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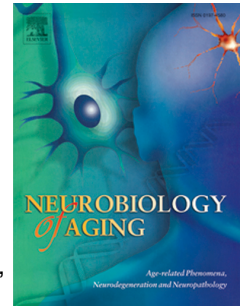
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Novel *VCP* mutations expand the mutational spectrum of frontotemporal dementia

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Key-words. Frontotemporal dementia; frontotemporal lobar degeneration; TDP-43; Paget's disease of bone; amyotrophic lateral sclerosis; valosin containing protein.

Abstract. *VCP* mutations are rare causes of autosomal dominant frontotemporal dementias associated with Paget's disease of bone, inclusion body myopathy and amyotrophic lateral sclerosis. We analyzed the *VCP* gene in a cohort of 199 FTD patients and identified 7 heterozygous mutations in unrelated families, including 3 novel mutations segregating with dementia. This expands the *VCP* mutation spectrum and suggest that although *VCP* mutations are rare (3.5% in this study) the gene should be analyzed even in absence of the full syndromic complex. Reporting genetic variants with convincing arguments for pathogenicity is important considering the large amount of data generated by next generation sequencing and the growing difficulties to interpret rare genetic variants identified in isolated cases.

1. Introduction Frontotemporal dementias (FTD) are neurodegenerative dementias displaying a considerable clinical, genetic and pathologic heterogeneity. Between 20 and 40% of cases are familial, *c9orf72* and *GRN* genes being, by far, the most common genetic causes of the disease. Many other genes, including valosin containing protein (*VCP*), contribute for a small proportion of familial cases (Watts et al., 2004). So far, more than 45 *VCP* mutations have been identified (Supplementary table S1). They are

responsible for a variable association of inclusion body myopathy (IBM), Paget's disease of bone (PDB),
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FTD and amyotrophic lateral sclerosis (ALS), known as IBMPFD or multisystem proteinopathy (MSP)
(Kim et al., 2013; Al-Obeidi et al., 2018). The common neuropathological substrate of *VCP* mutations is a
frontotemporal lobar degeneration (FTLD) with TDP43-immunoreactive inclusions according to the current
harmonised classification (Neumann et al., 2007; Mackenzie et al. 2011). In this paper, we have screened a
cohort of 199 FTD patients and identified three novel *VCP* mutations. We present the phenotypes of
mutation carriers and discuss the pathogenicity of the mutations in view of their predicted effect on protein
structure and the results of the *in silico* functional analyses.

2. Methods

2.1. Mutation screening and molecular analyses. We sequenced the *VCP* gene on DNA extracted from
lymphocytes by Sanger or exome sequencing in 199 unrelated FTD probands. The most frequent FTD, ALS
and MSP genes have been analyzed first by Sanger or exome sequencing (*MAPT*, *GRN*, *TBK1*, *SQSTM1*,
hnRNPA1, *hnRNPA2B1*, *TARDBP*, *CHMP2B*, *FUS/TLS*, *PFN1*, *UBQLN2*) or by repeat primed-PCR
(*c9orf72*), and mutation carriers were excluded from the screening. In three families (families 1, 2, 3)
carrying novel *VCP* mutations, DNA of affected relatives was also analyzed, when possible. In family 1,
DNAs of a proband's sib (III-2) and an affected relative (II-3) were available for genetic analyses (Figure 1).
In family 2 no affected relatives could be sampled for genetic analyses. One affected relative (III-2) was
analyzed in family 3 (Figure 1). Written informed consent for clinic-genetic studies was obtained for all
FTD probands and relatives. This study was approved by the local ethical committee (AP-HP-Paris).

3. Results

3.1. Molecular analyses. We identified 7 heterozygous missense mutations in 7 unrelated probands (7/199,
3.5% after exclusion of other FTD mutations). Four probands carried already known mutations
(p.Arg93Cys, p.Pro137Leu, p.Arg159His, p.Ala439Pro, supplementary table S1), 3 probands (families 1, 2
and 3) carried novel *VCP* mutations. A c.374G>A mutation (exon 4, NM_007126), leading to a
p.Gly125Asp substitution, was identified in the proband III-1 of family 1, as well as in two affected relatives
(II-3, III-2, Figure 1A) and an obligate transmitter (II-2). A c.353C>T, p.Pro118Leu mutation (exon 4) was

identified in proband of family 2. A c.296T>A, p.Val99Asp (exon 3) was identified in the proband (III-1) and one affected relative (III-2) in family 3. The three novel substitutions affected conserved residues across species, were located in the N-terminal domain of the protein and were absent from public databases of controls (EVS, ExAC, 1000Genome, dbSNP). The 3 mutations were predicted to be probably damaging (Wintervar) or deleterious (PolyPhen, Mutation Taster, SIFT) using four *in silico* prediction softwares (Supplementary table S2). No mutations were found in other MSP, FTD and ALS genes, thus strongly supporting that the p.Val99Asp, p.Pro118Leu and p.Gly125Asp are disease-causing mutations.

3.2. Probands and families description. The proband III-1 of family 1 (Figure 1A), carrying a c.374G>A, p.Gly125Asp mutation, was a French Caucasian subject who insidiously developed, at age 54, apathy and socially inappropriate behaviors. At the age of 57, he manifested familiarity, loss of empathy, anosognosia, grasp reflex and imitation behaviors. The neuropsychological evaluation disclosed impaired attention, planning abilities, working memory and mental flexibility. His score on the MMSE was 27/30, he scored 14/18 on the frontal assessment battery (FAB) and 6/20 on the Wisconsin card sorting test (WCST), with 2 criteria. The total recall score of the free and cued recall test was 47/48. Brain MRI showed bilateral mesial frontal and perisylvian atrophy, with left predominance (Supplementary Figure S1A, 1B); ^{99m}Tc-ECD SPECT revealed bilateral mesial frontal hypoperfusion. A behavioral FTD was diagnosed. The patient did not develop clinical symptoms of PDB nor IBM, but was not specifically investigated to detect asymptomatic PDB. He died at age 61. A brain post-mortem examination was performed that revealed marked cranial vault thickening evocative of PDB, and confirmed the diagnostic of FTLD. A proband's sib (III-2) was affected by PDB at the age of 50. In his early sixties, he developed delusional ideas and progressive behavioral disorders with apathy, agitation and impulsiveness. MRI showed fronto-temporal atrophy. A diagnosis of probable FTD was made. Proband's affected parent (II-2) and grandparent (I-1) also had PDB; they presented cognitive and behavioral disorders suggestive of FTD and leading to dementia. They died at age 77 and 74, respectively. Two other relatives (II-3, II-4) developed dementia and died at age 66 and 65.

The proband III-1 of family 2 (Figure 1B), who carried a c.353C>T, p.Pro118Leu mutation, developed progressive behavioral disorders at age 58, characterized by apathy, eating disorders (bulimia) and loss of social conducts. Neuropsychological testing revealed executive dysfunctions, impaired set-shifting on the WCST (1 criterion), reduced verbal fluencies (4 animals and 2 words beginning with the letter “P” in 2 minutes) but spared episodic memory. ^{99m}Tc-ECD SPECT showed marked frontal hypoperfusion, confirming the diagnosis of bvFTD. The patient was lost to follow up. A proband’s sib (III-2) developed verbal semantic disorders at age 64, associated with logorrhea, disinhibition, aggressiveness and executive dysfunctions. Brain MRI and ^{99m}Tc-ECD SPECT revealed marked, predominantly left, temporal lateral involvement at the age of 69. Serum alkaline phosphatases were normal. He died at age 70. Their affected parent (II-2) had a history of leg fracture and died of dementia associated with muscular weakness at the age of 53. Two sibs of the affected parent (II-3, II-4) also developed early-onset dementia, before the age of 60. There was no personal or familial history of PDB or myopathy, although not specifically investigated.

The proband III-1 of family 3 carried a c.296T>A, p.Val99Asp mutation (Figure 1C). He developed progressive behavioral disorders at the age of 65 (irritability, familiarity, joviality and rituals). At age 70, his score on the MMSE was 24/30, on the MDRS was 92/144 and 7/18 on the FAB. Brain MRI showed predominantly left, frontotemporal atrophy. ^{99m}Tc-ECD SPECT showed marked, mostly left, fronto-temporo-parietal hypoperfusion. He had no muscular symptoms or clinical PDB. He later developed delusions, and died at age 74. An affected sib (III-2) had seizures and cognitive disorders at age 66, characterized by executive dysfunction (FAB score: 14/18) and categorial fluency deficit (animals) (12, norms: 18±4). MMSE (30/30) and confrontation naming (14/15) were normal. Their affected parent (II-2) had a history of multiple bone fractures since age 62, and later developed gait disorders (age 63), dementia (age 71) and seizures (age 75). He died at age 78. A parent’s sib (II-3) had a bone fracture at age 62, and a PDB was confirmed by bones radiography and elevated serum alkaline phosphatases. He had dementia at age 72, and died at age 76.

4. Discussion. We report 3 novel *VCP* mutations segregating with FTD in 3 unrelated families. Their pathogenicity is highly probable since they affect highly conserved residues, they are predicted to be

damaging by *in silico* prediction softwares, and because of the absence of any other MSP and FTD disease-

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causing mutations. Furthermore, the variable association of FTD and PDB in patients of families 1 and 3 was highly suggestive of MSP, even in the absence of IBM or ALS. Although absent or not clinically detected in the patients described in this study, IBM is also one of the core features of MSP, present in 90% of mutation carriers (Al-Obeidi et al., 2018, supplementary Table S1). IBM, FTD, PDB and ALS can be variably associated in the same patient or within families; this inter- and intra-familial clinical variability could be partly due to the effect of genetic (or other) disease phenotype modifiers. *VCP* is the most frequent gene involved in this syndromic association, followed by *SQSTM1* gene (Le Ber et al., 2013). Mutations in the prion-like domains of two other genes coding for heterogeneous nuclear ribonucleoproteins, *hnRNPA1* and *hnRNPA2B1*, are only rarely involved (Kim et al., 2013). Proband's phenotypes are in line with the well-recognized heterogeneity of *VCP* mutations, which only rarely manifest with the full symptomatology of MSP. Patients of this study were mainly affected by a behavioral variant of FTD, but the phenotype of patient III-2 in family 1 suggests that verbal semantic deficit can be early associated to behavioral disorders. The p97/VCP protein, encoded by *VCP* gene, is a member of the AAA-ATPase superfamily including four domains: an N-terminal binding domain, two AAA modules and a C-terminal domain (Watts et al., 2004). It is involved in diverse cellular activities such as transcription, cell cycle control, autophagy, membrane fusion and Golgi reassembly. It also acts as a chaperone, and plays a critical role both in endoplasmic reticulum-associated protein degradation (ERAD), which is fundamental in protein quality control, and in ubiquitin-proteasome degradation system, a major cellular process of protein clearance (Meyer and Wheil, 2014). The p.Val99Asp, p.Pro118Leu and p.Gly125Asp mutations are all located in the N-terminal domain of the protein, where the vast majority of disease-causing mutations have been identified. This domain is responsible for binding to many cofactors, adaptors and ubiquitinated target proteins, similarly to the vast majority of other deleterious *VCP* mutations. It is thus possible that mutations altering this domain prevent p97/VCP to bind to its natural substrates and to perform its chaperone role in the ubiquitin-proteasome pathway (Tang and Xia, 2016). Functional studies are needed, however, to definitely confirm the pathogenicity and to validate the biological impact of these mutations, as well as identifying additional MSP families carrying these mutations.

5. Conclusion. This study expands the mutational spectrum of *VCP*, including three novel mutations

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affecting for the first-time codons 99, 118 and 125. Although the relative frequency of mutations is low, this study shows that *VCP* should be analyzed even in patients with isolated FTD. Finally, reporting genetic variants with convincing arguments for pathogenicity (particularly familial segregation) is important considering the huge amount of data generated by next generation sequencing, and the growing difficulties to interpret rare genetic variants identified in isolated cases.

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Figure and legend

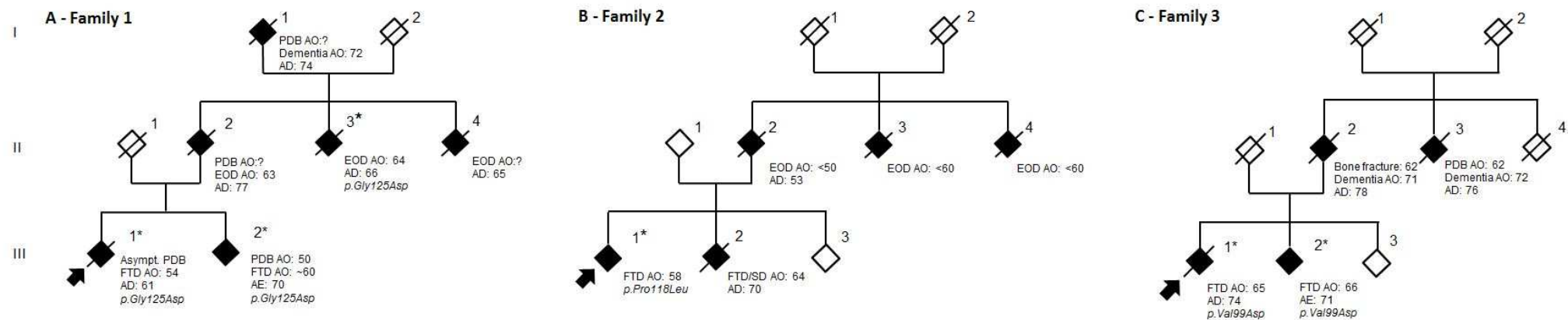


Figure 1. Pedigrees of the families. Individuals affected by FTD and/or PDB are indicated with black diamonds. The probands are indicated by an arrow. Pedigrees have been slightly simplified and gender has been masked to preserve confidentiality. Age at onset (AO), at last examination (AE) or at death (AD) are indicated for each affected individual. Mutation carriers are indicated by a star. A: family 1; II-2 is an obligate transmitter. B: family 2. C: family 3. Asympt. PDB: asymptomatic Paget disease of bone; EOD: Early onset dementia; FTD: frontotemporal dementia; PDB: Paget's disease of bone; SD: semantic dementia; ?: unknown data.

Highlights**Novel *VCP* mutations expand the mutational spectrum of frontotemporal dementia**

- 1) The frequency of *VCP* mutations is 3.5% in FTD.
- 2) Three novel missense mutations p.Gly125Asp, p.Pro118Leu and p.Val99Asp and their associated phenotypes are reported.
- 3) *VCP* mutations should be searched for even in FTD cases without the complete syndrome of multisystem proteinopathy.

Novel VCP mutations expand the mutational spectrum of frontotemporal dementia**Verification**

All the authors declare that they have no actual or potential conflicts of interest with this work.

No authors' institutions have contracts relating to this research through which they or any other organization may stand to gain financially now or in the future.

There are no other agreements (of authors or their institutions) that could be seen as involving a financial interest in this work.

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We assure that the data contained in the manuscript being submitted have not been previously published, have not been submitted elsewhere and will not be submitted elsewhere while under consideration at *Neurobiology of Aging*.

This study was approved by the local ethical committee (AP-HP-Paris).

All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

Yours sincerely,

Dario Saracino

Isabelle Le Ber

and all the co-authors