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Brief communication

A truncating mutation in Alzheimer's disease inactivates neuroligin-1 synaptic function

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ABSTRACT

Neuroligins (NLs) are cell-adhesion proteins that regulate synapse formation and function. Neuroligin 1 (NL1) promotes the formation of glutamatergic synapses and mediates long-term potentiation in mouse models. Thus, altered NL1 function could mediate the synaptic and memory deficits associated with Alzheimer's disease (AD). Here, we describe a frameshift mutation, c.875_876insTT, in the neuroligin 1 gene (*NLGN1*) in a patient with AD and familial history of AD. The insertion generates a premature stop codon in the extracellular domain of NL1 (p.Thr271fs). Expression of mutant NL1 shows accumulation of truncated NL1 proteins in the endoplasmic reticulum. In hippocampal neurons, the p.Thr271fs mutation abolishes the ability of NL1 to promote the formation of glutamatergic synapses. Our data support a role for inactivating mutations in *NLGN1* in AD. Previous studies have reported rare mutations in X-linked *NLGNL3* and *NLGNL4* genes in patients with autism, which result in the inactivation of the mutant alleles. Therefore, together with a role in neurodevelopmental disorders, altered NL function could underlie the molecular mechanisms associated with brain diseases in the elderly.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative illness characterized by progressive memory loss in the patients. Increasing evidence suggests that synapses are a main pathological target in AD (Selkoe, 2002; Spires-Jones and Hyman, 2014). Indeed, synapse loss is the best pathological correlate of cognitive dysfunction in AD patients (DeKosky and Scheff, 1990; DeKosky et al., 1996; Masliah et al., 2001; Terry et al., 1991). Therefore, perturbation of synaptic function is a key event for memory decline in AD.

Neuroligins (NLs) are postsynaptic proteins that regulate excitatory and inhibitory synapse formation and function by trans-synaptic interaction with their presynaptic receptors, such as neurexins (Dean et al., 2003; Scheiffele et al., 2000; Sudhof, 2008). In humans, NLs are coded by 5 genes located in autosomic (*NLGN1* and *NLGN2*) and sexual chromosomes (*NLGN3*, *NLGN4*, and *NLGN4Y*). NLs have received especial attention since the identification of mutations in patients with autism spectrum disorders (ASDs). Bourgeron et al. identified 1 missense and 1 frameshift mutation in X-linked *NLGN3* and *NLGN4* in 2 pairs of brothers with ASD, respectively (Jamain et al., 2003). Following this initial finding, rare mutations in *NRXN1*, *NLGN3*, and *NLGN4* have been described in patients with neurodevelopmental diseases, including autism and intellectual disability (Camacho-Garcia et al., 2012; Laumonnier et al., 2004; Lawson-Yuen et al., 2008; Rabaneda et al., 2014; Yan et al., 2005; Zhang et al., 2009). Although the number of identified mutations in *NLGN* genes is low, functional characterization of the mutant NL3 and NL4 proteins has helped to identify a convergent disease mechanism. Autism-associated mutations in *NLGN3* and *NLGN4* often induce the accumulation of the mutant proteins in the endoplasmic reticulum (ER) and impair their synaptogenic activity (Chih et al., 2004; Chubykin et al., 2005; Zhang et al., 2009). Therefore, mutations in *NGLN* genes are so far restricted to neurodevelopmental disorders.

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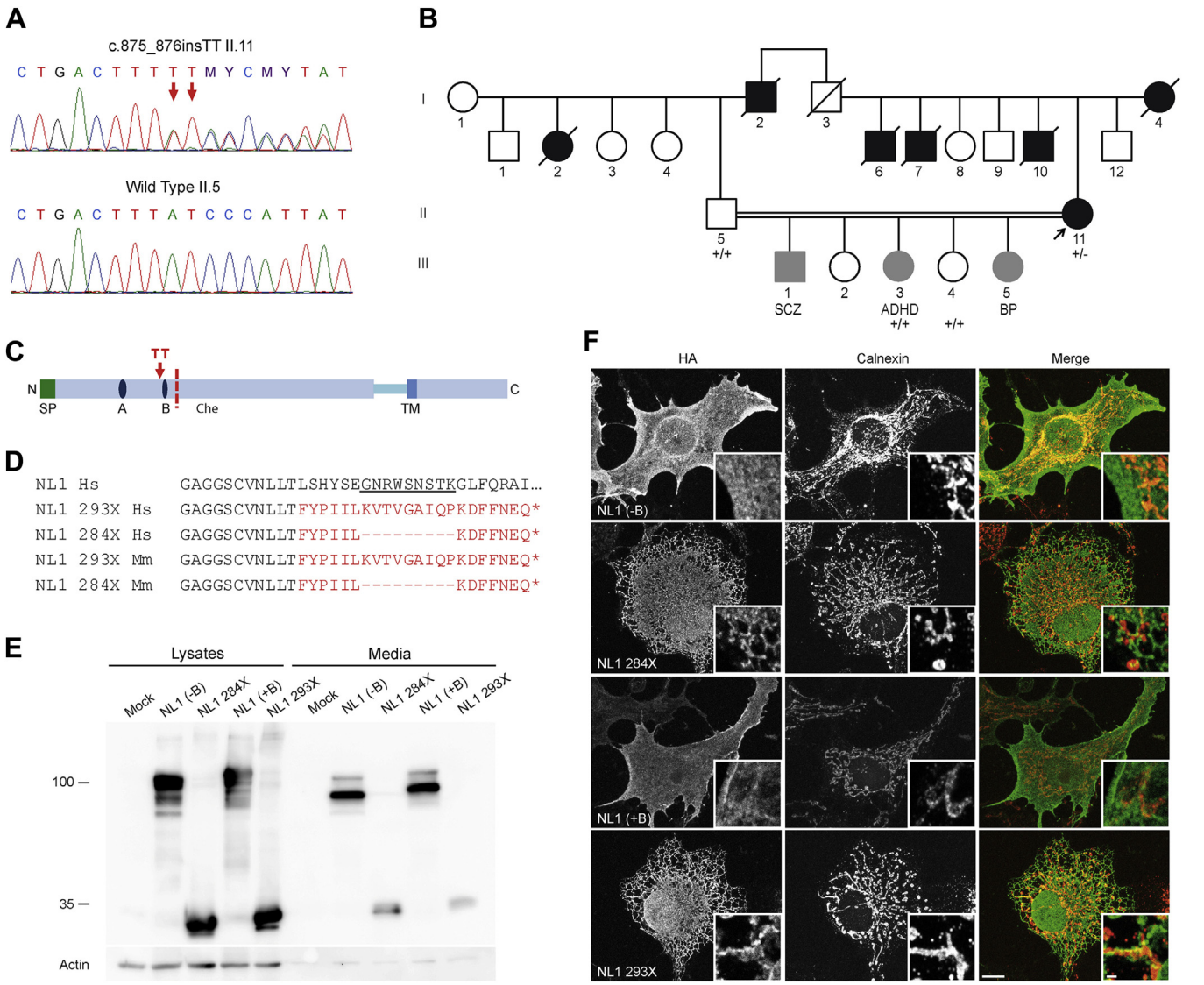

Fig. 1. Characterization of the c.875_876insTT mutation in *NLGN1* identified in AD. (A) Sequence chromatograms from polymerase chain reaction-amplified genomic DNA of the *NLGN1* gene from the patient (II.11) and her spouse (II.5). The insertion is marked by arrows. (B) Pedigree of the family and segregation of the identified *NLGN1* mutation in the participating family members. Black circles/squares: female/male patients with AD; gray symbols: family members diagnosed with neurodevelopmental disorders. Clinical diagnosis is indicated below each family member. The genotype status is indicated under each symbol. (C). Schematic diagram of NL1 protein. The predicted stop codon generated by the insertion is indicated as a vertical red dashed line. (D). Sequence alignment of wild-type and mutant NL1 proteins. The residues corresponding to the splice site B are underlined. Mutant residues for NL1 284X and NL1 293X are shown in red. (E) Expression of HA-tagged wild-type and mutant NL1 proteins in total cell lysates and in the cell media of transfected N2A cells. An anti-actin antibody was used as control. (F) Accumulation of NL1 mutants in the ER. COS cells transfected with wild-type or mutant NL1 proteins were costained with HA antibodies (green in the colocalization) and with the ER marker calnexin (red in the colocalization), as indicated. Mutant NL1 284X and NL1 293X proteins do not show surface localization and accumulate in the ER, as shown by the overlap with calnexin. Scale bars: 20 μ and 2 μ (insets). Abbreviations: A and B, alternatively spliced exons; AD, Alzheimer's disease; ADHD, attention-deficit/hyperactivity disorder; BP, bipolar disorder; ChE, cholinesterase domain; NL1, neuroligin 1; NLGN1, neuroligin 1 gene; SCZ, schizophrenia; SP, signal peptide; TM, transmembrane region.

NL1 localizes at glutamatergic postsynaptic terminals, and expression of NL1 in neurons promotes the formation of glutamatergic synapses (Chih et al., 2005; Scheiffele et al., 2000; Song et al., 1999). Moreover, NL1 and neurexins are substrates for presenilins (PS), a γ-secretase component frequently mutated in familial AD (Peixoto et al., 2012; Saura et al., 2011; Suzuki et al., 2012). On the basis of these data, defects in NL1 function might underlie synaptic and memory deficits associated with AD (Bie et al., 2014; Martinez-Mir et al., 2013; Sindi et al., 2014). However, the role of *NLGN* genes in AD is unclear because no mutations have been described in patients. Here, we report a frameshift mutation in *NLGN1* gene in a familial case of AD. The frameshift mutation truncates the protein at the extracellular domain, induces ER accumulation, and inhibits the induction of glutamatergic synapses. These data extend the role for mutations in *NLGN* genes to AD.

2. Subjects and methods

The coding region of the *NLGN1* gene was sequenced in a group of 192 patients with AD using HaloPlex target enrichment (Agilent Technologies, USA) and a MiSeq sequencing platform (Illumina, USA). The c.875_876insTT *NLGN1* mutation was confirmed by Sanger sequencing in the index case (for a full clinical description of the patient, refer to Supplementary Material).

For transfection in N2A and COS cells, cultures were transfected with Lipofectamine 2000 (Invitrogen, USA) and analyzed