Supplementary Case Report 1

The proband is a Polish 62-year-old right-handed man, without family history, who was diagnosed with a severe myopathy affecting proximal and distal muscles of the four-limbs and axial muscles. Muscle biopsy showed dystrophic changes associated with rimmed vacuoles. Genetic study identified a missense pathogenic variant, c.271A>C, p.Asn91His in exon 3, in the *VCP* gene at the age of 47 years. By the age of 61 he was wheelchair bound and has developed scapular winging, cataracts, and a reduced respiratory function (FVC 2,9 litres, 79% predicted).

The patient's highest educational level was primary education, he worked as a chainsaw in the woods and had cardiovascular risk factors such as diabetes type 2, hypertension, and hypercholesterolemia.

Since the age of 60, he complained about slowly progressive problems in concentration, mild forgetfulness and orientation in time and place. In addition, his relatives complained both about the progressive memory deficits as well as agitation, irritability, and languaje problems. A neuropsychological evaluation, one year later, revealed signs of short-term and long-term auditory memory deficits, executive function deficits in the field of resource searching (verbal fluency) and auditory attention deficits. The linguistic functions in the field of speech production and understanding, the ability to read and the perception of complex visual material were preserved. He scored 22 in the Mini-mental state examination.

A brain MRI showed multiple, diffuse vascular changes in the white matter of both brain hemispheres (Fazekas 1) and slight widening of the choroid fissure bilaterally in the medial temporal lobe compatible with a medial temporal lobe atrophy score of one. A mild dementia syndrome was diagnosed and treatment with Donepezil was introduced.

By the age of 64.5 years, the patient turned bedridden and was completely dependent for the activities of daily living, mainly due to the muscle weakness, showing a Barthel index of 30/100.

At age 66 the patient died due to a myocardial infarction in the context of a hospital admission due to a severe SARS-CoV-2 pneumonia.

Supplementary Materials and Methods

This is a multicentre international descriptive retrospective study collecting clinical and genetic data obtained on routine clinical care visits, from patients with a genetically confirmed diagnosis of MSP. Fifty-two centres from 24 countries participated in the study and contribute to the data collection.

In order to try to standardize variant interpretation, which was performed differently within individual countries, all variants were centrally reviewed by an experienced geneticist from the John Walton Muscular Dystrophy Centre using the criteria suggested in the American College of Medical and Genomic Genetics [28] as a guide. Variant nomenclature was based on transcript reference NM_007216.3. Inclusion criteria were: (i) patients being heterozygous pathogenic (P)/likely pathogenic (LP) variant in the *VCP* gene and (ii) enough data available in the clinical notes to answer some of the following clinical questions: age of disease onset, genetic diagnosis and clinical progression, signs/symptoms at onset, clinical progression, ambulatory status and ancillary test results if performed. Patients carrying a pathogenic variant but who did not have any clinical symptoms of disease at last appointment are also described but not included in the analysis. Patients who harboured a variant of unknown significance (VUS) were included if disease causality was supported by in silico analysis and clinical judgment based on item (ii) of the inclusion criteria and/or the pedigree indicated a dominant disease. Once the clinical data was collected for all the patients and family history, the ACMG criteria of each variant were recalculated (https://www.medschool.umaryland.edu/Genetic_Variant_Interpretation_Tool1.html/).

Study approval was obtained from the Newcastle upon Tyne Hospitals Register Audit, Newcastle, UK (project number 10833, Caldicott Approval: 7918). Institutional Review Boards approvals were obtained from the LMU Klinikum at Ludwig-Maximilians University in Munich, Germany (project 21-0071) and from the Washington University School of Medicine Institutional Review Board, USA (n° 201103416) and the Johns Hopkins Hospital Institutional Review Board, Baltimore, USA (n° 00288171). These ethics committees catalogued the present study as an audit as it was collecting deidentified retrospective data of patients with VCP. In these cases, there is no need for patients to sign a consent form.

Data sources

Participating centres completed a survey for each of their patients. The following data were collected: age of disease onset, age at last assessment, age at genetic diagnosis, genetic mutation and type of variant (missense, nonsense, frame shift, aberrant splicing or other non-specified), effect of the variant identified on protein, presence and pattern of muscle weakness, cardiac and respiratory impairment, cognitive impairment described as clinically compatible with mixed cognitive impairment, Alzheimer disease or FTD, presence of extrapyramidal disorders (defined as the presence of resting and/or action tremor, rigidity and bradykinesia, dystonic postures and movements, apraxic movements -ideomotor, limb-kinetic-, and postural instability [29]), signs of upper motor and/or lower motor neuron involvement, presence of PDB, presence of polyneuropathy, ambulatory status and family history. Results of the following ancillary tests were collected if available: spirometry, electromyography and nerve conduction studies, echocardiography, muscle biopsy, muscle magnetic resonance imaging (MRI), serum creatine kinase (CK) and alkaline phosphatase values (ALP). The data on muscle biopsy and muscle magnetic resonance imaging will be fully reported in separate papers.

To evaluate disease progression, we calculated the median times from disease onset to ambulation with assistance (ambulation with a stick/cane), to wheelchair for outdoor and indoor

activities, or to being confined to bed. The age of loss of ambulation was defined as the age when patients required a full-time wheelchair for both outdoor and indoor activities.

Cardiac involvement was defined by a left ventricular ejection fraction lower than 55%, presence of morphological abnormalities in the ventricular walls as detected by echocardiography or the existence of cardiac conduction defects evaluated by an electrocardiogram.

A severe respiratory impairment was defined as a vital force capacity (FVC) less than 50% of predicted or by the use of assisted ventilation. The use of non-invasive or invasive ventilation assistance at last assessment was recorded as well as the age at which it was started.

In addition, the following disease landmarks in years were collected: age at genetic diagnosis (age at a genetic test confirming a variant in the VCP gene), time to genetic diagnostic (difference between the age at a genetic diagnosis and the age of disease onset), age at last clinical assessment (in years as noted in clinical records), time of disease progression (difference between the age at last clinical assessment or death and the age of disease onset), age of death (as recorded in clinical notes) and time to death (difference between the age of death and the age of disease onset).

Statistical analysis

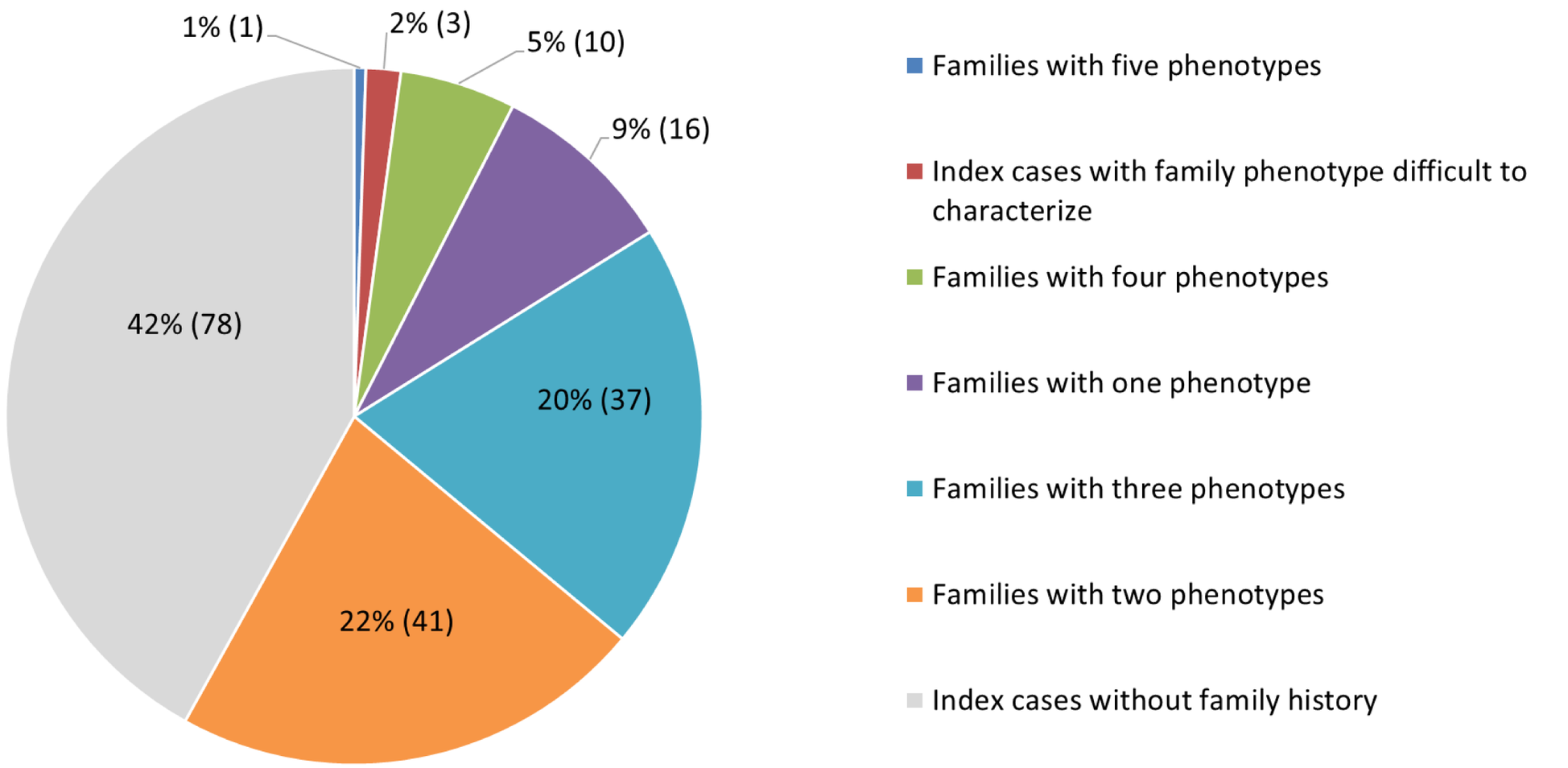
Quantitative variables were analysed using the Kolmogorov-Smirnov or Shapiro-Wilko tests to assess normal distribution. Data was expressed as number and percentage for categorical variables, and as mean ± SD or as median and first and third interquartile and minimum and maximum for quantitative variables as appropriate. The Chi-squared or Fisher exact tests were used for the association between signs/symptoms and the four most frequent mutation types, with Bonferroni correction for multiple comparisons. Analysis of covariance, with adjustment for age at last assessment, was used to compare means of signs/symptoms onset among the four most frequent mutation types.

We performed a two-step analysis to select which variables were associated with being a full-time wheelchair user/confined to bed or death. First, a Chi-squared or Fisher exact test as appropriate was used for the association between signs/symptoms and each outcome. Pearson correlation was used to examine the correlations between the age of full-time wheelchair user/confined to bed or the age of death with the ages of the variables associated with those outcomes. Second, those variables that showed a significantly different distribution among groups (considered as $p<0.05$) were included in a binary logistic regression analysis (enter method). Following that, a Cox proportional hazard regression model (enter method) was performed to identify the variables associated with a risk of being a full-time wheelchair user/confined to bed or death. Finally, a Kaplan Meier survival analysis was carried to determine the time to being a full-time wheelchair user/confined to bed or death by the variables identified through the previous analysis. The level of significance allowed was $p<0.05$. Statistics analysis was performed using SPSS software version 20 from IBM.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Supplementary Fig1. Phenotype's frequency reported in the families included in the study (n=186).



	Phenotype combinations	Frequency of families
Families with one phenotype (n=16)	IBM	12
	ALS	2
	LMN	1
	FTD	1
Families with two phenotypes (n=41)	IBM and ALS	11
	IBM and FTD	10
	IBM and PDB	5
	FTD and ALS	4
	FTD and PNP	3
	IBM and extrapyramidal disorders	3
	PNP and extrapyramidal disorders	1
	IBM and PNP	1
	PDB and ALS	1
	IBM and spinocerebellar degeneration	1
	IBM and spastic paraparesis	1
Families with three phenotypes (n=37)	FTD, PDB and IBM	21
	FTD, ALS and IBM	8
	FTD, PDB and ALS	2
	FTD, IBM and PNP	1
	PDB, IBM and PNP	1
	ALS, Huntington disease and FTD	1
	FTD, extrapyramidal disorders and ALS	1
	FTD, PDB and spastic paraparesis	1
	FTD, extrapyramidal disorders and IBM	1
Families with four phenotypes (n=10)	IBM, PDB, FTD and ALS	6
	FTD, extrapyramidal disorders, PDB and IBM	1
	FTD, PDB, ALS and PNP	1
	FTD, PDB, ALS and Spastic paraplegia	1
	FTD, extrapyramidal disorders, PDB and ALS	1
Families with five phenotypes (n=1)	FTD, extrapyramidal disorders, PDB, IBM and ALS	1
Index cases with family phenotype difficult to characterize (n=3)	The index case had IBM and gait disturbances were reported in the family	3

A total of 186 families were included in the study. The 42% (78/186) were index cases without family history and the 58% (108/186) were families in which the index cases and at least one relative were included in this study. The pie chart shows the frequency of families by number of phenotypes and the table below details the phenotypes combinations.

ALS = Amyotrophic Lateral Sclerosis; FTD= Fronto-Temporal Dementia; IBM= Inclusion Body Myopathy; LMN= Lower Motor Neurone Signs; PDB= Paget's Disease of the Bone

Supplementary Table 1. Number of patients, families, and variants by country.

Country	Patient frequency	Family frequency	Variants per-country	Most frequent variant	Frequency of families in which the most frequent variant was identified
USA	47 (20.1%)	38 (20.4%)	17	c.464G>A	17
France	33 (14.1%)	23 (12.4%)	13	c.463C>T	6
UK	26 (11.1%)	20 (10.8%)	11	c.464G>A	7
Japan	25 (10.7%)	22 (11.8%)	14	c.277C>T	4
Spain	23 (9.8%)	19 (10.2%)	10	c.464G>A	6
Germany	15 (6.4%)	15 (8.1%)	6	c.464G>A	9
Italy	14 (6.0%)	10 (5.4%)	7	c.463C>T and c.277C>T	3 on each
Finlandia	10 (4.3%)	5 (2.7%)	4	c.410C>T	2
Brasil	9 (3.8%)	7 (3.8%)	6	c.464G>A	2
Belgium	7 (3.0%)	5 (2.7%)	4	c.476G>A	2
Netherland	4 (1.7%)	3 (1.6%)	2	c.464G>A	2
Poland	3 (1.3%)	2 (1.1%)	2	c.1159A>C and c.271A>C	1 on each
Korea	3 (1.3%)	2 (1.1%)	2	c.463C>T and c.464G>A	1 on each
Chile	3 (1.3%)	3 (1.6%)	3	c.464G>A, c.464G>A and c.648A>G	1 on each
Greece	2 (0.9%)	2 (1.1%)	1	c.476G>A	1
India	2 (0.9%)	2 (1.1%)	2	c.1988A>G and c.265C>G	1 on each
China	1 (0.4%)	1 (0.5%)	1	c.464G>A	1
Hungary	1 (0.4%)	1 (0.5%)	1	c.196G>A	1
Australia	1 (0.4%)	1 (0.5%)	1	c.464G>A	1
Scotland	1 (0.4%)	1 (0.5%)	1	c.1106T>C	1
Sweden	1 (0.4%)	1 (0.5%)	1	c.286C>G	1
Serbia	1 (0.4%)	1 (0.5%)	1	c.383G>C	1
Austria	1 (0.4%)	1 (0.5%)	1	c.476G>A	1
Canada	1 (0.4%)	1 (0.5%)	1	c.572G>A	1
Total	234 (100.0%)	186 (100.0%)			

Supplementary Table 2.1 List of variants in the *VCP* gene identified in the VCP international study.

DNA variant	Protein variant	Exon	Patient frequency	Family frequency	ACMG classification*	gnomAD	ACGM evidence criteria	ACGM re-classification**
c.464G>A	p.Arg155His	5	67 (28.63%)	53	P	absent	PS4, PP5, PM1, PM2, PM5, PS3, PP1-M	Pathogenic
c.463C>T	p.Arg155Cys	5	26 (11.11%)	18	P	absent	PS3, PS4, PM1, PM2, PM5, PP1-M, PP3	Pathogenic
c.476G>A	p.Arg159His	5	18 (7.69%)	17	P	2/251462	PS4, PM1, PM2, PM5, PP1-M, PP3	Pathogenic
c.277C>T	p.Arg93Cys	3	17 (7.26%)	14	P	absent	PS4, PM1, PM2, PM5, PP1-M, PP3, PP5	Pathogenic
c.572G>A	p.Arg191Gln	5	13 (5.56%)	10	P	4/251484	PS3, PS4, PM2, PM5, PP1-M, PP3, PP5	Pathogenic
c.475C>T	p.Arg159Cys	5	9 (3.85%)	7	P	1/251438	PS4, PM1, PM2, PP1-M, PP3, PP5, PM5	Pathogenic
c.410C>T	p.Pro137Leu	4	7 (2.99%)	2	P	absent	PS3, PP1-M, PM5, PP3, PP5	Pathogenic
c.290G>A	p.Gly97Glu	3	4 (1.71%)	2	P	absent	PS4, PM1, PM2, PP1-M, PP3, PP5	Pathogenic
c.374G>A	p.Gly125Asp	4	4 (1.71%)	2	VUS	absent	PM2, PP1-M, PS4, PP3	Likely pathogenic
c.648A>G	**p.Ile216Met**	6	4 (1.71%)	3	VUS	absent	PS4, PP1, PM2, PP3	Likely pathogenic
c.1106T>C	**p.Ile369Thr**	10	3 (1.28%)	3	VUS	absent	PS4, PM2, PP3	Likely pathogenic
c.1159A>C	p.Asp387His	10	3 (1.28%)	2	VUS	absent	PS4, PM2, PP1, PP3	Likely pathogenic
c.1160A>C	p.Asn387Thr	10	3 (1.28%)	2	VUS	1/251492	PS4, PM2, PP1, PP3	Likely pathogenic
c.572G>C	p.Arg191Pro	5	3 (1.28%)	2	LP	absent	PS4, PM2, PM5, PP3, PP5, PP1	Pathogenic
c.1160A>G	p.Asn387Ser	10	2 (0.85%)	2	VUS	absent	PS4, PM2, PP3	Likely pathogenic
c.1315G>C	p.Ala439Pro	11	2 (0.85%)	1	VUS	absent	PS4, PM2, PP1, PP3	Likely pathogenic
c.1315G>T	p.Ala439Ser	11	2 (0.85%)	1	VUS	absent	PM2, PP1, PP3	VUS - not enough evidence
c.268A>G	**p.Asn90Asp**	3	2 (0.85%)	1	LP	absent	PP1, PM1, PM2, PP3	Likely pathogenic
c.283C>T	p.Arg95Cys	3	2 (0.85%)	2	P	1/251464	PS3, PS4, PM1, PM2, PP1-M, PP3, PP5	Pathogenic
c.376A>T	p.Ile126Phe	4	2 (0.85%)	2	VUS	absent	PM2, PP3, PS4	Likely pathogenic
c.383G>C	p.Gly128Ala	4	2 (0.85%)	2	LP	absent	PM2, PP3, PP5, PS4	Likely pathogenic
c.383G>T	p.Gly128Val	4	2 (0.85%)	2	LP	absent	PS4, PM2, PM5, PP3	Likely pathogenic
c.451A>G	p.Ile151Val	5	2 (0.85%)	2	P	1/31382	PS3, PM1, PM2, PP3, PP5, PS4	Pathogenic
c.722T>G	**p.Ile241Ser**	7	2 (0.85%)	2	VUS	absent	PS4, PM2, PP3	Likely pathogenic
c.785C>G	p.Thr262Ser	7	2 (0.85%)	1	VUS	absent	PP1, PM2, PP3, PS4	Likely pathogenic
c.1057A>G	**p.Ile353Val**	9	1 (0.43%)	1	VUS	absent	PM2	VUS - not enough evidence
c.1105A>T	**p.Ile369Phe**	10	1 (0.43%)	1	VUS	absent	PM2, PP3	VUS - not enough evidence
c.1696-3C>T	p.(?)	intron 13 of 16, position 509 of 511	1 (0.43%)	1	LB	10/249 922	BS2	VUS - not enough evidence
c.196G>A	**p.Glu66Lys**	3	1 (0.43%)	1	VUS	absent	PM2, PP3	VUS - not enough evidence
c.1984C>T	p.Arg662Cys	14	1 (0.43%)	1	VUS	1/249512	PS4, PM2, PP3	Likely pathogenic
c.1988A>G	**p.Lys663Arg**	14	1 (0.43%)	1	VUS	absent	PM2, PP3	VUS - not enough evidence
c.2345G>C	p.GLy782ALa	17	1 (0.43%)	1	VUS	absent	PM2	VUS - not enough evidence
c.265C>G	**pArg89Gly**	3	1 (0.43%)	1	LP	absent	PM2, PM1, PP3	VUS - not enough evidence
c.259G>T	p.Val87Phe	3	1 (0.43%)	1	VUS	absent	PP3, PM2	VUS - not enough evidence
c.266G>A	p.Arg89Gln	3	1 (0.43%)	1	LP	absent	PM2, PP3, PM1	VUS - not enough evidence
c.271A>C	p.Asn91His	3	1 (0.43%)	1	P	absent	PM1, PM2, PM5, PP3	Likely pathogenic
c.271A>T	p.Asn91Tyr	3	1 (0.43%)	1	P	absent	PM1, PM2, PP5, PP3	Likely pathogenic
c.286C>G	**p.Leu96Val**	3	1 (0.43%)	1	LP	absent	PM1, PM2, PP3, PP5	Likely pathogenic

c.367G>A	**p.Val123Met**	4	1 (0.43%)	1	VUS	absent	PM2, PP3	VUS - not enough evidence
c.376A>G	p.Ile126Val	4	1 (0.43%)	1	VUS	absent	PM2, PP3	VUS - not enough evidence
c.382G>T	p.Gly128Cys	4	1 (0.43%)	1	LP	absent	PM2, PM5, PP3	VUS - not enough evidence
c.431_432delGAinsAC	**p.Arg144His**	4	1 (0.43%)	1	VUS	absent	PP3, PM2	VUS - not enough evidence
c.463C>A	p.Arg155Ser	5	1 (0.43%)	1	P	absent	PM1, PM2, PM5, PP3, PP5	Likely pathogenic
c.463C>G	**p.Arg155Gly**	5	1 (0.43%)	1	P	absent	PM1, PM2, PM5, PP3	Likely pathogenic
c.464G>C	p.Arg155Pro	5	1 (0.43%)	1	P	absent	PM1, PM2, PM5, PP5, PP3	Likely pathogenic
c.469G>C	p.Gly157Arg	5	1 (0.43%)	1	P	absent	PM1, PM2, PP3, PP5	Likely pathogenic
c.472A>G	p.Met158Val	5	1 (0.43%)	1	P	absent	PM1, PM2, PM5, PP3, PP5	Likely pathogenic
c.473T>C	**p.Met158Thr**	5	1 (0.43%)	1	P	absent	PM1, PM2, PM5, PP3	Likely pathogenic
c.474G>T	p.Met158Ile	5	1 (0.43%)	1	P	absent	PM1, PM2, PM5, PP3, PP5	Likely pathogenic
c.478G>A	p.Ala160Thr	5	1 (0.43%)	1	P	absent	PM2, PM1, PP3	VUS - not enough evidence
c.490A>C	**p.Lys164Gln**	5	1 (0.43%)	1	LP	absent	PM1, PM2, PP3	VUS - not enough evidence
c.593T>G	p.Leu198Trp	6	1 (0.43%)	1	LP	absent	PM2, PP3, PP5	VUS - not enough evidence
c.625T>G	**p.Cys209Gly**	6	1 (0.43%)	1	VUS	absent	PM2, PP3	VUS - not enough evidence
c.697A>G	**p.Ile233Val**	6	1 (0.43%)	1	VUS	2/251480	PM2	VUS - not enough evidence
c.784A>G	p.Thr262Ala	7	1 (0.43%)	1	VUS	absent	PS4, PM2, PP3	Likely pathogenic
c.80T>C	**p.Ile27Thr**	2	1 (0.43%)	1	VUS	absent	PM2, PP3	VUS - not enough evidence
c.-249C>T	n/a	UTR	1 (0.43%)	1	VUS	absent	PM2,	VUS - not enough evidence

*As stated by Varsome, as of May 2022.
**Calculated using all genetic data available for this study.
Novel or not previous clinically characterized variants are highlighted in bold.
Variants that have been upgraded from VUS to LP/P based on the ACGM re-classification are marked in light blue.
The number of patients in this table extends to 235 and the numbers of families to 187 because one extra patient is included. The extra patient, carrying variant c.697A>G, p.Ile233Val, was excluded from the final analysis due to not fulfilling the inclusion criteria but the variant is reported in this table for information purposes.

Supplementary Table 2.2 Clinical information of the VUS and novel variants identified in the VCP International Study.

DNA m (Protein m)	Families	Patients				Family History	Family segregation
		Sex	ALA (y)	AOO (y)	Phenotypes		
c.374G>A (p.Gly125Asp)	1	M	67	56	IBM	ALS IBM	Positive
		M	79	70	ALS		
		F	61	58	IBM		
	2	M	53	45	PNP	None	Not available
c.648A>G (p.Ile216Met)	1	M	57	42	IBM FDT	D IBM	Positive
		M	55	45	Mild cognitive impairment		
	2	M	58	50	IBM PNP	AD	Not available
	3	M	68	55	IBM	None	Not available
c.1106T>C (p.Ile369Thr)	1	F	71	NA	IBM PDB	D ALS	Not available
	2	M	55	50	IBM MND	MND D	Not available
	3	M	66	60	IBM	None	Not available
c.1159A>C (p.Asp387His)	1	M	39	37	MND	None	Not available
	2	M	46	44	IBM	D PDB M	Positive
		M	51	47	IBM		
c.1160A>C (p.Asn387Thr)	1	M	59	56	ALS FTD PNP	D PDB ALS PK	Positive
		M	65	60	ALS PDB FTD		
	2	M	68	62	IBM MND PDB FTD	FDT	Positive
c.1160A>G (p.Asn387Ser)	1	M	61	54	IBM	None	Not available
	2	F	82	68	IBM	None	Not available
c.1315G>C (p.Ala439Pro)	1	M	43	40	IBM FTD PDB	D PDB M	Positive
	2	F	49	46	IBM + FDT isolated		
c.1315G>T (p.Ala439Ser)	1	M	67	53	IBM PDB PNP	IBM PDB PNP	Positive
		M	70	62	IBM		
c.268A>G (p.Asn90Asp)	1	F	39	26	PDB ALS Squizofrenia	ALS	Positive
		F	44	35	Dys PDB Squizorenia		
c.376A>T (p.Ile126Phe)	1	M	53	48	IBM	None	Not available
	2	M	60	57	IBM	Father with gait disturbances not further details	Not available
c.722T>G (p.Ile241Ser)	1	M	60	53	IBM PDB	D ALS IBM	Positive
	2	M	67	55	MND	IBM	Not available
c.785C>G (p.Thr262Ser)	1	M	63	59	IBM	D	Positive
		M	67	62	IBM		
c.1057A>G (p.Ile353Val)	1	M	67	64	ALS	None	Not available
c.1105A>T (p.Ile369Phe)	1	F	57	38	IBM	IBM PK	Positive
c.196G>A (p.Glu66Lys)	1	M	56	51	IBM FDT	None	Not available
c.1984C>T (p.Arg662Cys)	1	M	72	56	IBM FDT	None	Not available
c.1988A>G (p.Lys663Arg)	1	M	39	37	MND	None	Not available

c.2345G>C (p.GLy782Ala)	1	F	44	42	PNP	None	Not available
c.265 C>G (p.Arg89Gly)	1	M	42	37	MND	None	Not available
c.259G>T (p.Val87Phe)	1	M	65	47	IBM MND PNP DYS	None	Not available
c.286C>G (p.Leu96Val)	1	M	68	58	IBM	None	Not available
c.367G>A (p.Val123Met)	1	M	52	50	IBM	PK	Not available
c.376A>G (p.Ile126Val)	1	M	73	71	IBM FTD	None	Not available
c.431_432delGAinsAC (p.Arg144His)	1	M	60	46	IBM DYS	D ALS IBM	Positive
c.463C>G (p.Arg155Gly)	1	F	43	38	IBM	None	Not available
c.473T>C (p.Met158Thr)	1	M	52	44	IBM PDB MND	D PDB	Not available
c.490A>C (p.Lys164Gln)	1	M	62	51	IBM PDB	Spastic paraparesis	Not available
c.625T>G (p.Cys209Gly)	1	F	50	38	Spastic paraparesis	D PDB ALS	Not available
c.697A>G (p.Ile233Val)	1	M	NA	NA	PDB	Unknown	Not available
c.784A>G (p.Thr262Ala)	1	M	63	50	IBM PDB	D PDB PK	Positive
c.80T>C (p.Ile27Thr)	1	M	63	52	IBM FDT	None	Not available
c.-249C>T n/a	1	M	61	38	PNP DFT	PK PNP	Not available

The table shows the patients that harboured a VUS, and novel or not previous clinically characterized variants at the moment of enrolment in the VCP study. The main patient phenotype, the phenotypes reported within their families and segregation analysis information is reported.
Novel or not previous clinically characterized variants are highlighted in bold
Abbreviations: ALA: age at last assessment. AOO: age of onset. D: Dementia. Dys: dysautonomia. FDT: Fronto temporal dementia. MND: Motor Neuron Disease. PDB: Paget Disease of the Bone. IBM: Inclusion Body Myopathy. AD: Alzheimer Disease. n/a: not available

Supplementary Table 3. Patients ages at sign/ symptom development

Sign/ symptom	Age data availability/ Patients with the sign or symptom	Mean (SD) [y]	Min - Max [y]
Proximal lower limb weakness	179/207	48.1± 09.7	18 - 77
Proximal upper limb weakness	152/181	49.5± 10.2	18 - 77
Distal lower limb weakness	122/166	49.9± 09.0	24 - 72
Distal upper limb weakness	98/139	52.1± 09.2	30 - 77
Axial weakness	71/106	51.8± 10.3	30 - 80
Scapular wigning	23/110	51.2± 10.7	35 - 77
Respiratory impairment	57/91	54.1± 10.9	32 - 80
PDB	50/64	48.7± 10.0	25 - 69
Dysautonomia	17/42	52.2± 10.2	35 - 71
LMN	30/46	48.5± 10.9	29 - 70
Cramps	31/42	48.5± 10.4	25 - 70
Dysphagia	34/37	55.7± 11.5	31 - 79
Depression	23/32	53.7± 11.0	40 - 77
PNP	23/35	52.0± 08.2	38 - 65
FTD	25/33	58.0± 07.5	40 - 71
UMN	16/28	50.4± 10.8	30 - 75
Drop Head	10/28	53.7± 11.9	37 - 71
Facial weakness	3/25	45.3± 05.7	39 - 50
Mix cognitive impairment	6/25	49.7± 10.7	35 - 66
Camptocormia	4/17	54.0± 07.6	46 - 61
Dysarthria	13/16	51.9± 12.4	31 - 80
Cardiac impairment	9/17	49.7± 09.2	36 - 62
Catarats	9/14	64.2± 10.8	41 - 79
Extrapyramidal disorders	5/8	55.2± 06.8	48 - 66

PDB: Paget Disease of the Bone. LMN: Lower Motor Neuron signs. PNP: polyneuropathy. FTD: Fronto-Temporal Dementia. UMN: Upper Motor Neuron signs

Supplementary Table 4. Frequency of signs/symptoms in the four most frequent variants.

	c.464G>A, p.Arg155His	**c.463C>T, p.Arg155Cys**	**c.476G>A, p.Arg159His**	**c.277C>T, p.Arg93Cys**
N	67	26	18	17
Gender (male/female, %)	42/25 (62.69%)	11/15 (42.31%)	16/2 (88.89%)	12/5 (70.59%)
Frequency of families/ total relatives/families in which only the proband was included/ families in which the proband and other relative were included	53/14/46/7	18/8/13/5	17/1/16/1	14/3/11/3
Age of onset (Number of patients with data available /total) [mean, SD, minimum, maximum, median in years]	66/67 [42.6 ± 6.7, 29-56, 42.0]	25/25 [37.8 ± 7.5, 22-54, 38.0]	18/18 [50.8 ± 7.5, 35-65, 51.5]	17/17 [52.3 ± 5.6, 41-63, 52.0]
Age at last assessment (Number of patients with data available/total) [mean, SD, minimum, maximum, median in years]	67/67 [54.6 ± 7.9, 37-77, 54.0]	26/26 [50.0 ± 6.3, 41-67, 49.5]	18/18 [63.5 ± 9.2, 45-82, 63.0]	17/17 [64.2 ± 5.3, 56-77, 64.0]
Time of disease progression (Number of patients with reported data/total) [mean, SD, minimum, maximum, median in years]	66/67 [12.0 ± 6.8, 1-31, 11.0]	25/26 [12.3 ± 5.9, 5-26, 13.0]	18/18 [12.6 ± 7.5, 1-30, 11.5]	17/17 [11.7 ± 7.6, 2-28, 9.0]
Alive	48/60 (80.00%)	17/23 (73.91%)	11/15 (73.33%)	9/10 (90.00%)
Age of death (Number of patients death/ Number of patients with data available) [mean, SD, minimum, maximum, median in years]	12/12 [61.4 ± 7.0, 45-72, 63.3]	6/6 [59.0 ± 7.7, 50-69, 56.0]	3/4 [69.8 ± 6.6, 63-76, 70.3]	1/1 [64.63]
Proximal upper limb weakness	54/63 (85.71%)	24/24 (100.00%)	13/18 (72.22%)	12/15 (80.00%)
Proximal lower limb weakness	60/67 (89.55%)	25/25 (100.00%)	16/18 (88.89%)	16/17 (94.12%)
Distal upper limb weakness	37/63 (58.73%)	21/25 (84.00%)	11/18 (61.11%)	11/15 (73.33%)
Distal lower limb weakness	41/63 (65.08%)	21/25 (84.00%)	14/17 (82.35%)	11/15 (73.33%)
Facial Weakness	7/60 (11.67%)	2/24 (8.33%)	2/18 (11.11%)	1/15 (6.67%)
Camptocormia	28/59 (47.46%)	19/24 (79.17%)	8/18 (44.44%)	4/15 (26.67%)
Drop Head	7/62 (11.29%)	6/25 (24.00%)	3/18 (16.67%)	3/15 (20.00%)
Muscle Cramps	15/61 (24.59%)	4/23 (17.39%)	3/18 (16.67%)	3/16 (18.75%)
Scapular Wigning	28/59 (47.46%)	19/24 (79.17%)	8/18 (44.44%)	4/15 (26.67%)
Axial weakness	31/60 (51.67%)	18/23 (78.26%)	7/15 (46.67%)	6/11 (54.55%)
Alzheimer disease	0/66 (0.00%)	0/26 (0.00%)	0/18 (0.00%)	0/17 (0.00%)
Mixed cognitive impairment	5/66 (7.58%)	7/26 (26.92%)	2/18 (11.11%)	1/17 (5.88%)
FTD	7/66 (10.61%)	4/26 (15.38%)	2/18 (11.11%)	4/17 (23.53%)
PDB	17/66 (25.76%)	9/26 (34.62%)	3/17 (17.65%)	7/16 (43.75%)
Depression	12/55 (21.82%)	5/26 (19.23%)	1/16 (6.25%)	2/15 (13.33%)
PNP	9/66 (13.64%)	4/26 (15.38%)	1/18 (5.56%)	5/17 (29.41%)
Dysphagia	11/62 (17.74%)	5/25 (20.00%)	1/18 (5.56%)	3/15 (20.00%)
Dysarthria	6/61 (9.84%)	3/24 (12.50%)	0/18 (0.00%)	0/14 (0.00%)
Dysautonomia	14/53 (26.42%)	6/19 (31.58%)	2/18 (11.11%)	2/16 (12.50%)
UMN	11/61 (18.03%)	2/25 (8.00%)	2/17 (11.76%)	3/16 (18.75%)
LMN	13/62 (20.97%)	6/25 (24.00%)	2/17 (11.76%)	5/16 (31.25%)
Extrapyramidal disorders	0/62 (0.00%)	1/26 (3.85%)	3/18 (16.67%)	0/15 (0.00%)
Cataracts	3/49 (6.12%)	1/24 (4.17%)	2/17 (11.76%)	2/15 (13.33%)
Cardiac impairment	7/67 (10.45%)	1/26 (3.85%)	0/18 (0.00%)	1/17 (5.88%)
Dilated cardiomiopathy	2/67 (2.99%)	0/26 (0.00%)	0/18 (0.00%)	1/17 (5.88%)
Hypertrophy cardiomaiopathy	2/67 (2.99%)	0/26 (0.00%)	0/18 (0.00%)	0/17 (0.00%)
Respiratory symptoms	27/63 (42.86%)	8/24 (33.33%)	6/18 (33.33%)	6/17 (35.29%)
Effort disnea	18/62 (66.67%)	7/24 (87.50%)	4/18 (66.67%)	1/16 (16.67%)
Nocturnal Hipoventilation	14/62 (51.85%)	2/22 (25.00%)	1/17 (16.67%)	4/17 (66.67%)
Repeated respiratory infections	3/63 (11.11%)	1/24 (12.50%)	1/18 (16.67%)	1/16 (16.67%)
FVC < 80%	15/34 (55.56%)	7/15 (87.50%)	4/7 (66.67%)	3/9 (50.00%)
FVC < 50%	4/34 (14.81%)	5/15 (62.50%)	0/7 (0.00%)	0/9 (0.00%)
Part time NIV	0/66 (0.00%)	0/26 (0.00%)	0/18 (0.00%)	0/17 (0.00%)
Full time NIV	0/66 (0.00%)	0/26 (0.00%)	0/18 (0.00%)	0/17 (0.00%)
Walk independently	18/66 (27.27%)	6/25 (24.00%)	9/18 (50.00%)	6/15 (40.00%)
Walk with aids	16/66 (24.24%)	8/25 (32.00%)	4/18 (22.22%)	5/15 (33.33%)
Wheelchair outdoors user	15/66 (22.73%)	2/25 (8.00%)	2/18 (11.11%)	2/15 (13.33%)
Wheelchair outdoors and indoors user	15/66 (22.73%)	7/25 (28.00%)	3/18 (16.67%)	1/15 (6.67%)
Confined to bed	2/66 (3.03%)	2/25 (8.00%)	0/18 (0.00%)	1/15 (6.67%)

Order of data shown: frequency of patients with the sign/ symptom; frequency of patients with reported data; percentage of sign/symptom present.
FTD: Fronto-Temporal Dementia. LMN: lower motor neuron signs. UMN: upper motor neuron signs. NIV: non invasive ventilation. PDB: Paget Disease of the Bone. PNP: polyneuropathy.

Supplementary Table. 5: Correlation between age of sign/symptom onset with age of full-time wheelchair use/confined to bed.

Pearson correlation	Age of full-time wheelchair use/confined to bed
Age at first symptoms (n=43)	0.82*
Age of distal lower limb weakness onset (n=33)	0.82*
Age of proximal lower limb weakness onset (n=41)	0.82*
Age of axial weakness onset (n=20)	0.81*
Age of dysautonomia onset (n=7)	0.99*
Age of FTD onset (n=7)	0.93*
Age of FVC<50% onset (n=9)	0.94*

*$p < 0.01$
Numbers in brackets represent patients in which both conditions were presented and data about age of onset were available.

Supplementary Table 6. Binary logistic regression analysis for being a full-time wheelchair user or confined to bed.

Parameters	B	Standard Error	Wald	Degree of freedom	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
FTD	-19.65	17819.73	0.00	1	1.00	0.00	0.00	.
FVC ≥ 80%			5.75	4	0.22			
FVC less 50%	4.22	1.80	5.44	1	0.02	68.19	1.97	2363.28
FVC 50 – 59%	2.27	1.933	1.39	1	0.24	9.76	0.22	431.35
FVC 60 – 69%	3.55	1.77	4.03	1	0.05	34.99	1.09	1125.84
FVC 70 – 79%	3.34	2.08	2.56	1	0.11	28.37	0.47	1702.85
Age at first symptoms	0.06	0.06	1.07	1	0.30	1.06	0.95	1.20
Distal lower limb weakness	-2.46	1.60	2.34	1	0.13	0.08	0.00	1.98
Axial weakness	-0.71	0.85	0.69	1	0.40	0.49	0.09	2.60
Dysautonomia	1.99	1.14	3.030	1	0.08	7.32	0.78	69.04
Constant	-27.74	16523.55	0.000	1	1.00	0.00		

Method selection used in the model: Enter, p= 0.04.

FTD: Fronto-Temporal Dementia. FVC: Vital Force Capacity.

Supplementary Table 7. Cox regression analysis for being a full-time wheelchair user or confined to bed.

Parameters	B	Standard Error	Wald	Degree of freedom	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
FVC ≥ 80%			6.65	4	0.16			
FVC less 50%	1.83	0.76	5.85	1	0.02	6.24	1.42	27.51
FVC 50 – 59%	0.83	0.76	1.18	1	0.28	2.29	0.52	10.17
FVC 60 – 69%	-0.19	1.12	0.03	1	0.86	0.83	0.09	7.46
FVC 70 – 79%	0.68	0.90	0.58	1	0.45	1.98	0.34	11.52
FTD	-0.76	1.24	0.38	1	0.54	0.47	0.04	5.26
Distal lower limb weakness	-1.00	0.82	1.48	1	0.22	0.37	0.07	1.84
Axial weakness	-0.01	0.57	0.00	1	0.99	0.99	0.33	3.01
Dysautonomia	-0.03	0.67	0.00	1	0.96	0.97	0.26	3.56
Age at first symptoms	-0.03	0.04	0.49	1	0.48	0.97	0.91	1.05

Method selection used in the model: Enter, Chi-square 20.05, p= 0.03.

FTD: Fronto-Temporal Dementia. FVC: Vital Force Capacity.

Supplementary Table. 8: Correlation between age of sign/symptom onset with age of death.

Pearson correlation	**Age of death**
Age at first symptoms (n=36)	0.71*
Age of full-time wheelchair user (n=14)	0.84*
Age of FTD onset (n=8)	0.95*
Age FVC ≤ 70% (n=6)	0.94*
Age dysphagia onset (n=10)	0.97*

*$p < 0.01$
Numbers in brackets represent patients in which both conditions were present and data about age of onset were available.

Supplementary Table 9. Binary logistic regression analysis for death.

Parameters	B	Standard Error	Wald	Degree of freedom	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
FTD	1.08	0.92	1.39	1	0.24	2.94	0.49	17.68
FVC ≥ 80%			6.12	4	0.19			
FVC less 50%	2.53	1.24	4.15	1	0.04	12.55	1.10	143.23
FVC 50 – 59%	1.62	1.32	1.51	1	0.22	5.07	0.38	67.20
FVC 60 – 69%	2.89	1.25	5.32	1	0.02	18.05	1.54	210.98
FVC 70 – 79%	2.16	1.25	3.01	1	0.08	8.68	0.76	99.62
Dysphagia	0.25	0.84	0.09	1	0.77	1.28	0.25	6.57
Drop head	-0.39	0.95	0.17	1	0.68	0.67	0.11	4.30
Full time wheelchair/confined to bed	1.88	0.88	4.62	1	0.03	6.56	1.18	36.46
Age at first symptoms	0.06	0.04	2.93	1	0.09	1.06	0.99	1.14
Constant	-7.22	2.10	11.82	1	0.00	0.00		

Method selection used in the model: Enter, Chi-square 19.25, p= 0.04.

FTD: Fronto-Temporal Dementia. FVC: Vital Force Capacity.

Supplementary Table 10. Cox regression analysis for variables associated with death.

Parameters	B	Standard Error	Wald	Degree of freedom	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
FVC ≥ 80%			7.11	4	0.13			
FVC less 50%	3.51	1.45	5.85	1	0.02	33.29	1.94	570.65
FVC 50 – 59%	2.46	1.48	2.76	1	0.10	11.74	0.64	214.44
FVC 60 – 69%	2.99	1.31	5.20	1	0.02	19.91	1.52	260.73
FVC 70 – 79%	1.68	1.40	1.43	1	0.23	5.34	0.34	82.77
FTD	2.28	0.86	7.08	1	0.01	9.76	1.82	52.29
Age at first symptoms	0.08	0.03	6.92	1	0.01	1.08	1.02	1.14
Dysphagia	1.43	0.91	2.44	1	0.12	4.16	0.70	24.90
Drop head	-1.90	1.10	2.96	1	0.09	0.15	0.02	1.30
Full time wheelchair user/confined to bed	0.31	0.87	0.13	1	0.72	1.36	0.25	7.57

Method selection used in the model: Enter, Chi-square 25.30, p= 0.003.

FTD: Fronto-Temporal Dementia. FVC: Vital Force Capacity.