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Familial Alzheimer's disease coding mutations reduce *Presenilin-1* expression in a novel genomic locus reporter model

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ABSTRACT

We have generated a physiologically relevant bacterial artificial chromosome (BAC)-based genomic DNA expression model to study *PS1* gene expression and function. The *PS1-WT*-BAC construct restored γ-secretase function, whereas the mutant *PS1* BACs demonstrated partial to complete loss of enzymatic activity when stably expressed in a *PS* double knock-out clonal cell line. We then engineered *WT* and mutant human *PS1-BAC-Luciferase* whole genomic locus reporter transgenes, which we transiently transduced in mouse and human non-neuronal and neuronal-like cells, respectively. *PS1 ΔE9* and *C410Y* FAD were found to lower *PS1* gene expression in both cell lines, whereas *PS1-M146V* showed a neuron-specific effect. The nonclinical γ-secretase inactive *PS1-D257A* mutation did not alter gene expression in either cell line. This is the first time that pathogenic coding mutations in the *PS1* gene have been shown to lower *PS1* gene expression. These findings may represent a pathologic mechanism for *PS1* FAD mutations independent of their effects on γ-secretase activity and demonstrate how dominant *PS1* mutations may exert their pathogenic effects by a loss-of-function mechanism.

1. Introduction

Familial Alzheimer's disease (FAD) cases are clinically and neuropathologically similar to late-onset AD and are caused by autosomal-dominant mutations in *Presenilin 1 (PS1)* (Sherrington et al., 1995), *Presenilin 2 (PS2)* (Levy-Lahad et al., 1996), and *Amyloid Precursor Protein (APP)* genes (Goate et al., 1991). PS1 protein undergoes auto-endoproteolysis within its large intracellular loop to generate an amino terminal fragment (NTF) and a carboxy terminal fragment (CTF) (Thinakaran et al., 1996), which form the catalytic core of the γ-secretase enzyme complex (Li et al., 2000), which is critical for proteolytic processing of APP and Notch proteins into the APP intracellular domain and Notch intracellular domain (NICD), respectively (De Strooper et al., 1998). Both endoproteolysis and γ-secretase activity are dependent on the presence of two highly conserved aspartate residues located at amino acid positions 257 and 385 of the PS1 protein (Wolfe et al., 1999).

During the past decade it has become increasingly clear that PS1 has many different cellular functions. These include amyloid beta (Aβ) peptide production and calcium signalling, both of which have been shown to play a major role in the etiology of AD. AD is therefore likely caused by the culmination of toxic effects of mutant PS1 protein, which impair γ-secretase enzymatic activity, leading to overproduction of amyloidogenic Aβ species as well as defective calcium signalling as a result of impaired PS1 calcium leak channel activity. The mechanism through which *PS1* FAD mutations are proposed to exert their effects will, therefore, depend on the pathway being studied.

Notch signalling is not only required for neuronal differentiation during neurogenesis but also for neuronal survival (Handler et al., 2000). The production of NICD is significantly reduced in *PS1* knockout (KO) and completely abrogated in *PS1/PS2* double KO (*PS* dKO) cells (De Strooper et al., 1999; Herreman et al., 2000). The *PS1* FAD mutations, especially *C410Y*, have been shown to result in reduced Notch proteolysis (Song et al., 1999) and impair endoproteolytic cleavage of PS1 (Murayama et al., 1999). When transfected into *PS1*-deficient cells, *PS1* FAD mutant transgenes are unable to restore Notch signalling (Kumar-Singh et al., 2006; Levitan et al., 1996; Song et al., 1999). With respect to NICD production, therefore, *PS1* FAD mutations exhibit a partial or complete loss of function phenotype. Although the loss of NICD production leads to region-specific loss of neurons susceptible in AD, it is not sufficient to produce all aspects of this disease as it occurs in humans (Saura et al., 2004).

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PS-deficient cells have abnormally high levels of endoplasmic reticulum (ER) calcium compared with *PS* wild-type (WT) cells. This observation was also made in *PS*-deficient cells transfected with *PS1* FAD mutations but not with the proteolytically inactive artificial *PS1* D257A mutation. Specifically, the FAD mutations had reduced calcium leak from the ER and because PS can form a pore with the ability to pass current through, PS were suggested to act as the major calcium leak channel in the ER (Tu et al., 2006). With respect to calcium leak channel activity, therefore, *PS1* FAD mutations exhibit a partial or complete loss of function phenotype.

Recently, familial cases of autosomal-dominant acne inversa (AI) were reported and found to be attributable to nonsense/frame-shift mutations in the *PS1* gene and the loci of its γ-secretase cofactors. Although FAD cannot be excluded at this stage, no AI patient has yet been found to have symptoms of FAD. The dominant penetrance of AI appears to be the result of haploinsufficiency (Wang et al., 2010), whereas the *PS1*-FAD mutations are thought to act through a dominant-negative mechanism (Kelleher and Shen, 2010). Specifically, it has been shown that FAD mutant PS1 proteins assemble into γ-secretase complexes exhibiting lower activity rates, which result in the production of longer and more amyloidogenic Aβ fragments (Kakuda et al., 2006; Wolfe, 2009). The lowered PS1 γ-secretase enzyme activity leads to overproduction of toxic Aβ, leading to an overall dominant-negative phenotype.

Previous attempts to identify associations between *PS1* gene expression and AD have produced mixed results. For example, although the results of an earlier study showed an association of the -48C/T polymorphism with AD (Theuns et al., 2006), the more recent much larger results from genome-wide association studies did not replicate this finding (Gerrish et al., 2012). In another study, a very limited region (only ∼0.3 kb) of the *PS1* promoter region was cloned into a basic luciferase vector and both the *PS1* -48C polymorphism and *PS1* -280G FAD promoter mutation were found to lower *PS1* gene expression (Theuns et al., 2003). However, interpretation of findings from these promoter fragment studies is limited by the small size of the *PS1* promoter region studied. Indeed, the TATA-less *PS1* promoter exhibits a scattered regulatory genetic sequence typical of house-keeping genes (Pastorcic and Das, 1999; Rogaev et al., 1997), which includes several CpG islands and two consensus sites for multiple start site element downstream (i.e., MED-1) required for transcription from TATA-less promoters (Kornberg, 2007; Stamatoyannopoulos, 2010). During normal aging, *PS1* mRNA levels show significant reduction in brain regions specifically affected by AD (Theuns et al., 2000).

In this study we have used our bacterial artificial chromosome (BAC)-based approach for functional analysis of physiological gene expression (Lufino et al., 2007). In addition, BACs provide an excellent platform for recombineering of large genomic constructs to study DNA sequence changes associated with human disease (Muyrers et al., 2000, 2001). When the *iBAC* method is used, genes up to 150 kb can be expressed from their native promoters at physiologic levels in a wide range of cell types (Wade-Martins et al., 2003).

Here we have generated and used two cell culture models to study *PS1* in a physiologically relevant manner from *iBAC-PS1* vectors carrying the 121-kb wild-type or mutant *PS1* genomic locus. The *iBAC-PS1-WT* construct restored γ-secretase function to *PS* dKO cells, whereas the mutant *PS1* vectors demonstrated partial to complete loss of enzymatic activity, confirming the loss of function mechanism of FAD mutations. *PS1* is abundantly expressed in neurones; and expression patterns of *PS1* differ among neuronal and non-neuronal (for example, glial) cells (Kovacs et al., 1996; Lee et al., 1996; Page et al., 1996). Therefore, in our study, transient and physiologic expression of *PS1*-WT and mutant genomic BAC reporter transgenes was performed in both mouse *PS* dKO and human neuronal-like SHSY5Y cells. *PS1-ΔE9 (S290C)* and *C410Y* FAD mutations were found to lower *PS1* gene expression in both cell lines, whereas *PS1-M146V* had a neuron-specific effect on *PS1* gene expression. The artificial nonclinical *PS1-D257A* mutation, which we confirmed as lacking *PS1*-dependent γ-secretase activity, did not alter gene expression in either cell line. This is the first time that pathogenic coding mutations in the *PS1* gene have been shown to lower *PS1* gene expression and is consistent with previous findings that PS1 mutant protein has reduced function. Overall, we describe a novel mechanism through which *PS1* coding mutations may exert their pathogenic effects, independent of their effects on γ-secretase activity.

2. Methods

2.1. Vector construction

BAC *CTD-2635D18* was subcloned by homologous recombination to obtain *iBAC-PS1-WT*. For this the vector backbone of *pCYPAC2*, including its low-copy number plasmid replicon, lox P site, and kanamycin resistance gene (*Kmr*), were amplified by polymerase chain reaction (PCR) via use of the Bio-X-Act Long enzyme (Bioline, Taunton, MA, USA) and recombination primers 5′ end homology primer *ET1*:

5′TTGGGAAAATAAGCCTCATCTAAACATTGTATACCTGCTTACACTTATTTGCATTaaaatcatttaattggtggtgctgc-3′ and the 3′ end homology primer *ET2:*

5′AGTTCACAACAGGGTTCATGCTCCCGTGGGAATCTAATGCTGCCACTGACCTGACattgacccggaacccttaatataac-3′ (capital letters indicate *PS1* BAC sequence; lower letters indicate *pCYPAC2* plasmid sequence). Recombineering between the *pCYPAC2* long-range PCR product and CTD-2635D18 was performed in *Escherichia coli* using the Red/ET recombination plasmid *pSC101BADgbatet* and the RpsL-neo selection/counter selection cassette (Genebridges, Dresden, Germany) according to the manufacturer's instructions. *iBAC-PS1-WT* was used to generate *iBAC-PS1-(2A)-Luc* by a 2-step recombineering technique involving *pSC101BADgbatet* and the RpsL-Chl selection/counter selection cassette. The mutant *iBAC-PS1* and *iBAC-PS1-2A-Luc* (*M146V*, *D257A*, Δ*E9*, and *C410Y*) were generated by recombineering technique involving *pSC101BADgbatet* and the RpsL-Chl selection/counter selection cassette. Specifically, a ∼500-bp region containing the mutation site from *iBAC-PS1-WT* was PCR amplified using Bio-X-Act Long and cloned into *pBlueScript* (Stratagene) before introducing the mutation by site-directed mutagenesis. The WT region in each mutant PAC was replaced with the mutant sequence in the second round of recombineering. All vectors were verified by restriction enzyme digestion, PCR and sequencing.

pEFGEEZ was generated by routine plasmid cloning methods. The *hygroR-2A peptide-eGFP* fragment was generated by replacing the translational stop signal from *hygromycin R* (*hygroR*) sequence with the 2A peptide sequence followed by *eGFP* coding sequence and subsequent cloning into *pGL4-EF1-RLuc* (Promega) downstream of the EF1 promoter and in place of the Renilla luciferase gene. *Sbf-I* RE-end primers amplified the *EF1-hygroR-2A-eGFP-SV40 polyA* expression cassette, which was subsequently cloned into *Sbf-I* site of *pEHHZ* to generate *pEFGEEZ*. *PS1* PACs were retrofitted to *pEFGEEZ* using Cre/*LoxP* recombination (Novagen) according to manufacturer's instructions.

2.2. Cell culture, HSV-1 amplicon production, and infection

PS MEFs, Vero 2-2 cells and G16-9 cells were cultured in Dulbecco modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco BRL), 100 U/mL penicillin, and streptomycin (Sigma-Aldrich, St.