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Diagnosis and Management of Classical Homocystinuria in Brazil: A Summary of 72 Late-Diagnosed Patients

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Abstract

This study described a broad clinical characterization of classical homocystinuria (HCU) in Brazil. This was a cross-sectional, observational study including clinical and biochemical data from 72 patients (60 families) from Brazil (South, n = 13; Southeast, n =

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37; Northeast, n = 8; North, n = 1; and Midwest, n = 1). Parental consanguinity was reported in 42% of families. Ocular manifestations were the earliest detected symptom (53% of cases), the main reason for diagnostic suspicion (63% of cases), and the most prevalent manifestation at diagnosis (67% of cases). Pyridoxine responsiveness was observed in 14% of patients. Only 22% of nonresponsive patients on treatment had total homocysteine levels <100 μmol/L. Most commonly used treatment strategies were pyridoxine (93% of patients), folic acid (90%), betaine (74%), vitamin B12 (27%), and low-methionine diet + metabolic formula (17%). Most patients diagnosed with HCU in Brazil are late diagnosed, express a severe phenotype, and poor metabolic control. Milder forms of HCU are likely underrepresented due to underdiagnosis.

Keywords
classical homocystinuria, CBS deficiency, homocysteine, pyridoxine responsiveness, diagnosis

Introduction

Classical homocystinuria (HCU; OMIM 236200) is an inborn error of methionine metabolism caused by deficient activity of cystathionine β-synthase (CβS; EC 4.2.1.22). Cystathionine β-synthase deficiency leads to massive accumulation of homocysteine and methionine and low levels of cysteine.[1,2] Homocystinuria was first described in 1962[3]; since then, many advances in treatment and early diagnosis, including newborn screening, have improved prognosis dramatically.[4–6] However, diagnosis and management of HCU is still a major challenge in developing countries, where newborn screening is unavailable and access to health-care services is often poor and unequal.[7]

From a clinical standpoint, the classic signs of HCU are lens dislocation, thromboembolism, mental retardation, psychiatric disorders, osteoporosis, and marfanoid features.[8] Established treatment strategies include supplementation of pyridoxine (CβS cofactor), folic/folinic acid, betaine, and a methionine-restricted diet supplemented with an essential amino acids admixture free of methionine.[9] Novel therapies with chaperones and enzyme replacement are currently under development.[10–12] Usually, patients who respond to pyridoxine supplementation exhibit a milder phenotype and have a better prognosis.[8]

The worldwide prevalence of HCU is estimated to be 1:100 000.[13] However, several mild and late presentation phenotypes have been described,[14–16] raising the issue of whether HCU is largely underdiagnosed. Newborn screening for HCU is performed in some countries with high incidence of the disease, such as Ireland and Qatar.[5,17] Usually, newborn screening is performed by the determination of methionine on filter paper. However, this method has a large percentage of false negatives (up to 50%), since pyridoxine-responsive HCU may not present with hypermethioninemia in the first days of life.[18,19] In Brazil, the prevalence of HCU is unknown, and the disease is not included in the National Neonatal Screening Program.

Brazil is a very large country with over 200 million inhabitants, and the country is characterized by intense admixture.[20,21] The country also has one of the world's largest publicly funded health-care systems, the Unified Health System (*Sistema Único de Saúde*), which was established to provide equitable and comprehensive care to all users. However, betaine and the methionine-free amino acid formulation are not available through Unified Health System, and few centers across the country offer biochemical testing for diagnosis and management of this condition. Furthermore, several factors make diagnosis and management of HCU within the Brazilian health system a major challenge; hence, a substantial number of patients with this treatable condition are believed to remain undiagnosed and thus untreated. Within this context, the present study sought to establish a broad clinical characterization of HCU in Brazil through a survey on diagnosis and management of a representative patient population that is being followed at several centers nationwide.

Materials and Methods

The present study was approved by the local research ethics committee. Collection procedures for the study were conducted only after participants or their caregivers had agreed to take part in the investigation and provided written informed consent.

Patients

The study sample comprised 72 Brazilian patients with a diagnosis of HCU, from 60 different families. Diagnosis was in general made on clinical symptoms followed by clear elevations in homocysteine and methionine. Mutation analyses was performed in 35 of the 72 patients.[22] Families from all 5 regions of Brazil were represented: South (n = 13), Southeast (n = 37), Northeast (n = 8), North (n = 1), and Midwest (n = 1). Patients were recruited through contact with physicians involved in care and/or research activities at medical genetics centers across the country. A structured questionnaire containing queries regarding diagnosis, consanguinity, treatment strategies, metabolic control, and current health condition of patients with HCU was sent to 15 medical centers that had agreed to participate in the study. Clinical data regarding diagnosis were available only for 28 patients.

All 72 patients had delayed diagnoses: 62 patients had been diagnosed after clinical suspicion and biochemical findings consistent with HCU (hyperhomocysteinemia and hypermethioninemia), and the remaining 10 had been diagnosed on family screening. Each of the participating centers used a different protocol for determination of pyridoxine responsiveness. For the purposes of this study, patients were classified as

Table 1. Classical Homocystinuria in Brazil: A Summary of Clinical Findings at Diagnosis.[a]

	Total (N = 72)	Pyridoxine Responsive		P
		Yes (n = 10)	No (n = 61)	
Current age, years	19 (5-45)	23 (14-35)	18 (5-45)	.120
Age at first symptom onset, years	5 (0-20)	2 (0.2-15)	5 (0.7-20)	.316
Age at diagnosis, years	10 (1-39)	11 (4-34)	9 (1-39)	.545
Systems affected at diagnosis, %				
Ocular	72	50	75	.100
CNS	60	70	59	.497
Skeletal	60	40	54	.401
Vascular	15	20	14	.625

Abbreviation: CNS, central nervous system.
[a]N = 72, data expressed as the median (range) or percentage. In 1 patient, pyridoxine responsiveness could not be determined, data from this patient were used alone in the whole-group analysis.

responsive if they achieved homocysteine levels <50 μmol/L on pyridoxine alone or pyridoxine + folic acid (regardless of the number of weeks since testing). All other patients were classified as nonresponsive to pyridoxine.

Target total homocysteine levels on treatment were set according HCU guidelines,[9] which were <50 μmol/L for pyridoxine-responsive patients and <100 μmol/L for nonresponsive patients. Treatment adherence was determined by the subjective impressions of the care team at each medical center.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows, version 18.0 (SPSS Inc, Chicago, Illinois). Asymmetrically distributed variables were expressed as the median (range). The Mann-Whitney *U* test (continuous variables) or χ^2 test (categorical variables) was used to assess between-group differences. Values of $P < .05$ were considered significant.

Results

Of the 72 patients included, 55% were male. Two patients were already deceased at the time of inclusion in the study (presumably due to thromboembolic events). The patients belonged to 60 families; parental consanguinity was reported in 25 (42%) families. Median age at assessment was 19 years. The youngest patient was aged 5, and the oldest was 45. Regarding pyridoxine responsiveness, 61 (85%) patients were classified as nonresponsive and 10 (13.8%) as responsive. In 1 patient, pyridoxine responsiveness was not reported/determined.

Journey to Diagnosis

The very first symptom noticed by families and/or physicians was visual impairment (mainly due to *ectopia lentis*) in 53% of the cases, followed by developmental delay (22% of cases), seizures (11% of cases), and isolated thromboembolic episodes (9.5% of cases). Table 1 reports clinical features at diagnosis for the sample as a whole and stratified by pyridoxine responsiveness.

The median time elapsed between symptom onset and diagnosis was 5 years (maximum, 34 years). One-third of the patients had 3 or more systems already affected at the time of diagnosis. The main clinical findings leading to investigation of HCU are represented in Figure 1. Eye disease, the most prevalent symptom at diagnosis (67% of cases), accounted for 63% of referrals for HCU investigation.

Management

Current clinical and biochemical data were available for 44 patients, of whom 7 were responsive and 37 were nonresponsive to pyridoxine. The median length of follow-up was 6 years (range, 0-27 years). Table 2 describes clinical manifestations and biochemical control in this group of patients. Ocular manifestations were more prevalent among pyridoxine-nonresponsive patients (71% vs 97%, $P = .01$). *Ectopia lentis* was the most common complication in our sample, affecting 91% of patients at the time of study inclusion.

Pyridoxine-responsive patients had significantly lower total homocysteine levels at study inclusion ($P < .001$). Only 22% of nonresponsive patients achieved target total homocysteine levels (<100 μmol/L) on treatment, while all responsive patients (n = 7) had total homocysteine <50 μmol/L. Treatment adherence was reported as appropriate in 44% of patients.

Regarding treatment strategies, 93% of patients were on pyridoxine supplementation, 90% on folic acid, 74% on betaine, 27% on vitamin B12, and only 17% on a low-methionine diet + metabolic formula.

Discussion

The present report provides the largest clinical profile of patients with HCU ever studied in Brazil to date. Clinical data of 72 patients (60 unrelated) from 15 medical genetics centers across Brazil were analyzed. Most families lived in the South and Southeast regions of the country. These regions are home to 57% of the country's population (http://www.ibge.gov.br/) and, compared to other regions of Brazil, have higher rates of access to health-care services and procedures. The fact that patients from other regions, particularly the North (n = 1) and Midwest (n = 1), were underrepresented suggests high rates of HCU underdiagnosis and/or limited access to care in these regions.[7]

A high proportion of pyridoxine-nonresponsive patients with HCU was found in our study (85%). Nonresponsive patients usually present a more severe phenotype, have more complications, and younger ages .[6,8,23] In our study, this proportion exceeded rates described worldwide of approximately 50%.[8] In countries where the proportion of nonresponsive patients is disproportionately high, such as Qatar and Ireland,

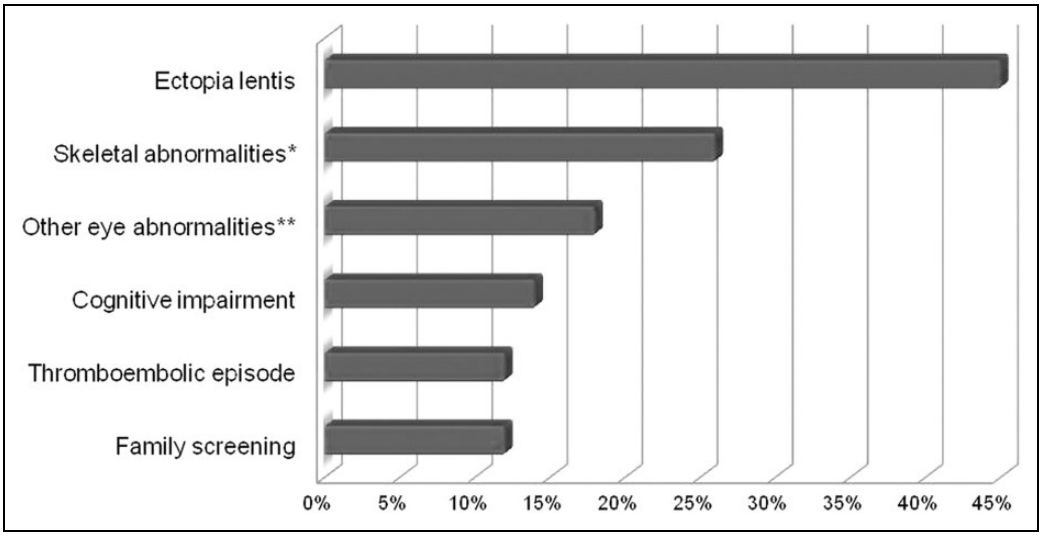

Figure 1. Main reasons for clinical suspicion of classical homocystinuria in our sample (N = 72). *Including marfanoid habitus, **Other than *ectopia lentis*.

Table 2. Classical Homocystinuria in Brazil: Clinical and Biochemical Profile of Patients on Treatment at the Time of Study Inclusion.[a]

		Pyridoxine Responsive		
	Total (n = 44)	Yes (n = 7)	No (n = 37)	*P*
Current age, years	16	19 (14-35)	16 (5-37)	.182
Homocysteine, μmol/L	168	19 (14-34)	212 (9-455)	<.001
Methionine, μmol/L	131	27 (23-650)	218 (6-881)	.065
Clinical manifestations, %				
Ocular	93	71	97	.013
CNS	70	86	68	.318
Skeletal	61	57	62	.803
Vascular	25	14	27	.475

Abbreviation: CNS, central nervous system.
[a]n = 44, data expressed as the median (range) or percentage; reference ranges: homocysteine, 5-15 μmol/L; methionine, 5-30 μmol/L.

specific genotypes are highly prevalent and account for these discrepancies .[24,25] In a previous study of our group, we explored in more depth the genotype of 35 patients with HCU who are also included in the current study.[22]

Our results show there is no single molecular basis for the high prevalence of HCU nonresponsive phenotypes in Brazil. In fact, the most prevalent mutation in that study (p.Ile278Thr) is a pyridoxine-responsive mutation. Another genetic study of 14 Brazilian patients with HCU showed similar results.[26] These findings rather suggest underdiagnosis of pyridoxine-responsive patients in Brazil who express milder phenotypes with fewer symptoms and later onset of clinical presentation.[6,8] In our study, no significant differences in age at symptom onset or age at diagnosis were found between pyridoxine-responsive and nonresponsive patients; however, we believe our analysis was underpowered because of the low number of pyridoxine-responsive patients (n = 10 vs n = 61, respectively).

The high prevalence of ocular manifestations at diagnosis and their predominant contribution to diagnostic suspicion reinforce the importance of eye disease in HCU. *Ectopia lentis* is usually the earliest manifestation of HCU, occurring in half of all untreated patients by age 10 years and in over 90% by age 24 years.[8] No significant difference in the prevalence of ocular manifestations at diagnosis was found between responsive and nonresponsive patients (75% vs 50%, $P = .10$), although the lack of significance could also be explained by the small sample size. However, this finding is consistent with previous reports in the literature.[8]

Skeletal and neurologic manifestations were also highly prevalent at diagnosis, affecting more than half of patients of our cohort. The lower prevalence of vascular events at diagnosis is consistent with the natural history of HCU, in which such manifestations usually occur in general at a later age.[8] However, vascular disease may have been underrepresented due to the high lethality of thromboembolic events. The relative large time gap between symptom onset to diagnosis (median, 5 years) and the presence of multiple clinical manifestations attest to the difficulty in establishing a definitive diagnosis in these patients.

The main strategies and goals of HCU treatment have been recently established in the first guidelines for the diagnosis and management of CβS deficiency.[9] Early diagnosis and early treatment are the key to prevent clinical manifestations and improve prognosis.[4,18]In the present study, current clinical and biochemical data were obtained from 44 patients in treatment. There was a clear difference in metabolic control between the groups: Responsive patients had low and even near-normal homocysteine and methionine levels, whereas most nonresponsive patients had persistently high homocysteine levels (>100 μmol/L) despite multiple treatment strategies. This difficulty in achieving metabolic control in pyridoxine-nonresponsive patients has been reported elsewhere.[18,27,28]

The high rate of betaine supplementation and comparatively low use of methionine-restricted diet in the nonresponsive patients may be attributed to several factors: (1) difficulties in obtaining the metabolic formula, which is expensive and not provided by the Unified Health System in Brazil;[29] (2) low adherence to dietary methionine restriction, particularly in patients with a late diagnosis; and (3) limited training of health-care professionals in dietary prescription. In a European survey of 181 patients with pyridoxine-nonresponsive HCU, 66% were on dietary treatment, that is, twice as many as in the present study.[30] Homocystinuria guidelines clearly state that betaine should not be considered a first-line treatment for HCU-nonresponsive patients but used as adjunct treatment in those who cannot achieve target levels of homocysteine by other means.[9] While dietary therapy dramatically reduces methionine and homocysteine and normalizes cysteine, betaine supplementation reduces homocysteine but increases methionine levels.[31,32] In animal models, betaine is less effective at preventing clinical manifestations,[33] and its efficacy declines over time.[34] In humans, there are no studies of the long-term efficacy of betaine supplementation alone in HCU.

In conclusion, this study provides the first broad clinical characterization of HCU in Brazil. All patients described here were late diagnosed, and most expressed a severe phenotype associated with nonresponsiveness to pyridoxine, early and multisystem clinical manifestations, and poor metabolic control. Limitations of this study include the underrepresented number of patients responsive to pyridoxine, and the number of patients coming from some regions of the country. We believe that our findings will contribute to the development of protocols and guidelines to improve diagnosis and management of HCU in Brazil.

Authors' Note

Soraia Poloni and Giovana Weber Hoss contributed equally to this article. Soraia Poloni, Giovana W. Hoss, Fernanda Sperb-Ludwig, Taciane Borsatto, and Ida V. D. Schwartz made substantial contributions to the conception and design, acquisition of data, analysis, and interpretation of data; Maria Juliana R. Doriqui, Emilia K.E.A Leão, Charles M. Lourenço, Chong A. Kim, Carolina F. M. Souza, Helio Rocha, Marcia Ribeiro, Carlos E. Steiner, Carolina A. Moreno, Pricila Bernardi, Eugenia Valadares, Osvaldo Artigalas, Gerson Carvalho, Hector Y. C. Wanderley, Ney Boa Sorte, and Luiz C. Santana-da-Silva made substantial contributions to the acquisition of data and were involved in revising the manuscript. Ida V. D. Schwartz and Henk J. Blom were involved in the analysis and interpretation of data and critically revising the manuscript for important intellectual content. All authors have given final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Declaration of Conflicting Interests

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