Modeling Familial British Dementia in Transgenic Mice

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The chromosome 13 linked amyloidopathies familial British dementia (FBD) and familial Danish dementia (FDD) are caused by mutations in the C-terminus of the *BRI2* gene. In both diseases, novel peptides are deposited in amyloid plaques in the brain. Several laboratories have attempted to model these diseases in BRI2 transgenic mice with limited success. While high expression levels of BRI protein were achieved in transgenic lines, no ABri-amyloidosis was observed in aged mice. This review discusses the strategies chosen and problems experienced with the development of FBD/FDD models and suggests novel approaches to model the diseases in murine models.

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INTRODUCTION

Murine models have been used extensively to investigate the molecular mechanisms of several amyloidoses, including Alzheimer disease (AD) (10, 32) tauopathies (17), synucleinopathies (11) and prion diseases (39). Since the identification of the first familial AD linked mutation (8), many groups have generated models that develop some but not all the pathologies of Alzheimer disease (3, 4, 7, 13, 16, 22, 23, 35). A complete murine model of AD that exhibits all of the behavioral, biochemical and pathological hallmarks of the disease including senile plaques, neurofibrillary tangles and cell loss still remains elusive. However, mice that replicate some aspects of the disease have been invaluable in understanding the pathogenesis of AD, identifying the affect of mutations and modifier genes on pathology and phenotype (15), and are currently being used to test potential AD therapeutics (18).

Given the clinical and pathological similarities between the chromosome 13-linked amyloidopathies, familial British dementia (FBD) and familial Danish dementia (FDD) and Alzheimer disease, the development of a murine model that replicated FBD or FDD would be extremely valuble in providing insight into the pathogenesis of not only those diseases, but also AD. Several independent groups have used their experience in developing transgenic AD models to attempt to generate FBD and FDD mouse models, with unexpected results. This review will focus on 2 sets of mice developed independently in our laboratories, and the problems experienced with the development of FBD/FDD models.

TRANSGENE DESIGN

BRI2 and its mouse homolog *mBRI2* are expressed pan-neuronally throughout the brain and also in glial cells (33, 38). The disease-linked mutation in FBD is a T→A transversion in the stop codon of *BRI2* that results in a 33 base pair extension to the open reading frame (38). The novel amyloidogenic peptide, ABri, is cleaved by furin or a furin like protease (19, 20) from the extended C terminus of BRI2 (Figure 1A). Amyloid pathology was observed throughout FBD affected brains, with plaques most densely distributed in the hippocampal formation and cerebellar cortex (14, 38).

Several groups have attempted to express human mutant *BRI2* (*mtBRI2*) and wild-type *BRI2* (*wtBRI2*) transgenes in mice under pan-neuronal heterologous promoters such as the mouse prion promoter (MoPrP) and mouse Thy-1.2 promoter element, since the endogenous *BRI2* promoter has not yet been identified or characterized. The MoPrP promoter was chosen to drive transgene expression (Figure 1A) since it is expressed in a pattern similar to *BRI2* and *mBRI2*, with pan-neuronal expression at highest levels in the hippocampus, cerebellum and cortex (2). It had also been used successfully by several groups, including our own, to achieve high levels of overexpression and induce pathology in mutant APP, tau, synuclein and Npc1 transgenic models (4, 8, 23, 25). High levels of expression proved to be a critical factor in inducing fibrillar Aβ deposition in transgenic models of AD (7, 16, 21). In consequence, we anticipated that a similar threshold would be required in BRI2 transgenic mice. Similarly wild-type or mutant human *BRI2* cDNA transgenes were expressed by the well characterized neuronal Thy-1.2 promoter element that has previously been used to model cerebral amyloidosis and cerebral amyloid angiopathy in transgenic mice (3, 12, 26, 29, 35).

TRANSGENE AND PROTEIN EXPRESSION

Analysis of 8 mutant and 2 wild-type MoPrP-BRI2 lines, initially generated on an outbred B6/D2/SW background, revealed high levels of transgene expression. A single human *BRI2* mRNA band (3.15 Kb) was detected by northern blots in both the mutant and wild-type transgenic lines (Figure 1B). The highest expressing mutant BRI2 transgenic line expressed human *BRI2* mRNA at approximately 2½ times endogenous *BRI2* expression. The highest expressing wild-type *BRI2* transgenic line expressed human *BRI2* mRNA at 1½ times endogenous *BRI2* expression. The expression of the human *BRI2* did not appear to affect the expression of the mouse *BRI2* transcripts. In situ hybridization revealed that the *BRI2* transgene has a similar ex-

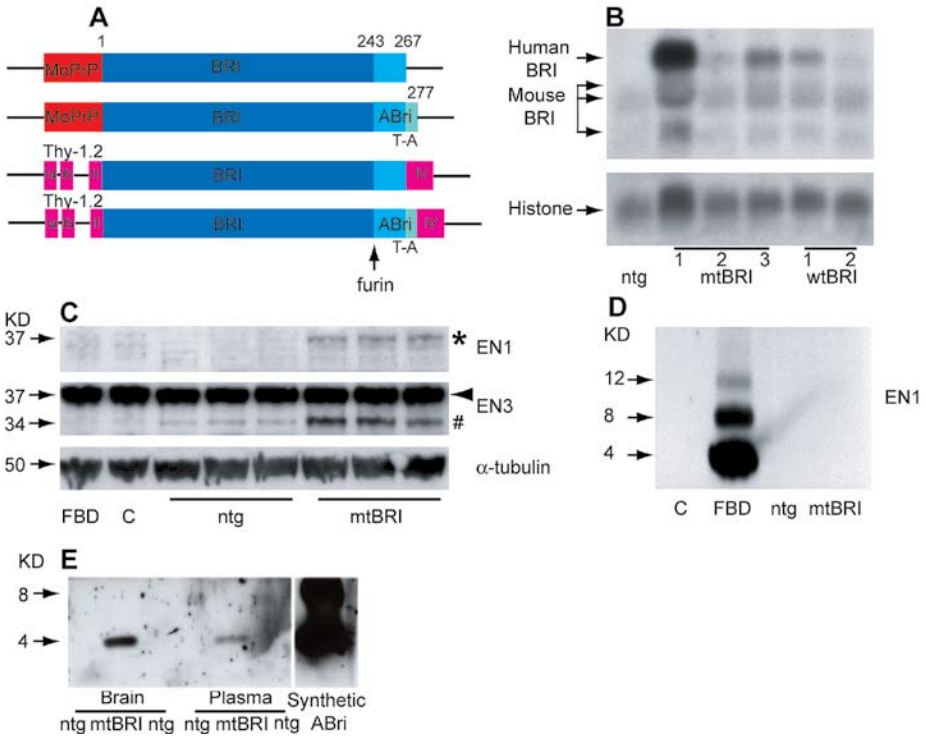

Figure 1. *Mutant BRI expression in transgenic mice.* **A.** Human mutant and wild-type *BRI2* transgene constructs. Corresponding amino acids, the FBD mutation and furin cleavage site are marked. The BRI antibodies described in this review are specific to the following peptides; EN3; BRI 229-241, EN1; BRI 267-277, 338; BRI 268-277, 1130; BRI 265-277, C2; BRI 1-19, C4; BRI 267-277. **B.** Northern blot of total brain RNA from 3 MoPrP-mtBRI2 and 2 MoPrP-wtBRI2 lines showing mouse and transgenic human *BRI2* mRNA expression. Multiple mouse transcripts are due to differential polyadenylation. **C.** Full length mutant BRI2 was detected in MoPrP-mtBRI2 mouse brain extracts, but not in non-transgenic (ntg) mouse brain extracts, FBD or control (C) human brain extracts (*). Full length wild-type BRI was detected in all samples (arrowhead). BRI N-terminal fragments were elevated in MoPrP-mtBRI2 mouse brain extracts (#). **D.** ABri peptides were detected in FBD human brain extracts but not in control, non-transgenic or MoPrP-mtBRI2 mouse brain extracts using EN1. **E.** Low levels of ABri were detected by IP-western with antibody 338 from plasma and total brain homogenates from Thy1.2-mtBRI2 mice but not non-transgenic mice.

pression pattern to that previously described for this promoter (2). High expression was observed in the hippocampus, cerebellum, cortex, and specific nuclei in the thalamus, hypothalamus and pons. A similar pattern and level of expression was seen between males and females, and in all lines.

Whole brain protein extracts were analyzed by western blot using an antibody specific for BRI(EN3) (33). There was no difference in full length BRI(37 KD) between non-transgenic and transgenic mice. However, the levels of the N-terminal fragment (34 KD) were elevated by up to 3.5-fold over endogenous levels in the highest expressing mutant line and 2.5-fold over endogenous levels in the highest expressing wild-type line (Figure 1C). Increased levels of N-terminal BRI protein corresponded to levels of human *BRI2* mRNA in the different lines. Full-length mutant BRI2 (37 KD) was detected in the mutant lines by an antibody specific to ABri (EN1) (Figure 1C). However, the same antibody did not detect full-length mutant BRI2 in FBD human brain samples. SDS insoluble proteins were subsequently extracted using 70% formic acid. In FBD human brain, a ladder of bands (4, 8, 12 KD) corresponding to monomers, dimers, and trimers of the 4-KD ABri peptide was detected by EN1. However, ABri was not detected in whole brains from hetero- or homozygous mice or dissected brain regions of BRI2 transgenic mice up to 24 months of age. Similar results were obtained upon analysis of Thy-1.2-mtBRI2 transgenic mice on a B6D2 background, although low levels of ABri were detected by immunoprecipitation (IP) from plasma and whole brain extracts of 12½-month-old Thy-1.2-mtBRI2 mice (Figure 2E), suggesting that BRI2 cleavage and ABri secretion can occur in this model, but at very low levels.

MUTANT *BRI2* ACCUMULATED IN CELLS BUT WAS NOT SECRETED

Mutant BRI2 transgenic mice (MoPrP-mtBRI2 and Thy-1.2-mtBRI2) had specific accumulation of intracellular mutant BRI2 immunoreactivity, but no extracellular deposits were detected. The same antibodies EN1, 338, and 1130 have been used successfully to immunostain ABri plaques in FBD tissue sections (1, 38) and the 4-KD ABri protein using Western blots (Figure 1D). The mutant BRI2 protein accumulation had a granular (in larger cells) or punctate (in smaller, more closely packed cells) localization in the cytoplasm of predominantly neuronal cells, sometimes extending into neuronal processes. Nuclei were not specifically stained. The pattern of cellular immunoreactivity clearly followed the distribution of transgene mRNA detected by in situ hybridization (Figure 2). In MoPrP-mtBRI2 mice, the strongest immunostaining occurred in the granular cell layers of the hippocampus and cerebellum, the mitral cell later of the olfactory bulb and in the cortex. In Thy-1.2-mtBRI2 transgenic mice, the strongest immunostaining occurred in the pyramidal cells of cortical layer V. Granular wild-type BRI2 immunoreactivity was detected in human brain tissue in a very similar pattern to that seen in these mutant BRI2 transgenic mice (1). Holton and colleagues noted that pyramidal cells in the hippocampus were delicately stained around cell margins in FBD brains (14). This may be the normal intracellular distribution of BRI2 (wild-type and mutant). Further immunohistochemistry using antibodies specific to human or mouse, wild-type and mutant BRI2 is required to determine whether the granular immunoreactivity is normal or pathogenic, and whether the immunoreactivity is due to full-length mtBRI2 or ABri.

Ultrastructural localization of the mutant protein in MoPrP-mtBRI2 mice was determined by pre-embedding immunoelectron microscopy. ABri immunoreactivity was associated with the stacked membranes in the cytoplasm surrounding the nucleus and also in the neuronal processes. It was most likely that the mutant BRI2 protein was situated in the endoplasmic reticulum or the Golgi network. No immunoreactivity was observed in endosomal vesicles, suggesting the protein was not in the secretory pathway. However, immunoreactive cells

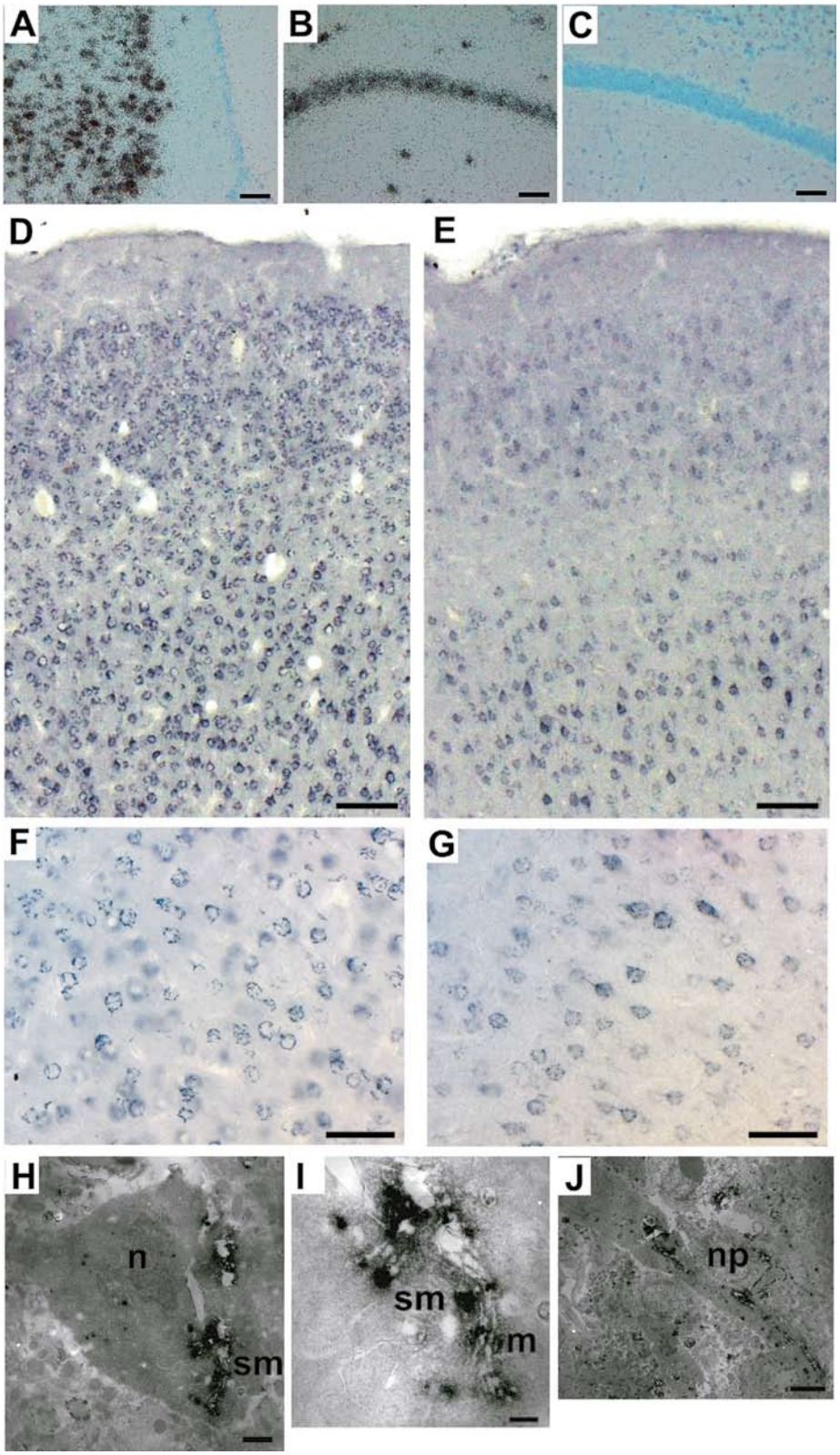

Figure 2. *Intracellular mutant BRI2.* Granular mutant BRI2 immunoreactivity follows mRNA expression and is associated with stacked membranes. Human *BRI2* mRNA in MoPrP-mtBRI2 (**A, B**) and non-transgenic (**C**) mice. **A.** Frontal cortex, (**B, C**) granular layer of CA1 region of hippocampus. Mutant BRI2 immunoreactivity (338 antibody) in frontal cortex of MoPrP-mtBRI2 (**D, F**) and Thy1.2-mtBRI2 (**E, G**) mice. Intracellular staining has a granular appearance. Pre-embedding immunoelectron microscopy (**H, I, J**) on MoPrP-mtBRI2 mice showed mutant BRI is associated with stacked membranes (sm) adjacent to the nucleus (n) and in neuronal proceses (np). Scale bars represent **A, B, C**; 60 µm, **D, E**; 100 µm, **F, G**; 50 µm, **H**; 1 µm, **I**; 300 nm, **J**; 3 µm.

appeared normal and healthy, and were identical to cells examined from wild-type and non-transgenic mice.

WHY IS ABRI NOT DEPOSITED?

The BRI2 transgenic mice described here express *BRI2* mRNA and protein at high levels, greater than 3-fold over endogenous, however no ABri peptide was detected and no amyloid deposition, extracellular accumulation or other pathology such as cell loss was observed. There are several possible explanations for these results, each of which merit further investigation in pursuit of developing a model of FBD.

The levels of expression achieved in these mice may not be sufficient to reach a threshold that induces amyloid deposition. Several initial attempts at modeling Alzheimer disease in transgenic mice failed due to subthreshold levels of Aβ production (21, 27). AD transgenic models that exhibit amyloid deposition have either high levels of APP expression (7, 16) or produce high levels of Aβ due to multiple mutations (4). However, high levels of transgene expression are not always needed to induce protein aggregation; mutant tau transgenic mice develop Thioflavin-S positive neurofibrillary tangles and neuronal loss with overall transgene expression levels equal to endogenous tau levels (23), although pathology first occurs in motor neurons in the spinal cord that have high localized expression of the Tau transgene. In addition, BRI-Aβ42 mice that express a fusion construct comprised of the BRI2 protein with Aβ1-42 fused to the C-terminus in place of ABri, have lower levels of transgene expression (1.4-fold over endogenous *BRI2* compared to 2.5-fold over endogenous *BRI2* levels in MoPrP-mtBRI mice) but have robust Aβ production and deposition (28). The differences between BRI-Aβ42 mice which produce and deposit Aβ and mtBRI2 mice which produce and deposit little or no ABri may be due to: *i)* peptide aggregation characteristics, *ii)* differential degradation, and *iii)* different proteolysis of precursors.

In mtBRI2 mice, ABri may be cleaved from the BRI2 protein and cleared quickly from neurons so that it was only present in the brain tissue at levels below the threshold for detection for the EN1 (25ng) or C4 antibodies. Alternatively, if ABri aggregates slowly or requires a high threshold for aggregation, then it will not accumulate.

However, full length, uncleaved BRI2 is present in MoPrP-mtBRI2 transgenic mice but not in the diseased human tissue (Figure 1C), suggesting that BRI2 is cleaved at a slower rate in transgenic mice than in humans. In addition, in vitro studies suggested that ABri aggregates in a similar manner to Aβ (6, 34) and FBD cases have a very high amyloid burden (14, 38) showing that under the right conditions, ABri is clearly highly amyloidogenic. More data is required on the kinetics of ABri aggregation compared to Aβ aggregation to investigate this possibility.

Human BRI2 may be aberrantly processed in these transgenic mice. Full length mutant BRI2 protein was detected in MoPrP-mtBRI2 and in Thy1.2-mtBRI2 transgenic mice, but not in FBD human brain (Figure 1C). This indicated that the processing or turnover of the human BRI2 protein in the human brain may occur at a different rate, or in a different manner to processing in the mouse brain. It also demonstrated that the mutant human protein remained unprocessed, at least in part, in the transgenic mice. The reduced processing of human BRI2 in Thy 1.2- and MoPrP-mtBRI2 transgenic mice described here may be due to mouse furin or furin-like proteases inefficiently cleaving human transgenic BRI2 proteins. Previous transgenic mice that exogenously expressed the different furin substrate, human protein C (HPC) in mammary glands (37) required co-expression of furin for processing of HPC to mature forms (5, 31). Also, the furin cleavage site in BRI2 is sub-optimal (IQKR compared to RXXR), which may further reduce processing to ABri (30). In contrast, Aβ appears to be efficiently cleaved from the BRI-Aβ constructs in both in vitro (22) and in MoPrP-BRI-Aβ transgenic mice (28). However, this fusion protein is not endogenous and has different 1′ and 2′ amino acids at the furin cleavage site, therefore may interact differently with mouse furin.

Inefficient mtBRI2 proteolysis could also be due to saturation of mouse furin by the transgenic protein. Another possibility is that mouse furin and human BRI2 could reside in different subcellular compartments within the cell. This is unlikely to be the case as several experiments have shown that furin is active not only in the TGN, but also in several secretory pathways and in endosomes (36). BRI2 is glycosylated in the transgenic mice following a similar pattern to that in humans (data not shown), suggesting that in both systems BRI2 must be present in the ER and TGN. Also, BRI2 has been localized to the membrane fraction of the cell in transfected N2a cells (20), as well as in the MoPrP-mtBRI2 transgenic mice.

CO-EXPRESSION OF HUMAN FURIN AND MUTANT BRI

In an attempt to promote ABri production, we elevated levels of furin in the brains of the MoPrP-mtBRI2 transgenic mice, using adeno-associated virus (AAV) technology, with a view to increasing the rate of cleavage of the mutant BRI2 protein. As furin plays a role in several pathways involved in development and disease (36), global upregulation of furin throughout the CNS may cause additional associated unwanted phenotypes. Consequently, we chose a viral approach and stereotaxically injected neuronal specific associated adenovirus serotype 1 (AAV1) that contained human furin cDNA into the hippocampus of MoPrP-mtBRI2, MoPrP-wtBRI2 and non-transgenic mice. Mice were harvested one or 3 months after induction of human furin expression. Brains were analyzed for human furin mRNA expression by in situ hybridization, immunohistochemically for human furin protein expression, and for mutant BRI2 processing in the hippocampus by immunoblotting.

AAV is an efficient technique that induces high levels of both human furin mRNA and protein in discrete areas of the hippocampus, including the CA1 pyramidal cells, interneurons of the stratum radiatum and stratum oriens and the dentate gyrus (Figure 3A, 3B). Additionally, human furin expression altered BRI2 processing. Full length BRI2 (37KD) was detected by EN3 in MoPrP-mtBRI2 mice injected with empty AAV1, non-transgenic mice injected with AAV1-human furin, and also in MoPrP-mtBRI2 transgenic mice injected with AAV1-human furin. The N terminal fragment of BRI (34KD) was elevated in the mtBRI2 transgenic mice injected with empty AAV1 compared to the non-transgenic mice, as expected. However, the N terminal fragment was no longer detectable in the mutant BRI2 transgenic mice after injection of AAV1-human furin (Figure 3C).

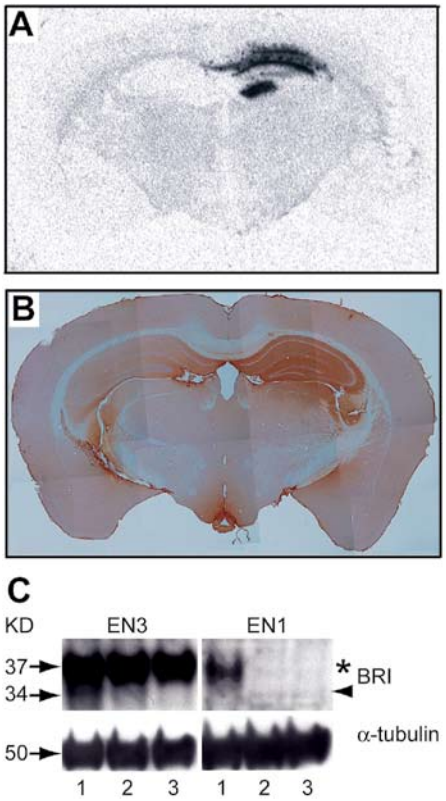

Figure 3. *Human furin expression in MoPrP-mtBRI2 mice alters mutant BRI proteolysis.* **A.** Human furin mRNA expression in the hippocampus of MoPrP-mtBRI2 mice injected with furin-AAV into the right hippocampus. **B.** Human furin protein expression in the hippocampus of MoPrP-mtBRI2 mice injected with furin-AAV into the right hippocampus. **C.** BRI protein expression in the right hippocampus. 1; MoPrP-mtBRI2 mouse injected with empty AAV, 2; ntg mouse injected with furin AAV, 3; MoPrP-mtBRI2 mouse injected with furin AAV. Human mutant BRI2 was cleaved by human furin (*), and N-terminal fragments of BRI were cleared (arrowhead).

Full length mutant human BRI2 (37 KD) was detected by the anti-ABri antibody, EN1, in the MoPrP-mtBRI2 mice injected with empty AAV1 but not in the non-transgenic mice injected with AAV1-human furin. However, no full length BRI2 was detected in the MoPrP-mtBRI2 transgenic mice after injection with AAV1-human furin. This suggested that mutant human BRI2 was cleaved when human furin was locally overexpressed using the AAV1 system. In addition levels of N terminal BRI2 normally elevated in transgenic mice, returned to basal levels. However, no ABri peptides were detected on western blots probed with EN1, and no extracellular ABri deposits were detected by immunohistochemistry. This data suggests that

BRI2 processing is compromised in the BRI2 transgenic mice.

CONCLUSION

The quest to develop a model of FBD continues 6 years after the mutation was discovered, although several groups have attempted to generate wild-type and mutant BRI2 transgenic mice. Mice expressing high levels of human mutant BRI2 do not appear to produce significant amounts of the ABri peptide and aberrant processing of human mutant BRI2 by mouse furin or furin-like proteases may block ABri production. Alternatively, properties of the ABri peptide may inhibit its extracellular aggregation and deposition. Further study of the ABri peptide may provide insights into plaque development as human co-factors or chaperones may be missing in mouse models.

"Synthetic" approaches that elevate ABri concentrations or amyloidogenicity, modulate the furin cleavage site or express efficiently cleaved ABri fusion constructs may prove effective strategies to generate mice that copy some of the hallmarks of FBD. "Endogenous" approaches including knocking-in the FBD mutation into m*BRI2* or expressing a genomic transgene under the regulation of the endogenous *BRI2* promoter may also be effective. Despite the difficulties encountered in generating murine models of FBD and FDD, the previous generation of AD research has demonstrated how essential this tool will be to understanding the pathogenesis of these diseases.

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