The Cerebellum (2019) 18:781–790
https://doi.org/10.1007/s12311-019-01038-0

ORIGINAL PAPER

Increased Diagnostic Yield of Spastic Paraplegia with or Without Cerebellar Ataxia Through Whole-Genome Sequencing

Aryun Kim[1] · Kishore R. Kumar[2,3,4,5] · Ryan L. Davis[2,3] · Amali C. Mallawaarachchi[6] · Velimir Gayevskiy[2] · Andre E. Minoche[2] · Zachary Walls[2,7] · Han-Joon Kim[8] · Mihee Jang[9] · Mark J. Cowley[2,10,11] · Ji-Hyun Choi[8] · Chaewon Shin[12] · Carolyn M. Sue[3,4] · Beomseok Jeon[8]

Published online: 18 May 2019

Abstract

Inherited disorders of spasticity or ataxia exist on a spectrum with overlapping causative genes and phenotypes. We investigated the use of whole-genome sequencing (WGS) to detect a genetic cause when considering this spectrum of disorders as a single group. We recruited 18 Korean individuals with spastic paraplegia with or without cerebellar ataxia in whom common causes of hereditary cerebellar ataxia and hereditary spastic paraplegia had been excluded. We performed WGS with analysis for single nucleotide variants, small insertions and deletions, copy number variants (CNVs), structural variants (SVs) and intronic variants. Disease-relevant variants were identified in *ABCD1* ($n = 3$), *CAPN1* ($n = 2$), *NIPA1* ($n = 1$) and *PLA2G6* ($n = 1$) for 7/18 patients (38.9%). A 'reverse phenotyping' approach was used to clarify the diagnosis in individuals with *PLA2G6* and *ABCD1* variants. One of the *ABCD1* disease-relevant variants was detected on analysis for intronic variants. No CNV or SV causes were found. The two males with *ABCD1* variants were initiated on monitoring for adrenal dysfunction. This is one of only a few studies to analyse spastic-ataxias as a continuous spectrum using a single approach. The outcome was improved diagnosis of unresolved cases for which common genetic causes had been excluded. This includes the detection of *ABCD1* variants which had management implications. Therefore, WGS may be particularly relevant to diagnosing spastic ataxias given the large number of genes associated with this condition and the relatively high diagnostic yield.

Keywords Whole-genome sequencing · Spastic · Ataxia · Cerebellar · Hereditary spastic paraplegia · Diagnosis

Aryun Kim and Kishore R. Kumar contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12311-019-01038-0) contains supplementary material, which is available to authorized users.

✉ Kishore R. Kumar
kkum4618@uni.sydney.edu.au

[1] Department of Neurology, Chungbuk National University Hospital, Cheongju-si, South Korea

[2] Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

[3] Department of Neurogenetics, Kolling Institute, University of Sydney Faculty of Medicine and Health Northern Clinical School and Royal North Shore Hospital, St Leonards, NSW, Australia

[4] Department of Neurology, Royal North Shore Hospital, Northern Sydney Local Health District, St Leonards, NSW, Australia

[5] Molecular Medicine Laboratory, Concord Hospital, Sydney, NSW, Australia

[6] Division of Genomics and Epigenetics, Garvan Institute of Medical Research, Sydney, NSW, Australia

[7] Faculty of Engineering & Information Technologies, The University of Sydney, Sydney, NSW, Australia

[8] Department of Neurology, Movement Disorder Center, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, South Korea

[9] Department of Neurology, Presbyterian Medical Center, Jeonju, South Korea

[10] St Vincent's Clinical School, UNSW Sydney, Sydney, NSW, Australia

[11] Children's Cancer Institute, UNSW Sydney, Sydney, NSW, Australia

[12] Department of Neurology, Kyung Hee University Medical Center, Seoul, South Korea

Introduction

The autosomal dominant cerebellar ataxias, autosomal recessive cerebellar ataxias, and hereditary spastic paraplegias (HSP) were traditionally considered as separate clinicogenetic disease entities [1–3]. Each disorder is associated with a large number of disease genes; variants in nearly 40 genes are currently associated with autosomal dominant cerebellar ataxia [2], a further 50 are associated with autosomal recessive cerebellar ataxia [3], and more than 80 genes have been associated with HSP [4].

It is now recognised that ataxias and hereditary spastic paraplegias have common causative genes that result in overlapping phenotypes and disease mechanisms [1]. A Movement Disorders Society task force identified 69 genes causing the 'ataxia-spasticity syndrome' [1] and recommended that they be considered as a spectrum disorder. With the vast causative genetic landscape of spasticity and ataxia syndromes, next-generation sequencing (NGS) technologies may be useful to diagnose unsolved cases by considering them as two extremes of a continuous spectrum.

Three main NGS-based applications currently exist: (i) whole-genome sequencing (WGS), (ii) whole-exome sequencing (WES), and (iii) targeted gene-panel sequencing [5]. Gene-panel sequencing is a commonly used application but requires the correct genes to be considered in a panel. WES can sequence DNA coding for proteins, which accounts for 1% of the entire human genome, and is enriched for pathogenic mutations [6]. However, WGS offers potential advantages over WES including improved ability to detect copy number variants (CNVs), structural variants (SVs), and intronic variants, as well as more uniform coverage [7]. While WGS is more expensive than WES, there is evidence that it provides a higher diagnostic return and can provide future value by serving as a comprehensive dataset that can be interrogated and re-interrogated in the future [8].

Genetic pleiotropy, a phenomenon by which the same mutation can produce different diseases, is another reason to consider WES or WGS over gene-panel sequencing in the diagnosis of inherited spasticity and ataxia. For example, studies have used WES to demonstrate that complex HSP cases show genetic heterogeneity encompassing genes associated with amyotrophic lateral sclerosis, hereditary neuropathy, neurodegeneration with brain iron accumulation, disorders of glycogen metabolism and spasticity-parkinsonism [9, 10]. We recently used WGS in early onset HSP to show mutations in known HSP genes, as well as in genes outside of traditional HSP panels [11].

There are limited studies using WES or WGS to investigate disorders of spasticity and ataxia as a single disorder using a common approach [12]. We report the use of WGS to identify genetic causes of spastic paraplegia with or without ataxia in 18 undiagnosed probands from South Korea. We show that WGS is an effective diagnostic tool to screen many known disease genes in overlapping disease phenotypes.

Patients and Methods

Eighteen patients from unrelated Korean families with undiagnosed spastic paraplegia with or without cerebellar ataxia/spastic ataxia spectrum were enrolled by the Movement Disorder Center at Seoul National University Hospital in Seoul, South Korea. All patients provided written informed consent according to the Declaration of Helsinki. The objectives and procedures of the study were approved by the institutional review board (Seoul National University Hospital: reference number 1601-048-733). Routine investigations were performed in all patients including testing for vitamin B12, vitamin E, ceruloplasmin, serum copper, human immunodeficiency virus, human t-lymphotropic virus and treponema pallidum. We performed brain and cervical spinal cord magnetic resonance imaging (MRI) at 1.5 T or 3 T for all participants. In addition, we tested mutations for *SPAST* and *ATL1* in individuals with a predominant HSP phenotype. Individuals with predominant ataxia phenotypes were further tested for spinocerebellar ataxia (SCA) 1, 2, 3, 6, 7, 8, and 17, as well as dentatorubropallidoluysian atrophy (Supplementary Table 1).

WGS was performed on DNA extracted from blood at the Kinghorn Centre for Clinical Genomics using the Illumina HiSeq X sequencing platform, as previously described [11, 13, 14]. Data analysis was performed using the GATK Best Practices pipeline, as previously reported [15]. Variant prioritisation was performed using Seave [16], by considering variant occurrence in population databases (including gnomAD [17]), medium and high variant impact in keeping with Gemini Database Schema criteria [18], in silico prediction of variant impacts (including SIFT [19] and Polyphen2 [20]), and analysis using VarSome (http://varsome.com) [21]. We initially considered a large set of genes associated with disorders of spasticity and ataxia that we created (Supplementary Table 2). In those individuals that returned a negative outcome for the initial gene set, we also interrogated the Illumina TruSight One Sequencing Panel of 4802 disease-associated genes, which may be considered as a 'clinical exome' [22]. We identified copy number variants (CNVs) and structural variants (SVs) using ClinSV (Minoche et al., manuscript in preparation and as described [7]). We analysed the WGS data using *Introme* (Gayevskiy et al., manuscript in preparation, also see https://github.com/KCCG/introme) to prioritise the intronic variants that are likely to disrupt existing splice sites or create new ones. Classification of variants was performed according to ACMG 2015 criteria [23], with variants falling just short of 'likely pathogenic' considered as 'variants of uncertain significance (VUS)

(favour pathogenic)' [24]. We confirmed candidate variants by Sanger sequencing in new blood samples from the patient and any first-degree relatives to check segregation with disease. Coverage of WGS was compared to WES datasets as previously described [7].

Results

The individuals studied had average age at onset 35.5 (range 5–75), age at examination 45.9 (11–76), and were 13 males and 5 females (Supplementary Table 1). They had 13 ataxia predominant phenotype and 5 spastic paraplegia predominant phenotype.

We identified disease-relevant variants in 38.9% (7/18) of individuals screened. Disease-associated variants were identified in the *ABCD1* ($n = 3$), *CAPN1* ($n = 2$), *NIPA1* ($n = 1$) and *PLA2G6* ($n = 1$) genes (Tables 1 and 2) from our panel, with no further diagnoses detected on analysis of the clinical exome based on genes targeted by the Illumina TruSight One panel, or from testing for CNVs or SVs using ClinSV. One of the variants in *ABCD1* was detected on analysis for intronic variants affecting splicing using *Introme*. We considered as diagnosed two individuals who were compound heterozygous for *CAPN1* variants; this included a VUS-favour pathogenic variant *in trans* with a pathogenic or likely pathogenic variant in both cases.

ABCD1 Variants

A novel variant in ATP-binding cassette sub-family D member 1 gene *ABCD1* (NM_000033.3:c.(644T>C); NP_000024.2 p.(Leu215Pro)) was found in a 58-year-old man (pt.A in Table 2, III-1 in Fig. 1a) presenting with a gait impairment, bladder disturbance and erectile dysfunction since the age of 20 years. His symptoms progressed slowly, and he had a cystostomy and walked with a cane by 46 years of age. His mother had a gait abnormality in her thirties but was not available for assessment. On neurological examination, he had cerebellar dysarthria, upper limb ataxia, mild weakness in both legs, hyperreflexic knee jerks, absent ankle jerks and hypoesthesia to temperature worse in his right leg. His brain and spinal cord MRI showed mild cerebellar atrophy and slightly increased T2 signal in both parieto-occipital white matter (Fig. 2a, b). His initial nerve conduction study (NCS) at the age of 46 was normal, but a follow-up NCS at the age of 58 showed a sensorimotor polyneuropathy. Results from WGS prompted testing of plasma very long-chain fatty acids (VLCFA), which were found to be elevated [C26:0 = 1.608 μmol/L (Ref range 0–1.310), C26:0/C22:0 = 0.099 (Ref range 0–0.023) and C24:0/C22:0 = 1.839 (Ref range 0–1.390], consistent with the diagnosis of adrenomyeloneuropathy. There was no evidence of adrenal involvement on reassessment of this individual to warrant treatment with corticosteroids; however, he was instituted on periodic re-evaluation of adrenocortical function at six monthly intervals as recommended [31].

A woman with a spastic paraparesis phenotype (pt.B in Table 2, Fig. 1b) was found to carry a novel, likely pathogenic variant in *ABCD1* (NM_000033.3:c.(346_348dupGGA), NP_000024.2:p.(Gly116dup). Unfortunately, she was not available for plasma VLCFA testing.

An *ABCD1* variant (NM_000033.3:c.1866-10G>A), previously reported as pathogenic [25, 26], was also identified in a man (pt.C in Table 2, Fig. 1c) with a spastic ataxia phenotype. This intronic variant was initially overlooked due to our primary filters being focused on coding and essential splice site variants but was identified using the *Introme* analysis. This variant was known to be associated with human disease, with previously reported functional analysis demonstrating that it results in the creation of an upstream splice acceptor site that causes abnormal splicing [25, 32]. Results from WGS in this patient also prompted testing of plasma very long-chain fatty acids (VLCFA), which were found to be elevated [C26:0 = 2.101 μmol/L (Ref range 0–1.310), C26:0/C22:0 = 0.092 (Ref range 0–0.023) and C24:0/C22:0 = 2.007 (Ref range 0–1.390], consistent with the diagnosis of adrenomyeloneuropathy. The rapid adrenocorticotropic hormone (ACTH) stimulation test was normal, and he was instituted on monitoring for adrenal dysfunction.

NIPA1 Variant

We identified a variant in the magnesium transporter *NIPA1* gene (*NIPA1*) (NM_144599.4:c.(316G>A); NP_653200.2:p.(Gly106Arg)) that had previously been reported in the literature [27, 28], in a 41-year-old woman with a 25-year history of slow progressive gait disturbance and bladder involvement (pt.D in Table 2, III-3 in Fig. 1d). Neurological examination showed upper limb hyperreflexia, lower limb spasticity and weakness, distal loss of vibration sense, and an ataxic gait. Her brain and spinal cord MRI were normal. Given the variant was absent in both her parents, it was classified as de novo.

CAPN1 Variants

A 25-year-old man (pt.E in Table 2, III-1 in Fig. 1e) presented with a progressive ataxic gait since the age of 19 years old. On examination, he had gaze-evoked nystagmus, slow saccades, cerebellar speech, dysphonia, truncal titubation, dysmetria in both hands, lower limb weakness and spasticity, and a spastic and ataxic gait. His brain MRI showed cerebellar atrophy (Fig. 2c), and brain positron emission tomography (PET) imaging showed mild hypometabolism in bilateral temporal lobes and the cerebellum. He did not respond to treatment with dantrolene, clonazepam, and baclofen. WGS showed novel compound

Table 1 Summary of disease-relevant variants identified on WGS

Individual identified	Family history	Gene, OMIM disease, mode of inheritance	Variant	Previously reported	ACMG variant classification/comments	Relevant gene included in the list of spastic-ataxia genes from Synofzik and Schule [1]
Pt.A	Y	*ABCD1*, ALD, XR	chrX:g.152991365T>C NM_000033.3:c.644T>C NP_000024.2:p.(Leu215Pro)	No	Pathogenic—PS3, PM1, PM2, PP2, PP3	Yes
Pt.B	Y	*ABCD1*, ALD, XR	chrX:g.152991066C>CGGA NM_000033.3:c.346_348dupGGA NP_000024.2:p.(Gly116dup)	No	Likely pathogenic—PM1, PM2, PM4	Yes
Pt.C	Y	*ABCD1*, ALD, XR	chrX:g.153008665G>A NM_000033.3:c.1866-10G>A	Yes [25, 26]	Likely pathogenic—PS3, PM2, PP5, BP4	Yes
Pt.D	N	*NIPA1*, SPG6, AD	chr15:g.23060816C>T (het) NM_144599.4: c.316G>A NP_653200.2:p.(Gly106Arg)	Yes [27, 28]	Pathogenic—PS1, PM2, PP3, PP5	No
Pt.E	N	*CAPN1*, SPG76, AR (CH)	chr11:g.64972259T>G (het) NM_005186.3:c.1271T>G NP_005177.2:p.(Met424Arg)	No	Variant of uncertain significance (favour pathogenic)—PM2, PM3, PP3	Yes
			chr11:g.64977798TGGACCA>T (het) NM_005186.3:c.1943-5_1943delACCAGG	No	Pathogenic—PVS1, PM2, PM4	Yes
Pt.F	Y	*CAPN1*, SPG76, AR (CH)	chr11:g.64953664T>G (het) NM_005186.3:c.614T>G NP_005177.2:p.(Leu205Arg)	No	Variant of uncertain significance (favour pathogenic)—PM2, PM3, PP3	Yes
			chr11:g.64956194C>T (het) NM_005186.3:c.1142C>T NP_005177.2:p.(Ala381Val)	Yes [29]	Likely pathogenic—PS1, PM2, PP3	Yes
Pt.G	N	*PLA2G6*, PLAN, AR (CH)	chr22:g.38541592G>T (het) NM_003560.2:c.278C>A NP_003551.2:p.(Pro93His)	No	Likely pathogenic—PM2, PM3, PP2, PP3	Yes
			chr22:g.38516874T>C (het) NM_003560.2:c.1634A>G NP_003551.2:p.(Lys545Arg)	No, different missense change at this amino acid residue previously determined pathogenic [30]	Likely pathogenic—PM1, PM2, PM5, PP2, PP3, PP5	Yes

SCA spinocerebellar ataxia, *ALD* adrenoleukodystrophy, *SPG* spastic paraplegia, *PLAN* PLA2G6-associated neurodegeneration, *AD* autosomal dominant, *He* heterozygous, *XR* X-linked recessive, *AR* autosomal recessive, *CH* compound heterozygous

Table 2 Summary of clinical and laboratory findings of the individuals with disease relevant variants

Proband	Pt.A	Pt.B	Pt.C	Pt.D	Pt.E	Pt.F	Pt.G
Gene, variant	*ABCD1* c.644T>C, p.(Leu215Pro)	*ABCD1* c.346_348dupGGA, p.(Gly116dup)	*ABCD1* c.1866-10G>A	*NIPA1* c.316G>A. (p.Gly106Arg)	*CAPN1* c.1271T>G, p.(Met424Arg) c.1943-5_1943delACCAGG	*CAPN1* c.614T>G, p.(Leu205Arg) c.1142C>T, p.(Ala381Val)	*PLA2G6* c.278C>A, p.(Pro93His) c.1634A>G, p.(Lys545Arg)
Age (year)	58	68	26	44	27	29	30
Onset (year)	20	59	20	16	19	16	14
Gender	M	F	M	F	M	F	M
Nystagmus	N	N	N	N	Y	N	Y
Dysarthria	N	N	N	N	Y	Y	N
Spasticity	Y	Y	Y	Y	Y	Y	Y
Weakness, leg/arm	Y/N	N/N	Y/N	Y/N	Y/N	Y/Y	Y/Y
Hyperreflexia	Y	Y	Y	Y	Y	Y	Y
Babinski sign	Y	Y	N	Y	Y	Y	Y
Ankle clonus	N	N	N	Y	Y	Y	N
Dysmetria	Y	N	N	N	Y	Y	Y
Ataxic gait	Y	Y	Y	Y	Y	Y	Y
Truncal titubation	N	N	N	N	Y	Y	N
Amyotrophy	N	N	N	N	N	Y	N
Sensory loss	Y	Y	Y	N	N	N	Y
Bladder symptom	Y	N	Y	Y	N	N	N
Other features					Slow saccades	Postural tremor	Epilepsy, cognitive impairment
NCS/SEP/MEP	Abnormal[a]/NA	Normal/normal/abnormal[b]	Normal/abnormal[c]/normal	NA	Normal	NA	NA/abnormal[c]/normal
Fundoscopy	Normal	Normal	Normal	Normal	Normal	Normal	Normal
MRI	Cerebellar atrophy Mild white matter change	Mild white matter change	Normal	Normal	Cerebellar atrophy, thin corpus callosum	Pontocerebellar atrophy, cervical cord atrophy	Cerebellar atrophy, claval hypertrophy, iron deposition in BG

M male, *F* female, *Y* yes, *N* no, *NA* non-available data, *NCS* nerve conduction study, *SEP* somatosensory evoked potential, *MEP* motor evoked potential, *BG* basal ganglia

[a] Sensorimotor polyneuropathy in the right arm and bilateral legs

[b] Central conduction defect in bilateral posterior tibial nerve motor evoked potentials

[c] Central conduction defect in both median and posterior tibial somatosensory evoked potentials

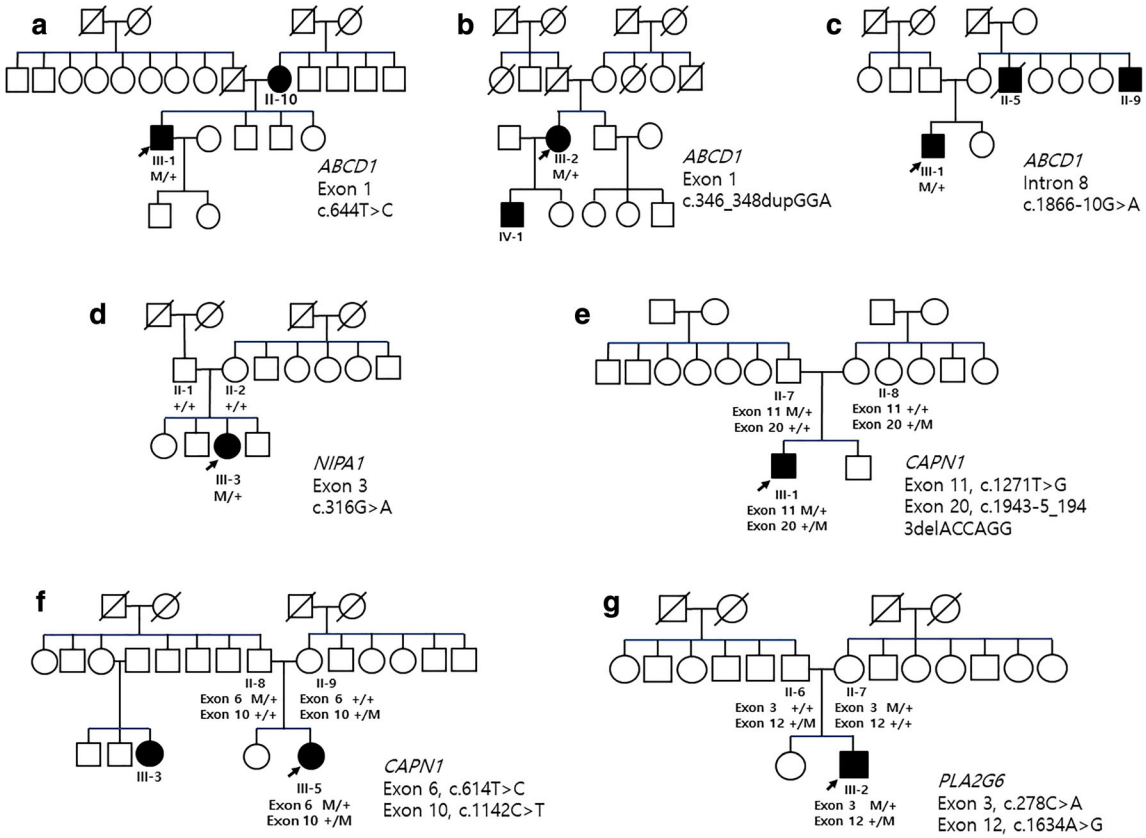

Fig. 1 Pedigrees of the individuals with disease relevant variants. Pedigree charts of pt.A (**a**), pt.B (**b**), pt.C (**c**), pt.D (**d**), pt.E (**e**), pt.F (**f**), and pt.G (**g**)

heterozygous variants in the calpain-1 catalytic subunit gene *CAPN1* (NM_005186.3:c.(1271T>G), NP_005177.2:p.(Met424Arg) and NM_005186.3:c.(1943-5_1943delACCAGG)). The c.1943-5_1943delACCAGG variant would delete the canonical splice acceptor site and the first nucleotide of exon 20, likely resulting in exon

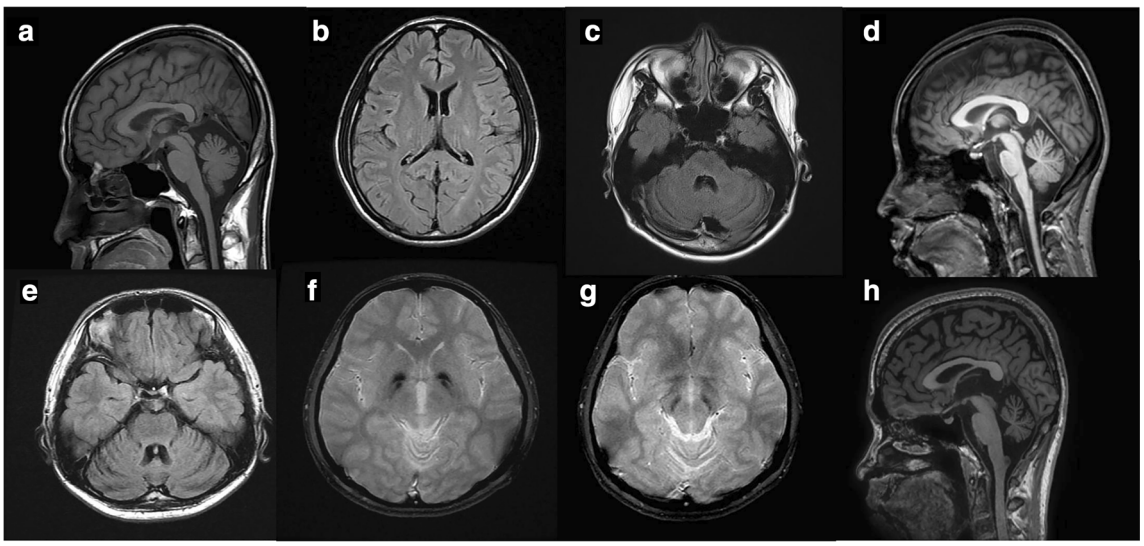

Fig. 2 Neuroimaging findings in individuals with disease relevant variants. Sagittal T1-weighted MR (**a**) shows mild atrophy of cerebellum and cervical cord (Pt.A). Axial FLAIR MRI (**b**) showing slightly increased signal involving the parieto-occipital white matter (Pt.A). Axial T1-weighted MRI (**c**) shows cerebellar atrophy (Pt.E). Sagittal T2-weighted MRI (**d**) shows pontocerebellar atrophy (Pt.F). Axial FLAIR MRI (**e**) shows cerebellar atrophy, and axial GRE T2-weighted images (**f**, **g**) disclose iron deposition involving globus pallidus and substantia nigra (Pt.G). Sagittal T1-weighted MRI (**h**) shows cerebellar atrophy and claval hypertrophy (Pt.G). MRI magnetic resonance imaging, FLAIR fluid-attenuated inversion recovery, GRE gradient recalled echo

skipping or a frameshift and premature truncation of the protein.

Biallelic *CAPN1* variants were also implicated in the phenotype of pt.F (III-5 in Fig. 1f). We identified a missense variant (NM_005186.3:c.(1142C>T), NP_005177.2:p.(Ala381Val)) that has been reported in association with progressive spastic ataxia [29], and a novel missense variant (NM_005186.3:c.(614T>G); NP_005177.2:p.(Leu205Arg)). This individual presented with dysarthria at 13 years of age, developed an ataxic gait at 18 years of age, which was followed by progressive lower limb weakness. She finally became wheelchair dependent in her late twenties. Her cognition was normal. One of her cousins (III-3 in Fig. 1d) in her paternal line had an undiagnosed gait disturbance starting in her twenties but was not available for assessment. Neurological examination revealed cerebellar speech, mild weakness of both arms, distal lower limb amyotrophy, moderate weakness and spasticity in both legs, truncal titubation, limb ataxia, and impairment of vibration sense in the feet. Her brain MRI showed pontocerebellar atrophy (Fig. 2d).

PLA2G6 Variants

A 30-year-old man (pt.G in Table 2, III-2 in Fig. 1g) presented with a slowly progressive gait disturbance, epilepsy, and mild cognitive dysfunction at 14 years of age. Neurological examination showed increased gain on upward smooth pursuit, right and up beating nystagmus after head shaking, and weakness in both arms and legs, especially in the distal lower limbs. There was bilateral lower limb spasticity, hyperreflexic knee jerks and absent ankle jerks. He had upper limb ataxia and impairment of vibration sense and proprioception in the lower limbs. He did not develop features of parkinsonism until his last visit at 30 years of age. WGS analysis identified novel variants in the calcium-dependent phospholipase A2 gene *PLA2G6*, (NM_003560.2:c.(278C>A); NP_003551.2:p.(Pro93His) and (NM_003560.2:c.(1634A>G); NP_003551.2:p.(Lys545Arg)) that were shown to be biallelic. The latter variant affects the site of a previously described amino acid substitution (p.Lys545Thr) that was reported in association with neurodegeneration and brain iron accumulation type 2A (NBIA2A) [30]. This prompted re-evaluation of his brain MRI findings to reveal cerebellar atrophy and iron deposition in bilateral basal ganglia and substantia nigra (Fig. 2f–h), which are typical features of PLA2G6-Associated Neurodegeneration (PLAN) [33].

Variants of Uncertain Significance

We detected VUS in five individuals (Supplementary Table 3). Of note, we detected a heterozygous variant (NM_001080414.3:c.(1391G>A); NP_001073883.2:p.(Arg464His)) in the gene *CCDC88C* (SCA40) in a 36-year-old man with a 2-year history of a progressive cerebellar disorder. This variant was originally reported as a cause of cerebellar ataxia. However, the allele frequency of this variant for East Asians in the gnomAD database (0.003641) was considered too high to be disease-causing for a rare autosomal dominant disorder.

A canonical acceptor splice site variant (NM_014846.3:c.2668-1G>A) was identified in the WASH complex subunit 5 gene *WASHC5* (SPG8) in one individual. Loss of function variants in the gene have been reported to be pathogenic in an autosomal dominant manner [34]. However, the variant was present in the proband's asymptomatic father and was therefore classified as a variant of uncertain significance.

Using ClinSV, we detected a deletion involving the 5′ untranslated region of *NIPA1* in two individuals (pt.1 and pt.E, Supplementary Table 4). This deletion was detected in 1 out of 500 individuals from our Medical Genome Reference Bank control database and 2% of "1000 genomes project" samples, and so was not considered a high impact variant.

Coverage of WGS Compared to WES Datasets

Firstly, we showed that on the splice variant in *ABCD1* (NM_000033.3:c.1866-10G>A) may have been missed on WES, given that it fell below a critical threshold of 15X mapped read depth [35] for one of two WES datasets (Supplementary Table 5). Furthermore, we compared coverage of all pathogenic/likely pathogenic obtained from ClinVar [36] from our HSP and ataxia gene panel between WGS and WES in-house datasets, using methods as previously described [7]. We showed that only a very low number of variants fell below 15 reads for the WGS dataset (reads with coverage < 15: 12 of 29517) in comparison to the WES datasets (reads with coverage < 15: 1861 of 29517 and 2809 of 29517, Supplementary Fig. 1). This suggests that WGS misses far fewer clinically relevant variants in comparison to WES.

Discussion

In this study, we used WGS to detect disease-relevant variants in 38.9% (7/18) of patients. This is a surprisingly high diagnostic rate given common causes of spasticity, and ataxia had been excluded in this previously undiagnosed cohort. Using high-throughput sequencing by considering disorders of spasticity and ataxia as a single entity can help to improve the diagnostic yield. Consistent with this concept, a recent report showed good diagnostic efficacy (29%) when using gene panel sequencing in individuals with ataxia and spasticity disorders [12].

Mutations in *NIPA1* (SPG6, HSP-NIPA1 [37]) typically cause a pure phenotype of HSP that can be complicated by peripheral neuropathy, spinal cord atrophy, epilepsy,

dysarthria, dystonia, and arm involvement [38]. To our knowledge, there are no previous reports of *NIPA1* mutations causing both spasticity and ataxia. Our findings suggest that mutations in this gene should be considered as a cause of spastic ataxia.

CAPN1 mutations have been described independently as a cause of both HSP [39] and ataxia [29] (HSP/ATX-CAPN1 [37]). Our findings are consistent with recent reports highlighting that *CAPN1* variants can cause spastic-ataxia phenotypes [40–42]. We suggest that CAPN1-related disorders should be considered along with genes such as *SPG7* [43, 44], as exemplars of spastic ataxia spectrum disorders.

Mutations in *PLA2G6* were originally described as a cause of dystonia-parkinsonism [45], but the phenotype has now broadened. Our findings support *PLA2G6* as a cause of spastic ataxia spectrum phenotype and are in keeping with recent studies implicating this gene as a cause of HSP, highlighting the broad clinical spectrum of PLA2G6-related disorders [46].

For three individuals, we attributed the spastic-ataxia spectrum phenotype to *ABCD1* variants. For two men (pt.A and pt.C), the finding of an *ABCD1* variant prompted the treating clinician to test for aberrant VLCFs, the findings of which supported a diagnosis of adrenomyeloneuropathy [31]. One of these men (Pt.C) was found to have a known splice site mutation in *ABCD1* detectable on analysis for splice variants. This supports the utility of WGS for detailed interrogation of intronic variants. The genetic findings led to the diagnosis of adrenomyeloneuropathy, which prompted monitoring for adrenal dysfunction which is potentially treatable with corticosteroids. A female carrier of an *ABCD1* variant (patient B) was assessed as having a myelopathy with age-dependent penetrance which is a known manifestation of X-linked adrenoleukodystrophy in women [47]. This individual was not available for VLCFA testing, which is abnormal for plasma or skin fibroblasts in 85% of female carriers [31]. In another individual (pt.G), the findings of biallelic *PLA2G6* variants prompted detailed re-evaluation of the brain MRI, revealing typical radiological features of PLAN. For four individuals (pt.A, pt.B, pt.C, and pt.G), results of WGS investigations prompted clinical re-evaluation that led to a change in diagnosis. In these scenarios, we used the genetic data to direct phenotyping as part of the diagnostic pathway, in what could be termed a 'reverse phenotyping' approach [48]. It can be argued that these disorders should have been suspected and diagnosed with directed genetic testing. However, our findings are in keeping with previous studies using WES which have shown that unanticipated rare disorders are relatively frequent in cerebellar ataxia and spastic paraplegia [49]; this may be particularly relevant for atypical or forme fruste clinical presentations. While routine testing of plasma VCLFA in individuals with HSP may be worthwhile, our experience is that this is not a universal practice; in fact, there are examples in the literature where *ABCD1* variants were detected on exome sequencing rather than by VCLFA testing [50]. Furthermore, we suggest that the MRI findings of NBIA may be easily overlooked unless clinically suspected.

To date, it has been difficult to select the best approach for genetic diagnosis of inherited disorders of spasticity and ataxia. It is clinically challenging to distinguish between these disorders, which have previously been investigated with distinct gene panels. There are a large number of possible disease genes involved, and there has been a high rate of gene discovery, making this a diagnostic challenge [43]. In this study, we identified a variant in a gene not included in a list of 69 genes considered relevant to ataxia-spasticity [1]. This highlights the difficulty of tailoring inclusive gene lists to detect unanticipated findings and emphasises the limited indication of restrictive gene lists. We did not identify any pathogenic CNV or SV in known disease genes in this cohort; however, this does not exclude the possibility of detecting these mutations in a larger sample. Furthermore, CNVs/SVs are known to be important in these disorders; for example, there is a high frequency of deletions in *SPAST* for autosomal dominant HSP [51]. WGS allowed us to interrogate non-coding regions of the genome, identifying a known splice variant leading to an additional diagnosis. In total, there remain 11/18 cases without an identifiable cause (11/18). This may be due to variants in genes not currently implicated in human disease, variants that we currently cannot interpret, or repeat expansion disorders that we did not test for (e.g., SCA8). Our study supports investigating spastic-ataxia disorders using WGS to exploit comprehensive consideration of the large panel of genes associated with these overlapping disorders. The major limitation of the study is the small sample size with only one intronic disease-relevant variant detected. Arguably all other cases can be potentially picked up by WES with good coverage; however, our coverage data suggests that WGS is more sensitive than WES for detecting disease-relevant variants. While WGS is expensive, we anticipate that the costs will fall over time and that the benefits of this approach are yet to be fully exploited—this could be further explored by a cost-effectiveness study.

Conclusion

Inherited disorders of spasticity and ataxia have overlapping genes and phenotypes. Individuals with both spasticity and ataxia frequently present to the clinic and pose a diagnostic challenge to clinicians. Our study supports taking a unified approach to genetic diagnosis, considering these conditions on a spectrum and investigating genetic variation using a genome-scale approach, such as WGS.

Acknowledgements DNA for this study was provided by the Seoul National University Hospital Human Biobank, a member of the National Biobank of Korea, which is supported by the Ministry of

Health and Welfare. All samples derived from the National Biobank of Korea were obtained with informed consent under institutional review board-approved protocols. We would like to thank Dr. Sarah Kummerfeld, Garvan Institute of Medical Research, for reviewing the manuscript.

Funding Whole-genome sequencing was funded by a University of Sydney Kickstarter Grant awarded to KRK. RLD and MJC are supported by Early-Mid Career New South Wales Health Fellowships. CMS is supported by a National Health and Medical Research Council Practitioner Fellowship.

Compliance with Ethical Standards

All patients provided written informed consent according to the Declaration of Helsinki. The objectives and procedures of the study were approved by the institutional review board (Seoul National University Hospital: reference number 1601-048-733).

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This article does not contain any studies with animals performed by any of the authors.

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