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A novel function of Huntingtin in the cilium and retinal ciliopathy in Huntington's disease mice

Alice Karam [a,b,c,d], Lars Tebbe [e], Chantal Weber [a,b,c,d], Nadia Messaddeq [a,b,c,d], Laurette Morlé [g], Pascal Kessler [a,b,c,d], Uwe Wolfrum [e,f], Yvon Trottier [a,b,c,d,*]

[a] Institute of Genetics and Molecular and Cellular Biology, 67404 Illkirch, France
[b] Centre National de la Recherche Scientifique, UMR7104, 67404 Illkirch, France
[c] Institut National de la Santé et de la Recherche Médicale, U964, 67404 Illkirch, France
[d] Université de Strasbourg, 67000 Strasbourg, France
[e] Cell and Matrix Biology, Institute of Zoology, Johannes Gutenberg University of Mainz, 55122 Mainz, Germany
[f] Focus Program Translational Neurosciences (FTN), Johannes Gutenberg University of Mainz, 55122 Mainz, Germany
[g] Centre de Génétique et de Physiologie Moléculaires et Cellulaires, CNRS UMR 5534, Université Claude Bernard Lyon 1, Villeurbanne, Lyon, 69622, France

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ABSTRACT

Huntington's disease (HD) is a neurodegenerative disorder caused by the toxic expansion of polyglutamine in the Huntingtin (HTT) protein. The pathomechanism is complex and not fully understood. Increasing evidence indicates that the loss of normal protein function also contributes to the pathogenesis, pointing out the importance of understanding the physiological roles of HTT. We provide evidence for a novel function of HTT in the cilium. HTT localizes in diverse types of cilia — including 9 + 0 non-motile sensory cilia of neurons and 9 + 2 motile multicilia of trachea and ependymal cells — which exert various functions during tissue development and homeostasis. In the photoreceptor cilium, HTT is present in all subciliary compartments from the base of the cilium and adjacent centriole to the tip of the axoneme. In HD mice, photoreceptor cilia are abnormally elongated, have hyperacetylated alpha-tubulin and show mislocalization of the intraflagellar transport proteins IFT57 and IFT88. As a consequence, intraflagellar transport function is perturbed and leads to aberrant accumulation of outer segment proteins in the photoreceptor cell bodies and disruption of outer segment integrity, all of which precede overt cell death. Strikingly, endogenous mouse HTT is strongly reduced in cilia and accumulates in photoreceptor cell bodies, suggesting that HTT loss function contributes to structural and functional defects of photoreceptor cilia in HD mouse. Our results indicate that cilia pathology participates in HD physiopathology and may represent a therapeutic target.

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by CAG repeat expansion in the *HTT* gene, which encodes an expanded polyglutamine (polyQ) at the N-terminus of Huntingtin (HTT). PolyQ expansion confers dominant toxic properties to mutant HTT (mHTT). However, loss of normal protein function also contributes to the pathogenesis, pointing out the importance of understanding the physiological roles of HTT (Cattaneo et al., 2005).

HTT is a large ubiquitously expressed protein associated with vesicles, microtubules and several cellular organelles (Trottier et al., 1995; Caviston and Holzbaur, 2009). HTT builds a network of interactions with plus- and minus-end-directed microtubule motor-associated proteins (Li et al., 1995; McGuire et al., 2006; Rong et al., 2006; Caviston et al., 2007) as well as with actin-based motors (Pal et al., 2006). Cumulative evidence suggests an emerging role for HTT as integrator of vesicle transport along the cellular cytoskeleton. In pathological situations, mHTT alters axonal transport and vesicle trafficking in multiple animal model systems (Gunawardena et al., 2003; Gauthier et al., 2004; Trushina et al., 2004; Colin et al., 2008; Her and Goldstein, 2008). The underlying molecular mechanisms are not yet clear, but they may involve mHTT aggregation-dependent depletion of motor proteins and loss of HTT normal function (Borrell-Pages et al., 2006; Caviston and Holzbaur, 2009).

HD primarily causes brain degeneration, however, other tissues are affected. Tests for retinal increment thresholds show evidence for retinal pathology in HD patients (Paulus et al., 1993). Consistently, the R6/1 and R6/2 mouse models of HD (Mangiarini et al., 1996), which express the first exon of the human *HTT* gene containing a polyQ expansion (mHTT-exon-1), cause brain and retinal degeneration (Helmlinger et al., 2002; Petrasch-Parwez et al., 2004; Abou-Sleymane

* Corresponding author at: Institute of Genetics and Molecular and Cellular Biology, 67404 Illkirch, France. Fax: +33 3 88653246.
E-mail address: Yvon.Trottier@igbmc.fr (Y. Trottier).
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et al., 2006; Batcha et al., 2011). The retinal pathology is characterized by primary dysfunction of cone and rod photoreceptors, which showed shortening and disorganization of their outer segments, whereas cell loss is limited. These anomalies correlate with progressive transcriptional repression of photoreceptor specific genes. However, the mechanisms underlying the retinal degeneration in HD are largely unknown.

Since HTT is involved in microtubule-dependent intracellular trafficking, it was hypothesized that mHTT-exon-1 might disrupt the function of the photoreceptor cilium (Batcha et al., 2011). Indeed, the assembly, maintenance and function of outer segments are strictly dependent on intraflagellar transport (IFT) along the photoreceptor cilium. The photoreceptor cilium is a vulnerable organelle, the defects of which could compromise photoreceptor functions and survival. About a quarter of genes known to cause photoreceptor degeneration are associated with ciliary structure and functions (Wright et al., 2010).

In this study, we report that Htt is a component of a large variety of mouse cilia, including the photoreceptor cilium. In R6/2 retina, photoreceptor cilia show numerous structural and functional defects correlating with a strong reduction of mouse Htt in cilia. Our data indicate that cilia pathology accounts for photoreceptor degeneration in R6/2 retina.

Material and methods

Animals

Experiments were performed using hemizygous R6/2 transgenic and wild type littermate mice from the same breeding colony (B6CBATg (HDexon1)62Gpb/3J) maintained at the IGBMC in compliance with National Animal Care Guidelines (European Commission directive 86/609/CEE; French decree no. 87-848). The breeders, males and ovary-transplanted females, were from the same strain, obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Genotyping was as described in Mangiarini et al. (1996). The absence of the Pdeb (rd1) was assessed by PCR according to Gimenez and Montoliu (2001). All testing was performed during the light phase of a 12-h light-dark cycle.

Histological and electron microscopic analysis

Eyes were pierced with a thin needle in the cornea and fixed by immersion in 2.5% glutaraldehyde and 2.5% formaldehyde in cacodylate buffer (0.1 M, pH 7.4) as in Yefimova et al. (2010). Briefly, after 10 min, lens and cornea were removed and eyecups were fixed overnight in the same fixative and washed in cacodylate buffer for 30 min. Eyes were then post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h at 4 °C, dehydrated through graded ethanol (50, 70, 90 and 100%) and propylene oxide for 30 min each and embedded in Epon 812. Semi-thin (2 μm) sagittal sections were cut with an ultramicrotome (Leica Ultracut UCT), stained with toluidine blue, and histologically analyzed by light microscopy. Ultra-thin (70 nm) sections were contrasted with uranyl acetate and lead citrate and examined at 70 kV with a Morgagni 268D electron microscope. Images were captured digitally by a Mega View III camera (Soft Imaging System) and contrast was linearly adjusted for display purposes.

Immunofluorescence microscopy and TUNEL

Enucleated eyes of adult mice were mounted and oriented in Shandon CRYOMATIX, stabilized by quick immersion in liquid nitrogen and frozen on dry ice. They were sectioned in 10 μm slices on a Leica cryostat CM 3050S. Retinal sections were fixed using 4% formaldehyde. After phosphate-buffered saline (PBS) wash, retinal sections were permeabilized with 0.3% Triton X-100 and blocked by incubation with blocking solution (3% normal goat serum (NGS) and 1% BSA in PBS) for 1 h at room temperature. Retinal sections were incubated for 1 h at room temperature with the following primary antibodies: 1259 is an affinity-purified rabbit polysera (pAb) detecting 1-82aa of human and mouse HTT (dilution 1:20) (Lunkes et al., 2002); 2166 (also named 4C8) is a mouse monoclonal antibody (mAb) recognizing 414-503aa of human and mouse HTT (1:100) (Lunkes et al., 2002); biotinylated 2166 (biot 2166) (1:100, gift from Mustapha Oulad-Abdelghani, IGBMC); EM48 is a rabbit pAb raised against the first 256 aminoacids of HTT without the polyQ and polyproline stretches (1:100) (Li et al., 1999); 2B4 is a mAb recognizing 50–64aa of human HTT (1:100) (Dehay et al., 2007); mouse acetylated α-tubulin mAb (1:500; clone 6-11B-1, Sigma Aldrich); rabbit centrin3 pAb (1:500) (Trojan et al., 2008); mouse γ-tubulin mAb (1:1000, clone GTU-88, Sigma Aldrich); rabbit pericentrin pAb (1:500, Eurogentec, France); chicken RP1 pAb (1:1000) (Liu et al., 2002); rabbit IFT57 (1:200) and IFT88 pAb (1:200) (Pazour et al., 2002; Sedmak and Wolfrum, 2010); rabbit PCM1 pAb (1:300, gift from Andreas Merdes, Center for biological development, Toulouse, France); rabbit Optineurin pAb (1:300, ab23666, Abcam), mouse arrestin1 SCT-128 mAb (1:50) (Smith et al., 2011), mouse rhodopsin c-terminus mAb (1/250, clone 1D4, MAB5356, Merck Millipore). Cy3-streptavidin (Fisher scientific, SAS) or secondary antibodies goat anti-mouse IgG coupled to Alexa 594 (1/800) and goat anti-rabbit IgG coupled to Alexa 488 (1/800) were incubated in 3% NGS and 1% BSA in PBS for 1 h at room temperature. Control experiments included samples where the primary antibody was omitted. Nuclei were counterstained with DAPI. Slides were incubated in Draq5 (1/1000) and mounted in Dako mounting medium (Dakocytomation). TUNEL labeling was performed as described in Yefimova et al. (2010). Images were acquired with an epifluorescent microscope (Leica DM 4000) or a confocal laser scanning microscope (Leica TCS SP2 AOBS — Leica Microsystems) equipped with a UV Laser (351 nm) and a Plan-Neofluar 63×, NA 1.3 oil objective. Projections of optical slices (z-axis step 0.24 μm) are shown. Images were processed by Leica confocal software and adjusted for contrast and brightness (linear adjustment) using Fiji/Image J and Adobe photoshop CS4 (Adobe Systems, San Jose, USA). Figures were arranged using Adobe illustrator and Figure J in Fiji (Mutterer and Zinck, 2013).

Mouse tracheas, brains and testes (P14) were either pre-fixed overnight at 4 °C in 4% formaldehyde prepared in PBS or fixed after cryosection (post-fixed) in 4% formaldehyde for 10 min at room temperature. Pre-fixed samples were embedded in 7.5% gelatin and 15% sucrose in PBS and cryo-sectioned (12 μm). Pre- and post-fixed sections were permeabilized for 10 min in 10% NGS, 0.3% Triton, and PBS, and incubated for 1 h (pre-) or overnight (post-) at 4 °C in 3% NGS and 1% BSA in PBS, with 1259 pAb (1/20) and acetylated tubulin mAb (1/400). Secondary antibodies goat anti-mouse IgG coupled to Alexa 594 (1/800) and goat anti-rabbit IgG coupled to Alexa 488 (1/800) were incubated in 3% NGS and 1% BSA in PBS for 1 h at room temperature. Slides were observed on confocal microscopes (Zeiss LSM510 or Leica TCS SP2 AOBS).

Quantification of cilia length and immunofluorescence intensity

Co-immunostaining of AcTub/pericentrin and Cen3/gTub ciliary markers was performed on 9- and 12-week-old wild type and R6/2 mouse retina and cilia length was measured using Fiji/ImageJ. Quantification of HTT-2166 immunofluorescent staining in the IS of R6/2 mice and wild type littermates (12-week-old) was performed using the Metamorph software (Molecular Devices Inc., Sunnyvale, USA). Quantification of HTT biotinylated 2166 immunofluorescent staining in the ONL R6/2 mice and wild type littermates (12-week-old) was performed using a custom-made Fiji Macro. Fluorescent images were acquired with the same excitation and emission integration settings using confocal microscopy. Data are expressed as mean ± SD (n = 3–4 mice/age/genotype). Student's *t* test was used for single comparisons.

Immunoelectron microscopy analysis

For immunoelectron microscopy we applied previously introduced protocol for pre-embedding labeling (Maerker et al., 2008; Sedmak