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Characteristics Of Patients Participating In A Study Of Homocystinuria Due To Cystathionine Beta-synthase Deficiency

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Background

Homocystinuria (HCU) caused by cystathionine beta-synthase (CBS) deficiency is the most common genetic disorder of sulfur metabolism and affects visual, skeletal, vascular, and central nervous systems. CBS deficiency impairs the conversion of homocysteine (Hcy) to cystathionine, leading to increased plasma levels of Hcy and methionine (Met) and decreased levels of cysteine and cystathionine and ensuing pathophysiology (Morris et al. 2017, Mudd et al. 1985). Treatment consists of supplementation with B-vitamins (vitamins B6, B12, folate) and/or betaine, and/or restriction of the intake of the essential amino acid Met through a diet that is very low in natural protein to reduce the precursor load on the trans-sulfuration pathway. For patients on dietary treatment, supplementation with a Met-free L-amino acid mixture is also advised (Morris et al. 2017). Study CBS-HCY-NHS-01 is a comprehensive, longitudinal, natural history study documenting the clinical course of patients with HCU on current therapy.

Methods

Fifty-five (55) patients, ages 5–53 at enrollment, with genetically confirmed HCU, are being assessed every 6 months over a 3-year period. An interim analysis of baseline and longitudinal characteristics, including demographics, medical and family histories, medications, dietary information, ophthalmologic findings, plasma total Hcy (tHcy) and other sulfur metabolites, and laboratory values was performed. Patient data from 1 to 5 visits were available. Because Study CBS-HCY-NHS-01 is ongoing, this analysis was performed using a data cut-off date of 05 July 2019. Skeletal abnormalities and cognitive impairments in these patients were also assessed and these data are reported in separate posters (0874 and 0853, respectively).

Results

Patient Characteristics (Table 1):

- Fifty-five patients with HCU were enrolled in this natural history study. The median time on study was 12.2 months.
- The disease affects both sexes similarly (55% males, 45% females).
- HCU patients in study are young (median age: 21.0 years old); 42% are pediatric patients (<18 years of age) and 58% are adult patients.
- Median BMI of this population is in the normal range (21.5 kg/m^2).
- Half of the patients (51%; 65% of pediatric; 41% of adult) were diagnosed in the first year of life (<1 year of age).
- 36% of patients had a family history of HCU.
- The median plasma tHcy level was 95 µM (74 µM for pediatric patients; 104 µM for adult patients), consistent with underlying undertreated disease.
- 95% of pediatric patients had tHcy greater than the upper limit of normal (ULN) and 82% had tHcy levels >50 µM (82%). The tHcy levels of almost half (45%) of the pediatric patients were >100 µM.
- All (100%) adult patients had tHcy levels greater than the ULN, 93% had tHcy levels >50 µM, and 77% had tHcy >100 µM (77%).
- Based on 3-day diet diaries recorded prior to each clinic visit, most patients (93%; 95% pediatric; 91% adult) were following a natural protein-restricted diet and most were taking a Met-free L-amino acid mixture (58%; 74% pediatric; 47% adult).
- Most patients (83%; 91% of pediatric; 76% of adult) were taking B vitamins supplements.
- Most patients (85%; 87% of pediatric; 84% of adult) were taking betaine.

Table 1. Patients demographic and baseline characteristics

	All patients
Age at enrollment, median [range]	21.0 [5-53]
Pediatric (<18 years), N (%)	23 (42%)
Adult (≥18 years), N (%)	32 (58%)
Male / Female, %	55% / 45%
Race, N (%)	
White	51 (93%)
African-American	3 (6%)
Ethnicity, N (%)	
Hispanic or Latino	6 (11%)
Non-Hispanic	46 (84%)
Adult weight, median [range], kg	80.5 [55-138]
Adult height, median [range], kg	176 [148-200]
BMI, median [range], kg/m^2	21.5 [15.0-49.5]
Diagnosed at <1 year of age, N (%)	
Yes	28 (51%)
No	25 (46%)
Have family history of HCU, N (%)	
Yes	20 (36%)
No	35 (64%)
Adult patients' highest education level	
Some high school	14%
High school graduate	21%
Some college/college graduate	41%
Master's degree	14%
Professional degree	3%
Unknown	7%
tHcy at diagnosis,† median [range], µM	
Adults (≥18 years)	100 [5-364]
Pediatric (<18 years)	144 [35-298]
Natural protein-restricted diet, N (%)	38 (93%)
Adults (≥18 years)	20 (91%)
Pediatric (<18 years)	18 (95%)
Met-free L-amino acid mixture, N (%)	32 (58%)
Adults (≥18 years)	15 (47%)
Pediatric (<18 years)	17 (74%)
B-vitamin supplements, N (%)	40 (83%)
Adults (≥18 years)	19 (76%)
Pediatric (<18 years)	21 (91%)
Betaine supplement, N (%)	41 (85%)
Adults (≥18 years)	21 (84%)
Pediatric (<18 years)	20 (87%)
Time on study, median [range], months	12.2 [0.03$, 24.8]

† Historic tHcy data were available for 33 patients only, self-reported.
$ One subject has only undergone Visit 1.

Physical Abnormalities:

The following physical abnormalities were reported in this population at any time in the study:

- Three patients reported thrombotic events in their medical history (CVA at 42 years of age, blood clots in lungs at undisclosed age, dural sinus thrombosis at 2 years of age).
- A large majority of patients (69%) reported ophthalmologic deficits, most commonly myopia and hyperopia. Of note, 2 pediatric patients (<18 years of age) had cataracts and 1 had retinal pigmentosa (Table 2). No patient had uveitis or corneal abrasions.
- Historically, patients have often been diagnosed due to ectopia lentis. In the largest retrospective survey conducted to date, 85% of HCU patients had developed this condition by the age of 20 (Mudd et al. 1985). In contrast, only 19% of adult and 9% of pediatric patients in this managed population has this condition.

Table 2. Ophthalmic deficits

	All Patients N = 55	Pediatric (5-17 years) N = 23	Adult (≥18 years) N = 32
Any ocular deficit	38 (69%)	16 (70%)	22 (69%)
Ectopia lentis/ dislocation of the lens	8 (15%)	2 (9%)	6 (19%)
Cataract	6 (11%)	2 (9%)	4 (13%)
Retinal degeneration	1 (2%)	0 (0%)	1 (3%)
Retinal pigmentosa	1 (2%)	1 (4%)	0 (0%)
Hyperopia	11 (28%)	7 (30%)	4 (13%)
Myopia	25 (63%)	9 (39%)	16 (50%)

Mutations in CBS Gene:

Analysis of the mutations in the CBS gene showed 26 unique mutations identified in 48 patients, with 16 patients apparently homozygote and 32 patients apparently compound heterozygotes (Table 3).

Table 3. Mutations in the CBS gene

DNA	Protein
c.(689del)	p.(Leu230Argfs*39)
c.(209+1G>A)	p.(?)
c.(1126G>A)	P.(Asp376Asn)
c.(752T>A)	P.(Leu251Gln)
c.(808_810del)	p.(Glu270del)
c.(442G>A)	p. (Gly148Arg)
c.(536_553del)	p.(Asp179_Leu184del)
c.(700G>A)	p.(Asp234Asn)
c.(1106G>C)	p.(Arg369Pro)
c.(325T>C)	p.(Cys109Arg)
c.(361C>T)	p. (Arg121Cys)
c.(1006C>T)	P.(Arg336Cys)
c.(785C>T)	p.(Thr262Met)
c.(1058C>T)	P.(Thr353Met)
c.(1039G>A)	P.(Gly347Ser)
c.(770C>T)	p.(Thr257Met)
c.(.153_165del)	P.(Arg51Serfs*27)
c.(1224-2A>C)	p.(?)
c.(919G>A)	p.(Gly307Ser)
c.(1330G>A)	p.(Asp444Asn)
c.(833T>C)	p.(Ile278Thr)
c.(1152G>C)	P.(Lys384Asn)
c.(1339C>T)	P.(Pro447Ser)
c.(488A>G)	P.(Tyr163Cys)
c.(624G>A)	p.(Trp208*)
c.(738del)	P.(Lys274Serfs*2)

tHcy Values:

Determination of tHcy levels at each visit (Table 4) revealed that:

- Most patients (96%) have plasma tHcy levels 5 to 40 times the ULN of 14 µM for >12 years of age and the ULN of 9.6 µM for 0-12 years of age.
- Intra-subject variability was moderate. Overall, patients with low tHcy levels at Visit 1 tended to have low tHcy levels throughout the study (and vice-versa) (Pearson's R = 0.6294). Large between-visit variabilities observed in some patients may be attributed to a change of diet/therapy compliance between visits.

Table 4. tHcy levels during study

Patients	N	Median (µM)	Minimum (µM)	Maximum (µM)
All	52	95	2	402
Pediatric (< 18 years)	22	74	2	361
Adult (≥ 18 years)	30	104	10	402

Laboratory Values:

Determination of various laboratory values during the study (Table 5 and Figure 1) showed the following abnormalities:

- High tHcy and Met, and low cystathionine and total cysteine levels, which are hallmarks of the disease, were observed despite most patients following standard of care (protein-restricted diet, B-vitamins, supplements, betaine).
- Methionine levels >1000 µM were observed in 11% of patients (14% pediatric, 10% adult) and ≥600 µM in 33% of patients (36% pediatric, 33% adult).
- High betaine, vitamin B12, and B6 levels were observed as expected as most patients take those supplements.
- Dimethylglycine (DMG) levels above the ULN were observed in 76% of patients, which could lead to a higher risk of acute myocardial infarction in some patients (Svingen et al. 2013).
- ALT levels above the ULN were observed in 37% of patients (52% pediatric; 28% adult).
- Creatinine levels below the lower limit of normal (LLN) were observed in 43% of patients (74% pediatric, 21% adult), and may be due to low muscle mass secondary to protein restriction.
- hsCRP levels above the ULN were observed in 35% of patients tested (N = 40; only tested in patients ≥13 years of age, only observed in patients ≥18 years of age).
- Low Protein C activity levels were observed in 28% of patients, and low fibrinogen levels were observed in 31% of patients (N = 29; both only tested in patients ≥13 years of age, only observed in patients ≥18 years of age).

Normal levels were observed for:

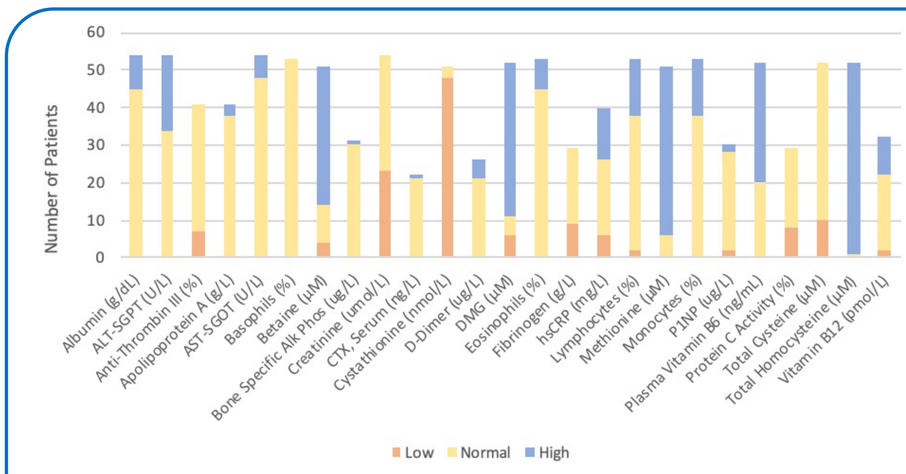
- AST levels (89% of patients), all patients tested.
- Anti-thrombin III (83% of patients) and apolipoprotein A (93% of patients). Only patients ≥13 years of age were tested for these laboratory parameters.
- The following osteoporosis biomarkers: bone-specific alkaline phosphatase (97% of patients), serum CTX (95% of patients), P1NP (87% of patients). Only patients >18 years of age were tested for these laboratory parameters.

Table 5. Selected abnormal laboratory values (all laboratory ranges are age-adjusted)

	All patients	Pediatric (<18 years)	Adults (≥18 years)
tHcy >ULN	51 (98%)	21 (95%)	30 (100%)
tHcy >50 µM	46 (88%)	18 (82%)	28 (93%)
tHcy >100 µM	33 (63%)	10 (45%)	23 (77%)
Methionine >ULN	45 (88%)	20 (91%)	23 (85%)
Methionine >1,000 µM	6 (11%)	3 (14%)	3 (10%)
Methionine ≥600 µM	18 (33%)	8 (36%)	10 (33%)
Cystathionine <LLN	48 (94%)	20 (95%)	26 (93%)
DMG >ULN	41 (79%)	19 (86%)	20 (71%)
ALT >ULN	20 (37%)	12 (52%)	8 (28%)
Creatinine <LLN	23 (43%)	17 (74%)	6 (21%)
hsCRP* >ULN	14 (35%)	0 (0%)**	13 (45%)**
hsCRP* >2x ULN	10 (25%)	0 (0%)**	9 (31%)**
Protein C activity* <LLN	8 (28%)	0 (0%)	8 (28%)
Fibrinogen* <LLN	9 (31%)	0 (0%)	9 (31%)

**hsCRP, Protein C activity and fibrinogen were only tested in patients ≥13 years of age.*
***Age was missing for one patient.*

Figure 1. High/normal/low patient laboratory values



Colored bars indicate the number of patients with a high/normal/low value.
ALT-SGPT= alanine aminotransferase - serum glutamic-pyruvic transaminase
AST-SGOT = aspartate aminotransferase - serum glutamic-oxaloacetic transaminase
DMG = dimethylglycine; hsCRP = hypersensitive C-reactive protein
P1NP = procollagen type 1 N-terminal propeptide

Discussion

- Individuals enrolling into this study are similar to previously described cohorts of patients with HCU.
- Despite being seen at centers of excellence and being prescribed a natural protein-restricted diet and/or a Met-free L-amino acid mixture and supplements, many patients have:
  - plasma tHcy values 5 to 40 times the ULN for tHcy
  - hypermethioninemia (≥600 µM) in 33% of patients (36% pediatric, 33% adult)
  - ocular deficits
  - signs of inflammation, protein metabolism and/or liver dysfunction.
- Hypermethioninemia, known to cause metabolic encephalopathy, is common in this population (Allen et al. 2019).
- Skeletal fragility and significant cognitive impairments were also observed in these patients. These data are reported in Posters 0874 and 0853, respectively.
- These data indicate that current diet and therapeutic interventions are poorly effective and/or that most patients are not able to remain compliant, leading to high tHcy levels, even in patients being frequently monitored at centers of excellence.
- In particular, these data also point out that betaine does not result in appropriate control of the tHcy levels in HCU and, therefore, is not in itself adequate treatment for HCU, contrary to common belief.
- New laboratory markers identified in this study may be useful in clinical trials to determine the efficacy of new treatments.

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