Arginase 1 deficiency presenting as complicated hereditary spastic paraplegia.

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ABSTRACT

Introduction: Argininemia or arginase deficiency is a metabolic disorder caused by pathogenic variants in *ARG1* and consists of a variable association of progressive spastic paraplegia, intellectual disability, and seizures. Hereditary spastic paraplegia (HSP) is a group of inherited diseases whose main feature is a progressive gait disorder characterized by lower limb spasticity. This study presents 7 patients with arginase 1 deficiency from 6 different families, all with an initial diagnosis of complicated HSP.
Methods: We evaluated the clinical data of 7 patients belonging to six independent families who were diagnosed with hyperargininemia in a neurogenetics outpatient clinic.
Results: All patients had lower limb spasticity and six had global developmental delay. Five individuals had intellectual disability and two had epilepsy. Psychiatric abnormalities were seen in two patients. In two participants of this study, MRI disclosed thinning of the corpus callosum. Molecular diagnosis was made by whole exome sequencing. All variants were present in homozygosis; we identified two novel missense variants, one novel frameshift variant, and one previously published missense variant.
Discussion: Clinical diagnosis of early onset complicated hereditary spastic paraplegia was made in all patients. Two patients were initially suspected of having SPG11 due to thinning of the corpus callosum. As argininemia may present with a highly penetrant phenotype of spastic paraplegia associated with additional symptoms, this disease may represent a specific entity amongst the complicated HSPs.

INTRODUCTION

Argininemia or arginase 1 deficiency (OMIM #207800) is a metabolic disorder caused by pathogenic variants in *ARG1*. Arginase 1 catalyzes the final step in the urea cycle and presents clinically with episodic hyperammonemia of variable degrees that can be severe enough to cause encephalopathy or death (Iyer et al. 1998). Individuals with untreated pathogenic variants in *ARG1* commonly have slowing of linear growth at the age of one to around three years, followed by spasticity in lower limbs, plateauing of cognitive development, and subsequent loss of developmental milestones, progressing to loss of ambulation, complete loss of bowel and bladder control, severe intellectual disability and, often, seizures. This rare inborn error of urea cycle has an estimated incidence of 1:950,000 live births (Summar et al. 2013).

Hereditary spastic paraplegia (HSP) is a group of inherited diseases whose main feature is a progressive gait disorder characterized by lower limb spasticity. This heterogeneous group of diseases is commonly divided in pure or complicated HSPs. Complicated HSP involves spasticity and other symptoms such as ataxia, cognitive impairment, movement disorders or other neurologic syndromes.

This study presents 7 patients with arginase 1 deficiency from 6 different families. In all cases, the initial diagnosis was complicated HSP.

METHODS

We reviewed the clinical data of 7 patients who were diagnosed with arginase deficiency in a Neurogenetics Outpatient Clinic. The local ethics committee approved the study and written informed consent was obtained from all families. Metabolic tests, including tandem mass spectrometry for amino acids, were performed on 3 patients, and diagnosis was established by the finding of biallelic pathogenic variants in *ARG1* (using as reference the transcript ENST00000368087) by whole-exome sequencing (WES), performed by UCL Institute of Neurology, Queen Square, London or by Mendelics Analise Genômica, São Paulo, Brasil.

RESULTS

The main clinical findings of the 7 patients are summarized in table 1 and the main genetic results are disclosed in table 2.

All patients were born from consanguineous parents. There was only one male patient in this series. The age of onset ranged from one to seven years. All patients had lower limb spasticity. Six patients had global developmental delay. The only individual without developmental delay (patient II) had a later onset of symptoms at 7 years of age. Five patients had intellectual disability and only two patients had epilepsy. Two patients had prominent neuropsychiatric features, presenting as social withdrawal. Only one patient had recurrent vomiting.

Brain MRI was normal in four patients. Two patients had thinning of the corpus callosum, one of them also presented some T2-hyperintensity and T1-hypointensity in the forceps minor of the corpus callosum, resembling the “ears of the lynx sign”, but changes were milder than it is commonly described in the classic presentation of this sign (Figure 1). One patient had periventricular white matter

hyperintensities (see Patient VI, below). Serum ammonia levels were mildly elevated in two patients. One patient (I) had demyelinating polyneuropathy, and one (III-1) had axonal polyneuropathy.

We identified one novel missense variant, one novel frameshift variant and two previously published missense variants. All patients were homozygous for missense variants in *ARG1*, except patient IV, who was homozygous for a deletion that led to a frameshift mutation.

The novel missense variant in *ARG1* (ENST00000368087, c.3G>A; p.Met1?) is rare in population databases (GnomAD and Abraom), occurred in a position of maximal conservation across all species and is predicted to be pathogenic by two *in silico* models (view table 2). This variant, which leads to a loss of the translation start codon, was present in one patient with a clinical picture compatible with arginase deficiency.

The novel frameshift variant (c.646_649delCTCA; p.Leu216Alafs*4) found in patient V has never been reported in the literature. This variant is very rare in population databases (GnomAD and Abraom) and it is predicted to be pathogenic by *in silico* analysis, leading to premature termination of translation.

Patient I

A 27-year-old man, born from consanguineous parents, developed social withdrawal and frequent falls due to progressive lower limb spasticity when he was 4 years old. At age of 5, he presented cognitive impairment and astatic seizures. He has been wheelchair-bound since the age of 20. Brain MRI, performed at the age of 16, disclosed thinning of the corpus callosum, especially the genu, T2-hyperintensity and T1-hypointensity in the forceps minor of the corpus callosum, resembling a milder form of the “ears of the lynx” sign (Figure 1), which raised the suspicion of SPG11. His serum ammonia level, measured at the age of 16, was 86 μmol/L (NR: 11-32 μmol/L). Arginine serum levels were not measured. WES identified a homozygous *missense* variant in *ARG1* (c.404C>T; p.Thr134Ile), previously reported (Huemer *et al*. 2016) and submitted to ClinVar (variation/802268) by our group. This patient was treated only with dietary protein restriction since the age of 18 and had no significant improvement after treatment.

Patient II

A 34-year-old woman, born from consanguineous parents, developed spastic gait from the age of 7, with slowly progressive tiptoe walking. There was also slowly progressive cognitive impairment. At the age of 14 an orthopedic surgery for tendon stretching was performed. She became wheelchair-bound at age 23. Her brain MRI performed at the age of 16 disclosed thinning of the corpus callosum and SPG11 was suspected (no image available). Serum ammonia level was normal (24μmol/) at the age of 33 and arginine serum levels were not measured. WES identified a homozygous *missense* variant in *ARG1* (c.3G>A; p.Met1?). The patient was treated with protein restriction since the age of 33 with no significant clinical change after treatment.

Patients III-1 and III-2

Patient III-1 was a 46-year-old woman with parental consanguinity. At the age of 1 she was noted to have frequent falls and delayed language ability, followed by progressive lower limbs spasticity, and she became wheelchair-bound by the age 14 . Her serum ammonia levels were normal (28 μmol/L - NR: 11-32 μmol/L). Her sister, patient III-2 was a 30-year-old woman who first presented frequent falls and cognitive slowing at the age of 3 years. Progression of gait impairment and spasticity was slow, and she was wheelchair-bound by the age of 20. Her serum ammonia levels were normal (30μmol/L - NR: 11-32 μmol/L). The clinical suspicion was of a complicated phenotype of HSP. In both cases, arginine serum levels were never measured. Both sisters underwent WES and had the same *missense* homozygous variant in *ARG1* (c.404C>T; p.Thr135Ile) found in case 1. They were treated with protein restriction and had no significant improvement after treatment.

Patient IV

A 16-year-old female with parental consanguinity and early history of frequent vomiting and global developmental delay in her first year of life, as well as a spastic gait with frequent falls. At the age of 5, she presented epileptic seizures. As she had persistent liver enzyme abnormalities, a liver biopsy was performed and evidenced glycogen deposition. Blood amino acid determination by tandem mass spectrometry in Guthrie paper showed elevated blood arginine (performed at the age of 12), ammonia levels were not measured before treatment. WES found a novel *frameshift* homozygous variant in *ARG1* (c.646_649delCTCA; p.Leu216Alafs*4). This patient was treated with protein restriction and sodium benzoate 20% (85mg/kg/day) since the age of 12 and had resolution of recurrent vomiting, ammonia levels under 35μmol/L (NR: 11-32 μmol/L) and no further clinical worsening or complications.

Patient V

An 11-year-old girl, with parental consanguinity, had a history of tiptoe walking and frequent falls that began at the age of 3, progressing with bilateral spasticity. She also had elevated liver enzymes and arginine levels 2 to 3 times above the normal upper limit. Her serum ammonia levels were 37 μmol/L (NR: 11-32 μmol/L) at the age of 5. WES found a homozygous *missense* variant in *ARG1* (c.923G>A; p.Arg308Gln). This variant has already been reported (Carvalo *et al*. 2012; Giovani *et al*, 2021). This patient was treated with protein restriction since the age of 5 with stability of spasticiy and no further complications. Sodium benzoate 20% (85mg/kg/day) was started but had to be discontinued due to epigastralgia.

Patient VI

A 4-year-old girl with parental consanguinity, born after premature labor at 29 weeks of pregnancy, with several neonatal complications. She had global developmental delay and had received a diagnosis of cerebral palsy. At the age of 9 months, she developed repetitive vomiting. At the age of 3 years, she presented with epileptic seizures. In diagnostic evaluation, we found high transaminases and amino acid chromatography showed elevated serum arginine (895 at the age of 1 year and 4 months and 141,2 at the age of 2 years and 7 months - NR: 30-250 μmol/L). She underwent a liver biopsy that evidenced glycogen deposition. Her serum ammonia levels were 42 μmol/L (NR: 11-32

μmol/L) at the age of 2. Her brain MRI, performed at the age of one, disclosed periventricular leukomalacia. WES found the same *missense* homozygous variant in *ARG1* found in case V

(c.923G>A; p.Arg308Gln). This patient was treated with protein restriction since the age of 2 and had normalization of serum ammonia levels and no further complications.

Table 1 – Clinical and paraclinical findings in seven patients with argininemia

Patient	I	II	III-1	III-2	IV	V	VI
Gender	M	F	F	F	F	F	F
Age of onset *	4	7	1	3	1	3	1
Developmental delay	+	-	+	+	+	+	+
Intellectual disability	+	+	+	+	+	-	-
Spasticity	+	+	+	+	+	+	+
Epilepsy	-	-	-	-	+	-	+
Other clinical features	Social isolation	-	-	-	vomiting	-	-
Brain MRI	CC thinning	CC thinning	nl	nl	nl	nl	Periventricular hyperintensities
Ammonia levels (μmol/L)**	86	24	29,5	30	28	45,7	22,7
Arginine levels	NP	NP	NP	NP	Elevated	Elevated	895
Polyneuropathy	Demyelinating	-	Axonal	-	-	-	-

CC : Corpus callosum, MRI : Magnetic Resonance Imaging (MRI)
*Measured by years
** Reference values: 11-32 μmol/L

Table 2 – Summary of variants, pathogenicity prediction scores, previous reports and ACMG classification

Patient	I	II	III-1	III-2	IV	V	VI
cDNA change	c.404C>T	c.3G>A	c.404C>T	c.404C>T	c.646_649delCTCA	c.923G>A	c.923G>A
Protein change	p.Thr134Ile	p.Met1Ile	p.Thr134Ile	p.Thr134Ile	p.Leu216Alafs*4	p.Arg308Gln	p.Arg308Gln
Gnomad_exome	0	2	0	0	1	2	2
Gnomad_genome	0	3	0	0	1	3	3
Polyphen2_HVAR_pred	0,420 (Benign)	0,00 (Benign)	0,420 (Benign)	0,420 (Benign)	Probably Damaging (0,999)	Probably Damaging (1,00)	Probably Damaging (1,00)
Mutation Taster_score	Disease Causing	Disease Causing	Disease Causing	Disease Causing	Disease Causing	Disease Causing	Disease Causing
PROVEAN_pred	Deleterious (-4,555)	Neutral (-1,164)	Deleterious (-4,555)	Deleterious (-4,555)	Deleterious (-10,155)	Deleterious (-2.580)	Deleterious (-2.580)
CADD_phred	23,1	23,6	23,1	23,1	32	27,6	27,6
Abraom_freq	0	0	0	0	0	3	3
Previously published?	Huemer, 2016	no	Huemer, 2016	Huemer, 2016	no	Carvalho, 2012	Carvalho, 2012
ACMG classification	Likely Pathogenic	Likely Pathogenic	Likely Pathogenic	Likely Pathogenic	Pathogenic	Pathogenic	Pathogenic

Genome reference = GRCh38
Transcrpt = ENST00000368087

DISCUSSION

Arginase deficiency is a rare urea cycle disorder (UCD) which main clinical symptom is progressive spastic paraplegia that begins in the first decade of life. The disease becomes symptomatic during early childhood and is characterized by progressive spasticity predominantly in the lower limbs, progressive mental impairment, growth retardation, and periodic episodes of hyperammonemia.

In this series of arginase deficiency, the clinical picture of all patients was fairly typical, with spastic paraparesis beginning before 7 years of age. All patients presented with at least one additional clinical feature – namely, intellectual disability, developmental delay, or epilepsy – that allowed their classification as complicated hereditary spastic paraplegia. Seizures occurred in 3 out of 7 patients, and psychiatric symptoms in 2 patients.

All patients in this series were treated with dietary protein restriction and only one (patient IV) with sodium benzoate. Patients who started treatment early had clinical improvement or stabilization and improvement in ammonia levels. Conversely, patients who started treatment in late adolescence or adulthood had no significant clinical response, although the results from this case series are not adequate to assess a significant correlation between age at treatment start and prognosis.

In contrast to other disorders of the urea cycle, arginase deficiency may not be associated with hyperammonemic encephalopathy in the neonatal period (Cederbaum et al. 2004). While 5 out of 7 patients in this study had normal ammonia levels, 2 patients had levels slightly higher than normal. Ammonia levels are variable in argininemia and usually not as high as in other urea cycle defects (Scaglia et al. 2006). The fact that we have performed few measurements of ammonia and most of them in a late stage of disease may explain why we have found normal levels in most patients.

Diagnosis was probably delayed in many cases in this series due to limitations in access to biochemical tests in remote locations of a continental and underdeveloped country like Brazil. Additionally, test performance of plasma amino acids analysis is quite variable, as for instance thin layer chromatography is still in practice in some parts of the country, giving the false assurance that amino acids result is normal. This scenario is changing with introduction of new and more reliable techniques, as tandem mass spectrometry, which is sensitive for arginine detection and is becoming increasingly available, and more recently, with the expansion of next generation sequencing as a primary and convenient way to investigate genetic disorders. So, late diagnosis can be explained by both lack of access caused by geographic isolation and lack of lab resources for a correct diagnosis, a situation that probably is common in many parts of the world, particularly in underdeveloped countries. In this sense, stressing that arginase deficiency causes spastic paraplegia and differs from other urea cycle disorders because of its more chronic and relentless progression is important for clinical practitioners.

Liver enzymes were elevated in two patients, and it was an important clue that led to further investigation in these cases.

No abnormal neuroradiological findings were shown in 4 patients. Thinning of corpus callosum was observed on MRI in patients I and II. Periventricular leukomalacia was observed in patient VI, which had several neonatal complications and a previous diagnosis of cerebral palsy. It is not clear if arginase deficiency was the only factor contributing to spasticity in this patient, although progression of spasticity supports a role for argininemia in its etiology.

In the few studies reporting neuroimaging in arginase deficiency, variable cerebral and mild cerebellar atrophy were frequent findings (Huemer et al. 2016). Other neuroradiological findings may include signal changes in the posterior putamen and insular cortex, corticospinal tract abnormalities on diffusion tensor imaging (DTI), global brain edema (especially in neonatal-onset patients), ischemic changes on T2 and diffusion-weighted images, basal ganglia involvement followed by delayed myelination, ulegyria, and cystic lesions (Dorum et al. 2021).

All reported patients were homozygous for likely pathogenic or pathogenic variants in *ARG1.* Most patients were from small cities in the Brazilian countryside where consanguineous marriages are relatively common, and an increased incidence of autosomal recessive disorders has been previously reported in some of these populations (Rangel et al. 2018).

Patient IV, who had a novel frameshift variant, had earlier onset disease and more severe clinical manifestations. A similar study (Jain-Ghai et al. 2011) suggested that neonatal presentation of arginase deficiency is commonly due to loss of function variants, such as nonsense, splice site or frameshift. The classic later onset arginase deficiency patients had at least one missense variant, however, further studies are needed to confirm any genotype–phenotype correlations (Jain-Ghai et al. 2011).

Spasticity represents a common finding in many inborn errors of metabolism (IEM), Although symptoms usually present at birth in urea cycle disorders, this is not the case in arginase deficiency, which first symptoms are often noted between 2 and 4 years. These symptoms can be a variable combination of progressive spastic paraplegia, intellectual disability, and seizures (Panza et al. 2019). Disease progression can be quite slow, and it is noteworthy that four of the patients in the present series

had their diagnosis confirmed more than 20 years after symptom onset. A recently published large cohort of childhood-onset hereditary spastic paraplegia showed that the fourth most common cause was related to *ARG1* biallelic pathogenic variants (Giordani et al. 2021).

Clinical diagnosis of early onset complicated hereditary spastic paraplegia was made in all patients in this series. Two patients were initially suspected to have SPG11 due to thinning of the corpus callosum. As argininemia may be characterized by a highly penetrant phenotype of spastic paraplegia, associated with additional symptoms, this disease may represent a specific entity amongst the complicated HSPs.

The constellation of clinical and imaging findings in argininemia may support the assumption that this disease should be included in the group of complex HSPs.

ADDITIONAL INFORMATION

Ethics Statement

Patients were recruited with their informed and written consent and the research was approved by the USP's Faculty of Medicine Research Ethics Committee.

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Data Deposition and Access

Pathogenic and likely pathogenic variants in *ARG1* included in this manuscript have been submitted to ClinVar database:
p.Thr134Ile, SCV001137233.1
p.Leu216Alafs*4, SCV001137234.1
p.Arg308Gln, SCV001137235.1
p.Met1Ile, SCV002576286.1

Author Contributions

Fernando Freua, MD: Data collection, text writing and revision.

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Lúcia Inês Macedo-Souza, MSc, PhD: Text writing and revision.

Clarissa Bueno, MD, PhD: Text writing and revision.

Leandro Tavares Lucato, MD, PhD: Text writing and revision.

David S Lynch, MD, PhD: Text revision.

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Competing Interest Statement

The authors have declared no competing interest.

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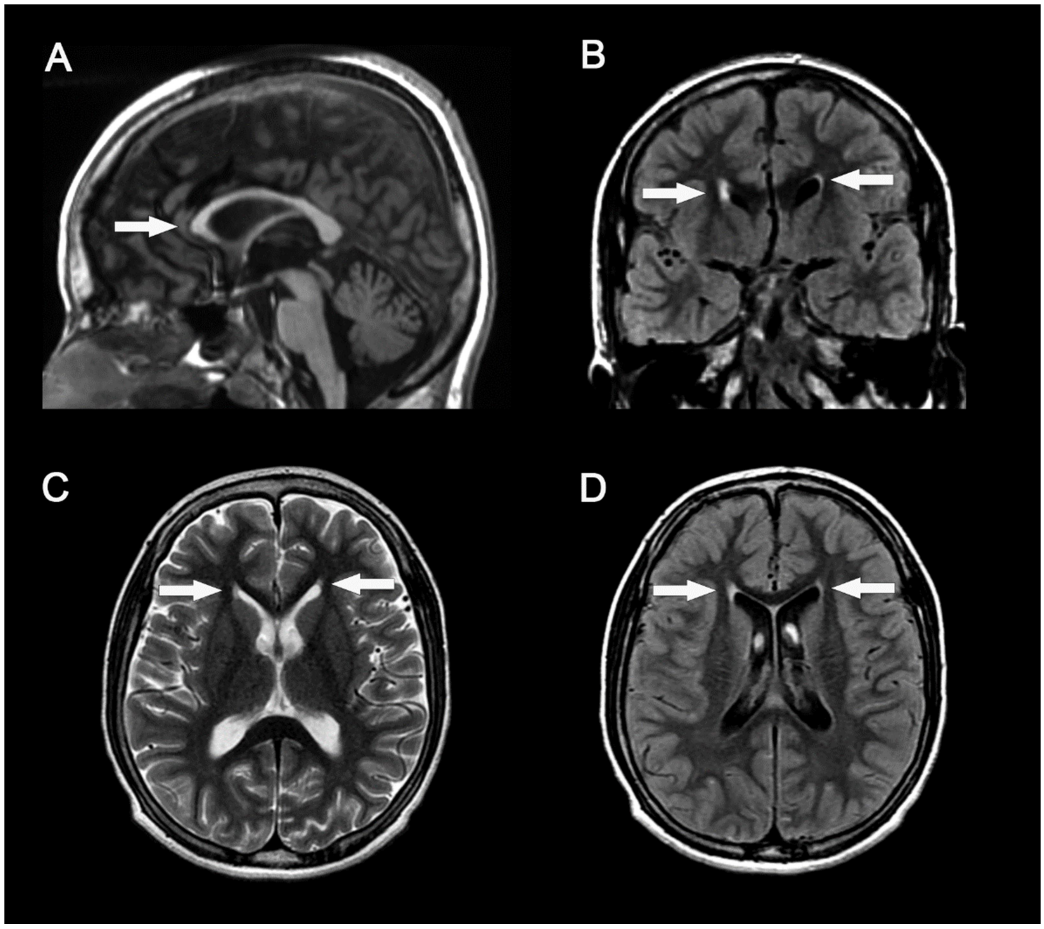
FIGURE LEGENDS

Figure 1. Brain MRI from patient I. Sagittal T1-weighted image (A), reformatted from a 3D sequence, demonstrates thinning of the corpus callosum, especially the genu (arrow). Coronal FLAIR image (B) discloses hyperintensity in the forceps minor, more evident to the right (arrows). Axial T2-weighted (C) and axial FLAIR (D) images show periventricular hyperintensities. The whole picture resembles a milder form of the “ears of the lynx” sign.



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