Platform Presentation #O06

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

Janet Thomas[1], Tiziano Pramparo[2], Kimberly A. Chapman[3], Ying Chen[2], Can Ficicioglu[4], Harvey Levy[5], Sagar A. Vaidya[2], Steve Rodems[2], Ellen Crushell[6]

[1]University of Colorado School of Medicine and The Children's Hospital of Colorado, Aurora, CO; [2]Travere Therapeutics, San Diego, CA; [3]Children's National Medical Center, Washington, DC; [4]The Children's Hospital of Philadelphia, Philadelphia, PA; [5]Harvard Medical School, Boston, MA and Boston Children's Hospital, Boston, MA; [6]Children's Health Ireland at Temple St, Dublin, Ireland

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TRAVERE THERAPEUTICS

DISCLOSURES AND ACKNOWELDGMENTS

Disclosures

- **JT:** 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
- **SAV:** Employee and stockholder, Travere Therapeutics, Inc
- **SR:** Employee and stockholder, Travere Therapeutics, Inc
- **EC:** Investigator for Travere Therapeutics, Inc

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INTRODUCTION

Introduction

- Classical homocystinuria (HCU) is a slowly progressive rare autosomal recessive disorder caused by pathogenic variants in the cystathionine β-synthase *(CBS)* gene, which leads to elevated homocysteine (Hcy) in the body[1]
- HCU is characterized by cognitive impairment, ectopia lentis and/or severe myopia, skeletal abnormalities (excessive height and length of the limbs), and thromboembolic vascular complications; current management focuses on controlling total Hcy (tHcy) levels[1]
- The most common pathogenic variants representing half of all HCU alleles reported worldwide are p.Ile278Thr, p.Gly307Ser, p.Thr191Met, and p.Trp323Ter[2]
  - Some patients with HCU may be responsive to pyridoxine (vitamin B6) and may be able to maintain normal tHcy levels through B6 therapy[1]
  - p.Ile278Thr is an established B6-responsive allele
- The relationship between genotype and tHcy levels is not well understood

Objective

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

1. Sacharow SJ, et al. Homocystinuria Caused by Cystathionine Beta-Synthase Deficiency. Updated May 18, 2017. In: Adam MP, et al, eds. *GeneReviews®*. University of Washington, Seattle; 1993-2023. Accessed February 2023. https://www.ncbi.nlm.nih.gov/books/NBK1524; **2.** Weber Hoss GR, et al. *Mol Genet Genomic Med.* 2020;8(6):e1214.

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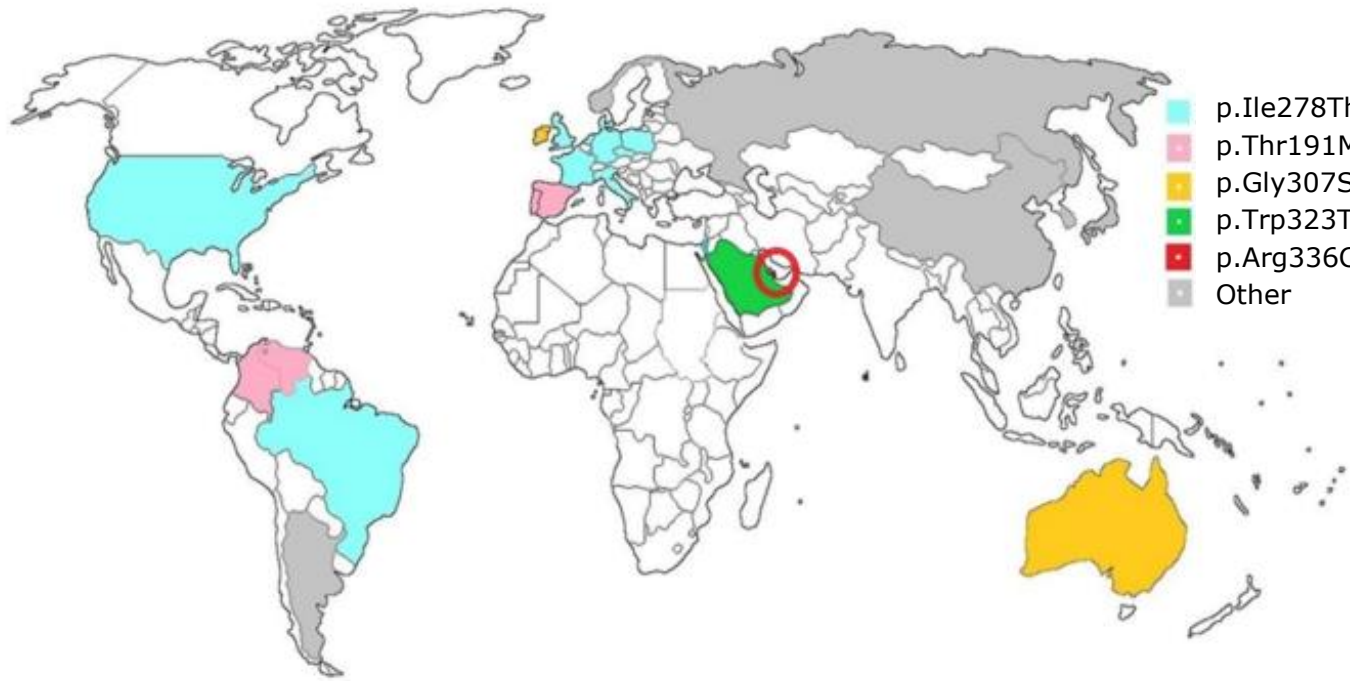
BACKGROUND

Common Pathogenic *CBS* Gene Alleles for HCU Differ Globally

- There is some variation present when looking at which pathogenic alleles of the *CBS* gene are most common geographically

Most Common Pathogenic *CBS* Alleles by Country



The most prevalent variant in the world (p.Ile278Thr) is the most common in The Netherlands (allelic frequency: 55%), Poland (36%), Germany (33%), England (29%), Italy (29%), Denmark (20%), Czech Republic and Slovakia (20%), USA (19%), Israel (18%), France (17%), and Brazil (16%). The variant p.Thr191Met is the most common in Colombia (73%), Spain (44%), Portugal (23%), and Venezuela (20%). In Ireland (66%) and Australia (22%) the most common variant is p.Gly307Ser. The variant p.Trp323Ter is the most common in Saudi Arabia (77%), and in Qatar (highlighted by the red circle) the most common variant is p.Arg336Cys (97%). Other prevalent mutations are c.700_702delGAC in Korea (20%), c.1224-2A>C in Russia (27%), p.Arg121His and p.Lys441Ter in Japan (16% each one), p.Arg125Gln in China (15%), p.Ala226Thr in Argentina (22%), and p.Arg266Lys in Norway (34%).

Figure from Weber Hoss GR, et al. *Mol Genet Genomic Med.* 2020;8(6):e1214.

CBS, cystathionine β-synthase; HCU, classical homocystinuria.
Weber Hoss GR, et al. *Mol Genet Genomic Med.* 2020;8(6):e1214.

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 years) with a total of 14 visits
- Enrolled patients will have clinically documented diagnosed HCU based on the presence of elevated levels of tHcy and either enzymatic and/or genetic confirmation of HCU
- Nonparametric 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

HCU, classical homocystinuria; tHcy, total homocysteine.

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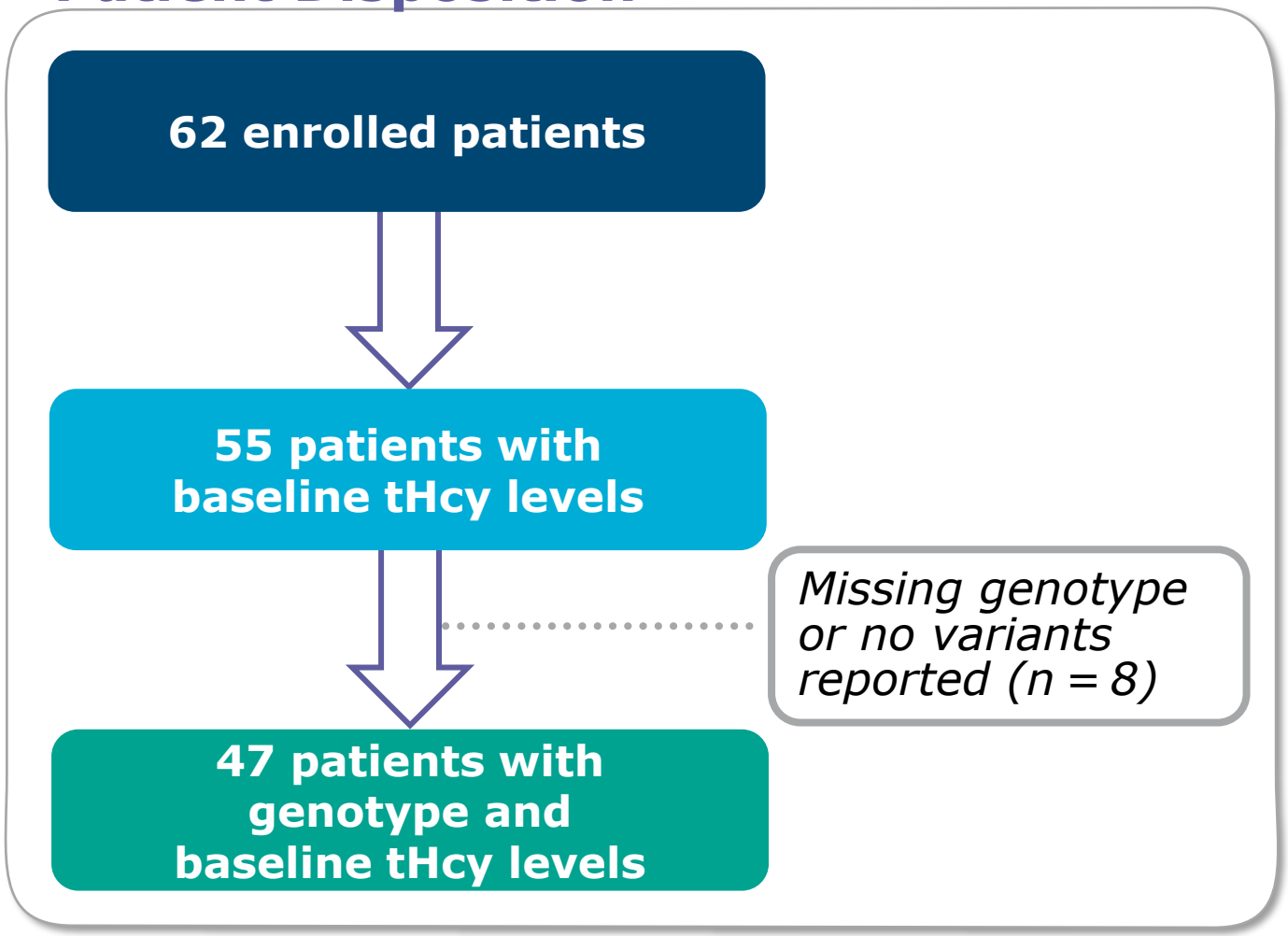
- As of July 15, 2021, a total of 62 patients with HCU were enrolled, of whom 55 (89%) had tHcy measurement at enrollment (baseline visit), 51 (82%) had *CBS* genotype information, and 47 (76%) had both

Patient Disposition



CBS, cystathionine β-synthase; HCU, classical homocystinuria; tHcy, total homocysteine.

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RESULTS

Patient Baseline Demographics and tHcy 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.
F, female; M, male; SD, standard deviation; tHcy, total homocysteine.

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RESULTS

Alleles Identified in Our Cohort

- We identified 30 unique alleles in 53 patients screened for *CBS* variants
  - Two patients were negative for rare variants, and 2 patients were heterozygous for pathogenic variants, thus lacking biallelic confirmation of disease
- Fifty percent (10/20) of homozygous patients carried the Irish allele c.919G>A (p.Gly307Ser), consistent with enrollment design
- We identified three B6-responsive alleles: c.833T>C (p.Ile278Thr), c.1058C>T (p.Thr353Met), and c.1152G>C (p.Lys384Asn)
- The variant p.Cys109Arg, which is not commonly reported, was highly prevalent in our cohort

*High prevalence in Irish population †High prevalence in Dutch, German, and Italian populations; these data include a few patients with no tHcy levels information.

CBS, cystathionine β-synthase; p.?, no information available on the predicted protein change; tHcy, total homocysteine.

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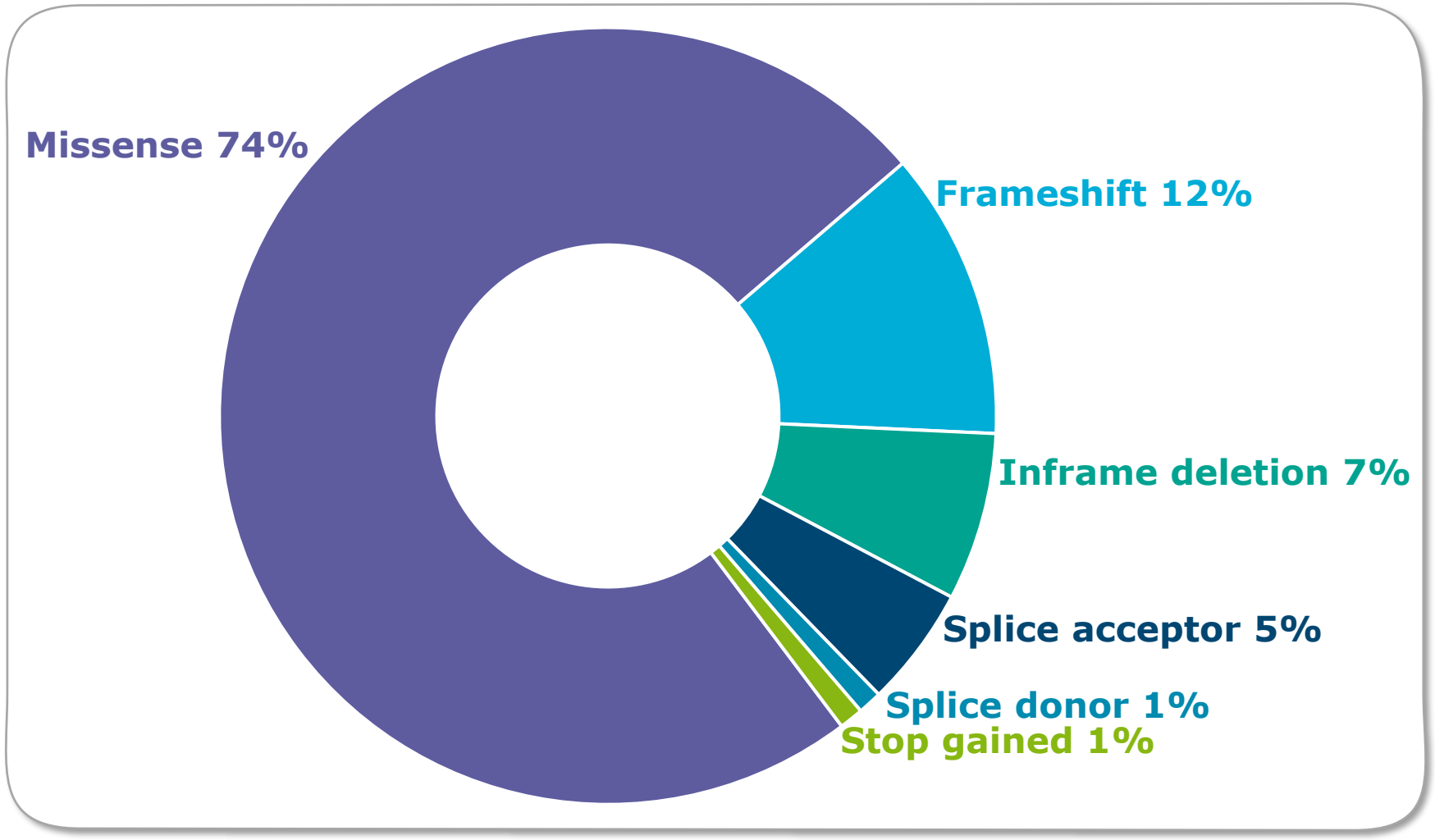
DNA change	Protein change	Count	Homozygous
c.919G>A	p.Gly307Ser*	30	10
c.325T>C	p.Cys109Arg	7	1
c.833T>C	p.Ile278Thr†	7	0
c.1224-2A>C	p.?	5	2
c.536_553del	p.Asp179_Leu184del	5	0
c.738del	p.Lys247SerfsTer22	5	0
c.700G>A	p.Asp234Asn	4	2
c.829-78_1146-273delins469	p.?	4	2
c.1006C>T	p.Arg336Cys	3	0
c.1039G>A	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**

RESULTS

Proportion of Variant Consequences Identified in Our Patient Cohort

Most variants detected were missense, which is consistent with the literature



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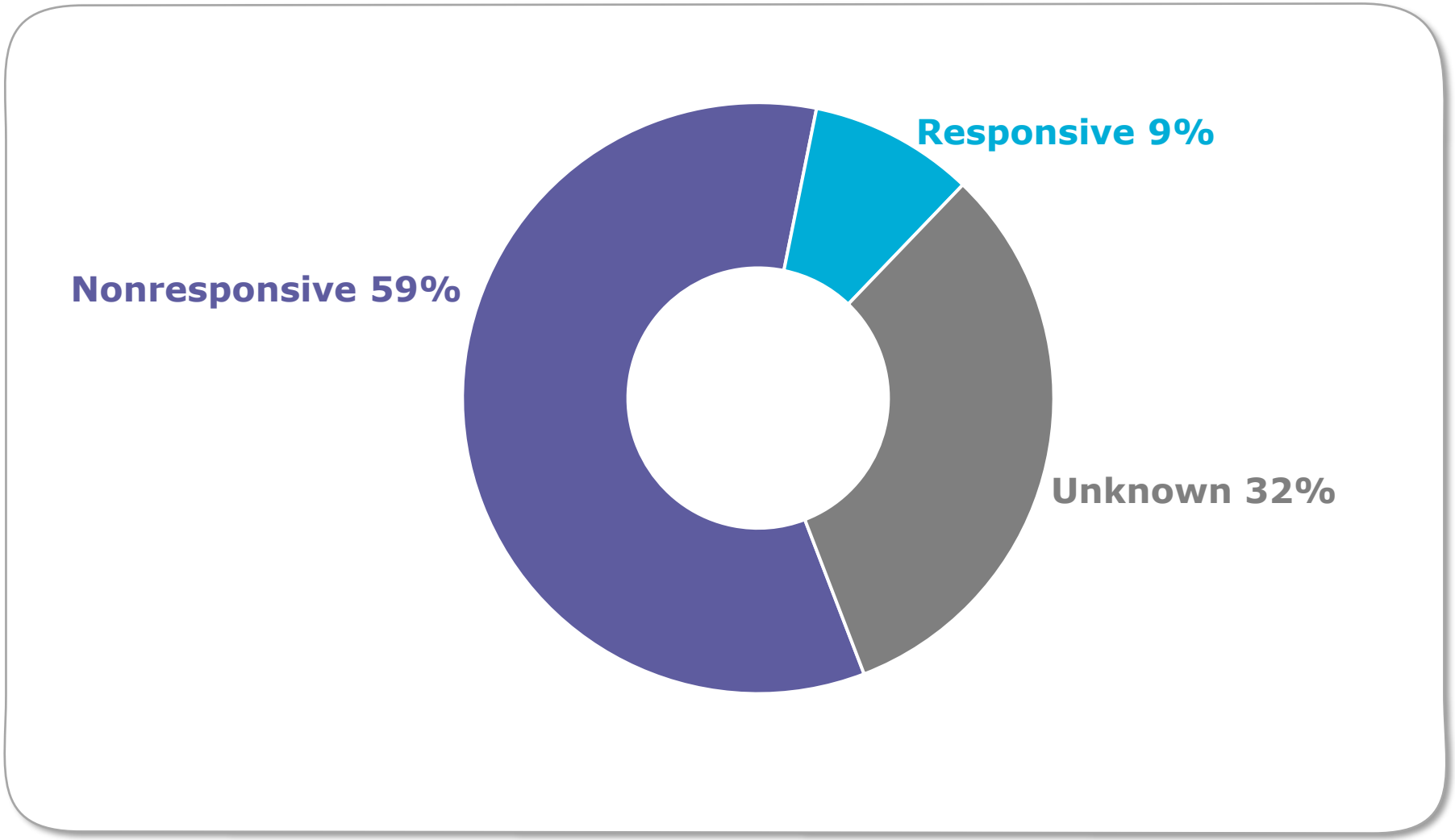
Allele B6-Responsive Status

Most alleles identified were B6-nonresponsive based on the number of pyridoxine (non)responsive entries in LOVD and additional supporting literature information



Leiden Open Variation Database (LOVD v.3.0, www.lovd.nl), updated as of October 12, 2021.

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RESULTS

Association of HCU Genotypes With tHcy Levels

Patients were grouped based on features related to their *CBS* variants/genotype and compared with all remaining patients in order to identify significant differences in 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. P-values from Wilcoxon rank sum one-sided exact tests.
CBS, cystathionine β-synthase; HCU, classical homocystinuria; ns, not significant ($p > 0.05$); tHcy, total homocysteine.

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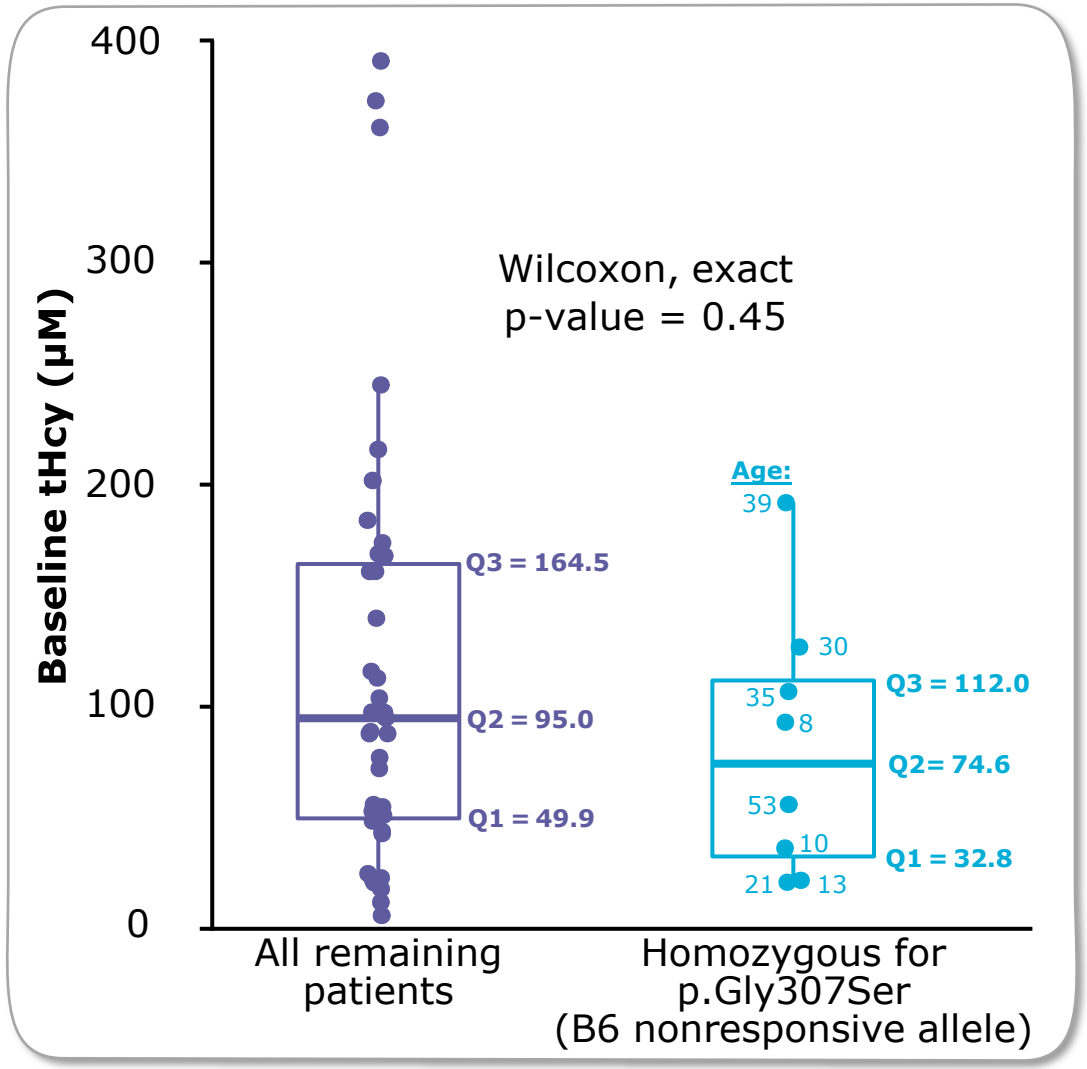
RESULTS

Homozygous p.Gly307Ser Are Not Associated With Higher tHcy 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.
tHcy, total homocysteine.

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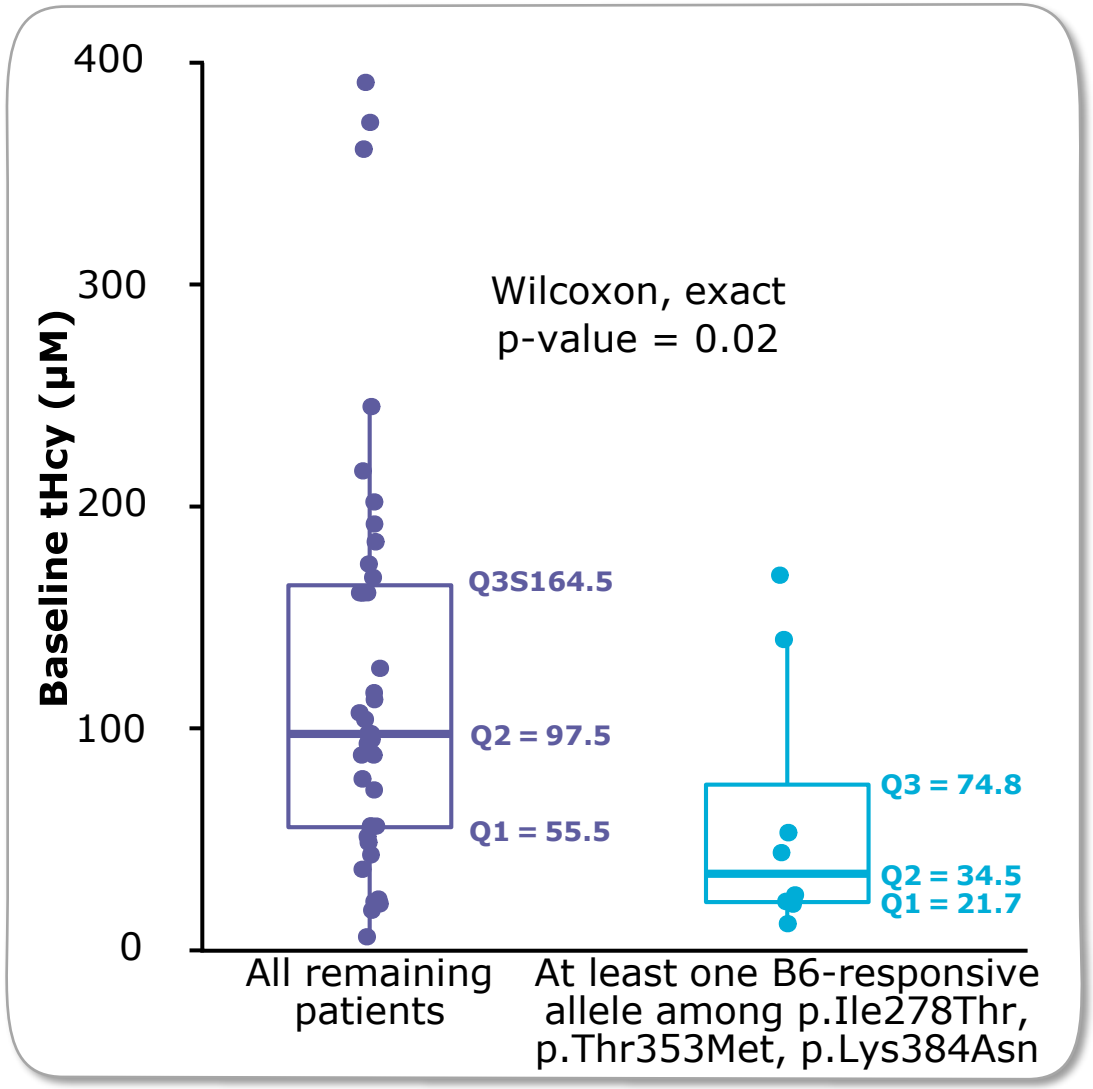
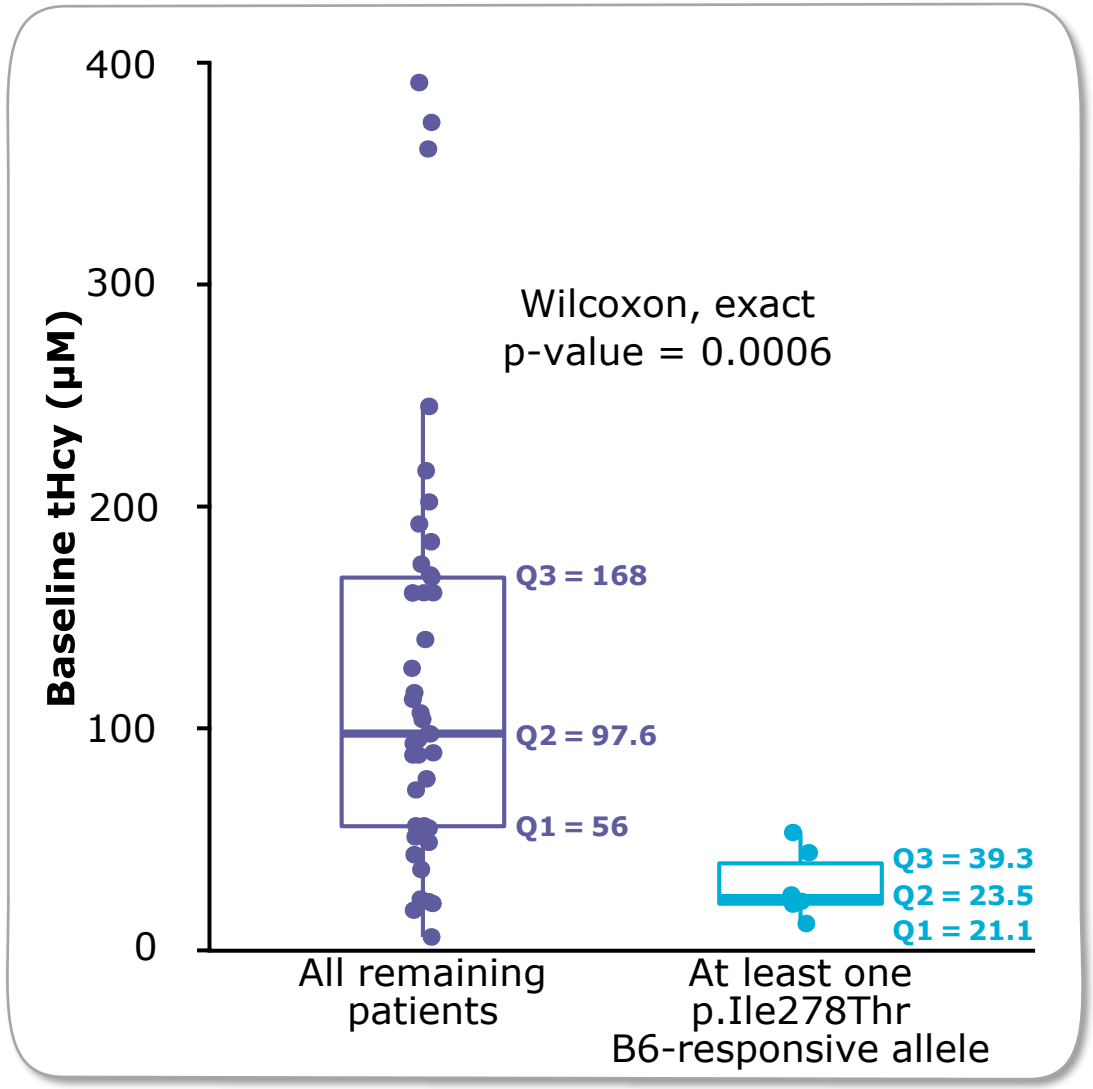
RESULTS

B6-Responsive Alleles Are Associated With Lower Levels of tHcy





P-values are from one-sided tests. Quartiles labeled as Q1, Q2, Q3.
tHcy, total homocysteine.

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LIMITATIONS

- tHcy levels were not available at the time of diagnosis and pretreatment, and were measured on their first study visit or ‘baseline’ visit
- Thus most patients may have already been 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 the US, UK, and Ireland
- Limited sample size for a genetic association study

SoC, standard of care; tHcy, total homocysteine.

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CONCLUSIONS

- Two of the four most common pathogenic *CBS* gene 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 with SoC treatment
- Heterozygosity for the B6-responsive allele p.Ile278Thr was associated with lower tHcy levels, consistent with literature evidence

CBS, cystathionine β-synthase; SoC, standard of care; tHcy, total homocysteine.

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Questions?

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