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New variants and genotype–phenotype correlation in *KIF5A* mutation: the contribution of a large Italian cohort

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ABSTRACT

Background Variants in the Kinesin-family member 5A (*KIF5A)* gene are associated with a range of motor diseases, and a strong correlation between the protein domains (motor, stalk and tail) and the clinical phenotype has been proposed. However, several studies have reported exceptions contributing to a complex genotype–phenotype correlation in recent years. Further studies are needed to improve our knowledge about the prevalence of *KIF5A* variants and their genotype–phenotype correlation.

Methods 390 patients (220 hereditary spastic paraplegia, 80 Charcot-Marie-Tooth disease type 2 and 90 amyotrophic lateral sclerosis) have been selected for next-generation sequencing Clinical Exome.

Results Five patients have been found to carry causative variants in the *KIF5A* gene. Of these, three are familiar cases, and two are sporadic. Segregation analysis was performed on the familiar probands. The five patients with pathogenic variants represent 4% of the studied population, and the clinical and genetic analysis of these five families allowed us to examine different scenarios.

Some of these data support the hypothesis of a complex correlation between domains and disease.

Conclusion These data confirm the complex genotype–phenotype correlation, both in terms of clinical heterogeneity associated with a specific domain and variability within the members of the same family, but also suggest a strong genotype–phenotype correlation, both intrafamiliar and interfamiliar, produced by a few variants.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Variants in the Kinesin-family member 5A (*KIF5A)* gene are associated with a range of motor diseases, including hereditary spastic paraplegia, Charcot-Marie-Tooth disease type 2 and amyotrophic lateral sclerosis. Previous studies have suggested a correlation between specific protein domains (motor, stalk and tail) of KIF5A and the resulting clinical phenotype, but exceptions to this correlation have also been reported, highlighting the complexity of the genotype–phenotype relationship.

WHAT THIS STUDY ADDS

⇒ This study identifies causative variants in the *KIF5A* gene in 5 out of 390 Italian patients (4%), including three familial and two sporadic cases. It provides new insights into the complex genotype–phenotype correlation, demonstrating that certain variants, such as p.Tyr276Cys in the motor domain, are consistently associated with specific phenotypes across different families, while others, like p.Leu957del in the tail domain, present with unexpected clinical manifestations.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These findings underscore the need for comprehensive genetic analysis in patients with overlapping neurological syndromes, as well as the importance of personalised patient management. Improved understanding of the genotype–phenotype relationship in KIF5A-related disorders could inform the development of more precise diagnostic criteria and therapeutic strategies.

INTRODUCTION

Kinesin-family member 5A protein (KIF5A), a kinesin superfamily member, is a molecular motor protein essential for intracellular transport. The intricate structure of KIF5A is essential for its function as a microtubule-based motor protein.[1] The motor domain generates the force required for movement, while the stalk domain provides structural stability and facilitates dimerisation. The tail domain determines the specific cargo transported by KIF5A, influencing various cellular processes. Its role in transporting cargo along microtubules is crucial for various cellular processes, including neuronal development, axonal transport and synaptic function.[2] Mutations in the *KIF5A* gene have been implicated in human neurological disorders.[3 4]

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The first clinical phenotype to have been identified so far consists of hereditary spastic paraplegia (HSP) type 10 (SPG10). The clinical phenotype is a 'pure' HSP, which is clinically characterised by lower limb distal muscle weakness and spasticity associated with brisk deep tendon reflexes, sphincter disturbances and profound sensory loss, reflecting the isolated degeneration of the corticospinal tract.[5 6] Missense mutations in the same gene, however, may also manifest with a 'complex' HSP phenotype, in which the spastic paraplegia is typically associated with additional motor, sensory, cognitive disturbances, ataxia, polyneuropathy, epilepsy, skeletal abnormalities, upper limb amyotrophy and optic atrophy.[7–9] More recently, a specific phenotype associated with *KIF5A* genetic mutation has been described to resemble the clinical manifestation observed in Charcot-Marie-Tooth type 2 (CMT2). These mutations affect the motor domain and result in chronic axonal motor and sensory polyneuropathy, leading to progressive loss of sensation, muscle weakness, loss of deep tendon reflexes and skeletal deformities (ie, pes cavus, foot deformities).[10] By contrast, when gene mutations hit the cargo domain, the clinical phenotypes are typically characterised by combined degeneration of upper motor and lower motor neurons. This leads to muscle weakness, atrophy and characteristic neurophysiological findings, thus resembling an amyotrophic lateral sclerosis (ALS)-like phenotype.[11]

The phenotypic manifestations of KIF5A-related disorders range from mild to severe, and disease onset typically ranges from early childhood to late adulthood.[5] This phenotypic heterogeneity is likely influenced by a combination of factors, including the specific *KIF5A* mutation, the affected cell type and the stage of development at which the mutation occurs.

Genotype–phenotype correlation is exceptionally complex in KIF5A-related disorders. Although, as previously reported, there is a correlation between the domain and the disease, several papers report exceptions to this scenario. These phenotypes are not necessarily limited to one domain and may even differ within family members sharing the same variant, resulting in a broad clinical spectrum due to *KIF5A* variants. For example, variants in the stalk domain of *KIF5A* most often result in complex HSP phenotype but can also be associated with ALS.[5 12] In this scenario, understanding the complex relationship between *KIF5A* genotype and phenotype is essential for elucidating disease mechanisms and developing targeted therapies.

In this work, we report the prevalence of *KIF5A* variants among 390 Italian patients affected by HSP, CMT2 and ALS to improve our knowledge about the prevalence of *KIF5A* variants and their genotype–phenotype correlation, reporting clinical and genetic findings in five patients and three pedigrees with apparent autosomal dominant inheritance due to *KIF5A* variants.

MATERIAL AND METHODS

Patients

390 patients were recruited from the IRCCS Neuromed Institute in Pozzilli (IS), Italy, and the Department of Human Neurosciences at Sapienza University of Rome, Italy. Among these patients, 220 exhibited a clinical phenotype consistent with HSP, 80 with CMT2 and 90 with ALS. The inclusion criteria for all patients were: (1) a clinical presentation indicative of HSP, CMT2 or ALS; and (2) exclusion of sporadic, non-genetic causes for their symptoms. Each patient underwent a comprehensive clinical examination and an instrumental assessment. Specifically, all patients performed a brain and spinal cord MRI, followed by neurophysiological evaluations, including nerve conduction studies (NCSs) and electromyography of both upper and lower limbs. In addition, patients with HSP and ALS were further assessed using somatosensory-evoked potentials and motor-evoked potentials (MEP) in both upper and lower limbs. Written informed consent was obtained from all participants following the Helsinki Declaration, and the local ethics committee approved the study.

DNA extraction

According to standard procedures, genomic DNA was isolated from peripheral blood leukocytes (QIAamp DNA Blood Mini Kit—QIAGEN).

Next-generation sequencing panel

The next-generation sequencing (NGS) analysis was performed using the KAPA HyperCap Heredity Panel Clinical Exome (Hoffmann-La Roche, Basel), 10 Mb capture target panel covering 3332 genes strongly associated with hereditary disease research, including genes for neurological and neurodegenerative diseases, on Illumina NextSeq 550 System (San Diego, California, USA). All coding exons of the RefSeq transcripts of the genes and 15 bp of the flanking introns were targeted, for which the target region is extended 860 bases upstream of the ATG start codon to include the nerve-specific promoter region. 99% of the coding exons were sequenced with a minimal read depth of 30×.

GenomeUp software (https://platform.genomeup.com) was used for data analysis. It provides automated annotation (Best Practices workflows of GATK V.4.1 for germline variant calling), alignment of sequence reads to the reference genome GRCh37/hg19 and selection of potentially pathogenic variants. The Integrative Genomics Viewer V.2.3 directly evaluated the data sequence. The Sanger sequencing ABI 3130xl Genetic Analyzer (Applied Biosystems) performed the mutation resequencing and segregation analysis.

As an Italian control population, we used the frequencies of a cohort of 6223 subjects analysed in our laboratory from 2015 to today. All these analyses also include the sequencing of the *KIF5A* gene, and all identified variants classified as pathogenic or likely pathogenic are not present among these subjects.

Data analysis and variant interpretation

Each variant was classified according to the 2015 ACMG (American College of Medical Genetics and Genomics) Association for Molecular Pathology guidelines. The classification was based on a combination of criteria grouped into different levels of pathogenicity evidence (very strong, strong, moderate and supporting). The categories used were: pathogenic, likely pathogenic, variant of uncertain significance, likely benign, benign.[13] To this aim, public databases were used (VarSome https://varsome.com; GnomAD https://gnomad.broadinstitute.org).

Segregation analysis

To evaluate the possibilities of a common ancestor for variant p.Tyr276Cys, we performed a segregation analysis in families 671 and 824 using seven short tandem repeat (STR) markers flanking the mutation. Specifically, three markers were located centromeric to the mutation (D12S1663, D12S85, D12S368), and four were telomeric (D12S83, D12S313, D12S326, D12S1708). The analysis revealed a shared haplotype consisting of the following alleles: 205 bp at D12S368, 111 bp at D12S83, 145 bp at D12S313, 210 bp at D12S326 and 176 bp at D12S1708. Based on the informativity and relative positioning of the STR markers, these results strongly support the hypothesis that the two patients share a common ancestor.

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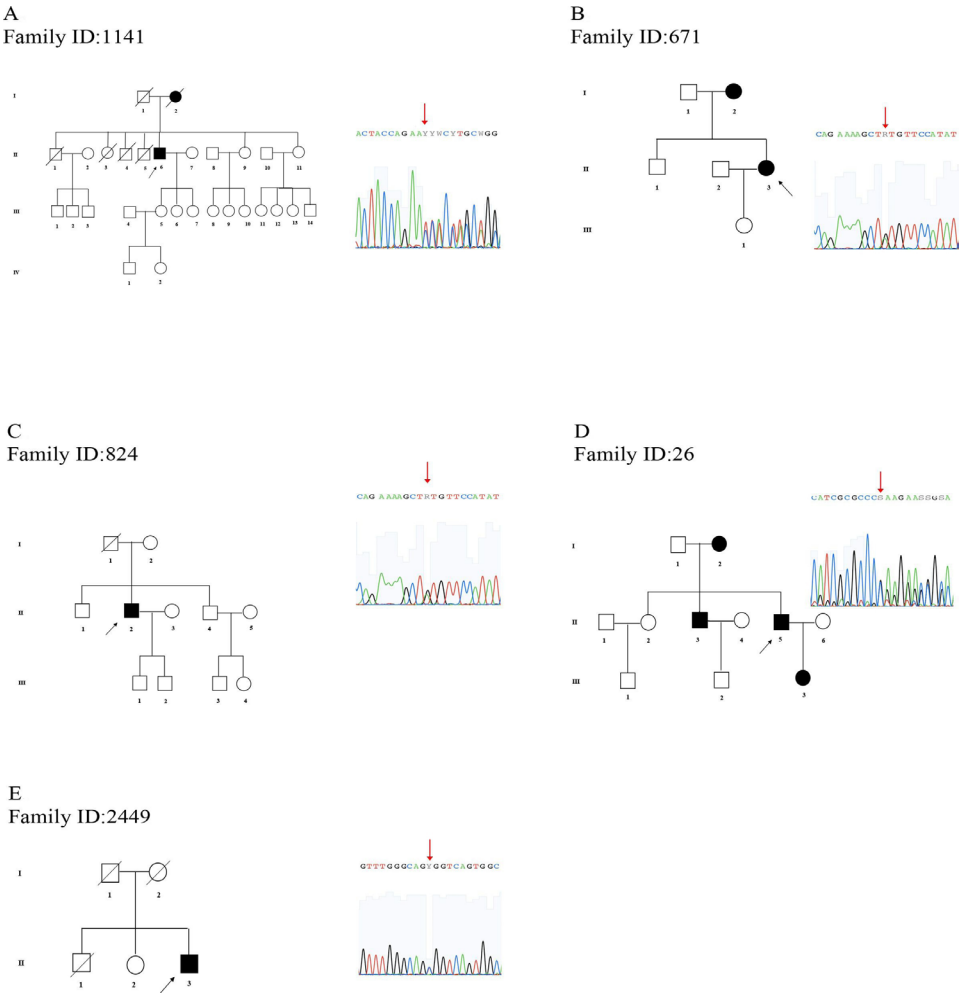

Figure 1 Pedigree and electropherograms of class 4 and 5 mutations identified by next-generation sequencing (NGS). (A) Family ID:1141, heterozygous deletion: c.2868_2870del, p.(Leu957del), rs575223790; (B) Family ID:671, heterozygous missense: c.827A>G, p.(Tyr276Cys), rs121434443; (C) Family ID:824, heterozygous missense: c.827A>G, p.(Tyr276Cys), rs121434443; (D) Family ID:26, heterozygous frameshift: c.1233dup, p.(Glu412ArgfsTer15) ; (E) Family ID:2449, heterozygous missense: c.967C>T, p.(Arg323Trp), rs1012819766.

RESULTS

Cohort description and genetic analysis

We recruited 390 patients (220 HSP, 80 CMT2 and 90 ALS) at the IRCCS Neuromed, Pozzilli, Italy, and the Department of Human Neurosciences, Sapienza University of Rome, Italy. They underwent NGS analysis by Clinical Exome.

A cohort of five patients (three familiar and two sporadic) with a clinical phenotype compatible with HSP or hereditary axonal neuropathy resembling typical CMT2 (one female, four males; mean age±SD 58.5±14.0, range 51–81) were identified. In three familiar probands, a segregation analysis has been performed (Families ID:1141; 671; 26).

In detail, variants have been found in (1) Motor domain Families ID:671 (II:3 and I:2), Families ID:824 (II:2) and Families ID:2449 (II:3); (2) Tall domain Families ID:1141 (II:6) and (3) Stalk domain Families ID:26 (I:2, II:5, II:3).

Family ID:1141

The proband (II:6), a 67-year-old male (figure 1A), onset at 56, presented with marked lower limb weakness with greater severity in the distal segments. The patient did not manifest increased muscle tone but reported moderate impairment of proprioception with markedly reduced deep tendon reflexes. His clinical syndrome underwent further worsening along with disease progression, showing a subtle relapsing-remitting profile. Electrophysiological testing showed motor distal latency prolongation ≥50% above the upper limit of typical values of bilateral tibial and left peroneal nerves, alongside bilateral depression of sensory nerve action potentials (SNAPs) amplitude in sural nerves. Moreover, slight prolongation of F waves of the tibial nerves was reported. No motor blocks were evident. Accordingly, the diagnosis of chronic inflammatory demyelinating polyneuropathy (CMT) was suggested, and the patient received treatment with IVIG for 5 days (2 g/kg for the first cycle, 1 g/kg for the second and third cycle after 1 month each). Due to the long history of disease and the absence of response to immunotherapy, the patient was scheduled for genetic testing.

Molecular analysis identified the new heterozygous variant c.2868_2870del; p.(Leu957del); rs575223790 in the Tail domain, considered as likely pathogenic (Class 4) on ACMG parameters (online supplemental table 1).

The variant is not present in his sisters (II:9, II:11), who are reported as neurologically healthy. Analysis has not been performed on the mother, who died at 88, and reported on anamnesis with marked lower limb weakness.

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Family ID:671

The proband (II:3), 67-year-old male (figure 1B), onset at 56, presented marked lower limb spasticity associated with increased muscle tone and pathologically enhanced deep tendon reflexes. Also, she reported distal limb paraesthesia since the onset of muscle weakness. NCSs showed axonal neuropathy with reduced SNAPs from sural nerves. MEPs recorded from lower limbs were compatible with altered central conduction time (CCT) with desynchronised responses compatible with severe damage of the corticospinal tract.

Molecular analysis identified the known heterozygous variant:[9] c.827A>G; p.(Tyr276Cys); rs121434443, considered as pathogenic (Class 5) (online supplemental table 1). This variant is present in her mother (I:2), studied at the age of 92 years when she was severely affected by lower limb muscle weakness associated with a severe increase in muscle tone and distal paraesthesia. Unfortunately, we were not able to perform diagnostic examinations (NCS and MEPs), and thus we can only infer she was affected by spastic paraparesis associated with axonal neuropathy. The variant is absent in her healthy brother (II:1) and daughter (III:1).

Family ID:824

The proband (II:2), a 56 years old male (figure 1C), onset at 54 years old, presented with spastic paraparesis with lower limb weakness and increased deep tendon reflexes. Over the following years, he also developed distal limb paraesthesia, and NCS revealed a sensory neuropathy; MEPs from the lower limbs were also altered. He was treated with symptomatic therapy for the relief of spasticity and paraesthesia.

Molecular analysis identified the known heterozygous mutation:[9] c.827A>G; p.(Tyr276Cys); rs121434443 considered pathogenic (Class 5) (online supplemental table 1). Family members were not available for testing.

Family ID:26

The proband (II:5), a 53-year-old male (figure 1D), presented with symptom onset at age 51. His clinical presentation included spastic paraparesis, a pathological gait, reduced lower limb muscle strength and lower urinary tract symptoms. NCS revealed axonal neuropathy with reduced SNAPs, while MEPs indicated altered CCT. He was managed with symptomatic therapies.

His older brother (II:3) was clinically evaluated at age 57 and exhibited predominant lower limb spasticity. Unfortunately, objective instrumental testing could not be performed. Their mother (I:2) was assessed at age 81 and followed for 5 years. Her clinical phenotype included distal lower limb muscle weakness, severe spasticity, deep tendon hyper-reflexia and bilateral Achilles tendon clonus without sensory symptoms. Instrumental diagnostic testing was not conducted, and she received only symptomatic treatments (online supplemental table 1). In this family, molecular analysis identified a novel heterozygous mutation, c.1233dupC; p.(Glu412ArgfsTer15) considered pathogenic (Class 5), in the proband (II:5), his brother (II:3) and their mother (I:2). This mutation was not detected in the proband's unaffected sister (II:2).

Family ID:2449

The proband (II:3), a 65-year-old male (figure 1E), presented from the age of 53 with a progressive weakness of the lower limbs, severely affecting his walking skills. The clinical examination identified symmetric spasticity of the lower limbs, brisk reflexes and bilateral Babinski signs. Moreover, the patient complained of frequent urinary urgency. Molecular analysis identified the known heterozygous mutation:[14] c.967C>T; p.(Arg323Trp); rs1012819766 considered as pathogenic (Class 5) (online supplemental table 1). Family members were not available for testing.

Segregation analysis for p.Tyr276Cys

To investigate the potential presence of a common ancestor between the families ID:671 and ID:824 carrying the same new variant p.Tyr276Cys, we performed a segregation analysis using seven STR markers flanking the mutation. Specifically, three markers were located centromeric to the mutation (D12S1663, D12S85, D12S368), and four were telomeric (D12S83, D12S313, D12S326, D12S1708). The analysis revealed a shared haplotype consisting of the following alleles: 205 bp at D12S368, 111 bp at D12S83, 145 bp at D12S313, 210 bp at D12S326 and 176 bp at D12S1708. Based on the informativity and relative positioning of the STR markers, these results strongly support the hypothesis that the two patients share a common ancestor.

DISCUSSION

We selected 220 patients with HSP, 80 patients with CMT2 and 90 patients with ALS for this study. These patients were analysed using an NGS Clinical Exome panel comprising more than 300 genes responsible for neurological and neurodegenerative disorders, including *KIF5A*.

Causative variants were identified in a cohort of five patients (three familial and two sporadic). Segregation analysis was performed on three familiar probands. Therefore, 5 out of 390 (4%) were carriers of a pathogenic variant in the *KIF5A* gene. The frequency of mutations in *KIF5A* is complex and challenging to establish, as most papers report frequencies concerning specific diseases. For example, the frequency of *KIF5A* mutations in an Italian population is 8.8% among patients with SPG, 10% in a French cohort and about 3% in a CMT small cohort.[9 10] However, these data are based on the possibility that some patients manifest complex phenotypes. To date, no validated data exist regarding the combined frequencies of *KIF5A* mutations in HSP, CMT2 and ALS.

The *KIF5A* gene contains three domains: the Motor, Stalk and Tail domains, with a recurrent genotype–phenotype correlation for variants in different domains.[15]

Variants in the Motor domain are primarily responsible for clinical phenotypes related to HSP and axonal polyneuropathy resembling CMT2.[10] To date, only one patient affected by ALS has been reported.[16] This study supports these data. Patients I:2 and II:3 of Family ID:671 and II:2 of Family ID:824 are carriers of variants p.Tyr276Cys (figure 2). They all present a clinical phenotype characterised by variable degrees of lower limb muscle weakness associated with spasticity, stance imbalance and gait abnormalities. Additionally, they presented axonal polyneuropathy with distal paraesthesias, various degrees of amyotrophy and loss of deep tendon reflexes. This clinical phenotype aligns with previous work,[9] reporting *KIF5A* mutations as a significant cause of complex HSP in France. Segregation analysis supports the hypothesis that this new variant, identified in families ID:671 and ID:824 from central Italy, shares a common ancestor.

We observed a homogeneous clinical presentation in p.Tyr276Cys carriers across families ID:671 and ID:824. This finding suggests the presence of relevant genotype–phenotype correlation for specific genomic variants of *KIF5A*, including p.Tyr276Cys. Conversely, the last patient (II:3 of Family

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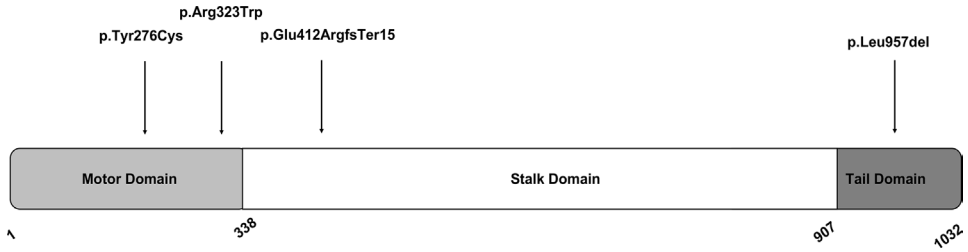

Figure 2 *KIF5A* variants reported in our study. The *KIF5A* gene spans the region of ~3659 kb of genomic DNA and contains 29 exons (RefSeq NM_004984.4). Mutations detected in this study are shown with an arrow. Motor Domain (1–338 amino acids), Stalk Domain (338–907 amino acids) and Tail Domain (907–1032 amino acids) are highlighted.

ID:2449), who presents the p.Arg323Trp variant,[14] presented with a pure HSP phenotype with isolated pyramidal signs.

Variants in the Tail domain are more frequently associated with the ALS phenotype.[17] Patient II:6 of Family ID:1141 presented the new variant p.Leu957del in the Tail domain (figure 2). His clinical phenotype consisted of marked lower limb weakness, with greater severity in the distal segments. Although the patient did not manifest increased muscle tone, he reported moderate impairment of proprioception with markedly reduced deep tendon reflexes. His clinical syndrome worsened as the disease progressed, and neurophysiological examination was consistent with chronic sensorimotor axonal neuropathy. This domain is essential for KIF5A's interaction with several binding partners, including TRAK2 (Trafficking Kinesin 2). TRAK2 is a key protein that regulates mitochondrial transport along microtubules, a process crucial for maintaining cellular energy metabolism and overall cellular function. The p.Leu957del mutation may disrupt the normal binding between KIF5A and TRAK2, impairing the efficient transport of mitochondria. Given the critical role of mitochondrial function in neurons—especially in the context of neurodegenerative diseases—such a disruption could contribute to phenotypes associated with KIF5A mutations, including ALS and sensorimotor polyneuropathy. This mutation may lead to mitochondrial dysfunction, compromising energy production and cellular integrity and further worsening the neurodegenerative processes observed in patients with mutations in the KIF5A Tail domain.

These data contribute to a better understanding of the genotype–phenotype correlation in the Tail domain. Although further confirmation in a large cohort is required, mutations in this specific region seem to be more frequently associated with ALS and, less frequently, with the peripheral nervous system conditions, such as the sensorimotor polyneuropathy observed in our patient.

Variants within the Stalk domain of *KIF5A* are relatively rare and less described. A recent literature review by de Boer and colleagues[5] selected 22 cases with stalk variants, most often resulting in complex HSP/CMT2 phenotype, which can also be associated with ALS.[11] This suggests that variants in the Stalk domain are not bound to a specific phenotype. Among these patients, symptom onset varies from childhood to adulthood, and common additional symptoms for HSP included involvement of the upper limbs, sensorimotor polyneuropathy and foot deformities. These clinical symptoms are atypical progressive motor syndromes KIF5A-related.[18–21]

Family ID:26 (I:2, II:5, II:3) carries the new c.1233dupC missense mutation (figure 2). This variant is classified as pathogenic (Class 5) (online supplemental table 1). Furthermore, the mutation exhibits a familial distribution, being present in the proband (II:5), his affected mother (I:2) and brother (II:3), but absent in his unaffected sister (II:2). The variant is responsible for disease in the three affected family members, all manifesting spasticity of the lower limbs associated with brisk deep tendon reflexes and muscle weakness. Specifically, we observed a clinical phenotype characterised by subacute disease progression, manifesting combined HSP and CMT2 features. However, each family member showed an independent clinical syndrome with variable disease severity. The mother and the brother showed isolated lower limb weakness and spasticity, whereas the proband presented more complex features with axonal polyneuropathy and lower urinary tract symptoms. Therefore, the clinical presentation linked to this mutation is inconsistent across affected family members, indicating significant symptom variability. The c.1233dupC missense occurs near the beginning of the coiled-coil region within the stalk domain of *KIF5A*. The coiled-coil region is a structural feature that plays a critical role in the formation of the stalk, which is essential for the proper assembly and function of the KIF5A motor protein complex. The stalk region is responsible for mediating interactions between KIF5A and other cellular components, such as the microtubules and cargo proteins, facilitating the motor protein's ability to transport cellular materials.[22]

The present study enhances the current understanding of the prevalence of *KIF5A* variants in the Italian population and clarifies the genotype–phenotype correlation. Through comprehensive clinical and genetic analysis of five patients and segregation analysis of three pedigrees, we have elucidated significant genotype–phenotype correlations, particularly highlighting the complexity and variability of KIF5A-related disorders. Some of these data support the hypothesis of a complex correlation between domains and disease. For instance, p.Leu957del in the Tail domain, primarily associated with ALS, may also be linked to sensorimotor neuropathy. Additionally, c.1233dupC in the Stalk domain, identified in three affected family members, produces combined HSP and CMT2 phenotype.[23] However, each member presented an independent clinical syndrome with variable disease severity. This also adds to the literature regarding variants in the Stalk domain, which are rare and less described.

This study also suggests a strong genotype–phenotype correlation, both intrafamiliar and interfamiliar, produced by a few variants, such as the p.Tyr276Cys in the Motor domain, which was evaluated in three patients of two different families. In all patients, this variant is responsible for a phenotype characterised by spasticity, axonal polyneuropathy with distal paraesthesias, varying degrees of amyotrophy and loss of deep tendon reflexes.

Longitudinal studies involving larger cohorts and extensive family studies are necessary to further elucidate the intrafamilial variability observed in KIF5A-related disorders. These studies could provide insights into the progression of symptoms and promote the development of management strategies. In

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this scenario, understanding the complex relationship between *KIF5A* genotype and phenotype is essential for elucidating disease mechanisms and developing targeted therapies.

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Competing interests None declared.

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Ethics approval This study involves human participants and was approved by IRCCS Neuromed Ethics Committee Number of approvation: CGM-01. Participants gave informed consent to participate in the study before taking part.

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