no)	3:3	6:0	0.181
tHcy (μmol/L)	80.0 (45.5–97.8)	–	
Met (μmol/L)	287.1 (40.0–460.1)	–	
Daily intake			
-Calories (kcal)	1349.3 (1308.2–1863.3)	1863.0 (1220.0–2493.0)	0.631
-Calories (kcal/kg)	24.4 (19.1–37.2)	24.7 (18.5–35.5)	0.749
-Protein (g)	41.7 (28.1–102.6)	82.9 (56.1–140.7)	0.150
-Protein (g/kg)	0.8 (0.4–1.5)	1.0 (0.9–1.8)	0.418
-Carbohydrates (g)	262.5 (219.8–304.3)	225.5 (148.2–292.5)	0.423
-Dietary fiber (g)	20.7 (16.5–29.5)	20.1 (13.6–28.4)	0.749
-Sucrose (g)	1.8 (0.2–8.3)	1.5 (0.8–10.1)	0.631
-Fat (g)	30.5 (17.2–40.2)	59.0 (38.8–89.5)	**0.025**
–Saturated (g)	8.8 (3.1–10.3)	20.3 (14.1–29.7)	**0.004**
–Monounsaturated (g)	5.7 (1.7–10.7)	16.9 (14.0–25.2)	**0.004**
–Polyunsaturated (g)	4.8 (2.2–9.6)	8.8 (5.3–16.0)	0.150
–Omega-3 (g)	0.2 (0.2–0.4)	0.3 (0.1–0.4)	0.629
–Cholesterol (mg)	32.4 (7.8–48.8)	223.0 (186.2–301.9)	**0.004**
-Vitamins			
-A (mcg)	372.3 (64.0–1324.6)	180.3 (97.4–534.7)	0.522
-Pyridoxine (mg)	1.4 (0.6–2.2)	1.2 (0.5–1.9)	0.749
-Choline (mg)	102.9 (47.6–233.7)	197.8 (131.4–397.8)	0.150
-Total folate (mcg)	179.4 (130.7–276.2)	349.1 (190.0–405.0)	0.109
-B12 (mcg)	3.0 (0.1–5.7)	4.5 (2.7–7.9)	0.200
-C (mg)	139.0 (33.9–209.0)	82.6 (43.5–110.3)	0.262
-D (IU)	16.2 (2.9–22.7)	58.5 (36.4–128.5)	**0.004**
-E (IU)	2.4 (0.8–3.5)	4.4 (2.6–7.5)	0.109
-K1 (mcg)	43.8 (28.6–75.0)	23.3 (13.5–68.0)	0.262
-Minerals			
-Calcium (mg)	602.6 (300.9–2577.43)	671.4 (400.4–973.0)	0.749
-Iron (mg)	9.7 (8.2–42.8)	13.3 (9.3–21.5)	0.748
-Magnesium (mg)	164.1 (111.7–419.0)	220.3 (166.2–276.8)	0.522
-Phosphorus (mg)	517.1 (379.3–1483.1)	962.8 (799.0–1426.5)	0.200
-Potassium (mg)	1500.7 (1116.6–2621.1)	1800.1 (1539.4–2652.8)	0.337
-Selenium (mcg)	37.1 (23.2–56.5)	98.8 (57.4–146.5)	**0.004**
-Zinc	7.1 (4.4–17.8)	12.7 (7.7–19.4)	0.423

HCU: classical homocystinuria; BMI: body mass index; tHcy: total homocysteine; Met: methionine; –: not measured.
Numerical variables summarized as median (interquartile range) and compared using the Mann-Whitney *U* test. Categorical variables were compared using Fisher's exact test. Significant p-values (<0.05) highlighted in bold.
[a] Formula and diet, but not vitamin supplementation, were taken into account for analysis.
[b] In the previous 6 months.

2.4. 16S profiling data analysis

The 16S rRNA reads were analyzed following the recommendations of the Brazilian Microbiome Project [21], for an efficient removal of sequencing artifacts that might exacerbate biases due to the presence of chimeric sequences and sequence errors. Briefly, an Operational Taxonomic Unit (OTU) table was built using the UPARSE pipeline [22]. Reads were truncated at 200 bp and quality-filtered with a maximum expected error of 0.5. Filtered reads were dereplicated and singletons were removed. The sequences were clustered into OTUs at a 97% cutoff, following the UPARSE pipeline. Taxonomic classification was carried out in QIIME version 1.9.1 [23], based on the UCLUST method, against SILVA ribosomal RNA gene database version v1327 [24] with 80% boundary confidence. Downstream analyses were performed with dataset rarefy to the minimum library size [25,26] in the R software environment [27], using the *phyloseq* [28], *vegan* [29], and *ALDEx2* [30,31] packages. Differential abundant microbes were selected based on the effect size rather than on *p*-values only, as proposed by Gloor et al. [31]. Spearman correlation was used to evaluate the association between HCU biochemical markers (tHcy and Met levels) and OTU richness.

2.5. Statistical analysis

Statistical analysis of clinical data among groups was carried out in PASW Statistics for Windows software (VVs18.0, 2009; SPSS Inc., Chicago, IL, USA). For comparison between groups, continuous variables were analyzed using the Mann–Whitney *U* test. Categorical variables were compared using Fisher's exact test ($p \leq 0.05$).

3. Results

Six unrelated HCU patients and six healthy individuals were included in the study (Tables 1 and 2). All patients had been diagnosed late and were unresponsive to pyridoxine. All were on a Met-restricted diet and presented with hyperhomocysteinemia. Oral

Table 2
Characteristics of patients with HCU included in the study.

Patient	H1[b]	H2	H3	H4	H5	H6
Age (years)	4	19	23	28	28	41
Age at treatment onset (years)	4	13	2	8	6	14
Sex (M/F)	M	M	M	F	M	M
Genotype	p.Glu176Lys/ p.Val533Gly	p.Thr191Met/ c.209+1delG	p.Ile95Thr/ p.Ile95Thr	p.Asp376Asn/p.828ins104, 737del9	p.Asn149fs/ p.Asn149fs	p.Gly85Arg/ p.Gly85Arg
Consanguinity	No	No	Yes	No	Yes	Yes
Pyridoxine responsiveness	No	No	No	No	No	No
tHcy (μmol/L)	48.6	102.6	36.2	96.3	75	85
1-year tHcy median (μmol/L)	-	110.1	40.5	117.1	146.9	168.6
Met (μmol/L)	29.5	690.2	43.5	217.1	357.2	383.5
Metabolic control[a] (good/poor)	Good	Poor	Good	Poor	Poor	Poor
Clinical manifestations						
-Ocular	Yes	Yes	Yes	Yes	Yes	Yes
-Skeletal	Yes	Yes	Yes	No	Yes	Yes
-CNS	Yes	No	Yes	Yes	Yes	Yes
-Vascular	No	No	Yes	No	Yes	No
Current treatment						
-Met-restricted diet	Yes	Yes	Yes	Yes	Yes	Yes
-Metabolic formula	Yes	No	No	No	Yes	Yes
-Pyridoxine	Yes	Yes	Yes	Yes	No	Yes
-Betaine	No	Yes	Yes	No	Yes	Yes
-Folate	Yes	Yes	Yes	Yes	Yes	Yes
-Vitamin B12	Yes	No	No	Yes	Yes	Yes

HCU: classical homocystinuria. tHcy: total homocysteine. Met: methionine. CNS: central nervous system.
[a] Metabolic control was defined on the basis of 1-year tHcy medians before the stool sample collection. If the tHcy level was within target, metabolic control was considered good. As all the patients were nonresponsive to pyridoxine, the target tHcy level was <100 μmol/L for the whole sample [3].
[b] The patient had been under treatment for 4 months. The patient had only one measure of tHcy and Met under treatment.

supplementation of folate (n = 6) and pyridoxine (n = 5), oral Betaine intake (n = 4), and intramuscular vitamin B12 supplementation (n = 4) were reported only in the HCU group. Only two patients had good metabolic control (patients H1 and H3, Table 2).

Nutritional analysis (Table 1) revealed differences in the intake of some nutrients between patients and controls. Overall fat (p = 0.025), saturated fat (p = 0.004), monounsaturated fat (p = 0.004), cholesterol (p = 0.004), vitamin D (p = 0.004), and selenium (p = 0.016) intake was lower in HCU patients compared to controls.

3.1. Microbiota composition and the correlation between gut microbiota and HCU biochemical markers

Nine known bacterial phyla were detected within all samples (Fig.1). The most dominant was *Bacteroidetes* (HCU: 62.5%; controls: 55.2%), followed by *Firmicutes* (HCU: 32.7%; controls: 39.1%) and *Proteobacteria* (HCU: 2.1%; controls: 5.1%). Alpha diversity analysis (Fig. 2) showed no difference between HCU patients and controls, neither in richness nor in evenness. The lack of difference in diversity between HCU and controls suggests that HCU treatment does not have an effect on gut bacterial diversity. Differences in gut composition between treatments were verified by Principal Coordinates Analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA). Beta diversity analysis is dependent upon distances of dissimilarities between samples; here, the Bray-Curtis and Binary distances were used. Irrespective of the distance applied, the beta diversity analysis showed no difference between HCU patients and controls, as observed by PCoA (Fig. 3) and PERMANOVA.

The association between HCU gut microbiota (number of OTUs), tHcy and Met is shown in Fig. 4. Microbial richness had no correlation with tHcy (R = -0.43, $p = 0.42$) and Met levels (R = -0.77, $p = 0.1$).

3.2. Biomarker analysis

Although no differences in overall alpha and beta diversity were detected between HCU patients and controls, significant associations between HCU patients and specific bacteria might still exist. Such associations were tested by modeling the data as a log-ratio transformed probability distribution rather than counts. Differential abundant microbes analysis indicated that one OTU closely related with the *Eubacterium coprostanoligenes group* was increased in HCU patients (Table 3). On the other hand, four OTUs closely related with *Alistipes* (2 OTUs), *Family XIII UCG-001*, and *Parabacteroidetes* were increased in controls (Table 3).

4. Discussion

To the best of our knowledge, this is the first study to characterize the gut microbiota of HCU patients. Surprisingly, our data suggest the microbiota of HCU patients and controls does not differ regarding diversity.

Dysbiosis can be defined as any perturbation to the structure of complex commensal communities [32]. Dysbiosis can contribute to the onset of chronic disease in one of three general ways: 1) pathogens and their functions can be acquired or opportunistically overgrow (gain of function dysbiosis); 2) health-protective bacteria and their functions may be lost or suppressed (loss of function dysbiosis), and 3) a combination of loss and gain of function dysbiosis [33]. Studies on the relationship between the gut microbiota and other IEM are still scarce and have shown that treated phenylketonuria (PKU) [34] and hepatic glycogen storage diseases (GSDs) patients have dysbiosis [18]. Even though the genetic and dietary aspects of these IEMs are different from HCU, both GSD and PKU patients had decreased alpha and beta diversity and distinct microbiota composition when compared to healthy subjects. We expected that the gut microbiota profile of patients with HCU

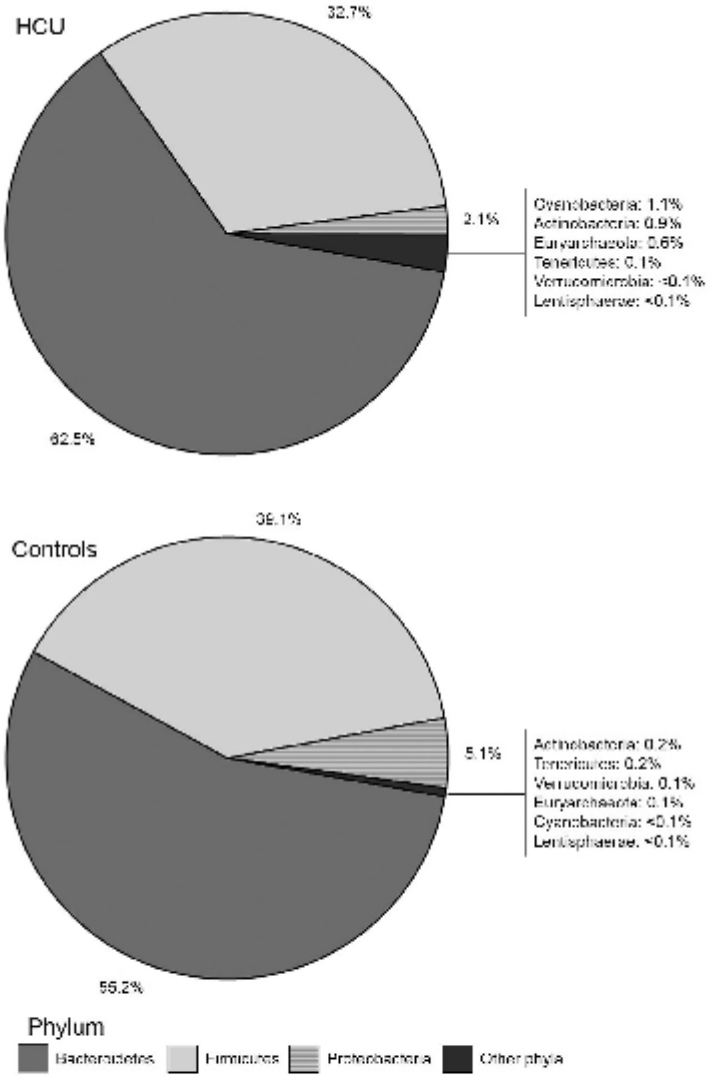

Fig. 1. Frequencies of phyla found in HCU patients (n=6) and healthy controls (n=6). The panel represents the average abundance per group. HCU: classical homocystinuria.

would be similar to that of patients with PKU, as treatment for both diseases involves a restrictive diet (in PKU, there is a restricted intake of phenylalanine and in HCU, of Met) and use of a metabolic formula to supplement nutrients. Nevertheless, we found no differences in alpha nor beta diversity in HCU patients when compared to controls. We also found no association between gut microbiota and biochemical markers in HCU patients.

Our finding of lower intake of cholesterol, fat (saturated and monounsaturated), vitamin D, and selenium in HCU patients is mainly explained by their Met-restricted diet, which excludes or restricts many foods from animal origin as well as nuts and beans, which are sources of these nutrients [35]. It is important to point out, however, that the intake of these nutrients was not below dietary recommendations; that most patients, actually, were not fully compliant to the dietary treatment (and, so, showed a bad metabolic control); and that the metabolic formula for HCU includes supplementation of vitamin D and selenium (at the time of inclusion in the study, only 3 out of the 6 patients were taking the

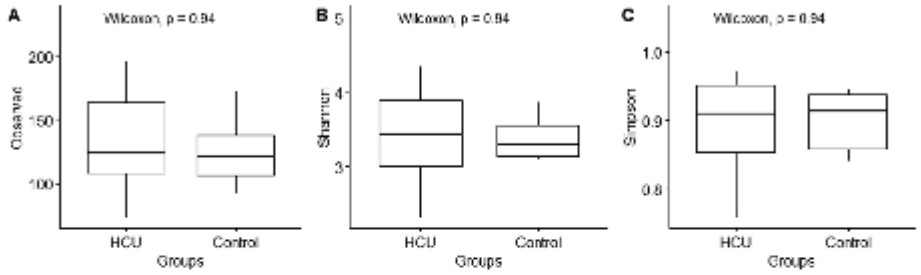

Fig. 2. Alpha diversity measurements of microbial communities in HCU patients (n=6) and controls (n=6). Each panel represent one alpha diversity measure: (A) Observed index estimates the amount of unique OTUs found (richness). (B) Shannon index accounts for richness (count) and evenness (distribution). (C) Simpson index accounts for both richness and evenness.

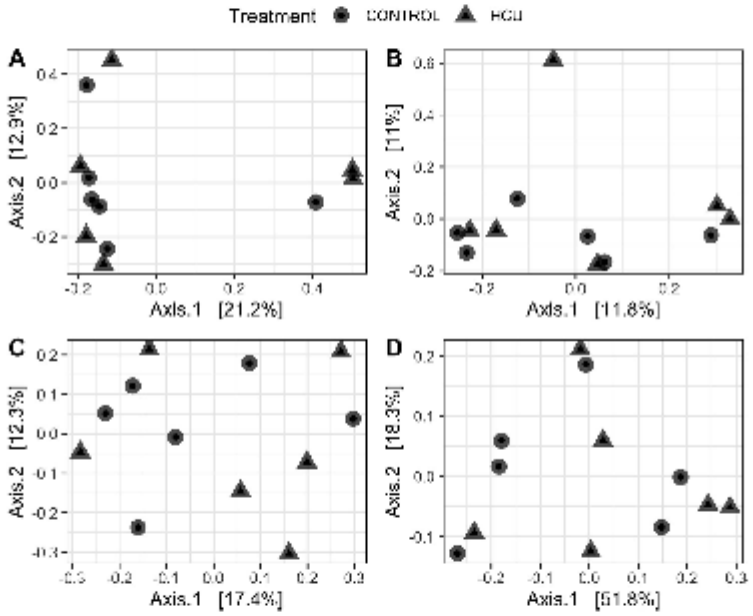

Fig. 3. Principal coordinates analysis (PCoA) representing the comparison of microbial communities in HCU patients and controls. PCoA base on (A) Bray-Curtis dissimilarity (F-value = 0.937; R^2 = 0.085; p-value = 0.453). (B) Binary distance (F-value = 0.966; R^2 = 0.088; p-value = 0.671). (C) Unweighted UniFrac (F-value = 0.212; R^2 = 0.895; p-value = 0.72). (D) Weighted UniFrac (F-value = 0.824; R^2 = 0.076; p-value = 0.453). HCU: classical homocystinuria.

formula, but only one had a good metabolic control). Regarding other nutrients, no differences were found between the groups. The most probably explanation for this finding is also the poor adherency to the Met-restricted diet.

A unique feature of HCU treatment is the supplementation of high doses of B vitamins [3], and the supplementation of dietary nutrients related to one-carbon metabolism has shown a role in modulating the gut microbiota[36–39]. Therefore, vitamin supplementation may be involved in the diversity profile found in patients with HCU. Furthermore, studies in mice have shown possible beneficial effects of a Met-restricted diet, such as decreased intestinal permeability, inflammation, and oxidative stress [40,41].

Gurwara et al. [36] reported that B vitamins, involved in one-carbon metabolism, were associated with variations in microbiota profile: high dietary intake of folate, pyridoxine, and vitamin B12 was associated with an increase in richness and evenness. Interestingly, our study found no difference in richness or in evenness. In addition, Gurwara et al. [36] found that high intake of pyridoxine, vitamin B12, and folate was associated with an increased abundance of *Verrucomicrobia* and *Alistipes*, while our study found a decreased abundance of *Alistipes* in patients with HCU. Furthermore, low abundance of *Alistipes* in the human gut microbiota is known to be associated with better dietary quality [42]. As HCU patients have a restricted diet, they are not expected to have a high

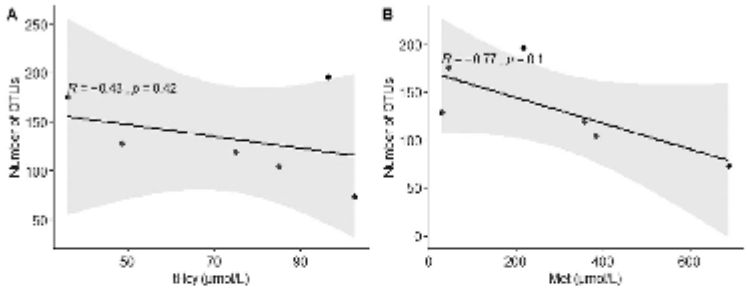

Fig. 4. Correlation between gut microbiota and biochemical markers. Spearman correlations between HCU gut microbiota richness (total number of OTUs), (A) tHcy and (B) Met levels.

Table 3
Microbial biomarkers differentiating HCU patients from healthy controls.

Closest microbial relative	Median of the centered log-ratio of the number of sequences		Effect size	p-Value
	HCU (n = 6)	Controls (n = 6)		
Eubacterium coprostanoligenes group	7.037	−1.271	1.026	0.051
Alistipes[a]	4.197	11.723	−1.107	**0.021**
Alistipes[a]	−1.262	3.263	−1.138	**0.044**
Family XIII UCG-001	0.622	5.299	−1.252	**0.020**
Parabacteroidetes	2.147	8.660	−1.308	**0.009**

HCU: classical homocystinuria.
Positive values of effect size indicate greater abundance in the HCU patients, whereas negative values indicate greater abundance in controls.
As the data were centered log-ratio transformed, OTUs with extremely low abundance appear to be negative.
Significant p-values (<0.05) highlighted in bold.
[a] Two different OTUs corresponding to the same genus were identified, but identification at the species level was not possible.

dietary quality, and this was a surprising finding. However, some important differences must be noted: (a) the Gurwara et al. study [36] was performed only in men between the ages of 50 and 75; (b) bacterial samples were obtained by colonoscopy, not from stool samples; and (c) their criterion of high or low intake of the analyzed vitamins was exclusively dietary, while our patients with HCU were taking high supplemental doses of these vitamins.

The relationship between gut microbiota and the CNS has been widely described in the literature; however, its mechanisms are not fully understood. The gut-brain axis is bidirectional [43], and a number of studies have associated gut microbiota profile with neurodegenerative diseases [9] and neuropsychiatric disorders [8]. In this study, we were unable to evaluate CNS manifestations in light of microbiome profile, both due to the small sample size and because, in patients with HCU, CNS manifestations may be secondary to the toxic effects of high Hcy levels [44]. Nevertheless, Hcy itself is able to disrupt the blood-brain barrier [45], and it is plausible that the microbiota might be a contributor to CNS manifestations in this condition due to a myriad of immune-cellular mechanisms [46]. This relationship must be elucidated further to understand how the gut microbiota may be related to CNS manifestations.

Our study found an increase in the genus *Eubacterium coprostanoligenes group* in HCU patients. This bacterial genus has been associated with anxiety disorder [47], psychosocial stress [48], and cholesterol metabolism [49,50]. Although there is lack of data in the literature, much because it is a bacterial genus not yet cultivable. In addition, we found a decrease in the genera *Parabacteroides* and *Family XIII UCG-001*. The decrease in the genus *Parabacteroides* was related to lower intake of milk and dairy products [42], and, indeed, the diet of HCU patients restricts intake of these types of foods. Despite little information in the literature, an increase in *Family XIII UCG-001* abundance has been described as a neuroprotective biomarker in chronic social defeat stress-induced depressive-like behavior in mice treated preventively with probiotics [51].

This was the first study designed to characterize the gut microbiota in HCU patients under treatment. As HCU is a rare disease, we were only able to enroll a small number of patients. This, and the cross-sectional design which precludes any causal inference were the main limitations of our study.

5. Conclusion

Our data suggest that the diversity of gut microbiota is similar in patients with HCU and healthy controls, despite differences in some genera. The gut microbiota profile found in HCU patients is probably a sum of several factors, such as diet and treatment; host genetics may be related to differences in microbial ecology and even to the presence of bacterial genera still little described in the literature. Future studies on the gut microbial composition of HCU patients are needed to confirm these findings and to investigate the association of gut microbiota with treatment regimens and biochemical features of HCU.

Author contributions

Gustavo M. Rizowy developed and designed the project, collected and analyzed data, and drafting and proofreading the manuscript.

Soraia Poloni helped collect and analyzed data, and drafting and proofreading of the manuscript.

Karina Colonetti developed and designed the project, collected

and analyzed data, and drafted the manuscript.

Karina C. Donis helped collect data and draft the manuscript.

Priscila T. Dobbler helped collect and analyze data.

Sandra Leistner-Segal developed and designed the project, analyzed data, and drafted the manuscript.

Luiz Fernando W. Roesch took part in the development and review of the project, data collection and analysis, and drafting and proofreading of the manuscript.

Ida Vanessa D. Schwartz was in charge of development and review of the project, data analysis, and drafting and proofreading of the manuscript.

Declaration of competing interest

None.

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12.3 Artigo publicado durante o período do doutorado “Cytokines levels in late-diagnosed Classical Homocystinuria patients”

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Cytokines levels in late-diagnosed Classical Homocystinuria patients

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Classical homocystinuria (HCU; CβS deficiency) is characterized by a blockage in homocysteine (Hcy) degradation, resulting in Hcy and methionine accumulation and cysteine deficiency. Studies in healthy and chronically ill individuals have found positive associations between proinflammatory cytokines and plasma total homocysteine (tHcy) [1–3], suggesting a role for immunomodulation in HCU pathogenesis. Therefore, we aimed to investigate 20 inflammatory cytokines in plasma of poorly controlled HCU patients and healthy controls.

The study sample comprised 9 late-diagnosed HCU patients and 10 age and gender-matched healthy controls from South Brazil. tHcy, cysteine, methionine, S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) were measured in plasma by LC-MS/MS. The cytokines quantification assay was performed through EMD Millipore's MILLIPEX® MAP Human Cytokine kit, accordingly manufacturer's instruction. All samples were measured in duplicates for 20 cytokines (Table 1). Measurements with divergence ≥30% between duplicates

Table 1
Homocysteine-related metabolites and cytokine levels in HCU patients and controls.

		Patients (n = 9)	Controls (n = 10)	p
		Median (range)	Median (range)	
Homocysteine-related metabolites (μmol/L)	Met	165 (22–777)	24 (14–30)	0.007
	Hcy	130 (17–300)	6.7 (5.7–12)	< 0.001
	Cys	158 (67–297)	223 (174–241)	0.014
	SAM	756 (99–3264)	82 (69–107)	< 0.001
	SAH	135 (18–591)	19 (10 – 23)	0.002
Pro-inflammatory cytokines (pg/mL)	IL-1α	0.05 (0.01–1.25)	0.24 (0.01–30.77)	0.252
	IL-1β	0.79 (0.39–1.83)	0.57 (0.37–1.05)	0.743
	IL-6	1.03 (0.45–2.05)	1.12 (0.47–11.56)	0.653
	IL-8	2.07 (0.58–15.60)	1.58 (1.12–18.14)	0.624
	IL-17	1.96 (1.04–5.48)	4.09 (1.23–12.94)	0.102
	TNF-α	7.92 (3.62–14.81)	9.17 (3.92–13.67)	0.287
	TNF-β	0.01 (0.00–1.94)	0.02 (0.00–119)	0.617
	MCP-1	254 (185–1014)	267 (216–357)	0.935
	IP-10	277 (170–1855)	489 (264–1764)	0.153
	GRO	829 (197–2473)	704 (303–1014)	0.595
	MDC	554 (299–1288)	527 (295–759)	0.744
	MIP-1α	1.00 (0.54–3.62)	2.24 (0.66–34.80)	0.077
	MIP-1β	18.61 (1.01–37.62)	19.57 (1.84–68.80)	0.327
	VEGF	156.71 (0.01–376)	255.33 (1.32–704)	0.102
	GM-CSF	3.62 (1.06–15.20)	3.01 (1.77–13.67)	0.744
	IFN-γ	3.62 (1.67–9.72)	10.92 (4.94–82.04)	0.007
Anti-inflammatory cytokines (pg/mL)	IL-4	0.48 (0.04–2.83)	0.68 (0.16–26.46)	0.327
	IL-10	0.75 (0.27–1.39)	0.79 (0.41–3.29)	0.935
	IL-13	0.11 (0.02–9.17)	0.15 (0.02–78)	0.368
	G-CSF	13.67 (7.69–46.01)	16.83 (7.69–135.66)	0.653

Met: methionine; Hcy: homocysteine; Cys: cysteine; SAM: S-Adenosylmethionine; SAH: S-Adenosylhomocysteine.

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were excluded from data analysis (n = 1).

Hcy-lowering treatment (pyridoxine 7/9, folic acid 8/9, betaine 7/9, methionine-restricted diet 3/9) was prescribed; but only 3/9 patients had tHcy < 100 μmol/L (target level). Most patients (7/9) were pyridoxine nonresponsive. Because of the high concentrations of tHcy with a wide range we consider this an ideal group to explore the potential relation between cytokines and tHcy. Cytokines plasma levels were similar in patients and controls, with the exception of IFN-γ, which was three-fold reduced ($p = .007$) in patients (Table 1). In line an inverse association of Hcy and SAM with IFN-γ was found ($r - 0.487$ and $r - 0.537$; $p < .05$).

To our knowledge, only one study had previously evaluated cytokines in HCU patients. Keating et al. measured 16 cytokines in plasma of HCU patients, and found that patients with tHcy > 150 μM, ($n = 5$) had increased levels of several pro-inflammatory cytokines (IL-1a, IL-6, TNF-α, IL-17 and IL-12), while well controlled patients (Hcy < 86.1 μM, n = 5) had not [4]. The authors provided no information about which patients received treatment. IFN-γ was not evaluated in this study. In previous studies, reduced IFN-γ levels have shown anti-inflammatory properties [5,6].

In summary, our study provides no evidence of increased inflammatory cytokines in HCU patients on treatment, despite poor metabolic control. Hcy may even show anti-inflammatory properties like glutathione [7], what could explain the finding of lowered IFN-γ. The potential impact of Hcy-lowering treatment on cytokines requires further study.

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12.4 Trabalho selecionado para apresentação oral para XXVI Jornada de Jóvenes Investigadores AUGM.