ELSEVIER

Neurobiology of Aging 33 (2012) 2231.e15–2231.e30

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Identification of Alzheimer disease-associated variants in genes that regulate retromer function

Badri N. Vardarajan[a], Sophia Y. Bruesegem[b,1], Michael E. Harbour[b,1], Peter St. George-Hyslop[b,c], Matthew N.J. Seaman[b,*], Lindsay A. Farrer[a,d,*]

[a] Department of Medicine (Biomedical Genetics), Boston University School of Medicine, Boston, MA, USA

[b] University of Cambridge, Departments of Clinical Biochemistry and Clinical Neurosciences, Cambridge Institute for Medical Research, Addenbrookes Hospital, Cambridge, UK

[c] Tanz Centre for Research in Neurodegenerative Diseases, Department of Medicine, University of Toronto, Toronto, Ontario, Canada

[d] Departments of Neurology, Ophthalmology, Genetics & Genomics, Epidemiology, and Biostatistics, Boston University Schools of Medicine and Public Health, Boston, MA, USA

Received 9 December 2011; received in revised form 13 March 2012; accepted 30 April 2012

Abstract

The proteolytic processing of amyloid precursor protein (APP) to generate the neurotoxic amyloid β (Aβ) peptide is central to the pathogenesis of Alzheimer disease (AD). The endocytic system mediates the processing of APP by controlling its access to secretases that cleave APP. A key mediator of APP localization is SorL1—a membrane protein that has been genetically linked to AD. The retromer complex is a conserved protein complex required for endosome-to-Golgi retrieval of a number of physiologically important membrane proteins including SorL1. Based on the prior suggestion that endocytosis and retromer sorting pathways might be involved, we hypothesized that variants in other genes in this pathway might also modulate AD risk. Genetic association of AD with 451 polymorphisms in 15 genes encoding retromer or retromer-associated proteins was tested in a Caucasian sample of 8309 AD cases and 7366 cognitively normal elders using individual single nucleotide polymorphism (SNP)- and gene-based tests. We obtained significant evidence of association with *KIAA1033* (VEGAS $p = 0.025$), *SNX1* (VEGAS $p = 0.035$), *SNX3* ($p = 0.0057$), and *RAB7A* (VEGAS $p = 0.018$). Ten *KIAA1033* SNPs were also significantly associated with AD in a group of African Americans (513 AD cases, 504 control subjects). Findings with four significant *SNX3* SNPs in the discovery sample were replicated in a community-based sample of Israeli-Arabs (124 AD cases, 142 control subjects). We show that Snx3 and Rab7A proteins interact with the cargo-selective retromer complex through independent mechanisms to regulate the membrane association of retromer and thereby are key mediators of retromer function. These data implicate additional AD risk genes in the retromer pathway and formally demonstrate a direct link between the activity of the retromer complex and the pathogenesis of AD.

Keywords: Alzheimer disease; Retromer; Genetic association; Snx1; Snx3; Rab7A; KIAA1033

1. Introduction

The localization of membrane proteins to discrete and specific compartments within eukaryotic cells is governed by a complex interplay of protein–protein interactions in which a sorting motif(s) in the cytoplasmic tail of a membrane protein is recognized by membrane-associated "coat" proteins to direct the respective membrane proteins into a tubule or vesicle for transport to another compartment. A failure in the fidelity of sorting processes can lead to a range of pathologies. Sometimes the failure occurs when a sorting

* Corresponding author at: Biomedical Genetics L320, Boston University School of Medicine, 715 Albany Street, Boston, MA 02118, USA or Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Addenbrookes Hospital, Cambridge, CB2 0XY, UK. Tel.: +1-617-638-5393 or +44-1223-763225; fax: +1-617-638-4275 or +44-1223-762640.

E-mail address: farrer@bu.edu (L.A. Farrer) or mnjs100@cam.ac.uk (M.N.J. Seaman).

[1] These authors contributed equally to this work.

0197-4580/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2012.04.020

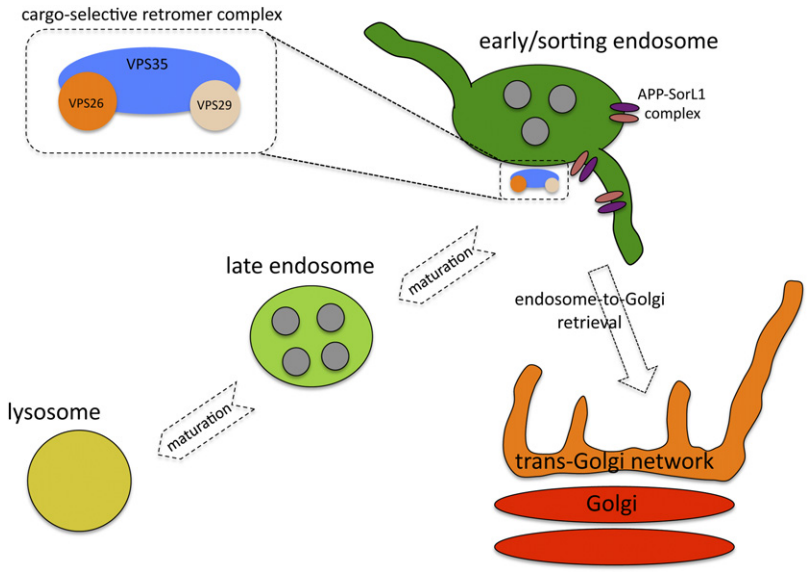

Fig. 1. Schematic diagram of the endocytic pathway and the role of retromer in sorting APP and SorL1. The SorL1 protein associates with APP. The cargo-selective retromer complex interacts with SorL1 to direct the APP-SorL1 complex into an endosome-to-Golgi retrieval pathway. Aberrant APP localization to late endosomal compartments increases processing to the neurotoxic Aβ peptide.

motif is mutated—a notable example being the mutation of the NPXY motif identified as causal in familial hypercholesterolemia by Brown and Goldstein (Anderson et al., 1977). Alternatively the molecular machinery that recognizes sorting motifs is at fault, for example, patients with deficient adapter protein-3 (AP-3) function in Hermansky-Pudlack syndrome (Dell'Angelica et al., 1999).

There has been a growing appreciation recently of the importance of correct protein sorting in regulating the processing of amyloid precursor protein (APP) and therefore the proteins that function in mediating localization to the post-Golgi endocytic system have been of great interest to studies of the underlying causes of late-onset Alzheimer disease (AD). Recently the retromer complex, an endosomally localized protein complex, has been implicated in regulating APP processing (Fig. 1) (Burd, 2011; Sullivan et al., 2011).

The retromer complex is a conserved endosome-associated protein complex that was first identified in yeast as essential for the endosome-to-Golgi retrieval of the carboxypeptidase Y (CPY)-sorting receptor, Vps10p. The studies first conducted in yeast revealed that retromer comprises five proteins, (encoded by vacuole protein sorting—VPS—genes) that are arranged into two functionally distinct subcomplexes; a cargo-selective trimer of Vps35p, Vps29p, and Vps26p and a structural complex proposed to drive vesicle or tubule formation made of a dimer of the yeast sorting nexin proteins, Vps5p and Vps17p (Seaman et al., 1998). The retromer complex is conserved across all eukaryotes, underscoring its vital role in mediating endosomal protein sorting (Koumandou et al., 2011).

Since retromer was first identified in yeast, studies in a variety of systems have identified cargo proteins that require retromer for their localization, and accessory proteins that function with retromer in endosomal protein sorting. For example, the small GTPase Rab7A associates with the cargo-selective retromer complex to mediate its localization to endosomes (Rojas et al., 2008). Other retromer-associated proteins include Tre, Bub2, cdc16 domain, family member 5 (TBC1D5), a rab GTPase-activating protein, Eps15 homology domain-containing protein-1 (EHD1), and the Wiskott-Aldrich syndrome homologue (WASH) complex (Gokool et al., 2007; Harbour et al., 2010; Seaman et al., 2009).

Membrane proteins that depend on retromer for their proper localization, and therefore are considered to be retromer cargo proteins, now include the cation-independent mannose 6-phosphate receptor (CIMPR); Wntless, a protein required for the secretion of the Wnt morphogen; divalent metal-ion transporter 1 (DMT1), a divalent cation transporter; and the Vps10-family members sortilin and SorL1 (also known as SorLA) (Arighi et al., 2004; Canuel et al., 2008; Eaton, 2008; Nielsen et al., 2007; Seaman, 2004, 2007; Tabuchi et al., 2010). Since SorL1 has been shown to associate with APP and regulate the processing of APP (Lee et al., 2008a), it has been of interest to studies directed at understanding the pathology of AD. The pathophysiological importance of the physical association of SorL1 and its four type I membrane homologs (sortilin, sorCS1, sorCS2, and sorCS3) with APP has been elevated by the identification of AD-linked single nucleotide polymorphisms (SNPs) in the genes encoded by these loci (Lee et al., 2007; Reitz, unpublished results; Reitz et al., 2011a, 2011b; Rogaeva et al., 2007).

Retromer is required to mediate the localization of SorL1, and loss of retromer results in increased production