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ORIGINAL COMMUNICATION

CHMP2B mutations are rare in French families with frontotemporal lobar degeneration

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Abstract Two C-truncating *CHMP2B* (chromatin modifying protein 2B) mutations were recently found in Danish and Belgian families with autosomal dominant forms of frontotemporal lobar degeneration (FTLD). In addition, few *CHMP2B* missense mutations of uncertain pathogenic role were reported in several families with FTLD or FTLD associated with motoneuron disease (FTLD-MND). In order to determine the genetic contribution of *CHMP2B* mutations in FTLD and FTLD-MND families, we analyzed the *CHMP2B* gene in 198 French probands with familial FTLD and FTLD-MND. One *CHMP2B* missense variant was found in a proband with familial FTLD (0.8%). The pathogenic role of CHMP2B missense variants is unclear, however the pSer194Leu substitution, located in the C-terminal domain of the protein, was predicted to alter the stability of the protein by in silico analyses. We conclude that *CHMP2B* mutations represent a rare cause of familial FTLD and they are not implicated in familial FTLD-MND in French patients. The previously reported C-truncating *CHMP2B* mutations may be private to the Danish and Belgian pedigrees.

Keywords *CHMP2B* · FTLD · ALS · MND · ESCRT-III

Introduction

Frontotemporal lobar degeneration (FTLD) is the second cause of degenerative dementia after Alzheimer's disease [1]. FTLD is inherited as an autosomal dominant disorder in 30–50% of cases [2, 3]. In 2005, a splice site mutation in the *CHMP2B* (chromatin modifying protein 2B) gene was identified in a large Danish family with FTLD linked to the pericentromeric region of chromosome 3 (FTD3) [4].

The members of the French research network on FTD and FTD/MND are given in the Appendix.

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CHMP2B is a 213-amino-acid protein that contains two coiled-coil domains (residues 1–50, 120–150), a large Snf-7 domain (residues 16–178) and C-terminal domain (Supplementary figure 1a). It is expressed in the brain including the hippocampus, frontal and temporal lobes and cerebellum, in all neuronal populations [4]. CHMP2B is a subunit of the ESCRT-III complex (endosomal sorting complex required for transport-III), which is implicated in the endosomal–lysosomal pathway. This pathway sorts transmembrane proteins for either recycling by the Golgi complex or for degradation by the lysosome through the late endosomal pathway. The latter requires internalization of transmembrane proteins into multiple vesicular bodies (MVBs) [5].

The function of CHMP2B is largely regulated by its C-terminal domain, which normally keeps CHMP2B in the closed inactive state in the cytosol by masking the CHMP2B domains which interact with other ESCRT-III subunits. Upon activation, CHMP2B protein polymerizes with other ESCRT-III subunits to form ESCRT-III complex on the endosomal membrane [5, 6]. In addition, the C-terminal domain is required for dissociation of ESCRT-III by its interaction with the AAA-ATPase Vps4 (vacuolar protein sorting 4) after MVBs are formed [7].

To date, only two putatively pathogenic *CHMP2B* mutations have been identified. The first is a splice site mutation resulting in two C-truncated transcripts (p.Met178ValfsX2 and p.Met178LeufsX30), which segregates in a large Danish family with FTLD [4]. Another C-truncating mutation (p.Gln165X) was recently found in a Belgian family with FTLD [8]. The Danish and Belgian mutations both lead to a truncated CHMP2B protein of 36–49 amino-acids, respectively, in the C-terminal region of the protein [4, 8]. Further mutation analyses in various populations reported rare *CHMP2B* missense variants in few FTLD and FTLD-MND families but failed to identify the reported C-truncating mutations [8–12]. In the present study, we performed a systemic screen for *CHMP2B* mutations in a large number of French families with FTLD or FTLD-MND in order to accurately evaluate their genetic contribution to these phenotypes.

Materials and methods

Patients

A total of 198 unrelated patients with familial FTLD, including 130 patients with pure FTLD (without MND) and 68 patients with FTLD-MND, were recruited through a national clinical and genetic research network. Most of the families were French, four were African, and one was Russian. The diagnosis of FTLD was based on the revised Neary criteria [13]; the diagnosis of MND was based on the El Escorial criteria [14]. The mean age at onset in the overall population of patients was 59.4 ± 9.5 years (17–77). The mean age at onset in the patients with pure FTLD was 59.2 ± 9.8 years (17–76) and the mean age of onset in the patients with FTLD-MND was 59.7 ± 9 years (38–77).

Gene sequencing analysis

Blood samples were obtained after the patients gave a signed consent. Genomic DNA was extracted from peripheral blood. All exons and approximately 50-bp intron–exon boundaries of the *CHMP2B* gene were amplified by standard polymerase chain reaction in a total volume 25 µl (concentration of DNA 80 ng, every primer 0.4 µM, and DNA polymerase 0.5 U). Amplification products were purified using 1 U of Antarctic phosphatase (New England Biolabs, Ipswich, MA) and 1 U of exonuclease1 (New England Biolabs). Followed by a single bidirectional sequencing using the Big Dye Terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA) and ABI3730 automated sequencer (Applied Biosystems). In addition, 175 French neurologically healthy controls were also analyzed using the same protocol for all the exons. This study was approved by the Medical Research Ethics Committee of Assistance Publique Hôpitaux de Paris (AP-HP) and the ethics committee of the Salpêtrière hospital. The genotype and allelic frequencies of genomic variant was compared between the patients and the controls with a Chi-square and a Yates's correction for the small samples.

In silico analyses

The putative effect of the missense variant on CHMP2B stability and function were evaluated by in silico analyses with the software PolyPhen (http://genetics.bwh.harvard.edu/pph/), SNPs3D (http://www.snps3d.org) and PANTHER (http://www.pantherdb.org/tools/csnpScoreForm.jsp). PolyPhen predicts the difference in fitness between wild-type and mutant amino acid to either benign, possibly damaging, probably damaging with a score ranging form 0% (predicted benign) to 100% (predicted deleterious) [15]. The *SNPs3D* software uses the Support Vector Machine and data from 15 parameters (structure based) or five parameters (alignment based) to analyze substitutions. The output score is called the svm profile. Negative svm profiles are indicative of deleterious and positive profiles are indicative of neutral [15]. The *PANTHER* software estimates the likelihood of a functional effect from a single amino acid substitution as a subPSEC (substitution position-specific evolutionary conservation) score. *PANTHER*

subPSEC scores are continuous values from 0 (neutral) to about −10 (most likely to be deleterious). A cut-off of −3 corresponds to a 50% probability that a score is deleterious. The probability that a given variant will cause a deleterious effect on protein function is estimated by $P_{\text{deleterious}}$, such that a subPSEC score of −3 corresponds to a $P_{\text{deleterious}}$ of 0.5 [15].

Results

Molecular analyses

A novel heterozygous variant p.Ser194Leu (c.581C>T) was found in the exon 6 of the *CHMP2B* gene in one patient with pure FTLD (supplementary figure 1). The Serine194 is highly conserved among species (supplementary figure 1c). The segregation of the variant with the disease could not be studied, but this variant was not identified in controls.

A missense variant, p.Thr72Met, was found in the exon 3 in a patient, but it does not segregate with the disease in the family. It is therefore probably not pathogenic. Two other novel polymorphisms, p.Arg69Gln (c.206G>A) and p.Ser187Asn (c.560G>A), were found in one control each in the exons 3 and 6, respectively. No other missense variants were found in the controls. The other genomic variants were found with the same frequency in the patients and the controls (supplementary table 1).

In silico analyses

The three software programs predicted that the variant p.Ser194Leu has a damaging effect on the stability of CHMP2B structure. The PolyPhen software predicted a damaging effect (score 100%). A deleterious effect was also predicted with the PANTHER (subPSEC −3.36513, $P_{\text{deleterious}}$ 0.59028) and SNPs3D software (score −0.05).

Phenotype of the proband

The variant p.Ser194Leu was found in a proband with FTLD (Supplementary figure 1d). This patient had behavioral disorders at age 50. He progressively developed memory disorders, apathy, indifference, perseverations, and motor stereotypies. Parkinsonian symptoms, laterocollis and visual hallucinations were present at age 55. The MMSE score was 24, the Mattis dementia rating scale score was 108. He had severe amyotrophy but no clinical or electromyographic signs of motor neuron disease. He was severely demented at age 64. Brain MRI showed marked temporal atrophy, predominantly affecting the right side. Brain SPECT revealed bilateral frontal hypoperfusion. His mother and two maternal grand-aunts had behavioral disorders and were institutionalized in a psychiatric hospital (Supplementary figure 1d).

Discussion

We analyzed the *CHMP2B* gene in a large series of French patients with familial FTLD and FTLD-MND. We found a novel *CHMP2B* missense variant, p.Ser194Leu, in a proband with familial FTLD (0.8%, 1/130) but no *CHMP2B* mutations in patients with familial FTLD-MND. Several missense variants were previously identified in patients with FTLD, FTLD-MND, or isolated MND, but their pathogenicity is not clearly established [8–12]. Similarly, we cannot establish the pathogenicity of this variant but in silico analyses using three different software programs consistently predicted that p.Ser194Leu variant has a damaging effect on the stability of the CHMP2B protein. However, recent studies have shown that truncating mutations [16] as well as three missense mutations (p. Ile29Val, p.Gln206His, p.Thr104Asn) [17] confer a specific aberrant endosomal phenotype in transfected cell lines, the later study providing more functional evidence for pathogenicity of *CHMP2B* missense mutations.

Furthermore, the Ser194 is a highly conserved residue localized in the functional C-terminal region of the protein. The Danish and Belgian *CHMP2B* mutations both lead to a truncated protein of 36–49 amino-acids in the C-terminal region, or to an aberrant C-terminal sequence [4]. It is unknown how C-truncating *CHMP2B* mutations lead to neurodegeneration but it is largely believed to be due to dysfunctional ESCRT-III complex which impairs multivesicular bodies (MVB) formation [4, 8]. The acidic C-terminal domain of CHMP2B normally interacts with its basic N-terminal domain, thereby closing the protein and inhibiting its properties to bind to the endosomal membrane and to other proteins of ESCRTIII complex. CHMP2B normally shifts from a closed inactive soluble state in the cytosol to an open activated state on the endosomal membrane. The Danish and Belgian C-truncating mutations both abolish the autoinhibitory role of the C-terminal domain, maintaining CHMP2B in the active state and subsequently bound to other components of ESCRT-III complex on the endosomal membrane [5]. In addition, CHMP2B recruits the AAA-ATPase Vps4 through its C-terminal region, which lead to the active dissociation of the complex ESCRT-III and to the inactivation of CHMP2B [5, 6]. The C-truncating mutations could therefore also disrupt the dissociation of the ESCRT-III complex by blocking its interaction with the

AAA-ATPase Vps4, resulting in impaired formation of multiple vesicular bodies [8]. It has been shown that the overexpression of both the Danish and the Belgian C-truncated CHMP2B in rat PC12 cells resulted in the appearance of abnormally enlarged and dysmorphic endosomes [4, 8]. Similarly, the p.Ser194Leu variant, which is located in the C-terminal domain of the protein could abrogate the binding of the protein to Vps4 and the autoinhibitory effect of the C-terminal domain of CHMP2B protein as do the reported C-truncating mutations [4, 8].

Despite the rare contribution of *CHMP2B* mutations to FTLD, their discovery is important to link FTLD to other neurodegenerative conditions associated with abnormal endosomes like Alzheimer's disease [18]. The reported *CHMP2B* splice site mutation seems to be private to the Danish FTD3 family. Interestingly, FTD3 represents a unique pathologic subtype of FTLD-U as it was found to be TDP-43 negative [19]. Furthermore, contrary to most FTLD-U cases negative to TDP-43, FTD3 is not immunoreactive to FUS [20]. The presence of TDP-43 and FUS-negative ubiquitine-positive inclusions would further reinforce the pathogenic role of the p.Ser194Leu mutation in our patient.

In conclusion, our study confirms that *CHMP2B* mutations are a rare cause of familial FTLD in the French population. It indicates that *CHMP2B* mutations are either not or rarely implicated in familial FTLD-MND cases. These results are in accordance with previous studies in different Caucasian populations [8–12]. Our results indicate that screening for *CHMP2B* mutations should be taken into consideration only after the exclusion of *PGRN*, *MAPT* and *VCP* mutations in cases with familial FTLD.

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Conflict of interest statement The authors declare that they have no conflicts of interest.

Appendix

The French clinical and genetic research network on FTD/FTD-MND includes: Alexis Brice (Hôpital de la Salpêtrière, Paris), Frédéric Blanc (Hôpitaux Civils, Strasbourg), William Camu (CHU Gui de Chauliac, Montpellier), Françoise Clerget-Darpoux (Hôpital Paul Brousse, Villejuif), Philippe Corcia (CHU Tours), Mira Didic (CHU La Timone, Marseille), Bruno Dubois (Hôpital de la Salpêtrière, Paris), Charles Duyckaerts (Hôpital de la Salpêtrière, Paris), Marie-Odile Habert (Hôpital de la Salpêtrière, Paris), Véronique Golfier (CHU Rennes), Eric Guedj (CHU Marseille), Didier Hannequin (CHU Charles Nicolle, Rouen), Lucette Lacomblez (Hôpital de la Salpêtrière, Paris), Isabelle Le Ber (Hôpital de la Salpêtrière, Paris), Richard Levy (CHU St Antoine, Paris), Vincent Meininger (Hôpital de la Salpêtrière, Paris), Bernard-François Michel (CH Sainte-Marguerite, Marseille), Florence Pasquier (CHU Roger Salengro, Lille), Catherine Thomas-Anterion (CHU Bellevue, Saint-Etienne), Michèle Puel (CHU Rangueil, Toulouse), François Salachas (Hôpital de la Salpêtrière, Paris), François Sellal (CH Colmar), Martine Vercelletto (CHU Laennec, Nantes), Patrice Verpillat (Hôpital de la Salpêtrière, Paris).

References

1. Neary D, Snowden J, Mann D (2005) Frontotemporal dementia. Lancet Neurol 4:771–780
2. Chow TW, Miller BL, Hayashi VN, Geschwind DH (1999) Inheritance of frontotemporal dementia. Arch Neurol 56(7):817–822
3. Rosso SM, Donker Kaat L, Baks T, Joosse M, de Koning I, Pijnenburg Y, de Jong D, Dooijes D, Kamphorst W, Ravid R, Niermeijer MF, Verheij F, Kremer HP, Scheltens P, van Duijn CM, Heutink P, van Swieten JC (2003) Frontotemporal dementia in The Netherlands: patient characteristics and prevalence estimates from a population-based study. Brain 126(Pt 9):2016–2022
4. Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, Nielsen JE, Hodges JR, Spillantini MG, Thusgaard T, Brandner S, Brun A, Rossor MN, Gade A, Johannsen P, Sørensen SA, Gydesen S, Fisher EM, Collinge J (2005) Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. Nat Genet 37(8):806–808
5. Shim S, Kimpler LA, Hanson PI (2007) Structure/function analysis of four core ESCRT-III proteins reveals common regulatory role for extreme C-terminal domain. Traffic 8(8):1068–1079
6. Zamborlini A, Usami Y, Radoshitzky SR, Popova E, Palu G, Gottlinger H (2006) Release of autoinhibition converts ESCRT-III components into potent inhibitors of HIV-1 budding. Proc Natl Acad Sci USA 103:19140–19145
7. Stuchell-Brereton MD, Skalicky JJ, Kieffer C, Karren MA, Ghaffarian S, Sundquist WI (2007) ESCRT-III recognition by VPS4 ATPases. Nature 449:740–744
8. Van der Zee J, Urwin H, Engelborghs S, Bruyland M, Vandenberghe R, Dermaut B, De Pooter T, Peeters K, Santens P, De Deyn PP, Fisher EM, Collinge J, Isaacs AM, Van Broeckhoven C (2008) CHMP2B C-truncating mutations in frontotemporal lobar degeneration are associated with an aberrant endosomal phenotype in vitro. Hum Mol Genet 17(2):313–322
9. Cannon A, Baker M, Boeve B, Josephs K, Knopman D, Petersen R, Parisi J, Dickison D, Adamson J, Snowden J, Neary D, Mann D, Hutton M, Pickering-Brown SM (2006) CHMP2B mutations are not a common cause of frontotemporal lobar degeneration. Neurosci Lett 398(1–2):83–84
10. Momeni P, Rogaeva E, Van Deerlin V, Yuan W, Grafman J, Tierney M, Huey E, Bell J, Morris CM, Kalaria RN, van Rensburg SJ, Niehaus D, Potocnik F, Kawarai T, Salehi-Rad S, Sato C, St

George-Hyslop P, Hardy J (2006) Genetic variability in CHMP2B and frontotemporal dementia. Neurodegener Dis 3(3):129–133
11. Parkinson N, Ince PG, Smith MO, Highley R, Skibinski G, Andersen PM, Morrison KE, Pall HS, Hardiman O, Collinge J, Shaw PJ, Fisher EM MRC, Proteomics in ALS Study; FReJA Consortium (2006) ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). Neurology 67(6):1074–1077
12. Rizzu P, van Mil SE, Anar B, Rosso SM, Kaat LD, Heutink P, van Swieten JC (2006) CHMP2B mutations are not a cause of dementia in Dutch patients with familial and sporadic frontotemporal dementia. Am J Med Genet B Neuropsychiatr Genet 141B(8):944–946
13. Neary D, Snowden J, Gustafson L, Passant U, Stuss D, Black S, Freedman M, Kertesz A, Robert PH, Albert M, Boone K, Miller BL, Cummings J, Benson DF (1998) Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 51(6):1546–1554
14. Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron Diseases (2000) El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 1(5):293–299
15. Sunyaev S, Ramensky V, Koch I, Lathe W, Kondrashov AS, Bork P (2001) Prediction of deleterious human alleles. Hum Mol Genet 10(6):591–597
16. Urwin H, Authier A, Nielsen JE, Metcalf D, Powell C, Froud K, Malcolm DS, Holm I, Johannsen P, Brown J, Fisher EM, van der Zee J, Bruyland M, FReJA Consortium, Van Broeckhoven C, Collinge J, Brandner S, Futter C, Isaacs AM (2010) Disruption of endocytic trafficking in frontotemporal dementia with CHMP2B mutations. Hum Mol Genet 19(11):2228–2238
17. Cox LE, Ferraiuolo L, Goodall EF, Heath PR, Higginbottom A, Mortiboys H, Hollinger HC, Hartley JA, Brockington A, Burness CE, Morrison KE, Wharton SB, Grierson AJ, Ince PG, Kirby J, Shaw PJ (2010) Mutations in CHMP2B in lower motor neuron predominant amyotrophic lateral sclerosis ALS. PLoS One 5(3):9872
18. Nixon RA (2005) Endosome function and dysfunction in Alzheimer's disease and other neurodegenerative diseases. Neurobiol Aging 26(3):373–382
19. Holm IE, Englund E, Mackenzie IR, Johannsen P, Isaacs AM (2007) A reassessment of the neuropathology of frontotemporal dementia linked to chromosome 3. J Neuropathol Exp Neurol 66(10):884–891
20. Holm IE, Isaacs AM, Mackenzie IR (2009) Absence of FUS-immunoreactive pathology in frontotemporal dementia linked to chromosome 3 (FTD-3) caused by mutation in the CHMP2B gene. Acta Neuropathol 118(5):719–720