The role of CHMP2B in frontotemporal dementia

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Abstract

Mutations in the CHMP2B (charged multivesicular body protein 2B) gene that lead to C-terminal truncations of the protein can cause frontotemporal dementia. CHMP2B is a member of ESCRT-III (endosomal sorting complex required for transport III), which is required for formation of the multivesicular body, a late endosomal structure that fuses with the lysosome to degrade endocytosed proteins. Overexpression of mutant C-terminally truncated CHMP2B proteins produces an enlarged endosomal phenotype in PC12 and human neuroblastoma cells, which is likely to be due to a dominant-negative effect on endosomal function. Disruption of normal endosomal trafficking is likely to affect the transport of neuronal growth factors and autophagic clearance of proteins, both of which could contribute to neurodegeneration in frontotemporal dementia.

Introduction

FTD (frontotemporal dementia) is the second most common form of primary degenerative dementia after Alzheimer’s disease [1], and displays clinical, neuropathological and genetic variation. Approx. 40% of FTD cases are familial, with a first-degree relative also affected [1,2]. FTD-causing mutations have been found in the MAPT (microtubule-associated protein tau) [3] and PGRN (progranulin) [4,5] genes. MAPT mutations account for approx. 10% of FTD cases [6] and those in PGRN cause a similar amount [5,7]. Mutations in CHMP2B (charged multivesicular body protein 2B) are a much rarer cause of FTD [8], as are mutations in VCP (valosin-containing protein), which cause FTD associated with inclusion body myopathy and Paget’s disease of bone [9]. Neuropathologically, FTDs are categorized as having primary tau pathology, tau-negative FTLD-U (frontotemporal dementia with ubiquitinated inclusions) or DLDH (dementia lacking distinct histopathology) [10]. In most cases of FTLD-U, the major ubiquitinated protein has been identified as TDP-43 (TAR-DNA-binding protein-43) [11], although the role of this protein in the pathogenesis of FTD is still unclear.

FTD3 (FTD linked to chromosome 3)

FTD3 is an autosomal dominant presenile dementia. It is associated with a large pedigree originating from the Jutland region of Denmark. FTD3 has an onset of 48–67 years and duration of 3–21 years. The clinical phenotype includes changes in personality, disinhibition, dyscalculia and hyperorality, speech disturbances progressing to a non-fluent aphasia, and dystonic posturing [12,13]. FTD3 is a TDP-43-negative FTLD-U, characterized by generalized symmetrical frontal and temporal cortical atrophy, variable numbers of small round ubiquitin-positive neuronal cytoplasmic inclusions in the dentate gyrus and sparse ubiquitin-positive inclusions in frontal and temporal cortical neurons [14]. Ubiquitin-positive inclusions were also immunoreactive for p62, also called SQSTM1 (sequestosome 1) [14], which has a role in the targeting of inclusion bodies for degradation by autophagy [15–17].

CHMP2B mutations in FTD

Linkage analysis on the Jutland pedigree identified a critical region of 15.5 Mb, and sequencing of all known genes in this region found a disease segregating mutation in CHMP2B [8,18]. This G>C transition in the 5′ acceptor splice site of exon 6 produces two aberrant transcripts: CHMP2BIntron5 and CHMP2BIntron6, which were shown by RT (reverse transcription)–PCR to be expressed in FTD3 patient brain, and not in controls [8]. CHMP2BIntron5 retains intron 5 and CHMP2BIntron6 uses a cryptic splice site 10 bp into exon 6, resulting in a 10 bp deletion and a sequence frameshift (Figure 1). The proteins predicted from these splice variants are both C-terminal truncations of 36 amino acids (Figure 2). CHMP2BIntron5 has a novel C-terminal valine residue resulting from translation of intron 5, the second codon of which is a stop codon. CHMP2BIntron6 replaces the C-terminal 36 amino acids with 29 residues of nonsense sequence which has no homology with any known sequence. CHMP2B mutations remain a rare cause of FTD, since most further screens of other populations have not identified mutations [18–20].

A CHMP2B nonsense mutation (C493T) predicting replacement of a glutamine residue with a stop codon (CHMP2BQ165X) has been found in an unrelated familial
Figure 1 | CHMP2B splice variants
Schematic view of CHMP2B genomic DNA, position of the splice site mutation (marked with an asterisk) and the resulting transcripts. ORF, open reading frame.

Figure 2 | Multiple alignment of CHMP2Bs
Wild-type CHMP2B, the predicted isoforms produced by CHMP2B mutations and CHMP2B homologues from other species were aligned, showing that the protein is highly conserved in evolution. A black background indicates identical amino acids, and a grey background indicates a conservative amino acid substitution. The novel C-termini of CHMP2BItron5 and CHMP2BΔ10 are underlined.

Belgian FTD patient, but was not present in an additional 459 Belgian controls [21]. Expression of the aberrant transcript was detected by RT–PCR in lymphoblast cells from the index patient [21]. CHMP2BQ165X represents an even more severe C-terminal truncation than the FTD3 mutant proteins, of 49 amino acids (Figure 2).

CHMP2B is a member of the ESCRT (endosomal sorting complex required for transport) III, which has roles in sorting proteins in the endocytic pathway [22], autophagy [23,24], viral budding [25] and cytokinesis [26,27]. The role of CHMP2B in the endosome–lysosome and autophagy pathways, and their relevance to neurodegeneration are discussed further below.

CHMP2B and endosomal trafficking
In the endocytic pathway, proteins from the cell membrane are internalized, or endocytosed, in vesicles which converge on early endosomes from which they are recycled back to the cell membrane, or are trafficked via late endosomes to the lysosome for degradation. A subset of late endosomes are termed multivesicular bodies as they contain a number of ILVs (intraluminal vesicles) which are formed by invagination of the outer endosomal membrane. Cargo proteins that are destined for degradation are sorted into these ILVs, so that, upon fusion with the lysosome, they are delivered to the lysosomal lumen and degraded by hydrolases. ESCRT-III, and the other ESCRTs, I and II, provide the cellular machinery for
the sorting of proteins into ILVs and are required for efficient lysosomal degradation of proteins (reviewed in [22]).

Overexpression of CHMP2B mutant proteins produces an enlarged endosomal phenotype in PC12 cells and the human neuroblastoma cell lines SK-N-SH and SH-SY-5Y [8,21]. Whereas wild-type CHMP2B was expressed diffusely throughout the cytoplasm, CHMP2BItron5 and CHMP2BQ165X formed discrete puncta. These aberrant CHMP2B puncta were shown to co-localize with the late endosomal marker CD63, suggesting that they represent enlarged late endosomes. Interestingly, CHMP2BQ165X also translocated to the nucleus in 78% of transfected cells [21].

These data are consistent with overexpression data on other related CHMPs. CHMPs comprise a family of highly structurally similar coiled-coil proteins with basic positively-charged N-terminal halves and acidic negatively-charged C-terminal halves [28]. Shim et al. [29] found that deletion of one predicted α-helical domain from the C-terminus of CHMPs (a similar truncation to CHMP2BItron5) caused a redistribution from diffuse cytosolic expression to localization on enlarged vacuoles. Deletion of a further α-helix (a similar truncation to CHMP2BQ165X) resulted in enlarged vacuoles and nuclear translocation of CHMP3, CHMP4A and CHMP2A.

It has been suggested that the negatively charged C-terminus of CHMPs functions as an autoinhibitory domain, interacting with the positively charged N-terminus to keep the protein in a ‘closed’ conformation which masks the membrane-binding and polymerization properties of the N-terminus [29–32]. Deletion of the C-terminus would therefore be predicted to produce a constitutively active protein which is likely to polymerize and accumulate on endosomal membranes, leading to a dominant-negative inhibition of the ESCRT pathway. This is likely to be exacerbated by the fact that the C-terminus of CHMP2B is also necessary for binding of Vps4 (vacular protein sorting 4) [33,34], which is required for the active dissociation of ESCRTs from the endosomal membrane. CHMP2BItron5, CHMP2B410 and CHMP2BQ165X lose this Vps4-binding domain, which would be predicted to prevent dissociation from the endosomal membrane and thus could explain the endosomal localization of the CHMP2B mutant proteins.

In summary, C-terminal truncation has a clear effect on the normal function of CHMP2B, and a dominant-negative action of the mutant proteins may explain the pathogenesis of FTD caused by CHMP2B mutations. Two cellular processes which are likely to be affected by endosomal dysfunction, and impairment of which may contribute to neurodegeneration, are growth factor trafficking and autophagy.

**Endosomal trafficking and neuronal growth factors**

Endosomal trafficking provides a system for the transport of neuronal growth factors, or neurotrophins, within a cell. Secreted growth factors act as signalling molecules between target tissues and innervating cells, influencing cell survival, proliferation and differentiation. Neuronal growth factors, as well as their receptors and associated signalling molecules, are known to be trafficked through endosomes (reviewed in [35]). Dysfunction of this process could potentially lead to a lack of trophic support to the cell and aberrant cellular signalling. Dysfunction of endosomal trafficking, caused by ESCRT depletion, has been shown to inhibit the normal degradation of EGF (epidermal growth factor) and its receptor, EGFR [36,37].

Null mutations in PGRN [4,5] are a more common cause of FTD than CHMP2B mutations, accounting for approx. 10% of cases [38]. PGRN acts as a neuronal growth factor; increasing survival time and maximal neurite outgrowth in rat motor and cortical neurons [39]. It will therefore be of interest to examine the potential role of CHMP2B in the endosomal trafficking of growth factors, including PGRN, and to determine whether there is any interaction between these two disease proteins, or between the pathways in which they act.

**CHMP2B and autophagy**

As well as functioning in the endosomal degradative pathway, CHMP2B is also likely to have a role in macroautophagy, commonly referred to as autophagy. Autophagy is a bulk degradation mechanism for long-lived proteins and organelles, whereby cytoplasmic material is sequestered within double-membrane vesicles (autophagosomes) which eventually fuse with lysosomes, where the autophagocytosed material is degraded [40]. Autophagosomes also undergo fusion with late endosomes to form amphisomes [41,42], suggesting an important role for endosomes in autophagic degradation. This is particularly relevant to neuronal survival, since impairing autophagy in mouse brain leads to neurodegeneration [43,44].

Recent data has shown that functional ESCRT complexes are required for autophagic fusion and clearance, since depletion of ESCRT proteins inhibits the fusion of autophagosomes with endosomes and/or lysosomes and impedes autophagic degradation of disease proteins such as polyglutamine-expanded huntingtin [23,24]. Significantly, overexpression of CHMP2BItron5 causes accumulation of ubiquitin and p62-positive structures, increased numbers of autophagosomes and impaired fusion of autophagosomes with lysosomes in cell models and Drosophila [23,45]. Accumulation of ubiquitin- and p62-positive structures is also observed in FTD3 patient brain [14], indicating that impaired autophagy may be a contributing factor to cell death in FTD3.

**Future directions**

Overexpression of CHMP2B mutant proteins in cell culture causes endosomal abnormalities consistent with a toxic gain-of-function or dominant-negative mode of action. It will be important to confirm these phenotypes in patient tissue and address whether the loss of one normal allele of CHMP2B also plays a role in the disease process. The development and study of CHMP2B transgenic and knockout mice will help dissect this issue. It is clear that the ESCRTs perform a number of roles within the cell, so the continued analysis
of the normal function of CHMP2B will also be important in revealing further functions which may contribute to neuronal dysfunction and death.

Conclusions

New insights have been gained into the functions of CHMP2B by studying the C-terminal truncations associated with disease. A number of potential pathways could contribute to the neurodegeneration observed in FTD3, including impaired endosomal trafficking, which may have an impact on transport of and signalling by neuronal growth factors, and impairment of autophagic fusion and clearance. Further investigation of these pathways will lead to a better understanding of the pathophysiology of FTD and eventual development of therapeutics.

Acknowledgments

We thank the Frontotemporal Dementia Research in Jutland Association (FReJA) for their continued collaboration, the FTD3 family for their ongoing support of this research and Ray Young for illustrations.

Funding

This work is supported by the Medical Research Council.

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Received 11 September 2008
doi:10.1042/BST0370208