

















The continuously evolving phenotype of succinic semialdehyde dehydrogenase deficiency

Natalia Alexandra Julia-Palacios¹  | Oya Kuseyri Hübschmann²  |
 Mireia Olivella³  | Roser Pons⁴  | Gabriella Horvath⁵  | Thomas Lücke⁶  |
 Cheuk-Wing Fung⁷  | Suet-Na Wong⁷ | Elisenda Cortès-Saladelafont^{1,8}  |
 M. Mar Rovira-Remisa⁸ | Yilmaz Yıldız⁹ | Saadet Mercimek-Andrews^{10,11}  |
 Birgit Assmann²  | Galina Stevanović¹² | Filippo Manti¹³  |
 Heiko Brennenstuhl^{2,14}  | Sabine Jung-Klawitter²  | Kathrin Jeltsch² |
 H. Serap Sivri⁹  | Sven F. Garbade²  | Àngels García-Cazorla¹ |
 Thomas Opladen² 

Correspondence

Thomas Opladen, Center for Pediatric and Adolescent Medicine Department I, Division of Pediatric Neurology and Metabolic Medicine, Heidelberg University, Medical Faculty Heidelberg, Heidelberg, Germany.
 Email: thomas.opladen@med.uni-heidelberg.de

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Abstract

The objective of the study is to evaluate the evolving phenotype and genetic spectrum of patients with succinic semialdehyde dehydrogenase deficiency (SSADHD) in long-term follow-up. Longitudinal clinical and biochemical data of 22 pediatric and 9 adult individuals with SSADHD from the patient registry of the International Working Group on Neurotransmitter related Disorders (iNTD) were studied with *in silico* analyses, pathogenicity scores and molecular modeling of *ALDH5A1* variants. Leading initial symptoms, with onset in infancy, were developmental delay and hypotonia. Year of birth and specific initial symptoms influenced the diagnostic delay. Clinical phenotype of 26 individuals (median 12 years, range 1.8–33.4 years) showed a diversifying course in follow-up: 77% behavioral problems, 76% coordination problems, 73% speech disorders, 58% epileptic seizures and 40% movement disorders. After ataxia, dystonia (19%), chorea (11%) and hypokinesia (15%) were the most frequent movement disorders. Involvement of the dentate nucleus in brain imaging was observed together with movement disorders or coordination problems.

Natalia Alexandra Julia-Palacios, Oya Kuseyri Hübschmann, Àngels García-Cazorla and Thomas Opladen contributed equally to this study.

Take-home message: Our findings from a comprehensive longitudinal study on patients with succinic semialdehyde dehydrogenase deficiency (SSADHD) highlight the evolving nature of the disease phenotype over time and expand the genotypic spectrum. We were able to observe diverse clinical manifestations, particularly in relation to movement disorders. The study underlines the importance of continuous clinical monitoring and emphasizes the use of *in silico* approaches.

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Short attention span (78.6%) and distractibility (71.4%) were the most frequently behavior traits mentioned by parents while impulsiveness, problems communicating wishes or needs and compulsive behavior were addressed as strongly interfering with family life. Treatment was mainly aimed to control epileptic seizures and psychiatric symptoms. Four new pathogenic variants were identified. In silico scoring system, protein activity and pathogenicity score revealed a high correlation. A genotype/phenotype correlation was not observed, even in siblings. This study presents the diversifying characteristics of disease phenotype during the disease course, highlighting movement disorders, widens the knowledge on the genotypic spectrum of SSADHD and emphasizes a reliable application of in silico approaches.

KEYWORDS

evolving phenotype, genetic spectrum, in silico analyses, long-term follow-up, SSADH deficiency

1 | INTRODUCTION

Succinic semialdehyde dehydrogenase deficiency (SSADHD; OMIM #271980) is the most frequent inherited disorder of γ -aminobutyric acid (GABA) metabolism and is caused by homozygous or compound heterozygous variants in the *ALDH5A1* gene (610045) on chromosome 6p22. Its impairment leads to the accumulation of large quantities of GABA and γ -hydroxybutyric acid (GHB; 4-hydroxybutyric), especially in the central nervous system (CNS).

GABA, the CNS major inhibitory neurotransmitter, is formed from glutamate by pyridoxal-5-phosphate-dependent glutamate decarboxylase. It is catabolized into succinic acid through the sequential action of two mitochondrial enzymes, GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH). Succinic acid enters the Krebs cycle and is converted to 2-oxoglutarate, which further leads to the synthesis of glutamate. In the absence of SSADH, the accumulation of succinic semialdehyde results in its conversion to GHB.

High concentrations of GABA and GHB, are detectable in cerebrospinal fluid (CSF) and urine of individuals with SSADHD and are related to the neurological manifestation of the disease, but this is not a known pathophysiological explanation as far as the literature states, and there still is need of further study. In addition, recent evidence suggests that multiple metabolic perturbations may be associated with the pathophysiology including involvement of the mitogen-activated protein kinases (MAPK) pathway affecting the myelination,¹ dysregulation of autophagy and oxidative stress via mTOR pathway² and astrogliosis and myelin-related phospholipid reduction.³

In SSADHD, the first symptoms appear at an average age of 11 months (range 0–44 months). However, the

mean age at diagnosis was reported as 6.6 years.^{4,5} In 10% of patients, the diagnosis was made after the first decade, or even in the sixth decade. Phenotypic spectrum is broad, and no genotype–phenotype relationship has been described to date.⁶ The disease presents with a wide spectrum of symptoms: hypotonia, nonprogressive ataxia, hyporeflexia, developmental delay, expressive language impairment, obsessive-compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD), sleep disturbances and epilepsy.^{5,7–9} Despite the fact that the treatment of SSADHD currently relies mainly on a symptomatic approach,⁵ various therapeutic strategies aimed at directly addressing the underlying deficiency have been developed in animal models such as *Aldh5a1* homozygous knockout mouse model. These strategies include liver-directed adenoviral¹⁰ approaches and systemic injection of recombinant SSADH proteins, as demonstrated by Vogel et al.¹¹ Moreover, the recent development of a novel SSADHD mouse model capable of on-demand restoration of SSADH offers significant implications for proof-of-concept studies and represents a promising advancement towards the clinical translation of this research.^{12,13} Since the initial description in 1981, about 450 patients have been reported. However, due to the nonspecific clinical presentation that overlaps with various neurological disorders, SSADHD is likely underdiagnosed. Consequently, a high level of clinical suspicion is required to initiate the diagnostic work-up, consisting of the determination of GHB concentration in urine, followed by mutation analysis. To date, over 52 pathogenic variants have been identified.^{6,14}

This current study presents a standardized longitudinal evaluation of patients with SSADHD enrolled in the patient registry of the “International Working Group on

Neurotransmitter related Disorders (iNTD)".¹⁵ It focuses on the disease-specific initial clinical and biochemical presentation, highlights the diversifying evolving phenotype during the disease course and widens the knowledge on the genotypic spectrum of SSADHD.

2 | METHODS

The iNTD patient registry study is a multicenter, uncontrolled, non-randomized, open, unblinded observational study, registered German Clinical Trials Register, <https://www.drks.de>, DRKS00007878. The iNTD patient registry is web-based and password-protected (<https://www.intd-registry.org>). Patients' data is collected after written informed consent is obtained by physicians at each participating iNTD center. After a baseline visit, longitudinal follow-up visits are performed annually.

Exclusion criteria applied in the iNTD patient registry are severe comorbidities in the newborn period, e.g., severe intraventricular hemorrhage (III–IV), birth weight <1500 g, kernicterus, Down syndrome, and embryo-fetal disease due to maternal alcohol or drug abuse.

2.1 | Data description

Gestational age (GA) was calculated based on completed weeks of gestation. Preterm pregnancy was defined as delivery before 37 completed (<37 + 0) weeks of gestation.¹⁶ Small for gestational age (SGA) was defined as birth weight (BW) below the 10th percentile (perc). Microcephaly was defined as head circumference at birth (BHC) below the 3rd perc.¹⁷ Symmetrical intrauterine growth restriction (sIUGR) referred to BW, BHC and birth length (BL) below 10th perc.¹⁸ The current age in Table S1 is as of June 2022.

The description of clinical symptoms was available in the medical history and clinical examination form in a controlled vocabulary. Localization and type of muscular symptoms as well as types, frequency and length of epileptic seizures were specified. Free text boxes were provided for additional symptoms and observations.

Cranial MRIs were performed in the respective study centers. Standardized imaging reports form of the iNTD registry was analyzed.

Behavior and emotional characteristics were assessed using a self-report (for patients >13 years) or a parental report form, as previously described.¹⁹

Selective screening described diagnostic procedures initiated after the onset of symptoms while high-risk family screening was undertaken due to an affected individual in

the family before the onset of disease-related signs or symptoms.

The variant description was according to the Human Genome Variation Society (HGVS) guidelines and refers to transcript ID NM_001080.3 for *ALDH5A1*.

2.2 | Statistical analysis

Statistical analyses were performed in R (version 4.0.2). Standard deviation scores (SDSs) for anthropometric variables at birth were computed according to Fenton and Kim.²⁰ Numeric variables were compared between two independent groups with Wilcoxon–Mann–Whitney (WMW) test, or *t*-test with Welch correction, setting $p < 0.05$ as significant. Classification and regression trees (CART) were used to identify constellations of symptoms that might have an impact on the age at diagnosis or diagnostic delay (= age at diagnosis – age at initial symptom).

Frequent combinations of behavioral traits were displayed as combinations of two to a maximum of four traits according to ECLAT algorithm implemented in R package ‘arules’.²¹ We used the eclat algorithm to mine frequent two or three set of traits, and report most 30 sets of item-sets and their frequency (*support*).

2.3 | In silico analysis

SIFT,¹⁵ Polyphen-2,²² REVEL²³ and CADD²⁴ predictors were used to predict the functional effect of *ALDH5A1* missense variants. The molecular models of missense mutations were based on SSADH, 2w8n X-ray crystal structure²⁵ at 2.0 Å of resolution. The molecular models of the mutant proteins were energy minimized using GROMACS.²⁶ Pymol3²⁷ was used to visualize the effect of the variants on the protein structure and function.

3 | RESULTS

3.1 | Description of the study population

Between January 1, 2015, and May 15, 2020, 429 individuals were enrolled in the iNTD patient registry. Thirty-one patients (17 male, age range 1.5–33.4 years) from 27 families had the diagnosis of SSADHD and one patient of GABA-T deficiency. The latter is not the objective of this study. Twenty five out of 31 patients with SSADHD were born in Europe, five in Asia and one in North America. Consanguinity was reported in five cases (SSADHD_06, _09, _22, _28, and _29). Four families with two affected children each were registered (Table S1).

There was one further patient with one affected sibling who was not enrolled in the iNTD registry study.

3.2 | Initial presentation and diagnostic work-up

Only one premature newborn was registered (35 weeks of gestation). SGA was reported in 29% (8/28) of patients while 5% (1/20) had a BL below the 10th perc. 13% (2/16) had a BHC <10th perc. but none showed microcephaly. sIUGR was not observed. Hyperbilirubinemia and feeding problems were the most frequent postnatal problems.

The most common initial symptom was developmental delay followed by truncal hypotonia with or without limb hypotonia, (60% of cases truncal hypotonia was accompanied by limb hypotonia) (Figure 1A). Behavioral problems (autistic traits [$n = 3$], hyperactivity [$n = 1$]), speech disorders (affecting expressive language skills, $n = 2$) and gastrointestinal problems (bowel and feeding problems, $n = 2$) were entered commonly as additional initial symptoms (Table S1). Symptom onset occurred in infancy ($n = 26$, median 6 months, mean 8.9 months, range 1–24 months). Only one patient (SSADHD_02) presented with developmental and feeding problems in the neonatal period.

All children were diagnosed via selective screening after being symptomatic except three patients detected via high-risk family screening. The median age at the time of diagnosis was 21 months ($n = 30$, mean 46.6 months, SD

48.3 months, range 2 days–16 years). The confirmation of suspected diagnosis was established by determination of GHB in urine and/or genetic analysis of the *ALDH5A1* gene. GHB concentration in urine was increased in 17/18 cases (normal range 0–14 nmol/L, measured concentrations in the range of 5.8–1535 nmol/L, mean 419 nmol/L, median 336 nmol/L, SD 398 nmol/L. $n = 16$). GHB concentration in the normal range (5.8 nmol/L) was detected in an individual SSADHD_21, who was diagnosed at the age of 2 days because of an index patient (SSADHD_20) in the family (Table S1). GABA concentration in CSF was elevated in three cases that were tested (normal range 0–0.01 and 0–1 $\mu\text{mol/L}$, age-dependent references, measured concentrations in the range of 2.16–270 $\mu\text{mol/L}$, mean 98.85 $\mu\text{mol/L}$, median 24.40 $\mu\text{mol/L}$, SD 148.63 $\mu\text{mol/L}$). Homovanillic acid concentration was in normal range in two cases and elevated in one case. 5-hydroxyindoleacetic acid was decreased ($n = 1$), increased ($n = 1$) or in normal range ($n = 1$).

The median diagnostic delay was 17 months (mean 34 months, SD 45 months, range –11.5 to 182.7 months). Age at diagnosis as well as the diagnostic delay was influenced by specific initial symptoms: developmental delay or truncal hypotonia was associated with later age at diagnosis (3.6 years, WMW-test, $p = 0.19$) and with a longer diagnostic delay (37 months, WMW-test, $p = 0.16$) than presentation with epilepsy, limb hypotonia or sleep problems (diagnostic age 1.5 years, diagnostic delay 12 months, Figure 2A,B). Patients born before 2005 had a statistically significant longer latency to diagnosis (mean

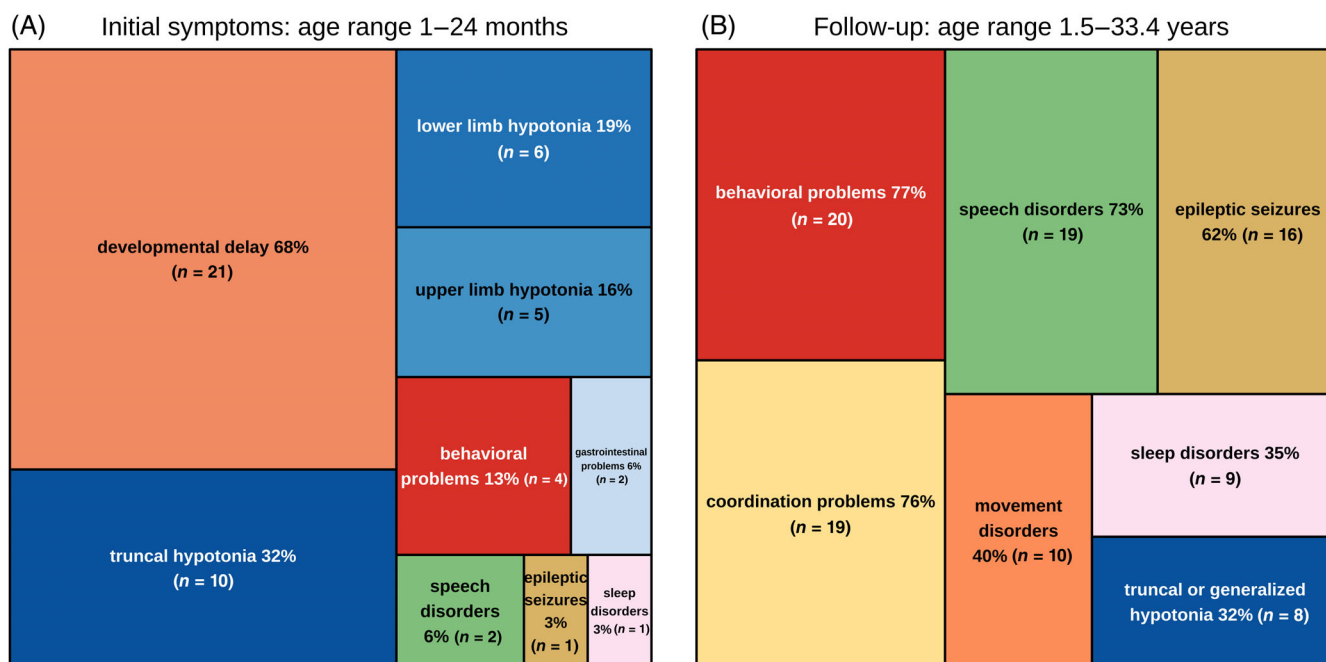


FIGURE 1 The frequency of initial symptoms (A, $n = 31$) and symptoms in follow-up (B, $n = 26$).

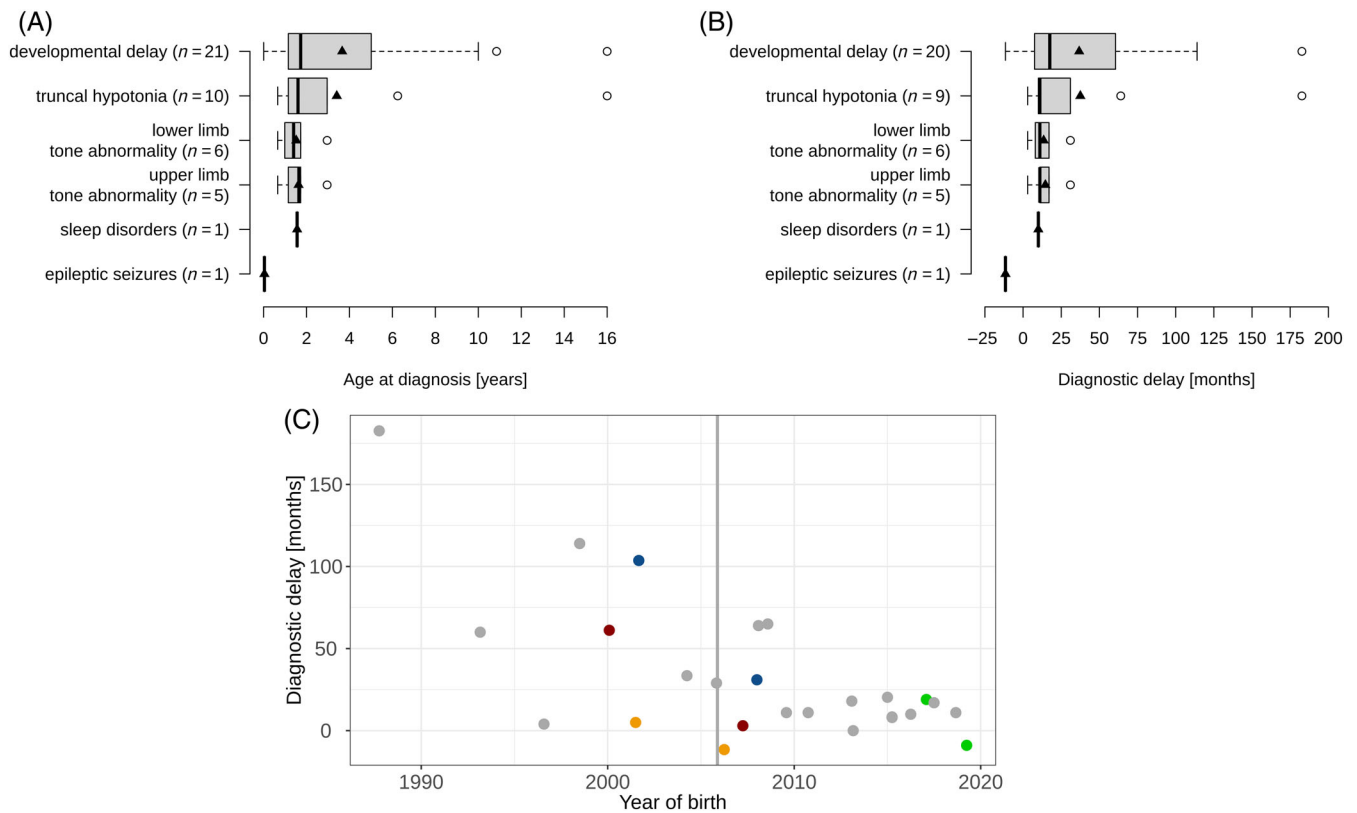


FIGURE 2 Initial symptoms influence the age at diagnosis (A) and diagnostic delay (B). Age at diagnosis was higher (A, WMW-test, $p = 0.19$) and time point of the diagnosis later in life (B, WMW-test, $p = 0.16$) in patients presenting with developmental delay and truncal hypotonia. n , number of patients. (C) Year of birth showed a significant effect on diagnostic delay; bold line: significant, WMW-test, $p = <0.05$. Sibling pairs are coloured respectively.

65.9 months, SD 58.5 months) than those born after 2005 (mean 16 months, SD 21.6 months; $p = 0.029$, WMW-test, Figure 2C).

3.3 | Evolving phenotype

Follow-up neurological examinations were available in 17 pediatric and 9 adult individuals ($n = 26$, 84% of total study population), the vast majority ($n = 21$) older than 5 years (median 12 years, mean 12.3 years, SD 7.77 years, range of 1.8–33.4 years, Figure 1B, Table S1). A remarkably high frequency of movement disorders (dystonia [$n = 5$], chorea [$n = 3$], tremor [$n = 1$], hypokinesia [$n = 4$], bradykinesia [$n = 1$], dyskinesia [$n = 1$], athetosis [$n = 1$] or coordination problems (ataxia [$n = 8$], clumsiness [$n = 6$], gait disturbance [$n = 12$]) were observed in 22/25 cases (Table 1 and Table S1). In 3/5 cases, dystonia resolved in follow-up. Hypokinesia was transient in 2/4 cases, stable in one case and worsened in the case. Differing evolution was also observed for chorea: transient ($n = 1$), stable under medication ($n = 1$) and stable with onset in infancy ($n = 1$) (Table 1). In

21 patients (out of 22) that presented with developmental delay initially, the affection of developmental aspects was documented as scattered in multiple domains such as behavioral, psychiatric, speech and motor disorders with growing age (Figure 1B). Behavioral problems (including autism, obsessive disorder, aggression, ADHD), were observed in 20/26 cases, and an impairment of language development – mainly affecting the expressive domain – in 19/26. Nearly all patients had special educational needs according to their age (Table S1).

Data about behavioral traits were available in 14 parental reports in the last visit (median 9.7 years, mean age 11.7 years, SD 7.5 years, range 1.6–28.7 years). Among 33 traits, short attention span (78.6%, $n = 11$, mild/moderate $n = 6$, severe $n = 5$) and distractibility (71.4%, $n = 10$, mild/moderate $n = 6$, severe $n = 4$) were the most frequently mentioned ones while impulsiveness (5/6), problems communicating wishes or needs (4/7) and compulsive behavior (4/8) were addressed as strongly interfering with family life (Figure 3A,B). Eight (57%) patients presented with more than 9 behavioral traits, two with 5–9 and four with 2–4. Distractibility and/or

TABLE 1 Characterization of movement disorders.

Pat_ID	Age of onset	Type of movement	Description of movement	Severity (onset and evolution)	Brain MRI (age)	Specific treatment for movement disorder spectrum
SSADHD_10	14 years	Action tremor	Intentional action tremor affecting hands	Mild → stable	AV 10 years: Increased T2-weighted signal of GP, and nucleus dentatus	No
SSADHD_14	10 years	Bradykinesia, hypokinesia, rigidity, hypomimia	Generalized	Moderate at onset but progressed over the last year without a clear trigger. Currently is severe and limits the ambulation (non-ambulant) with secondary joint retractions	AV 8 and 11 years: Increased T2-weighted signal of GP, white matter and nucleus dentatus. Increased T2-weighted signal and atrophy of cerebellar cortex and corpus medullare	2 months under treatment with Ldopa/carbidopa (300 mg L dopa per day), without improvement
SSADHD_19	1.8 years (chorea)	Dystonia, chorea	N/A	Mild dystonia and moderate chorea → dystonia disappeared	N/A	No
SSADHD_20	3.5 years	Dystonia	Action dystonia affecting upper limbs (palms and fingers)	Mild → resolved	Normal	No
SSADHD_21	N/A	Dystonia	Action dystonia affecting limbs	Moderate → N/A	AV 7 m: Increased T2-weighted and FLAIR signal of GP. Increased T2-weighted signal of subcortical white matter, with increase diffusion on ADC mapping	No
SSADHD_22	N/A	Hypokinesia	N/A	Mild → resolved	AV 12.1: T2 hyperintensity in GP and nucleus dentatus	No
SSADHD_25	1 year	Dystonia, dysdiadochokinesia, clumsiness	Generalized dystonia	Marked dysdiadochokinesia, and moderate dystonia → stable	AV 15 years: Persistence of a nodular-impressive T2 or FLAIR signal elevation in the transition from the globus pallidus on the right to the posterior limb of the inner capsule. The previously described small nodular subcortical T2 signal elevations on both sides of the high frontal area also show no relevant dynamics in the course. Finally, the symmetrical, punctate iron deposits in the basal ganglia are also unchanged in the course	No
SSADHD_28	3 years	Hypokinesia, chorea	Paroxysmal hypokinesia but frequent. Chorea affecting hands	Mild hypokinesia and chorea → hypokinesia is stable, and chorea has disappeared	Normal	No

TABLE 1 (Continued)

Pat_ID	Age of onset	Type of movement	Description of movement	Severity (onset and evolution)	Brain MRI (age)	Specific treatment for movement disorder spectrum
SSADHD_29	2.5 years	Hypokinesia, dystonia	Axial and peripheral hypokinesia and dystonia affecting wrists and ankles	Mild hypokinesia and dystonia → resolved	AV 9.7: T2 hyperintensity in GP, thinned corpus callosum, T2 hyperintensity and atrophy of cerebellar cortex	No
SSADHD_31	3 years 3 months after regression	Dyskinesia, athetoid movement, chorea	Facial dyskinesic and athetoid movement mainly over 4 distal extremities associated choreiform movement	Mild dyskinesia, athetoid movement and chorea → stable and decreased with clonazepam, tetrabenazine and chloral hydrate	AV 2.2 years: Increased T2-weighted signal of GP, nucleus dentatus and brainstem, with corpus callosum involvement	Clonazepam, tetrabenazine and chloral hydrate

Note: Pairs of siblings in bold.

Abbreviations: ADHD, attention deficit hyperactivity disorder; AV, age at visit; N/A, not available.

short attention were accompanied by impulsiveness, compulsive behavior or problems understanding other people's feelings (Figure 4).

Epilepsy (16/26) and sleep disorders (9/26) were reported less frequently than expected. The most common reported seizure type was generalized tonic-clonic ($n = 6$) followed by myoclonic ($n = 3$), complex partial ($n = 2$), atonic seizures ($n = 1$). Of patients with generalized tonic-clonic seizures, two have a history of myoclonic seizures and one absence seizure before 3 years of age. Age of onset of generalized tonic-clonic seizures was 10 years or older ($n = 5/6$). EEG findings were available in eight patients, depicted in Table S1.

One patient (SSADH_21) had a severe phenotype with developmental regression at the age of 9 months in the context of an infection. He had a known homozygous variant present in other six individuals in our cohort, c.1226G>A, same as his older brother with a milder phenotype. Another patient (SSADHD_31) showed significant neurological regression after an episode of status epilepticus and became wheelchair bound.

We classified clinical endpoints have been considering the presence of sleeping problems, psychiatric symptoms, epilepsy, fine motor disturbances, movement disorders, coordination problems, speech disorder and absent speech. There was no statistically significant association between occurrence of these symptoms and gender, diagnostic delay or age of diagnosis in the cohort of patients with longitudinal follow-up.

3.4 | Brain imaging

Reports of 23 MRIs from 21 patients including three follow-up investigations were available, depicted in Table S1. T2 hyperintensity of globus pallidus ($n = 8$), involvement of infratentorial structures (T2 hyperintensity of dentate nucleus [$n = 9$], T2 hyperintensity and atrophy of corpus medullare [$n = 3$] and of cerebellar cortex [$n = 3$]) and corpus callosum involvement ($n = 7$) were the most frequent findings. In five cases, no abnormalities were described (3 described in follow-up, Table S1).

3.5 | Variants in *ALDH5A1*

23 different variants in the *ALDH5A1* gene have been identified in our study: thirteen missense, two truncating, two splice site, four frameshift and two deletion variants (Table S2). In 12 out of 26 different families, patients were compound heterozygous (Table S2). The most common missense variants were NM_001080.3:c.278G>T, p-Cys93Phe, found in six families (seven individuals, six

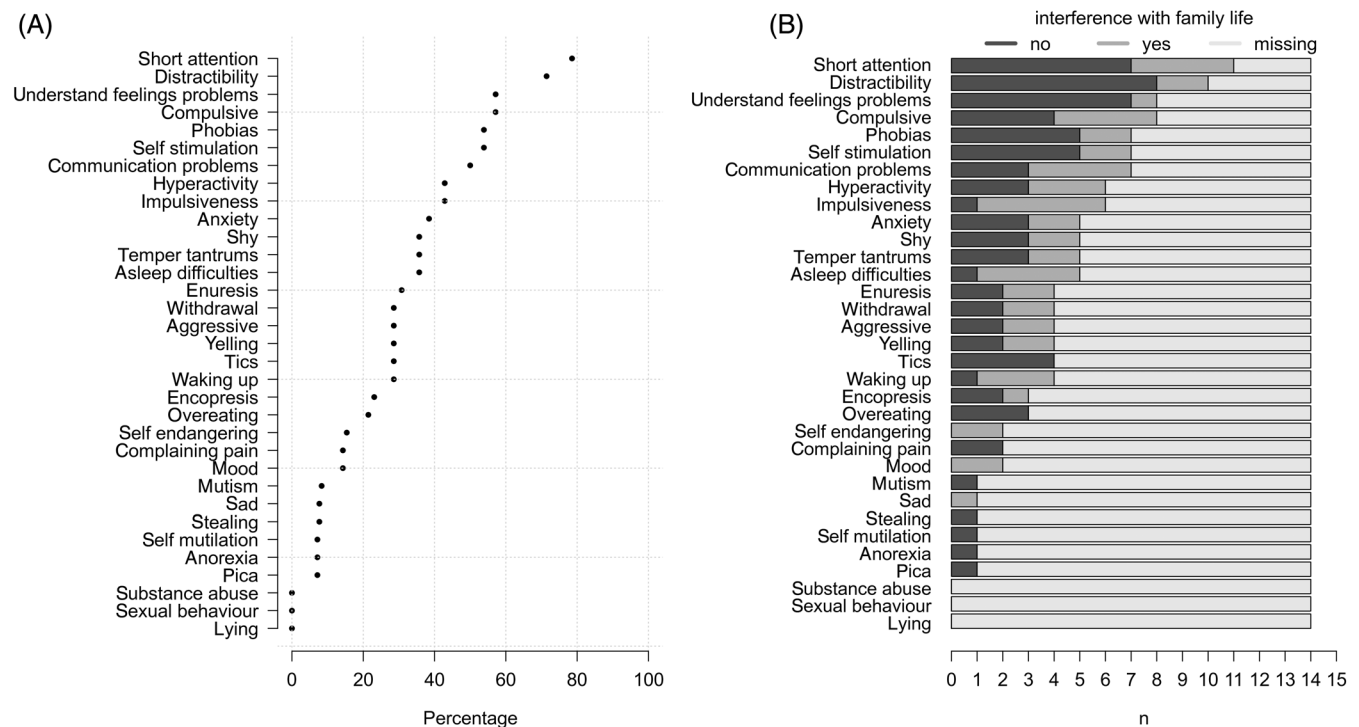


FIGURE 3 (A) Frequency of behavioral traits reported by the parents ($n = 14$). Problems understanding feelings stands for problems understanding other people's feelings. Communication problems refers specifically to problems communicating wishes or needs. (B) Behavioral traits interfering with family life. yes: interfering with family life; no: not interfering with family life; missing: data missing.

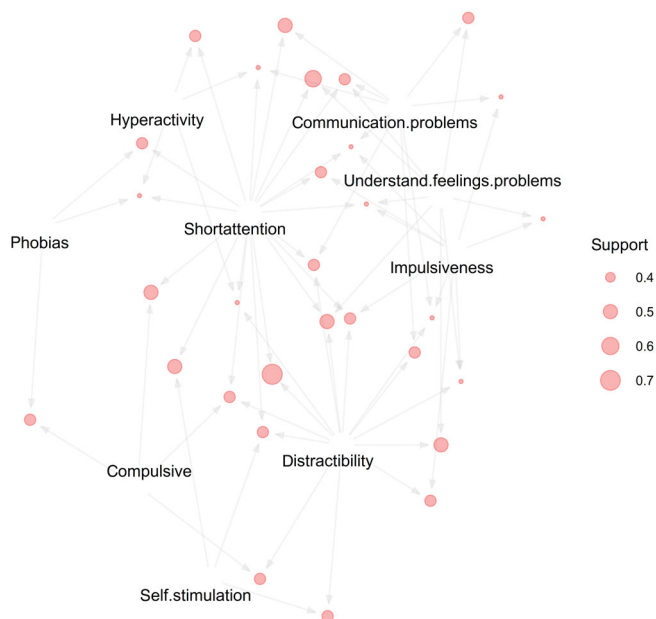


FIGURE 4 Visualization of 30 traits and their strongest associations (n of reports = 14). Circle size corresponds to frequency (or support): strongest associations have traits 'short attention' and 'distractibility', whereas 'phobias' and 'compulsive' have a support of 0.4. There are also some frequent three item sets, e.g., 'compulsive', 'short attention' and 'distractibility' with a support of 0.4.

compound heterozygous), four from Europe, one from North America and one from Asia and NM_001080.3: c.1226G>A, p.Gly409Asp detected in six families (seven individuals, six homozygous) from different European countries.

Four new pathogenic variants were identified: NM_001080.3:c.113_114insGCCCG, p.Gln43ArgfsTer50, NM_001080.3:c.439-2A>G, deletion 9 kb: ISCN: arr 6p22 (24.491.940–24.500.970)x1 and deletion of exon 1.

3.6 | In-silico analysis of missense variants

Truncating, frameshift, splice site, and large deletion variants were predicted to completely abolish protein activity. In order to assess the pathogenicity of missense variants, variant predictors for reported variants ($n = 13$) were used. SIFT,²⁸ Polyphen-2,²² CADD²⁴ and REVEL²³ variant predictors forecasted all variants as pathogenic, except for the previously reported p.His180Tyr variant (Table 2).^{29–37} Molecular models for missense variants were constructed to assess the effect of the mutations on the structure and function of SSADH. Figure 5 shows the SSADH structure with the identified missense variants:

TABLE 2 *ALDH5A1* missense variants and corresponding pathogenicity scores.

Nucleotide change	Protein change	Domain	Experimental enzyme activity	Polyphen-2	Sift	CADD	REVEL	Molecular modeling	Published
NM_001080.3: c.278G>T	p.Cys93Phe	NAD binding domain	3%	1	0.63	31	0.638	Affects protein stability	31
NM_001080.3: c.515G>A	p.Arg172His	NAD binding domain	NA	1	0.13	28.7	0.746	Located at the interacting loop. Affects Oligomerization	32
NM_001080.3: c.526G>A	p.Gly176Arg	Oligomerization domain	<1%	1	0	26.7	0.953	Located at the interacting loop. Mutation to Arg completely avoids Oligomerization due to a steric clash with the interacting subunit	31
NM_001080.3: c.538C>T	p.His180Tyr	Oligomerization domain	82.5	0	1	8.62	0.125	Located at the interacting loop. Involved in subunit interaction, mutation to Tyr affects oligomerization	33
NM_001080.3: c.587G>A	p.Gly196Asp	NAD binding domain	NA	1	0	24.3	0.958	Local structural distortion, steric clash	34
NM_001080.3: c.608C>T	p.Pro203Leu	NAD binding domain	NA	1	0	32	0.936	Interaction with NAD. Mutation to Leu avoid NAD binding	36
NM_001080.3: c.728T>C	p.Leu243Pro	NAD binding domain	<1%	1	0	27.1	0.931	Disrupts secondary structure according to Brennenstuhl 2020	35
NM_001080.3: c.763A>G	p.Asn255Asp	NAD binding domain	NA	1	0.05	24.2	0.851	Local Structural change	38
NM_001080.3: c.803G>A	p.Gly268Glu	NAD binding domain	<1%	1	0	24.3	0.865	Mutation to Glu avoids NAD binding	31
NM_001080.3: c.916G>A	p.Glu306Lys	NAD binding domain	NA	1	0	31	0.889	Mutation to Lys avoids NAD binding	37
NM_001080.3: c.1226G>A	Gly409Asp	Catalytic domain	<1%	1	0	26.2	0.936	Changes loop conformation	31
NM_001080.3: c.1558G>C	p.Gly520Arg	Oligomerization domain	NA	1	0.01	31	0.959	Steric clash, affects protein folding	40
NM_001080.3: c.1597G>A	p.Gly533Arg	Oligomerization domain	<1%	1	0.01	31	0.946	Located at the interacting loop. Involved in subunit interaction, mutation to Arg affects oligomerization	31

Note: Polyphen-2, SIFT, CADD and REVEL predictors were used to assess the pathogenicity of the variants. Molecular modeling of the variants shows the effect of the variant on protein structure and function.

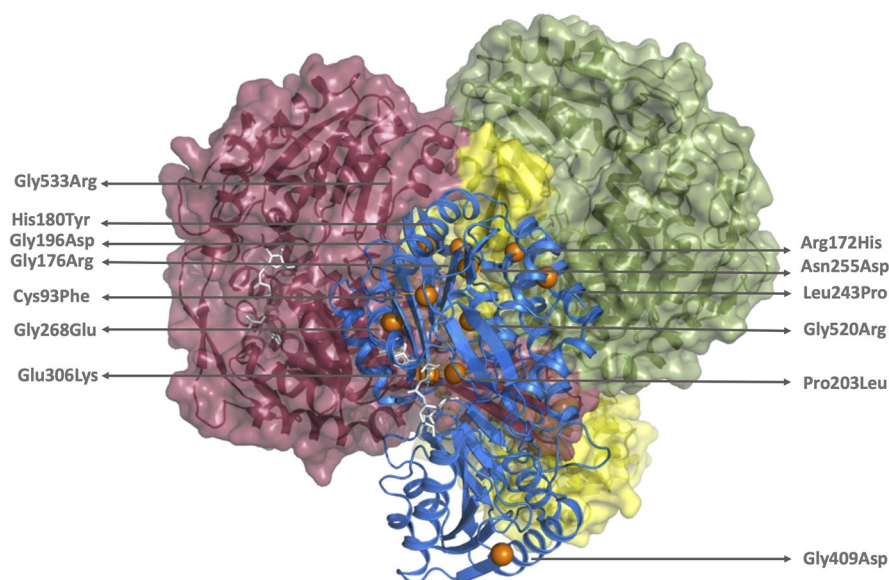


FIGURE 5 SSADH tetrameric structure. Subunits are colored blue, pale red, green, and yellow. Variants are shown as orange spheres. NAD (nicotinamide adenine dinucleotide) molecule is shown in white.

eight missense mutations are located in the NAD binding domain, four in the oligomerization domain, and one in the catalytic domain.

The molecular models of previously reported missense variants shows that p.Cys93Phe, p.Gly196Asp, p.Leu243Pro, p.Asn255Asp, p.Gly409Asp and p.Gly520Arg induce a structural change that affects protein folding or conformation. p-Pro203Leu, p.Gly268Glu and p.Glu306Lys avoid NAD binding affecting the overall protein activity. Finally, p-Arg172His, p.Gly176Arg, p.His180Tyr and p.Gly533Arg are located at the subunit interacting loop, affecting oligomerization. p.His180Tyr is predicted as nonpathogenic, in accordance with a high enzymatic activity³¹ and its high frequency in Gnomad.³⁸ This variant is found in combination with a variant that results in a complete loss of protein activity (loss of exon one) and consequently, all expressed subunits present p.His180Tyr variant, affecting subunit interaction. This is in accordance with previous studies,²⁹ describing a SSADH patient harboring variants p.His180Tyr and p.Pro182Leu at the oligomerization domain, that presented catalytic activity. The computational predictions of the effect of SSADH missense variants agree with Tokatly Latzer et al.,³⁹ for the coincident variants.

3.7 | Genotype/phenotype correlation

Figure S1 presents the occurrence of clinical endpoints regarding the most prevalent *ALDH5A1* variants in the cohort of patients with longitudinal follow-up.

The most common pathogenic variants in this cohort were NM_001080.3:c.278G>T, p.Cys93Phe (seven individuals; one homozygous), NM_001080.3:c.1226G>A, p.Gly409Asp (seven individuals, six homozygous) and

NM_001080.3:c.621delC, p.Ser208ValfsTer3 (three individuals; one homozygous, two heterozygous with NM_001080.3:c.278G>T, p.Cys93Phe). Speech disorder, fine motor abnormalities, coordination problems and psychiatric symptoms were common in all groups. Individuals with NM_001080.1:c.803G>A, p.Gly208Glu or NM_001080.3:c.278G>T, p.Cys93Phe presented with a heterogeneous spectrum of symptoms. No sleeping problems and epileptic seizures were observed during follow-up visits in three individuals who were homozygous for the NM_001080.3:c.1226G>A, p.Gly409Asp variant. Three individuals with NM_001080.3:c.621delC, p.Ser208ValfsTer3 variant did not present any movement disorders.

We could not observe any genotype/phenotype correlation regarding the occurrence of disease-related signs and symptoms, including four sibling pairs in our study population (Table S1). Due to the relatively small number of patients, no statistical analyses could be performed.

3.8 | Treatment approaches

Treatment of epileptic seizures and behavioral problems were the main clinical features for the medical management in our patient cohort. For seizures, levetiracetam ($n = 6$), vigabatrin ($n = 3$), clobazam ($n = 3$) and ketogenic diet ($n = 1$); for behavioral problems, methylphenidate ($n = 3$), antipsychotics (risperidone [$n = 3$], olanzapine [$n = 2$], quetiapine [$n = 2$] and haloperidol [$n = 1$]) were used. In addition, three individuals were treated with taurine (range of 10–140 mg/kg/day). Tetra-benzazine and chloral hydrate was used to control choreatic movements (SSADHD_31). Levodopa/carbidopa was

applied for rigid-hypokinetic syndrome (SSADHD₁₄) without any clear improvement after 2 months of treatment (Table 1).

4 | DISCUSSION

SSADHD is a neurometabolic disorder with onset in infancy-early childhood and evolving clinical features over time. This study on 31 patients with SSADHD (27 unpublished cases and 26 with follow-up examinations during their adolescence and adulthood) from the iNTD patient registry widens the knowledge on the phenotypic spectrum of this disease, presents the diversifying symptoms during disease course and expands the number of disease-causing variants. We report a remarkably high occurrence of movement disorders, coordination, speech and behavioral problems. We also demonstrate the application of *in silico* analysis based on molecular modeling and protein stability to classify the pathogenicity of a variant.

In the context of early clinical presentation, the individuals in our study, the vast majority born in Europe, showed an elevated rate of SGA (29%) compared to the SGA prevalence in Europe (4.6%–15.3%).⁴⁰ In contrast to previous reports, prematurity was not detected as a frequent neonatal problem in our study.⁷

Age at diagnosis, in our study cohort younger than reported before (3.9 years vs. 6 years⁴), and diagnostic delay were affected by different initial symptoms of the patients. The timely diagnosis after birth by three individuals because of an affected sibling had an influence on this observation. However, together with our observation of a tendency to a significantly shortened diagnostic delay in correlation with more recent birth dates, it can be assumed that the broadened availability of (genetic) diagnostic tools,⁴¹ especially the implementation of next-generation sequencing-based diagnostics, and higher awareness of SSADHD have a positive impact on the length of the diagnostic process.

Starting in late childhood and with increasing frequency during adolescence and adulthood, epilepsy was reported as co-morbid in half of the patients with SSADHD.^{8,42–44} In our cohort, the frequency of seizures aligns with findings from previous studies. Additionally, to date five sudden unexplained death in epilepsy (SUDEP)-related deaths of SSADHD deficient patients have been documented, all occurring in adults and in the context of worsening seizure control⁴²; however, no instances of SUDEP were observed in our cohort. *ALDH5A1* variants reported in four SUDEP cases were c.1015-2A>C; c.1005C>A (p.Asn335Lys), c.1226G>A (p.Gly409Asp), and c.608C>G (p.Pro203Arg).^{6,43} These variants were also identified in eight individuals in the

present study; however, only one of them is older than 12 years. Therefore, the probability of SUDEP in this group of patients cannot be ruled out at the current stage.

Short attention span and distractibility also occurred predominantly in our study cohort,^{4,6,45} although impulsiveness, problems communicating wishes or needs and compulsive behavior were the strongest aspects interfering with family life. Our study confirms a uniform approach in different countries and reveals that the major goal of the treatment is the management of seizures and different severe behavioral disturbances mentioned above.

Brain imaging results revealed a broad spectrum ranging from normal finding to increased T2-weighted MRI signal affecting globus pallidus, cerebellar dentate nucleus, and subthalamic nucleus with variable cerebral and cerebellar atrophy, complementary to previous reports.⁴⁶ Involvement of cerebellar dentate nucleus was observed together with movement disorders (action tremor, hypokinesia and dystonia/chorea) or coordination problems. However, in case of globus pallidum involvement and atrophy of corpus medullare, both clinical and radiological signs were also observed independently from each other.

We observed a remarkably high rate of occurrence of movement disorders in our study population (40%, e.g., dystonia, chorea, tremor or hypokinesia). Movement disorders in SSADHD deficiency have been scarcely reported, apart from previously described ataxia. In particular, choreoathetosis, dystonia, and myoclonus have been described only in a few individuals with earlier-onset and more severe disease manifestation.^{14,47–49} Clinical presentation with acute onset of generalized hypotonia and choreiform movement following upper-respiratory tract infection is one of these reported early severe manifestations.^{50,51} However, the vast majority of the patients in our study did not present an early severe onset or any clinical regression but developed different types of movement disorders over time. These movement disorders were classified as mild or moderate. Transitory or stable courses were observed commonly, except in one case with severe progression of hypokinesia. The mildness and transient character of the movement disorders are most likely the reason that no specific drug treatment was indicated in most cases. This evolving spectrum of the phenotype should be taken into consideration of differential diagnoses in children presenting with movement disorders discussed above together with developmental delay, impairment of expressive language, coordination problems, behavioral abnormalities, or epilepsy.

Mechanisms that cause movement disorders in SSADHD have not been elucidated. Its pathophysiology is predominantly shaped by the supra-physiological

accumulation of two key metabolites: GHB and GABA. The increase in GHB could have two parallel effects: on one hand, GHB inhibits presynaptic dopamine release with subsequent alterations in dopaminergic transmission,⁵² a central neurotransmitter affected in many movement disorders. On the other hand, at non-physiologic levels (i.e., SSADHD) GHB acts predominantly at the GABA_B receptor.^{53,54} Its tonic activation leads to a compensatory down-regulation of pre- and postsynaptic GABA_B receptor-mediated function. Together with GHB, GABA itself could contribute to the movement disorders phenotype in SSADHD. GABA provides strong inhibition to the striatum by directly inhibiting the striatal medium spiny projection neurons (MSNs) through activation of GABA_A receptors. The increased GABA concentrations found in SSADHD may result in a tonic inhibition of MSNs, contributing both to the aforementioned down-regulation of GABA_B receptors and to a decreased dopamine release. Both dopamine and GABA are crucial neurotransmitters in the basal ganglia. We hypothesize that GHB and GABA dysregulation will result in altered dopamine homeostasis in midbrain cell groups, underlying, in some way, the aggravation of movement disorders observed in SSADHD.

Additional mechanisms are likely to contribute to the complex clinical picture. A recently published hypothesis on the role of dopamine D2 receptors (D2R) in cerebellum in regulation of social behavior highlights a new aspect of underlying pathophysiology,⁵⁵ considering the cerebellar involvement in SSADHD. Cutando et al showed that D2R deletion or overexpression does not affect cerebellar motor function but sociability and preference for social novelty.⁵⁵ As further relevant mechanisms should be considered the impairment in mitochondrial degradation⁵⁶ and elevation of reactive oxygen species in both patients and in the murine SSADHD model, implicating oxidative stress as well as defects in autophagy,² mediated by altered signaling through the mTOR. Since globus pallidus is metabolically very active with high mitochondrial energy demand, it is especially vulnerable towards oxidative damage and impaired autophagy. That susceptibility is consistent with neuroimaging findings observed in SSADHD⁵ and may contribute, in part, to the motor phenotype observed in SSADHD deficient patients.

The analysis of genetic variants, including 12 missense variants, revealed a high correlation between the applied in silico scoring system, protein activity and pathogenicity. Molecular modeling of mutant proteins provides an excellent approach to understand how a variant causes loss of enzymatic activity due to conformational changes, inhibition of NAD binding or defective oligomerization. This approach is able to predict also how the effect of a

variant with a high enzymatic activity (i.e. variant p-His180Tyr in this study) can be pathogenic due to its effect in the oligomerization domain.³⁶ In line with previous reports, no overall correlation between gene variant and phenotype could be observed in our cohort,⁵⁷ even in siblings. The exact cause of this variability despite a similar genetic background is not yet known. Due to the relatively small number of patients in individual groups with recurrent variants and the heterogeneity of the clinical presentation, no correlation analyses could be performed. The potential of computational approaches for clinical use can be improved by (i) defining specific clinical endpoints based on disease severity (ii) application in larger patient cohorts and (iii) combined evaluation with underlying molecular mechanisms. Functional studies of new pathogenic variants reported in this study are subject of further publications.

Although having some limitations due to the small study group size and missing availability of results of standardized neurodevelopmental testing, our study underlines the relevance of a longitudinal and standardized approach and the value of international collaborations to study rare disorders.

In conclusion, our results widen the knowledge on the phenotypic spectrum of SSADHD, highlight the evolving symptomatic characteristics over time and the occurrence of movements disorders, discuss therapeutic approaches, and emphasize a reliable application of in silico approaches.

AUTHOR CONTRIBUTIONS

Natalia Alexandra Julia-Palacios: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data, study concept or design; analysis or interpretation of data; additional contributions: writing-original draft. Oya Kuseyri Hübschmann: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data, study concept or design; analysis or interpretation of data; additional contributions: writing-original draft, project administration, visualization. Mireia Olivella: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data; additional contributions: writing-original draft, software, visualization. Roser Pons: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Gabriella Horvath: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Thomas Lücke: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Cheuk-Wing Fung: drafting/revision of the manuscript for content, including

medical writing for content; major role in the acquisition of data. Suet-Na Wong: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Elisenda Cortès-Saladela-font: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. M. Mar Rovira-Remisa: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Yılmaz Yıldız: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Saadet Mercimek-Andrews: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Birgit Assmann: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Galina Stevanović: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Filippo Manti: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Heiko Brennenstuhl: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Sabine Jung-Klawitter: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Kathrin Jeltsch: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data, project administration. H. Serap Sivri: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. Sven F. Garbade: analysis or interpretation of data; additional contributions: formal analysis, methodology, software, visualization. Àngels García-Cazorla: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data, study concept or design; additional contributions: writing-original draft, project administration. Thomas Opladen: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data, study concept or design; additional contributions: writing-original draft, project administration.

AFFILIATIONS

¹Inborn Errors of Metabolism Unit, Department of Neurology, Institut de Recerca Sant Joan de Déu and CIBERER-ISCIH, Barcelona, Spain

²Center for Pediatric and Adolescent Medicine Department I, Division of Pediatric Neurology and Metabolic Medicine, Heidelberg University, Medical Faculty Heidelberg, Heidelberg, Germany

³Bioinformatics and Medical Statistics Group, University of Vic-Central University of Catalonia, Vic, Spain

⁴First Department of Pediatrics, Aghia Sofia Hospital, University of Athens, Athens, Greece

⁵Division of Biochemical Genetics, Department of Pediatrics, BC Children's Hospital, University of British Columbia, Vancouver, British Columbia, Canada

⁶St. Josef-Hospital, University Children's Hospital, Ruhr-University Bochum, Bochum, Germany

⁷Department of Pediatrics and Adolescent Medicine, The Hong Kong Children's Hospital, Hong Kong, Hong Kong

⁸Unit of Inherited Metabolic Diseases and Child Neurology, Department of Pediatrics, Hospital Germans Trias i Pujol, Badalona and Faculty of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

⁹Division of Pediatric Metabolism, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Turkey

¹⁰The Hospital for Sick Children, Toronto, Ontario, Canada

¹¹Department of Medical Genetics, Faculty of Medicine and Dentistry, Women and Children's Health Research Institute, Stollery Children's Hospital, University of Alberta, Edmonton, Alberta, Canada

¹²Clinic of Neurology and Psychiatry for Children and Youth, School of Medicine, University of Belgrade, Belgrade, Serbia

¹³Unit of Child Neurology and Psychiatry, Department of Human Neuroscience, Università degli Studi di Roma La Sapienza, Rome, Italy

¹⁴Institute of Human Genetics, Heidelberg University, Medical Faculty Heidelberg, Heidelberg, Germany

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CONFLICT OF INTEREST STATEMENT

Natalia Alexandra Julia-Palacios has received support for attending meetings from PTC therapeutics. Roser Pons has served as advisory board member and has received honoraria or research support from PTC Therapeutics, Genesis and Novartis Gene Therapy and serves as consultant from Teva and Neurocrine. Elisenda Cortès-Saladelafont has received honoraria from Takeda and Lucane Pharma as a speaker, for lectures and travel and congress expenses. Angels García-Cazorla has received honoraria for lectures from PTC Therapeutics International GT, Immedica, Recordati Rare Diseases Foundation and Nutricia, she is a co-founder of the Hospital Sant Joan de Déu start-up “Neuroprotect Life Sciences”. Thomas Opladen received travel support from GC Pharma, South Korea. The authors confirm independence from the sponsors; the content of the article has not been influenced by the sponsors. Oya Kuseyri Hübschmann, Mireia Olivella, Gabriella Horvath, Thomas Lücke, Cheuk-Wing Fung, Suet-Na Wong, M. Mar Rovira-Remisa, Yilmaz Yıldız, Saadet Mercimek-Andrews, Birgit Assmann, Galina Stevanović, Filippo Manti, Heiko Brennenstuhl, Sabine Jung-Klawitter, Kathrin Jeltsch, H. Serap Sivri and Sven F. Garbade declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data sets in the registry are not publicly available. Data is owned by the members of the iNTD and its availability for specific research purposes is subject to the consent of the iNTD executive board and members. All requests for raw and analyzed data for specific research purposes will be reviewed by the iNTD executive board and respective iNTD members within 72 h.

ETHICS STATEMENT


iNTD registry study was approved by the Institutional Research Ethics Board (IRB) Heidelberg University Hospital (S-471/2014) on December 22, 2014, and subsequently by all contributing centers. All procedures followed were in accordance with the Helsinki

Declaration of 1975, as revised in 2013. Written informed consent was obtained from all study patients or their legal guardians. This study was approved by all participating iNTD members. iNTD is an independent academic organization without any involvement or sponsorship from industry.

INFORMED CONSENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study. Proof that informed consent was obtained must be available upon request.

ORCID

Natalia Alexandra Julia-Palacios  <https://orcid.org/0000-0002-2560-3970>

Oya Kuseyri Hübschmann  <https://orcid.org/0000-0001-6576-6566>

Mireia Olivella  <https://orcid.org/0000-0002-6035-3399>

Roser Pons  <https://orcid.org/0000-0001-6749-1906>

Gabriella Horvath  <https://orcid.org/0000-0003-2573-2489>

Cheuk-Wing Fung  <https://orcid.org/0000-0002-0105-1673>

Elisenda Cortès-Saladelafont  <https://orcid.org/0000-0002-8604-2230>

Saadet Mercimek-Andrews  <https://orcid.org/0000-0001-8396-6764>

Birgit Assmann  <https://orcid.org/0000-0003-0458-953X>

Filippo Manti  <https://orcid.org/0000-0002-2012-8410>

Heiko Brennenstuhl  <https://orcid.org/0000-0002-6909-0003>

Sabine Jung-Klawitter  <https://orcid.org/0000-0001-8851-672X>

H. Serap Sivri  <https://orcid.org/0000-0001-8260-9984>

Sven F. Garbade  <https://orcid.org/0000-0002-7420-4757>

Thomas Opladen  <https://orcid.org/0000-0003-4349-7662>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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