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Investigating the genetic basis of hereditary spastic paraplegia and cerebellar Ataxia in Pakistani families

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Abstract

Background Hereditary Spastic Paraplegias (HSPs) and Hereditary Cerebellar Ataxias (HCAs) are progressive neurodegenerative disorders encompassing a spectrum of neurogenetic conditions with significant overlaps of clinical features. Spastic ataxias are a group of conditions that have features of both cerebellar ataxia and spasticity, and these conditions are frequently clinically challenging to distinguish. Accurate genetic diagnosis is crucial but challenging, particularly in resource-limited settings. This study aims to investigate the genetic basis of HSPs and HCAs in Pakistani families.

Methods Families from Khyber Pakhtunkhwa with at least two members showing HSP or HCA phenotypes, and who had not previously been analyzed genetically, were included. Families were referred for genetic analysis by local neurologists based on the proband's clinical features and signs of a potential genetic neurodegenerative disorder. Whole Exome Sequencing (WES) and Sanger sequencing were then used to identify and validate genetic variants, and to analyze variant segregation within families to determine inheritance patterns. The mean age of onset and standard deviation were calculated to assess variability among affected individuals, and the success rate was compared with literature reports using differences in proportions and Cohen's h.

Results Pathogenic variants associated with these conditions were identified in five of eight families, segregating according to autosomal recessive inheritance. These variants included previously reported *SACS* c.2182 C > T, p.(Arg728*), *FA2H* c.159_176del, p.(Arg53_Ile58del) and *SPG11* c.2146 C > T, p.(Gln716*) variants, and two previously unreported variants in *SACS* c.2229del, p.(Phe743Leufs*8) and *ZFYVE26* c.1926_1941del, p.(Tyr643Metfs*2). Additionally, *FA2H* and *SPG11* variants were found to have recurrent occurrences, suggesting a potential founder effect within the Pakistani population. Onset age among affected individuals ranged from 1 to 14 years (M = 6.23, SD = 3.96). The diagnostic success rate was 62.5%, with moderate effect sizes compared to previous studies.

Conclusions The findings of this study expand the genotypic and phenotypic spectrum of HSPs and HCAs in Pakistan and emphasize the importance of utilizing exome/genome sequencing for accurate diagnosis or support

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accurate differential diagnosis. This approach can improve genetic counseling and clinical management, addressing the challenges of diagnosing neurodegenerative disorders in resource-limited settings.

Keywords Hereditary Spastic Paraplegias, Hereditary Cerebellar Ataxias, Neurodegenerative disorders, Spastic ataxia, Pakistani families

Background

Hereditary cerebellar ataxias (HCAs) and hereditary spastic paraplegias (HSPs) constitute subtypes of neurodegenerative disorders under the umbrella of spinocerebellar degenerative disorders. These conditions involve the progressive degeneration of cerebellar Purkinje cells, impacting spinocerebellar tracts for ataxias and corticospinal tracts for spastic paraplegia [1]. Clinical classifications distinguish them based on specific symptoms, with ataxias characterized by gait and limb ataxia, coordination loss, and oculomotor disturbances, while spastic paraplegia manifests as lower limb spasticity and weakness. Additionally, the disorders are categorized as pure or complex, depending on the presence of additional symptoms such as polyneuropathy, dementia, and tremor [2]. Classifying disorders within HSPs presents challenges due to intricate inclusion/exclusion criteria. Genetic variants leading to spastic paraparesis, and ataxia are recognized as HSPs syndromes, whereas ataxia associated with corticospinal tract deficits is categorized as spinocerebellar ataxia, not HSPs [3]. Among the 81 genetic forms of HSPs, 28 exhibit alternative phenotypes, complicating diagnosis. Disease-specific gene panels in genetic testing emphasize accurate clinical classification, influencing diagnostic yield and complicating test planning [4, 5]. This classification relies on clinical and genetic features rather than neuropathological ones [3]. The challenge is worsened in populations with limited access to advanced diagnostic tests, highlighting the importance of understanding the genetic aspects of HSPs and HCAs for precise diagnosis and effective management. This is especially crucial in regions facing financial constraints and geographical impediments, as observed in populations like Pakistan, where access to advanced clinical diagnostic facilities is constrained.

Recent advances in genomic technologies, such as whole exome sequencing (WES), have provided new insights into the genetic basis of these disorders, though significant challenges remain in translating these findings into clinical practice in resource-limited settings. These technologies offer the potential to identify novel genetic variants and improve diagnostic accuracy. However, the integration of these findings into routine clinical practice is hindered by factors such as limited availability of advanced diagnostic tools and expertise in certain regions [6].

Both HSPs and HCAs exhibit significant genetic heterogeneity, with numerous causative genes identified [5, 7]. Common genes for autosomal recessive HSPs include *ZFYVE26*, *B4GALNT1*, *DDHD1*, *REEP1*, *NT5C2*, *AP4M1*, *AP4S1*, *DDHD2*, *CYP2U1*, and *TFG*, contributing to the diverse spectrum of manifestations observed in these neurodegenerative disorders. Cognitive decline and intellectual disability are newly identified features associated with certain gene variants [8, 9]. HSPs, often linked with genes implicated in HCAs such as *GRID2*, *PNPLA6*, *GBA2*, *SLC52A2*, *COG5*, *SLC25A46*, *SYNE1*, and *TSEN54*, display a broad spectrum of phenotypes. Genetic analysis reveals that *FA2H* gene variants in HCAs encompass leukoencephalopathy and neurodegeneration with iron brain accumulation, highlighting the varied manifestations within a single gene. Clinical presentations such as leukoencephalopathy, hypogonadotropic hypogonadism, and chorioretinal dystrophy overlap in both HSPs and HCAs [10, 11]. This clinical overlap is evident in sequence variants, with some cases initially presenting as spastic paraplegia before evolving into a cerebellar syndrome [12].

Despite significant progress, gaps remain in our understanding of the full spectrum of genetic variants and their phenotypic implications in different populations. Limited studies in Pakistan have explored the genetic basis of HSPs and HCAs within familial contexts. Recent investigations identified homozygous variants in genes like *ZFYV26*, *CYP2U1*, and *BICD2* in Pakistani families with HSPs, revealing diverse phenotypic manifestations [13, 14]. Previous studies, such as those by Saddi et al. [15] and Zaman et al. [16], identified novel, recurrent, and previously reported pathogenic variants in genes like *ZFYVE26*, *SACS*, *BICD2*, *ALS2*, *B4GALNT1*, *FA2H*, *APTX*, and *SETX*, contributing to the understanding of overlapping phenotypes in neurodevelopmental disorders within Pakistani families. Additionally, HCAs were associated with genes such as *GRM1*, *ERCC8*, *FA2H*, *APTX*, *SETX*, *ZFYVE26*, *SACS*, *BICD2*, *ALS2*, and *B4GALNT1*. Notably, over six additional genes (*ATL1*, *FA2H*, *GJC2*, *AP4E1*, *ALDH18A1*, and *ATP13A2*) were mapped in Pakistani families exhibiting HSPs and HCAs phenotypes. These findings emphasize the importance of genetic studies in unraveling the complexities of neurodegenerative disorders in the region, offering valuable insights for targeted diagnostics and potential therapeutic interventions [14, 15, 17].

This study aims to address the existing knowledge gap by exploring the genetic basis of lower limb weakness and ataxia in Pakistani families through WES and Sanger

Sequencing. By identifying novel and recurrent genetic variants in key neurodegenerative genes, the study seeks to contribute to the understanding of HSPs and HCAs within this specific population. The identification of biallelic pathogenic variants in *SACS*, *FA2H*, *ZFYVE26*, and *SPG11* genes expands the genotypic and phenotypic spectrum of HSPs and HCAs in Pakistan, offering valuable insights for targeted diagnostics and potential therapeutic interventions.

Methods

Study design

This study was designed to investigate the genetic basis of neurodegenerative disorders in families from Khyber Pakhtunkhwa, Pakistan, specifically focusing on HSP and HCA. Ethical approval was obtained from the Ethical Committee and Advanced Studies and Research Board (ASRB) at KUST, Pakistan. The data were sourced from clinical assessments and genetic analyses conducted between January 2022 and September 2023. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Informed written consent was obtained from both adult participants and parents of affected children involved in the study.

Inclusion and exclusion criteria

Families were eligible for inclusion if they met the following criteria:

Inclusion criteria

- Resided in Khyber Pakhtunkhwa, Pakistan.
- Had at least two family members exhibiting phenotypes consistent with either HSP or HCA.
- No prior genetic analysis or provision of blood/DNA samples for analysis.

Exclusion criteria

- Families who had previously undergone genetic testing or provided blood/DNA samples for analysis.

The families were identified through initial clinical examinations by local neurologists in districts' hospitals in Khyber Pakhtunkhwa, focusing on the proband. The proband's clinical features, alongside findings from clinical investigations—when available—and indications of a potential genetic neurodegenerative disorder, determined their eligibility for referral for genetic analysis [18].

Outcome measures

Clinical data collection

- Detailed clinical profiles were created for each proband and their affected family members. This included the diverse manifestations of HSP or HCA.
- Comprehensive family histories and age of onset were documented, and pedigrees were constructed to trace the inheritance patterns within families.

Genetic analysis

Genomic DNA was extracted from blood samples by the ReliaPrep™ kit (Blood gDNA Miniprep System, Promega) and the MagMAX DNA Multi-Sample Ultra 2.0 Kit, by applying the automated KingFisher Apex system (Thermo Scientific), as well as the standard Phenol-Chloroform method. WES was performed on DNA from individuals IV:6 in family-1, IV:3 in family-2, IV:5 in family-3, IV:2 in family-4, III:1 in family-5, VI:6 in family-6, VI:2 in family-7 and V:3 in family-8 (NextSeq500; Illumina, Twist Human Core Exome targeting)//(HiSeq; Illumina, Agilent Sureselect human Whole Exome v6 targeting). Analysis involved read alignment (BWA-MEM v0 0.7.17), mate-pairs fixed, and duplicates removed (Picard v2.15), InDel realignment and base quality recalibration (GATK v3.7.0), single nucleotide variant and InDel detection (GATK Haplotype Caller), variant annotation (Alamut batch v1.11) and read depth assessment (GATK Depth of Coverage). Copy number variants (CNVs) were detected using SavvyCNV. For all platforms a minimum read depth and coverage of 20x was achieved for 90–95% of the exome. Variants with <5 reads and/or a frequency $>0.5\%$ in public genome databases including the Genome Aggregation Database (gnomAD v2.1.1) and the 1000 Genomes Project were excluded. Homozygous and compound heterozygous variants that were exonic and non-synonymous, synonymous with predicted splicing impact or intronic at ± 6 nucleotides from splice sites were prioritized for further analysis based on pedigrees of affected families, maintaining focus on autosomal recessive inheritance patterns. Variants were then assessed for clinical correlation with the affected individuals' phenotype using the HGMD (http://www.hgmd.cf.ac.uk/ac/search.php), PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), and OMIM (https://www.ncbi.nlm.nih.gov/omim/) databases to create a list of genetic variants as potentially causative for further analysis. The pathogenicity of genetic variants was assessed according to the standards and guidelines set by the American College of Medical Genetics and Genomics (ACMG) [19]. Polymerase Chain Reaction (PCR) and Sanger sequencing were utilized to confirm candidate variant segregation with disease phenotypes in family members. Allele-specific primers, designed

using primer3 software (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) were employed to amplify the genomic DNA containing candidate variants, and sequenced by Source BioScience LifeSciences (https://www.sourcebioscience.com/) or Beijing Tsingke Biotech Co., Ltd. (https://tsingke.com/pages/about-us-1) and assessed with chromatogram viewer software Chromas Lite version 2.6.6. Only variants co-segregating with disease in all family members were considered causative for HSP or HCA.

Statistical analysis

The mean age of onset and standard deviation were calculated to characterize the variability in the affected individuals. To evaluate the efficacy of the genetic diagnostic approach, the success rate of the current study was compared with those reported in the literature. Specifically, studies by Riso et al. [20], which reported a 46.3% success rate, and Lan et al. [21], which reported a 73% success rate, were used for comparison. The difference in proportions and Cohen's h were calculated to quantify the effect size and assess comparative effectiveness.

Results

A total of eight families were recruited for the study based on the inclusion criteria. In addition, another family, identified during the study period through a proband, was excluded because it had provided blood samples for another research group for analysis. A total of 21 affected individuals (16 males and 5 females) from recruited 8 families were included in this study. The age of onset among the affected individuals across the eight families ranged from 1 to 14 years. The mean age of onset was calculated to be 6.23 years, with a standard deviation of 3.96 years. A genetic diagnosis was established in 62.5% of the families presenting with HCA or HSP phenotypes. Comparatively, Riso et al. (2021) reported a success rate of 46.3%, and Lan et al. (2022) reported 73%. The difference in proportions between this study and Riso et al. (2021) was 0.162, while the difference compared to Lan et al. (2022) was −0.105. Cohen's h was calculated to assess the effect size: h ≈ 0.172 for comparison with Riso et al. (2021) and h ≈ −0.128 for comparison with Lan et al. (2022). These values indicate moderate differences in success rates compared to previous studies.

Table 1 presents organized clinical data on HSPs or HCAs in probands from families where a genetic diagnosis was established, providing an overview of the observed clinical profiles. Table 2 lists the pathogenic variants identified in five families with features of complex HSP or HCA, along with ACMG classification and genomic database allele frequencies. The table in the supplementary data presents descriptions of three families and a list of variants that were analyzed for segregation, but a genetic diagnosis could not be established. Figure 1 displays the pedigrees of families and the segregation of pathogenic variants in those families where causal genetic variants for HSP and HCA were identified. It was observed that parents of affected individuals in Families 1 to 4 practiced consanguineous marriages, while parents of affected individuals in Family-5 practiced tribal endogamy, leading to autosomal recessive disease inheritance.

Family-1

Family −1, as illustrated in Fig. 1A, includes three affected siblings, all presenting with a severe and progressive neurological disorder. The proband, identified as a 15-year-old female (IV:6), initially showed signs of early delayed motor development. Over time, her condition worsened, leading to quadriparesis. Additionally, she exhibited cerebellar features, such as ataxia and dysarthria. Her condition progressed significantly, resulting in complete paralysis by the age of 15.

Her siblings, labeled as IV:1 and IV:2, displayed clinical features similar to those of the proband. The uniformity in their clinical features includes early motor delays, progressive muscle weakness leading to quadriparesis, muscle atrophy, foot drop, and cerebellar dysfunctions like ataxia and dysarthria. The progression of the disease in all three siblings' points towards a severe, likely hereditary, neurological disorder that significantly impairs motor functions and coordination, culminating in paralysis.

In the proband of family-1, WES identified a novel homozygous frameshift variant [Chr13(GRCh38): g.23341649del, NM_014363.6: c.2229del, p.(Phe743Leufs*8)] in exon 10 of the *SACS* gene (Fig. 1B). Sanger sequencing confirmed this variant segregation within the family (Fig. 1A). As this variant is located in the last exon, it is predicted to escape nonsense mediated decay (NMD), despite exon 10 being large, containing 3851 amino acid residues and comprising 84% of the SACS protein [22]. This variant was not observed in population databases including gnomAD (v2.1.1, v4.0), and 1000 genomes project, with no homozygous individuals. According to ACMG criteria, the p. (Phe743Leufs*8) variant is predicted to be pathogenic (Table 2).

Family-2

In family-2, depicted in (Fig. 1A), the male proband (IV:3), aged 20 years, was clinically diagnosed with Charcot-Marie-Tooth (CMT) disease. This diagnosis was confirmed through nerve conduction studies, which revealed chronic sensory and motor demyelinating peripheral polyneuropathy. Additionally, there was evidence of secondary axonal loss, indicating that the nerve fibers themselves were also degenerating.

Table 1 Clinical features of five Pakistani families presenting with HSPs or HCAs

	Family 1	Family 2	Family 3	Family 4	Family 5
Gene	*SACS*	*SACS*	*FA2H*	*ZFYVE26*	*SPG11*
Variant(s)	c.2229del; p.(Phe743Leufs*8)	c.2182 C>T; p.(Arg728*)	c.159_176del; p.(Arg53_Ile58del)	c.1926_1941del; p.(Tyr643Metfs*2)	c.2146 C>T; p.(Gln716*)
Zygosity	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous
Condition (OMIM #)	Spastic ataxia, Charlevoix-Saguenay type (270550)	Spastic ataxia, Charlevoix-Saguenay type (270550)	Spastic paraplegia 35, autosomal recessive (612319)	Spastic paraplegia 15, autosomal recessive (270700)	Spastic paraplegia 11, autosomal recessive (604360)
Neurological					
Muscle wasting	++	+	++	+	++
Lower limb weakness	++	++	++	++	+
Upper limb weakness	++	+	+	+	+++
Spasticity	NK	NK	NK	+	+
Tendon reflexes	_	+++	NK	+++	NK
Gait abnormalities	Ataxia	NK	++	Spastic/scissoring	+
Cerebellar ataxia	++	+	+	+	+
Nystagmus	NK	NK	NK	-	NK
Dysarthria	++	NK	NK	-	NK
Pes cavus	NK	++	NK	NK	+
Tone	NK	NK	NK	Normal	NK
Foot drop	+++	NK	NK	+	++
Peripheral neuropathy	NK	+	+	-	NK
Nerve conduction studies/ MRI	NK	Chronic sensory and motor demyelinating peripheral polyneuropathy with secondary axonal loss	MRI brain: Leukodystrophy: bilateral symmetrical abnormal signal intensity areas involving cerebral white matter	MRI cervical spine: NAD	NK
Intellectual disability	-	-	+	-	++
Motor delay	++	NK	NK	NK	NK
Other	-	-	-	Elevated CK Mild colitis	-

Abbreviations: + present, ++ medium, +++ Severe, - absent, NK not known, NAD no abnormalities detected, CK creatine kinase, MRI magnetic resonance imaging

Table 2 Pathogenic variants identified in five families with features of complex HSP or HCA, ACMG classification and genomic database allele frequencies

Family ID	Gene	Nucleotide Variant	Protein Variant	ACMG Classification	Allele Frequency gnomAD (4.0, 2.1.1)	Novelty status	Previously reported in Ethnicities
Family-1	*SACS*	c.2229del	p.(Phe743Leufs*8)	Pathogenic (PVS1, PM2, PM3_supporting)	Absent	Current study	
Family-2	*SACS*	c.2182 C>T,	p.(Arg728*)	Pathogenic (PVS1, PM2, PM3_supporting)	0.00001597	PMID: 18465152 PMID: 26539891	Dutch, Turkish
Family-3	*FA2H*	c.159_176de,	p.(Arg53_Ile58del)	Likely pathogenic (PM1, PM2, PM3_supporting, PM4, PP1_moderate)	0.000007933	PMID: 33246395 PMID: 20104589 PMID: 37510308	Pakistani
Family-4	*ZFYVE26*	c.1926_1941del,	p.(Tyr643Metfs*2)	Pathogenic (PVS1, PM2 PM3_supporting)	Absent	Current study	
Family-5	*SPG11*	c.2146 C>T	p.(Gln716*)	Pathogenic (PVS1, PM2, PM3_supporting)	0.000004957	PMID: 20390432 PMID: 27217339	Indian, Pakistani

A paternal cousin, identified as IV:15, displayed similar clinical features, suggesting a familial link to the disorder. The cousin had progressive quadriparesis from birth. Hyperreflexia was also noted, alongside muscle wasting in both the upper and lower limbs. Additionally, the cousin exhibited pes cavus.

WES of individual IV:3 identified a homozygous nonsense variant [Chr13(GRCh38): g.23353788G>A, NM_014363.6: c.2182 C>T, p.(Arg728*)] in exon 9 of the *SACS* gene (Fig. 1B), which segregated within the family as expected for an autosomal recessive condition (Fig. 1A). The p.Arg728* variant was previously

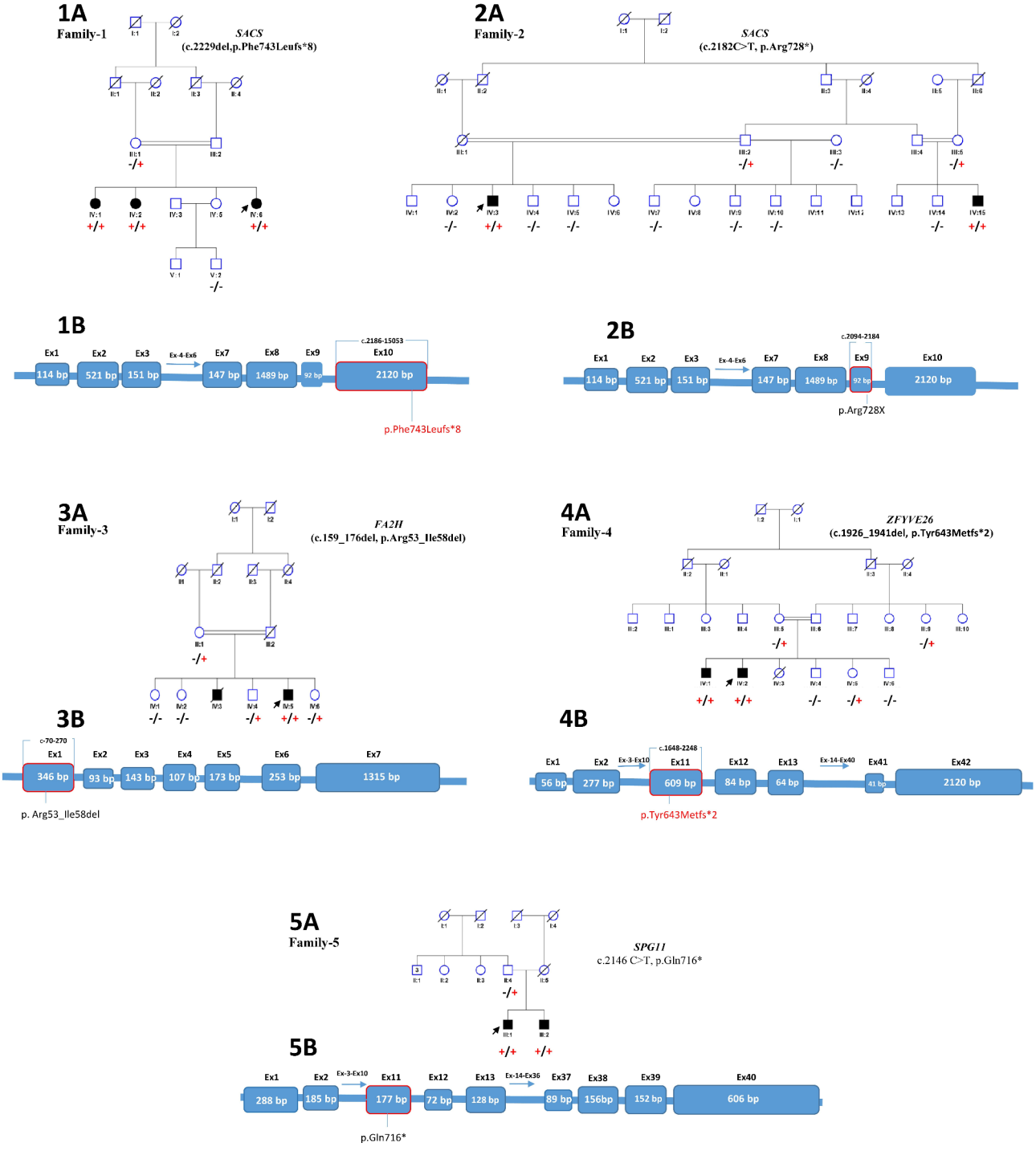

Fig. 1 Pedigrees of the five Pakistani families investigated, and genetic findings. (**A**) Pedigrees showing segregation of the pathogenic variants identified in each individual. Arrow symbols indicate WES screened samples, and the pedigree symbols represent genotyping results: (+/-) heterozygous carrier, (+/+) homozygous affected, and (-/-) homozygous wildtype. (**B**) Schematic diagrams of the *SACS*, *FA2H*, *ZFYVE26*, and *SPG11* genes showing the location of previously reported pathogenic variants in black and those identified by WES in the current study in red

identified in the literature as pathogenic and associated with an HSP phenotype, as the truncated SACS protein is predicted to undergo NMD [23]. With a minor allele frequency (MAF) of 0.00001597 in gnomAD v4.0 and no homozygous individuals detected, this variant is classified as pathogenic according to ACMG criteria (Table 2).

Family-3

In family-3, illustrated in (Fig. 1A), individual IV:5 exhibited significant neurological symptoms starting at a young age. By the age of 6 years, IV:5 began experiencing difficulty walking. This symptom was accompanied by muscle wasting and progressive weakness in both upper and lower limbs, with the lower limbs being more severely affected. Additionally, IV:5 suffered from peripheral sensation loss. The proband's gait was ataxic, indicating a lack of coordination and unsteady movement. This, along with other cerebellar signs, pointed to dysfunction in the cerebellum. MRI neuroimaging showed bilateral symmetrical periventricular abnormal white matter signal intensity suggestive of a leukodystrophy.

Unfortunately, another affected sibling (IV:3) with similar clinical presentation died at the age of 4 years from complications. This sibling exhibited comparable symptoms, suggesting a genetic or hereditary basis for the condition affecting these siblings. The early onset and severe progression of symptoms in both siblings highlight the aggressive nature of the disorder.

A homozygous inframe deletion [Chr16(GRCh38): g.74774583_74774600del, NM_024306.5: c.159_176del, p.(Arg53_Ile58del)] in exon 1 of the *FA2H* gene (Fig. 1B) was identified by WES as the underlying cause of the disease in the proband, and segregation was confirmed for all living family members (Fig. 1A). The p.(Arg53_Ile58del) variant causes an inframe deletion of 7 amino acids within a highly conserved region, affecting the protein structure and resulting in a distinct molecular configuration. This variant has been previously associated with HSP in Pakistani families [17, 24].

It is rare, with a MAF of 0.000007933 in gnomAD v4.0, and no homozygous individuals have been observed. According to ACMG criteria, this variant is classified as likely pathogenic (Table 2).

Family-4

In family-4, as illustrated in (Fig. 1A) the proband is a 22-year-old male (IV:2) who has experienced progressive lower limb weakness and pain starting at the age of 14. Based on clinical assessments, he was diagnosed with HSP. Upon clinical examination, IV:2 exhibited several hallmark features of HSP. He had a scissoring gait. IV:2 also had foot drop. Further examination revealed lower limb weakness with muscle power graded at 4-/5 bilaterally. Spasticity and severe hyperreflexia, accompanied by an increase in muscle tone and sensation, were noted in the lower limbs. Laboratory tests showed a mildly elevated creatine kinase level (240U/L), suggesting some degree of muscle damage or stress. However, MRI imaging of the cervical spine was normal, ruling out cervical spinal cord lesions as a cause of his symptoms. In addition to the neurological symptoms, IV:2 had recurrent episodes of diarrhea. A colonic biopsy revealed a mild lymphocytic infiltration in the lamina propria, leading to a diagnosis of mild colitis. This gastrointestinal involvement is noteworthy as it adds a layer of complexity to his clinical picture, although it is not typically associated with HSP.

The proband's brother, identified as IV:1, exhibited a similar clinical presentation, supporting the diagnosis of HSP within the family. Overall, the case of IV:2 and his brother IV:1 illustrates the progressive and multifaceted nature of HSP, characterized by lower limb spasticity and weakness, specific gait abnormalities, and additional complications such as mild colitis.

Given the pattern of inheritance and symptoms, the siblings in the fourth family likely have a form of HSP and an additional complication of mild colitis.

WES of IV:2 revealed a novel homozygous frameshift variant, [Chr14 (GRCh38): g.67798323_67798338del, NM_015346.4: c.1926_1941del, p.(Tyr643Metfs*2)], in exon 11of the *ZFYVE26* gene (Fig. 1B), shared by affected sibling IV:1 and segregating within the family in an autosomal recessive manner (Fig. 1A). The p.Tyr643Metfs*2 variant results in a truncated ZFYVE26 protein, which is predicted to undergo NMD [22]. It is absent from gnomAD (v2.1.1, 4.0) with no homozygous individuals, and it is classified as pathogenic based on ACMG criteria (Table 2).

Family-5

In family-5, depicted in (Fig. 1A), two male siblings, identified as III:1 and III:2, both began experiencing progressive lower limb weakness at the age of 10 years. Their clinical presentations, although similar in onset, have evolved with some differences by the time they reached their late teens. The progressive nature of their conditions, with an early onset at around 10 years and worsening symptoms by their late teens, suggests a genetic neuromuscular disorder.

By the age of 17, the proband individual III:1 has developed severe lower limb weakness and spasticity. Due to the severity of his lower limb impairment, he now relies on a wheelchair for mobility. In addition to his lower limb issues, III:1 has milder upper limb weakness. Another notable feature in his clinical presentation is the clawing of the hands.

The sibling, III:2, presents a somewhat different progression of symptoms. At 17 years old, he exhibits

severe gait ataxia. Like his brother, III:2 also experiences progressive muscle weakness, though the description emphasizes his gait abnormalities over other specific limb weaknesses.

Considering the pattern of inheritance (two male siblings affected) and the described symptoms, a potential diagnosis could involve a form of HSP with additional ataxic features or a hereditary neuropathy that includes motor and sensory components.

A homozygous variant [Chr15(GRCh38):g.44918627G>T, NM_025137.4:c.2146 C>T, p.(Gln716*)] in exon 11 of the *SPG11* gene (Fig. 1B) was identified in the proband (III:1). Segregation studies confirm an autosomal recessive inheritance pattern of the *SPG11* variant (Fig. 1A). This variant, predicted to undergo NMD, has been reported as pathogenic in the literature associated with HSP with thin/absent corpus callosum [25, 26]. The *SPG11* p.(Gln716*) variant has a MAF of 0.000004957 (gnomAD v4.0), with no homozygous individuals and it is classified as pathogenic based on ACMG criteria (Table 2).

Discussion

The genetic exploration of HSPs and HCAs in Pakistani families presents considerable challenges, primarily due to the complexities in classifying these neurodegenerative disorders. The difficulty in distinguishing between HSPs and spastic ataxias based solely on clinical features complicates the diagnostic process, particularly in resource-limited settings with limited access to advanced diagnostic tests [27]. The primary aim of this study was to investigate the genetic basis of HSPs and HCAs to improve diagnosis and management, especially in regions with limited healthcare resources. This study successfully identified novel, recurrent, and previously reported pathogenic variants in the *SACS*, *FA2H*, *ZFYVE26*, and *SPG11* genes in five Pakistani families from Khyber Pakhtunkhwa. These families exhibited variable but overlapping clinical phenotypes of HSPs and HCAs. The genetic diagnosis success rate in this study was 62.5%, which is higher than the 46.3% reported by Riso et al. (2021) but slightly lower than the 73% reported by Lan et al. (2022) indicate moderate variability, underscoring the importance of considering population-specific factors and methodologies in interpreting these results [20, 21]. These findings contribute to understanding the genetic landscape of HSPs and HCAs and highlight the importance of next-generation sequencing combined with clinical assessments for accurate diagnosis.

Families 1 and 2, exhibiting HCA phenotypes, homozygous variants p.(Phe743Leufs*8) and p.(Arg728*) within the *SACS* gene were identified as the underlying cause of the disease. The p.(Arg728*) variant was previously reported in a Dutch patient [28] and a Turkish patient [29] with biallelic familial inheritance. Biallelic *SACS* variants are associated with Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS, OMIM 270550). ARSACS is a complex neurodegenerative disorder characterized by early childhood onset of cerebellar ataxia, pyramidal tract signs, and peripheral neuropathy, often resulting in wheelchair-bound patients with preserved cognitive function [30]. Initially recognized in Quebec, ARSACS has also been reported in individuals of Dutch, Turkish, and Brazilian origin [31]. Additionally, ARSACS has been reported in Pakistani families [15, 16]. These reports highlight the presence of ARSACS in diverse populations, underscoring its broader geographic and ethnic distribution beyond its originally recognized demographic. The presence of peripheral neuropathy in CMT, accompanied by diminished conduction velocities (CVs), could indicate a potential link to ARSACS. Distinctive MRI features in the pons and alterations in retinal nerve fiber structure could serve as diagnostic markers for ARSACS [32]. However, overlooking these indicators may result in misdiagnosis as CMT. Vill et al. reported patients initially labeled with CMT but later confirmed to have ARSACS. Similarly, the proband in a Pakistani family investigated in the current study was initially diagnosed with CMT with axonal loss, exhibiting clinical features consistent with ARSACS. This emphasizes the importance of thorough differential diagnostic evaluation, including genetic testing and comprehensive clinical investigations, to accurately distinguish between these conditions. Shared characteristics included muscular atrophy, quadriparesis, gait abnormalities, and high foot arches, consistent with features previously reported in individuals carrying *SACS* gene variants [23].

Variants in the *SACS* gene disrupt the normal production or function of sacsin expressed from this gene [31]. The mutated *SACS* gene expresses a dysfunctional sacsin/DNAJC29 protein, which normally plays a crucial role in maintaining the health and function of nerve cells, particularly in the cerebellum and spinal cord [33]. Sacsin, one of the largest human proteins, consists of 4579 amino acids and features a modular structure with a ubiquitin-like (UBL) domain that interacts with the proteasome. SiRNA-mediated sacsin knockdown experiments suggest sacsin's protective role against mutant ataxin-1 and its potential modulation of the effects of other ataxia-related proteins [34]. Among the 392 variants in the *SACS* gene reported in HGMD, 65% have been associated with ataxia, including spastic ataxia, ARSACS, and cerebellar ataxia. The remaining 35% of the variants have been linked to various neurodevelopmental disorders such as CMT, ataxia, intellectual disability and epilepsy. In addition, among the 392 *SACS* variants, 172 different types lie in the last exon 10 that is predicted to escape NMD,

although evidence that variants in exon 10 can result in loss of function of Sacsin [35]. This provides support for the pathogenicity of a novel frameshift variant c.2229del, p.(Phe743Leufs*8) in exon 10, that is predicted to escape NMD although this variant results in the loss of 85% of the SACS protein and is classified as likely pathogenic according to ACMG criteria. This expands the genotypic spectrum of known variants causing ARSACS and supports the pathogenicity of variants in exon 10.

In family- 3, the proband exhibited features of HSP with intellectual disability. MRI neuroimaging at six years revealed white matter abnormalities, suggestive of leukodystrophy associated with a rare homozygous *FA2H* variant c.159_176del, p.(Arg53_Ile58del). The identified *FA2H* variant, (p.Arg53_Ile58del), has previously been detected in families from various regions of Pakistan, including Khyber Pakhtunkhwa, exhibiting similar presentations of complex HSP [15, 16]. Its recurrence in another Pakistani family in our study suggests a potential founder effect within this population. Among the studies conducted in Pakistan, only one has reported that patients with HSP also had leukodystrophy, similar to our findings [24]. Interestingly, the HGMD reports 88 variants in the *FA2H* gene, with 52% associated with HSP and only 8% contributing to leukodystrophy. The overestimation of HSP cases in HGMD could be due to underreporting, as MRI and genetic testing are not routinely performed in most clinical settings, leading to diagnoses based solely on clinical features. Importantly, white matter lesions on MRI typically signify leukodystrophies, but late-onset cases may lack these, leading to misdiagnosis [36–38] and may also contribute to the overestimation of HSP cases. As there is no effective HSP treatment, advancements in leukodystrophy therapies offer hope for improvements [33, 34]. Early differentiation between the two conditions and understanding the *FA2H* variants' link to these conditions are vital for prognosis and early treatment for other pedigree members, emphasizing the importance of incorporating genetic analysis findings into differential diagnostic assessments [39]. Fatty acid 2-hydroxylase plays a crucial role in myelination, and alongside the absence of 2-hydroxylated sphingolipids in *FA2H*-deficient mice, emphasizes its importance in understanding the shared phenotypes of leukodystrophy and HSP observed in humans with *FA2H* variants [40].

The proband in family- 4 displayed features of HSP with cerebellar ataxia, with a novel variant, c.1926_1941del, p.(Tyr643Metfs*2) identified in the *ZFYVE26* gene, established as the cause of disease. Biallelic *ZFYVE26* variants are associated with autosomal recessive spastic paraplegia-15 (SPG15) [41], and confirmed as a cause of HSP across diverse populations [42–44], including Pakistani families with HSP cause by different *ZFYVE26* variants [14], and our findings expand the genotypic spectrum within this population. In this study, we observed mild colitis confirmed on colonic biopsy in the affected brothers, which is a previously unreported feature of SPG15. It's important to note that while this association suggests a potential link between SPG15 and colitis, it does not establish causation, as other factors such as shared environmental influences could contribute. Further validation of this association in a larger number of families is necessary to confirm its significance. HGMD reports a total of 85 variants in the *ZFYVE26* gene causing various neurodegenerative disorders, with 45% confirmed to be causative for HSP, and none previously associated with colitis. The *ZFYVE26* gene encodes a protein that features a FYVE zinc finger binding domain, playing a crucial role in the maturation of autophagosomes. Extensive studies have revealed that ZFYVE26 interacts with BECN1 and its associated proteins, including PIK3C3, UVRAG, and RUBCN, which collectively serve as major regulators of autophagy and endocytosis. The disruption of these interactions in the presence of biallelic *ZFYVE26* loss-of-function variants results in the accumulation of immature autophagosomes and impairs autophagosome-lysosome fusion in cells derived from individuals with SPG15 [45]. Studies involving *ZFYVE26* knockout mice have provided additional insights, demonstrating the accumulation of large intraneuronal deposits containing lysosomal markers. This accumulation is accompanied by axonal degeneration and the progressive loss of both cortical motor neurons and cerebellar Purkinje cells [46]. Disrupted function, observed in SPG15 individuals and knockout mice, highlights the critical importance of *ZFYVE26* in neuronal homeostasis, providing insights into the pathogenic mechanisms of HSP.

WES in family- 5 identified a previously reported homozygous variant (c.2146 C>T, p.(Gln716*) in the *SPG11* gene as the cause of complex HSP with cerebellar ataxia and intellectual disability [47]. *SPG11* has over 300 documented variants, with more than 75% associated with HSP and the remaining 25% reported in other neurodegenerative disorders with overlapping features. Biallelic variants in *SPG11* cause autosomal recessive spastic paraplegia-11 (SPG11, MIM #604360), characterized by spasticity, cognitive impairment, and peripheral neuropathy, with structural brain abnormalities including thinning or absence of the corpus callosum (HSP-TCC) [3, 48]. The symptoms of *SPG11* can vary, and the gradual onset of these symptoms may lead to diagnostic delays [49, 50] and MRI neuroimaging is useful to detect corpus callosal abnormalities. The patients described in this study exhibited features of HSP with ataxia and intellectual disability. Due to logistical constraints, MRI neuroimaging could not be performed for these individuals who reside in rural Pakistan. This emphasizes the importance of genetic studies in identifying disease-associated

variants to confirm diagnoses, especially given the diverse phenotypic manifestations associated with *SPG11* variants. The *SPG11* gene encodes spatacsin, a large protein implicated in autophagic lysosome reformation [51]. Fibroblasts from patients with biallelic *SPG11* variants exhibit defective autophagosome-to-lysosome fusion, linking genetic defects in autophagy to HSP [52].

In conclusion, this study provides valuable insights into the genetic underpinnings of HSPs and HCAs in Pakistani families, particularly in regions with limited diagnostic resources. By employing WES and Sanger Sequencing, key pathogenic variants were identified, enhancing the understanding of the genetic diversity associated with these disorders. The findings emphasize the importance of accurate genetic diagnosis and differential diagnosis for effective clinical management and genetic counseling, especially in resource-limited settings. Future research should aim to include a larger cohort from the Khyber Pakhtunkhwa region to further validate and expand these findings. Additionally, functional studies are needed to elucidate the mechanisms of the identified variants and to develop targeted diagnostic tools and therapeutic strategies. These efforts will help improve patient outcomes and enhance our understanding of these complex neurodegenerative disorders.

Supplementary Information

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Supplementary Material 1

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Author contributions

Clinical data collection, collation, and analysis: AA, ANA, IU, LER, ELB and SS; Genetic testing and data analysis: AA, ANA, MY, NK, NU, NV, LER, ELB, AHC and SS; Manuscript writing: AA, ANA, NK and SS; Manuscript revision; LER, ELB and AHC; Study supervision and coordination: NK and SS. All authors read and approved the final manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are available in the ClinVar repository, with the accession numbers i.e., SCV004812218, SCV004812219, SCV004812220, SCV004812221 and SCV004174820.1.

Declarations

Ethical approval and consent to participate

The study was approved by the Ethical Committee of Kohat University of Science and Technology. The study was conducted in accordance with Declaration of Helsinki. Informed written consent was obtained from the participating members of the families and the parents of the minor children.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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