Human Genomics

RESEARCH

Open Access

Expanding the repertoire of loss-of-function variants in *HACE1* causing complex spastic paraplegia: literature review and recommendations on clinical management

Hammad Yousaf[1], Sajid Ali[1], Ahad Yousuf Moulvi[2], Lubaba Bintee Khalid[1], Iram Javed[3], Rafia Zafar Ghumman[4], Cindy Colson[5], Shahnaz Ibrahim[6], Salma Zia[1], Muhammad Musawer Khan[7], Perrine Brunelle[5], Juan Pablo Trujillo-Quintero[8], Anna Ruiz[8], Salman Kirmani[9], Henry Houlden[10], Mathias Toft[11,12], Ambrin Fatima[1,13*†] and Zafar Iqbal[12*†]

Abstract

Background *HACE1* encodes a HECT domain and ankyrin repeat containing protein regulating several small GTPases. This protein is involved in several important functions, such as cell division, protein ubiquitination, and localization. Biallelic variants in *HACE1* have been implicated in spastic paraplegia and psychomotor retardation with or without seizures (MIM: 616756). Previously, 32 patients of various ethnicities have been reported with different types of variants; loss-of-function (LoF), missense, and indels.

Results Here, we studied five unrelated families of diverse ethnicities with eight cases of 4.5 years to 31 years of age. Our cases presented global developmental delay, dysarthria, intellectual disability, limb spasticity, and seizures. Exome sequencing identified biallelic/ compound heterozygous loss-of-function variants in *HACE1* in all families (Family A: *HACE1* (NM_020771.4): c.2628-1G > C p.(Ile877Tyrfs*58); Family B and C: c.1396 C > T p.(Gln466*); Family D: c.2242 C > T p.(Arg748*)/c.152 C > G p.(Ser51*); Family E: c.355G > T p.(Glu119*)). All variants co-segregated with the phenotype in respective families and are classified as pathogenic by the ACMG criteria.

Conclusion This study adds eight new cases of *HACE1* related spastic paraplegia from five unrelated families to the literature, bringing the total to 40 cases from 22 families. By integrating clinical data from our cohort with the published reports, we defined the phenotypic spectrum linked to *HACE1* disruption, noting key overlaps and

†Ambrin Fatima and Zafar Iqbal contributed equally to this work.

*Correspondence:
Ambrin Fatima
ambrin.fatima@aku.edu
Zafar Iqbal
zafar.iqbal@ous-research.no; iqbal.z.phd@gmail.com

Full list of author information is available at the end of the article

variability, and highlighted gaps in reporting clinical features. It also provides clinical management recommendations and calls for standardized phenotypic documentation to strengthen genotype–phenotype correlations.

Keywords Exome sequencing, Spastic paraplegia, SPPRS, *HACE1*. genotype-phenotype correlation

Introduction

Hereditary spastic paraplegias (HSPs) are a group of highly heterogeneous conditions with overlapping clinical features which make them challenging to diagnose and manage. HSPs are estimated to affect 0.1–9.6 individuals in every 100,000 individuals around the globe depending on the demographics and inheritance patterns [1–3]. These disorders are characterized by lower limb spasticity, hyperreflexia, and extensor plantar reflex [4]. Depending on the underlying etiology, HSPs can co-present spastic paraparesis, peripheral neuropathy, cognitive impairment, epilepsy, cerebral ataxia, spasticity, dystonia, dysarthria, dysphagia, and ocular manifestations in complicated forms of the disease [5, 6]. In addition, the age of onset and severity of the disease is determined by the underlying pathology [7]. Clinical heterogeneity and coexisting conditions in HSPs can at least partly be attributed to the massive genetic heterogeneity with over 80 genes reported for monogenic forms of HSPs in all inheritance patterns [6–9]. Thus, genetic heterogeneity, overlapping clinical findings, and debilitating consequences on the affected individuals and families make HSP a challenging group of disorders for diagnosis and management.

Belonging to HSPs, Spastic paraplegia and psychomotor retardation with or without seizures (SPPRS, MIM: 616756) is a rare disorder that is caused by recessive variations affecting *HACE1* function. So far, 32 patients from 17 unrelated families have been reported with recessive variations, predominantly loss-of-function, as the cause of SPPRS [10–17]. With the infantile onset of the disease, common presentation includes progressive lower limb spasticity, psychomotor developmental delay affecting motor coordination, speech, and cognition, muscle hypotonia, and ocular anomalies. Some cases show neonatal to infantile myoclonic seizures. Variable incidence of neuroanatomical anomalies associated with *HACE1* disruption has been observed which includes hypoplastic corpus callosum, cerebral atrophy, delayed myelination, and diminished white matter volume [10–13].

HECT domain and Ankyrin repeat Containing E3-ubiquitin-protein ligase 1 (*HACE1*) encodes for a 130 kDa protein consisting of 909 amino acids that regulates ubiquitination and proteasomal degradation of target proteins. It is a ubiquitously expressed gene with a predominant expression in the brain [18–20]. HACE1 targets several small GTPases including Ras-related C3 botulinum toxin substrate 1 (RAC1), and cyclin c, tumour necrosis factor receptor (TNFR), and optineurin (OPTN) to mediate brain development and maintenance [19, 21–23]. RAC1 along with other small GTPases have been shown to cause neurodevelopmental disorders including intellectual disability and atypical synaptic plasticity [24–26].

Here we report five unrelated families harboring recessive *HACE1* variants as the likely cause of the observed phenotype, adding eight new cases to the 32 previously published, bringing the total to 40. This represents the largest SPPRS cohort to date, expanding the clinical and mutational spectrum of *HACE1*-related disorders and reinforcing the role of E3 ubiquitin ligases in neurodevelopment.

Materials and methods

Research subjects' recruitment and DNA extraction

The current study was approved by the IRB of the Aga Khan University (Approval letter no. 2021–65141-9346). The cohort was assembled through GeneMatcher [27], and subsequent data/information was shared according to the Declaration of Helsinki. Neurologist evaluated cases in each family and clinical information was collected. Detailed history was collected from patients and/or parents/guardians in case of minors. After informed consent, peripheral blood was drawn from affected individuals, parents, and healthy siblings. DNA extraction was performed at each collaborating laboratory using standard protocols.

Genetic investigations

Family A: Exome sequencing (ES) was performed for individual IV:1 at the Novogene Co., Ltd (Cambridge, UK). In brief, Agilent SureSelect Human All Exome V6 (Agilent Technologies, Santa Clara, CA, USA) was used to capture the exome and subsequent paired-end (PE150) sequencing was performed on an Illumina platform, NovaSeq 6000 (Illumina, Santa Clara, CA, USA). The detailed methodology for ES and variant prioritization was performed as described earlier [28, 29]. Functional annotation of the variants was carried out by Annotate Variation (ANNOVAR) [30]. Variant filtering was performed by FILTUS [31]. MAF of < 0.01, absence of variants in homozygous state in the control population, and the phenotypic relevance of the genes implicated in human diseases with patient phenotypes was also considered while filtering the data.

Family B: ES was performed for individual IV:1 and IV:4 as described elsewhere [32, 33], and the bioinformatics filtering strategy included screening for only exonic

and donor/acceptor splicing variants. In accordance with the pedigree and phenotype, priority was given to rare variants [< 0.01 in public databases] that were fitting a recessive (homozygous or compound heterozygous) or a *de novo* model and/or variants in genes previously linked to neuropathy, intellectual disability and other neurological disorders.

Family C: Library preparation and exome enrichment were performed by using TWIST Human Comprehensive Exome kit according to the manufacturer's procedure. Enriched libraries were sequenced on MGI T7 instrument and by using PE150-FCL cartridges according to the manufacturer's specifications. Raw data was subjected to GATK best practices workflow at Intergen Genetic Diagnostic Centre Ankara/Turkey. Raw reads were aligned to hg38 using BWA MEM 0.7.17 [34]. Sorting, duplicate marking and base recalibration steps were performed subsequently by GATK4 [35]. Variant Call was made using 2 separate algorithms. GATK UnifiedGenotyper and GATK HaplotypeCaller were both used to complement each other [35]. Low quality variants from both sets were eliminated based on strand bias, read depth and call quality parameters using GATK SelectVariants option [35].

Family D: Exome enrichment was performed using the KAPA HyperExome (Roche) and sequenced using paired end sequencing (2 × 150 bp) at 90x in a HiSeq Illumina platform. The capture and sequencing was performed at centre nacional d'anàlisi genómica (CNAG) in Barcelona, Spain. Following sequencing, downstream bioinformatics analysis was done to identify single nucleotide variants and small insertions and deletions (indels). Variant filtering was performed based on the following parameters: minimum coverage depth > 10, variants present in coding or splicing sequences, number of reads with the variant for heterozygotes > 30% (for stop and frameshift variants, number of reads with the variant for heterozygotes > 20%). All variants without an effect on the protein (synonymous variants) were excluded. CNVs were predicted using the eXome-Hidden Markov Model (XHMM) software [36] and ExomeDepth software [37].

Family E: Exome library preparation was done using the DNAprep with enrichment (Illumina®) kit. Trio-based ES was performed with an Illumina NovaSeq6000 (Illumina®) with paired-end reads of 2 × 150 bp, according to the manufacturer's instructions. Reads were aligned and mapped to the human reference genome sequence hg38 (GRCh38) and variants were called using Dragen® (Illumina). Annotations and filtering was performed by an in-house software ANATOLE2®.

Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines [38]. Segregation of the variants was confirmed by Sanger sequencing in all available individuals of the families except Family B, as both affected individuals in this family were sequenced (Figure S1). PCR primers and conditions used for variant verification are available on request.

RNA isolation, RT-PCR analysis and sequence confirmation of mutant transcript

To evaluate the impacts of *HACE1*: c.2628-1G > C variant on splicing, we extracted total RNA from venous blood stored in PAXgene® Blood RNA Tube using Trizol (ThermoFisher Scientific). After assessing RNA quality and integrity, RevertAid First Strand cDNA Synthesis kit (Thermo Scientific Cat# K1622) was used for reverse transcription according to the manufacturer's protocols. We purified RT-PCR amplified product using GeneJET PCR Purification Kit (Thermo Scientific Cat# K0702) and Sanger sequenced the purified product. RT-PCR thermocycling conditions and primers are available on request.

Results

Individuals harboring LoF variants in *HACE1* show spastic paraplegia, developmental delay and seizures

Family A is a Pakistani family consisting of three affected females aged 24 years, 22 years, and 12 years, who are born to a consanguineous couple (Fig. 1). All affected females presented spastic paraplegia, delayed developmental milestones including sitting, standing, walking, and speech. Seizures were observed in IV:1 and IV:2, while IV:3 did not show any signs of seizures. Seizures started as early as six months of age with each episode lasting as long as 10 minutes. Individual IV:2 and IV:3 showed brain trophy. Furthermore, we noted that individual IV-3 showed a milder phenotype as compared to the other individuals in this family. She was followed up from birth. She was cognitively and developmentally better than her two siblings and initially showed motor and speech delay. Her lower limbs started developing spasticity. The calf muscles were tight and treated with botulinum toxin injections along with supportive orthosis. Her last botulinum toxin treatment was given 3 years ago. The patient also underwent serial casting to improve her spasticity and contractures in legs. She can only stand with supporting devices and does not walk at all. Speech onset has been observed, with normal upper limb movements and intact grip strength. She is treated with baclofen for her spasticity and did not have any seizures. IV-1 and IV:2 also showed dyskinetic movements (Table 1, Table S1).

Family B also has a Pakistani origin with two affected males born to first-degree related parents (Fig. 1). The patients were aged 13.5 years and 4.5 years at the time of last visit to the clinic, respectively. Both patients show spasticity, speech anomalies, and global developmental delay including unachieved unaided walking (Table 1,

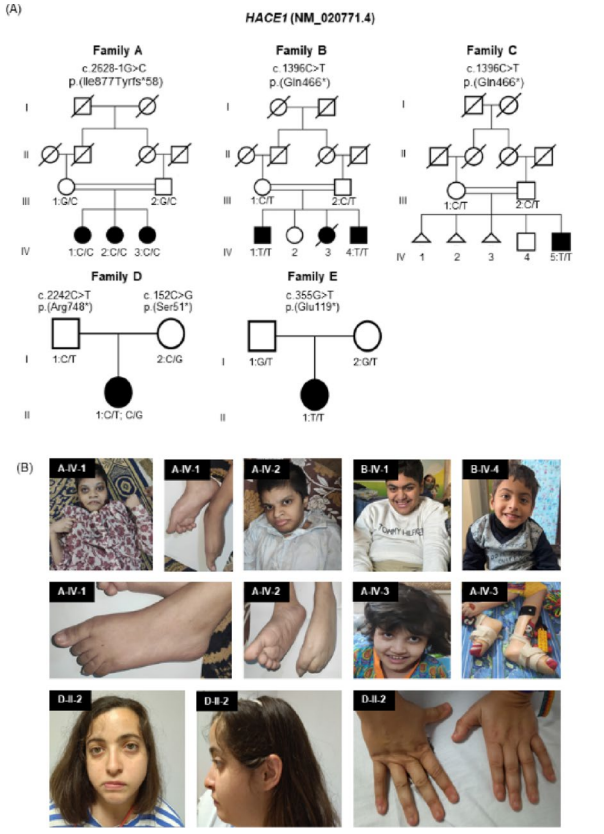

Fig. 1 Pedigrees of the five families carrying *HACE1* variants. **A** Pedigrees of Families A–E. Filled symbols represent affected individuals. Identified variants are written on top of pedigrees. Genotypes are shown for each tested individual below the symbols. **B** Clinical photographs of the cases with recessive *HACE1* variants

Table S1). A computed topography (CT) scan showed bilateral hyperdense basal ganglia in IV:1 and brain atrophy in individual IV:4. Although EEG for individual IV:1 performed at the age of 2 months showed no interictal epileptic discharges, both individuals have experienced seizures starting in the neonatal period. In addition to neonatal seizures, individual IV:4 experienced a second episode at the age of four years. Interestingly, the cases IV:1 and IV:4 had a female sibling (IV:3) who died of a similar clinical presentation for which no other clinical or biological material was available for assessment.

Family C concerns a male child of 10 years, born to first-cousin Sindhi parents, with a history of three miscarriages and a 13-year-old healthy male sibling (Fig. 1). The proband was born at full-term via cesarean section following an unremarkable pregnancy with good APGAR score. No perinatal complications were reported. At 2 months of age, he started to experience seizures with each episode lasting between 10 and 15 minutes. Antiseizure treatment with levetiracetam was efficacious. EEG at 3 years of age showed bilateral temporo-occipital slightly dominant slow waves. He continued to experience seizures till 5 years of age. In addition, he exhibited global developmental delay, achieved sitting at 2 years, crawling at 4 years, and cannot walk unaided even for few steps (Table 1, Table S1). Motor function was severely affected, with the child being classified as Gross Motor Function Classification System (GMFCS) level 5, later improving to level 4 after a year-long course of intensive physiotherapy. At the most recent follow-up, the patient remains seizure-free and off medication. Although motor function improved slightly with therapy, the child continues to face significant global developmental challenges.

Family D is of Caucasian descent, consisting of a female patient (31 years) born to non-consanguineous parents (Fig. 1). With unremarkable gestational history, patient (II-1) showed delayed psychomotor development, spasticity with mild ID, hypotonia, and generalized tonic-clonic seizures (starting at 16 years). The seizures were managed with valproate and levetiracetam treatment. EEG at 29 years was normal. In addition, the patient showed skeletal and facial anomalies including thoracolumbar scoliosis and lumbar hyperlordosis. Brain MRI showed thinning of the corpus callosum and mild dilation of the lateral ventricles (colpocephaly) (Table 1, Table S1).

Belonging to the Caucasian ethnicity residing in France, Family E includes a female child (II-1) of unrelated parents with unremarkable family and gestational history (Fig. 1). Clinical history includes spastic paraplegia, delayed developmental milestones, and mild intellectual disability (ID). The patient cannot walk unaided and can only speak few words. She showed myopathy, hypotonia, and positive Babinski sign. Furthermore, there is no history of seizures. Electrocardiogram, MRI, and nerve conduction investigations were normal. Muscle histology was also found to be normal. In addition, we also observed myopia, recurrent pyelonephritis, and obesity (Table 1, Table S1).

Exome sequencing identified biallelic LoF variants in *HACE1* as the most plausible cause of spastic paraplegia in 8 individuals

Following the data filtering criteria at each collaborating center in this study, LoF variants in *HACE1* emerged as the most likely cause of the disease in the five investigated families. In family A, a novel splice acceptor site variant, *HACE1* (NM_020771.4): c.2628-1G>C, p.(Ile877Tyrfs*58) segregated with the phenotype (Fig. 1, Figure S1). Family B and C harbors a homozygous stop variant in *HACE1*: c.1396C>T, p.(Gln466*) that segregates with the disease (Fig. 1). This variant is classified as pathogenic in ClinVar (accession no. RCV000661911.4). The proband in family D harbors compound heterozygous variants *HACE1*: c.2242C>T, p.(Arg748*)/c.152C>G, p.(Ser51*) inherited from each parent (Fig. 1, Figure S1).

Table 1 Clinical features of the cases reported with *HACE1* variants

Clinical features	Family A			Family B		Family C	Family D	Family E	Family 1 (44)		Family 2 (10)	
***HACE1*(NM_020771.4)**	c.2526-1G>C, p.Ile877Tyrfs*58 (Homozygous)			c.1396C>T, p.Gln466* (Homozygous)		c.1396C>T, p.Gln466* (Homozygous)	c.2242C>T, p.Arg748*/ c.152C>G, p.Ser51*	c.355G>T, p.Glu119* (Homozygous)	Family A CHR6 c.1990C>T, p.Arg664*(Homozygous)	Family A CHR6 c.1990C>T, p.Arg664*(Homozygous)	Family PKMR285 (c.350T> C, p.Leu117Ser (Homozygous)	
Origin	Pakistan (Consanguineous Marriage)			Pakistan (Consanguineous Marriage)		Pakistan (Consanguineous Marriage)	Caucassian/ (Non Consanguineous Marriage)	French Caucassian (Non Consanguineous Marriage)	Saudi Arabia (Consanguineous Marriage)	Saudi Arabia (Consanguineous Marriage)	Pakistan	Pakistan
Sex, Age	IV-1 Female 24 years	IV-2 Female 22 years	IV-3 Female 12 years	IV-1 Male 13.5 years	IV-4 Male 4.5 years	IV-1 Male10 years	II-1 Female 31 years	II-1 Female 10 years	Female 11 months	Female 10 years	Female 25 years	Male 23 years
Hypotonia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Developmental Delay	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Epilepsy, Seizures	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	n/a	Yes	Yes
Spasticity (Lower Limbs)	n/a	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	n/a	Yes	Yes
Spasticity (Upper Limbs)	Yes	No	No	Yes	Yes	Yes	No	Yes	No	n/a	Yes	Yes
Speech Delay	Yes	Yes	Yes	Yes	n/a	Yes	Yes	Yes	n/a	n/a	Yes	Yes
Dysmorphic Faical Features	n/a	Yes	Yes	No	No	No	Yes	No	No	n/a	No	No
Intellectual Disability	Yes	Yes	Yes	Yes (Mild)	Yes (Mild)	Yes (Mild)	Yes	Yes	n/a (too young to assess)	n/a	Yes	Yes
Head Size	Normocephaly	Microcephaly (42.5 at 11 months is the 3rd percentile), normal now	Normocephaly (33.7 cm at 7 days is 13th percentile)	Normocephaly	Normocephaly	Normocephaly	Normocephaly	Normocephaly	Microcephaly	n/a	n/a	n/a
Skeletal/digit anomalies	Scoliosis, Metarsus varus deformity in one foot	Scoliosis/ plagiocephaly	Scoliosis/ plagiocephaly brachycephaly	No	No	Clubbing, Brachycepahly	Thoracolumbar scoliosis, lumbar hyperlordosis.	No	n/a	n/a	n/a	n/a

Table 1 (continued)

Clinical features	Family A			Family B		Family C	Family D	Family E	Family 1 (44)		Family 2 (10)	
MRI/CT/similar	n/a	Mild atrophic changes in the supratentorial compartment.	Diffuse brain atrophy with ventriculomegaly, thinned out corpus callosum and bilateral calcification in globus pallidi.	Bilateral hyperdense basal ganglia	Brain Atrophy	MRI: Mild atrophy of bilateral frontal lobes. EEG: Bilateral temporo-occipital slightly dominant slow waves	Thinning of the corpus callosum and mild dilation of the lateral ventricles (colpocephaly)	Normal	Normal	n/a	n/a	n/a
Recurrent infections	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	n/a	n/a	n/a
Eye	Normal	Normal	Normal	Normal	Normal	Normal	High myopia	Myopia	Normal	n/a	n/a	n/a
Gait anomalies	Non ambulatory	Non ambulatory	n/a	Non ambulatory	Non ambulatory	Wobbly gait with support	n/a	Non ambulatory	No	n/a	n/a	n/a
Age of onset- What was observed	Seizures at 6 months	Developmental Delay, Seizures at 11 month	n/a	n/a	n/a	Seizures at 2 months	n/a	n/a	2 months (Referred to neurologist at 11 months)	n/a	n/a	n/a
Any other	n/a	Heart: Left ventricle dilation and systolic dysfunction which recovered in 2017.	n/a	n/a	n/a	n/a	n/a	n/a	Delayed tooth eruption, hemangioma involving the right eyelid and forehead, and congenital heart defect	CP	None	None

Table 1 (continued)

Clinical Features	Family 3 (13)	Family 4 (9)		Family 5 (14)	Family 6 (15)			Family 7, 8 (22)			Family 9 (11)
***HACE1*(NM_020771.4)**	Family A exon 7 del/entire *HACE1* deletion	Family 4 Patient 6 and 7 c.2581G>C, p.Ala861Pro (Homozygous)		Family A c.240C>A, p.Cys80* (Homozygous)	Family A c.2212-1G>A/c.2212-1G>A			Family A c.625C>T, p.Gln209* (Homozygous)	Family B c.994C>T, p.Arg332*(Homozygous)		Family A c.1110G>A, p.Trp370* (Homozygous)
Origin	Russian (Non Consanguineous Marriage)	Turkey (Consanguineous Marriage)		Pakistan (Non Consanguineous Marriage)	n/a (Consanguineous Marriage)			Saudi Arabia (Consanguineous Marriage)	Turkey (Consanguineous Marriage)		India (Consanguineous Marriage)
Sex, **Age**	Male 2.8-years	Male 17 years	Male 8 years	Male 10 years	Male	Female	Female	Female 5 years 4 months	Female 4 months	Male 6 months	Male 11 years
Hypotonia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Developmental Delay	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Epilepsy, **Seizures**	No	Yes	No	n/a	Yes	Yes	Yes	No	No	No	Yes (Myoclonic seizures in limbs)
Spasticity (Lower Limbs)	Yes (more severe)	Yes	Yes	Yes (Paresis of the lower extremities with rigidity)	Yes (Dystonia)	Yes (Dystonia)	Yes (Dystonia)	n/a	n/a	n/a	Yes (Hypertonia and exaggerated deep tendon reflexes)
Spasticity (Upper Limbs)	Yes	No	No	n/a	n/a	n/a	n/a	n/a	n/a	n/a	Yes (Hypertonia and exaggerated deep tendon reflexes)
Speech Delay	Yes	Yes	Yes	Yes	Yes	n/a	n/a	Yes	Yes	Yes	Yes
Dysmorphic Faical Features	Yes (Coarse face)	Yes	Yes	n/a	Yes (Broad forehead and hypertelorism)	Yes (Broad forehead and hypertelorism)	Yes (Broad forehead and hypertelorism)	Yes	Yes	Yes	n/a
Intellectual Disability	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	n/a (too young to assess)	n/a (too young to assess)	Yes
Head Size	n/a	Normocephaly	Normocephaly	n/a	n/a	n/a	n/a	Microcephaly	Macrocephaly	Macrocephaly	Normocephaly
Skeletal/ digit anomalies	Mild hypotrichosis	Camptodactyly of fourth and fifth fingers (unsure which patient)	Camptodactyly of fourth and fifth fingers (unsure which patient)	n/a	n/a	n/a	n/a	Scoliosis, skeletal abnormalities, and ulnar deviation of the wrists and small feet	small feet	small feet	Lumbar lordosis, proximal femoral focal dysplasia
MRI/ CT/ similar	Ventriculomegaly, cerebral atrophy	Normal	Normal	Diffuse cortical atrophy and an arachnoid cyst in the right temporal lobe	Cerebral cortical atrophy and hypoplastic corpus callosum			Microcephaly and brachycephaly, hypoplastic corpus callosum and likely brainstem abnormality; small sella with ectopic neurohypophysis; mild ventriculomegaly	Normal	Normal	Hypoplastic corpus callosum, possible neuroenteric cyst. EEG: well-defined α-activity at 9–10 Hz
Recurrent infections	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Eye	Long eyelashes (optahlomogical findings n/a)	Long eyelashes	Long eyelashes	n/a	Eye movement deficit and hypertelorism	Eye movement deficit and hypertelorism	Eye movement deficit and hypertelorism	Retinal Dystrophy	Strabismus of the right eye	Bilateral strabismus	Heterochromia of the iris
Gait anomalies	n/a	n/a	n/a	n/a	No	No	No	n/a	n/a	n/a	Broad-based, crouched gait
Age of onset- What was observed	5 months	1-2 months	1-2 months	First Months of life	Less than 1 year	Less than 1 year	Less than 1 year	n/a	4 months	6 months	n/a
Any other	None	None	None	3-methylglutaconic aciduria	Regression, Downturned mouth	Regression, Downturned mouth	Regression, Downturned mouth	Bilateral Sensioneural hearing loss reported at 4 years	Wide spaced nipples	Wide spaced nipples	Hypopigmented Hairs

Yes: Present; No: Absent; n/a: Not available/ applicable

Table 1 (continued)

Clinical features	Family 10 (16)	Family 11 (17)		Family 12, 13 (39)							
***HACE1*(NM_020771.4)**	Family A c.1990C>T, p.Arg664* (Homozygous)	Family A c.402+5G>A/ c.402+5G>A		Family A c.655C>T, p.Arg219*(Homozygous)					Family B c.2242C>T, p.Arg748*/ c.2019_2020ins, p.Pro674Phefs*5		
Origin	n/a (Consanguineous Marriage)	Syria (Consanguineous Marriage)		Pakistan (Consanguineous Marriage)					Germany (Non Consanguineous Marriage)		
Sex, **Age**	Female	Female	Female	Male 18 years	Male 15 years	Male 22 years	Male 19 years	Female 3 years	Female 10 years 11 months	Male 7 years 8 months	Male 7 years 8 months
Hypotonia	n/a	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Developmental Delay	n/a	n/a	n/a	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Epilepsy, **Seizures**	n/a	n/a	n/a	Yes (Myoclonic and tonic–clonic)	Yes (Myoclonic and Tonic Clonic)	Yes	Yes (Tonic-Clonic)	Yes (Myoclonic Epilepsy)	No	Yes (Myoclonic Seizures, Focal)	No
Spasticity (Lower Limbs)	Yes	n/a	n/a	Yes (Bilateral spasticity-progressive)	Yes (Bilateral spasticity-progressive)	Yes (Bilateral spasticity-progressive)	Yes (Bilateral spasticity-progressive)	Yes (Bilateral spasticity-progressive)	Yes (Dystonia)	n/a	Yes (Bilateral Spasicity)
Spasticity (Upper Limbs)	n/a	n/a	n/a	No	Yes (Dystonic)	No	No	Yes (Increased Tone and hyperreflexia)	Yes (Dystonia)	Yes (Dystonic Posturing)	No
Speech Delay	n/a	Yes	Yes	Yes	Yes	Yes	Yes	Yes	n/a	Yes	Yes
Dysmorphic Faical Features	n/a	No	No	No	No	No	No	No	Yes	Yes	Yes
Intellectual Disability	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Head Size	n/a	n/a	n/a	Macrocephaly	Macrocephaly	Macrocephaly	Macrocephaly	Microcephaly	Normocephaly	Normocephaly	Normocephaly
Skeletal/digit anomalies	n/a	n/a	n/a	n/a	Left talipes equinovarus and Kyphosis	Erb's palsy Right subtalar fusion age 9 years	Calcaneovalgus deformity of feet	Kyphosis/ Bilateral Hip dislocation	Lumbar lordosis	Pes planus	Mild Talipes Equinovaris

Table 1 (continued)

Clinical features	Family 10 (16)	Family 11 (17)		Family 12, 13 (39)							
MRI/CT/similar	n/a	n/a	n/a	Cerebral atrophy and marked atrophy of the frontal and temporal lobes (CT scan)	n/a	Gene-realised Cerebral Atrophy (CT)	Ventriculo-megaly	Prominent generalised cerebral and brain stem atrophy disproportion between grey and white matters.	Hypoplastic Corpus Callosum	Hypoplastic Corpus Callosum	Ventriculo-megaly
Recurrent infections	n/a	Yes	Yes	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Eye	n/a	n/a	n/a	Divergent strabismus	Divergent strabismus	Right divergent strabismus, bilateral myopia, right macular hypoplasia, retinal dystrophy	Divergent strabismus, myopia, bilateral retinal dystrophy	Severe myopia, left divergent squint, sluggish pupil responses, retinal dystrophy	n/a	n/a	Convergent strabismus, bilateral myopia, retinal dystroph
Gait anomalies	n/a	Ataxia	Ataxia	Non ambulatory	Non ambulatory	Calliper aided short distance walk	10–15 steps with rollator	Non ambulatory	Unstable Waddling Gait	Unstable Waddling Gait	Unstable Waddling Gait
Age of onset- What was observed	n/a	n/a	n/a	birth or by 3–4 months of age	birth or by 3–4 months of age	birth or by 3–4 months of age	birth or by 3–4 months of age	birth or by 3–4 months of age	birth or by 3–4 months of age	birth or by 3–4 months of age	birth or by 3–4 months of age
Any other	Neurogenic bladder	None	None	Hypogenitalism, doubly incontinent	Doubly incontinent	Hypogenitalism/ Bilateral sensiouneural hearing loss, doubly incontinent	Hypogenitalism, doubly incontinent, EEG normal	Bilateral sensorineural loss (40 dB), doubly incontinent, EEG: focal and generalised spike-like discharges. Nerve conduction studies normal.	EEG normal	EEG: consistent with myoclonic epilepsy	EEG normal

Yes: Present; No: Absent; n/a: Not available/applicable

Both variants are classified as pathogenic in ClinVar via accession # RCV000578801 and RCV002287613 respectively. In addition, the p.(Arg748*) has been previously reported in compound heterozygosity with an insertion [39]. A homozygous pathogenic *HACE1*: c.355G>T, p.(Glu119*) variant predicted to cause premature truncation segregates with disease in family E (Fig. 1, Figure S1). This variant is also listed in ClinVar as a pathogenic variant (RCV002274460).

All protein truncating variants were classified as pathogenic according to the ACMG classification [38] meeting PVS1, PM2, PP5 criteria and were absent in homozygous state in gnomAD database (Table 2). Given the clinical presentation of the proband(s) in each family and phenotypic overlap with the *HACE1* implicated autosomal recessive disease- spastic paraplegia and psychomotor retardation with or without seizures (MIM#616756), the identified LoF variants are the most plausible cause for the primary phenotype in the investigated families.

Splice site variant c.2628-1G>C in *HACE1* leads to partial deletion of exon 24

To study the effects of *HACE1*: c.2628-1G>C variant on splicing in family A, we performed RT-PCR analysis that showed a shortened product as compared to wildtype control sample. To further confirm this, we sequenced the wildtype and mutant RT-PCR product that showed 16 bp frameshifting deletion of exon 24 leading to HACE1: p.(Ile877Tyrfs*58), thus providing the evidence for splicing defects (Fig. 2A).

Discussion

Clinical heterogeneity, diverse forms of paraplegia, and unexplained variability in disease severity are challenging hallmarks of spastic paraplegia cases [9]. Here, the use of genetics, combined with clinical information comes worthwhile to provide diagnosis, management, and devise preventive measures. This study was conducted to provide diagnosis and possible further clinical insights. We investigated eight cases from five unrelated families of south Asian and Caucasian origin (Table 1, Table S1). ES identified biallelic pathogenic LoF variants in *HACE1*, including a consensus splice acceptor site variant in one family. The splice site c.2628-1G>C variant leads to aberrant splicing and partial skipping of exon (Fig. 2A), while the protein truncating variants potentially lead to nonsense mediated decay of the mRNA. Studies have shown that 1/3rd of Mendelian disorders are caused by LoF variants that generate premature termination codons leading to nonsense-mediated mRNA decay [42]. Based on identification of pathogenic variants in *HACE1* and phenotypic resemblance to the *HACE1* associated

Table 2 Details of the identified *HACE1* variants in current study

	Family A	Family B and C	Family D		Family E
Variant details					
HACE1 (NM_020771.4)	c.2628-1G>C p.(Ile877Tyrfs*58)	c.1396C>T p.(Gln466*)	Parent 1: c.2242C>T p.(Arg748*)	Parent 2: c.152C>G p.(Ser51*)	c.355G>T p.(Glu119*)
Zygosity	Homozygous	Homozygous	Compound heterozygous		Homozygous
Genomic coordinates (hg19)	chr6-105177640-C>G	chr6-105232873-G>A	chr6-105198317-G>A	chr6-105298851-G>C	chr6-105291145-C>A
Allele frequency					
gnomAD (v4.1.0)	Absent	6.20e-6	2.48e-6	Absent	Absent
Homozygotes (v4.1.0)	Absent	Absent	Absent	Absent	Absent
Variant classification					
ACMG classification	Pathogenic	Pathogenic	Pathogenic	Pathogenic	Pathogenic
ACMG criteria	PVS1, PM2, PP5	PVS1, PM2, PP5	PVS1, PM2, PP5	PVS1, PM2, PP5	PVS1, PM2, PP5
ClinVar classification	n/a	Pathogenic	Pathogenic	Pathogenic	Pathogenic
ClinVar accession no.	Absent	RCV000661911.4	RCV000207215 RCV000578801	RCV002287613	RCV002274460.
In silico predictions					
Meta[a] (40)	12	8	8	12	12
BayesDel[b] addAF (41)	PVS	PS	PS	PVS	PVS
BayesDel[b] noAF (41)	PS	PS	PS	PS	PS

n/a: not applicable; PVS: Pathogenic very strong; PS: Pathogenic Strong

[a]Meta determines pathogenicity based on the combined evidence from multiple other in-silico predictors. Note: Engines are assigned a prediction points score based on the strength of the calibrated prediction. Supporting: 1 point. Moderate: 2 points. Strong: 4 points. Very Strong: 8 points

[b]BayesDel is more accurate than PolyPhen2, SIFT, FATHMM, LRT, Mutation Taster, Mutation Assessor, PhyloP, GERP++, SiPhy, CADD, MetaLR, and MetaSVM

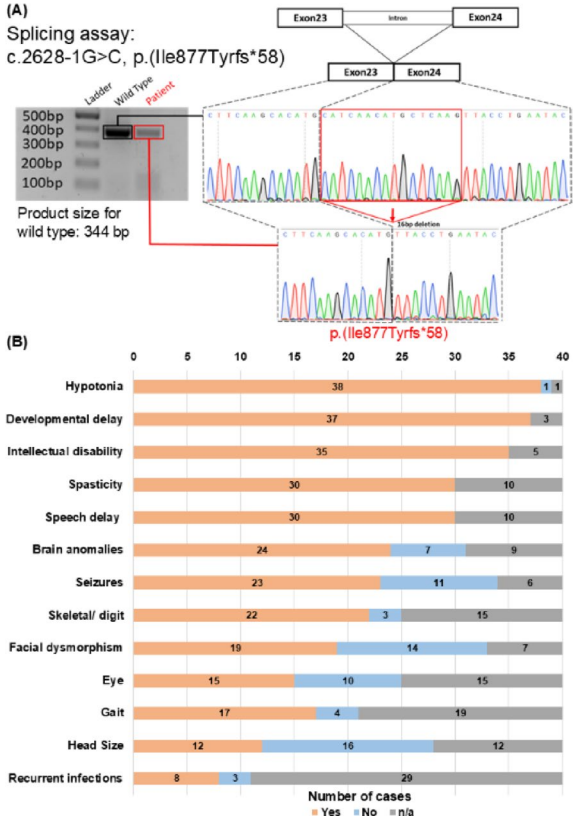

Fig. 2 Splicing assay and clinical spectrum of *HACE1*-related Spastic Paraplegia across reported cases. **A** Splicing assay for the NM_020771.4: c.2628-1G>C variant confirms aberrant splicing leading to frameshift [p.(Ile877Tyrfs*58)] in protein. Left: Electropherogram from the RT PCR showing DNA ladder (line 1), wild type sample (line 2), and patient sample (line 3). The expected PCR product size for wild-type transcript is 344 bp. Right: Schematic of the deletion breakpoint and deleted 16 bps from exon 24 following defective splicing. Red outlined box spans the 16 deleted bases. **B** Bar graph depicting the frequency of various clinical features observed in individuals with SPPRS. The x-axis represents the number of cases, while the y-axis lists the clinical manifestations. Each feature is categorized by whether it was present (Yes), absent (No), or no information available (n/a) in each individual. Hypotonia, developmental delay, intellectual disability, and spasticity were among the most frequently reported findings

disease-SPPRS, it can be concluded that these LoF variants are the most likely cause of spastic paraplegia in our cohort.

Review of literature: genotype to phenotype correlation

The ultimate goal in the field of genetic medicine is to establish genotype-phenotype association to shed insights into the underlying mechanisms and predict disease outcomes, prognosis, counselling, and personalized management in some cases [43]. By corroborating the clinical information from our cohort with published literature and genetic data, we consolidated the phenotypic outcomes associated with *HACE1* disruption, highlighting both similarities and differences in disease presentation. So far, 12 studies have reported *HACE1* related recessive disease in 17 families consisting of 32 patients (15 females and 17 males) with the age ranging from 4 months to 31 years. These families originated from Pakistan (n = 4), Saudi Arabia (n = 2), Türkiye (n = 2), Russia, India, Syria, Germany, United Kingdom, Algeria (n = 1 each), while no origin information was available for 3 families. The current study reports eight additional cases from five unrelated families raising the total number of cases to 40 and from 22 families (Table 1). Overall, cases with SPPRS presented a constellation of symptoms consisting of neurodevelopmental, neuroanatomical, and neuromuscular manifestations. The hallmark phenotypes included lower limb spasticity (30/30 cases; n/a = 10 cases), developmental delay (37/37; n/a = 3), speech disturbances (30/30; n/a = 10), intellectual disability (35/35; n/a = 5), and hypotonia (38/39 cases; n/a = 1) (Table 1; Fig. 2). In the 40 patients so far, the genotypic spectrum included biallelic nonsense variants (n = 9), biallelic splice site variants (n = 3), compound heterozygous variants (n = 3 nonsense in trans with n = 2 frameshifting and n = 1 nonsense), biallelic in-frame deletion (n = 1), gross deletion (n = 1), and biallelic missense variants (n = 2). These variants span throughout the protein length of 909 amino acids affecting ankyrin and HECT domains without any trend of mutation hotspots [11, 13, 19, 20].

In contrast to these common clinical presentations, some patients in the literature show differences in clinical presentation that included neuroanatomical anomalies, seizures, skeletal malformations and abnormal head size. Neuroanatomical anomalies as seen on brain MRI predominantly showed corpus callosum hypoplasia (n = 11), cerebral and/or cortical atrophy (n = 9), ventriculomegaly (n = 5) co-occurring together in 24/31 (77%) cases for which the information is known. We hypothesize that the localization and functional consequence of the altered variant on protein function might have some effect on the manifestation or severity of the neuroanatomical and clinical features. We noticed that all patients with LoF/deletion variants (for which the neuroanatomical information is known) show structural brain anomalies with few exceptions as seen in patients with p.(Arg664*) and p.(Arg332*) variants [22, 44]. This should be interpreted with caution as the information for 10 cases is missing (Table 1). Similarly, epilepsy and facial dysmorphism were observed in 23/34 (67%) and 19/33 cases (57%), respectively with no specific association with the type/position of variant (LoF vs. missense) (Table 1). Furthermore, we observed that out of the 520 data points pertaining to most common features related to SPPRS cases reported so far, no information is available for 141 of them, which makes it difficult to establish accurate genotype-to-phenotype correlations. (Fig. 2B; Table 1).

Although reports of cases with homozygous *HACE1* variants are on the rise, the overall clinical spectrum, disease manifestations, and genotype-phenotype correlations remain poorly characterized. Considering the missing information, recognizing the exceptions, and small number of individuals reported overall, we recommend further studies on larger cohorts with detailed standardized phenotyping such as using Human Phenotype Ontology (HPO) terms. This will aid to reach a consensus on the phenotypic outcomes and early phenotypic markers of the disease to improve diagnosis, devise timely palliative and preventive strategies.

Recommendation on clinical management

SPPRS predominantly manifests as neurodevelopmental disorders such as lower limb spasticity, developmental anomalies including cognitive and speech anomalies, hypotonia, and seizures along with neuroanatomical malformations as seen through imaging. Similar to other rare inherited disorders, the development of gene therapy/targeted therapies is still in its infancy and would not be an option for everyone, especially in the developing world. Therefore, the management mainly relies on palliative care.

As evident from the gathered clinical data from all cases with *HACE1* related disorder including the current study, the early clinical features include spasticity and developmental delay that occurs during early months to first year of life (Table 1). In such cases, regular follow-up with a paediatric neurologist is important to monitor overall health and growth, focusing on preventing serious complications.

Although seizures are not universally observed but seem to be manageable with routine anti-seizure drugs such as levetiracetam, valproate and carbamazepine. Brain imaging through MRI and CT scan should be performed, as neuroanatomical malformations have been observed in nearly 80% of the individuals so far (Table 1).

For the management of spasticity, medications such as baclofen, tizanidine, trihexiphenidyl or botulinum toxin may be used. Early initiation of physical and occupational therapy along with mobility aids is vital to improve motor function and reduce spasticity. Speech therapy should also begin early to support communication and cognitive development. An ophthalmological evaluation at diagnosis is recommended to assess visual acuity and eye movement disorders.

Importantly, genetic counselling plays a pivotal role in the management and prevention of SPPRS, particularly in low- and middle-income countries, where consanguineous marriages are more prevalent. Genetic counselling can help at-risk families understand inheritance patterns and make informed reproductive decisions, ultimately reducing the recurrence of such rare inherited disorders.

In conclusion, while these recommendations offer a comprehensive approach to managing SPPRS, we acknowledge the limitations posed by the small number of reported cases. As such, these suggestions may not be universally applicable to all patients. A tailored, multidisciplinary assessment is essential to address the individual needs and variability in clinical presentation. Further studies with larger cohorts will be necessary to establish more robust and reliable clinical guidelines for the management of *HACE1*-related disorders.

Conclusion

This study reports eight cases with recessive LoF variants in *HACE1,* aiming to consolidate the clinical and genetic spectrum of *HACE1*-related recessive spastic paraplegia and provides recommendations on the clinical management. We also performed a thorough literature review to highlight the gaps in reporting clinical findings. Further, this study emphasizes the need for detailed uniform clinical data collection and reporting to achieve consensus clinical outcomes and establish genotype-to-phenotype correlation which is a mainstay in the field of genetic medicine.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40246-026-00914-1.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

The authors sincerely thank the patients and their families for their participation and trust in this research.

Author contributions

HY, AYM, and RZG drafted the original manuscript. AF and ZI reviewed and edited. HY, SA, LBK, RZG, CC, IJ, SI, SZ, MMK, PB, AR, JPTQ, SK, HH, MT, AF, and ZI contributed the research subjects and data acquisition. IJ, SI, and SK assessed the patients clinically. AF, ZI supervised the study. All authors read and approved the submitted version.

Funding

Open access funding provided by University of Oslo (incl Oslo University Hospital). This study was funded by the Higher Education Commission of Pakistan grants, P-No. 16914 and P-No. 80161 (to AF), ICGEB grant CRP/PAK21-05_EC (to AF). Z.I. was supported by a grant from the South-Eastern Norway Regional Health Authority.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval for this study was granted by the Institutional Review Board of respective collaborating institutes. Informed consent was obtained from all participants or their legal guardians for participation.

Consent for publication
Written informed consent to publish clinical data was obtained from the patients or their legal guardians.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi, Pakistan
[2]Ziauddin University, Karachi, Pakistan
[3]Department of Paediatric Neurology, Children Hospital and Institute of Child Health, Faisalabad, Pakistan
[4]Islam Medical College, Sialkot, Pakistan
[5]CHU Lille, Univ. Lille, Institut de Génétique médicale, ULR7364 RADEME, Lille 59000, France
[6]Department of Paediatrics and Child Health, The Aga Khan University, Karachi, Pakistan
[7]Bolan Medical College, Quetta, Pakistan
[8]Center for Genomic Medicine, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí (I3PT-CERCA), Universitat Autònoma de Barcelona, Sabadell, Spain
[9]Division of Women & Child Health, Aga Khan University, Karachi, Pakistan
[10]Department of Neuromuscular Diseases, University College London, Queen Square, Institute of Neurology, London, UK
[11]Institute of Clinical Medicine, University of Oslo, Oslo, Norway
[12]Department of Neurology, Oslo University Hospital, Oslo, Norway
[13]Centre for Regenerative Medicine and Stem Cells Research, The Aga Khan University, Karachi, Pakistan

Received: 6 August 2025 / Accepted: 7 January 2026
Published online: 11 February 2026

References

1. Shribman S, Reid E, Crosby AH, Houlden H, Warner TT. Hereditary spastic paraplegia: from diagnosis to emerging therapeutic approaches. Lancet Neurol. 2019;18(12):1136–46.
2. Meyyazhagan A, Orlacchio A. Hereditary spastic paraplegia: an update. Int J Mol Sci. 2022;23(3):1697.
3. Ruano L, Melo C, Silva MC, Coutinho P. The global epidemiology of hereditary ataxia and spastic paraplegia: a systematic review of prevalence studies. Neuroepidemiology. 2014;42(3):174–83.
4. Salinas S, Proukakis C, Crosby A, Warner TT. Hereditary spastic paraplegia: clinical features and pathogenetic mechanisms. Lancet Neurol. 2008;7(12):1127–38.
5. Fink JK. Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. Acta Neuropathol. 2013;126(3):307–28.
6. Boutry M, Morais S, Stevanin G. Update on the genetics of spastic paraplegias. Curr Neurol Neurosci Rep. 2019;19(4):18.
7. Schüle R, Wiethoff S, Martus P, Karle KN, Otto S, Klebe S, et al. Hereditary spastic paraplegia: clinicogenetic lessons from 608 patients. Ann Neurol. 2016;79(4):646–58.
8. Koh K, Ishiura H, Tsuji S, Takiyama Y. JASPAC: Japan spastic paraplegia research consortium. Brain Sci. 2018;8(8):153.
9. Sager G, Turkyilmaz A, Ates EA, Kutlubay B. *HACE1, GLRX5*, and *ELP2* gene variant cause spastic paraplegies. Acta Neurologica Belgica. 2022;122(2):391–9.
10. Usmani MA, Ghaffar A, Shahzad M, Akram J, Majeed AI, Malik K, et al. A missense variant in *HACE1* is associated with intellectual disability, epilepsy, spasticity, and psychomotor impairment in a Pakistani Kindred. Genes. 2024;15(5):580.
11. Hariharan N, Ravi S, Pradeep BE, Subramanyam KN, Choudhary B, Srinivasan S, et al. A novel loss-of-function mutation in *HACE1* is linked to a genetic disorder in a patient from India. Hum Genome Variation. 2018;5(1):1–4.
12. Akawi N, McRae J, Ansari M, Balasubramanian M, Blyth M, Brady AF, et al. Discovery of four recessive developmental disorders using probabilistic genotype and phenotype matching among 4,125 families. Nat Genet. 2015;47(11):1363–9.
13. Kovalskaia VA, Zabnenkova VV, Petukhova MS, Markova ZG, Tabakov VY, Ryzhkova OP. Previously undescribed gross *HACE1* deletions as a cause of autosomal recessive spastic paraplegia. Genes. 2022;13(12):2186.
14. Ugarteburu O, Sanchez-Viles M, Ramos J, Barcos-Rodriguez T, Garrabou G, Garcia-Villoria J et al. Physiopathological bases of the disease caused by *HACE1* mutations: alterations in Autophagy, mitophagy and oxidative stress response. J Clin Med. 2020;9(4).
15. Issa MY, Chechlacz Z, Stanley V, George RD, McEvoy-Venneri J, Belandres D, et al. Molecular diagnosis in recessive pediatric neurogenetic disease can help reduce disease recurrence in families. BMC Med Genomics. 2020;13(1):68.
16. Alfares AA. Applying filtration steps to interpret the results of whole-exome sequencing in a consanguineous population to achieve a high detection rate. Int J Health Sci (Qassim). 2018;12(5):35–43.
17. Reuter MS, Tawamie H, Buchert R, Hosny Gebril O, Froukh T, Thiel C, et al. Diagnostic yield and novel candidate genes by exome sequencing in 152 consanguineous families with neurodevelopmental disorders. JAMA Psychiatry. 2017;74(3):293–9.
18. Coorens TH, Guillaumet-Adkins A, Kovner R, Linn RL, Roberts VH, Sule A, et al. The human and non-human primate developmental GTEx projects. Nature. 2025;637(8046):557–64.
19. Torrino S, Visvikis O, Doye A, Boyer L, Stefani C, Munro P, et al. The E3 ubiquitin-ligase *HACE1* catalyzes the ubiquitylation of active Rac1. Dev Cell. 2011;21(5):959–65.
20. Anglesio MS, Evdokimova V, Melnyk N, Zhang L, Fernandez CV, Grundy PE, et al. Differential expression of a novel ankyrin containing E3 ubiquitin-protein ligase, *Hace1*, in sporadic Wilms' tumor versus normal kidney. Hum Mol Genet. 2004;13(18):2061–74.
21. Stankiewicz TR, Linseman DA. Rho family GTPases: key players in neuronal development, neuronal survival, and neurodegeneration. Front Cell Neurosci. 2014;8:314.
22. Nagy V, Hollstein R, Pai T-P, Herde MK, Buphamalai P, Moeseneder P, et al. *HACE1* deficiency leads to structural and functional neurodevelopmental defects. Neurol Genet. 2019;5(3): e330.
23. Mulherkar S, Uddin MD, Couvillon AD, Sillitoe RV, Tolias KF. The small GTPases RhoA and Rac1 regulate cerebellar development by controlling cell morphogenesis, migration and foliation. Dev Biol. 2014;394(1):39–53.
24. Scheffner M, Kumar S. Mammalian HECT ubiquitin-protein ligases: biological and pathophysiological aspects. Biochim Et Biophys Acta (BBA)-Molecular Cell Res. 2014;1843(1):61–74.
25. Zamboni V, Armentano M, Berto G, Ciraolo E, Ghigo A, Garzotto D, et al. Hyperactivity of Rac1-GTPase pathway impairs neuritogenesis of cortical neurons by altering actin dynamics. Sci Rep. 2018;8(1):7254.
26. Tejada-Simon MV. Modulation of actin dynamics by Rac1 to target cognitive function. J Neurochem. 2015;133(6):767–79.
27. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat. 2015;36(10):928–30.
28. Yousaf H, Rehmat S, Jameel M, Ibrahim R, Hashmi SN, Makhdoom EUH, et al. A homozygous founder variant in *PDE2A* causes paroxysmal dyskinesia with intellectual disability. Clin Genet. 2023;104(3):324–33.
29. Yousaf H, Fatima A, Ali Z, Baig SM, Toft M, Iqbal Z. A novel nonsense variant in *GRM1* causes autosomal recessive spinocerebellar ataxia 13 in a consanguineous Pakistani family. Genes. 2022;13(9):1667.
30. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38(16):e164–e.
31. Vigeland MD, Gjotterud KS, Selmer KK. FILTUS: a desktop GUI for fast and efficient detection of disease-causing variants, including a novel autozygosity detector. Bioinformatics. 2016;32(10):1592–4.
32. Mencacci NE, Kamsteeg E-J, Nakashima K, R'Bibo L, Lynch DS, Balint B, et al. De Novo mutations in *PDE10A* cause childhood-onset chorea with bilateral striatal lesions. Am J Hum Genet. 2016;98(4):763–71.
33. Efthymiou S, Salpietro V, Malintan N, Poncelet M, Kriouile Y, Fortuna S, et al. Biallelic mutations in neurofascin cause neurodevelopmental impairment and peripheral demyelination. Brain. 2019;142(10):2948–64.
34. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010;26(5):589–95.
35. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the genome analysis toolkit best practices pipeline. Curr Protoc Bioinf. 2013;43(1110):11. 0 1 – 0 33.
36. Fromer M, Moran JL, Chambert K, Banks E, Bergen SE, Ruderfer DM, et al. Discovery and statistical genotyping of copy-number variation from whole-exome sequencing depth. Am J Hum Genet. 2012;91(4):597–607.

37. Plagnol V, Curtis J, Epstein M, Mok KY, Stebbings E, Grigoriadou S, et al. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. Bioinformatics. 2012;28(21):2747–54.
38. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. Genet Sci. 2015;17(5):405–23.
39. Hollstein R, Parry DA, Nalbach L, Logan CV, Strom TM, Hartill VL, et al. *HACE1* deficiency causes an autosomal recessive neurodevelopmental syndrome. J Med Genet. 2015;52(12):797–803.
40. Kopanos C, Tsiolkas V, Kouris A, Chapple CE, Albarca Aguilera M, Meyer R, et al. VarSome: the human genomic variant search engine. Bioinformatics. 2019;35(11):1978–80.
41. Feng BJ. Perch: a unified framework for disease gene prioritization. Hum Mutat. 2017;38(3):243–51.
42. Mort M, Ivanov D, Cooper DN, Chuzhanova NA. A meta-analysis of nonsense mutations causing human genetic disease. Hum Mutat. 2008;29(8):1037–47.
43. Nuzhdin SV, Friesen ML, McIntyre LM. Genotype–phenotype mapping in a post-GWAS world. Trends Genet. 2012;28(9):421–6.
44. Alzaidan H, Alluhaybi B, Albulayhid NA, Al-Jabr KH, Alotaibi FT, Alqahtani A et al. Autosomal recessive spastic paraplegia and psychomotor retardation with or without seizures: a case report from Saudi Arabia. Cureus. 2024;16(5).

Publisher's note