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Defining the clinical, molecular and imaging spectrum of adaptor protein complex 4-associated hereditary spastic paraplegia

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Bi-allelic loss-of-function variants in genes that encode subunits of the adaptor protein complex 4 (AP-4) lead to prototypical yet poorly understood forms of childhood-onset and complex hereditary spastic paraplegia: SPG47 (*AP4B1*), SPG50 (*AP4M1*), SPG51 (*AP4E1*) and SPG52 (*AP4S1*). Here, we report a detailed cross-sectional analysis of clinical, imaging and molecular data of 156 patients from 101 families. Enrolled patients were of diverse ethnic backgrounds and covered a wide age range (1.0–49.3 years). While the mean age at symptom onset was 0.8 ± 0.6 years [standard deviation (SD), range 0.2–5.0], the mean age at diagnosis was 10.2 ± 8.5 years (SD, range 0.1–46.3). We define a set of core features: early-onset developmental delay with delayed motor milestones and significant speech delay (50% non-verbal); intellectual disability in the moderate to severe range; mild hypotonia in infancy followed by spastic diplegia (mean age: 8.4 ± 5.1 years, SD) and later tetraplegia (mean age: 16.1 ± 9.8 years, SD); postnatal microcephaly (83%); foot deformities (69%); and epilepsy (66%) that is intractable in a subset. At last follow-up, 36% ambulated with assistance (mean age:

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8.9 ± 6.4 years, SD) and 54% were wheelchair-dependent (mean age: 13.4 ± 9.8 years, SD). Episodes of stereotypic laughing, possibly consistent with a pseudobulbar affect, were found in 56% of patients. Key features on neuroimaging include a thin corpus callosum (90%), ventriculomegaly (65%) often with colpocephaly, and periventricular white-matter signal abnormalities (68%). Iron deposition and polymicrogyria were found in a subset of patients. *AP4B1*-associated SPG47 and *AP4M1*-associated SPG50 accounted for the majority of cases. About two-thirds of patients were born to consanguineous parents, and 82% carried homozygous variants. Over 70 unique variants were present, the majority of which are frameshift or nonsense mutations. To track disease progression across the age spectrum, we defined the relationship between disease severity as measured by several rating scales and disease duration. We found that the presence of epilepsy, which manifested before the age of 3 years in the majority of patients, was associated with worse motor outcomes. Exploring genotype-phenotype correlations, we found that disease severity and major phenotypes were equally distributed among the four subtypes, establishing that SPG47, SPG50, SPG51 and SPG52 share a common phenotype, an 'AP-4 deficiency syndrome'. By delineating the core clinical, imaging, and molecular features of AP-4-associated hereditary spastic paraplegia across the age spectrum our results will facilitate early diagnosis, enable counselling and anticipatory guidance of affected families and help define endpoints for future interventional trials.

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Abbreviations: AED = anti-epileptic drugs; AP-4 = adaptor protein complex 4; HSP = hereditary spastic paraplegia; MAS = Modified Ashworth Scale; SPRS = spastic paraplegia rating scale

Introduction

The hereditary spastic paraplegias (HSP) are a group of more than 80 neurodegenerative diseases that lead to progressive neurological decline (Blackstone, 2018). Collectively the HSPs present the most common cause of inherited spasticity and associated disability. Bi-allelic loss-of-function variants in genes that encode subunits of the adaptor protein complex 4 (AP-4) lead to prototypical yet poorly understood forms of complex HSP in children, called AP-4-associated HSP (AP-4-HSP) (Verkerk *et al.*, 2009; Abou Jamra *et al.*, 2011; Moreno-De-Luca *et al.*, 2011; Ebrahimi-Fakhari *et al.*, 2018*a*). This includes four different conditions: SPG47 (*AP4B1*, OMIM #614066), SPG50 (*AP4M1*, OMIM #612936), SPG51 (*AP4E1*, OMIM #613744), and SPG52 (*AP4S1*, OMIM #614067). The molecular mechanism in all four diseases is a loss-of-function of the AP-4; hence they are thought to share a similar clinical phenotype.

AP-4 belongs to a family of adaptor proteins (AP-1–AP-5) that are evolutionarily conserved heterotetrameric protein complexes. The adaptor protein complexes facilitate the selective incorporation of transmembrane cargo proteins into vesicles and mediate their intracellular trafficking. AP-4 is composed of four subunits (β4, ε, μ4 and σ4) that form an obligate complex (Hirst *et al.*, 1999). AP-4 is known to mediate protein trafficking from the trans-Golgi network to early (Burgos *et al.*, 2010; Toh *et al.*, 2017) and late endosomes (Aguilar *et al.*, 2001). The autophagy protein ATG9 has emerged as an important cargo of AP-4 and mislocalization of ATG9A is a robust indicator of AP-4 deficiency including in patient-derived fibroblasts and induced pluripotent stem cell-derived neurons (Behne *et al.*, 2020). While the molecular pathology of AP-4 deficiency remains largely unknown, AP-4-HSP is becoming an increasingly recognized form of complex HSP with a thin corpus callosum (da Graca *et al.*, 2018). Published reports, however, consist of single case reports or small case series only, and no studies have systematically delineated the spectrum of the disease or its progression.

Here, we report a detailed cross-sectional analysis of clinical, radiographic, and molecular features of 156 patients from 101 families with AP-4-associated HSP. We define a core set of clinical and imaging features, delineate disease manifestations and progression in a standardized manner, and describe the molecular spectrum.

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Materials and methods

Patients with AP-4-HSP

To systematically document the clinical presentation and natural history of AP-4-related HSP we developed an international cohort (www.CureAP4.org). Inclusion criteria include a clinical diagnosis of AP-4-HSP and the presence of bi-allelic variants in *AP4B1*, *AP4M1*, *AP4E1* or *AP4S1*. By 15 July 2019, 156 patients from 101 families were enrolled. A few patients were identified through the DECIPHER (Firth *et al.*, 2009) and CentoMD (Trujillano *et al.*, 2017) databases and subsequently referred by the treating clinician. Written informed consent for participation was obtained. For patients who were lost to follow-up, a waiver of consent was used. This study was approved by the Institutional Review Board at Boston Children's Hospital (#10-02-0053 and #A00033016-1).

Clinical data

A cross-sectional analysis of patients enrolled in the AP-4-HSP Registry was undertaken using the AP-4-HSP Natural History Study Questionnaire (Supplementary material). For 46 previously reported patients from 23 families (Verkerk *et al.*, 2009; Moreno-De-Luca *et al.*, 2011; Najmabadi *et al.*, 2011; Kong *et al.*, 2013; Lamichhane, 2013; Tuysuz *et al.*, 2014; Hardies *et al.*, 2015; Karaca *et al.*, 2015; Langouet *et al.*, 2015; Tan *et al.*, 2016; Tessa *et al.*, 2016; Accogli *et al.*, 2018; Ebrahimi-Fakhari *et al.*, 2018*b*), no follow-up data were available, and here a detailed review of published information was conducted. The Spastic Paraplegia Rating Scale (SPRS) (Schule *et al.*, 2006) was used to assess 37 patients, and total and spasticity subscores (Karle *et al.*, 2013) were calculated. The Modified Ashworth Scale (MAS) was used in 28 patients. A SPATAX-EUROSPA Disability Score (Chrestian *et al.*, 2017), ranging from 0 (no disability) to 7 (severe disability/confined to bed), and a Four Stage Functional Mobility Score (1 = mild symptoms walking without an aid; 2 = walking without aid but unable to run; 3 = walking with aid; and 4 = wheelchair-dependent) (Erichsen *et al.*, 2009) were assigned for 39 and 85 patients, respectively. Brain imaging was performed on 104 patients.

Genetic testing

In the majority of cases, clinical exome sequencing was employed to detect variants in *AP4B1*, *AP4M1*, *AP4E1* and *AP4S1* (75%). A multi-gene panel (9%) or Sanger sequencing (14%) was performed in a subset of patients. A chromosomal microarray detected microdeletions covering *AP4E1* in four patients. Variants were annotated using the gnomAD database (http://gnomad.broadinstitute.org) and interrogated *in silico* to predict damaging effects by calculating Combined Annotation Dependent Depletion (CADD) scores (Kircher *et al.*, 2014).

Statistical analysis

Statistical analysis was performed with GraphPad Prism version 6.0 (GraphPad Software, Inc.) and IBM SPSS Statistics Version 25. Descriptive statistics are provided for demographic and clinical variables. Frequency count and percentages are provided for categorical variables. Mean, standard deviation (SD), and ranges were calculated for continuous variables. For data shown in Fig. 5F and G, a linear (red line) and non-linear (blue line) regression analysis were performed, and a Pearson correlation coefficient was calculated for linear regression. One-way ANOVA with Tukey correction (data shown in Fig. 5I and J) or an unpaired *t*-test (data shown in Fig. 5K) were used to determine the significance of differences between conditions. $P < 0.05$ was considered significant, denoted with an asterisk.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Results

Detailed clinical characterization of AP-4-HSP defines a set of core clinical features

Demographic and anthropometric features

This study includes 156 patients from 101 families with AP-4-HSP; 83 patients are reported here for the first time, and new or follow-up data are reported on 27 previously published patients (Fig. 1A and Supplementary Fig. 1). All patients had a genetically confirmed diagnosis of AP-4-HSP. Demographic data are summarized in Fig. 1, and clinical data are presented on a cohort level in Table 1 and on an individual level in Supplementary Fig. 1. Patients were recruited from over 50 medical centres with the majority of families based in Europe (32%), the Middle East (25%), North America (15%) and North Africa (15%) (Supplementary Table 1 for details). In our cohort, *AP4M1*-associated SPG50 was the most common subtype, accounting for about 38% of patients, closely followed by *AP4B1*-associated SPG47 in 34% of patients (Fig. 1A). *AP4S1*-associated SPG51 and *AP4E1*-associated SPG52 were less common (15% and 13%, respectively, Fig. 1A). The mean age at last follow-up was 11.4 ± 8.3 years (SD) while the mean age at diagnosis was 10.2 ± 8.5 years (SD) (Fig. 1B and Table 2). The latter varied considerably, ranging from 1 month to 46 years, and in general patients diagnosed since 2015 were diagnosed at a younger age due to the increased availability of multi-gene panels and exome sequencing. When compared to the age at onset of symptoms, a significant delay in diagnosis was found, averaging around 10 years (Table 2). Consanguinity was reported in two-thirds of patients although this varied between subtypes. More than one family member was affected in 67% of patients (Table 2). No aggregation or association with other neurological disease was found upon assessment of a three-generation family history. Patients originated from 30 countries and were of different ethnic backgrounds (Fig. 1C, D and Supplementary

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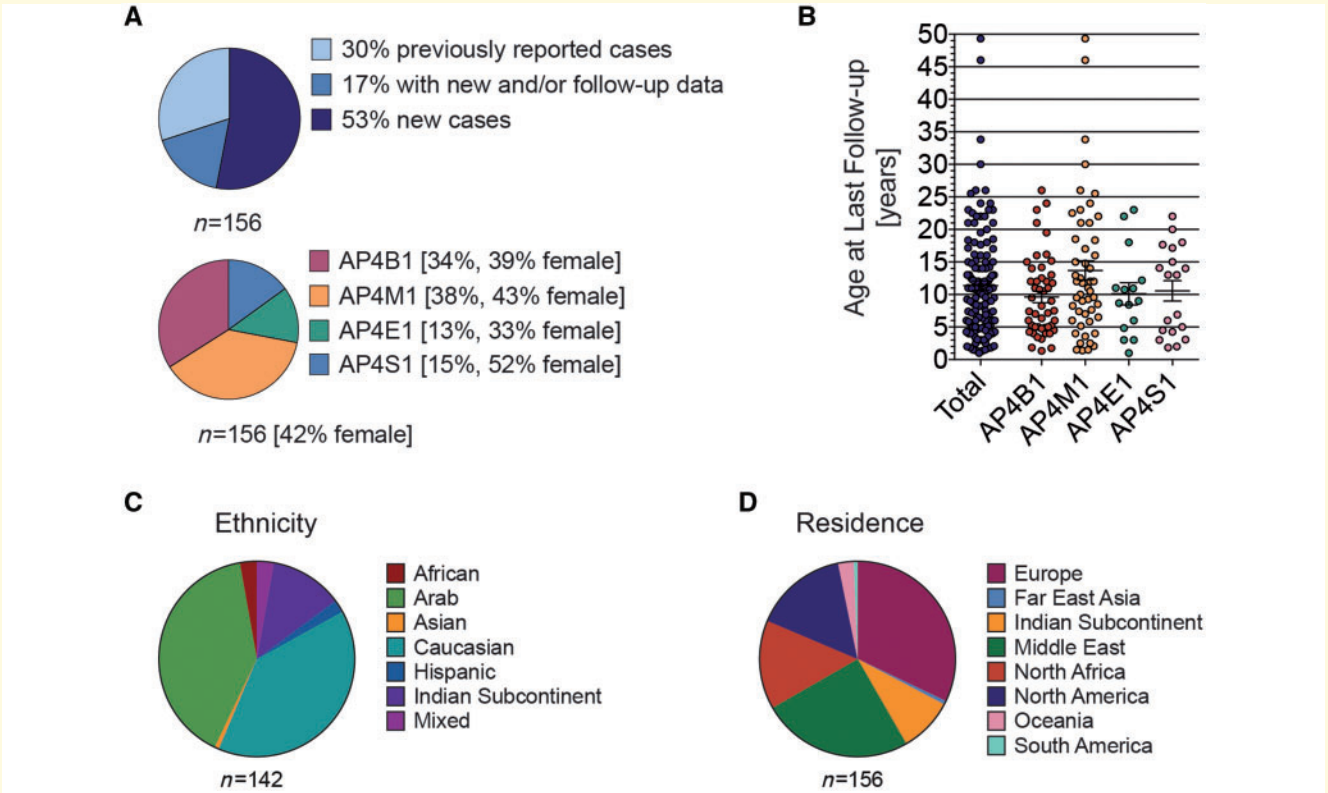


Figure 1 Demographic data of the study cohort. (**A**) A total of 156 patients were enrolled in the study, the majority carrying bi-allelic variants in *AP4B1* and *AP4M1*. (**B**) Most patients were under the age of 18 years at the last follow-up with only a few adult patients reported. (**C** and **D**) Enrolled patients cover all major ethnicities with the majority being Caucasian or of Arab descent. Most families are located in Europe, the Middle East, North America and North Africa.

Table 1). This includes, although is not limited to, regions with high rates of consanguinity.

Clinical features were assessed using a standardized questionnaire (Supplementary material) developed for the purpose of this study. A set of core clinical and imaging features is defined based on their presence in more than 50% of individuals with AP-4-HSP (Box 1). Most patients were born at term (92%). There was no aggregation of any specific prenatal or neonatal complications although ventriculomegaly was detected prenatally in eight patients, and hypotonia and poor feeding in the neonatal period were noted in 16% of patients. Fifteen per cent of patients required admission to a neonatal intensive care unit. Average birth weight across all patients was 3.10 ± 0.46 kg (SD). Review of anthropometric data at the last follow-up showed an average height at −1.69 SD (range −5.76 to +2.45 SD) and weight at −0.53 SD (range −4.8 to +5.0), respectively, according to sex- and age-appropriate CDC growth charts (Table 1 and Fig. 2A). A subset of patients showed significant growth failure with a height (35%) and/or weight (22%) below the −2 SD mark. Postnatal microcephaly has been described in AP-4-HSP (Verkerk *et al.*, 2009; Abou Jamra *et al.*, 2011; Hardies *et al.*, 2015; Ebrahimi-Fakhari *et al.*, 2018*b*), and we found postnatal

Box 1 Principal clinical and radiographic features of AP-4-HSP

Principal clinical features of AP-4-HSP	
Developmental delay / intellectual disability	100%
Motor delay	100%
Speech delay	99%
Mild neonatal or infantile hypotonia	89%
Spasticity	97%
Spastic diplegia	54%
Spastic tetraplegia	43%
Hyperreflexia	92%
Babinski sign	88%
Contractures	50%
Drooling	70%
Postnatal microcephaly	83%
Dysmorphic facial features	78%
Foot deformities	69%
Febrile seizures	62%
Epilepsy	66%
Episodes of stereotypic laughter	56%
Principal radiographic features of AP-4-HSP	
Thin corpus callosum	90%
Ventriculomegaly	65%
White matter loss/changes	68%

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Table 1 Summary of clinical features of AP-4-HSP

Anthropometric data			
Height	−1.69 ± 1.63 (mean, SD) [range −5.76 to +2.45, *n* = 79]		
Weight	−0.53 ± 1.77 (mean, SD) [range −4.8 to +5.0, *n* = 78]		
Head circumference	−2.80 ± 1.36 (mean, SD) [range −7.2 to +0.25, *n* = 114]		
Dysmorphology			
Microcephaly (*n* = 101/122): 83%	*AP4B1*: 84%, *n* = 36/43	*AP4M1*: 85%, *n* = 41/48	
	AP4E1: 85%, *n* = 11/13	*AP4S1*: 72%, *n* = 13/18	
Dysmorphic facial features (*n* = 78/100): 78%	*AP4B1*: 84%, *n* = 31/37	*AP4M1*: 66%, *n* = 23/35	
	AP4E1: 82%, *n* = 9/11	*AP4S1*: 88%, *n* = 15/17	
Development			
Developmental delay / intellectual disability (*n* = 135/135): 100%	*AP4B1*: 100%, *n* = 50/50	*AP4M1*: 100%, *n* = 51/51	Severe: 58%
	AP4E1: 100%, *n* = 15/15	*AP4S1*: 100%, *n* = 19/19	Moderate: 33%
			Mild: 9%
Developmental regression or progressive cognitive deficits (*n* = 39/95): 41%	*AP4B1*: 26%, *n* = 9/35	*AP4M1*: 51%, *n* = 20/39	
	AP4E1: 50%, *n* = 4/8	*AP4S1*: 46%, *n* = 6/13	
Age at onset of developmental delay (*n* = 93, months): 9.2 ± 7.5 (SD)	*AP4B1*: 6.9 ± 4.6 (SD), range: 2–24, *n* = 34	*AP4M1*: 11.3 ± 9.5 (SD), range 3–60, *n* = 42	
	AP4E1: 8.2 ± 3.1 (SD), range 3–12, *n* = 6	*AP4S1*: 9.2 ± 6.1 (SD), range 3–24, *n* = 11	
Delayed motor development (*n* = 132/132): 100%	*AP4B1*: 100%, *n* = 46/46	*AP4M1*: 100%, *n* = 49/49	
	AP4E1: 100%, *n* = 18/18	*AP4S1*: 100%, *n* = 19/19	
Delayed speech development (*n* = 124/125): 99%	*AP4B1*: 98%, *n* = 42/43	*AP4M1*: 100%, *n* = 49/49	
Non-verbal: 50%, (*n* = 54/109)	*AP4E1*: 100%, *n* = 15/15	*AP4S1*: 100%, *n* = 18/18	
Shy character (*n* = 31/96): 32%	*AP4B1*: 35%, *n* = 12/34	*AP4M1*: 32%, *n* = 12/38	
	AP4E1: 25%, *n* = 3/12	*AP4S1*: 33%, *n* = 4/12	
Stereotypic laughter (56/100): 56%	*AP4B1*: 53%, *n* = 19/36	*AP4M1*: 50%, *n* = 20/40	
	AP4E1: 82%, *n* = 9/11	*AP4S1*: 62%, *n* = 8/13	
Motor symptoms			
Neonatal hypotonia (*n* = 97/109): 89%	*AP4B1*: 87%, *n* = 39/45	*AP4M1*: 90%, *n* = 35/39	
	AP4E1: 100%, *n* = 10/10	*AP4S1*: 87%, *n* = 13/15	
Neonatal hypotonia progressing to spasticity (*n* = 104/116): 90%	*AP4B1*: 88%, *n* = 38/43	*AP4M1*: 92%, *n* = 43/47	
	AP4E1: 100%, *n* = 10/10	*AP4S1*: 81%, *n* = 13/16	
Spasticity (*n* = 130/134): 97%	*AP4B1*: 96%, *n* = 46/48	*AP4M1*: 98%, *n* = 50/51	
	Spastic diplegia: 61%	Spastic diplegia: 47%	
	Spastic tetraplegia: 35%	Spastic tetraplegia: 51%	
	AP4E1: 100%, *n* = 16/16	*AP4S1*: 95%, *n* = 18/19	
	Spastic diplegia: 50%	Spastic diplegia: 63%	
	Spastic tetraplegia: 50%	Spastic tetraplegia: 31%	
Hyperreflexia (*n* = 97/106)	92%		
Babinski sign (*n* = 80/91)	88%		
Drooling (*n* = 64/91)	70%		
Contractures (*n* = 49/98)	50%		
Foot deformity (*n* = 68/99)	69%		
Extrapyramidal movement disorders (*n* = 33/79)	42%		
Cerebellar signs (*n* = 26/76)	34%		
Swallowing dysfunction / aspiration (*n* = 18/63)	29%		
Seizures			
Seizures (*n* = 84/126): 67%	*AP4B1*: 65%, *n* = 32/49	*AP4M1*: 70%, *n* = 33/47	Focal: 29%
			Generalized: 63%
	AP4E1: 67%, *n* = 8/12	*AP4S1*: 61%, *n* = 11/18	Focal + generalized: 8%
Febrile seizures (*n* = 74/120): 62%	*AP4B1*: 69%, *n* = 31/45	*AP4M1*: 60%, *n* = 27/45	
	AP4E1: 36%, *n* = 4/11	*AP4S1*: 63%, *n* = 12/19	
Epilepsy (*n* = 69/105): 66%	*AP4B1*: 76%, *n* = 28/37	*AP4M1*: 62%, *n* = 24/39	
	AP4E1: 67%, *n* = 8/12	*AP4S1*: 53%, *n* = 9/17	
Status epilepticus (*n* = 24/65): 37%	*AP4B1*: 48%, *n* = 14/29	*AP4M1*: 24%, *n* = 6/25	
	AP4E1: 40%, *n* = 2/5	*AP4S1*: 33%, *n* = 2/6	
Response to AEDs (*n* = 64)	Complete 69%		
	Incomplete 31%		
Therapy with multiple AED or medically-refractory epilepsy (*n* = 67)	27%		

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Table 2 AP-4-HSP: summary of key demographic features and stratification based on genotype

AP-4-HSP Subtype	Age at onset, years	Age at diagnosis, years	Age at last visit, years	Consanguinity	Familial case
AP4B1-HSP: *n* = 53	0.6 ± 0.4 (*n* = 34)	7.8 ± 6.9 (*n* = 31)	9.6 ± 6.1 (*n* = 48)	60% (*n* = 50)	55% (*n* = 53)
AP4M1-HSP: *n* = 59	0.9 ± 0.8 (*n* = 42)	11.7 ± 10.0 (*n* = 44)	13.7 ± 10.6 (*n* = 50)	77% (*n* = 52)	78% (*n* = 59)
AP4E1-HSP: *n* = 21	0.7 ± 0.3 (*n* = 6)	10.3 ± 6.7 (*n* = 14)	10.1 ± 6.6 (*n* = 15)	90% (*n* = 20)	86% (*n* = 21)
AP4S1-HSP: *n* = 23	0.8 ± 0.5 (*n* = 11)	10.4 ± 7.3 (*n* = 13)	10.6 ± 6.8 (*n* = 19)	47% (*n* = 19)	48% (*n* = 23)
Total: *n* = 156	0.8 ± 0.6 (*n* = 93)	10.2 ± 8.5 (*n* = 102)	11.4 ± 8.3 (*n* = 132)	69% (*n* = 141)	67% (*n* = 156)

microcephaly in 83% of patients, while only a small subset was born microcephalic (8%). Microcephaly was mostly in a moderate range, in 50% patients between −2 SD and −4 SD, yielding an overall average of −2.80 SD (range −7.2 to +0.25, Fig. 2A). Dysmorphic facial features were described by clinicians in 78% of patients and included a broad range of features including a wide nasal bridge, bulbous nose, short philtrum and epicanthal folds in some. No uniform *gestalt* was discernible, however. Of note, a significant subset of patients (69%) were found to have foot deformities, most commonly pes equinovarus (54%), pes planus (22%) or deformities such as syndactyly (4%) or macrodactyly (4%) (Fig. 2B).

Developmental history and cognitive function

Most patients presented to medical attention because of early-onset global developmental delay. Delayed developmental milestones were noticed at an average age of 9.2 ± 7.5 months (SD) months (range: 2–60 months) (Table 1). Motor milestones were delayed with unsupported sitting (mean age: 17 ± 11 months, SD), crawling (mean age: 23 ± 11 months, SD), and standing (mean age: 34 ± 19 months, SD) achieved late (Table 1 and Fig. 2C). Eighty-five per cent of patients achieved walking with assistance at an average age of 37 ± 21 months (SD). Unsupported walking was achieved in a subset of patients (43%), a skill that was lost over time in most with only 11% ambulating independently at last follow-up. Thirty-six per cent of patients were able to walk with assistance at last follow-up, and 54% of patients required a wheelchair for any substantial distance. The latter occurred at an average age of 9.0 ± 5.3 years (SD) with the mean age of wheelchair-dependent patients being 13.4 ± 9.8 years (SD) at last follow-up. Fine motor skills were also universally delayed although not systematically assessed in this study. Speech development was prominently delayed in nearly all patients, and 50% remained non-verbal (Table 1). Behavioural problems including inattention and hyperactivity were frequently reported. Common character traits or behaviours reported previously in AP-4-HSP include a shy character (Abou Jamra *et al.*, 2011) and stereotypic episodes of laughter (Ebrahimi-Fakhari *et al.*, 2018*b*). We failed to identify a high rate of shyness in our cohort (32%), and many patients were reported to be quite social. Episodes of stereotypic laughter were found in a subset of patients although they did not seem to be a universal feature (56%). In older patients, intellectual disability was usually in the moderate to severe range. Loss of previously acquired skills or developmental regression and cognitive decline was reported in 41% of patients (Table 1).

Motor symptoms and movement disorders

Progressive spasticity is a key clinical feature of AP-4-HSP (Fig. 2D). The first motor symptom, however, was usually truncal and appendicular hypotonia, which was noted in the neonatal period or infancy in 89% of patients and was usually mild. Over time, muscle tone increased in the distal lower extremities leading to spastic diplegia (54% of patients, mean age: 8.4 ± 5.1 years, SD) with progression to spastic tetraplegia (43% of patients) in late childhood/adolescence (mean age: 16.1 ± 9.8 years, SD) (Fig. 2D). Along with spasticity, other pyramidal signs in a similar distribution were noted and included hyperreflexia and a Babinski sign (Fig. 2D and Table 1). Contractures developed over time and mainly involved the ankles and knees. Extrapyramidal movement disorders were less common although ataxia, dystonia and others were found in a subset of patients (Fig. 2E). Besides cerebellar ataxia, other cerebellar signs included dysarthria, nystagmus and dysdiadochokinesia in a small group of patients (Fig. 2F). Progressive motor symptoms were associated with swallowing dysfunction and sialorrhea. Aspiration with associated respiratory complications occurred in patients with advanced disease.

Seizures and epilepsy

Seizures (67%) including unprovoked seizures and seizures in the setting of fever were common in AP-4-HSP patients and 66% of patients qualified for a diagnosis of epilepsy based on the occurrence of two unprovoked seizures or seizures that required treatment with anti-epileptic drugs (AEDs) (Fig. 2G–I and Table 1). Seizure onset occurred in the first 3 years of life, including in infancy, though usually not in the neonatal period. Of note, a significant number of patients presented with status epilepticus (37%), often with their first febrile or unprovoked seizure (Fig. 2I). Severe or medically refractory epilepsy, defined as persistence of seizures despite therapy with two AEDs, was only seen in 27% (Table 1). The majority of patients experienced a significant reduction of seizures or complete cessation of seizures with standard AEDs (Table 1). Levetiracetam and valproate were the AED most commonly

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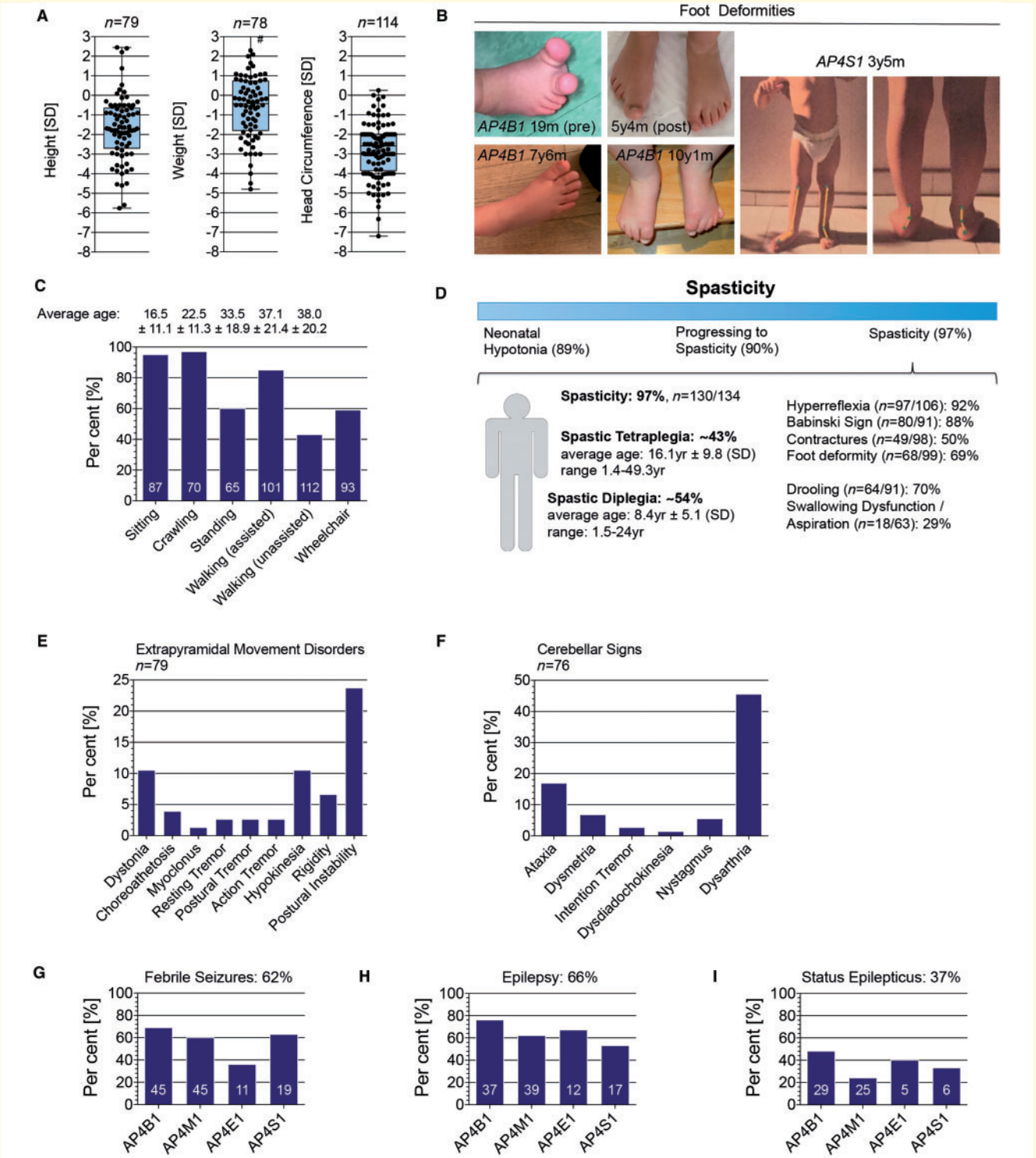


Figure 2 Key clinical features in AP-4-HSP. (**A**) Distribution of height, weight and head circumference according to sex- and age-appropriate CDC growth charts. A subset of patients shows significant growth failure with a height and/or weight under the −2 SD mark (hash symbol indicates two patients with a weight above +3 SD). Postnatal microcephaly is found in the majority of patients, most commonly in a moderate range between −2 SD and −4 SD. (**B**) Foot deformities may include macrodactyly (*top*, macrodactyly of the right foot shown before and after surgical debulking), and pes equinovarus as well as pes planus. (**C**) Developmental delay is a universal feature and motor milestones are often prominently delayed. The bar graph shows the percentage of patients who achieved a given motor milestone. The average age at which each milestone was achieved is shown above the graph. White numbers on bars indicate the number of individuals for whom information was available. (**D**) Motor symptoms evolve from mild hypotonia in infancy to spastic diplegia and later tetraplegia. Progressive spasticity is a hallmark feature of AP-4-HSP and accounts for a large part of the morbidity. (**E** and **F**) Extrapyramidal movement disorders and cerebellar signs are found in a subset of patients, often with advanced disease. (**G** and **H**) Seizures in AP-4-HSP include frequent seizures in the setting of fever as well unprovoked seizures leading to a diagnosis of epilepsy in about two-thirds of patients. (**I**) About 40% of all patients present with at least one episode of status epilepticus, often with their first seizure. White numbers on bars indicate the number of individuals for whom information was available.

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used although others were used as well and no single AED seemed specifically effective. Both focal and primary generalized seizures were common (Table 1). Seizure semiology was variable, and generalized seizures were tonic-clonic (51%), tonic (13%) or myoclonic (16%) in the majority of patients. Structure-related epilepsy occurred in patients with focal brain malformations (see below). Except in these patients and patients with refractory epilepsy during early childhood, seizures became less frequent over time and many patients were able to ultimately stop treatment with AEDs.

Other symptoms

Urinary and stool incontinence were commonly reported, including in older children. A short attention span and decreased perception of hazardous or noxious stimuli were anecdotally reported by several parents.

Treatment and developmental support

Most patients received physical therapy, occupational therapy and speech and language therapy. Depending on the available resources, the majority of children received an individualized education plan. In addition to AEDs, many patients were treated with anti-spasticity agents including oral baclofen or botulinum toxin injections.

Imaging features of AP-4-HSP

MRI characteristics of AP-4-HSP patients are summarized in Fig. 3. Thinning of the corpus callosum was found in the vast majority of patients with AP-4-HSP (90%). Thinning predominantly affected the posterior aspects of the corpus callosum, the splenium (Fig. 3). A complete agenesis of the corpus callosum was not found. Non-specific T_2 signal changes in the supratentorial white matter were common and were mainly present in the periventricular region. Ventriculomegaly was common (65%) and often presented as asymmetric colpocephaly, likely secondary to a loss of periventricular white matter volume. Global cerebral atrophy was seen in some patients (37%), including in toddlers and young children but seemed more common in older patients with significant disease progression (Fig. 3). Cerebellar atrophy was overall uncommon but evident in a subset of patients mainly with advanced disease. Less common imaging findings included symmetric iron deposition in the globus pallidus, found in a single family with *AP4M1*-related SPG50 (Fig. 3 and Roubertie *et al.*, 2018), and one individual with *AP4S1*-related SPG52 (Vill *et al.*, 2017). Three newly diagnosed patients who presented with early-onset seizures had bilateral symmetric polymicrogyria (Fig. 3).

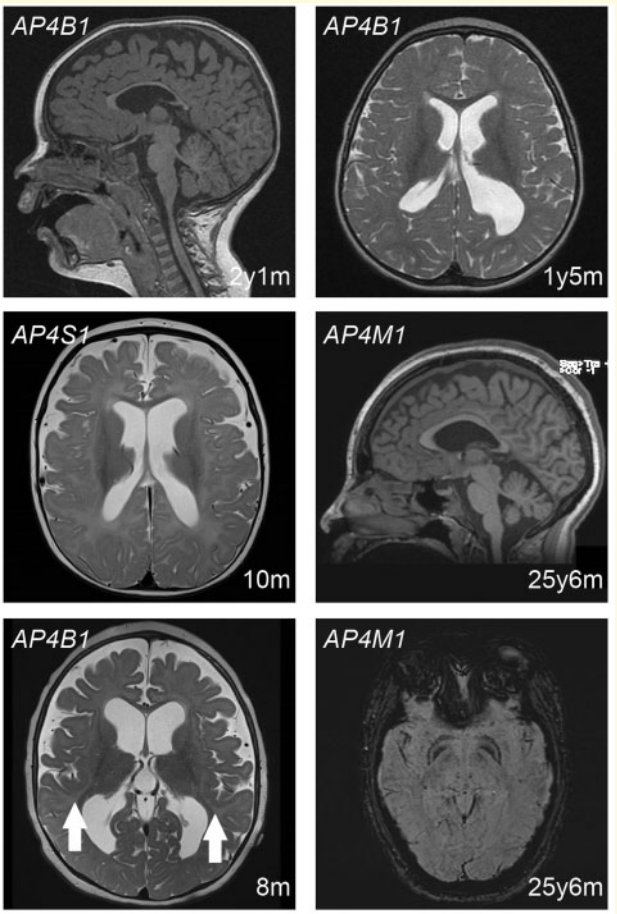


Figure 3 Key imaging features in AP-4-HSP. Brain MR images of AP-4-HSP patients [mean age: 7.4 ± 7.3 years (SD)]. Representative images of key imaging features are shown. *Top left*: Thinning of the corpus callosum, shown here on a sagittal T_1-weighted image, is found in 90% (94/104) of patients and is prominent in the posterior parts. Non-specific white matter abnormalities are noted in 68% (69/102). *Top right*: Ventriculomegaly, shown here on an axial T_2-weighted image, is common (65%, 67/103) and often presents as asymmetric colpocephaly. *Middle left*: Cortical atrophy, shown here on an axial T_2-weighted image, is more commonly found in patients with advanced disease but can be present in young patients as well. Cerebral atrophy is overall found in 37% of cases (38/102). *Middle right*: Cerebellar atrophy, shown here on a sagittal T_1-weighted image, is found in a subset of patients (23%, 20/89). *Bottom left*: Bilateral perisylvian polymicrogryria (arrows), shown on an axial T_2-weighted image. *Bottom right*: Bilateral symmetric hypointensity of the globus pallidus on axial susceptibility-weighted imaging is suggestive of iron accumulation in this previously reported patient with *AP4M1*-associated HSP (Roubertie *et al.*, 2018).

The molecular spectrum of AP-4-HSP

Most patients were identified through exome sequencing and variants in *AP4M1* and *AP4B1* were detected in the majority of families. A total of 75 unique variants were

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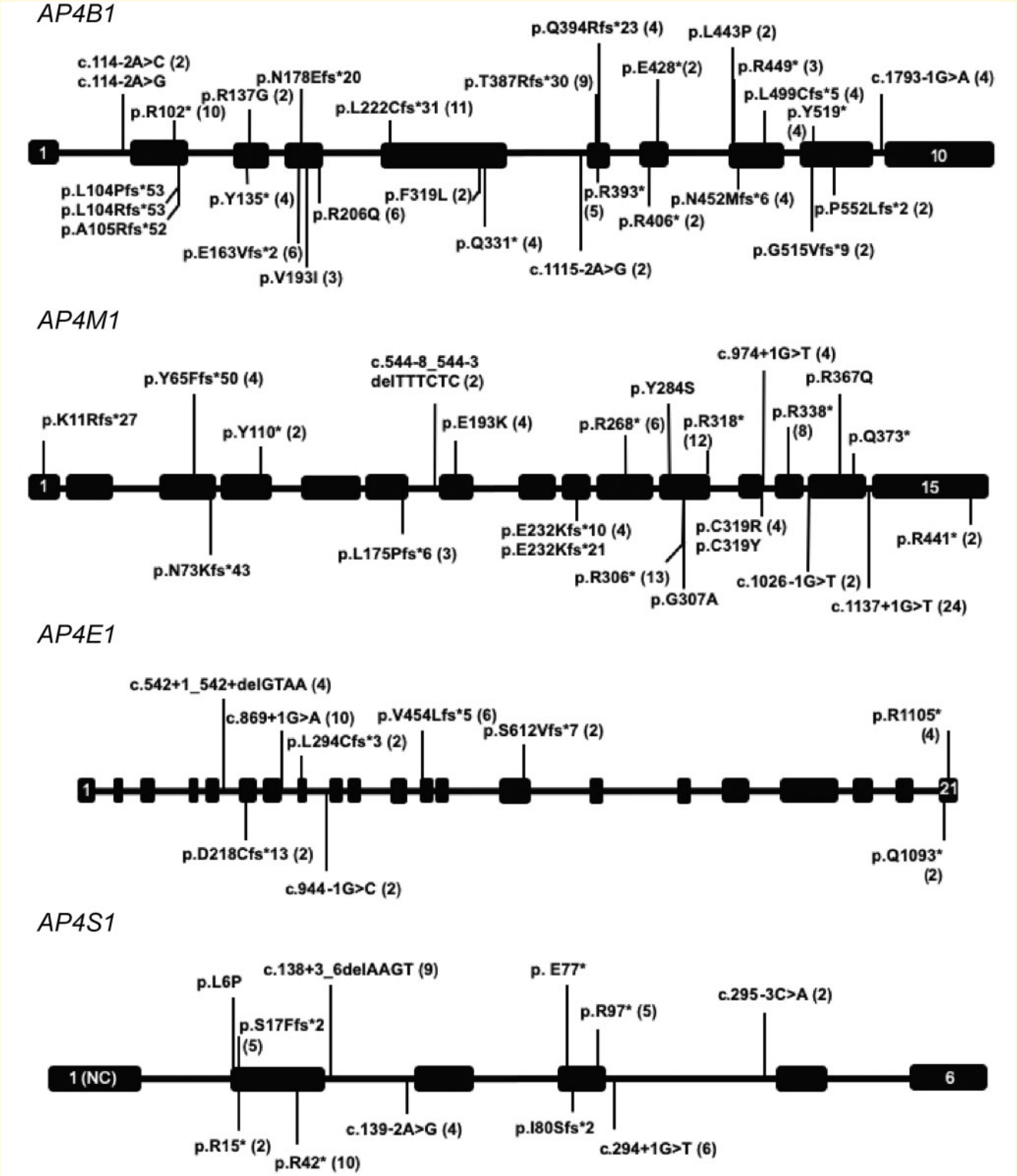


Figure 4 The molecular spectrum of AP-4-HSP. Schematic representation showing the four genes encoding subunits of the AP-4 (*AP4B1*, *AP4M1*, *AP4E1*, *AP4S1*) and the distribution of the reported variants. Parentheses indicate the number of alleles with that variant. Reference sequences: *AP4B1*: NM_001253852.1; *AP4M1*: NM_004722.3; *AP4E1*: NM_007347.4; *AP4S1*: NM_007077.4. NC = non-coding.

identified (Supplementary Table 2 and Fig. 4). All patients carried bi-allelic variants: 82% had homozygous variants, and 18% had compound heterozygous variants. Four patients showed bi-allelic microdeletions. The vast majority of variants were nonsense or frameshifting predicted to result in a truncated protein, and only a small number of

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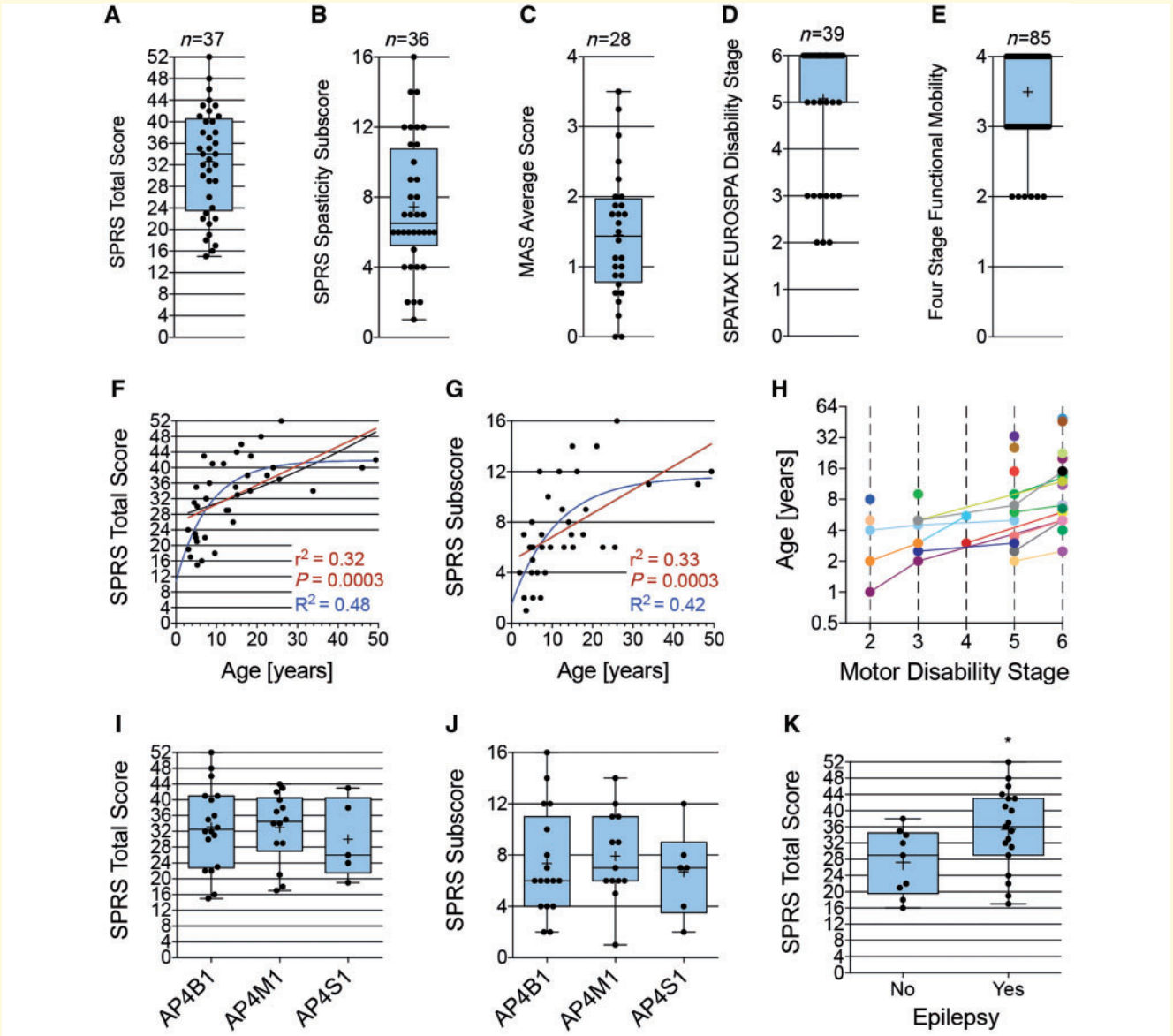


Figure 5 Clinical rating scales and disease progression in AP-4-HSP. Standardized assessment of disease severity using the (**A**) SPRS, (**B**) SPRS spasticity subscore (items 7–10 of the SPRS), (**C**) Modified Ashworth Scale (MAS), (**D**) SPATAX EUROSPA disability score (0 = no functional handicap, 1 = no functional handicap but signs at examination, 2 = mild, able to run, walking unlimited, 3 = moderate, unable to run, limited walking without aid, 4 = severe, walking with one stick, 5 = walking with two sticks or four-wheel walker, 6 = unable to walk, requiring wheelchair, 7 = confined to bed), (**E**) Four Stage Functional Mobility score (1 = mild symptoms walking without an aid; 2 = walking without aid but unable to run; 3 = walking with aid; and 4 = wheelchair dependent). The number of individuals with data available is indicated above each graph. Assessing disease severity across the age spectrum, we found a moderate but significant linear correlation between the (**F**) SPRS total score (n = 37) and (**G**) SPRS spasticity subscore (n = 36) with age as determined by Pearson correlation index for linear regression. The red line indicates a linear regression curve while the blue line indicated a non-linear (exponential) curve. (**H**) Longitudinal assessment of SPATAX-EUROPA disability stages in patients who achieved ambulation (n = 39). Each patient is shown in a different colour with lines between different time points. A general trend towards higher scores, indicating greater motor disability, with increasing age becomes apparent. (**I** and **J**) A subanalysis by genotype showed no significant difference in disease severity by (**I**) SPRS total score and (**J**) SPRS spasticity subscore. (**K**) Significantly higher SPRS scores are present in individuals with epilepsy (unpaired t-test, P = 0.04).

missense variants or splice-site variants were identified. Recurrent variants in *AP4B1* included the two truncating variants p.Arg102* in exon 2 and p.Leu222Cysfs*31 in exon 5 found on 10 and 11 alleles, respectively. Similarly, for *AP4M1* two truncating variants in exon 11 were common as was the c.1137+1G>T variant in a canonical splice site which was found on 24 alleles. Variants in *AP4B1* and *AP4M1* covered most exons while variants in *AP4E1* and *AP4S1* showed a predilection for early truncating variants. *In silico* prediction of pathogenicity using

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CADD scores (Supplementary Table 2) showed high scores for the majority of variants.

Clinical rating scales and disease progression

Standardized assessment of disease severity using rating scales showed a mean SPRS score of 32.6 ± 9.7 (SD, range 15–52) and the majority of patients (62%) had SPATAX disability scores of 6 (range 2–6) (Fig. 5A and D). The spasticity subscale of the SPRS, which is independent of ambulation, showed an average score of 7.4 ± 3.7 (SD, range 1–16) (Fig. 5B). The MAS showed an average score of 1.5 ± 0.9 (SD) (Fig. 5C). The Four Stage Functional Mobility confirmed that the vast majority of patients required assistance with ambulation (=level 3, 36%) or depended on a wheelchair for any significant distance (=level 4, 56%) (Fig. 5E). Assessing disease severity across the age spectrum, we found a moderate but significant correlation between the SPRS total score and SPRS spasticity subscore with age (Fig. 5F and G). This was also reflected in the longitudinal assessment of SPATAX-EUROPA disability stages in patients who achieved ambulation (Fig. 5H). A subanalysis of the four genes involved in AP-4-HSP showed no significant difference in disease severity, exemplified by SPRS total and spasticity subscores (Fig. 5I and J) or major clinical features (Table 1). Of note, we detected significantly higher SPRS scores in individuals with epilepsy (Fig. 5K), a finding that could not be accounted for by a difference in age since the age spectrum of patients with and without epilepsy was greatly overlapping [patients with epilepsy: 9.9 ± 6.3 years (SD) versus patients without epilepsy: 11.1 ± 6.9 years (SD)]. This indicates that the presence or absence of epilepsy, which manifested in the first 3 years of life in the vast majority of patients, is a prognostic indicator of later motor complications.

Discussion

In this study we detail the clinical and molecular signature of AP-4-HSP, establishing this condition as a paradigm of childhood-onset complex HSP associated with defective protein trafficking. Most of the enrolled patients were only diagnosed in the last 3 years, indicating that AP-4-HSP is likely under-recognized. From our data, we conclude that all four subtypes of AP-4-HSP share a common clinical phenotype, an 'AP-4 deficiency syndrome'. The core clinical features that should lead clinicians to suspect AP-4-HSP are summarized in Box 1. The combination of global developmental delay, progressive spasticity, seizures and a thin corpus callosum should raise the suspicion for AP-4-HSP although these features are also common to other forms of HSP (Stevanin *et al.*, 2008; Pensato *et al.*, 2014; Ebrahimi-Fakhari *et al.*, 2016, 2018*a, b*; Kara *et al.*, 2016). Episodes of stereotypic laughter, perhaps indicating a pseudobulbar affect (Wild *et al.*, 2003), are a peculiar and specific finding in a subset of patients and thus may help in establishing a differential diagnosis. Microcephaly, short statue in some and the high prevalence of foot deformities are also features that help distinguish AP-4-HSP from other conditions with developmental delay and spasticity. Prior to the availability of clinical exome sequencing and multi-gene panels that cover the AP-4 subunit genes, some of our patients had received a diagnosis of cerebral palsy. The slowly progressive nature of AP-4-HSP may be mistaken for a static disease course, at least initially, and AP-4-HSP should be added to the list of 'cerebral palsy mimics' (Leach *et al.*, 2014; Appleton and Gupta, 2019; Pearson *et al.*, 2019). The absence of prenatal or neonatal risk factors (i.e. prematurity, infections, birth complications), a history of consanguinity, and the progression of symptoms in mid to late childhood may help to distinguish AP-4-HSP from cerebral palsy clinically. The high prevalence of characteristic imaging findings (a posterior-dominant thinning of the corpus callosum and colpocephaly) may also be important distinguishing features although these have been reported in series of patients with a clinical diagnosis of cerebral palsy in the absence of comprehensive genetic testing (Kwong *et al.*, 2004; Bax *et al.*, 2006; Rana *et al.*, 2016).

In AP-4-HSP, the first reported manifestation is early developmental delay with prominent motor and speech delay. Motor milestones are usually delayed by several months and only a subset of patients ever achieved independent walking. Speech development is delayed, and 50% of patients remain non-verbal. Intellectual disability in older children is typically moderate to severe. Importantly, regression and loss of skills were reported in about half the patients. Anecdotally, loss of skills occurred transiently in young children in the setting of an intercurrent illness or with onset of seizures. However, true regression seems to occur later in patients with more advanced disease.

Delayed development and later regression are paralleled by progressive motor symptoms. Mild truncal hypotonia in infancy is retrospectively reported in the vast majority of patients and often coincides with delayed early motor milestones. Spasticity begins with an often largely symmetric diplegia in late infancy or toddler years. Over time, spasticity progresses and other pyramidal signs become apparent including hyperreflexia and a Babinski sign. Spasticity progresses to cause contractures, mainly of the ankles and knees, and by adolescence often involves the arms, leading to spastic tetraplegia. The average age for patients with spastic tetraplegia was 16.1 ± 9.8 years (SD) and although the youngest reported patient was 1.4 years old, the majority of patients were older than 10 years. This leads us to conclude that significant disease progression occurs between the age of 5 and 16 years. At last follow-up, most patients were able to ambulate with assistance, i.e. with a posterior walker, but about 60% used a wheelchair for significant distances. Of note, in addition to pyramidal symptoms, extrapyramidal movement disorders and cerebellar signs were reported in a subset of patients. Taken together with the finding of cognitive decline, this points to a neurodegenerative process that involves widespread brain

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areas, including the cerebral and cerebellar cortices, basal ganglia and corticospinal tracts. This is supported by the findings of cerebral and cerebellar atrophy on brain imaging in a subset of patients, mainly with advanced disease.

A standardized assessment using the SPRS (Schule *et al.*, 2006) revealed an average score of 33, which is in the moderate range. The SPRS is an established tool to track disease severity in HSP though it is only validated for individuals older than 9 years (Schule *et al.*, 2006). This poses a challenge for our patient population and may limit its interpretation. Nevertheless, a longitudinal assessment using the SPRS may reveal trends of progression and hence the reported values here serve as important baseline values. The SPRS spasticity subscore does not depend on ambulation and hence was included in a subanalysis. This subscore mirrored the total score, and both scores showed a moderate correlation with age, which may reflect disease progression. A stratification based on longitudinal data in future studies will help to define the SPRS as an indicator of disease progression in AP-4-HSP.

Standardized assessment of spasticity using the MAS (Bohannon and Smith, 1987) was available for a subset of patients not allowing us to draw any conclusions for the broader AP-4-HSP population at this point but establishing a starting point for longitudinal studies. The SPATAX-EUROSPA disability score was 6 in the majority of cases reflecting high rates of wheelchair-dependence. The Four Stage Functional Mobility Score confirmed the latter finding. Given the early age of wheelchair-dependence and the a priori lack of independent walking in most patients, both scores do not seem sensitive enough to track disease progress but help establish a level of functionality.

Complications related to progressive spasticity such as contractures, drooling and swallowing dysfunction were reported in patients with advanced disease. The latter, just like the pseudobulbar affect with unprovoked and stereotyped episodes of laughter, might point to involvement of corticobulbar tracts. We failed to detect high rates of sensory deficits (pain sensation, temperature sensation, proprioception, vibration sense) in our cohort but acknowledge that this is often difficult to assess particularly in children with developmental delay or intellectual disability.

Seizures and/or epilepsy are found in the majority of patients with seizure-onset commonly in the first three years of life. Importantly, many patients presented with status epilepticus with their first seizure. Seizures in the setting of fever were frequent in AP-4-HSP patients who both do and do not go on to develop epilepsy. Both focal and generalized seizures were common, and no clear dominant seizure semiology or EEG signature was found. In the majority of patients, seizures responded to standard AEDs and two or more agents were only needed in about a quarter of patients. Anecdotally it appears that seizures become less frequent with age, and in some patients AEDs were discontinued without seizure recurrence. Although polymicrogyria was rarely reported in our cohort (only in three patients), developmental brain malformations should be evaluated for, particularly in AP-4-HSP patients with seizures given the strong association with epilepsy (Leventer *et al.*, 2010; Shain *et al.*, 2013). Of note, the presence of epilepsy was correlated with higher SPRS scores at last follow-up and thus might be predictive of greater disease severity in the long-term.

On brain imaging, thinning of the corpus callosum was almost universal, making AP-4-HSP part of the spectrum of autosomal-recessive and complex HSPs that present with a thin corpus callosum (Kara *et al.*, 2016). The thinning prominently affects the posterior parts of the corpus callosum, the splenium. Ventriculomegaly, seen in the majority of patients, mostly involves the lateral ventricles and often presents as asymmetric colpocephaly. This points to a loss of periventricular white matter, i.e. loss of axons of the posterior corona radiata. Together with the finding of a thin corpus callosum, loss of periventricular volume points to a degeneration of the long projecting neurons, including the interhemispheric, corticobulbar and corticospinal tracts. Bilateral symmetric perisylvian polymicrogyria was found in three families with compound heterozygous variants in *AP4S1* (Patient 98) and *AP4B1* (Patient 86) or homozygous splice-site variants in *AP4S1* (Patient 64), respectively. It is interesting to speculate that AP-4 deficiency could possibly cause abnormalities during late neuronal migration or early cortical organization. The true prevalence of malformations of cortical development remains to be evaluated in larger series and AP-4 variants should be assessed in patients with polymicrogyria of unclear aetiology (Stutterd and Leventer, 2014). Interestingly, symmetric hypointense SWI signal in the globus pallidus, suggestive of iron deposition, was found in a previously reported family with *AP4M1*-related SPG50 and one individual with *AP4S1*-related SPG52 (Vill *et al.*, 2017; Roubertie *et al.*, 2018). Findings suggestive of iron deposition were not discovered in any other patients in our cohort although dedicated MRI sequences were not available in all scans examined. Interestingly, the three members of the family with *AP4M1*-related SPG50 and brain iron accumulation were among the oldest in our cohort. Thus, it remains to be seen if iron deposition in the basal ganglia is an age-dependent phenotype. Arguing against this is the young age of the male patient with *AP4S1* splice site mutations and brain iron deposition reported by Vill *et al.* (2017) and the absence of AP-4 variants in a cohort of patients with brain iron accumulation of unclear aetiology (S. Hayflick, personal communication). Cerebral atrophy, mainly in a fronto-temporal pattern, and cerebellar atrophy were described in patients with an average age of 11.3 ± 8.5 and 19.5 ± 13.5 years (SD), respectively. This argues that there might be some dependency of atrophic changes on age and disease progression although prominent cerebral atrophy was also seen in some of the youngest patients in our cohort (Fig. 3). Cerebellar atrophy, however, was rare in young patients. Genotype to imaging correlations and indicators of disease progression remain to be established through standardized and longitudinal imaging studies in AP-4-HSP patients of different age groups.

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Variants in the AP-4 subunit genes in our cohort were found in a wide range of different ethnicities. Consanguinity was reported in ~70% of patients but interestingly rates were lower for patients with *AP4B1*- and *AP4S1*-associated HSP while over two-thirds of patients with *AP4M1*- and *AP4E1*-associated HSP were of consanguineous parents, often of North African or Middle Eastern descent. *AP4M1* and *AP4B1* were most commonly mutated, together accounting for over 70% of patients. This is a larger percentage than expected simply based the relative size of the four different genes. At 1137 amino acids, AP4E1 is the largest of the four subunits so would be expected to have the most mutations under a simple loss of function model. Interestingly, variants were not homogenously distributed throughout the *AP4E1* gene, being absent from exons 15 through 20. The reason for this skewing of the variant distribution in *AP4E1* is unknown, but may reflect a distinct function of this region of the protein.

While the majority of AP-4 variants were private, we identified a number of seemingly recurrent variants. As an example, a homozygous c.617G>A [p.R206Q] variant in *AP4B1* was found in three families originating from the Jain community and the same discrete geographic within the state of Rajasthan, India, suggesting a possible founder effect. Most AP-4 variants identified were single nucleotide substitutions with a predicted truncating effect on the respective subunit protein (nonsense or frameshift). Canonical splice site variants were the third most common group. About 80% of patients had homozygous variants while compound heterozygous variants occurred in about 20%. The latter naturally occurred in many patients with no reported consanguinity. Microdeletions were found in a few patients and although rare, genetic investigations for AP-4-HSP should include techniques capable of detecting deletions.

Despite some variability, there was homogeneity concerning the core clinical features (Box 1), probably reflecting that most variants are predicted to cause at least partial loss of the AP-4 subunit protein rather than more subtle alterations of functionally critical domains. Stratification of disease severity by affected subunit did not reveal any clear correlation. This finding of a shared phenotype is supported by the presence of similar cellular phenotypes with loss of any of the AP-4 subunits, as recently demonstrated in patient-derived neurons (Behne *et al.*, 2020).

In summary, we here describe for the first time a detailed cross-sectional assessment of the clinical, imaging and molecular spectrum of AP-4-HSP. We define a core set of clinical features, delineate manifestations across the age spectrum, and explore genotype-phenotype correlations. Our results suggest that children with developmental delay, microcephaly, spasticity and a thin corpus callosum on imaging should undergo evaluation for AP-4-HSP to reduce the delay between presentation and diagnosis. Our findings also, for the first time, allow counselling of affected families. The development of this international cohort (www.CureAP4.org) and natural history study will enable us to understand disease progression more precisely and will define endpoints for future interventional trials.

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Competing interests

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Supplementary material

Supplementary material is available at *Brain* online.

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