Psychoneuroendocrinology (2008) 33, 863–873

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Altered hypothalamus–pituitary–adrenal gland axis regulation in the expanded CGG-repeat mouse model for fragile X-associated tremor/ataxia syndrome

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Received 10 January 2008; received in revised form 19 March 2008; accepted 26 March 2008

KEYWORDS
FXTAS;
Fmr1;
CGG-repeat;
Corticosterone;
HPA axis;
Inclusions

Summary
The human *FMR1* gene contains an unstable CGG-repeat in its 5′ untranslated region. The repeat length in the normal population is polymorphic (5–54 CGG-repeats). Individuals carrying lengths beyond 200 CGGs (i.e. the full mutation) show hypermethylation and as a consequence gene silencing of the *FMR1* gene. The absence of the gene product FMRP causes the fragile X syndrome, the most common inherited form of mental retardation. Elderly carriers of the premutation (PM), which is defined as a repeat length between 55 and 200 CGGs, can develop a progressive neurodegenerative syndrome: fragile X-associated tremor/ataxia syndrome (FXTAS). The high *FMR1* mRNA levels observed in cells from PM carriers have led to the hypothesis that FXTAS is caused by a pathogenic RNA gain-of-function mechanism. Apart from tremor/ataxia, specific psychiatric symptoms have been described in PM carriers with or without FXTAS. Since these symptoms could arise from elevated stress hormone levels, we investigated hypothalamic–pituitary–adrenal (HPA) axis regulation using a knock-in mouse model with an expanded CGG-repeat in the PM range (>98 repeats) in the *Fmr1* gene, which shows repeat instability, and displays biochemical, phenotypic and neuropathological characteristics of FXTAS. We show elevated levels of corticosterone in serum and ubiquitin-positive inclusions in both the

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doi:10.1016/j.psyneuen.2008.03.011

pituitary and adrenal gland of 100-week-old animals. In addition, we demonstrate ubiquitin-positive inclusions in the amygdala from aged expanded CGG-repeat mice. We hypothesize that altered regulation of the HPA axis and the amygdala and higher stress hormone levels in the mouse model for FXTAS may explain associated psychological symptoms in humans.

1. Introduction

The fragile X mental retardation gene 1 (*FMR1*), located on the X chromosome, harbors a CGG-repeat in its 5′ untranslated region. This repeat may become unstable upon transmission to the next generation. Different clinical outcomes occur depending on the length of this trinucleotide repeat. Normal individuals carry a repeat of up to 44 CGG units, which remains stable on transmission (Fu et al., 1991). Alleles with between 45 and 54 repeats are considered intermediate size alleles, which are associated with some degree of instability. Individuals with over 200 CGGs have the full mutation, which usually leads to methylation of both the promoter region and the CGG-repeat, and consequent transcriptional silencing of the gene. The absence of the gene product fragile X mental retardation protein (FMRP) is the cause of the mental retardation seen in fragile X patients (Verkerk et al., 1991). Individuals with the premutation (PM) have between 55 and 200 CGGs. Female carriers of the PM are at increased risk of developing premature ovarian failure (POF) (Sherman, 2000). PM carriers also are at risk for fragile X-associated tremor/ataxia syndrome (FXTAS), which has been observed in elderly men over age 50, and less often in female PM carriers (Hagerman et al., 2001; Hagerman and Hagerman, 2002; Jacquemont et al., 2003).

FXTAS is a progressive neurodegenerative disorder, believed to be the result of a pathogenic RNA gain-of-function mechanism, as PM carriers produce two- to eight-fold elevated levels of *FMR1* mRNA in their lymphocytes. While transcription is increased, translation is hampered, resulting in slightly lower FMRP levels in individuals with high CGG-repeat alleles within the PM range (Tassone et al., 2000a, b, 2007; Kenneson et al., 2001). Patients with FXTAS usually present with tremor and ataxia, but may develop other neurological symptoms such as Parkinsonism, autonomic dysfunction and peripheral neuropathy and may suffer from cognitive decline including formal dementia. Post mortem studies of brains from patients with FXTAS reveal intranuclear inclusions in neurons and astrocytes in multiple brain areas (Greco et al., 2002). These inclusions contain several proteins, including ubiquitin, heat shock proteins including αB-crystallin, the RNA-binding proteins hnRNP-A2 (heterogeneous nuclear ribonucleoprotein A2) and MBNL1 (muscle blind-like protein 1) and a number of neurofilaments, among which are lamin A/C (Iwahashi et al., 2006) and *FMR1* mRNA (Tassone et al., 2004). Very recently, Pur α has been identified as a component of the ubiquitin-positive inclusions in FXTAS brain (Jin et al., 2007). Proteins that are sequestered into the inclusions may be prevented from exerting their normal function, thus resulting in cellular dysfunction, ultimately leading to neurodegeneration (Rosser et al., 2002; Jin et al., 2007; Sofola et al., 2007).

Recent studies have documented that the abnormal elevation of *FMR1* mRNA is associated with increased psychological symptoms, such as anxiety, depression, and irritability in adult PM carriers, with or without symptoms of FXTAS, especially males (Jacquemont et al., 2004; Hessl et al., 2005; Bacalman et al., 2006; Bourgeois et al., 2007). These psychological symptoms could arise from elevated stress hormone levels, thus aberrant regulation of the hypothalamus–pituitary–adrenal (HPA) gland axis. More evidence suggestive of altered regulation of the HPA axis by the PM comes from the observation that ubiquitin-positive intranuclear inclusions are also present in the anterior and posterior lobes of the pituitary gland of patients with FXTAS (Louis et al., 2006; Greco et al., 2007). A link has been suggested between pituitary inclusions and dysregulated neuroendocrine function in patients with FXTAS. Increased follicle stimulating hormone (FSH) (Hundscheid et al., 2001; Sullivan et al., 2005; Greco et al., 2007) and decreased inhibin A and B levels in female PM carriers were reported even in those who are cycling normally, suggestive of early ovarian aging and ovarian compromise (Welt et al., 2004). Elevated levels of FSH have been found to reflect decreasing ovarian reserve (MacNaughton et al., 1992), which can be correlated to the risk of developing POF seen in female PM carriers. Interestingly, intranuclear inclusions have been reported in the testicles of two men with FXTAS; inclusions were present in the anterior and posterior pituitary gland of one of these for whom the pituitary gland was available (Greco et al., 2007). Finally, a reduced amygdala response has been reported in PM male carriers which may explain the etiology of psychological symptoms involving emotion and social cognition as well (Hessl et al., 2007).

An expanded CGG-repeat knock-in (CGG) mouse model has been generated (Bontekoe et al., 2001; Willemsen et al., 2003), by substituting the endogenous mouse $(CGG)_8$ with a human $(CGG)_{98}$. The CGG-repeat in the mouse model shows instability upon transmission to the next generation (Brouwer et al., 2007), similar to humans. Also, the CGG mice show elevated *Fmr1* mRNA levels (Brouwer et al., 2007), as well as ubiquitin-positive neuronal inclusions throughout the brain (Bontekoe et al., 2001; Willemsen et al., 2003). Aberrant behavior in mice was described by Van Dam et al. (2005), including mild learning deficits and increased anxiety.

We explored HPA axis physiology in expanded CGG-repeat mice for two reasons: (1) increased anxiety has been observed in our mouse model (Van Dam et al., 2005) and PM carriers, especially those with elevated *FMR1* mRNA experience more psychological distress (Hessl et al., 2005);