Computational and Structural Biotechnology Journal 19 (2021) 4265–4272

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

New structural variations responsible for Charcot-Marie-Tooth disease: The first two large *KIF5A* deletions detected by CovCopCan software

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ARTICLE INFO

Article history:
Received 3 May 2021
Received in revised form 19 July 2021
Accepted 28 July 2021
Available online 30 July 2021

Keywords:
NGS
Structural variations
CovCopCan
Charcot-Marie-Tooth
KIF5A

ABSTRACT

Next-generation sequencing (NGS) allows the detection of mutations in inherited genetic diseases, like the Charcot-Marie-Tooth disease (CMT) which is the most common hereditary peripheral neuropathy. The majority of mutations detected by NGS are single nucleotide variants (SNVs) or small indels, while structural variants (SVs) are often underdiagnosed. *PMP22* was the first gene described as being involved in CMT via a SV of duplication type. To date, more than 90 genes are known to be involved in CMT, with mainly SNVs and short indels described. Herein targeted NGS and the CovCopCan bioinformatic tool were used in two unrelated families, both presenting with typical CMT symptoms with pyramidal involvement. We have discovered two large SVs in *KIF5A*, a gene known to cause axonal forms of CMT (CMT2) in which no SVs have yet been described. In the first family, the patient presented with a large deletion of 12 kb in *KIF5A* from Chr12:57,956,278 to Chr12:57,968,335 including exons 2–15, that could lead to mutation c.(130-943_c.1717-533del), p.(Gly44_Leu572del). In the second family, two cases presented with a large deletion of 3 kb in *KIF5A* from Chr12:57,974,133 to Chr12:57,977,210 including exons 24–28, that could lead to mutation c.(2539-605_*36 + 211del), p.(Leu847_Ser1032delins33). In addition, bioinformatic sequence analysis revealed that a NAHR (Non-Allelic-Homologous-Recombination) mechanism, such as those in the *PMP22* duplication, could be responsible for one of the *KIF5A* SVs and could potentially be present in a number of other patients. This study reveals that large *KIF5A* deletions can cause CMT2 and highlights the importance of analyzing not only the SNVs but also the SVs during diagnosis of neuropathies.

1. Introduction

Thanks to next-generation sequencing (NGS) technique, we are able to detect pathogenic germline mutations and thus to improve the diagnosis of patients with inherited genetic diseases. Though nowadays, the majority of reported mutations are single nucleotide variants (SNVs), while structural variants (SVs) have rarely been described, probably because the analysis of NGS data is complicated and there are a few available analyzing tools.

Charcot-Marie-Tooth disease (CMT) is the most common hereditary peripheral neuropathy, characterized by damages in both

Abbreviations: ALS, Amyotrophic Lateral Sclerosis; CMT, Charcot-Marie-Tooth; CMT2, Charcot-Marie-Tooth type 2; CNV, Copy Number Variants; DSMA, Distal-Spinal-Muscular-Atrophy; HSP10, Hereditary-Spastic-Paraplegia-type-10; NAHR, Non-Allelic Homologous Recombination; NEIMY, Neonatal-Intractable-MYoclonus; NGS, Next Generation Sequencing; SNV, Single Nucleotide Variant; SV, Structural Variant.

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https://doi.org/10.1016/j.csbj.2021.07.037

motor and sensory peripheral nerves. *PMP22* duplication was the first mutation to be described in this disease, and is described as explaining 70–80% of the demyelinating form of the disease (CMT1) [1–5]. To date, more than 90 different genes have been reported to be involved in the disease [6]. The majority of the reported mutations in CMT patients consist of point mutations in the coding region of these genes, whereas structural variants (SVs) have only rarely been described [7,8].

KIF5A encodes the heavy chain of Kinesin I (ensembl: ENSG00000155980, UniProtKB-Q12840). This protein is part of a heterotetrameric Kinesin I complex consisting of two light chains and two heavy chains (Fig. 1A). Kinesin I, located in central and peripheral nervous system, is involved in the anterograde transport of various cargoes (RNA, mitochondria, neurofilaments) along microtubules in neurons [9]. The kinesin heavy chain subunit, encoded by *KIF5A*, presents three main domains: motor (amino acid residue 9–327), stalk (amino acid residue 328–906), and tail (amino acid residue 907–1032) (Fig. 1B). The motor domain possesses ATPase activity necessary to produce kinetic force, the stalk domain allows the dimerization with another kinesin heavy chain forming a coiled-coil interaction, and the tail interacts with the kinesin light chain and cargoes [10–12].

Missense mutations located on the Kinesin-I heavy chain motor domain were first described as mainly causing a rare form of Hereditary-Spastic-Paraplegia type 10 (HSP10; OMIM#604187) [13] but also Charcot-Marie-Tooth type 2 disease (CMT2; ORPHA:324611) [14]. The clinical symptoms of HSP observed in patients presenting with *KIF5A* mutations are pyramidal syndrome and sphincter disturbances, while CMT signs consist of lower and/or upper limb weakness with gait disturbances, sensory deficits, depressed tendon reflexes, articular deformations (*pes cavus* and scoliosis) and distal muscular atrophy [15,16]. In addition, other

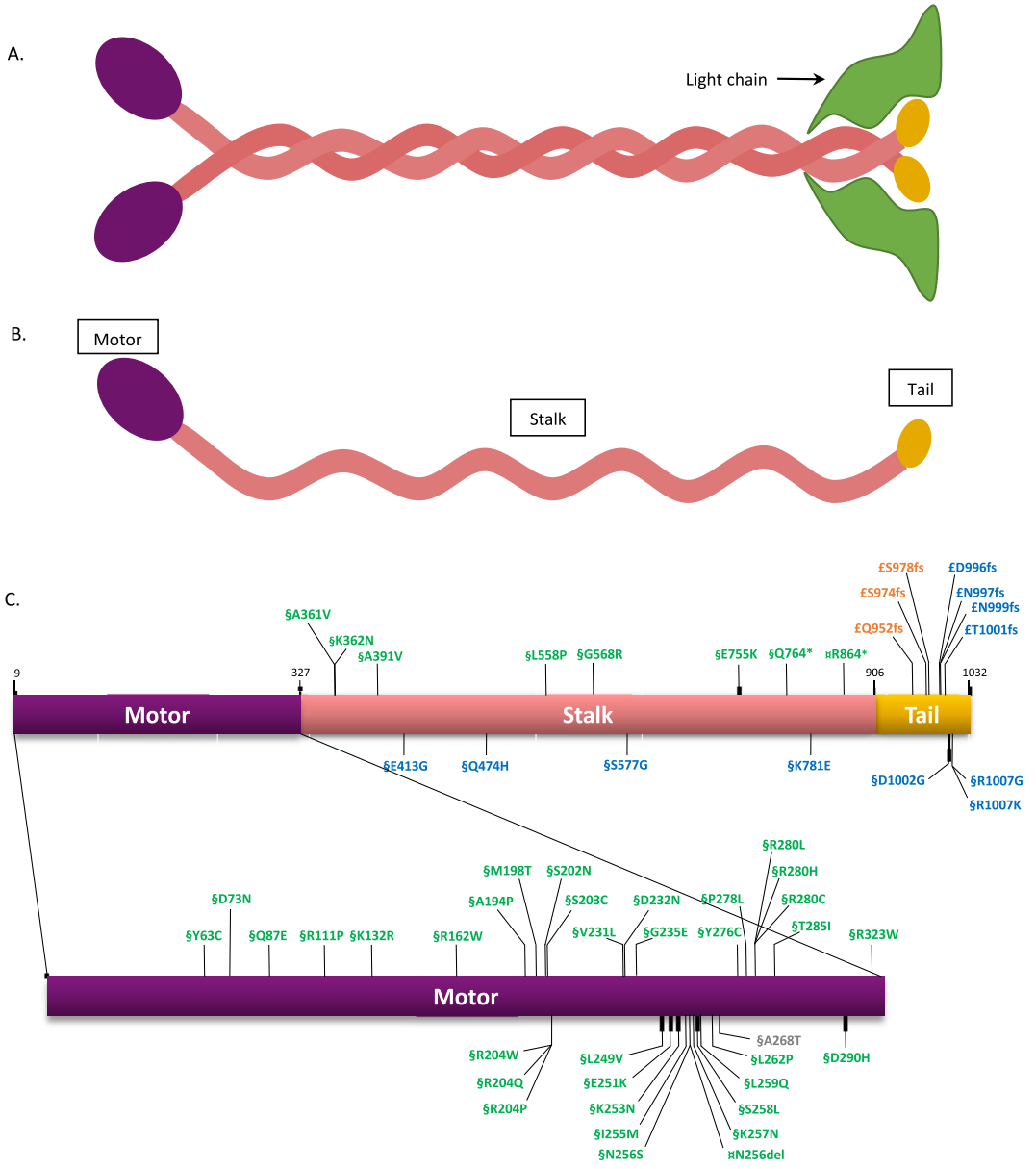


Fig. 1. A) Kinesin-I structure. Two heavy chains and two light chains (in green) form the heterotetramer of Kinesin-I. B) Kinesin-I heavy chain encoded by *KIF5A*. The heavy chain of Kinesin-I contains three domains: a motor domain (purple), a stalk domain (pink) and a tail domain (yellow). C) Location of known mutations in *KIF5A*. Functional domains are represented. Each mutation is indicated by the number of the affected amino-acid. CMT2 isolated or associated with HSP and isolated HSP are shown in green, ALS are shown in blue, NEIMY are shown in orange and DSMA are shown in grey. § = missense mutation; ¤ = deletion; £ = frame-shift mutations. 3′-UTR mutations, and splice mutations affecting the tail are not shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

KIF5A mutations have also been identified in a severe neonatal neurological syndrome called NEIMY (Neonatal-Intractable-MYo clonus; OMIM#617235) [17], in Amyotrophic-Lateral-Sclerosis (ALS, OMIM#617921) [18,19], and recently in Distal Spinal Muscular Atrophy (DSMA) (Fig. 1C) [20]. Thus, *KIF5A* mutations present a wide clinical spectrum exhibited in several diseases associated with an autosomal dominant mode of inheritance. Interestingly, to date, only SNVs or frameshift mutations have been described in *KIF5A*. To our knowledge, structural variations have never been discovered.

Herein, we describe the first two structural variants in *KIF5A* responsible for CMT symptoms associated with pyramidal syndrome in two distinct families. Targeted NGS and CovCopCan bioinformatic tool [21] revealed a 12 kb deletion in *KIF5A* from Chr12:57,956,278 to Chr12:57,968,335 including exons 2–15 in the first family and a 3 kb deletion in *KIF5A* from Chr12:57,974,133 to Chr12:57,977,210 including exons 24–28 in the second family. These SVs have not previously been described and highlight the importance of SV analysis of NGS data in the diagnosis of neuropathies.

2. Materials and methods

2.1. Patients

Family 1: The proband (A), a 60-year-old man presented axonal CMT syndrome associated with several HSP10 signs (Fig. 2A-Family 1). Family 2: The proband (B), a 70-year-old man, and his 47-year-old son (C) presented axonal CMT syndrome associated with atypical signs. The proband B's spouse was healthy (Fig. 2B-Family 2).

Peripheral blood was collected into EDTA tubes for each patient after informed consent was obtained according to the Declaration of Helsinki. DNA extraction was then performed using standard methods (Illustra-DNA-Extraction-kit-BACC3, GEHC). The study has been approved by the ethics committee of Limoges University Hospital (n° 387-2020-43).

2.2. Next generation sequencing (NGS)

NGS strategy was performed using a 93-gene custom panel designed for diagnosis of CMT and associated neuropathies (as described by Miressi et al. [22]). The amplified library was prepared with Ion-P1-HiQ-Template-OT2-200 kit (Ampliseq-Custom; Life Technologies), sequenced on Ion-Proton sequencer (Life-Technologies), and mapped to the human-reference-genome GHCh37.

2.3. Bioinformatics analysis

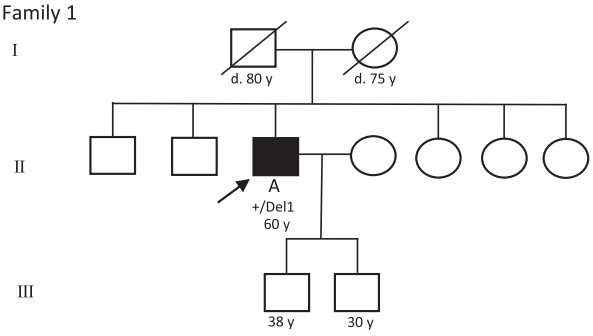
Variants were evaluated with Alamut-Visual-Interpretation Software v.2.11 (Interactive-Biosoftware, Rouen, France) using the NM_004984.4 reference sequence for the *KIF5A* gene. Databases such as gnomAD (https://gnomad.broadinstitute.org/), dbSNP135 (National-Center-Biotechnology-Information [NCBI], http://www.ncbi.nlm.nih.gov/projects/SNP/) and Clin-Var (www.ncbi.nlm.nih.gov/clinvar) were used as well.

Transposable elements were detected from the genomic sequence of the *KIF5A* using RepeatMasker software (http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker) with default settings. The graph was then generated with the Gviz package [23].

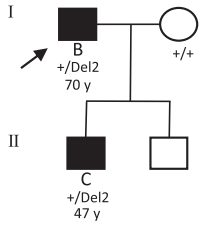
2.4. Structural variation detection

SVs were detected using Cov'Cop and CovCopCan analysis tools starting from the coverage file provided after NGS sequencing by Ion-Proton-sequencer [21,24]. Cov'Cop and CovCopCan software programs use a two-stage correction and normalization algorithm to identify unbalanced SVs, such as CNVs (Copy Number Variants), using NGS read depth. These software are based on the principle that in normal cases, both alleles should be amplified similarly within each amplicon. To the normal cases a theoretical score of 1 is attributed, whereas deletions or duplications are revealed by low (<0.5) or high (>1.5) scores respectively. At least three adjacent amplicons with altered values were required in order to highlight a

A. Family 1



B. Family 2



C.

	Motor Nerve Conduction Values (MNCV)						Sensory Nerve Conduction Values (SNCV)					
	Median		Fibular		Tibial		Median		Radial		Sural	
	Vel (m/s)	Amp (mV)	Vel (m/s)	Amp (mV)	Vel (m/s)	Amp (mV)	Vel (m/s)	Amp (μV)	Vel (m/s)	Amp (μV)	Vel (m/s)	Amp (μV)
Patient A	54.6	10.1	**33.3**	**1.1**	42.3	3.8	**44.6**	**17.0**	51.4	20.0	**36.2**	**4.2**
Patient B	51.6	9.7	**NR**	**NR**	**36.8**	**2.8**	**39.2**	**8.5**	**44**	**8.0**	**29.2**	**4.3**
Patient C	54.6	4.5	**40.2**	2.3	**40.7**	5.9	ND	ND	ND	ND	**35.7**	**1.8**

Normal values of MNCV: median (>49m/s; >4mV), fibular (>41m/s; >2mV), tibial (>41m/s; >3mV)
Normal values of SNCV: median (>53m/s; >20uV), radial (>48m/s; >20uV), sural (>41m/s; >6uV)

Fig. 2. Family pedigrees with segregation of the phenotypes and genotypes and neurophysiological recordings. A) Pedigree of family 1 and B) Pedigree of family 2. (d.) indicates the age of death, (+) indicates normal *KIF5A* allele and (Del1 or Del2) allele with a *KIF5A* partial deletion; Del1 corresponding to c.(130-943_1717-533del) including exons 2–15 and Del2 corresponding to c.(2539-605_*36 + 211del) including exons 24–28. C) Patients A, B and C neurophysiological recordings. Abnormal values are represented in bold (Vel: Velocity; Amp: Amplitude; NR: no response; ND: not determined).

CNV. CovCopCan software also allows graphical representation of data.

In order to delineate the exact deletion breakpoints, long range PCRs were performed using Taq-Polymerase-Phusion-hot-Start II (Qiagen), according to the manufacturer's recommendations. Several long range PCRs were tested using forward primers in exon 1 or all along intron 1 and reverse primer in exon 16 for Family 1 in order to optimize the detection of the deleted allele. Only one PCR was tested for family 2. The final following primers (Sigma Aldrich) were used: Int1-F (AGTCAGTCACCTATGATAGAG) and Ex16-R (GCCACAGTGAACTCCTCC) for Family 1; Int22-F (CTGCCCCTGTTGCCCCTATG) and Ex29-R (GCCGCTGGAGAATCTTGGTCT) for Family 2. Conditions of PCR were 98 °C 1 min, followed by 30 cycles [98 °C 1 min; 59 °C 30 sec (Fam 1) and 64 °C 30 sec (Fam 2); 72 °C 8 min (Fam 1) and 72 °C 4 min (Fam 2)] (See Supplementary data 1 for details on PCR results). PCR products were sequenced by the Sanger sequencing method using the Big Dye Terminator Cycle Sequencing Kit v2 (ABI Prism, Applied Biosystems), but also a walking primer strategy for Family 2 (See Supplementary data 1 for details on primers used to identified the breakpoints)

3. Results

3.1. Atypical clinical phenotype

Regarding Family-1 (Fig. 2A), patient A was a 60-year-old man, presenting gait disturbances and distal muscle wasting without objective motor weakness of the four limbs since the age of 26. He had *pes cavus* and pyramidal syndrome. This patient has also

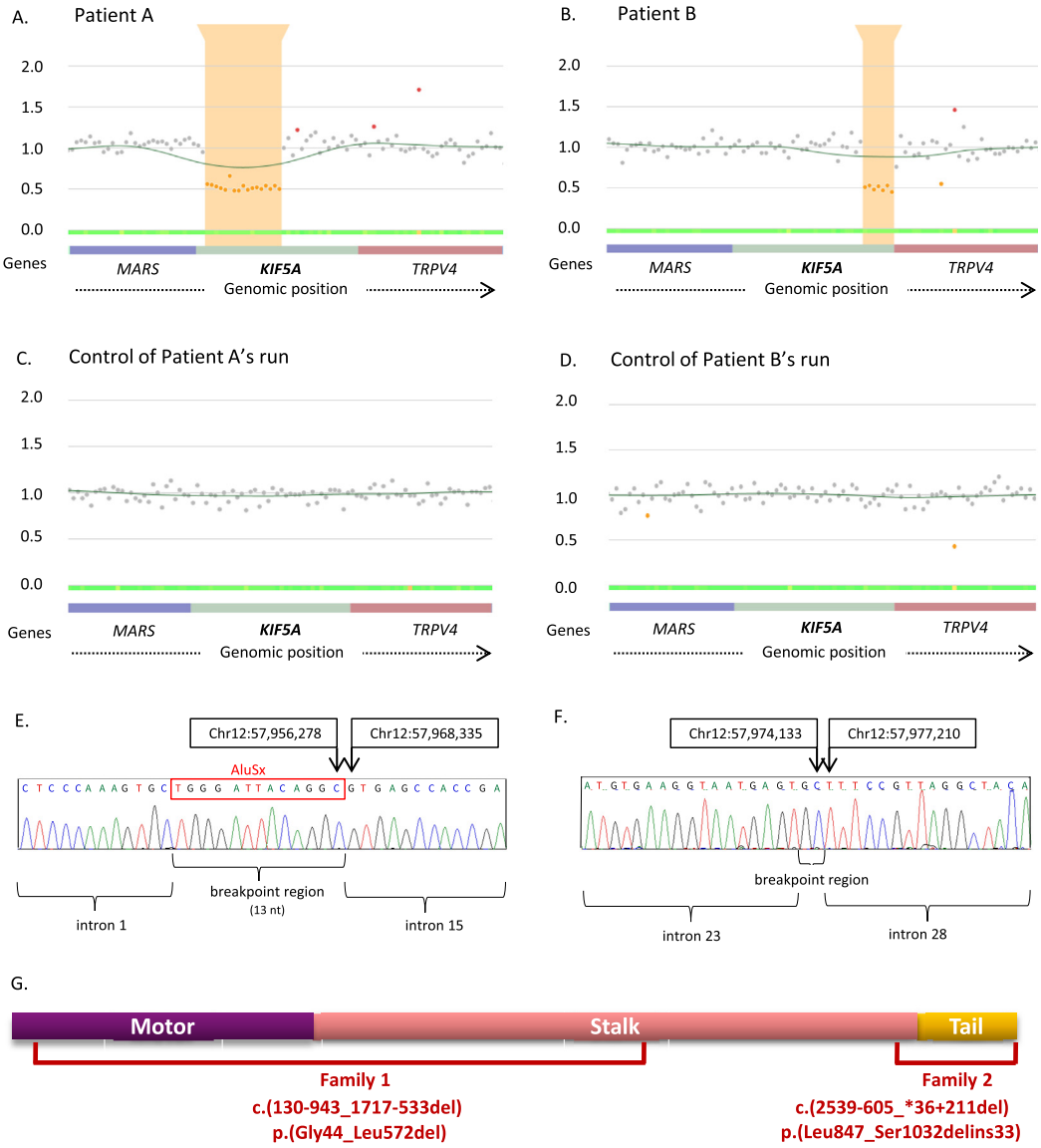


Fig. 3. CovCopCan analysis of *KIF5A* and surrounding genes on chromosome 12 and Sanger Sequencing results. A, B), C) and D) CovCopCan graphical representation of patients A and B analyses and controls samples respectively. Each dot corresponds to an amplicon, which are distributed along the x-axis in accordance with their genomic position. The y-axis corresponds to the normalized values of each amplicon. Grey dots correspond to values considered "normal" (value around 1), while red or orange dots show the amplicons' duplication (value around 1.5) or deletion (value around 0.5) respectively. The deletion areas are highlighted with yellow rectangles. E) *KIF5A* intron 1 and intron 15 Sanger sequencing results. Results reveal the break points at positions Chr12:57,956,278 and Chr12:57,968,335, corresponding to a 12,057 base pairs deletion. F) *KIF5A* intron 23 and intron 28 Sanger sequencing results. Results reveal the break points at positions Chr12:57,974,133 and Chr12:57,977,210, corresponding to a 3077 base pairs deletion. (G) Localization of both deletions on the protein encoded by *KIF5A*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

been evaluated using (i) the Charcot-Marie-Tooth Neuropathy Score (CMTN) with a value at 2/4, (ii) the Medical Research Council (MRC) with a score 100/100 and (iii) Overall Neuropathy Limitation Scale (ONLS) with a score 3/12. Electroneuromyography showed a sensorimotor axonal peripheral neuropathy with fasciculations on all four limbs (Fig. 2C). His large biological analysis remains normal, with a normal B12 level of 432 pmol/L (normal range: 187–885) and no diabetes (HbA1C: 5.2%, with normal value expected around 6%). Patient A's father deceased at 80 years old without showing any neurological symptoms, while his mother deceased at the age of 75, having presented cognitive disorders and a ventricular cyst. Neither of the proband's five siblings, nor his two sons (38 and 30 years old), presented with any neurological problems.

Regarding Family-2 (Fig. 2B), patient B was a 70-year-old man, presenting with gait disturbances with *musculum tibialis anterior* weakness for at least ten years, fingertip paraesthesia and mild tremor. Articular deformities with *pes cavus* and mild scoliosis were also noted. He can however still do 12 km long walks. Axonal CMT disease with sensorimotor axonal peripheral neuropathy (low amplitude, subnormal velocity) was suspected based on electroneuromyography (Fig. 2C). Furthermore, a pyramidal syndrome with brisk deep tendon reflexes in the lower limbs was reported, associated with sphincter disturbance. He had no siblings, and his parents were deceased without neurological symptoms. He had two sons, of whom, one (C) also presented CMT symptoms.

Patient C was a 47-year-old man, presenting an axonal CMT disease with gait disturbance since the age of 40. He complains of difficulties walking since several years but only describes one fall. He had *pes cavus,* and electroneuromyography showed a sensorimotor axonal peripheral neuropathy (Fig. 2C). This symptomatology was also associated with brisk deep tendon reflexes in the lower limbs and moderate tremor. Proband B's spouse was healthy, as was his second son. However, his DNA was not available.

None of the patients presented other sensory deficits (neither vision loss nor deafness) and they do not have medical history of medication/intoxication that could be related to their CMT symptoms.

3.2. Detection of structural variants using CovCopCan

Targeted NGS analysis of our 93-gene panel involved in peripheral neuropathies was performed on DNA from patients A and B DNA. CNVs detection was performed using the user-friendly CovCopCan tool and allow the discovery of two heterozygous deletions for both cases. For patient A, CovCopCan analysis revealed a deletion of 17 adjacent amplicons covering the genomic region Chr12:57,957,118-Chr12:57,966,569 including *KIF5A* exons 2–15 (Fig. 3A). Regarding patient B, a deletion of 7 adjacent amplicons was identified, covering the genomic region Chr12:57,974,529-Ch r12:57,977,002 including *KIF5A* exons 24–28 (Fig. 3B). In comparison, no variation has been observed in control patients (Fig. 3C and D), in which normal values were observed for *KIF5A*. No additional positive variant candidate to explain patients' phenotype (SNVs and short indels) has been identified for patients using the standard NGS analysis.

3.3. Configuration of structural variants

Long range PCR, from intron 1 to exon 16, was then performed on DNA from patient A (see supplementary data 1). We amplified and sequenced the deleted allele to identify the exact breakpoints positions at Chr12:57,956,278 and Chr12:57,968,335 (Fig. 3E), corresponding to a 12,057 base pairs deletion. This *KIF5A* deletion identified on patient A DNA starts in intron 1 at position c.130–943 and ends in intron 15 at position c.1717–533. On KIF5A protein, it would correspond to the whole kinesin I heavy chain motor domain and part of the stalk domain and could be named p.(Gly44_Leu572del) (Fig. 3G, Family-1).

The same strategy performed, from intron 22 to exon 29, on patient B DNA allowed to amplify (see supplementary data 1) and sequence the deleted allele. We identified the breakpoints positions at Chr12:57,974,133 and Chr12:57,977,210 (Fig. 3F), corresponding to a 3,077 base pairs deletion. The identical result was obtained for the son (C). This *KIF5A* deletion identified on patients B and C DNAs starts in intron 23 at position c.2539–605 and ends in exon 29 at position c.*36 + 211. On KIF5A protein, it would affect the end of the Kinesin-I heavy chain stalk domain and tail domain and potentially alter its activity and could be named p.(Leu847_Ser1032delins33) (Fig. 3G, Family-2).

3.4. Informatics analysis of sequences and detection of the recombination mechanism

Then we proceed to the detection of the transposable elements presented in the genomic sequence of the *KIF5A*, using RepeatMasker software. This analysis revealed that the breakpoints of the exons 2–15 deletion (patient A) are located in a region rich in Alu repeat elements (Fig. 4). The numerous Alu repeat sequences (AluSx and AluJb) in this region could promote an erroneous recombination and thus a large gene deletion due to a non-allelic homologous recombination (NAHR) event.

4. Discussion

In this paper, we highlight the importance of bioinformatic analysis of NGS data in order to detect new pathogenic structural variants. For the first time, we report here that large *KIF5A* deletions, detected in two different families, may be responsible for CMT atypical cases associated with pyramidal syndrome. These deletions are carried in an autosomal dominant manner. In the first family, the patient presented with a large deletion of 12 kb in *KIF5A*

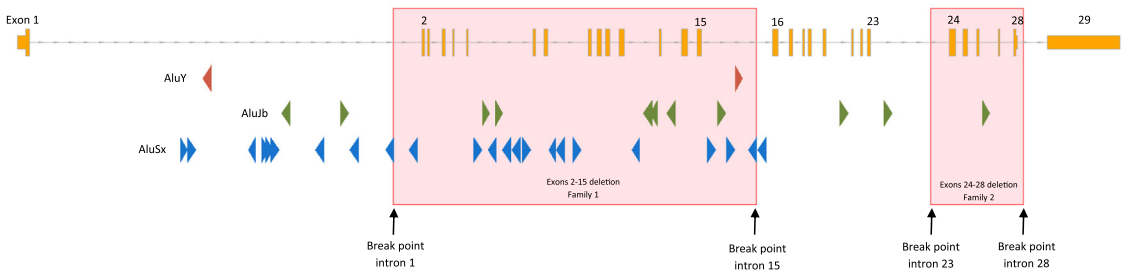


Fig. 4. Mapping of *KIF5A* structural variants. Orange rectangles represent *KIF5A* exons. Red, green and blue arrows represent transposable elements of Alu family: AluY, AluJb and AluSx respectively. Breakpoints of the deletion of Family 1 are situated in a region containing a large number of repetitive Alu sequences, promoting a mistaken recombination event via the NAHR mechanism. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

from Chr12:57,956,278 to Chr12:57,968,335, that could lead to mutation c.(130-943_c.1717-533del), including the deletion of exons 2 to15. In the second family, two cases presented with a large deletion of 3 kb in *KIF5A* from Chr12:57,974,133 to Chr12:57,977,210, that could lead to mutation c.(2539-605_*36 + 211del), including the deletion of exons 24 to 28. Both of the deletions were detected by using the CovCopCan analysis tool.

KIF5A large deletions have not been previously described. Indeed, to date, according to the Human-Gene-Mutation-Database (HGMD®) Professional 2020.3 (Qiagen), the published mutations mainly consist of SNVs leading to missense mutations, splicing alterations, premature stop codon and short deletions that may result in frame-shift. We showed here that *KIF5A* large deletions could be involved in atypical CMT cases.

It is important to remind that *KIF5A* encodes the heavy chain of kinesin I, which interacts with another heavy chain in order to form a homodimer. This homodimer interacts then with two light chains of kinesin I in order to form an active heterotetrametric complex. If one of this heavy chain is mutated but is still able to interact with another monomer including the wild type one (WT), only 25% of the dimer would be WT-WT and completely active. If the mutation has an effect on the motor domain or the cargoes attachment, this means that 75% of the remaining dimers (WT-Mutated and Mutated-Mutated) would have a disturbed activity. This kind of dominance mechanism has already been shown on other dimeric proteins such as TNSALP involved in hypophosphatasia [25,26].

In the previous studies, it has been shown that the clinical phenotype seems to be dependent on the mutated KIF5A domain as shown in Fig. 1, with severe phenotype observed when located in the tail domain and milder ones when located in motor or stalk domains. It is also interesting to note that indirectly some SNVs, previously described, such as splicing alterations, short deletions and premature stop codons could lead to a truncated protein, that could be compared to the deletions we described here. Interestingly, splicing mutations predicted or shown to result on exon deletion would lead to mild symptoms when located in the area coding the motor domain [27,28] and to a severe one when located in the area coding the tail domain ([18,19], for instance). In addition, small deletions leading to a frameshift have only been described, to our knowledge, in the same area (Exons 24 to 27) coding the tail domain (HGMD data) and are always associated to severe phenotype such as NEIMY and ALS. These observations could suggest that, as for point mutations (Fig. 1), functional domain deleted in KIF5A dictates the clinical phenotype.

In contrast, the rare described non-sense mutations, located in exons 20 or 24 [29,30], lead to CMT or HSP symptoms, as those observed in our patients. In these non-sense mutations cases, if the protein KIF5A is produced, the tail part would be missing and one could expect severe symptoms, while milder symptoms are observed. In these cases, mutated RNA are probably degraded by non-sense-mediated-decay mechanism (NMD), leading to the absence of the truncated heavy chain of Kinesin-I. The cell would then produce only normal KIF5A, that would create functional homodimer, but in a smaller proportion (50%). This could lead to a mild phenotype by a possible haplo-insufficiency effect.

In the cases, we described here, the deletions could result in (i) the production of a truncated protein or to (ii) in the complete absence of the heavy chain of Kinesin-I. (i) Regarding the first hypothesis, Alamut-Visual-Interpretation-Software was used in order to check for any reading frame alteration resulting from exons' deletion for both families. This analysis revealed that, for patient A (c.(130-943_1717-533del) including exons 2–15 deletion), assuming correct splicing occurs between exon 1 and 16, a correct reading frame could be preserved, but would produce a mutated heavy chain p.(Gly44_Leu572del) lacking almost all of its motor domain and part of its stalk domain (Fig. 3E). For patients B and C, the deletion c.(2539-605_*36 + 211del), including to exons 24 to 28, removes the last coding exon (exon 28) but not the non-coding exon 29. Again, if the splicing is performed correctly between exon 23 and 29, the translation of exon 29 would lead to 33 additional amino acids on a mutated heavy chain p.(Leu847_Ser1032delins33) in which the end of the stalk domain and the entire classic tail would be missing (Fig. 3E). For the first deletion, if the truncated heavy chain exists, it would probably not interact with the wild-type one due to the large loss of the stalk domain. Wild-type functional homodimer would be then present in smaller proportion (50%). This could lead to a mild phenotype comparable to those observed in patients harboring a non-sense mutation in which haplo-insufficiency could be suggested. For the second deletion, if the truncated heavy chain exists, it could have either a dominant negative effect by interacting with the wild type heavy chain and thus disrupting kinesin function, or an effect on itself (gain of function effect) due to the possible 33 additional amino-acids. This would be comparable to splicing mutations and the small deletions leading to a frameshift in the tail of KIF5A and to a severe phenotype (ALS, NEIMY). However, in our case (Family 2), the symptoms are milder suggesting a different pathophysiology mechanism. (ii) A more likely second hypothesis would be that the kinesin heavy chain derived from the deleted alleles would be absent (not produced or rapidly degraded). In this case, the deletions would also have a haplo-insufficiency effect, and would be classified as PVS1 according to the ACMG guidelines [31], since it corresponds to multi-exons deletions in a gene where loss of function can be a mechanism of the disease. Even if these deletions seem to be the cause of the patients' disease, their underlying pathophysiologies are difficult to decipher. Indeed, *KIF5A* being expressed mainly in the nervous system, it is difficult reach a conclusion due to the lack of nervous tissue availability.

In addition, regarding the appearance of these large deletions, we noticed that the parents of both patients A and B were deceased elderly without known neurological symptoms. Patients A and B thus seem to be the first members of their families presenting symptoms due to *KIF5A* partial deletion. We therefore hypothesize that these CNVs probably appeared *de novo*. Analysis of the exact breakpoints position of the deletion 2 (exons 24–28) did not allow us to detect any particular joint mechanism. Interestingly, for the first deletion (exons 2–15), a mechanism similar to that involved in *PMP22* duplication could be suspected. This kind of genomic alteration, due to a NAHR event, has already been described in several hereditary diseases including CMT disease, particularly in *PMP22* duplication [32]. In patient A, it seems that erroneous recombination occurred within the AluSx sequences (13 identical bp) via a NAHR mechanism. Because of the numerous repetitive elements present in this *KIF5A* region (Fig. 4), we believe that partial deletions in *KIF5A* could be currently underestimated, and we suggest searching for them systematically when performing NGS sequencing on CMT-patients who also harbor a pyramidal syndrome.

5. Conclusion

In conclusion, our approach using NGS data analysis to look for small nucleotide variants (SNVs or short indels) combined with investigation for large CNVs (using the CovCopCan tool for example) allowed us to highlight the existence of two different large structural variants in two distinct families. The clinical signs in these patients are in agreement with a variation of *KIF5A* and they did not present any other mutations in genes currently associated with CMT, leading to the conclusion that those structural variants are indeed the cause of their disease. In our cohort of 765 CMT

patients, we identified six patients with *KIF5A* variations, of whom two presented these SVs on *KIF5A*, *i.e.* in 33%, suggesting that this kind of variation is present in a significant manner. We conclude so that also large deletions in *KIF5A* are associated to CMT/HSP10 diseases.

We believe our strategy of looking systematically for SVs when performing NGS analysis could increase the diagnosis rate of patients suffering from neuropathies. This strategy could also be employed by molecular biologists and geneticists to improve all inherited diseases diagnosis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We would like to thank the technicians in the Biochemistry and Molecular Genetics Laboratory and the Sequencing and Bioinformatic platform at Limoges CHU, especially Emilie Guerin, Valentin Tilloy and Sophie Alain for their excellent technical support.

Author statement

All authors have reviewed and edited the manuscript.

IP: participated to the bioinformatics study, performed experiments and wrote most of the manuscript.

AP: participated to the bioinformatics study, performed experiments and proofread the manuscript.

AN: performed experiments and proofread the manuscript.

NB: performed experiments and proofread the manuscript.

PD: participated to the bioinformatics study and proofread the manuscript.

SB: performed experiments.

MF: reported clinical study.

GS: reported clinical study.

FD: reported clinical study.

CG: reported clinical study.

FF: performed critical revision of the manuscript.

FS: performed critical revision of the manuscript.

CM: planned the experiments, contributed to the interpretation of the results and proofread the manuscript.

ASL: initiated the work, designed and planned the experiments, analyzed the results and proofread the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2021.07.037.

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