

## **Supplementary Methods**

**Subjects.** Informed consent for research was obtained for all participating individuals. The study was approved by the Institute of Neurology/National Hospital for Neurology and Neurosurgery Research Ethics Committee and by the research ethics Committee for Viborg and North–Jutland Counties in Denmark. Blood samples were obtained from affected and unaffected family members and Danish non–blood relatives, usually spouses. Peripheral blood lymphocytes were used to generate cell lines by EBV transformation at European Collection of Cell Cultures (ECACC), CAMR, Wiltshire. Control DNA samples were obtained from the Centre d'Etude du Polymorphisme Humain (CEPH). Brain samples were obtained at autopsy from affected family members; control brain samples were obtained from the MRC Neurodegenerative Diseases Brain Bank at the Institute of Psychiatry, London.

**Haplotyping.** Fine mapping was performed using short tandem repeat (STR) markers, *D3S3581*, *D3S1577*, *D3S1595*, *D3S3671*, *D3S2386*, *D3S3690*, *D3S1536*, *D3S3634*, *D3S1603* and *D3S1271*. STR markers were typed using FAM fluorescently labelled forward primers (Sigma Genosys). Genotyping was carried out on an ABI model 377 sequencer (Applied Biosystems) and haplotypes constructed.

**PCR amplification of *CHMP2b*.** DNA was extracted from blood using Nucleon BACC2 DNA extraction kit (Amersham–Pharmacia). The acceptor splice site and start of exon 6 were amplified using the forward and reverse primers. Primers were also designed to flank regions outside the start and finish of exon 1-6 (Supplementary Table 1). PCR was carried out using 10ng genomic DNA, 35 cycles of 92°C for 30 sec, 55°C for 45 sec and 72°C for 1 min. PCR products were cleaned using Microclean (Microzone).

**Sequencing of *CHMP2b*.** Sequencing reactions were carried out in both forward and reverse directions using dynamic ET terminator chemistry (Amersham–Pharmacia) on MegaBACE 3000 instrument (Amersham–Pharmacia).

**Cell culture.** Lymphoblast cell lines from affected and unaffected family members (supplied by ECACC) were grown in RPMI medium supplemented with 10% fetal calf serum (FCS) and 2mM L–Glutamine. The cultures were maintained at a cell density of between  $3 \times 10^5$ – $2 \times 10^6$  cells/ml, with 5% CO<sub>2</sub> at 37°C.

**RNA extraction.** Total RNA was isolated from lymphoblasts of affected and unaffected family members using RNeasy® Midi prep (Qiagen) and RNA was extracted from brain tissue using RNeasy® Lipid Tissue Midi Prep (Qiagen).

**Reverse Transcription–Polymerase Chain Reaction (RT–PCR).** First strand cDNA synthesis was carried out using the Reverse Transcription System (Promega) according to the manufacturer’s standard protocol using human lymphoblast and brain total RNA. Two sets of primers were used to amplify, exons 4 to 6 and the entire ORF (Supplementary Table 1). RT–PCR fragments were gel extracted using QIAEXII DNA extraction kit (Qiagen).

**Cloning of *CHMP2b*.** The full–length coding region of wild–type and mutated forms of *CHMP2b* were cloned into the vector pCR®2.1 TOPO® (Invitrogen) and sequenced.

**Northern blot analysis.** A Human 12–lane Blot and a Human Brain Blot II (Biosciences) were hybridised with an [ <sup>32</sup>P]–labelled probe to the whole ORF of *CHMP2b*. The probe was made by random primed DNA labelling using Primer It II Random Primer labelling kit (Stratagene). The blots were incubated with the probe for 1 hr at 68°C in ExpressHyb solution (BD Biosciences). After washing, the blots were placed on intensifying screens with BioMax–MR–I–Film (Anachem) at –70°C for between 5–16 hrs prior to developing.

**In Situ hybridisation.** First strand cDNA synthesis was carried out using the Reverse Transcription System (Promega) according to the manufacturer's standard protocol on mouse whole brain total RNA samples. An 813bp fragment of mouse *CHMP2b* cDNA was amplified (Supplementary Table 1), cloned into the vector pSPT19 (Roche Applied Science) and used to synthesise DIG labeled sense and anti-sense ribo probes (Roche Applied Science) using the manufacturer's standard protocol. Normal mouse brains were mounted onto Superfrost slides (VWR International) using standard protocols. Samples were heat pre-treated in a microwave at 900 Watts for 25 mins cooled and hybridized with 70ng of sense or anti-sense riboprobe per slide on a Discovery Machine (Ventana Medical Systems Inc) according to standard operating protocols. Biotin conjugated anti-DIG antibody (Jackson ImmunoResearch) was incubated for 40 mins with substrate incubation for 6 hrs. The slides were then counterstained with nuclear fast red for 20 mins, washed, de-hydrated and coverslip mounted with Pertex (Meditex).

**Expression constructs.** c-myc tags were fused to the N-terminal of wild-type and mutant forms of *CHMP2b* and then cloned into the vector pLNCX2 (BD Biosciences). Complete ORFs including the N-terminal c-myc tag were checked by DNA sequencing in all constructs.

**Transient transfections.** PC12 cells were cultured in DMEM (Gibco) containing 4500mg/L glucose, 4mM L-glutamine, 1mM sodium pyruvate, 5% FCS (Invitrogen), 10% Horse Serum (Gibco), 10mM HEPES (Sigma) and 0.2U/ml Bovine serum (Sigma) at 37°C in a humid environment and 5% CO<sub>2</sub>. PC12 cells were seeded onto poly-L-lysine (1mg/ml) coated 22mm coverslips at a density of 1x10<sup>5</sup> cells per coverslip. After 12 hrs the cells were transfected with 4µl Lipofectamine2000 (Invitrogen) and 2µg construct DNA following manufacturer's instructions.

**Immunofluorescence.** PC12 cells were fixed in 4% paraformaldehyde (Sigma) for 20 mins, 48 hrs after transfection. The cells were washed in PBS, permeabilised in 0.2% Triton X-100 for 10 mins and blocked with 4% goat serum (Sigma) in PBS for 30 mins at 37°C. The cells were incubated with goat FITC conjugated polyclonal antibody to c-myc (Abcam) diluted in 1% goat serum at a 1:100 dilution for 1 hr at 37°C. The cells were washed in PBS, rinsed with ddH<sub>2</sub>O and mounted on glass slides in fluorescent mounting media (Dakocytomation) with 1 g/ml of DAPI (Sigma) to stain the nuclei. Where co-staining with CD63 was required, cells were blocked in 4% goat serum, washed in PBS and incubated with goat FITC conjugated polyclonal antibody to c-myc and mouse IgG<sub>1</sub> monoclonal against rat CD63 (BD Pharmingen). Both were diluted 1:100 in 1% goat serum, and incubated for 1 hr at 37°C. After incubation the cells were washed and incubated in Alexa Fluor 568 goat antibody against mouse IgG<sub>1</sub> at a 1:1000 dilution in 1% goat serum then incubated for 1 hr at 37°C. The cells were washed in PBS, rinsed with ddH<sub>2</sub>O and mounted on glass slides in fluorescent mounting media (Dakocytomation) with 1 g/ml of DAPI (Sigma) to stain the nuclei.

**Phenotypic evaluation.** To quantify changes in the cellular phenotype of the different CHMP2b isoforms at least 200 cells transfected with each CHMP2b construct i.e. positive for c-myc fluorescence, were scored for either a uniform expression pattern or a an aberrant punctate expression pattern (aberrant bodies were of variable size). The percentage of the aberrant punctate phenotype, displayed in at least 200 c-myc positive cells, was calculated for each CHMP2b isoform. For example CHMP2b<sup>Intron5</sup> had 135 (68%) c-myc positive cells showing aberrant bodies in their cytosol, whilst 65 (32%) cells showed a uniform expression of c-myc in the cytosol. Whereas cells c-myc positive for CHMP2b<sup>Wild-type</sup> had 10 (5%) cells displaying aberrant bodies whilst

203 (95%) cells showed a uniform expression for c-myc.

**Staining of the endocytic compartments with Dextran.** Transfected PC12 cells seeded on coverslips were incubated in Texas Red 10,000MW dextran (Molecular Probes) at a concentration of 0.1mg/ml for 3 hrs. After incubation, the cells were washed and incubated in fresh media for a further 2 hrs. Cells were then washed in PBS, and fixed in 4% paraformaldehyde (Sigma). The above protocol for immunofluorescence was then carried out.

**Confocal fluorescent microscope analysis.** Fluorescence images were obtained using a LSM 510 META confocal microscope (Carl Zeiss, Jena, Germany) equipped with a “plan-Apochromat” 63x/1.40 oil DIC objective at room temperature. Fluorescence was recorded at 488nm using 30mW Ar-laser for excitation or at 543nm using 1mW HeNE-laser for excitation. Image processing and editing were carried out with Zeiss LSM 5, Illustrator and Photoshop 6 software.

**Accession numbers.** Human *CHMP2b* mRNA GenBank NM\_014043.2. *CHMP2b* protein sequence NP\_054762.1. *CHMP2b* cDNA BC001553.1. *CHMP2b* ESTs; AK002180, AL080122, AF15184 and CR533456. *CHMP2b* orthologs; mouse NP\_081155.1, rat XP\_343996.1, chicken ENSGALP00000024944, pufferfish SINFRUP00000145644, zebrafish AAH4530.1 and *Drosophila* NP\_647947.1.