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Experience in the clinical and genetic diagnosis of a series of Algerian patients with hereditary spastic paraplegias

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Abstract

Hereditary spastic paraplegias (HSPs) are a heterogeneous group of neurodegenerative diseases that include more than 90 genetic forms. The diagnosis of HSPs is challenging owing to their exceptional clinical and genetic heterogeneity. Using whole exome sequencing (WES), we investigated the genetic and clinical features of 29 patients from 16 Algerian families diagnosed with HSPs. The prevalence of complicated forms was higher than that of pure forms, with interfamilial clinical variability. We identified pathogenic variants in 11 HSPs-causing genes with a diagnostic rate of 81.2%. The diagnosed families mainly presented an autosomal recessive inheritance pattern due to the high frequency of consanguineous marriages in Algeria. Five novel causative variants were identified in the homozygous state: *GBA2*:c.1351C>T/p.Arg451Ter in two sisters with the standard SPG46 phenotype; *SPG11*:c.6530_6534del in three siblings with a complicated HSP phenotype without thin corpus callosum; *HPDL*:c.862T>C/p.Tyr288His in a patient with complicated HSP; *AMFR*:c.707+1G>A in a patient with a complicated form extending the clinical spectrum of SPG89; and *SPAST*:c.1076T>C in two sisters with a pure HSP form overlapping with the classical SPG4 phenotype associated with dominant pathogenic variants in the same gene. In one family, reverse phenotyping revealed a case of Kufor-Rakeb syndrome after *ATP13A2* variants were identified, showing the importance of dialogue between clinicians and geneticists. This study is the first series of HSPs consisting exclusively of Algerian families. Our findings broaden the clinical and genetic spectrum of HSPs, confirm their high heterogeneity in Algeria, and underline the efficiency of WES as a diagnostic tool for genetically heterogeneous diseases.

Keywords: Hereditary spastic paraplegias, Diagnosis, Whole exome sequencing, Autosomal recessive inheritance, Consanguinity, Algeria

Statements and Declarations

Competing Interests: GS received a grant from Biogen, unrelated to this study from 2019 to 2023. All other authors have no relevant financial or non-financial interests to disclose.

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Ethics approval: All procedures performed in human participants were done in accordance with the ethical agreement of the **Mustapha Hospital ethics committee** (n° 17/CE/2025) and followed the 1964 Helsinki Declaration and its later amendments.

Consent: Informed written consent from all human subjects or their legal guardians, have been obtained prior to clinical examination and blood sampling, allowing the genetic testing and use of the data for scientific publication.

Availability of data and materials: Full dataset of candidate pathogenic variants identified in this can be found in ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>, accession numbers: SCV004847101, SCV004849363, SCV004933973, SCV004849374, SCV004849377, SCV005038742, SCV004934122, SCV004933975, SCV004849364, SCV004934093, SCV005367981, SCV004934094, SCV004934118, and SCV004849365).

Author contributions: MMK and GS conceived and designed the study. MIK, RBH, LAP, WAEK, and FM provided samples from patients and contributed to clinical, radiological, and biochemical data collection and interpretation. MMK, LGN and CD performed laboratory work. MMK, MGB, JMdeSA, GS and LGN contributed to the bioinformatics analysis. MMK drafted the manuscript. MMK and GS revised the manuscript. GS obtained dedicated grants for this study. All authors have read the manuscript, accepted its submission, and agreed to be responsible for all aspects of this work.

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1 Introduction

Hereditary spastic paraplegias (HSPs) are a large group of genetically heterogeneous diseases that result in broadly overlapping clinical features. The estimated prevalence is approximately 1.8 cases per 100,000 individuals worldwide and varies widely between geographical regions due to differences in the genetic make-up of populations and the methodological heterogeneity of studies [1]. HSPs exhibit a variable age at onset, spanning from early childhood to late adulthood, and affected patients primarily present with progressive spasticity and weakness in the lower limbs resulting from dysfunction of the pyramidal tract. Historically classified into pure and complicated (or complex) forms based on Harding's criteria [2], pure HSPs demonstrate pyramidal signs such as brisk reflexes, spasticity, and the Babinski sign (upward response of the plantar reflex), accompanied by sphincter disturbances and decreased vibration sense. In contrast, complicated forms encompass additional neurological and non-neurological manifestations, including intellectual disability, cognitive decline, cerebellar ataxia, peripheral neuropathy, epilepsy, and retinal degeneration, as well as extrapyramidal features like parkinsonism, dystonia. Complex forms often show brain magnetic resonance imaging (MRI) abnormalities [3, 4].

Genetically, all classical modes of inheritance have been described, including autosomal dominant (AD), autosomal recessive (AR), X-linked recessive (XLR), and mitochondrial (MT) inheritance patterns. The genetic classification of HSPs is based on the sequential numbering of chromosomal loci or specific genes as they were discovered, using the spastic paraplegia gene (SPG) designation. To date, up to 90 genetic forms have been defined and the number is constantly growing [4, 5]. The selection of a target gene based on clinical presentation is not always straightforward due to complex and imperfect genotype-phenotype correlations. Indeed, some genetic forms of HSPs are associated with both pure and complicated phenotypes. There is also considerable

phenotypic overlap between different HSPs forms and even with other neurodegenerative diseases, making it difficult to establish a rational diagnostic strategy. The advent of next-generation sequencing (NGS) has revolutionized the genetic diagnosis of HSPs. Although targeted sequencing gene panels are commonly used, mainly in countries with frequent AD inheritance because of the relative frequency of heterozygous *SPAST* and *KIF1A* variants, they are limited to known HSPs-causing genes and may become outdated given the rapid rate of gene discovery. Currently, there is a clear trend towards the use of whole exome sequencing (WES) with targeted analysis of suspected genes to reduce genetic turnaround time and cost for routine diagnostic applications [6-8].

In this study, we describe our experience in the diagnosis of HSPs using the WES strategy. We report both clinical and genetic findings in a series of 29 Algerian patients. Our findings add to the clinical and genetic variability associated with HSPs and highlight the utility of WES as a tool for the diagnosis of genetically heterogeneous diseases.

2 Materials and methods

2.1 Clinical Studies

This study comprised a cohort of 29 patients from 16 unrelated HSPs families originated from different regions of the country, who were referred to the neurology units of university hospitals in Algiers, Algeria. The clinical diagnosis was established by experienced neurologists and pediatricians in the field of neurodegenerative diseases. The patients were diagnosed with HSPs according to Harding's criteria [2]. A comprehensive evaluation was conducted for each patient, including a detailed medical history that encompassed perinatal events, developmental milestones, family history, and pedigree information, as well as a thorough neurological examination. The age at disease onset was set at the appearance of the first symptoms and was not necessarily related to the detection of pyramidal signs in the lower limbs. To exclude diseases that may mimic HSPs, a series of paraclinical investigations was performed based on clinical and/or neuroradiological indications. These investigations included brain and spinal MRI, electromyography, and an extensive biochemical workup of peripheral blood (vitamin B12, folic acid, vitamin E, lactic acid, ammonia, prolactin, lysosomal enzymes, and very long-chain fatty acids) and cerebrospinal fluid analysis (cell count, glucose, protein, and lactic acid levels). Whenever possible, siblings and other family members were also examined.

2.2 Molecular studies

2.1.1 DNA samples

Peripheral blood samples were collected from probands, parents, and, when available, other affected and/or unaffected family members, and genomic DNA was extracted using the salting-out method [9]. The study has been conducted in accordance with the World Medical Association's Declaration of Helsinki and was approved by the Mustapha Hospital ethics committee's under N° 17/CE/2025. Informed written consent from all human subjects or their legal representatives, have been obtained prior to clinical examination and blood sampling, allowing the genetic testing and use of the data for scientific publication. Subject anonymity was respected during the study and no identifiable information is available in the present manuscript.

2.1.2 Whole exome sequencing (WES)

WES was performed following standard procedures for 26 patients and six unaffected family members. Libraries were generated from DNA samples (100 ng of DNA) using two different exome capture kits: the SeqCap EZ MedExome Target Enrichment Kit (Roche NimbleGen, Inc., Madison, WI, USA) or the Human Core Exome Kit (Twist Bioscience, South San Francisco, CA, USA), with target capture sizes ranging from 33 to 47 Mb. Captured libraries were then sequenced on the Illumina NovaSeq™ 6000 Sequencing System (Illumina, Inc., San Diego, CA, USA), with >98% of the target regions covered at $\geq 20\times$ depth. Identity and quality assurance checks were performed for each sample. After data generation, the WES data were processed using the RD-Connect standardized analysis pipeline based on GATK v3.6 best practices [10] and using the human reference genome GRCh37 (hg19) for alignment and variant calling. Candidate variants were made available for analysis through the RD-Connect Genome-Phenome Analysis Platform (GPAP) [11].

2.2.3 Variant filtering parameters

Variant filtering was performed using the RD-Connect GPAP [11]. Putative disease-causing variants were identified by applying standard filtering criteria for rare diseases. The first priority was given to variants within a specific panel of HSPs-causing genes (Supplementary list 1) and following a disease inheritance pattern in accordance with the pedigree. If no candidate variants were identified, the analysis was extended to variants of the whole exome, considering all possible modes of transmission. Priority was also given to rare variants with a minor allele frequency (MAF) <0.01 in the Exome Sequencing Project (ESP) v0.0.30 [12], and gnomAD database v4.1 [13]. The impact of variants on the protein was classified as either high (for nonsense, frameshift, and splice donor/acceptor site variants) or moderate (for missense and in-frame variants) based on SnpEff predictions [14]. ClinVar [15] and *in silico* prediction tools, including Combined Annotation Dependent Depletion (CADD) (score ≥ 20) [16], Polymorphism Phenotyping v2 (PolyPhen-2) [17], Sorting Intolerant From Tolerant (SIFT) [18], and MutationTaster [19], were used to further assess the predicted pathogenicity of candidate variants. Missense and splice site variants were also analyzed using Alamut[®] Visual v.2.15 (Interactive Biosoftware, SOPHiA GENETICS), SpliceAI [20], and Pangolin [21] software, to predict their functional effects. Variants were ultimately described and classified according to the nomenclature guidelines of the Human Genome Variation Society (HGVS) [22] and the American College of Medical Genetics and Genomics (ACMG) [23].

2.2.4 Sanger sequencing validation

Bidirectional Sanger sequencing was applied to validate the candidate pathogenic variants and investigate their segregation with the disease phenotype in all available family members. PCR amplification was performed using the DreamTaq DNA Polymerase Kit (ThermoFisher Scientific, Waltham, MA, USA), followed by sequencing with GATC Eurofins on an ABI3730XL DNA sequencer (Applied Biosystems, Waltham, MA, USA). Primers used are listed in Supplementary Table 1. Sequence analysis was performed using the FinchTV v.1.4.0 software (Geospiza, Inc., Seattle, WA, United States), and the sequences were compared to the human reference genome GRCh37 (hg19) available in Ensembl genome browser.

2.2.5 Genetic data access

Full dataset of candidate pathogenic variants generated for this study can be found in ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) (accession numbers: SCV004847101, SCV004849363, SCV004933973, SCV004849374, SCV004849377, SCV005038742, SCV004934122, SCV004933975, SCV004849364, SCV004934093, SCV005367981, SCV004934094, SCV004934118, and SCV004849365).

3 Results

3.1 Medical history, clinical features and neuroimaging findings

A total of 29 patients from 16 unrelated families, including 16 males and 13 females (M/F ratio = 1.2), were recruited for this study. Among them, five cases (17.2%) were sporadic, and the remaining 24 (82.8%) had a positive family history. Consanguineous background was present in 14 families (87.5%), most frequently from cousin marriage (n = 8/14) (Supplementary Table 2, Supplementary Figures 1 and 2). Prominent interfamilial heterogeneity was observed in the clinical presentation, with differences in the age at onset, clinical features, rate of progression, severity of disability, and complexity of neurological manifestations. Conversely, intrafamilial heterogeneity was less pronounced, suggesting a degree of similarity between the affected members. The first symptoms were reported at an average age of 4.5 ± 4.9 years (range 0.75–16), and the mean disease duration was 18.9 ± 12.1 years (range 5–50) (Supplementary Table 2). Most patients displayed abnormal gait and walking difficulties (n = 26/29); other symptoms included frequent falls (n = 8/29), fatigability (n = 7/29), and lower limb weakness (n = 5/29). Delayed acquisition of motor milestones was observed in seven patients, two of whom had never achieved independent walking.

All patients fulfilled the diagnostic criteria for HSPs; nine patients (31%) presented with pure HSPs and 20 (69%) with complicated forms. Apart from the characteristic pyramidal signs, the other common clinical features comprised foot deformities (n = 11/29), scoliosis (n = 10/29), and sphincter disturbances (n = 6/29). In cases with complicated HSPs, the most frequent feature was intellectual disability (n = 17/20), followed by cognitive decline (n = 7/20), epilepsy (n = 5/20), cataracts (n = 5/20), dysarthria (n = 5/20), neuropathy (n = 5/20), and microcephaly (n = 4/20). Brain MRI revealed various abnormalities, including a thin corpus callosum (TCC) (n = 6/20), white matter hyperintensities (WMH) (n = 4/20), corpus callosum agenesis (n = 3/20), and cortical-subcortical atrophy (n = 3/20) (Figure 2 and Supplementary Table 2).

3.2 Genetic findings

Genetic diagnosis was obtained in 13 of 16 families, with an overall diagnosis rate of 81.2%. After filtering the WES data and validating putative disease-causing variants by Sanger sequencing, 13 candidate pathogenic variants were identified in 11 known HSPs genes. All these variants were unique to single families (Table 1, Figure 1, and Supplementary Figure 1). Eight variants were previously reported to be pathogenic (P). These included three missense variants, the heterozygous variants c.715C>T/p.Arg239Cys in *ATL1* (NM_015915.5) [24] and c.1252G>A/p.Glu418Lys in *SPAST* (NM_014946.3) [25], and the homozygous variant c.1978G>C/p.Asp660His in *DDHD2* (NM_001164232.1) [26]. In addition, five loss-of-function (LoF) variants were identified in the homozygous state: the nonsense variants c.774G>A/p.Trp258Ter in *ATP13A2* (NM_022089.2) [27], c.43C>T/p.Arg15Ter in *AP4SI* (NM_001128126.3) [28], and c.1168C>T/p.Arg390Ter in *CYP2U1* (NM_183075.2) [29], the splice donor site variant c.1137+1G>T/p.Ser342ArgfsTer65 in *AP4M1* (NM_004722.3) [30], and the frameshift variant c.733_734del/p.Met245ValfsTer2 in *SPG11* (NM_025137.3) [31].

The remaining five variants were novel, including three LoF variants with strong evidence of pathogenicity and two likely pathogenic (LP) missense variants according to the ACMG criteria [23]. A homozygous frameshift variant, c.6530_6534del/p.Asp2177AlafsTer3, was detected in *SPG11* (NM_025137.3) and co-segregated with the disease phenotype in three affected siblings. In *GBA2* (NM_020944.2), two affected sisters harbored the homozygous nonsense variant c.1351C>T/p.Arg451Ter (Figure 1 and Supplementary Figure 1). In addition, a single case carried a homozygous splice site variant c.707+1G>A in *AMFR* (NM_001144.6). The wild-type nucleotide at this position is highly conserved in the consensus sequence of the higher eukaryotes splice sites. This variant can therefore alter normal splicing and was predicted to be deleterious by standard *in silico* prediction tools (CADD and MutationTaster). Splice site prediction analysis revealed a disrupted wild-type donor site of intron 5, with scores dropping from 0.72 to 0 and from 0.7 to 0.05 using SpliceAI and Pangolin software, respectively. Alamut[®] Visual v.2.15 software predicted a high probability of exon 5 skipping. However, biological material from the patient was not available to test these predictions. This variant is extremely rare in the population, found only once in the heterozygous state in the gnomAD database v4.1 [allele frequency, AF = 6.844e-7]. Moreover, Sanger sequencing confirmed that the patient was homozygous for the variant, while the healthy parents and the brother were heterozygous carriers (Figure 1).

Of the novel missense variants, the homozygous c.862T>C/p.Tyr288His variant was identified in *HPDL* (NM_032756.4) in Family 1 and resulted in the replacement of a highly conserved tyrosine (present in 12 species), located in the vicinal oxygen chelate domain 2 of HPDL, with histidine (Figure 3). This variant was predicted to be disease-causing using standard *in silico* prediction tools (CADD, SIFT, PolyPhen-2, and MutationTaster) and was not reported in publicly available databases (ESP v0.0.30 and gnomAD v4.1) (Table 1). Notably, complete co-segregation between the variant and the disease phenotype was confirmed in this family. Our patient and two affected members from a distinct branch of the family were homozygous for the variant, whereas unaffected relatives were homozygous for the wild-type allele or heterozygous carriers (Figure 1 and Supplementary Figure 1).

Unexpectedly, a missense variant c.1076T>C/p.Ile359Thr was discovered in *SPAST* (NM_014946.3) in the homozygous state in family 8. *SPAST* variants are usually associated with a dominantly-inheritance pattern. This variant resulted in the substitution of a highly conserved isoleucine (found in 11 species) with threonine in the ATPase associated with various cellular activities (AAA) domain of spastin (Figure 3). Further evidence for the likely pathogenicity of this variant included its absence from reference databases of human genome variations (ESP v0.0.30 and gnomAD v4.1) and the results of *in silico* prediction tools (CADD, SIFT, PolyPhen-2, and MutationTaster) (Table 1). This homozygous variant was shared by two affected sisters from an extended and highly consanguineous family and was present in the heterozygous state in the unaffected parents. We can not exclude the presence of pyramidal signs that may appear later in life in the parents, however. Other family members from different branches of the family were affected; however, only one was available for segregation analysis and was also a homozygous carrier of the variant (Figure 1 and Supplementary Figure 1). No other candidate pathogenic variants were found in the genes known to be associated with HSPs or other neurogenetic disorders.

4 Discussion

The molecular diagnosis of HSPs is complex and represents a major challenge in clinical practice because of their exceptional clinical and genetic variability, making them among the most heterogeneous human diseases [7, 32]. Several approaches to genetic testing exist, and the most cost-effective and widely available is NGS [33].

The most commonly used NGS strategy is WES [34], which has been successfully used to date in the diagnosis of HSPs, either alone [35-37] or more frequently, in combination with other molecular approaches [38-42]. In this study, we used WES to investigate the clinical and genetic features of 29 patients with pediatric onset from 16 unrelated families originating from different regions of Algeria. To the best of our knowledge, our study is the largest cohort of HSPs investigated in Algeria and the first series consisting exclusively of Algerian families.

We achieved a genetic diagnosis in 81.2% of families (n = 13/16), which is relatively high compared to the overall diagnostic rate for HSPs, estimated at 40–70% [32]. Larger studies conducted on extensive patient cohorts have revealed diagnostic rates of approximately 29–49%, with lower success rates in simplex families [43-45]. Considering our stringent inclusion criteria and the limited number of selected families, most of which had a positive family history of HSPs (n = 11/16, 68.7%), we considered our findings to be consistent with expectations. Our series mainly comprised families with an AR inheritance pattern (n = 13/16, 81.2%), likely due to the high rate of consanguineous marriages in Algeria (11.5–56.3%) [46-50]. The phenotypic spectrum of AR-HSPs has been extensively reported, with the majority of cases exhibiting complicated forms, as often observed for patients with pediatric onset as this is the case in our series [43, 45, 51]. Similarly, we observed a higher prevalence of complicated forms than of pure forms across all families (n = 11/16, 68.7%) and specifically in AR-HSPs families (n = 10/13, 76.9%). These findings align with previous studies in countries with high consanguinity, such as Tunisia [52], Oman [53], Sudan [54, 55], and Turkey [56]. In contrast, in a cohort of Algerian and Portuguese families with AR inheritance, pure HSPs presentations were slightly predominant (53%) over complicated phenotypes (47%) [57], although MRI was not routinely used at that time, possibly leading to an overestimation of pure forms.

SPG11 is a major cause of complicated AR-HSPs, with most pathogenic variants introducing a premature termination codon, thus supporting the LoF mechanism as the underlying cause of the disease [58]. In the current study, we diagnosed *SPG11* caused by frameshift variants in two consanguineous families. In the first family, the sporadic patient F16.II:2 was homozygous for the common *SPG11* variant c.733_734del/ p.Met245ValfsTer2 [31, 59-68], with clinical and neuroimaging findings highly suggestive of the classic *SPG11* phenotype [58]. In addition, unlike other patients with the same variant, our patient had cataracts, a rare feature in *SPG11* [31, 43, 69]. In Family 11, we detected a novel homozygous deletion, c.6530_6534del/p.Asp2177AlafsTer3, in three affected siblings. Although no signs of peripheral neuropathy were observed in these patients, the characteristic clinical features of childhood onset, lower limb spasticity, learning difficulties with progressive cognitive decline, and intellectual disability were present. Disease progression was slow; only patient F11.II:3 required a walking aid and had normal brain and spinal cord MRI at the age of 20 years, but subsequent MRI scans were not available. Patients F11.II:4 and F11.II:6 had mild cervical myelopathy on spinal MRI, and only patient F11.II:4 showed brain MRI abnormalities, including the "ears of the lynx" sign, posterior parietal lobes and periventricular WMH. Interestingly, this patient did not present with TCC, a hallmark of *SPG11*, similar to some very rare cases described previously [70-77]. The etiology of TCC in *SPG11* patients remains unclear. An *in vivo* study in *SPG11* murine model revealed corpus callosum thinning after four months, prior to general brain atrophy [78], suggesting that thinning may occur even years after disease onset in humans. Thus, the neuroimaging findings of our patients do not exclude the possibility of later thinning of the corpus callosum. The variable timing of TCC manifestation could explain the presence of certain *SPG11* variants in patients with and without TCC at different ages [31, 70, 71, 73]. Therefore, *SPG11* variants alone may not fully explain the phenotypic spectrum, leaving some aspects to additional, yet unknown, genetic and/or environmental factors.

We also described a patient with complicated HSP in Family 1, harboring a novel homozygous missense variant, c.862T>C/p.Tyr288His, in *HPDL*. Several lines of evidence suggest its functional significance, including the variant's proximity to the predicted catalytic center, the high evolutionary conservation of the affected residue, its absence from reference databases, results of *in silico* prediction tools, and its segregation pattern within the family. Although no functional studies were conducted, a neighboring missense variant with a similar amino acid substitution, c.859T>C/p.Tyr287His, identified in another HSP patient, was shown to drastically reduce the enzymatic function of a homologous *HPDL* orthologue [79, 80], supporting the potential pathogenic nature of our variant. Clinically, *HPDL* variants have recently been associated with neurological disorders with a wide spectrum of clinical severity, ranging from severe congenital encephalopathy with no psychomotor development to adolescent-onset HSP (*SPG83*) [79-82]. In the present study, patient F1.III:1 had complicated HSP from the age of 12, characterized by spasticity, mild intellectual disability without language, social, or cognitive decline, and other variable features such as nystagmus, *pes planus*, and dysuria. The disorder was progressive and required the use of a walker. Sensory examination, nerve conduction studies, and EMG were normal. Brain MRI showed partial thinning of the corpus callosum in our patient, differing from typical *SPG83* cases but consistent with MRI features reported in other *HPDL*-associated diseases [79-81, 83, 84]. Therefore, our report extends the clinical spectrum of *SPG83* and its known *HPDL* variants. To date, no clear genotype-phenotype correlations

have been established to explain the wide range of clinical presentations linked to this single-exon gene. Future studies are required to clarify the physiological function of HPDL, the full spectrum of phenotypes, and the mechanisms of pathogenicity associated with HPDL deficiency.

In Family 13, we presented another single case of the recently discovered HSP form, SPG89, associated with pathogenic variants in *AMFR* [85]. Our patient carried the homozygous splice site variant c.707+1G>A, present in the heterozygous state in the unaffected parents and brother. Given the 100% evolutionary conservation of the +1G splice donor site position [86], this variant is expected to disrupt the consensus splice donor site of intron 5. However, its functional consequence remains untested in living cells. Deng et al. [85] reported 20 patients with AR-HSP from eight unrelated consanguineous families, most of whom had a pure form, while others showed a global developmental delay with mild intellectual disability or learning difficulties. In our study, patient F13.II:2 presented with a complicated infantile-onset form with delayed motor development, abnormal spastic gait, marked language delay, behavioral manifestations, learning and concentration difficulties, joint hyperlaxity, and dysmorphic features. Brain imaging revealed mild ectasia of pericerebral spaces. The novel splice variant identified in this study adds to the repertoire of *AMFR* variants and broadens the clinical spectrum of SPG89.

We also identified additional variants in five genes involved in complicated AR-HSPs: *GBA2* (SPG46), *AP4M1* (SPG50), *AP4S1* (SPG52), *DDHD2* (SPG54), and *CYP2U1* (SPG56), within families 2, 14, 6, 4, and 12, respectively. Overall, the clinical and neuroimaging features of the patients were comparable to those reported previously [8, 87, 88].

Pure forms were observed in only five families (n = 5/16, 31.3%) in our series (Families 7, 8, 9, 10, and 15). In three of them, we found variants in *ATL1* and *SPAST*, genes associated with the most prevalent forms of AD-HSPs, SPG3A and SPG4, respectively [89]. Despite consanguinity in Family 10, we detected the common *ATL1* missense variant c.715C>T/p.Arg239Cys in the heterozygous state [24, 25, 66, 90-100]. This variant was absent in the patient's unaffected parents, raising the possibility of either non-paternity or a *de novo* mutational event. The phenotype of our patient matched the standard clinical presentation and early age onset of SPG3A [101]. In Family 7, we identified the previously reported *SPAST* missense variant, c.1252G>A/p.Glu418Lys [25], which was found in several heterozygous family members with a classic SPG4 phenotype [102]. Notably, the individual F7.II:9 carried this variant, but remained asymptomatic at age 64, suggesting incomplete penetrance. Genetic penetrance is age-dependent, reaching 80% by the age of 50, and approximately 6% of *SPAST* mutations carriers remain asymptomatic, indicating possible effects of genetic and/or environmental modifiers [102, 103]. More importantly, we revealed a novel homozygous LP variant, c.1076T>C/p.Ile359Thr, in *SPAST*, in two affected sisters from Family 8. This result was unexpected as pathogenic variants are normally associated with AD-HSP. However, given the predicted damaging effect of this variant and the absence of other candidate pathogenic variants in WES-covered genes, we considered this variant a plausible genetic cause for this large consanguineous family. Furthermore, recent studies have shown rare cases with homozygous *SPAST* variants. In a Dutch family, the AR inheritance pattern was linked to a homozygous missense variant, c.1600C>G/p.Leu534Val, in two siblings with a pure HSP phenotype at the age of 39 years, while their heterozygous consanguineous parents remained asymptomatic at the ages of 68 and 73 years [104]. Cruz-Camino et al. [105] reported a French family in which two siblings, born to consanguineous and healthy parents, carried a homozygous nonsense variant, c.1634C>G/p.Ser545Ter, and presented an early-onset, complicated and severe form of the disease. Interestingly, 34 heterozygous relatives were either asymptomatic or showed clinical manifestations of SPG4 [105]. Another homozygous nonsense variant, c.153C>G/p.Tyr51Ter, was identified in a Pakistani patient displaying a complicated phenotype from the age of one year; her consanguineous parents were asymptomatic heterozygous carriers [106]. In this study, the patients showed a childhood-onset and gradually worsening pure HSP phenotype, with symptoms such as spastic tetraparesis, dysphagia, sphincter disturbances, *pes planus*, and other skeletal abnormalities. Patient F8.IV:2 had a more severe phenotype, characterized by head drooping and aggravated dysphonia leading to aphonia. Our patients were wheelchair-bound, whereas their heterozygous relatives were asymptomatic at the time of the evaluation. These findings suggest that homozygous *SPAST* variants cause a phenotype overlapping with that associated with dominant pathogenic variants in the same gene. Homozygous nonsense variants appear to be involved with early-onset complicated forms, whereas homozygous missense variants are associated with pure forms with variable onset. Further functional investigations are required to elucidate their impact on spastin function. Our study supports the recessive effect of certain *SPAST* variants with important implications for genetic diagnosis and counseling. Mixed inheritance patterns have been documented for other HSPs genes, including *ALDH18A1* (SPG9) [107], *ERLIN2* (SPG18) [108, 109], *KIF1A* (SPG30) [110, 111], and *REEP2* (SPG72) [112]. Some genes, such as *ATL1* (SPG3A) [113, 114] and *SPG7* [115], showed a subtle mixed inheritance pattern, along with a predominant one. The possibility of mixed inheritance patterns highlights the genetic heterogeneity of HSPs and their complex genotype-phenotype correlations.

To further complicate the overall picture, 28 genetic forms of HSPs have been assigned to alternative phenotypes in OMIM [7]. This is exemplified by *ATP13A2*, a gene responsible for Kufor-Rakeb syndrome [116], neuronal ceroid lipofuscinosis [117], and the complicated form of HSP, SPG78 [118]. These neurodegenerative disorders have overlapping features, making the diagnostic particularly challenging. In Family 3, we identified a known homozygous variant, c.774G>A/p.Trp258Ter, in *ATP13A2* [27]. Patient F3.II:6 was initially diagnosed with a complex HSP due to predominant spasticity, but subsequent molecular diagnosis and clinical re-examination—revealing early onset, parkinsonism, and supranuclear gaze palsy—led to a revised diagnosis of confirmed Kufor-Rakeb syndrome. This case underscores the diagnostic challenges posed by the highly complex and overlapping clinical presentations of *ATP13A2*-associated diseases. Our observations support the concept of a clinical continuum of connected phenotypes associated with *ATP13A2* and the proposal to adopt the term “*ATP13A2*-associated disorder” rather than separate allelic disorders with a different nomenclature or classification [119, 120].

Despite our best efforts, the genetic etiology remained inconclusive for three families (18.8%). In Family 5, we however identified a VUS in *USP8*, associated with SPG59, a rare form of HSP found only in two families [121, 122]. Our patient presented a phenotype similar to previously reported cases, with an additional feature of microcephaly. Although *in silico* prediction tools suggest the pathogenicity of this variant—likely through protein structure destabilization or functional domain disruption—the ACMG guidelines [23] indicate that there is insufficient evidence to classify it as pathogenic (Supplementary Table 3). Further functional studies are required to elucidate the exact effect of this variant on *USP8* function. In the other negative families (Families 9 and 15), we detected variants in genes not previously associated with disease (Supplementary Table 3), which could not be excluded using standard filtering criteria for rare diseases—a common problem when using NGS [123]. Even if some of these variants are involved in the disease, their corresponding genes have not yet been linked to HSPs, as the list of HSPs-causing genes is still incomplete [32]. Therefore, periodic re-evaluation of WES data is essential to investigate newly identified genes that may explain our negative cases in the future. The coupling with whole genome linkage analysis to identify regions of linkage or of homozygosity will also help to focus on a restricted series of variants. In addition, diagnostic challenges were compounded by the limited co-segregation analysis due to the pedigree structure of these families and the technical limitations of WES, including undetected variants in poorly covered or non-coding regions, as well as missed copy number variants, complex rearrangements, and trinucleotide repeat expansions. Overcoming these limitations requires complementary techniques such as multiplex ligation-dependent probe amplification, array comparative genomic hybridization, or whole genome sequencing [6, 124]. Alternatively, other mechanisms, including—digenic or oligogenic inheritance, genetic modifiers, uniparental isodisomy, imprinting defects, and gene/environment interactions—may be involved, calling for international collaborative efforts to achieve more accurate and comprehensive genetic diagnosis [125].

In conclusion, our study reports an analysis of a series of Algerian HSPs patients, highlighting variability in neurological and non-neurological symptoms and broadening the clinical spectrum of certain forms. Using WES, we identified genetic defects in 11 HSPs-causing genes, some of which were rare, and documented five novel variants. These results demonstrate the efficiency of WES as a diagnostic tool for genetically heterogeneous diseases and confirm the wide clinical and genetic heterogeneity of HSPs in Algeria. Beyond their diagnostic importance, our findings provide a basis for comprehensive investigation of the main HSPs genetic forms in Algeria and the exploration of population-specific features relevant to clinical care and genetic counseling.

Statements and Declarations

Competing Interests: GS received a grant from Biogen, unrelated to this study from 2019 to 2023. All other authors have no relevant financial or non-financial interests to disclose.

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Ethics approval: All procedures performed in human participants were done in accordance with the ethical agreement of the **Mustapha Hospital ethics committee** (n° 17/CE/2025) and followed the 1964 Helsinki Declaration and its later amendments.

Consent: Informed written consent from all human subjects or their legal guardians, have been obtained prior to clinical examination and blood sampling, allowing the genetic testing and use of the data for scientific publication.

Availability of data and materials: Full dataset of candidate pathogenic variants identified in this can be found in ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>, accession numbers: SCV004847101, SCV004849363, SCV004933973, SCV004849374, SCV004849377, SCV005038742, SCV004934122, SCV004933975, SCV004849364, SCV004934093, SCV005367981, SCV004934094, SCV004934118, and SCV004849365).

Author contributions: MMK and GS conceived and designed the study. MIK, RBH, LAP, WAEK, and FM provided samples from patients and contributed to clinical, radiological, and biochemical data collection and interpretation. MMK, LGN and CD performed laboratory work. MMK, MGB, JMdeSA, GS and LGN contributed to the bioinformatics analysis. MMK drafted the manuscript. MMK and GS revised the manuscript. GS obtained dedicated grants for this study. All authors have read the manuscript, accepted its submission, and agreed to be responsible for all aspects of this work.

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Table 1. Features of candidate pathogenic variants detected in this study using WES strategy

Family	Gene (Form)	Exon	cDNA change variant (Zygoty)	Protein change variant	ClinVar	CADD	SIFT	Polyphen-2	Mutation Taster	ESP v0.0.30 / gnomAD v4.1 frequency	ACMG classification	Reference
1	<i>HPDL</i> (SPG83)	1	c.862T>C (homo)	p.Tyr288His	–	26.9	D	D	D	0 / 0	LP (PM1, PM2, PP1, PP3)	This study
2	<i>GBA2</i> (SPG46)	8	c.1351C>T (homo)	p.Arg451Ter	–	38	–	–	A	0 / 0	P (PVS1, PM2, PP1)	This study
3	<i>ATP13A2</i> (SKR)	9	c.774G>A (homo)	p.Trp258Ter	P	36	–	–	A	0 / 0.0000065748	P (PVS1, PM2, PP5)	[27]
4	<i>DDHD2</i> (SPG54)	16	c.1978G>C (homo)	p.Asp660His	P	29.1	D	D	D	0.000077 / 0.00013258	P (PM1, PM2, PP1, PP3, PP5)	[26]
6	<i>AP4S1</i> (SPG52)	2	c.43C>T (homo)	p.Arg15Ter	P	37	–	–	A	0 / 0.000004	P (PVS1, PM2, PP1, PP5)	[28]
7	<i>SPAST</i> (SPG4)	10	c.1252G>A (hetero)	p.Glu418Lys	P/VUS	31	D	D	D	0 / 0	P (PM1, PM2, PP1, PP3)	[25]
8	<i>SPAST</i> (SPG4)	7	c.1076T>C (homo)	p.Ile359Thr	–	26.5	D	D	D	0 / 0	LP (PM1, PM2, PP1, PP3)	This study
10	<i>ATL1</i> (SPG3A)	7	c.715C>T (hetero)	p.Arg239Cys	P/LP	24.7	D	P	A	0 / 0.0000007	LP (PM1, PM2, PP3, PP5)	[24]
11	<i>SPG11</i> (SPG11)	35	c.6530_6534del (homo)	p.Asp2177AlafsTer3	–	–	–	–	–	0 / 0.0000007	P (PVS1, PM2, PP1)	This study
12	<i>CYP2U1</i> (SPG56)	3	c.1168C>T (homo)	p.Arg390Ter	P	40	–	–	A	0 / 0.00001	P (PVS1, PM2, PP1, PP5)	[29]
13	<i>AMFR</i> (SPG89)	–	c.707+1G>A (homo)	p.?	–	27.6	–	–	D	0 / 0.0000006844	P (PVS1, PM2)	This study
14	<i>AP4M1</i> (SPG50)	–	c.1137+1G>T (homo)	p.Ser342ArgfsTer65	P/LP	24.6	–	–	D	0 / 0.00001	P (PVS1, PM2, PP1, PP5)	[30]
16	<i>SPG11</i> (SPG11)	4	c.733_734del (homo)	p.Met245ValfsTer2	P	–	–	–	–	0 / 0.00006877	P (PVS1, PM2, PP5)	[31]

Abbreviations: A - Annotated as disease causing; D (SIFT) - Damaging; D (Polyphen-2) - Probably damaging; D (MutationTaster) - Disease causing; LB: Likely Benign; LP - Likely Pathogenic; P (Polyphen-2) - Possibly damaging; P (ClinVar; ACMG classification) - Pathogenic; VUS - Variant of Uncertain Significance; homo - Variant identified in the homozygous state; hetero - Variant identified in the heterozygous state; PM1 - Moderate / Functional Data; PM2 - Moderate / Population Data; PP1 - Supporting / Segregation Data; PP3 - Supporting / *In silico* Predictions; PP5 - Supporting / Reputable Source Data; PVS1 - Very Strong / Effect on Protein

Fig. 1 Pedigrees and genetic findings of families with identified candidate pathogenic variants. Pedigrees of 13 families with positive diagnosis, illustrating the variant carrier status of the affected (filled black symbols) and unaffected (unfilled symbols) individuals. The genotype of each tested individual for the identified variants is indicated (WT - wild-type allele; M - mutant allele). A double line between the parents indicates reported consanguinity. The asterisk denotes the individuals for whom WES was performed

Fig. 2 Neuroimaging findings of some genetically resolved cases. **(A)** Sagittal T1-weighted brain MRI revealing partial atrophy of the corpus callosum (patient F1.III:1; *HPDL*). **(B)** Sagittal T1-weighted brain MRI showing a thin corpus callosum (patient F2.II:2; *GBA2*). **(C)** Axial T2-weighted brain MRI disclosing mild frontal cortical-subcortical atrophy (patient F3.II:6; *ATP13A2*). **(D)** Sagittal T1-weighted and **(E)** coronal T2-weighted brain MRI, indicating a thin corpus callosum and minimal supratentorial cortical-subcortical atrophy (patient F4.II:1; *DDHD2*). **(F)** Sagittal T1-weighted and **(G)** axial T1-weighted brain MRI, revealing partial corpus callosum atrophy and bilateral colpocephaly (patient F6.II:2; *AP4S1*). **(H)** Axial FLAIR brain MRI showing the ears of the Lynx sign, posterior parietal lobes, and periventricular white matter hyperintensities; and **(I)** sagittal T2-weighted cervical spine MRI disclosing minimal cervical myelopathy (patient F11.II:4; *SPG11*). **(J)** Axial T2-weighted brain MRI and **(K)** axial computed tomography brain scan, indicating subcortical white matter hyperintensities and intracranial calcifications (patient F12.III:3; *CYP2U1*). **(L)** Sagittal T1-weighted and **(M)** axial FLAIR brain MRI, revealing partial agenesis of the corpus callosum and prominent dilation of the right lateral ventricle (patient F14.II:4; *AP4M1*). **(N)** Sagittal T1-weighted and **(O)** axial T2-weighted brain MRI, showing a thin corpus callosum, periventricular white matter hyperintensities, and frontoparietal cortical-subcortical atrophy (patient F16.II:2; *SPG11*). Arrows indicate the location of abnormalities

Fig. 3 Conservation analysis of amino acid sequences across different species. Amino acid alignments were generated using Alamut[®] Visual v.2.15 software (Interactive Biosoftware, SOPHiA GENETICS). Only novel missense variants are shown, with the altered amino acid position boxed

Fig 1

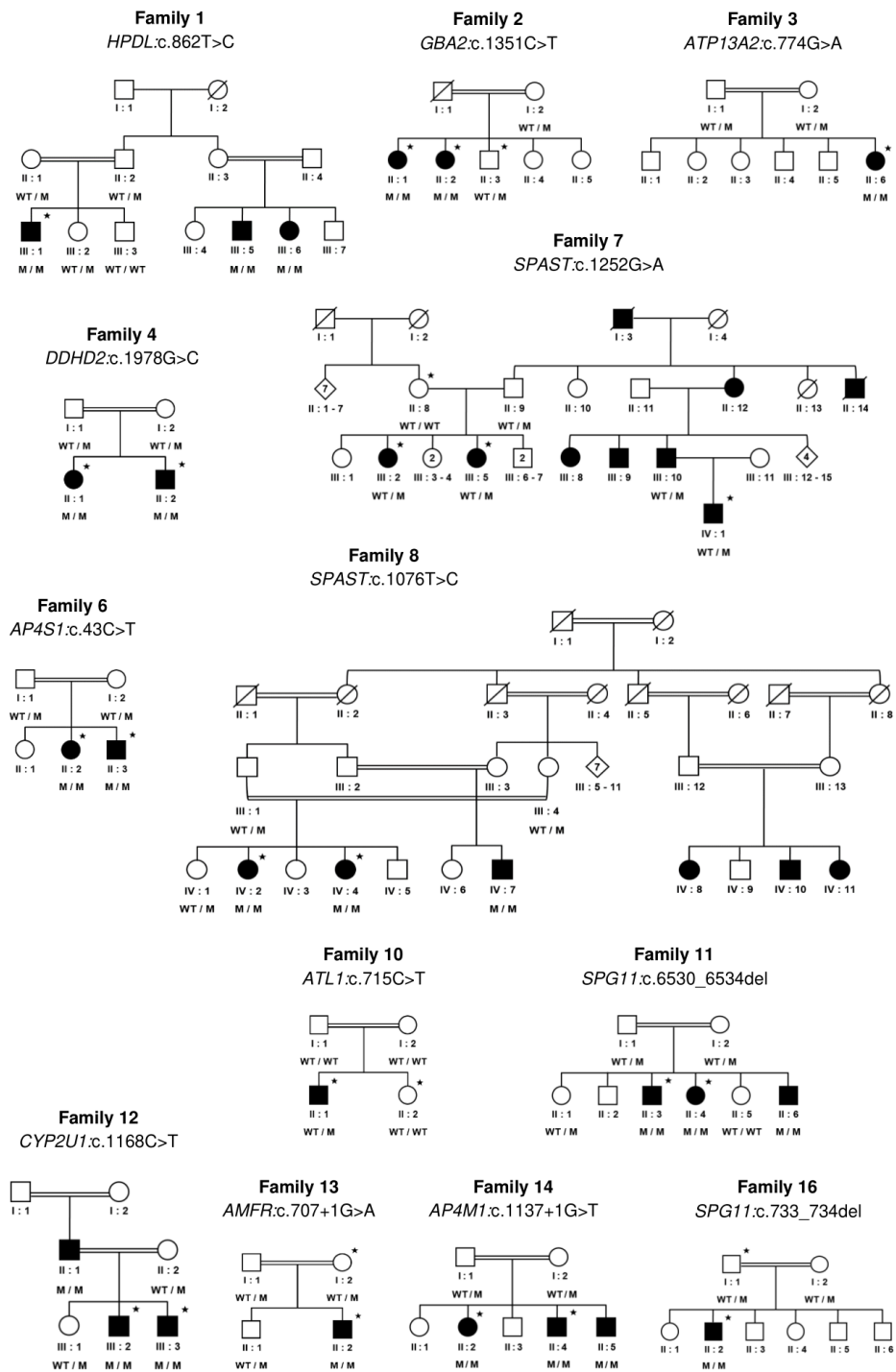


Fig 2

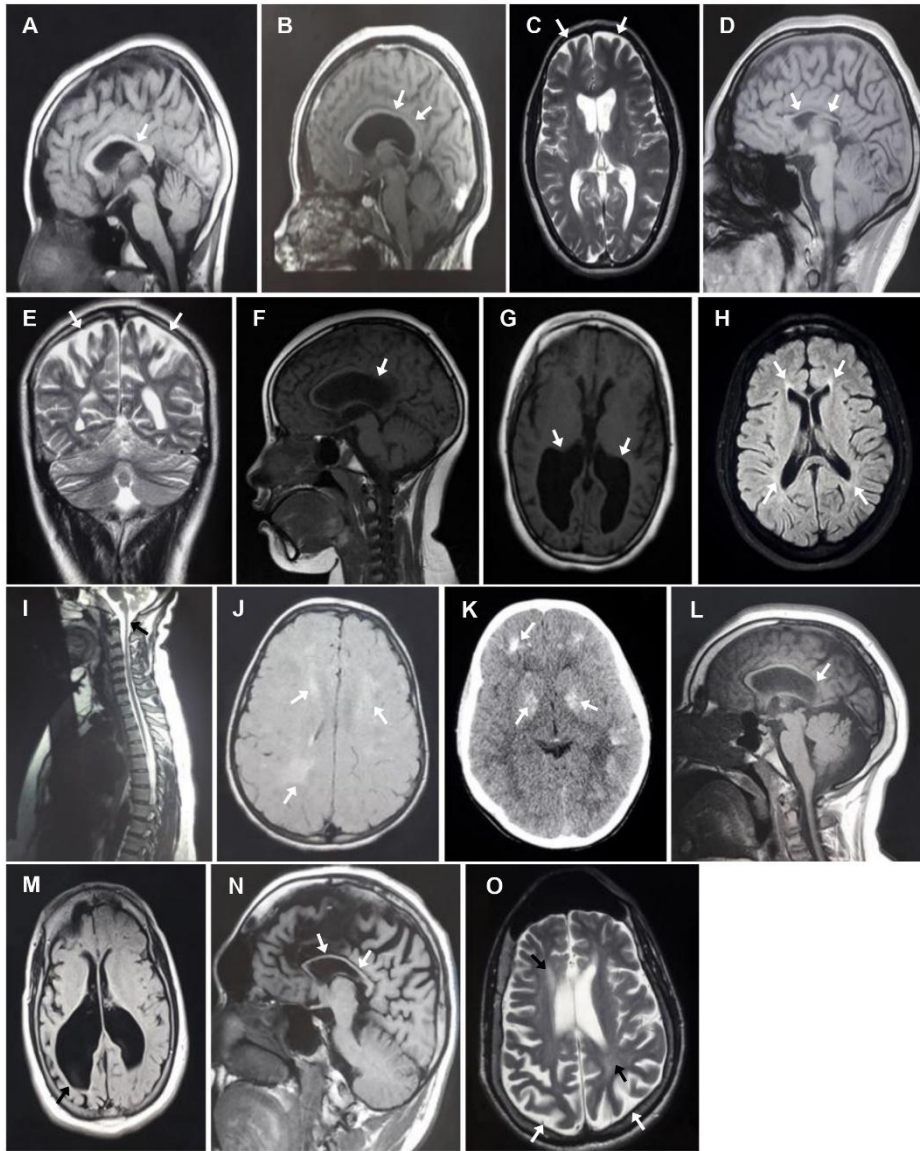


Fig 3

Family 1
HPDL:p.Tyr288His

Human	L	A	P	P	G	A	Y	Y	Q	Q	P	G	K	E	R
Chimpanzee	L	A	P	P	G	A	Y	Y	Q	Q	P	G	K	E	R
White-cheeked gibbon	L	A	P	P	G	A	Y	Y	Q	Q	P	G	K	E	R
Macaque	L	A	P	P	G	A	Y	Y	Q	Q	P	G	K	E	R
Rat	L	T	P	P	E	A	Y	Y	Q	Q	P	G	K	E	R
Mouse	L	T	P	P	E	A	Y	Y	Q	Q	P	G	K	E	R
Dog	L	A	P	P	E	A	Y	Y	Q	Q	P	G	K	E	R
Chicken	F	T	P	P	A	A	Y	Y	S	Q	G	G	K	E	R
Xenopus	A	S	Q	P	S	S	Y	Y	S	E	P	R	K	Q	K
Tetraodon	F	S	P	P	F	A	Y	Y	T	K	V	G	K	Q	H
Zebrafish	F	S	P	P	P	A	Y	Y	T	E	V	G	K	Q	Q
<i>C. elegans</i>	L	T	I	P	S	Q	Y	Y	D	N	L	E	E	R	K

Family 8
SPAST:p.Ile359Thr

Human	Q	A	L	Q	E	I	V	I	L	P	S	L	R	P	E
Chimpanzee	Q	A	L	Q	E	I	V	I	L	P	S	L	R	P	E
Rat	Q	A	L	Q	E	I	V	I	L	P	S	L	R	P	E
Mouse	Q	A	L	Q	E	I	V	I	L	P	S	L	R	P	E
Dog	Q	A	L	Q	E	I	V	I	L	P	S	L	R	P	E
Platypus	Q	A	L	Q	E	I	V	I	L	P	S	L	R	P	E
Chicken	Q	A	L	Q	E	I	V	I	L	P	S	L	R	P	E
Xenopus	Q	A	L	Q	E	I	V	I	L	P	S	I	R	P	E
Zebrafish	Q	A	L	Q	E	I	V	I	L	P	A	L	R	P	E
<i>Drosophila</i>	Q	A	L	Q	E	M	V	I	L	P	S	V	R	P	E
<i>C. elegans</i>	A	A	L	E	E	A	V	I	L	P	A	L	N	P	N
<i>S. cerevisiae</i>	Y	S	L	K	E	A	V	V	Y	P	F	L	R	P	D