Movement Disorders
CLINICAL PRACTICE

REVIEW

Autosomal Recessive Guanosine Triphosphate Cyclohydrolase I Deficiency: Redefining the Phenotypic Spectrum and Outcomes

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Abstract: Background: The *GCH1* gene encodes the enzyme guanosine triphosphate cyclohydrolase I (GTPCH), which catalyzes the rate-limiting step in the biosynthesis of tetrahydrobiopterin (BH4), a critical cofactor in the production of monoamine neurotransmitters. Autosomal dominant GTPCH (adGTPCH) deficiency is the most common cause of dopa-responsive dystonia (DRD), whereas the recessive form (arGTPCH) is an ultrarare and poorly characterized disorder with earlier and more complex presentation that may disrupt neurodevelopmental processes. Here, we delineated the phenotypic spectrum of ARGTPCHD and investigated the predictive value of biochemical and genetic correlates for disease outcome.

Objectives: The aim was to study 4 new cases of arGTPCH deficiency and systematically review patients reported in the literature.

Methods: Clinical, biochemical, and genetic data and treatment response of 45 patients are presented.

Results: Three phenotypes were outlined: (1) early-infantile encephalopathic phenotype with profound disability (24 of 45 patients), (2) dystonia-parkinsonism phenotype with infantile/early-childhood onset of developmental stagnation/regression preceding the emergence of movement disorder (7 of 45), and (3) late-onset DRD phenotype (14 of 45). All 3 phenotypes were responsive to pharmacological treatment, which for the first 2 must be initiated early to prevent disabling neurodevelopmental outcomes. A gradient of BH4 defect and genetic variant severity characterizes the 3 clinical subgroups. Hyperphenylalaninemia was not observed in the second and third groups and was associated with a higher likelihood of intellectual disability.

Conclusions: The clinical spectrum of arGTPCH deficiency is a continuum from early-onset encephalopathies to classical DRD. Genotype and biochemical alterations may allow early diagnosis and predict clinical severity. Early treatment remains critical, especially for the most severe patients.

The *GCH1* gene (OMIM: 2339109) encodes the enzyme guanosine triphosphate cyclohydrolase 1 (GTPCH), which catalyzes the first rate-limiting step of the tetrahydrobiopterin (BH4) biosynthesis.[1] BH4 is an essential cofactor for 3 key enzymes implied in dopamine, noradrenaline, epinephrine, and serotonin neurotransmitter biosynthesis.

Genetic variants of *GCH1* have been associated with both autosomal dominant (adGTPCH) and recessive (arGTPCH) patterns of inheritance, with different functional consequences on the structure and activity of the enzyme, ranging from dominant negative effect to haploinsufficiency, and with a wide, only partially overlapping spectrum of clinical presentations and

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outcomes. More than 300 variants have been associated with the 2 conditions, with very few variants shared between adGCH1 and arGCH1.[2]

The autosomal dominant GTPCH 1 deficiency (adGTPCH) (OMIM: 128230), the prototypical form of dopa-responsive dystonia (DRD),[3] is one of the first genetically characterized dystonias and the most common inherited dystonia in children and adults with a prevalence of 0.5 to 1.0 per million.[4]

On the contrary, autosomal recessive GTPCH deficiency (arGTPCH) (OMIM: 233910) is a rare metabolic disorder with a more complex presentation and earlier onset that may disrupt ongoing neurodevelopmental processes, leading to a neurodevelopmental disorder associated with dystonia parkinsonism.[5]

The genetic, clinical, and biochemical spectrum; disease course; and response to treatment of arGTPCH deficiency, despite being a long-recognized disease, are poorly characterized in the literature, with only 41 patients reported over time and largely incomplete metabolic data. To better define the clinical phenotype and outcome of arGTPCH deficiency, its genetic and metabolic correlates and their possible predictive value, we systematically reviewed the literature since the first description of the disease about 40 years ago. We also contribute to the resulting cohort with 4 previously unreported patients (Supporting Information S5.).

Patients and Methods

A systematic review of the literature, including papers published from the first identification of the disease up to November 2023, was performed by searching PubMed and Scopus English-language publications with descriptions of patients with arGTPCH deficiency. The following keywords were used for the research: "*GCH1*," "GTPCH1," "GTP cyclohydrolase," "DYT5," "Segawa disease," "L-dopa responsive dystonia," and "atypical phenylketonuria." We added data from 4 previously unreported patients from Sapienza University of Rome and Boston Children's Hospital to the cohort collected from the literature (Supporting Information S5.). The following data were extracted from literature and personal cases:

1. Clinical data of patients and their affected relatives: age at publication, gender, age at onset, clinical features at presentation, age and clinical status at the diagnosis, and response to treatment.
2. Biochemical markers: blood phenylalanine (Phe), cerebrospinal fluid (CSF) pterins (neopterin [Neo] and biopterin [Bio]/BH4), CSF homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) and 5-methylenetetrahydrofolate (5-MTHF), urine excretion of Neo and Bio, and GTP-CH activity.
3. *GCH1* genotype: the pathogenicity of each variant in the patient cohort described here was reevaluated due to conflicting interpretations reported in ClinVar and PNDdb databases (Supporting Information S4.). Variants were revised according to ACMG guidelines, ACGS best practice guidelines, and Clingen Recommendations for Sequence Variant Interpretation (Supporting Information S4.).[6–8]

The criteria for applied weight for each rule are reported in the Supporting Information.

Bioinformatic analysis was performed using AlamutVisualPlus_1.7.1 (SOPHiA Genetics).

The PP4 rule (patient's phenotype) was applied to all variants, at least with supporting weight. The weight was upregulated according to the severity of metabolic alterations reported for each patient/variant. However, when the only clinical phenotype was available (PP4 supporting), we tried to correct this bias by downregulating the weight of the rule for all those variants reported in different patients who presented a different PP4 weight. After the reclassification of variants, genotypes were categorized as follows: (1) "strong genotypes" denoted by the presence of pathogenic/likely pathogenetic variants in either homozygous or compound heterozygous states; (2) "mild genotypes" were identified in cases featuring 1 pathogenic/likely pathogenic variant alongside a variant of uncertain significance (VUS)/likely benign variant, or 2 VUS, either in homozygous or compound heterozygous state; and (3) "weak genotypes" were designated when characterized by the presence of VUS alongside likely benign variant.

Results

General Clinical, Genetic, and Biochemical Data

The literature search identified 35 articles (1984–2022)[9–43] describing 41 patients with arGTPCH deficiency. In addition, we included 4 unpublished cases. Our cohort consists of 45 patients (24 men/18 women/the sex was not reported for 3 individuals) from 38 families for whom informative clinical and/or biochemical and/or genetic data were available (Table 1; Tables S1. and S2.). Table S1. summarizes the clinical presentation and outcome of movement disorders (MD) according to the current terminology (papers in chronological order from publication). When we were uncertain, we reverted to the original terminology used by the authors.

The mean age at publication (available for 34 patients) was 10 years (range: 1 day to 47 years). The mean age at onset (available for 44 patients) ranged from 1 day to 39 years, with 11 of 44 patients with neonatal onset, 15 of 44 with infantile onset (up to age 1 year), 17 of 44 with childhood onset, and 1 with adult onset. Clinical signs and symptoms were reported in all patients and consisted mainly of hypokinetic and/or hyperkinetic MD. In the overall cohort, dystonia was the most frequently reported MD (33 of 45 patients), described as generalized in 13 patients and restricted to upper and/or lower limbs and/or head in 9 patients. Oculogyric crises (OGC) were described in 8 patients. Choreoathetotic/dyskinetic features were observed in 9 patients. Parkinsonian features, including hypokinesia/

TABLE 1 *Autosomal recessive GTPCH deficiency: genetic spectrum, biochemical features, and clinical phenotype of patients reported in the literature and in the present study*

Patient number	Reference	GCH1 genotype	Phenotype (a, b, c)	AOO	Biochemical data							
					Blood	CSF	CSF	CSF	CSF	CSF		
					Phe	HVA	5-HIAA	Neo	Bio	5-MTHF	Neo (U)	Bio (U)
1	9,10	–	a	2.5 mo	↑	↓	↓	↓	↓	–	↓	↓
2	11,12	p.Arg184His p.Arg184His	a	1 week	↑	–	–	↓	↓	–	↓	↓
3	13	–	a	Neonate	↑	–	–	–	–	–	↓	↓
4	14	–	a	Neonate	↑	–[a]	–[a]	–[a]	–[a]	–	↓	↓
5	15	–	a	Neonate	↑	–	–	↓	↓	–	↓	↓
6	16	p.Met211Ile p.Met211Ile	a	4 mo	ns: n -↑	↓	↓	↓	↓	–	–	–
7	17	p.Lys224Ter p.Pro23Leu	c	5 yr	n	–	–	–	–	–	–	–
8	18	p.Met221Thr Asn118ThrfsTer13	a	<6 mo	↑	–	–	↓	↓	–	–	–
9	18	p.Gly108Asp p.Lys224Arg	b	>12 mo	n	↓	↓	↓	↓	–	–	–
10	19	p.Val191Ile c.344-2A > G (p.)	c	–	n	–	–	–	–	–	–	–
11	19	p.Met211Val p.Met211Val	a	10 days	↑	↓	N (1yr) then ↓	–	–	–	↓	↓
12	20	p.Arg249Ser p.Arg249Ser	c	2 yr	n	–	–	–	–	–	n	
13	21	–	a	Birth	↑	↓	n	↓	↓	↓	↓	↓
14	22,23	p.Pro199Ala p.Pro199Ala	a	1 mo	n	–	–	–	–	–	–	–
15	22,23	p.Pro199Ala p.Pro199Ala	a	1 mo	n	–	–	–	–	–	–	–

(Continues)

TABLE 1 *Continued*

Patient number	Reference	GCH1 genotype	Phenotype (a, b, c)	AOO	Biochemical data							
					Blood	CSF	CSF	CSF	CSF	CSF		
					Phe	HVA	5-HIAA	Neo	Bio	5-MTHF	Neo (U)	Bio (U)
16	24	p.Pro69Leu p.Pro23Leu/ p.Met211CysfsTer4	c	2 yr	n	–	–	–	–	–	–	–
17	24	p.Pro69Leu p.Pro23Leu/ p.Met211CysfsTer4	c	2 yr	n	–	–	–	–	–	–	–
18	25	p.Ser223Arg p.Pro23Leu	c	7 yr	–	–	–	–	–	–	–	–
19	26	p.Pro23Leu p.Gln182Glu	b	3 yr	n	–	–		–	–	–	–
20	27	p.Val206Ala p.Val206Ala	a	Birth	n	↓	n	↓	↓ (BH4)	–	↓	↓
21	27	p.Val206Ala p.Val206Ala	a	Birth	n	n	n	↓	↓ (BH4)	–	↓	↓
22	28	p.Met211Val p.Lys224Arg	c	7 yr	–	–	–	–	–		–	–
23	28	p.Met211Val p.Lys224Arg	c	4 yr	–	–	–	–	–	–	–	–
24	29	p.Arg59Gly p.Arg198Trp	c	8 yr	n	–	–	–	–	–	–	–
25	30	Lys224Arg c.343 + 5G.C	a	<2 mo	n	n	↓	↓	n	–	–	–
26	30	Lys224Arg c.343 + 5G.C	a	<6 weeks	n	n	n	↓	↓	–	–	–
27	32	p. Ala73Asp p. Ala73Asp	a	4 mo	n	↓	↓	n	↓	–	↓	n

(Continues)

TABLE 1 *Continued*

Patient number	Reference	GCH1 genotype	Phenotype (a, b, c)	AOO	Biochemical data							
					Blood	CSF	CSF	CSF	CSF	CSF		
					Phe	HVA	5-HIAA	Neo	Bio	5-MTHF	Neo (U)	Bio (U)
28	32	p.Val204Ile p.Lys220GlnfsTer27	a	3 mo	n	↓	n	↓	↓	–	↓	↓
29	33	p.Val205Gly NC_000014.9:g.54902903_54902920del (18-bp *GCH1* promoter del)	a	<2 mo	n	–	–	–	–	–	–	–
30	34	p.Gln103His p.Gln103His	a	<3 mo	n	↓	↓	↓	↓ (BH4)	–	–	–
31	34	p.Gln103His p.Gln103His	a	7 days	n	–	–	–	–	–	–	–
32	35	p.Arg184His p.Arg235Trp	a	11 days (NS)	↑	↓	↓	↓	↓	–	↓	↓
33	36	p.Pro23Leu p.Phe104Leu	c	39 yr	–	–	–	–	–	–	–	–
34	36	p.Val204Ile p.Arg241Gln	c	4 yr	–	↓	↓	n	↓	–	–	–
35	37	p. Pro95Ser p. Lys224Arg	b	<18 mo	n	↓	↓	↓	↓	–	–	–
36	38	p.Arg184His p.Val204Ile	b	16 mo	–	–	–	–	–	–	–	–
37	39	p.Val205Ala p.Val205Ala	a	3 mo	↑	↓	↓	↓	↓	–	–	–
38	40	p.Lys224Arg p.Phe104Leu	c	9 yr	n	–	–	–	–	–	–	–
39	41	p.Val202Ile p.Val202Ile	b	7 mo	n	–	–	–	–	–	–	–

(Continues)

TABLE 1 *Continued*

Patient number	Reference	GCH1 genotype	Phenotype (a, b, c)	AOO	Biochemical data							
					Blood	CSF	CSF	CSF	CSF	CSF		
					Phe	HVA	5-HIAA	Neo	Bio	5-MTHF	Neo (U)	Bio (U)
40	42	p.Arg235Gly	a	<4 mo	↑	–	–	–	–	–	–	–
	43	p.Arg235Gly										
41	43	p.His153Tyr	c	6.6 yr	n	–	–	–	–	–	–	–
		p.His153Tyr										
42	Case 1	p.Arg170Gly	c	2 yr	n	n	n	↓	n	–	↓	↓
		p.Arg249Gly										
43	Case 2	p.Met221Thr	b	>12 mo	n	n	n	↓	↓	n	↓	↓
		p.Lys167Arg										
44	Case 3	p.Lys224Arg	b	9 mo	n	↓	n	↓	↓	n	–	–
		p.Ala74Pro										
45	Case 4	p.Glu183Lys	a	5 mo	↑	–	–	–	–	–	–	–
		p.Asp134Asn										

[a]Amniotic fluid 18th week of pregnancy (gestation) (nmol/L): HVA 29 (46–410), 5-HIAA 55 (44–918); Neo 4.0 (10.8–77.2); and Bio 2.2 (6.1–40.6).

Abbreviations: GTPCH, guanosine triphosphate cyclohydrolase I; AOO, age of onset; Phe, phenylalanine; CSF, cerebrospinal fluid; Neo, neopterin; Bio, biopterin; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-MTHF, 5-methylenetetrahydrofolate; U, urine; NS, newborn screening; BH4, tetrahydrobiopterin.

bradykinesia and tremor, were reported in 15 patients, 11 of whom had dystonic features. Hyperreflexia and/or signs of spasticity were observed in 13 patients. Comorbid hypotonia was described in 17 patients, whereas epileptic seizures were reported in 8 patients. Global developmental delay (GDD), developmental stagnation, or neurological regression was described in almost half of the child cohort.

Genotype was available in 40 of 45 patients (Table 1; Table S2.). Many variants reported in patients were deposited in the ClinVar and PNDdb databases with conflicting interpretations; therefore, we have reclassified the variants according to the ACMG guidelines[7] (Supporting Information S4.). Fifteen patients carried homozygous variants on the *GCH1* gene, whereas 25 were compound heterozygous (Supporting Information S4., third sheet). In 7 families, more than 1 affected sibling was diagnosed (Table S2.). Symptomatic heterozygosity was reported in 6 families (Table S1.: families 7, 23, 26, 27, 29, and 38), whereas 3 adults from 3 independent families with Parkinson's disease (PD) were not genetically tested. Twenty-three patients did not report a positive family history of MDs.

A complete biochemical characterization, including blood Phe, CSF HVA and 5-HIAA, and urine and CSF Neo and Bio, was available for only a small subgroup of patients (9 of 45) (Table S2.). Blood Phe was assessed in 39 of 45, CSF Neo and Bio in 21 of 45, CSF HVA and 5-HIAA in 19 of 45, and urine Neo and Bio in 15 of 45 patients. In 10 of 45, HVA, 5-HIAA, and pterins were tested in CSF only; in 7 of 45, pterins were the only metabolites analyzed (1 of 7 in CFS, 4 of 7 in urine, and 2 of 7 both in CSF and urine); in a single patient, CSF biogenic amine catabolites and pterins in urine were examined. Finally, in 15 of 45 patients, blood Phe was the only metabolite reported, and in 5 of 45 the diagnosis was based only on sequence analysis of *GCH1*. GTPCH enzymatic activity was measured in 10 patients.

Overall, low levels of CSF Neo or Bio were found in all 21 patients, and both were found in 17 of 21; low levels of urinary Neo and Bio were detected in 14 of 15 and 13 of 15 patients, respectively (both in 13 of 15); CSF HVA and 5-HIAA were reduced, respectively, in 14 of 19 (marginally in 2) and 11 of 19 (marginally in 3) subjects, both in 10 of 19.

Clinical Phenotypes, Metabolic Patterns, Genetic Correlates, and Outcome

The clinical spectrum of arGTPCH deficiency is broad and covers a continuum from early-onset devastating encephalopathies to isolated nondegenerative MDs (Table 1; Tables S1. and S2.). Based on the clinical presentation and outcome of patients reported to date, 3 distinct phenotypes can be outlined:

1. An early-infantile severe developmental and encephalopathic phenotype (severe developmental impairment, MDs, and epilepsy in some)
2. An early-onset neurodevelopmental phenotype with dystonia parkinsonism
3. A DRD overlapping with the adGTPCH deficiency phenotype but with a wider age range of onset

Focusing on the severity of the biochemical phenotype, 3 metabolic patterns can be delineated based on patients who underwent the most comprehensive diagnostic workup (16):

1. Subjects with hyperphenylalaninemia (HPHE), low CSF HVA and/or 5-HIAA levels, and low CSF and urinary Neo and Bio levels (when assessed) (5 subjects: 1, 6, 13, 32, and 37)
2. Subjects with normal blood Phe but reduced CSF HVA and/or 5-HIAA, CSF Neo and/or Bio, and/or both Neo and Bio in urine (8 subjects: 9, 20, 25, 27, 28, 30, 35, and 44)
3. Patients with altered pterins in CSF and/or urine as a unique diagnostic marker (4 subjects: 21, 26, 42, and 43).

In 2 patients, blood Phe was normal (patient 6) or slightly elevated (patient 8) at birth and peaked in the following months.

Phenotype a

Twenty-four of 45 patients (phenotype a in Table 1 and Table S1.) presented with severe forms of neonatal to early-infantile onset neurological manifestations. The clinical manifestations mainly included failure to thrive, shaking/trembling limb movements, hypokinesia, OGCs, mild to severe GDD, and congenital or acquired microcephaly in the most severely affected patients (patients 3, 4, 20, and 40). During the neonatal period or a few weeks later, trunk hypotonia (later leading to lack of trunk postural control) and limb rigidity were consistent neurological features in many subjects. Severe generalized dystonia and neurological deterioration developed at 3 to 4 months. Diurnal fluctuations occurred in 6 patients (14, 15, 25, 26, 28, and 45), and epileptic seizures and epileptic encephalopathy in 4 patients (patients 1, 2, 13, and 37). Whether untreated or treated late, this condition resulted in a disabling generalized dyskinetic or spastic-dyskinetic cerebral palsy with prominent gross- and fine motor impairment in 8 of 24 patients and neurodevelopmental derangement (GDD/intellectual disability [ID]) in 9 of 24 patients. In a few patients, the clinical follow-up was too short or poorly documented to ascribe them to a definitive outcome. Despite months or years of treatment delay, 7 of 24 patients escaped the most severe clinical outcome (patients 11, 14, 15, 20, 25, 27, and 28).

HPHE was detected in 13 of 24 subjects, the most severely and early affected in this cohort, with the worst outcomes if treated late or untreated. Otherwise, a few of them could benefit from a presymptomatic diagnosis and early treatment thanks to the newborn screening program for Phenylketonuria (PKU). A severe outcome was also reported in 2 of 11 subjects (patients 26 and 29) without HPHE.

Twelve patients from this subgroup underwent CSF study for assessment of biogenic amines and pterins: in 5 of them, dopamine and serotonin catabolites were low (only marginally in

some of them) as well as Neo and Bio, whereas in the rest the reduction involved HVA and pterins (patients 13, 20, and 28), pterins (patients 21 and 26), 5-HIAA and Neo (patient 25), and both the biogenic amines and Bio (patient 27). GDD/ID was almost constant in late-treated patients with HPHE, whereas motor disability and MDs were predominant in the others.

Genotype was available for 19 subjects (Table S2 and Supporting Information S4.).

Twelve patients were homozygous for pathogenic/likely pathogenic variants. Four patients were compound heterozygous for pathogenetic/pathogenic variants or pathogenetic/likely pathogenetic. According to our classification, 16 patients had a genotype characterized likely pathogenetic and/or pathogenetic variants ("strong genotypes"), of whom 12 were homozygous and 4 were compound heterozygous; only 3 patients were compound heterozygous of a pathogenetic variant and VUS ("mild genotypes"). Affected relatives among the heterozygous carriers were documented in patient 8 (DRD in maternal relatives carrying the p.Met221Thr transition) and patient 29 (DRD in maternal relatives carrying the p.Val205Gly transition). A genetically unconfirmed affected relative was reported in the maternal line of patients 14 and 15 (maternal aunt with PD at age 60, possibly carrying the p.Pro199Ala variant) and patient 45 (family members with mild DRD).

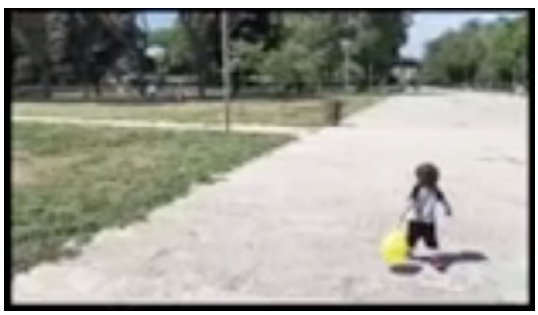
Phenotype b

Seven patients (phenotype b in Table 1 and Table S1.) presented with a late-infantile or early-childhood onset disorder, presenting after an asymptomatic period of normal or only mildly delayed development with global or motor developmental stagnation, irritability, hypotonia, hypokinesia, and neurological deterioration leading to multifocal to generalized dystonia (patient 43 in Videos 1–3), with or without pyramidal signs (Videos 1–3). In patient 9, delayed motor development and probably dystonic dysarthria anteceded the emergence of neurological regression and generalized dystonia by a few years. In 2 late-treated subjects (patients 9 and 36), the disease progressed to severe disabling generalized dystonia/spasticity with limb deformity, dysarthria, and relatively spared cognitive functions.

Four patients (patients 35, 39, 43, and 44) were treated 1 to 14 months (time of treatment initiation was not reported in 1 patient) after disease onset and experienced a dramatic response to treatment with near-normal neurological outcome (patient 39), a residual mild focal dystonia (patients 35 and 44), or a continuous improvement with residual motor delay after 6 months of therapy (patient 43, Video 3).

None of these patients had HPHE (Phe was not tested in patient 36). CSF was analyzed in 4 of these patients (patients 9, 35, 43, and 44): a mild decrease in biogenic amine metabolites and a marked reduction in Neo and Bio in CSF were found in patients 9, 35, and 44 (urine pterins were not evaluated), whereas

Video 1. Clinical status of patient 43 at presentation at 18 months of age with axial hypotonia, hypokinesia/bradykinesia, dystonia, and tremor. Marked irritability and sleep disturbance were also noted.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.14157



Video 2. Patient 43, 4 months after starting levodopa (L-dopa) therapy: marked improvement in signs and symptoms. He takes his first steps. Six months after L-dopa: he is able to walk independently.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.14157

Video 3. Patient 43, 11 months after starting levodopa therapy: the child is able to walk. He has no significant deficits.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.14157

an isolated decrease in pterins in either CSF or urine was found in patient 43.

Genotype characterization was available for all the patients. Six of 7 patients were compound heterozygous for missense variants in exons 1, 3, 5, and 6, of which 5 patients carried 2 pathogenic variants; 1 patient carried a pathogenic variant and the likely benign variant p.Pro23Leu. One patient (39) carried the homozygous pathogenic variant p.Val202Ile transition in exon 5 (Supporting Information S4.). Based on our reclassification system, 6 patients had a "strong genotype" (carriers of a pathogenic variant on both alleles) of which 1 was homozygous and 5 compound heterozygous, whereas 1 was considered with a "mild genotype" (compound heterozygosis of a likely benign and a pathogenic variant).

Affected relatives among the heterozygous carriers were reported for patient 9 (maternal grandfather with PD not genetically tested—possibly carrying p.Lys224Arg transition) and patient 36 (DRD in a paternal relative carrying the p.Arg184His variant).

Phenotype c

Fourteen patients (phenotype c in Table 1 and Table S1.) from 12 pedigrees presented with DRD parkinsonism emerging between the ages of 2 and 39 years. A typical DRD pattern with diurnal fluctuations was reported in 7 patients (Table 1 and Table S1.: patients 7, 12, 22, 23, 24, 38, and 42). Two siblings presented with delayed motor development during the first years of life (patients 16 and 17), anticipating DRD later in childhood; 1 adult patient presented at the age of 39 years with a progressive "loss of dexterity and slowness" of the right arm and dystonic posturing of the right foot, leading to asymmetric rigid-akinetic parkinsonism without tremor and severe right-foot fixed dystonia (patient 33); isolated cervical dystonia with diurnal fluctuation was the presenting symptom at the age of 2 years in 1 patient, 5 years before the rapid generalization of dystonia (patient 42).

All patients except patient 18, whose outcome is not detailed, experienced a complete and sustained response to the treatment, even when started months or years after the disease onset. ID was not reported in any of these patients.

Blood Phe was normal in all 9 subjects. Considering the patient's age at diagnosis and the country of origin, blood Phe can be considered normal in the remaining subjects, at least in the neonatal period. Two patients underwent CSF examination (patients 34 and 42): marginal reduction in HVA and 5-HIAA and reduction in Bio were found in 1 patient (patient 34), and low levels of CSF and urinary pterins with normal levels of biogenic amine catabolites in the other (patient 42).

Genotype characterization was available for all patients; 2 patients (patients 12 and 41) were homozygous for the p.Arg249Ser (exon 6) and His153Tyr (exon 3) transition, a VUS and a pathogenetic variant, respectively. All the other 12 patients were compound heterozygous for variants involving exons 1, 3, 5, and 6 and intron 1. Of the 12 compound heterozygote genotypes, 6 were characterized by pathogenic or likely pathogenic variants, 2 had 1 likely benign and 1 pathogenetic variants, 1 had a VUS and a likely pathogenetic variant, 1 had a VUS and a likely benign variant, and 2 had a likely benign and a VUS on 1 allele and a pathogenetic variant on the other allele.

According to our reclassification, 7 of 14 patients were characterized by "strong genotype" (1 homozygous, 6 compound heterozygous of pathogenic/likely pathogen variants); 6 patients had a "mild genotype" (1 homozygous, 2 heterozygous of pathogenetic/likely benign; 1 heterozygous of likely pathogenetic/VUS, and 2 heterozygous of VUS + likely benign/pathogenetic); and in 1 patient the genotype could be considered as "weak" (VUS/likely benign).

Affected relatives among the heterozygous carriers were reported for patients 33 (mother, 39 years, and daughter, 12y, with DRD carrying p.Phe104Leu missense variant) and 34 (father with PD with the p.Arg241Gln variant).

Response to Treatment

Although incomplete, information on drug therapy was available for all patients reported in the literature (Table S1.). Considering the entire cohort, the age of treatment initiation was reported in 36 patients, ranging from the prenatal (patient 31) and neonatal (patients 3, 21, 32) periods to 41 years (patient 33). All patients except 3 (patients 4, 5, 7, and) received levodopa (L-dopa) therapy. One patient was successfully treated with trihexyphenidyl (20 mg/day) in monotherapy (patient 7), whereas another was treated only with a Phe-restricted diet (no information on outcome, patient 5). One patient received no treatment and had an unfavorable neurological outcome (patient 4).

L-Dopa was usually administered with a peripheral decarboxylase inhibitor (PDI): carbidopa in the majority of patients and benserazide in 4 patients. Details of L-dopa treatment were not reported in 8 patients. L-Dopa/PDI doses, when reported, ranged from 0.5 to 20 mg/kg/day and from 8 to 800 mg/day. Most patients received adjunctive therapies. Eight HPHE patients and 3 non-HPHE patients were supplemented with BH4 (3–18.17 mg/kg/day), 11 with 5-hydroxytryptophan (1–10 mg/kg/day), 6 with a Phe-restricted diet, and 5 folate supplementation (up to 15 mg/day).

Some benefit from L-dopa treatment was described in 39 of 42 patients. A significant benefit was achieved in a subgroup of 16 patients, mainly from phenotypes b and c, who experienced complete or near-complete neurological recovery with normal cognitive development in 4 patients (patients 20, 27, 31, and 41). Conversely, only partial or transient benefit was reported in others, particularly in those early-onset late-treated patients with HPHE, who had residual mild–moderate (9 patients) to severe (3 patients) motor impairment and/or developmental disability. Unfavorable outcome/death was reported in 3 patients (patients 1–3) among the first diagnosed with this disease.

Finally, 6 patients developed L-dopa-induced dyskinesias, which were controlled by dose reduction or tapering or by the administration of amantadine in 2 patients (patients 16 and 17) and trihexyphenidyl in 1 (patient 9).

The delay in treatment in these patients was highly variable, and in 6 patients the delay was more than 1 year.

Conclusion

Due to blood Phe elevation and neonatal screening programs for PKU that had been implemented in many countries since 1964, arGTPCH deficiency could be identified a decade before the discovery of the autosomal dominant DRD with diurnal fluctuation described by Segawa,[3,12] which shares the same metabolic pathway.[9,10] The inefficacy of Phe intake restriction in preventing the neurological deterioration associated with HPHE suggested the involvement of a different metabolic pathway, such as the biosynthesis of the Phenylalanine Hydroxylase cofactor BH4, which was confirmed by targeted metabolic investigations.[9,10] It soon became clear that elevated blood Phe was not a reliable diagnostic marker for the disease,[16,17] further supported by the increasing number of patients with normal blood Phe reported over 40 years (Table 1; Table S2.).

ArGTPCH deficiency is an ultrarare (possibly underestimated) condition, currently classified as DYT/PARK-GCH1 (ar) among the genetic MDs.[44] Compared to the ad form, arDYT/PARK-GCH1 presents earlier with a variable neurodevelopmental derangement, a wider phenotypic spectrum, and a more severe outcome that is much more susceptible to delayed treatment. In recent reviews, it was either considered in the clinical spectrum of the dominant form[45] or clinically related to the other recessive defects of BH4 synthesis and regeneration.[2,46]

To outline the full phenotypic spectrum of this treatable condition, we performed a systematic review of all patients with arDYT/PARK-GCH1 reported in the literature since the disease was first identified, including 4 unpublished novel cases from our centers (Supporting Information S4.). We aimed to better characterize the clinical presentation, biochemical phenotype, and genotype and to explore their possible predictive value for the clinical outcome.

We identified 3 main clinical presentations: (a) an early-infantile encephalopathic phenotype resulting in profound irreversible neurodevelopmental impairment and disability that is not reversed by late treatment. This phenotype overlaps with that of other early-onset disorders of BH4 and biogenic amine synthesis[47]; (b) an early-childhood presentation after an asymptomatic period of a few months characterized by neurodevelopmental stagnation, regression, and dystonia parkinsonism if untreated or treated late, mimicking dyskinetic cerebral palsy with relatively preserved cognitive function; and (c) typical DRD parkinsonism, similar to what is observed in the dominant form, without neurodevelopmental impairment even though onset can be as early as 2 years of age, with excellent response to the treatment even after months or years after onset.

In parallel, based on the surrogate metabolic markers of GTPCH defect, we identified 3 biochemical patterns reflecting a gradient of enzymatic defect: the most severe pattern is due to the impairment of all 3 hydroxylases of aromatic amino acids (HPHE and depletion of biogenic amines in CSF), an intermediate pattern is limited to brain hydroxylase defect (depletion of biogenic amines in CSF), and a mild pattern has isolated pterin alterations, which is the shared, most reliable diagnostic biomarkers.

HPHE and depletion of biogenic amine in CSF were consistently associated with the most severe presentation (group a), with HPHE predicting a higher likelihood of ID when the treatment was delayed. Otherwise, HPHE gives these patients the advantage of a possible presymptomatic diagnosis and treatment.

Metabolic alterations were less consistent in patients presenting with neurodevelopmental dystonia of early-childhood onset or typical DRD presentations. The small number of patients with systematic metabolic investigations in group b does not allow us to distinguish them based on their metabolic profile.

Globally, as for the adGTPCH deficiency, the reduction in pterins (Neo > Bio) in CSF and urine remains the most consistent and reliable metabolic biomarker, regardless of the clinical phenotype.[48] According to current biochemical data, the prognostic value of biochemical alterations is based on their co-occurrence rather than on their quantitative alteration. How long the level of surrogate metabolites in CSF reflects the derangement of the altered homeostasis of neurotransmitters at the synapses remains to be established. A linkage between the level of biogenic amines in CSF at diagnosis and outcome has been suggested for Aromatic L-amino acid decarboxylase[49] and 6-pyruvoyl-tetrahydropterin synthase defects (cognitive and adaptive developments).[50] Systematic studies on this topic are not available in other neurotransmitter disorders so far.

Forty-one genetic variants were identified in 40 patients (Tables S2. and S3.): 34 were missense variants, 3 frameshift variants due to nucleotide deletions, 2 splicing variants (1 canonical), 1 nonsense, and 1 deletion affecting the promoter region. In 11 patients, the enzyme assay confirmed the reduction in GTPCH activity associated with their genotype.

Except for the p.His153Tyr and the p.Val205Ala transitions, all the homozygous variants associated with arGTPCH deficiency were also associated with adGTPCH deficiency (Table S3). Seven variants (p.Pro23Leu, p.Phe104Leu, p.Val204Ile, p.Arg184His, p.Met211Val, p.Met221Thr, and p.Lys224Arg) were observed in multiple unrelated patients. Except for p.Phe104Leu, which was exclusively present in group c, all other variants were detected across all phenotype groups. Our findings suggest that the greatest number of VUS is present in phenotype c, whereas pathogenic and likely pathogenic variants, although also present in phenotype c, are more common in phenotypes a and b.

Furthermore, individuals with a more severe phenotype (phenotype a) tend to have a higher occurrence of homozygous pathogenic or likely pathogenic variants, with 63% of patients with phenotype a being homozygous for pathogenic or likely pathogenetic variants. In this regard, we found the p.Met211Val and p.Lys224Arg variants were identified in compound heterozygosity within groups c and b, whereas they were in the homozygous state in patients of group a. Differently, we

frequently observed compound heterozygosity of pathogenic or likely pathogenic variants alongside variants classified as VUS or likely benign in phenotype c. In this context, the likely benign variant p.Pro23Leu (patients 7, 16, 17, 18, 19, and 33) was first reported in a patient with arDYT/PARK-GCH1 also carrying the p.Lys224Ter variant, and more recently in association with p.Phe104Lys variant.[36] In our cohort, the p.Pro23Leu was found in 6 patients with intermediate (1) and mild (5) phenotypes, and in the case of the latter group, on the same allele with mild VUS p.Pro69Leu in 2 brothers (patients 16 and 17). Although considered a benign variant due to its high frequency in the general population, a possible mild hypomorphic effect on the phenotype when associated with another hypomorphic variant could not be ruled out, as recently reported for a variant associated with a different neurological condition.[51]

In conclusion, the clinical spectrum of arGTPCH deficiency is broad and covers a continuum from early-onset devastating encephalopathies to classical DRD. Biochemical alterations, together with genetic data and clinical presentation, can guide the identification of the disease and definition of its prognosis. Early diagnosis and treatment remain essential to ensure a positive outcome for many of these patients. If altered, pterins in CSF and urine and neurotransmitters in CSF can provide a prompt diagnosis, although this cannot be ruled out if neurotransmitters are normal. Genetic diagnosis can shorten or support diagnostic procedures, although the pathogenicity of most variants is determined based on indirect criteria.

Author Roles

(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical analysis: A. Design, B. Execution, C. Review and critique; (3) Manuscript preparation: A. Writing of the first draft, B. Review and critique.

M.N.: 1A, 1B, 1C, 3A
M.T.: 1A, 1B, 1C, 3A
V.C.: 1A, 1B, 1C
C.C.: 3B
R.B.: 1B, 1C
G.R.: 1B, 1C
K.Y.: 1B, 1C
F.M.: 1B, 1C
F.P.: 3B
D.E.-F.: 3B
S.G.: 1A, 1B, 3A, 3B
V.L.: 1A, 1B, 3A, 3B

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Disclosures

Ethical Compliance Statement: Written informed consent to disclose clinical information was obtained from all patients or parents/guardians. Written informed consent for offline and online video distribution was obtained from parents of patient 43 and is available on request. We confirm that we have read the journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines. The authors confirm that the approval of an institutional review board was not required for this work.

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Supporting Information

Supporting information may be found in the online version of this article.

Table S1. Clinical presentation, outcome, and response to treatment of patients reported in literature and in the present study.
Table S2. Biochemical features of patients reported in literature and in the present study.
Table S3. Genotype–phenotype association in patients with GTPCH (guanosine triphosphate cyclohydrolase I) deficiency.
Supplementary Material S4. Variant reclassification: raw data.
Supplementary Material S5. Case vignettes (patients 42–45).