Journal of Human Genetics (2017) 62, 599–604

www.nature.com/jhg

ORIGINAL ARTICLE

Clinical diversity caused by novel *IGHMBP2* variants

Jun-Hui Yuan[1], Akihiro Hashiguchi[1], Akiko Yoshimura[1], Hiroshi Yaguchi[2], Koji Tsuzaki[3], Azusa Ikeda[4], Kenji Wada-Isoe[5], Masahiro Ando[1], Tomonori Nakamura[1], Yujiro Higuchi[1], Yu Hiramatsu[1], Yuji Okamoto[1] and Hiroshi Takashima[1]

Immunoglobulin helicase μ-binding protein 2 (*IGHMBP2*) gene is responsible for Charcot–Marie–Tooth disease (CMT) type 2S and spinal muscular atrophy with respiratory distress type 1 (SMARD1). From June 2014 to December 2015, we collected 408 cases, who referred to our genetic laboratory for genetic analysis, suspected with CMT disease or other inherited peripheral neuropathies (IPNs) on the basis of clinical manifestations and electrophysiological studies. Mutation screening was performed using Ion AmpliSeq Custom Panels, which comprise 72 disease-causing or candidate genes of IPNs. We identified novel homozygous or compound heterozygous variants of *IGHMBP2* in four patients. Three patients presented with childhood-onset axonal predominant sensorimotor polyneuropathies, whereas the other case was diagnosed with SMARD1, manifesting as low birth weight, weak cry, reduced spontaneous movement and developed respiratory distress 4 months after birth. We present the original report of CMT type 2S in Japan, and illustrate that recessive *IGHMBP2* variants account for ~ 1.6% of axonal CMT in our cohort.
Journal of Human Genetics (2017) **62,** 599–604; doi:10.1038/jhg.2017.15; published online 9 March 2017

INTRODUCTION

Charcot–Marie–Tooth (CMT) disease, the most common inherited peripheral neuropathy (IPN), consists of two major subtypes, demyelinating (CMT1) and axonal (CMT2), which can be distinguished by electrophysiological and nerve biopsy studies. CMT is typically characterized by progressive motor and sensory polyneuropathy, but may also present with significant clinical heterogeneity. Certain CMT disease-causing genes may also produce distinct IPN phenotypes, where they are, therefore, referred as separate disease entities. Distal hereditary motor neuropathy (dHMN), also known as distal spinal muscular atrophy (dSMA) predominantly affecting lower motor neurons, represents a typical IPN phenotype. To date, several genes have been linked to both CMT and dHMN/dSMA, comprising *GARS* (CMT 2D and dHMN 5A),[1] *AARS* (dHMN and CMT 2N),[2,3] *HSPB1* (CMT 2F and dHMN 2B),[4] *HSPB8* (dHMN 2A and CMT 2L),[5,6] *BSCL2* (dHMN 5A and CMT2),[7,8] *DNAJB2* (dHMN and CMT 2T),[9,10] *TRPV4* (dHMN 8 and CMT 2C),[11] *DYNC1H1* (CMT 2O and SMA with lower extremity predominance)[12,13] and *PLEKHG5* (dSMA 4 and intermediate CMT C).[14,15] Most recently, immunoglobulin helicase μ-binding protein 2 (*IGHMBP2*), a gene that has been classically linked to SMA with respiratory distress type 1 (SMARD1) as early as 2001,[16] was demonstrated as a novel causative gene of autosomal recessive axonal CMT (CMT2S).[17]

SMARD1 is a rare fatal disorder typically characterized by the development of respiratory failure within the first 6 weeks to 6 months of life, caused by diaphragmatic paralysis, and a severe infantile axonal neuropathy.[18] On the contrary, CMT2S patients harboring *IGHMBP2* mutations invariably develop childhood-onset axonal motor and sensory abnormalities with chronic progression.[17] In two multinational studies, *IGHMBP2* gene mutations reportedly account for 12.9% (11/85) of likely recessive CMT2 cases and 5.3% (3/57) of unresolved neuropathies.[17,19] However, no patient with *IGHMBP2* gene-related CMT has been reported in Japan. In the present study, using a next-generation sequencing system, we performed targeted resequencing in a large cohort of patients with CMT or other IPNs, and identified novel recessive *IGHMBP2* variants in four patients with CMT2S or SMARD1.

MATERIALS AND METHODS

Prescreening for duplication/deletion mutations of *PMP22* using fluorescence *in situ* hybridization, which is the only commercially available method in Japan, was negative for all cases with suspected CMT1 enrolled in the present study. From June 2014 to December 2015, we identified a cohort of 408 patients collected from all regions of Japan, who were clinically diagnosed with CMT (382 cases), dHMN (16 cases), hereditary sensory and autonomic neuropathy (6 cases) or hereditary neuropathy with liability to pressure palsies (HNPP; 4 cases), and referred to our genetic laboratory. Of these patients, eight were foreign residues of Japan. On the basis of electrophysiological analysis, the CMT patients were further classified into 257 axonal cases (median motor conduction velocity $\geqslant 38\ m\ s^{-1}$), 94 demyelinating cases (median motor conduction velocity $<38\ m\ s^{-1}$) and 31 unclassified cases

[1]Department of Neurology and Geriatrics, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima City, Japan; [2]Department of Neurology, The Jikei University Kashiwa Hospital, Chiba, Japan; [3]Department of Neurology, Kansai Electric Power Hospital, Osaka, Japan; [4]Kanagawa Children's Medical Center, Yokohama, Japan and [5]Division of Neurology, Department of Brain and Neurosciences, Faculty of Medicine, Tottori University, Yonago, Japan
Correspondence: Dr H Takashima, Department of Neurology and Geriatrics, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima City, Kagoshima 890-8520, Japan.
E-mail: thiroshi@m3.kufm.kagoshima-u.ac.jp
Received 17 November 2016; revised 8 January 2017; accepted 12 January 2017; published online 9 March 2017

Table 1 Clinical and genetic features of four patients

	Case 1	*Case 2*	*Case 3*	*Case 4*
Mutation	c.1034C>A; c.1783C>T	c.344C>T; c.1195G>A; c.1060+5G>C	c.826C>T; c.1702C>T	c.2759A>G
Protein	p.Ala345Glu; p.Arg595Trp	p.Thr115Met; p.Gly399Ser; splicing	p.Gln276*; p.Gln568*	p.Tyr920Cys
Parental consanguinity	–	–	–	+
Sex	Female	Male	Male	Male
Current age (years)	22	22	0.5	66
Age at onset (years)	4	13	0	Childhood
Primary symptom	Equinovarus foot	Difficulty of running	Low birth weight	Running slow
Muscle weakness (MMT)	Upper distal: 3; lower distal: 1~2	Proximal lower extremity: 4~5; distal lower extremity: 3~4+	Weak crying; poor head stability; rare spontaneous movement of legs	Lower distal: 0; steppage gait; flat foot
Muscle atrophy	–	Legs (left predominant)	/	Intrinsic hand muscle and legs
Pain sensation	Markedly decreased	Decreased	/	Decreased
Vibration sensation	Decreased	Decreased	/	Normal
Deep tendon reflexes	Decreased~absent	Decreased	Absent	Decreased~absent
Respiratory dysfunction	–	–	+	–
Autonomic neuropathy	–	–	/	Urine remaining

Abbreviations: MMT, manual muscle testing; –, negative; /, no record; +, positive.

(no data). In terms of inheritance pattern, the CMT patients could be grouped to likely autosomal dominant (113 cases), likely AR (35 cases), sporadic (221 cases) and X-link/unknown (13 cases).

The protocol of the present study was reviewed and approved by the Institutional Review Board of Kagoshima University. All patients and six family members provided written informed consents to participate in this study.

DNA extraction and targeted resequencing study

Genomic DNA of patients and family members was extracted from peripheral blood using QIAGEN Puregene Core Kit C (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol.

An Ion AmpliSeq Neuropathy-related gene Panel was designed online using Ion AmpliSeq Designer 3.6 (http://www.ampliseq.com/) to analyze the CDSs (±25 bp of intronic flanking regions) of 72 IPNs disease-causing or candidate genes. The final custom panel was composed of 1800 amplicons divided into two primer pools for a total of 338.29 kb of DNA. The panel covered 98.99% of the regions of interest.

For genomic DNA application, 10 ng samples were amplified using Ion AmpliSeq HiFi Master Mix (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) and Ion AmpliSeq Neuropathy-related gene Panel primer pools. Using the Ion AmpliSeq Library Kit v.2.0 and Ion Xpress Barcode Adapters 1–96 Kit (Life Technologies), libraries were prepared according to the manufacturer's instructions. Adaptor-ligated amplicon libraries were purified using the Agencourt AMPure XP System (Beckman Coulter Genomics, Danvers, MA, USA). A Qubit2.0 Fluorometer (Invitrogen, Life Technologies) was used to quantify the libraries by means of Qubit dsDNA HS Assay Kits (Invitrogen, Life Technologies, Eugene, OR, USA), and equimolar amounts of each library were pooled together and run on Ion Chef (Life Technologies) for emulsion PCR and loading onto a PI v2 chip (Thermo Fisher Scientifics, Life Technologies, Waltham, MA, USA). This chip was run on the Ion Proton sequencing system according to the manufacturer's procedures.

Data management and variant analysis

Using Torrent Suite Software 5.0 (Life Technologies), reads were aligned to the human reference genome (build GRCh37/hg19) and to the BED file designed using Ion AmpliSeq Designer (https://ampliseq.com/browse.action). DNA variants were called with Torrent Variant Caller plug-in software (Life Technologies). Read depth and the percentage of reads mapped to the target region (reads on target) were calculated using Coverage Analysis plug-in software (Life Technologies). The VCF file was annotated and filtered using CLC Genomics Workbench (Qiagen, Aarhus, Denmark). All variants were visually verified with the Integrative Genomics Viewer 2.3 and classified according to ACMG-AMP standards and guidelines.[20]

Variants were checked against the public databases, including Exome Sequencing Project, 1000 genomes project and Exome Aggregation Consortium database, which was referred to as a moderate evidence of pathogenicity (PM2). We also checked variants in non-disease population databases, comprising Human Genetic Variation Database (whole-exome sequencing variants of 1208 Japanese individuals) and Integrative Japanese Genome Variation Database (whole-genome sequencing variants of 2049 Japanese individuals). This result was attributed to a moderate level of strong evidence of pathogenicity (PS4-moderate). The pathogenicity prediction programs, PolyPhen2, SIFT, PROVEAN and MutationTaster, were used to evaluate variants not previously described (supporting evidence of pathogenicity, PP3). Candidate variants were validated by Sanger sequencing. Segregation studies of available family members were performed whenever possible (PM3).

RESULTS

Clinical features

Of the 408 unrelated patients, the Ion AmpliSeq Neuropathy-related gene Panel screening identified homozygous or compound heterozygous *IGHMBP2* variants in four patients. Major clinical features and genetic findings are summarized in Table 1. Results of nerve conduction studies (NCS) are listed in Table 2.

Case 1. A 22-year-old female, the second child of a non-consanguineous family, was born with an unremarkable perinatal history. No neurological symptoms were observed in other family members. She was able to walk independently from 1 year of age, whereas bilateral talipes equinovarus became apparent at 4 years of age. Ankle foot orthosis developed at 12 years of age. Cranial nerve examination and proximal muscle strength were normal, whereas distal muscle weakness was identified on manual muscle testing (MMT; normal, 5; upper extremities, 3; lower extremities, 1~2). Superficial pain and vibration sensation were decreased in the distal limbs. Deep tendon reflexes were markedly decreased in the upper limbs and absent in the lower limbs. No autonomic dysfunction was noted. NCS revealed

Table 2 Nerve conduction study of four patients

	Case 1			*Case 2*			*Case 3*			*Case 4*		
	Lat (ms)	*Amp (mv μv^{-1})*	*Vel (m s^{-1})*	*Lat (ms)*	*Amp (mv μv^{-1})*	*Vel (m s^{-1})*	*Lat (ms)*	*Amp (mv μv^{-1})*	*Vel (m s^{-1})*	*Lat (ms)*	*Amp (mv μv^{-1})*	*Vel (m s^{-1})*
Median nerve												
Motor (L)	/	/	/	4.5↑	5.5	42.0↓	3.8	1.2↓	26.2↓	/	5.1	39.8↓
Motor (R)	4.3	2.9↓	45.6↓	4.5↑	7.0	42.0↓	/	/	/	/	7.2	45.4↓
Sensory (L)	/	/	/	2.7	6.2↓	44.0↓	NR	NR	NR	/	/	57.4
Sensory (R)	NR	NR	NR	2.8	5.0↓	50.0	/	/	/	/	/	56.4
Ulnar nerve												
Motor (L)	/	/	/	3.1	7.5	55.0	3.0	1.1↓	25.1↓	/	/	/
Motor (R)	3.5	12.0	44.7↓	2.9	6.0↓	47.0↓	/	/	/	/	/	/
Sensory (L)	/	/	/	2.4	3.8↓	44.0↓	/	/	/	/	/	/
Sensory (R)	NR	NR	NR	2.5	0.9↓	37.0↓	/	/	/	/	/	/
Tibial nerve												
Motor (L)	/	/	/	NR	NR	NR	NR	NR	NR	NR	NR	NR
Motor (R)	NR	NR	NR	NR	NR	NR	/	/	/	NR	NR	NR
Sural nerve (L/R)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Abbreviations: Amp, amplitude; L, left; Lat, latency; /, not tested; NR, no response; R, right; Vel, velocity.

sensorimotor axonal polyneuropathy, particularly in the lower limbs. Sensory NCS revealed no response in the upper and lower limbs.

Case 2. This case was a 22-year-old, male, Chinese student. No consanguinity was reported between his parents; however, a similar foot deformity was observed in his mother. No concerns were recorded regarding his perinatal period and early development. He experienced difficulty running at 13 years old, and developed slowly progressive atrophy and weakness of the left leg at 17 years of age. Physical examination revealed bilateral hollow foot and brown pigmentation on the inner aspect of both hallexes. Muscle strength of the lower distal limbs was generally decreased to MMT 3~4+, with some involvement of the proximal muscles (gluteus maximus, 4+; quadratus femoris, 4; hamstring muscle, 4) and a positive Gower's sign. Lower extremity atrophy was also observed, particularly affecting the lower left leg. Pain and vibration sensation were decreased in both legs. Despite the absence of significant abnormalities during the heel-knee test, he was unable to heel-to-toe walk. No remarkable autonomic nervous dysfunction was observed. Deep tendon reflexes were preserved except the Achilles tendon reflex. Sural nerve biopsy revealed loss of large myelinated nerve fibers. Electrocardiography demonstrated normal sinus rhythm (65 beats per min) with early repolarization. Plain chest radiography was normal. NCS demonstrated severe axonal sensorimotor neuropathy of all extremities.

Case 3. This was a 6-month-old male who was the second child to healthy and unrelated parents. Intrauterine growth restriction was noted from 36 weeks gestation, and he was born at 39 weeks of gestation with a birth weight of 2156 g and an Apgar score of 6. Mediastinal emphysema was detected, and he was hospitalized for 16 days after birth. Newborn screening tests using tandem mass spectrometry and ALGO hearing screening were negative. After discharge, holotonia and weak cry were noted by his parents. At the age of 4 months, the patient was admitted to hospital owing to respiratory distress resulting from hand foot and mouth disease. Physical examination revealed weak crying, high-arched palate, head-lag, poor head stability and universal areflexia. Spontaneous movements of the upper limbs were observed; however, the legs were observed in a 'frog-leg' position. His emotional reactions and nonverbal communication were normal. He was subsequently intubated and mechanical ventilation was initiated. Thereafter, although extubation was completed, noninvasive positive pressure ventilation and pulmonary physiotherapy were performed because of his weak breathing. NCS was performed at the age of 6 months indicating severe axonal sensorimotor polyneuropathy, with no response observed in any tested sensory nerve.

Case 4. The patient was a 66-year-old male who was the second child of three siblings from a consanguineous family. He initially developed normally, before being noted to be running slower than other children in primary school. Later, gait disturbance was observed as a high school student. He reported no history of respiratory problems. Neurologic examination revealed no cervical nerve abnormalities and marked wasting of his intrinsic hand and lower extremities with an 'inverted champagne bottle' appearance. Steppage gait and bilateral flat feet were noted, whereas knee and Achilles tendon reflexes were negative. Distal hypotonus and mild stocking-type hypesthesia were identified, whereas vibration and position sensation were preserved. In addition, he also suffered incomplete urinary voiding. CT muscle scans demonstrated atrophy of the upper distal flexor muscle (right predominant) and generalized lower limb muscle atrophy with fatty degeneration. NCS demonstrated axonal predominant changes, particularly in the lower limbs. Muscle atrophy was also observed in lower limbs of his younger brother and developmental abnormalities in his elder sister. One of his father's sisters had been diagnosed with amyotrophic lateral sclerosis (ALS) and died at the age of 82 years in her own home.

Targeted resequencing

Among these cases, an average of 1,310,484 reads were obtained and mapped to the human genome reference 19 with 98.6% within the target region. Average coverage depth in the target region was 639.2 and 97.9% of regions had over 20-fold reads.

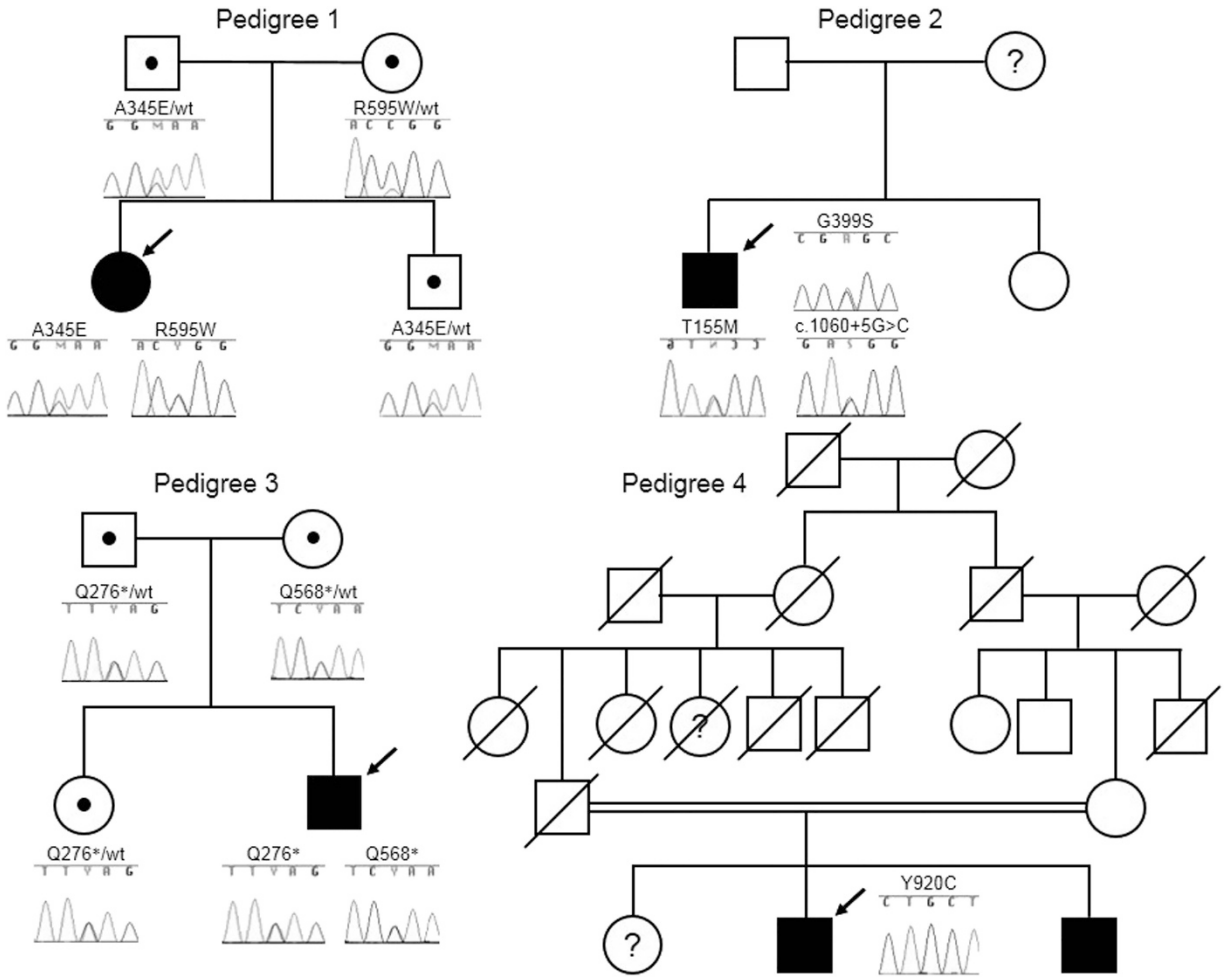

Figure 1 Segregation analysis and sequencing chromatogram of four pedigrees. Variants of case 1 (A345E and R595W) and case 3 (Q276* and Q568*) are inherited from their parents, respectively. Arrow: index case. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Within the *IGHMBP2* gene, we detected compound heterozygous variants in case 1, p.Ala345Glu and p.Arg595Trp (allele frequency 1.648e − 05 in Exome Aggregation Consortium) (PM2), both of which are absent in population database (PS4-moderate) and inherited from her father and mother, respectively (PM3). These variants were predicted to be disease causing using multiple programs (PP3), and were interpreted as likely pathogenic (3 moderate AND 1 supporting). In case 2, we identified three variants, comprising p.Thr115Met (8.653e − 4 in Exome Aggregation Consortium and 0.005 in Human Genetic Variation Database), p.Gly399Ser and c.1060+5G > C. The p.Thr115Met (pathogenic) has previously been reported twice in Chinese patients with the CMT2 phenotype,[21,22] whereas the other two novel variants were found to be disease causing or potentially responsible for splicing abnormalities with the use of prediction tools (PP3). Unfortunately, we were unable to obtain RNA samples or perform segregation analysis in this patient; thus, both variants were grouped of unknown significance (VUS; 2 moderate AND 1 supporting). We found two novel heterozygous nonsense variants in case 3, p.Gln276* and p.Gln568*, which were inherited from his father and mother, respectively, and were classified as pathogenic (1 very strong evidence AND 3 moderate). In case 4 from a consanguineous family, we identified a novel homozygous p.Tyr920Cys variant, which was found fulfilled the criteria of likely pathogenic (2 moderate AND 2 supporting) (Figure 1).

DISCUSSION

Using a targeted resequencing system, we screened 72 disease-causing and candidate genes in a cohort of 408 IPN patients, containing 257 cases suspected with axonal CMT, and identified recessive *IGHMBP2* variants in four patients with significant clinical diversity. Subsequently, we attempted to interpret these sequence variants according to ACMG-AMP guidelines.

IGHMBP2 encodes a ubiquitously expressed DNA/RNA helicase within the SF1 superfamily, encompassing a putative DNA helicase domain (amino acids 19–641) containing a DEAD/H box helicase and an AAA ATPase DEXDc region, a single-stranded nucleic acid-binding R3H motif (amino acids 726–784), and an AN1-like zinc-finger region (amino acid 897–940).[23,24] In previous studies of patients with SMARD1 or CMT2S, mutations of *IGHMBP2* have been found to be distributed through the whole protein except the R3H motif, and predominantly located in the helicase domain. Although case 3 with SMARD1 is the only case found to harbor nonsense mutations in the present study, there is no evidence that patients with SMARD1 or CMT2S are distinguishable at the genetic level, whereas a negative

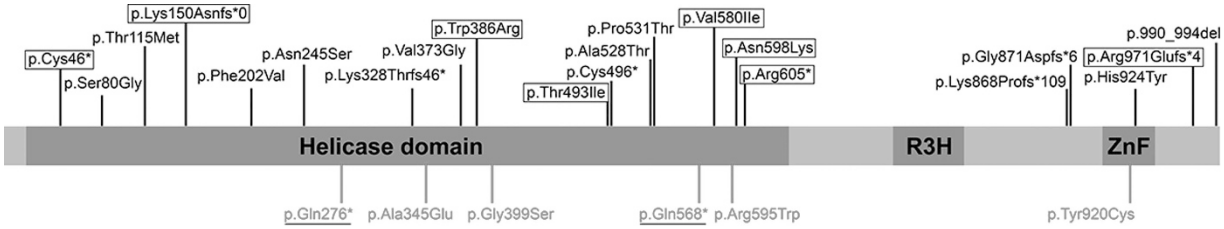

Figure 2 Schematic diagram of Immunoglobulin helicase μ-binding protein 2 (IGHMBP2) protein and variants. All reported *IGHMBP2* mutations leading to CMT2S are shown above the protein, whereas eight mutations could also result in spinal muscular atrophy with respiratory distress type 1 (SMARD1) phenotype (highlighted in boxes). All variants detected in this study associated with CMT2S or SMARD1 (underlined) are indicated under the protein, except the splice site variant with unknown significance, c.1060+5G>C. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

correlation between mutations at the protein level and clinical severity remains controversial.[17,19]

IGHMBP2 protein is involved in the regulation of pre-mRNA processing and transcription.[25] Interestingly, these functions are comparable to those of *GARS*, *AARS*, *HSPB1* and *HSPB8*, all of which were linked to both axonal CMT and dHMN phenotype.[1–6] In addition, senataxin, which contains a DNA/RNA helicase domain with strong homology to IGHMBP2, have been shown to be responsible for ataxia-oculomotor apraxia type 2 and ALS type 4.[26,27] Taken together, dysfunction of RNA processing pathways may selectively affect the peripheral nerve (axonal predominant), lower motor neuron or central nervous systems, and accordingly contribute to phenotypic variability. Future studies aimed at identifying factors that contribute to this selective susceptibility may further our understanding of the interaction between genotype and phenotype.

To date, 21 recessive *IGHMBP2* mutations have been reported in 17 pedigrees of CMT2S out of Japan.[17,19,21,28,29] Among these, eight mutations were also linked to the SMARD1 phenotype (Figure 2). In the present study, we identified novel compound heterozygous or homozygous *IGHMBP2* variants in the other three patients. These patients presented with childhood-onset sensorimotor polyneuropathy, manifesting as distal predominant muscle weakness and general hyporeflexia, without any respiratory dysfunction. Generally, respiratory involvement is unusual in adult-onset CMT but typically observed in patients with motor neuron disease, particularly ALS. It is noteworthy that an aunt of case 4 was clinically diagnosed with ALS; however, further clinical information or genetic studies were not performed because of her death. All three patients presented with axonal predominant sensorimotor polyneuropathy, which was comparable to the previously described patients with CMT2S. Among the variants in *IGHMBP2*, p.Thr115Met, as a rare variant with highest frequency in East Asia, has previously been linked to CMT2S, and our patient (case 2) is the third Chinese case associated with this *IGHMBP2* mutation. Meanwhile, we also detected two other VUS, a substitution from glycine to serine at highly conserved position of 399 and a possible splice donor site variant (c.1060+5G>C), which may result in aberrant splicing. Although pathogenicity prediction *in silico* tools indicated that both of the variants were deleterious, it is likely that only one of the identified VUS is disease causing.

SMARD1, also known as dSMA1 or dHMN6/HMN6, is a fatal form of infantile motor neuron disease. Affected patients usually die within 13 months of life, and only a small number of studies have reported patients presenting with milder phenotypes and surviving longer.[16,30,31] Case 3, harboring two compound heterozygous nonsense mutations, presented with a typical SMARD1 phenotype, including low birth weight, weak cry, predominant distal lower limb muscle weakness, areflexia and marked reduction of motor and sensory potential amplitudes. He developed respiratory distress at months of age and underwent intubation. Although extubation was completed thereafter, persistent respiratory support treatment remained necessary. While no significant autonomic dysfunction was recorded, caution should also be paid to cardiac function as cardiac arrhythmias have been reported in SMARD1 patients may be a potential lethal factor.[32–34] Recently, another gene, *LAS1L*, has been linked to the SMARD phenotype, and should be considered in *IGHMBP2* mutation-negative patients.[35]

It is noteworthy that genomic rearrangement mutations in *IGHMBP2* have been reported in either SMARD1 or CMT2S patients.[17,36,37] In the present study, we also detected multiple single heterozygous variants in other CMT patients (data not shown). Mutations in the other allele, such as large deletions, may have been overlooked because of limitations in sequencing techniques, regardless of target resequencing or Sanger sequencing. In this situation, multiplex ligation dependent probe amplification may be beneficial and has already been applied in previous studies of *IGHMBP2*.[34,37]

In conclusion, we present an original report of CMT2S patients caused by *IGHMBP2* variants in Japan. In our cohort, recessive *IGHMBP2* variants account for ~1.6% (4/257) of axonal CMT patients, the frequency of which appears lower than those of previous reports. In view of the significant clinical diversity observed, identification of potential genetic factors becomes essential in determining patient phenotypes. Moreover, further research is required to transform knowledge of contributory factors into therapeutic methods to ameliorate SMARD1 phenotypes and thus improve patient quality of life.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the patients and their family members for participation in the study. We thank Aya Ebina and Tomoko Onishi of our department for her excellent technical assistance, and Enago (http: //www.enago.jp) for the English language review. This study was supported, in part, by grants from research on the Nervous and Mental Disorders and Research Committee for Charcot–Marie–Tooth Disease, Neuropathy, Ataxic Disease and Applying Health and Technology of Ministry of Health, Welfare and Labour, Japan. This research is also supported by the research program for conquering intractable disease from Japan Agency for Medical Research and Development (AMED).

1 Antonellis, A., Ellsworth, R. E., Sambuughin, N., Puls, I., Abel, A., Lee-Lin, S. Q. *et al.* Glycyl tRNA synthetase mutations in Charcot–Marie–Tooth disease type 2D and distal spinal muscular atrophy type V. *Am. J. Hum. Genet.* **72**, 1293–1299 (2003).

2 Latour, P., Thauvin-Robinet, C., Baudelet-Mery, C., Soichot, P., Cusin, V., Faivre, L. *et al.* A major determinant for binding and aminoacylation of tRNA(Ala) in cytoplasmic alanyl-tRNA synthetase is mutated in dominant axonal Charcot–Marie–Tooth disease. *Am. J. Hum. Genet.* **86**, 77–82 (2010).
3 Zhao, Z., Hashiguchi, A., Hu, J., Sakiyama, Y., Okamoto, Y., Tokunaga, S. *et al.* Alanyl-tRNA synthetase mutation in a family with dominant distal hereditary motor neuropathy. *Neurology* **78**, 1644–1649 (2012).
4 Evgrafov, O. V., Mersiyanova, I., Irobi, J., Van Den Bosch, L., Dierick, I., Leung, C. L. *et al.* Mutant small heat-shock protein 27 causes axonal Charcot–Marie–Tooth disease and distal hereditary motor neuropathy. *Nat. Genet.* **36**, 602–606 (2004).
5 Irobi, J., Van Impe, K., Seeman, P., Jordanova, A., Dierick, I., Verpoorten, N. *et al.* Hot-spot residue in small heat-shock protein 22 causes distal motor neuropathy. *Nat. Genet.* **36**, 597–601 (2004).
6 Tang, B. S., Luo, W., Xia, K., Xiao, J. F., Jiang, H., Shen, L. *et al.* A new locus for autosomal dominant Charcot–Marie–Tooth disease type 2 (CMT2L) maps to chromosome 12q24. *Hum. Genet.* **114**, 527–533 (2004).
7 Windpassinger, C., Auer-Grumbach, M., Irobi, J., Patel, H., Petek, E., Horl, G. *et al.* Heterozygous missense mutations in BSCL2 are associated with distal hereditary motor neuropathy and Silver syndrome. *Nat. Genet.* **36**, 271–276 (2004).
8 Choi, B. O., Park, M. H., Chung, K. W., Woo, H. M., Koo, H., Chung, H. K. *et al.* Clinical and histopathological study of Charcot–Marie–Tooth neuropathy with a novel S90W mutation in BSCL2. *Neurogenetics* **14**, 35–42 (2013).
9 Blumen, S. C., Astord, S., Robin, V., Vignaud, L., Toumi, N., Cieslik, A. *et al.* A rare recessive distal hereditary motor neuropathy with HSJ1 chaperone mutation. *Ann. Neurol.* **71**, 509–519 (2012).
10 Gess, B., Auer-Grumbach, M., Schirmacher, A., Strom, T., Zitzelsberger, M., Rudnik-Schoneborn, S. *et al.* HSJ1-related hereditary neuropathies: novel mutations and extended clinical spectrum. *Neurology* **83**, 1726–1732 (2014).
11 Auer-Grumbach, M., Olschewski, A., Papic, L., Kremer, H., McEntagart, M. E., Uhrig, S. *et al.* Alterations in the ankyrin domain of TRPV4 cause congenital distal SMA, scapuloperoneal SMA and HMSN2C. *Nat. Genet.* **42**, 160–164 (2010).
12 Weedon, M. N., Hastings, R., Caswell, R., Xie, W., Paszkiewicz, K., Antoniadi, T. *et al.* Exome sequencing identifies a DYNC1H1 mutation in a large pedigree with dominant axonal Charcot–Marie–Tooth disease. *Am. J. Hum. Genet.* **89**, 308–312 (2011).
13 Harms, M. B., Ori-McKenney, K. M., Scoto, M., Tuck, E. P., Bell, S., Ma, D. *et al.* Mutations in the tail domain of DYNC1H1 cause dominant spinal muscular atrophy. *Neurology* **78**, 1714–1720 (2012).
14 Maystadt, I., Rezsohazy, R., Barkats, M., Duque, S., Vannuffel, P., Remacle, S. *et al.* The nuclear factor kappaB-activator gene PLEKHG5 is mutated in a form of autosomal recessive lower motor neuron disease with childhood onset. *Am. J. Hum. Genet.* **81**, 67–76 (2007).
15 Azzedine, H., Zavadakova, P., Plante-Bordeneuve, V., Vaz Pato, M., Pinto, N., Bartesaghi, L. *et al.* PLEKHG5 deficiency leads to an intermediate form of autosomal-recessive Charcot–Marie–Tooth disease. *Hum. Mol. Genet.* **22**, 4224–4232 (2013).
16 Grohmann, K., Schuelke, M., Diers, A., Hoffmann, K., Lucke, B., Adams, C. *et al.* Mutations in the gene encoding immunoglobulin mu-binding protein 2 cause spinal muscular atrophy with respiratory distress type 1. *Nat. Genet.* **29**, 75–77 (2001).
17 Cottenie, E., Kochanski, A., Jordanova, A., Bansagi, B., Zimon, M., Horga, A. *et al.* Truncating and missense mutations in IGHMBP2 cause Charcot–Marie–Tooth disease type 2. *Am. J. Hum. Genet.* **95**, 590–601 (2014).
18 Guenther, U. P., Varon, R., Schlicke, M., Dutrannoy, V., Volk, A., Hubner, C. *et al.* Clinical and mutational profile in spinal muscular atrophy with respiratory distress (SMARD): defining novel phenotypes through hierarchical cluster analysis. *Hum. Mutat.* **28**, 808–815 (2007).
19 Schottmann, G., Jungbluth, H., Schara, U., Knierim, E., Morales Gonzalez, S., Gill, E. *et al.* Recessive truncating IGHMBP2 mutations presenting as axonal sensorimotor neuropathy. *Neurology* **84**, 523–531 (2015).
20 Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J. *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **17**, 405–424 (2015).
21 Shi, C. H., Song, B., Luo, H. Y., Mao, C. Y., Shang, D. D., Cao, Y. *et al.* Recessive hereditary motor and sensory neuropathy caused by IGHMBP2 gene mutation. *Neurology* **85**, 383–384 (2015).
22 Luan, X., Huang, X., Liu, X., Zhou, H., Chen, S. & Cao, L. Infantile spinal muscular atrophy with respiratory distress type I presenting without respiratory involvement: Novel mutations and review of the literature. *Brain Dev.* **38**, 685–689 (2016).
23 Schultz, J., Milpetz, F., Bork, P. & Ponting, C. P. SMART, a simple modular architecture research tool: identification of signaling domains. *Proc. Natl Acad. Sci. USA* **95**, 5857–5864 (1998).
24 Maystadt, I., Zarhrate, M., Landrieu, P., Boespflug-Tanguy, O., Sukno, S., Collignon, P. *et al.* Allelic heterogeneity of SMARD1 at the IGHMBP2 locus. *Hum. Mutat.* **23**, 525–526 (2004).
25 Molnar, G. M., Crozat, A., Kraeft, S. K., Dou, Q. P., Chen, L. B. & Pardee, A. B. Association of the mammalian helicase MAH with the pre-mRNA splicing complex. *Proc. Natl Acad. Sci. USA* **94**, 7831–7836 (1997).
26 Moreira, M. C., Klur, S., Watanabe, M., Nemeth, A. H., Le Ber, I., Moniz, J. C. *et al.* Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. *Nat. Genet.* **36**, 225–227 (2004).
27 Chen, Y. Z., Bennett, C. L., Huynh, H. M., Blair, I. P., Puls, I., Irobi, J. *et al.* DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). *Am. J. Hum. Genet.* **74**, 1128–1135 (2004).
28 Wagner, J. D., Huang, L., Tetreault, M., Majewski, J., Boycott, K. M., Bulman, D. E. *et al.* Autosomal recessive axonal polyneuropathy in a sibling pair due to a novel homozygous mutation in IGHMBP2. *Neuromuscul. Disord.* **25**, 794–799 (2015).
29 Pedurupillay, C. R., Amundsen, S. S., Baroy, T., Rasmussen, M., Blomhoff, A., Stadheim, B. F. *et al.* Clinical and molecular characteristics in three families with biallelic mutations in IGHMBP2. *Neuromuscul. Disord.* **26**, 570–575 (2016).
30 Appleton, R. E., Hubner, C., Grohmann, K. & Varon, R. 'Congenital peripheral neuropathy presenting as apnoea and respiratory insufficiency: spinal muscular atrophy with respiratory distress type 1 (SMARD1)'. *Dev. Med. Child. Neurol.* **46**, 576 (2004).
31 Pierson, T. M., Tart, G., Adams, D., Toro, C., Golas, G., Tifft, C. *et al.* Infantile-onset spinal muscular atrophy with respiratory distress-1 diagnosed in a 20-year-old man. *Neuromuscul. Disord.* **21**, 353–355 (2011).
32 Grohmann, K., Varon, R., Stolz, P., Schuelke, M., Janetzki, C., Bertini, E. *et al.* Infantile spinal muscular atrophy with respiratory distress type 1 (SMARD1). *Ann. Neurol.* **54**, 719–724 (2003).
33 Rudnik-Schoneborn, S., Stolz, P., Varon, R., Grohmann, K., Schachtele, M., Ketelsen, U. P. *et al.* Long-term observations of patients with infantile spinal muscular atrophy with respiratory distress type 1 (SMARD1). *Neuropediatrics* **35**, 174–182 (2004).
34 Jedrzejowska, M., Madej-Pilarczyk, A., Fidzianska, A., Mierzewska, H., Pronicka, E., Obersztyn, E. *et al.* Severe phenotypes of SMARD1 associated with novel mutations of the IGHMBP2 gene and nuclear degeneration of muscle and Schwann cells. *Eur. J. Paediatr. Neurol.* **18**, 183–192 (2014).
35 Butterfield, R. J., Stevenson, T. J., Xing, L., Newcomb, T. M., Nelson, B., Zeng, W. *et al.* Congenital lethal motor neuron disease with a novel defect in ribosome biogenesis. *Neurology* **82**, 1322–1330 (2014).
36 Guenther, U. P., Schuelke, M., Bertini, E., D'Amico, A., Goemans, N., Grohmann, K. *et al.* Genomic rearrangements at the IGHMBP2 gene locus in two patients with SMARD1. *Hum. Genet.* **115**, 319–326 (2004).
37 Stalpers, X. L., Verrips, A., Poll-The, B. T., Cobben, J. M., Snoeck, I. N., de Coo, I. F. *et al.* Clinical and mutational characteristics of spinal muscular atrophy with respiratory distress type 1 in The Netherlands. *Neuromuscul. Disord.* **23**, 461–468 (2013).