Insights from the First Genetic Evaluation of a Longitudinal Natural History Study in Classical Homocystinuria (HCU)

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RESULTS

- As of July 15, 2021, a total of 62 HCU patients were enrolled, of which 55 (89%) had tHcy measurement at enrollment (baseline visit), 51 (82%) had *CBS* genotype information, and 47 (76%) had both (**Figure 1, Table 1**)
- We identified 30 unique alleles in 53 patients screened for *CBS* variants (**Table 2**)
  - Two patients were negative for rare variants, and 2 patients were heterozygous for pathogenic variants, thus lacking biallelic confirmation of disease
- 50% (10 out of 20) of homozygous patients carried the Irish allele c.919G>A (p.Gly307Ser), consistent with enrollment design (**Table 2**)
- We identified three B6-responsive alleles: c.833T>C (p.Ile278Thr), c.1058C>T (p.Thr353Met), and c.1152G>C (p.Lys384Asn) (**Table 2**)

Figure 1. Patient Disposition

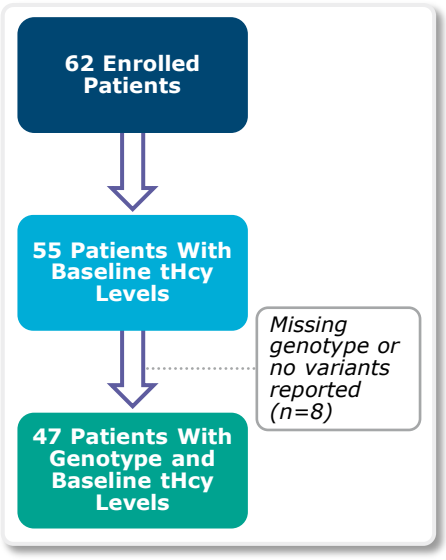


Table 1. Patient Baseline Demographics and Total Homocysteine Levels

Total available subjects, N (%)	62 (100)
Patient mean age, years (range)	22 (5-53)
Gender ratio M/F, n (%)	32/30 (52/48)
Baseline tHcy (µM)*, mean (SD)	109.6 (90.6)
Race, n (%)	
White	58 (94)
African American	3 (5)
Unknown	1 (1)
Ethnicity, n (%)	
Hispanic or Latino	6 (10)
Non-Hispanic	52 (84)
Unknown	4 (6)

*55 of the 62 patients enrolled had baseline tHcy levels.
SD, standard deviation; tHcy, total homocysteine.

Table 2. Alleles Identified in Our Cohort

DNA Change	Protein Change	Count	Homozygous
c.919G>A	p.Gly307Ser*	30	10
c.325T>C	p.Cys109Arg	7	1
c.833T>C	p.Ile278Thr†	7	0
c.1224-2A>C	p.?	5	2
c.536_553del	p.Asp179_Leu184del	5	0
c.738del	p.Lys247SerfsTer22	5	0
c.700G>A	p.Asp234Asn	4	2
c.829-78_1146-273delins469	p.?	4	2
c.1006C>T	p.Arg336Cys	3	0
c.1039G>A	p.Gly347Ser	3	0
c.1330G>A	p.Asp444Asn	3	1
c.1106G>C	p.Arg369Pro	2	1
c.361C>T	p.Arg121Cys	2	0
c.689del	p.Leu230ArgfsTer39	2	1
c.785C>T	p.Thr262Met	2	0
c.808_810del	p.Glu270del	2	0
c.1058C>T	p.Thr353Met	1	0
c.1126G>A	p.Asp376Asn	1	0
c.1136G>A	p.Arg379Gln	1	0
c.1152G>C	p.Lys384Asn	1	0
c.1339C>T	p.Pro447Ser	1	0
c.153_165del	p.Arg51SerfsTer27	1	0
c.209+1G>A	p.?	1	0
c.302T>C	p.Leu101Pro	1	0
c.362G>A	p.Arg121His	1	0
c.442G>A	p.Gly148Arg	1	0
c.488A>G	p.Tyr163Cys	1	0
c.624G>A	p.Trp208Ter	1	0
c.752T>A	p.Leu251Gln	1	0
c.770C>T	p.Thr257Met	1	0
Total		**100**	**20**

*High prevalence in Irish population. †High prevalence in Dutch, German, and Italian populations. These data include a few patients with no tHcy levels information. p.?, no information available on the predicted protein change.

- Most variants detected were missense, which is consistent with the literature (**Figure 2**)
- Most alleles identified were B6-nonresponsive based on the number of pyridoxine (non)responsive entries in LOVD and additional supporting literature information[3] (**Figure 3**)
- Patients were grouped based on features related to their *CBS* variants/genotype and compared to all remaining patients in order to identify significant differences in tHcy levels (**Table 3**)

Figure 2. Proportion of Variant Consequences Identified in Our Patient Cohort

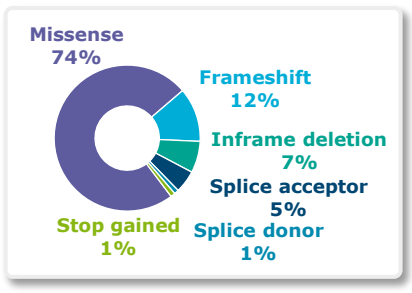


Figure 3. Allele B6-responsive Status[3]

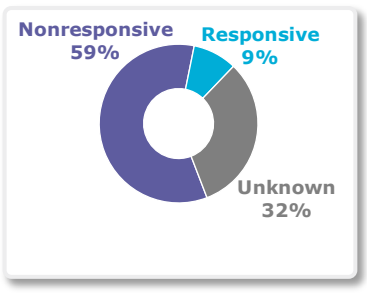
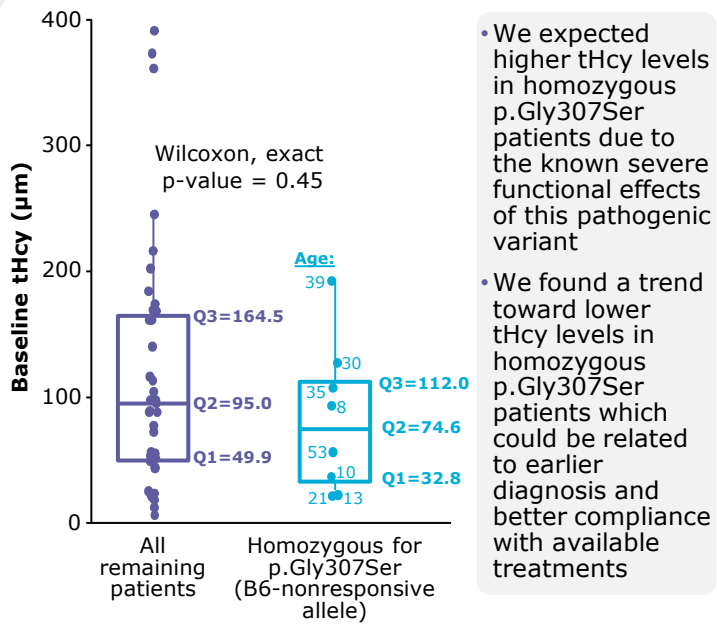


Table 3. Association of HCU Genotypes with tHcy Levels

Selected Genotypic Features	P-Value
Heterozygous for p.Ile278Thr B6-responsive variant	**0.0006**
Heterozygous for any B6-responsive variants*	**0.02**
Homozygous	ns
Homozygous for p.Gly307Ser	ns
Heterozygous for most prevalent variants†	ns
Homozygous for missense variants	ns
Homozygous for nonsense/frameshift	ns
Homozygous for pathogenic variants	ns
Homozygous for B6-nonresponsive	ns
Both variants in catalytic domain	ns

*p.Ile278Thr, p.Thr353Met, p.Lys384Asn. †Most prevalent includes p.Gly307Ser, p.Cys109Arg, p.Ile278Thr. ns, not significant (p>0.05). P-values from Wilcoxon rank sum one-sided exact tests.

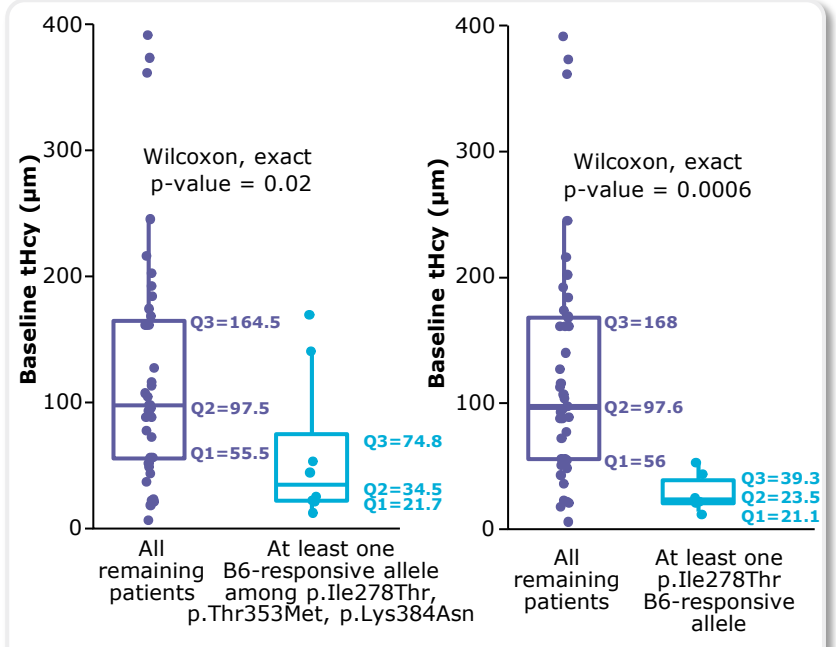
Figure 4. Homozygous p.Gly307Ser Are Not Associated With Higher Total Homocysteine Levels at Study Baseline Visit



- We expected higher tHcy levels in homozygous p.Gly307Ser patients due to the known severe functional effects of this pathogenic variant
- We found a trend toward lower tHcy levels in homozygous p.Gly307Ser patients which could be related to earlier diagnosis and better compliance with available treatments

P-value is from two-sided test. Numbers next to tHcy levels in homozygous patients indicate their age in years. Quartiles labeled as Q1, Q2, Q3.

Figure 5. B6-responsive Alleles are Associated With Lower Levels of Total Homocysteine



P-values are from one-sided tests.

CONCLUSIONS

- ✓ Two of the four most common pathogenic variants (p.Gly307Ser, p.Ile278Thr) were identified in our cohort
- ✓ The variant p.Cys109Arg, which is not commonly reported, was highly prevalent in our cohort
- ✓ High variability in tHcy levels was observed across patients and within the same genotype, including amongst patients homozygous for the Irish founder allele
- ✓ A trend toward lower tHcy levels was detected in homozygous p.Gly307Ser patients, possibly due to good compliance to SoC treatment
- ✓ Heterozygosity for the B6-responsive allele p.Ile278Thr was associated with lower tHcy levels, consistent with literature evidence

DISCLOSURES

EC: Investigator for Travere Therapeutics, Inc. **TP:** Employee and stockholder, Travere Therapeutics, Inc. **KAC:** Investigator for Travere Therapeutics, Inc. **YC:** Employee and stockholder, Travere Therapeutics, Inc. **CF:** Investigator for Travere Therapeutics, Inc. **HLevy:** Investigator and consultant, Travere Therapeutics, Inc. **JT:** Investigator for Travere Therapeutics, Inc. **SAV:** Employee and stockholder, Travere Therapeutics, Inc. **SR:** Employee and stockholder, Travere Therapeutics, Inc.

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1. Sacharow SJ, et al. Homocystinuria Caused by Cystathionine Beta-Synthase Deficiency. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. *GeneReviews®*. Seattle (WA) 1993. **2.** Weber Hoss GR, et al. *Mol Genet Genomic Med*. 2020;8(6):e1214. **3.** Leiden Open Variation Database (LOVD v.3.0, www.lovd.nl), updated as of October 12, 2021.

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INTRODUCTION

- Classical homocystinuria (HCU) is a slowly progressive rare autosomal recessive disorder caused by mutations in the *cystathionine β-synthase (CBS)* gene, which leads to elevated homocysteine in the body[1]
- HCU is characterized by developmental delay/intellectual disability, ectopia lentis and/or severe myopia, skeletal abnormalities (excessive height and length of the limbs), and thromboembolism[1]
- Most common pathogenic variants representing half of all HCU alleles reported worldwide are p.Ile278Thr, p.Gly307Ser, p.Thr191Met, and p.Trp323Ter[2]
  - p.Ile278Thr is an established B6-responsive allele
- Relationship between genotype and tHcy levels is not well-understood

Objective

- To describe the initial results of a genetic analysis in a cohort of HCU patients and the association of pathogenic *CBS* gene variants with tHcy levels

METHODS

- This natural history study is a prospective, longitudinal, multicenter, multinational assessment of disease severity in patients with HCU aged 5-65 conducted at 8 sites across the US, UK, and Ireland
- Each enrolled patient is being followed every 6 months over a period of 78-months (6.5-year) with a total of 14 visits
- Enrolled patients will have clinically documented diagnosed HCU based on the presence of elevated levels of total homocysteine and either enzymatic or genetic confirmation of HCU
- Non-parametric Wilcoxon rank sum exact tests were used to identify significant differences in tHcy levels between groups
- Statistical analyses and plots were done in R software environment v4.2.0

LIMITATIONS

- tHcy levels were not available at the time of diagnosis and pre-treatment and were measured on their first study or 'baseline' visit, thus most patients may have been already on an established SoC treatment (protein-restricted diet, B-vitamins, supplements, betaine), which could lead to lower tHcy levels
- Dietary compliance and use of concomitant medications such as betaine were not considered in this analysis and need further study
- There were no study sites outside of US, UK, and Ireland
- Limited sample size for a genetic association study

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