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ARG1 Variants in Arginase 1 Deficiency: Genetic Characterization of Participants in the Pegzilarginase Clinical Trials

Markey C. McNutt, MD, PhD[1]; Gillian Bubb, PhD[2]; Linda Neuman, MD[2]; Roberto T. Zori, MD[3]

[1]UT Southwestern Medical Center, Dallas, Texas; [2]Aeglea BioTherapeutics, Inc., Austin, Texas; [3]University of Florida, Gainesville, Florida

Background and Rationale

- Arginase 1 Deficiency (ARG1-D; OMIM 207800) is a rare autosomal recessive metabolic disorder caused by mutations in *ARG1* (HGNC 663) that impair enzymatic activity of arginase 1, thereby disrupting the cleavage of arginine to urea and ornithine in the final step of the urea cycle and resulting in accumulation of toxic levels of arginine[1,2]
  - Estimated prevalence is 1:726,000 (or 1.4 cases per million people) per a recent analysis of genetic population databases[3]
  - There are no approved therapies that effectively lower arginine levels. Current treatment relies on strict dietary protein restriction, which is inadequate to control plasma arginine and prevent deterioration over time[4-6]
- Persistent hyperargininemia in ARG1-D leads to progressive, debilitating neurologic manifestations including characteristic lower-limb spasticity as well as developmental delay, cognitive impairment, and seizures. Hyperammonemia may also occur, but is usually less common and less severe compared with other urea cycle disorders[1,2,4,7-10]
  - Manifestations generally develop in early childhood after a normal neonatal/infancy period
  - For reasons that remain to be elucidated, there is significant variability in age of onset, sequence of manifestations, rate of progression, and severity[1]
- More than 60 *ARG1* variants have been reported to date, and additional variants may exist. Understanding the potential relationships between genotype and clinical characteristics in ARG1-D may improve the understanding of this rare disease[9]
- Pegzilarginase is a novel investigational recombinant human enzyme therapy in development for ARG1-D that has demonstrated substantial arginine reduction in Phase 1/2[11] and pivotal Phase 3[12,13] clinical trials. The pegzilarginase clinical development program provided the opportunity to investigate *ARG1* variants in 2 cohorts of well-characterized patients with confirmed ARG1-D
- **The objective of this work was to assess the pegzilarginase trial population for any potential genotype/phenotype correlations**

Methods

Trial Designs

- Both clinical trials have been described in detail elsewhere
  - Phase 1/2 (NCT02488044 and NCT03378531) is a single-arm open-label study that comprised ascending- and repeated-dose periods and a long-term extension[11,14]
  - Phase 3 (NCT03921541; PEACE [Pegzilarginase Effect on Arginase 1 Deficiency Clinical Endpoints]) is a pivotal randomized double-blind, placebo-controlled trial with an ongoing long-term extension in which all patients receive open-label pegzilarginase[12,13,15]
- At baseline, patients were ≥2 years age and had a documented diagnosis of ARG1-D and elevated plasma arginine levels (on standard of care); PEACE inclusion criteria also required impairment on ≥1 clinical assessment
- In both trials, pegzilarginase is administered weekly in addition to patients' individualized standard-of-care disease management regimen

Data Collection and Assessment

- Population- and patient-level demographics, clinical characteristics, and *ARG1* variant data were summarized using descriptive statistics
- Data were assessed overall and by individual *ARG1* variant to identify potential relationships between disease characteristics and response to pegzilarginase after 24 weeks of treatment

Results

Patients

- A total of 48 patients were enrolled in the Phase 1/2 and Phase 3 clinical trials (**Table 1**)
- Patients were racially and ethnically diverse, with a broad age range (2–31 years at enrollment)
- Plasma arginine was markedly elevated at baseline despite dietary protein restriction (202–573 µmol/L)
- Substantial disease burden was evident, with most patients presenting with lower-limb spasticity and developmental delay or cognitive impairment. History of abnormal transaminases and/or hyperammonemia was also common
- Patients were heterogenous with regard to clinical characteristics
  - Age at onset of first manifestations was variable, ranging from 1–18 years
  - Clinical profiles were consistent with the understanding of the natural history of ARG1-D, with development and severity of key manifestations being variable from patient to patient

Table 1. **Key Demographics and Baseline Characteristics of Pegzilarginase Trial Patients**

Patient Information	Phase 1/2 (N=16)*	Phase 3 (N=32)*
Age at enrollment, y		
Mean ± SD	15.1±8.5	10.7±6.5
Median (range)	15.0 (5–31)	10.5 (2–29)
Sex, n (%)		
Male	5 (31.3)	19 (59.4)
Female	11 (68.8)	13 (40.6)
Race, n (%)		
White/Caucasian	11 (68.8)	14 (43.8)
Asian	2 (12.5)	6 (18.8)
Black/African	1 (6.3)	2 (6.3)
Multiple race	0	2 (6.3)
Other	2 (12.5)	6 (18.8)
Missing	0	2 (6.3)
Ethnicity, n (%)		
Hispanic or Latino	9 (56.3)	9 (28.1)
Not Hispanic or Latino	7 (43.8)	23 (71.9)
Age at initial manifestations, y		
Mean ± SD	10.8±5.8	3.0±2.4
Median (range)	7.1 (6–18)	2.0 (1–10)
Weight at baseline, kg		
Mean ± SD	28.4±15.7	33.4±16.0
Median (range)	20.6 (19–59)	31.0 (12–76)
Plasma arginine at baseline, µmol/L		
Mean ± SD	373.4±91.3	402.0±101.8
Median (range)	389.3 (238–566)	398.2 (202–573)
Key clinical characteristics, n (%)		
Lower-limb spasticity	12 (75.0)	21 (65.6)
Moderate to severe	9 (56.3)	12 (37.5)
Developmental delay or cognitive impairment	9 (56.3)	22 (68.8)
History of seizures	4 (25.0)	11 (34.4)
History of abnormal ALT and/or AST levels	9 (56.3)	22 (68.8)
History of hyperammonemia	7 (43.4)	18 (56.3)

ALT, alanine aminotransferase; AST, aspartate aminotransferase. *Cohorts represent distinct patient groups; no patients from Phase 1/2 were enrolled in the Phase 3 trial.

ARG1 Variants

- Patients were heterogeneous with regard to genotype (**Table 2**)
- A total of 37 unique *ARG1* variants were identified in the 47 patients with genotype data available (homozygous, 37 patients; compound heterozygous, 10 patients)
  - 23 unique variants were identified in homozygous patients and 14 were identified in compound heterozygous patients
  - 18 variants (in 29 patients) have been previously described in the literature and 19 are novel
  - Most variants (78.4%; n=29/37) were observed in single patients (homozygous, 15 patients; compound heterozygous, 7 patients)
- The 3 most common variants were c.466-1G>C (9 patients), c.61C>T (4 patients), and a novel variant, c.314_345delins20 (4 patients); all 3 were identified in both homozygous and compound heterozygous patients

Table 2. ***ARG1* Variants Identified in Pegzilarginase Trial Patients**

Known Variants		Novel Variants	
NM_000045.4(ARG1):	Total Patients, n (Homozygous/ Heterozygous)	NM_000045.4(ARG1):	Total Patients, n (Homozygous/ Heterozygous)
c.466-1G>C	9 (6 / 3)	c.314_345delins20	4 (3 / 1)
c.61C>T	4 (2 / 2)	c.749G>A	2 (2 / 0)
c.23T>A	3 (3 / 0)	c.23T>G	1 (1 / 0)
c.[703G>A; 708_712dup]	2 (2 / 0)	c.272-273insG	1 (1 / 0)
c.849delG	2 (2 / 0)	c.370G>T	1 (1 / 0)
c.93del	2 (2 / 0)	c.371A>G	1 (1 / 0)
c.[646_649del]	1 (1 / 0)	c.532G>C	1 (1 / 0)
c.130+1G>A	1 (1 / 0)	c.561-2A>T	1 (1 / 0)
c.425G>A	1 (1 / 0)	c.769G>C	1 (1 / 0)
c.695A>T	1 (1 / 0)	c.888del	1 (1 / 0)
c.700G>C	1 (1 / 0)	c.807_811delACTCT	1 (0 / 1)
c.802+2T>G	1 (1 / 0)	c.468-1G>C	1 (0 / 1)
c.892G>C	1 (1 / 0)	c.3G>A	1 (0 / 1)
c.603_604delTG	1 (0 / 1)	c.684delT	1 (0 / 1)
c.709G>A	1 (0 / 1)	c.611A>G	1 (0 / 1)
c.647_648ins32	1 (0 / 1)	c.787G>T	1 (0 / 1)
c.871C>T	1 (0 / 1)	c.92T>G	1 (0 / 1)
c.57+1G>A	1 (0 / 1)	c.683A>G	1 (0 / 1)
		c.151G>T	1 (0 / 1)

Phenotype/Genotype Variability

- No clear relationship between ARG1-D manifestations or severity and genotype was evident in the full cohort or for specific *ARG1* variants
- Among the subgroup of patients sharing the most common *ARG1* variant (c.466-1G>C):
  - All 9 patients reported their race and ethnicity as White and Hispanic/ Latino; they represented both sexes and a broad age range (2–31 years)
  - 6 patients were homozygous and 3 were heterozygous. In heterozygous patients, co-occurring variants were:
    - c.603_604delTG
    - c.314_345delins20
    - c.61C>T
  - Overall disease characteristics were generally consistent with the full cohort of 48 patients (**Table 3**)

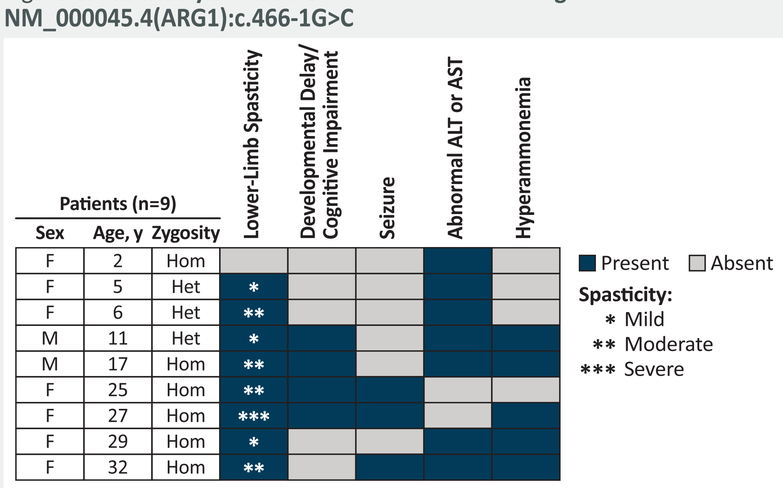
Table 3. **Comparison of Key Disease Characteristics Among Patients With NM_000045.4(ARG1):c.466-1G>C vs All Pegzilarginase Trial Patients**

Key Characteristics	c.466-1G>C (n=9)	Combined Cohort (n=48)*
Age range, y	2–31	2–31
Age range at onset of manifestations, y	1–18	1–18
Baseline plasma arginine range, µmol/L	243–446	202–573
Lower-limb spasticity, n (%)	8 (88.9)	33 (68.8)
Moderate to severe	5 (55.6)	21 (43.8)
Developmental delay or cognitive impairment, n (%)	4 (44.4)†	31 (64.6)
History of seizures, n (%)	3 (33.3)	15 (31.3)
History of abnormal ALT and/or AST levels, n (%)	7 (77.8)	31 (64.6)
History of hyperammonemia, n (%)	5 (55.6)	25 (52.1)

ALT, alanine aminotransferase; AST, aspartate aminotransferase. *Represents 37 unique *ARG1* variants among 47 patients; 1 patient did not have genotype information available. †One additional patient with the c.466-1G>C variant had notable speech delay but was not reported as having developmental delay.

- Similar to the heterogeneity observed in the genotypically diverse cohort, ARG1-D manifestations in the c.466-1G>C subgroup differed from patient to patient without a clear pattern (**Figure 1**)
  - Although the literature in this rare disease is limited, this observation is consistent with published case reports of patients with the c.466-1G>C variant that describe:
    - A patient (compound heterozygous with c.603_604delTG) identified through newborn screening and who remained asymptomatic through ≥2 years of follow-up[10]
    - A patient (homozygous) who presented at 11 years of age with learning difficulties, lower-limb spasticity, recurrent hyperammonemia, and no evidence of seizures[16]

Figure 1. **Variability of ARG1-D Manifestations Among Patients With NM_000045.4(ARG1):c.466-1G>C**

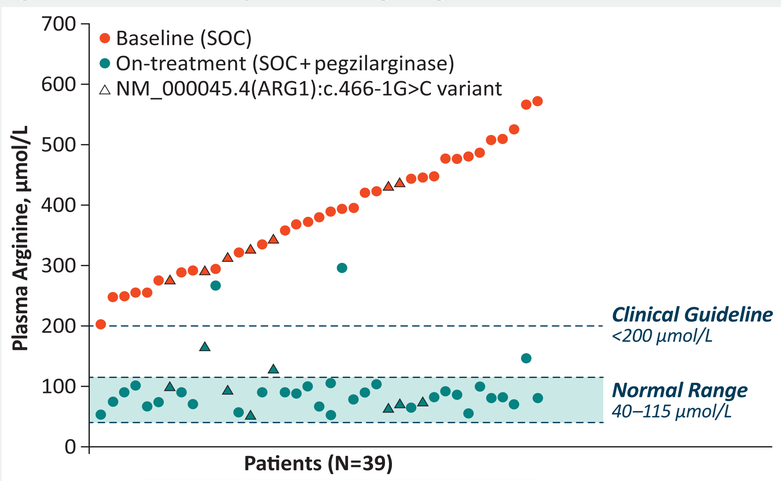


ALT, alanine aminotransferase; AST, aspartate aminotransferase; F, female; Het, heterozygous; Hom, homozygous; M, male.

Response to Pegzilarginase

- Among patients with genotype and plasma arginine data after 24 weeks of pegzilarginase (**Figure 2**):
  - Mean plasma arginine was markedly elevated at baseline, at 2-fold guideline-recommended levels and 3.5-fold the upper limit of normal
  - Pegzilarginase normalized mean plasma arginine levels; 90% of patients were not only within the guideline-recommended range but also below the upper limit of normal
- A broad range of genotypes was represented, with 31 *ARG1* variants among 39 patients (homozygous, 31 patients; compound heterozygous, 8 patients)
- On-treatment arginine levels were consistent regardless of *ARG1* variant and baseline plasma arginine, and there was no relationship between *ARG1* variant and arginine-lowering effect of pegzilarginase

Figure 2. **Individual Responses to Pegzilarginase at 24 Weeks**



pArg, µmol/L	Baseline	24 Weeks
Mean ± SD	381.5 ± 96.5	96.1 ± 49.5
Median (range)	380.3 (202–670)	86.4 (53–296)

pArg, plasma arginine; SOC, standard of care.

Conclusions

- Consistent with the literature and current understanding of ARG1-D, disease manifestations and severity were heterogeneous among participants of the pegzilarginase clinical trials, without a clear phenotype/genotype relationship. The arginine-lowering effect of pegzilarginase was observed across patients regardless of *ARG1* variant and clinical presentation
  - Patients were representative of the broader ARG1-D population and reflected diverse clinical presentations and genotypes
  - Genotypes included both known and novel *ARG1* variants; 19 of the 37 unique variants identified in these patients have not been previously described
  - This work characterized one of the largest cohorts of patients with this rare inherited metabolic disorder (48 patients across 2 clinical trials); however, the low numbers of patients overall or with a specific genotype limit the strength of conclusions that can be drawn from our findings

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