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ORIGINAL ARTICLES

VCP-related myopathy: a case series and a review of literature

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The valosin-containing protein (VCP), a widely expressed protein, controls the ubiquitin-proteasome system, endolysosomal sorting, and autophagy to maintain cellular proteostasis. Frontotemporal dementia (FTD), inclusion body myopathy, and Paget's disease of the bone (PDB) are all caused by dominant missense mutations in the VCP gene, which interfere with these mechanisms and cause a multisystem proteinopathy. We describe phenotypic and genetic findings of five patients with four different mutations in *VCP* gene (NM_007126): c.278G > A (p.R93H), c.463C > T (p.R155C), c.410C > T (p.P137L), c.464G > A (p.R155H), c.410C > T (p.P137L). We analysed the patient' biopsies, all characterized by a muscular phenotype, and we executed immunofluorescence staining to evaluate the presence of proteins: p62, VCP, desmin, myotilin, TDP-43. Eventually we performed a brief literature review to compare our cases with those already reported.
Our report strongly suggest that *VCP* gene mutations can be related with a predominant skeletal muscle phenotype without any central nervous system involvement, as occasionally reported in the literature. Particularly, our patient with R93H shows only myopathic involvement while this mutation has been described once associated only to Hereditary Spastic Paraplegia.
Further study will be necessary to understand such a broad and different clinical spectrum.

Key words: *VCP* mutations, muscular phenotype, biopsies, rimmed vacuoles

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Introduction

The *VCP* gene, on chromosome 9p13-p12, encodes for the valosin-containg protein (VCP/p97), a ubiquitously expressed protein belonging to the AAA+ (ATPases associated with various activities) protein family [1]. This protein is involved in several cellular functions as cell cycle regulation, DNA damage response and homotypic membrane assembly. Furthermore, VCP has a crucial role in cellular proteostasis being directly involved in endoplasmatic reticulum-associated degradation of protein (ERAD) [2] and Ubiquitin-proteasome system (UPS) processes [3]. Loss of VCP activity leads to the accumulation of ubiquitinated proteins and impaired ERAD [4,5].
Mutations in *VCP* gene, inherited in an autosomal dominant manner, may result in a multisystem degenerative disorder, affecting muscle, bone and brain as Inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia (IBMPFD) (MIM 167320) that show variable penetrance of its 3 main entities: the inclusion body myopathy, the Paget's disease of the bone (PDB) and the fronto-temporal dementia (FTD). Moreover, *VCP* mutations have also been associated to amyotrophic lateral sclerosis (ALS), distal myopathy, autosomal dominant Charcot-Marie-Tooth disease type 2Y and behavioural impairment and progressive non-fluent aphasia [6]. VCP- and TDP-43 positive aggregates have been documented in the cytoplasmic compartment of IBMPFD skeletal muscles,

although not specific since they have also been observed in a wide variety of neurodegenerative disorders including Parkinson's disease, Lewy body disease, Huntington's disease, amyotrophic lateral sclerosis and spinocerebellar ataxia type III [7].
Patients with *VCP* mutations, usually present in mid-adulthood with muscle weakness, sometimes associated with respiratory and cardiac muscle impairment, leading to life-threatening breathing difficulties and heart failure [8,6]. We here describe clinical, histological and molecular features of a small cohort of Italian patients with *VCP* mutations and a revision of the available literature.

Materials and methods

This is a retrospective study on 5 *VCP*-mutated patients (4 males, 1 female) and their follow-up at Fondazione IRCCS Istituto Neurologico Carlo Besta. All patients signed informed consent for publication.

Molecular analysis

Genomic DNA was extracted from the peripheral blood on Freedom Evo 100 (Tecan, Männedorf, Switzerland) by NucleoSpin blood Kit following the manufacturer's instructions (Macherey–Nagel, Düren, Germany). DNA quality and quantity were analysed by NanoDrop (Thermo Fisher, Foster City, CA, USA), gel electrophoresis, and fluorescence absorbance (Qubit® 2.0 Fluorometer; Thermo Fisher).
We performed a custom target gene panel testing for vacuolar, distal and myofibrillar myopathies by Next Generation Sequencing (NGS) approach, designed with Agilent's HaloPlex technology (Agilent Technologies Santa Clara, California) loaded on Illumina MiSeq sequencer. Sanger sequencing using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems), was performed to verify and validate the variants.

Muscle biopsy

Skeletal muscle biopsies were available for all patients except patient 3 and were obtained at the Fondazione IRCCS Istituto Neurologico Carlo Besta. Muscle tissues were frozen in liquid nitrogen-cooled isopentane and histological staining was performed on 8µm-thick cryosections.

Immunofluorescence

Immunohistochemical staining on 4% paraformaldehyde fixed sections was carried out using the following antibodies: anti-desmin (1:100 M0760, clone D33 mouse monoclonal DAKO), anti-TDP43 (1:200 10784-2-AP rabbit monoclonal Proteintech), anti-myotilin (1:100 mouse monoclonal Novacastra Leica), anti-p62 (1:100 gp62-c guinea-pig polyclonal; Progen), anti-VCP (1:100 MA3-004 mouse; ThermoScientific). Specific secondary Alexa 488/546/555 antibodies (1:1500; Invitrogen Life Technology) were used and visualized under a fluorescence microscope (Carl Zeiss AG, Oberkochen, Germany).

Results

Clinical, neurophysiological, histological, imaging and molecular features of included patients are summarized in Table I.

Genetic findings

Among the variants identified by NGS, only those in VCP were already reported in the literature as pathogenic and were correlating with the phenotypes in our patients. No other potential causative variants were found by NGS analysis. No other affected family members were available for segregation while the variants were absent in the healthy relatives tested.

Clinical features

The mean age of disease onset was 46 ± 5.8 years (range 40-54), with 4 patients presenting with lower limb muscle weakness, notably distal for patients 1, 3 and 5 and proximal in patients 2, while patient 4 presented with proximal upper limb muscle weakness. No familiarity for myopathy was reported in patient 2, 4 and 5, while patient 1's father had a myopathy with rimmed and patient 3 a sister with similar muscular symptoms.
At the last examination at a mean age of 51.6 ± 6.8 (range 59-43) years, the predominant pattern of muscle weakness included distal lower limb muscles in 3/5 patients, and scapular and pelvic muscles in remaining 2 cases. No cranial nerve involvement was observed, except for patient 4 showing mild tongue and orbicularis oculi muscle weakness. Notably, patients 2 and 4 showed Beevor's sign. Severity of motor dysfunction according to Walton and Gardner & Medwin scale (WGM) [9] was 4 in all patients, except for patient 2 that was unable to walk unassisted (WGM = 8).
Three out of 5 patients (60%) showed increased level of CK (within x 5 upper normal limit).
Heart involvement was reported only in 2/5 (40%) patients, with patient 1 presenting at the age of 61 years with mild atrial dilatation and diastolic dysfunction and patient 3 with hypertensive cardiopathy. Moreover, respiratory involvement requiring non-invasive ventilation (NIV) during night was reported only in patient 2 (20%) since the age of 54 years due to concomitant restrictive and obstructive pulmonary syndrome.
Different types of cancer were present in 3/5 (60%) patients.
Furthermore, patient 1 had also a mild lower limb sensory axonopathy. No patients showed evidence of FTD or central nervous system involvement and only patient 2 had PDB. Furthermore, no positive family history for FTD or PDB was reported, except for patient 3 whose father was affected by PDB.
All patients underwent electromyography showing always spontaneous activity with fibrillation and/or complex repetitive discharges; a myopathic pattern was found in 4 out of 5 patients, combined to neurogenic finding in 2 cases. An exclusively neurogenic pattern with myopathic signs was observed in patient 5.

Histology

The muscle biopsies were performed at a mean age of 49.2 ± 6.38 (42-58) and were undertaken in our centre for all patients but patient 3. Histological analysis showed mild to moderate myopathic changes with fibre degeneration/regeneration and rimmed vacuoles in all patients analysed, without rimmed vacuoles (Fig. 1 a-b).
Immunofluorescence staining with the selected antibodies showed comparable signals for TDP-43 (Fig. 1 c-d) and VCP (Fig. 1 g-h)

Table I. Summary of clinical features.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex/age at onset	M/54	M/49	F/46	M/40	M/41
VCP mutations	c.278G > A (p.R93H)	c.463C > T (p.R155C)	c.410C > T (p.P137L)	c.464G > A (p.R155H)	c.410C > T (p.P137L)
Symptoms at onset	Distal lower limb and axial muscle weakness	Proximal lower limb weakness	Distal lower limb weakness	Proximal upper limb weakness	Distal lower limb weakness
Pattern of weakness distribution at last examination (age)	Distal > proximal lower limbs > scapular asymmetric, left > right side (59y)	Scapular and pelvic girdle > distal plus Beevor's sign (54y)	Distal > proximal lower limbs > scapular asymmetric, left > right side (56y)	Scapular and pelvic > distal upper and lower limb plus Beevor's sign (43y)	Peroneal > pelvic and scapular (46y)
Scapular winging	No	Yes	No	Yes	NA
Walton and Gardner & Medwin at last examination	4	8	4	4	4
Cranial nerve involvement	No	No	No	Mild tongue and orbicularis oculi muscle weakness	No
Muscle biopsy (age)	Mild unspecific changes (53y); moderate myopathic changes with degeneration/regeneration, rimmed vacuoles and inflammatory cells (58y)	Moderate myopathic changes with degeneration/regeneration and rimmed vacuoles (53y)	Moderate myopathic changes with degeneration and inflammatory cells (48y)	Moderate myopathic changes with degeneration/regeneration and rimmed vacuoles (42y)	Mild myopathic and neuropathic changes, rimmed vacuoles in 1 fibre (45y)
EMG	Myopathic pattern plus SA (plus mild distal sensory axonopathy)	Myopathic and neuropathic pattern with SA	Myopathic pattern with SA	Myopathic and neuropathic pattern with SA	Neuropathic pattern with SA
Muscle imaging predominant pattern (age)	Asymmetric (R > L) fatty replacement of TA and MG with gadolinium enhancement and asymmetric (L > R) fatty replacement of AM and AL and SM and ST (MRI, 53y)	Severe fatty replacement of lumbar paravertebral, VL and VI and moderate of AM et AL (CT, 53y)	Fatty replacement of TA (L > R), mild substitution of AM and AL and SM (MRI, 54 years)	Severe fatty replacement of lumbar paravertebral, ileopsoas, MG, AM and VI (CT, 43y)	Severe fatty replacement of peroneal muscles (CT, 45y)
PDB/FTD	-/-	+/-	-/-	-/-	-/-
Familiarity for PDB/FTD	No	No	Father with PDB	No	NA
Fractures	Traumatic fractures	No	No	No	No
Cardiac involvement	Mild atrial dilatation and diastolic dysfunction	No	Hypertensive cardiopathy	No	NA
Respiratory involvement	No	Mixed restrictive/obstructive pattern; NIV during night started at 54y	No	No	NA
CNS involvement	No (normal NCT+ brain MRI)	No	Normal brain MRI. NCT with mild revocation memory and attention-executive dysfunction	No	No (normal brain MRI)
CK levels (normal range 38-174)	300 - 1032 U/L	455 - 536 U/L	214 U/L	Normal	NA
Death	No	55y – unknown reasons	No	No	No
Other simptoms	Well-differentiated papillary thyroid cancer	Vascular hypertension, Renal cell carcinoma; major depressive disorder; obstructive pulmonary disease	Depressive disorder; vascular hypertension; breast cancer	Vascular hypertension	NA

CNS: central nervous system, SA: spontaneous activity, TA: tibialis anterior, MG: medial gastrocnemius, ST: semitendinosus, SM: semimembranosus, AM: adductor magnus, AL: adductor longus, VL: vastus laterali, VI: vastus intermedius NCT: neurocognitive tests, CNS: central nervous system.

between patients and control and no desmin and myotilin positive aggregates (Fig.1 c-d/e-f). However, positivity for p62 (Fig. 1 e-f) was present in two muscle biopsies (patient 1 and 2) as also described in the literature (Tab. II).

Muscle imaging

The muscle imaging was performed through CT or MRI scans at calf and thigh levels at a mean age of 49.6 ± 9.4 (43-54) years, revealing fatty replacement, predominantly affecting adductor magnus and vastus intermedius and medialis in the thighs (Fig. 2) and tibialis anterior and medial gastrocnemius in the legs.

Discussion and conclusions

We here present 5 Italian patients affected by VCP-related myopathies. Our patients were characterized by distal lower or upper limb weakness at onset in 3 out of 5 cases, whereas remaining 2 subjects presented with predominant proximal upper or lower limb muscle weakness. Interestingly, the predominant pattern of weakness at onset was further maintained during the follow-up over the years. Two patients showed asymmetric weakness, that is present in VCP-related myopathies [10]. We also reported Beevor's sign in 2 patients, suggestive of selective lower abdominal muscles, never reported before in VCP-related myopathies; Beevor's sign is usually observed in late-onset Pompe disease and in facio-scapulo-humeral dystrophy [11,12]. So far, a genotype-phenotype correlation has only been reported in axonal Charcot-Marie-Tooth disease that has only been associated to the amino acid changes E185H, S171R and G87E mutations as well as spastic paraplegia that has been solely related to R93H and R159C mutations [13]. The most common mutations in the VCP gene are found within the N-terminal domain (exons 1-5), as showed in Figure 3. This domain is involved in the binding of the ubiquitin and other co-factors, such as UFD1 (ubiquitin recognition factor in ER associated degradation 1) and NPL4 (ubiquitin recognition factor), which are essential for UPS function. There are two other important domains that bind and hydrolase the ATP, the D1 and D2 domains. These domains are organized as two stacked rings with a central channel, whereas its regulatory N-domain is situated at the periphery of the D1 ring [14]. The complexity of VCP's diverse molecular functions is also expressed by the broad clinical variability caused by pathogenic variants in *VCP* as shown in Table II revising the literature. The P137L variant described by Palmio et al. [15] in 9 patients, presenting with a distal myopathy phenotype without proximal or scapular weakness, has been also found in 2 of our patients (patient 3 and patient 5) with lower limb distal weakness at onset. However, this variant was previously reported in a patient with initial distal weakness involving the ankle extensors and a progression to both proximal and distal upper limb muscles with marked scapular wings [16]. The R93H mutation found in patient 1, exhibiting lower limb and axial muscle weakness, was so far only been associated to Hereditary Spastic Paraplegia [17]. One of the *VCP* hotspots is codon 155, in which three frequent missense mutations are present, R155C, R155H and R155P. According to model predictions, the most deleterious is R155C because it involves major conformational changes in the ATP binding site, even though all three variants cause

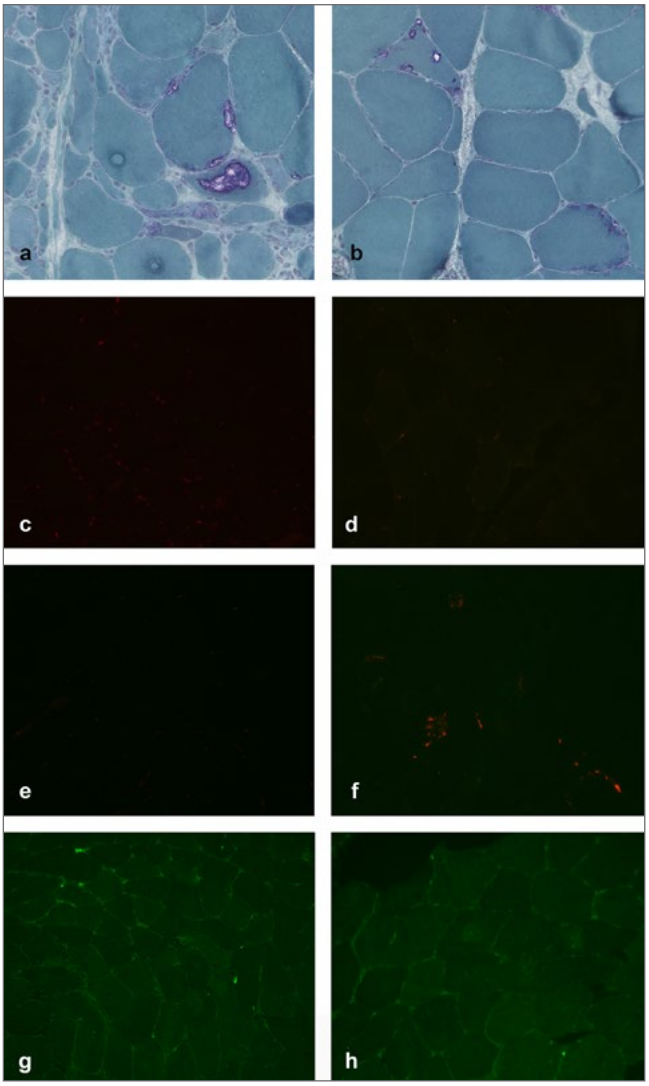

Figure 1. TRG staining (a-b) of patient 1. Desmin (green) and TDP-43 (red) double staining in the control tissue (c) and in the representative patient 1 (d); myotilin (green) and p62 (red) immunofluorescence in control (e) and patient 1 (f); VCP (green) staining in control (g) and patient 1's tissue (h).

a structural change affecting the ATP-ADP transition kinetics [18]. In fact, R155 interacts with the N387 which is located within the D1 domain that binds and hydrolyses ATP [19]. R93 and R155 are both surface-accessible residues located in the centre of cavities that may enable ligand-binding. The R155H variant present in patient 4, with mild increased parietal thicknesses of the left ventricle without cardiomyopathy, has been also reported in a patient with inclusion body myopathy and cardiomyopathy [20]. In patient 2 the mutation R155C is associated with Paget's disease (PDB). Moreover, the examination of the 31 cases reported in the literature and associated to the R155C mutation, reveals that 39% of them are inclusion body myopathy and 3% PDB only, while no patients present exclusively with FTD. Furthermore, 26% of patients show both inclusion body myopathy and PDB, 16% inclusion body myopathy and FTD, none has PDB and FTD, and 16% have inclusion body myopathy with PDB and FTD phenotypes [21].

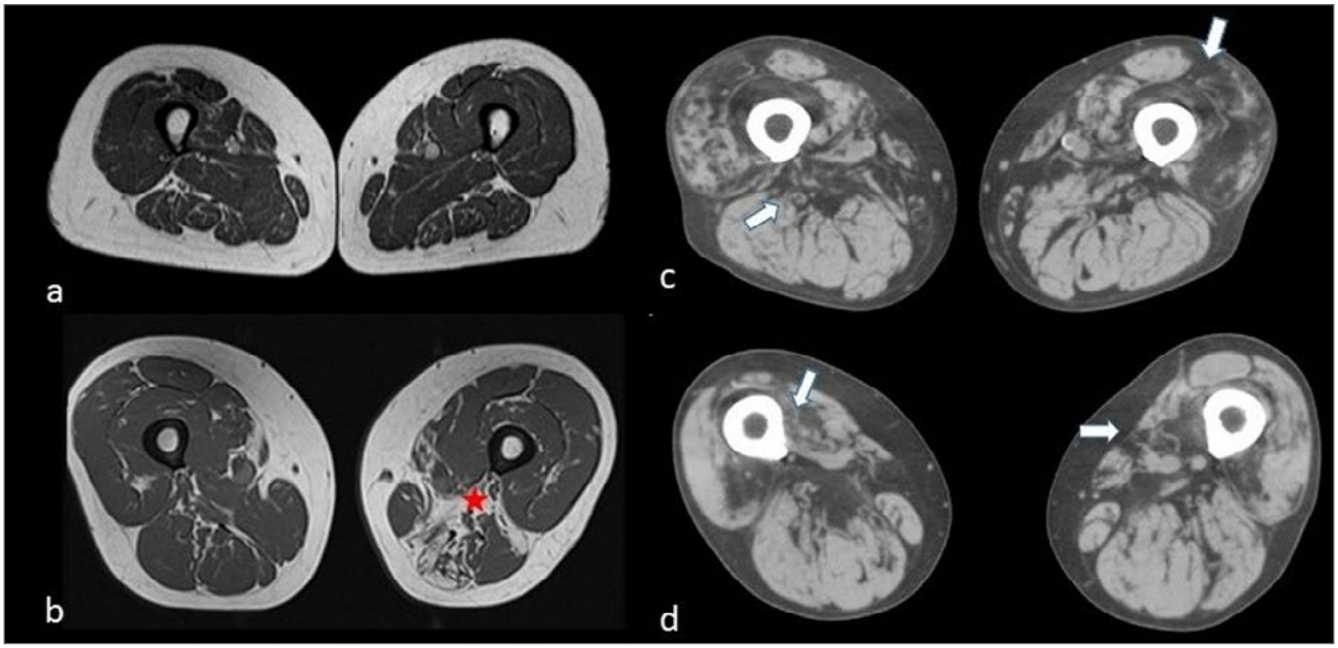

Figure 2. Muscle imaging at thigh level from patients 1-4. (a) T1-MRI axial images from patient 3 displayed minimal fatty degeneration in sartorious, semimembranous and semitendinosus muscles. (b) T1-MRI from patient 1 revealed asymmetric fatty changes of adductor magnus (red star), semimembranosus, semitendinosus and long head of biceps femoris on the left side and only initial changes of sartorious on both sides. The patient had a complete fatty replacement of tibialis anterior (data not shown). (c) CT scan from patient 2 demonstrating relevant symmetrical fatty changes mainly in vastus intermedius, medialis and adductor magnus (white arrows), while asymmetric changes are visible in the vastus lateralis, with the left side more involved. Gracilis muscles are involved on both side. (d) CT scan from patient 4, showing fatty changes mainly in vastus intermedius and medialis (white arrows), rectus, and adductor magnus.

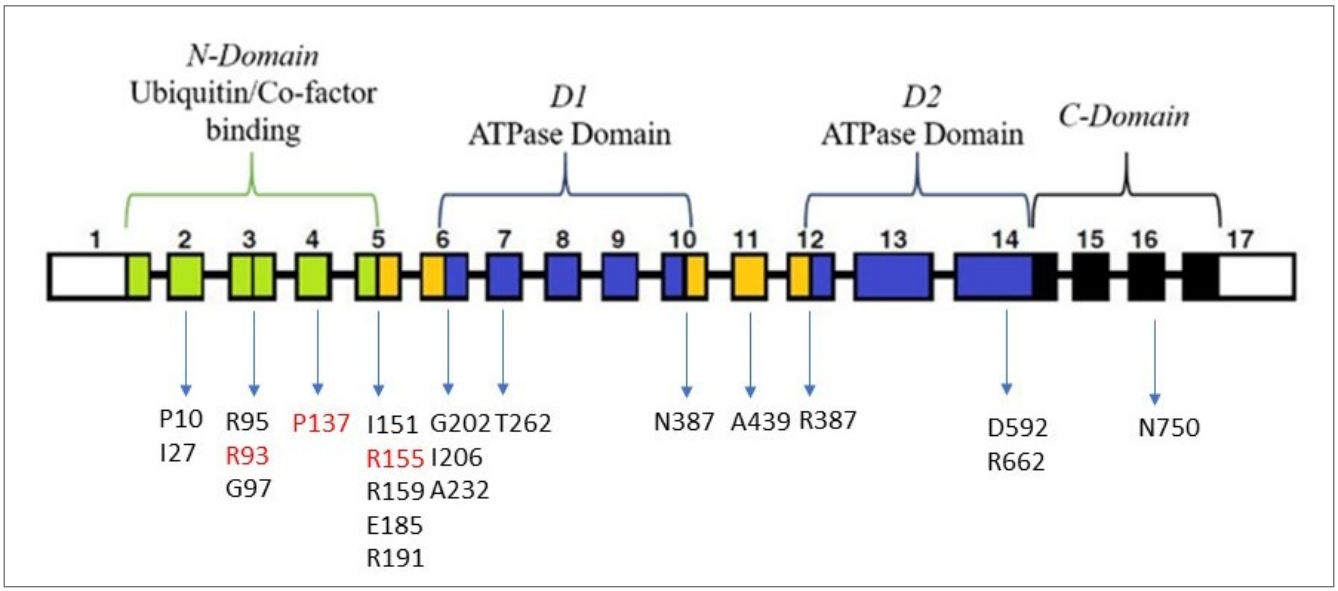

Figure 3. Representation of the *VCP* gene with its domains and the localization of all the reported mutations. Our identified mutations are highlighted in red.

It is known that VCP is overexpressed in many types of cancers probably due to its involvement in the DNA repair and stability as well as in the autophagy pathway for a proper proteostasis. However, despite 60% of our patients presented with cancer, no direct correlation with mutations in VCP has so far been reported, as also evident from the largest retrospective study published by the VCP International Study Group in 2022 [22] analyzing 225 patients with known VCP mutations. Our patient with Paget's disease has R155C mutation as other patients reported in the literature by Watts, Al-Obeidi, Figuroa, Guyant, Stojovic [16,21,23-25]. In contrast, FTD reported is associated with 6 differ-

Table II. Reported clinical features.

Reference	Affected patients	Prevalent phenotype	Ethnicity	Age at onset	Rimmed vacuoles at biopsy	VCP mutation (protein)	Neuroimaging (muscle or brain MRI) or electromyography	Immunohis-tochemistry	Cardiac involvement	Respiratory involvement	Walking ability
Ayaki et al. (2014) [29]	1M	sporadic ALS	Japanese	36	Not reported	M158V	CT scan showed osteolytic abnormalities and no brain atrophy. EMG: active and chronic denervation potentials	Not reported	Not reported	NIV dependent at 38 years old, died of respiratory failure at the age of 41	Not reported
Al-Obeidi et al. (2018) [21]	231 (118M, 113F)	IBM, PDB, FTD, ALS and Parkinson's disease.	European, Brazilian, Hispanic/ Apache, and African-American	Myopathy (43) PDB (41.2) FTD (55.9)	Muscle biopsy reports available for 115 of the symptomatic individuals. 46 (40%) out of 115 muscle biopsies showed rimmed vacuoles	R155H R155C R155P R191Q R159C R159H L198W R95G R93C A232E N387H G97E A160P G128A M158I	138 myopathic individuals underwent EMG studies: 45/138 (32,6%) had pure myopathic changes, 16/138 (11,6%) had neurogenic alteration and 19/138 (13,7%) both	Not reported	Not reported	Not reported	Independent
Bersano et al. (2007) [28]	1M	IBM+FTD	Italian	50	Yes	R159C	EMG: acute denervation in all examined muscles	VCP-positive aggregates, alpha B-crystallin, myotilin, desmin	Not reported	Not reported	Not reported
Bruno et al. (2021) [30]	3 (1M,2F)	Early onset FTD	Italian	40	Not reported	D395A	Brain MRI suggestive of FTD	Not reported	Not reported	Not reported	Not reported
de Bot et al. (2012) [31]	2 M	Slowly progressive spastic paraplegia and PDB	Dutch	55.5	Not reported	R159C	EMG: signs of active denervation, no myopathic changes, no neuropathy	Not reported	Not reported	Not reported	Not reported
DeJesus-Hernandez et al. (2011) [32]	1 F	Sporadic ALS	African American	68	Not reported	I151V	EMG examination showed acute and chronic denervation	Not reported	hypertension	NIV 19 months after onset of motor symptoms	Not reported
Figueroa-Bonaparte et al. (2015) [24]	42 (23M, 19F)	92.3% muscle weakness: 27% scapular/ pelvic, 21.6% proximal UL, 13.5% proximal LL, 24.2 % both distal/proximal UL and/or LL. PDB first symptom (one case)	English	42.05	9/17 (53%) biopsies revealed rimmed vacuoles	G202W A439G R155H R191Q R155C R93C	Not reported	Not reported	Not reported	Not reported	The mean time to loss of ambulation was 13.37 ± 6.6 years
Gang et al. (2016) [33]	3 (2M, 1F)	sIBM	Not reported	69	Not reported	I27V R159C	Not reported	Not reported	Not reported	Not reported	Not reported

Table II. *continues*

Gidaro et al. (2007) [34]	2 (1M, 1F)	Progressive myopathy	Italian	42.5	Yes	R155C	Muscle MRI of the LL showed focal areas of fatty replacement of the gastrocnemius, quadriceps, and biceps femoris	Not reported	Subjects II-1 and II-2 died of a myocardial failure	Not reported	Independent, but waddling gait
Gu et al. (2013) [35]	5 (3M, 2F)	IBMFD	Chinese	57.4	Not reported	G97E	Normal brain MRI	Not reported	Not reported	Not reported	Walking difficulty
Guyant-Maréchal et al. (2006) [25]	2 families	FTD in 100% (family 1), 70% (family 2). PDB more inconstant clinical feature	Northern-European	56.5	Subsarcolemmal rimmed vacuoles in II-5 and II-8	R93C R155C	Patients II-4, III-1 present myopathic alteration at EMG	Not reported	Not reported	Respiratory distress (mean duration of 15 years, range 11 to 18 years). Death in 3 patients	Not reported
Haubenberger et al. (2005) [36]	4 (1M, 3F)	Progressive proximal myopathy and PDB without dementia	Austrian	48.5	Rimmed vacuoles only in patient 4	R159H	Patient 2 EMG showed myopathic alterations	Not reported	Not reported	Not reported	Patients 1 and 4 lost ambulation
Hirano et al. (2015) [37]	1	Sporadic ALS, with later dementia	Japanese	65	Not reported	R487H	Marked atrophy of the frontal and temporal lobes by brain MRI	Not reported	Not reported	Not reported	Not reported
Hübbers et al. (2007) [19]	3	IBMPFD	Not reported	51.3	Yes	R93C R155C R155H	Frontal and temporal atrophy in brain MRI of patient II	VCP and ubiquitin-positive aggregates	Marked left ventricular dilatation and thickening of the left ventricular wall in patient II	Not reported	Not reported
Ikeda et al. (2020) [38]	1M	IBMPFD	Japanese	42	Yes	R155C	Cerebral MRI revealed bilateral frontal and temporal atrophy	Not reported	Not reported	Progressive respiratory involvement	Walker at 52, wheelchair at 55 years
Ikenaga et al. (2020) [20]	59 (28M, 31F)	53 IBM, 17 PDB, 8 patients with dementia, 6 with peripheral neuropathy, 4 with cardiomyopathy, 4 with cataracts, 2 with ALS, and 1 with parkinsonism	American English Australian Canadian Netherlands German New Zealander Brazilian Thai	43.4	Not reported	R155H R155C R159C R93C R159 R191Q G125D	Not reported	Not reported	Cardiomyopathy (R155H, R191Q)	20 patients had orthopnea, 7 patients used assisted ventilation or oxygen supplementation	Walking aid (n = 14), cane (n = 9), walker (n = 11), wheelchair (n = 5)
Inoue et al. (2017) [27]	2M	1 patient with ALS, and 1 with parkinsonism	Japanese	65	Yes	V87F I126V	EMG showed myopathic change	VCP and ubiquitin-positive aggregates	In patient 1 MIBG myocardial scintigraphy revealed reduced uptake	Patient 2 respiratory failure at age 65 years, death at age 66	Independent

Table II. *continues*

Jacquin et al. (2013) [39]	1M	IBMPFD	French	41	Yes	R155H	Spontaneous activity and both myopathic or neurogenic at EMG. Frontal and internal temporal atrophy at brain MRI	Not reported	Not reported	Not reported	Wheelchair dependent
Jerath (2019) [40]	3F	The proband present proximal LL and distal UL weakness	Caucasian	40	Not reported	R155H	Not reported	Not reported	Mildly abnormal cardiac stress test with mild ischemia of the anterior cardiac wall	Not reported	Independent
Kaleem et al. (2007) [41]	3	LOAD	Caucasian	Not reported	Not reported	R92H	Not reported	Not reported	Not reported	Not reported	Not reported
Koppers et al. (2012) [42]	2F	ALS	Not reported	55.5	Not reported	R159H I114V	Not reported	Not reported	Not reported	Not reported	Patient B: unable to walk
Kumar et al. (2010) [43]	6 (3M,3F)	1 myopathy 4 myopathy + PDB 1 IBMPFD	Australian	37	2 patients' muscle biopsies showed rimmed vacuoles	R155C L198W	EMG in 4 patients showed a myopathic pattern	TDP-43	Not reported	Not reported	Progressive difficulty getting out of chairs and walking up and down stairs
Lévensque et al. (2016) [44]	1	IBM	Not reported	60	Not reported	L386Q	Not reported	Not reported	Not reported	Not reported	Not reported
Nakamura et al. (2021) [45]	1F	HSP with PDB	Japanese	36	Not reported	R155C	Brain MRI was normal	Not reported	Not reported	Not reported	Independent
Neveling et al. (2013) [17]	1	HSP	Not reported	Not reported	Not reported	R93H	Not reported	Not reported	Not reported	Not reported	Not reported
Palmio et al. (2011) [15]	9 (6M, 3F)	3 patients with distal myopathy and rapidly progressive dementia	Finnish	46	3 patients' muscle biopsies showed rimmed vacuolar myopathy	P137L	Abnormal findings in anterior LL muscles from subtle to severe replacement by fatty connective tissue in all others	TDP-43 and p62 inclusions in rimmed vacuoles, granular cytoplasmic VCP in most fibres	Not reported	Not reported	Walk with a stick until the age of 50
Papadimas et al. (2017) [46]	4 (3M, 1F)	LL myopathy and FTD(II-1), dementia(I-2) classical ALS (II-2), behavioural symptoms (II-3)	Greek	62	Not reported	R159H	Brain MRI revealed frontal lobe atrophy. EMG showed diffuse myopathic changes and mild spontaneous activity. Muscle MRI showed extended atrophy and fatty degeneration	Not reported	Not reported	Not reported	Need of support

Table II. *continues*

Pellerin et al. (2020) [47]	3 (1M,2F)	Initial proximal and distal LL weakness with loss of ambulation, 12 years later distal UL weakness, later proximal arm and neck extension weakness	French Canadian	33.5	Biopsy of subjetc.2 revealed myopathic changes and scattered rimmed vacuoles	G156S	Brain MRI showed atrophy of the frontal and temporal lobes, slightly more significant over the left temporal lobe	TDP-43 immunoreactive cytoplasmic deposits, and numerous COX-reduced fibres	Not reported	Subject II.2 present orthopnea, Subjects II.1 and II.3 died of aspiration pneumonia	Subjects II.1 and II.3 became wheelchair-bound
Rohrer et al. (2011) [48]	2 (1F, 1M)	Male patient: deterioration in episodic memory and progressive behavioural disturbance later developing muscle weakness and tremor. Female patient: progressive speech disturbance	Japanese	63.5	Not reported	I27V	Male patient brain MRI showed marked symmetrical cerebral atrophy involving the frontal and parietal lobes. Female MRI was normal	Not reported	Not reported	Not reported	Not reported
Shi et al. (2008) [49]	2F	FTD and AD	Chinese	60.5	Not reported	T127A N401S	Brain MRI showed left temporal lobe atrophy at 58 years old, MRI at 62 years of age showed bilateral frontal and temporal lobe atrophy	Not reported	Not reported	Not reported	Not reported
Stojkovic et al. (2009) [16]	19 (11M 8F)	Early involvement of the proximal UL with scapular winging. Axial and LL muscles often affected. PDB observed in 8 and cognitive impairment in 9 patients	French and Spanish	42	Yes	P137L R155C R155S R155H A439S R159H G157R R191Q	Muscle MRI showing fatty degeneration of VL, VM, RF and gluteus. At the scapular level, fatty degeneration is observed on supraspinatus, infraspinatus and deltoid. EMG: acute denervation either a myopathic pattern or a mixed myogenic/ neurogenic pattern	Not reported	Not reported	Two patients required NIV and 7 died as a consequence of weakness and respiratory distress	10 patients wheelchair bound after a mean disease course of 9 years and 6 required canes for walking
van der Zee et al. (2009) [50]	2 families	FTLD, PDB	Belgian	54 (FTLD) 46 (PDB)	Not reported	R159H	By brain MRI corticosubcortical and cerebellar atrophy (P5), periventricular leukoencephalopathy (P2)	Not reported	Not reported	Not reported	Not reported

Table II. *continues*

Viassolo et al. (2008) [51]	4	IBMPFD	Italian	49	Rimmed vacuolar inclusion bodies in 3 biopsies	R155H	EMG of 2/4 patients: signs of diffuse acute and chronic denervation	Not reported	Not reported	Not reported	Independent
Watts et al. (2004) [23]	13 families	82% patients with myopathy, 49% PDB and 30% early-onset FTD	12 from the United States and 1 from Canada	42	Yes	R155H R155P R155C A232E R95G R191Q	Not reported	Not reported	Not reported	Not reported	Not reported
Watts et al. (2007) [52]	6	IBMPFD	Poland and American	40	4 patients showed rimmed vacuoles	N387H L198W	EMG: Myopathic (3 patients), Mixed myopathic-neurogenic (1 patient)	Not reported	Cases 6 and 7 died of cardiac failure	Case 6 died from respiratory failure	Losing the ability to walk within a few years of onset
Weihl et al. (2015) [53]	2	IBMPFD and Parkinson's Disease	Not reported	> 45	2 patients showed rimmed vacuoles	I27V R95C	EMG: Myopathic (2 patients)	Not reported	Not reported	Not reported	Not reported

PEG: percutaneous endoscopic gastrostomy; NIV: non-invasive ventilation; ALS: amyotrophic lateral sclerosis; PDB: Paget's disease of bone; CT: computed tomography; EMG: needle electromyography; IBM: inclusion body myopathy. FTD: frontotemporal dementia; IBMPFD: inclusion body myopathy with frontotemporal dementia; MUPs: motor unit potentials; Fibs: fibrillations at EMG; UL: upper limb; LL: lower limb; PSW: positive sharp waves; LOAD: late onset Alzheimer's disease.

ent mutations (R93H, R155C, R155H, R159C, D395A, R155C), as reported in the Table II.

Among the cases reported in the literature with clinical data, the patients showing respiratory involvement were the 8% (39/503). Amid these, around 30% (11/39) required NIV, as shown in the Table II. Notably, 14 patients died due to respiratory complications. Cardiac involvement was reported in about 20% of the cases (11/503). Our data are substantially in line with these results, suggesting that cardiac involvement and need of NIV are not specific and rare, in particular the former. In addition, about 256 patients underwent muscle biopsies and rimmed vacuoles have been detected in 106 samples (40%). Conversely, all our patients except patient 3, showed rimmed vacuoles at muscle biopsy.

For an in-depth study of the muscle tissue and correlation with the *VCP* mutations we performed immunofluorescence analysis to evaluate the expressions of p62, TDP-43 along with those of VCP, desmin and myotilin. In fact, desmin and myotilin have demonstrated to be sensitive diagnostic tools to depict pathological protein aggregation in MFM [26], while different studies have reported the presence of aggregates of p62, TDP43 and VCP in patients with mutation in *VCP* gene. Additionally, Inoue and colleagues [27] showed co-localization of VCP and ubiquitin positive inclusions both in the nucleus and the cytoplasm in 2 patients. Also, Bersano et al. [28] described a IBMPFD patient with the R159C mutation which biopsy was characterized by the presence of some fibres containing aggregates that were positive for VCP, alpha B-crystallin, myotilin or desmin. Finally, VCP and ubiquitin-positive cytoplasmic and nuclear inclusions were described in a patient with R155C mutation [18]. Conversely, in our patients, no significant positivity was observed for any of the tested proteins. The immunoassays of our patients were performed on quadriceps muscles, whereas in other works they used different muscles, e.g., Bersano et al. [28] biceps and the gastrocnemi and Hubbers et al. [19] biceps brachii, vastus lateralis, and tibialis anterior. Also, in one of the patients in the work of Bersano et al. no positivity for protein is shown, probably related to the lower severity of the phenotype at the time of biopsy. We could hypothesize that in our patients for the same reason, too, there is no signal in the muscle tissue.

Our report confirms that the R155C, P137L, R155H mutations present in our patients can be associated to a myopathic phenotype without any CNS involvement, as previously reported in the literature [21,15]. Indeed, all our patients have a predominant skeletal muscular phenotype. On the other hand, R93H mutation found in patient 1 has only been reported in the literature in association with HSP [17] and not causative of a myopathy.

Further studies are needed to better clarify disease natural history and genotype-phenotype correlations in VCP-related myopathies.

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Conflict of interest statement

The Authors declare no conflict of interest.

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Authors' contributions

EI: performed acquisition and analysis of data, revision of the literature and drafting of the manuscript; GR and FB: performed immunohistochemical analysis; SG and AC: acquired and analyzed genetic data; MC and LM: acquired and analyzed clinical data; SG, MC, LM and AR: revised the manuscript.

Ethical consideration

This study was approved by the Ethical Committee at the Fondazione IRCCS Istituto Neurologico C. Besta (project number 108/2020). Informed consents were obtained from the participant patients.

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