Increased Glyoxalase-1 Levels in Fkbp5 Knockout Mice Caused by Glyoxalase-1 Gene Duplication

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ABSTRACT  Fkbp5 is genetically linked to stress-related diseases. Fkbp5 knockout mice are available and widely used to explore the role of Fkbp5 in health and disease. We found that these mice carry a gene duplication of glyoxalase-1, which explains why glyoxalase-1 levels are increased in the Fkbp5 knockout mice.

In several genetic studies researchers linked FK506 binding protein 5 (Fkbp5) to stress-related diseases and phenotypes such as major depression, posttraumatic stress disorder, and recovery from psychosocial stress (Binder et al. 2004; Zimmermann et al. 2011; Klengel et al. 2013). In addition, Fkbp5 is also linked to treatment response in depression (Binder et al. 2004; Lekman et al. 2008). To elucidate the role of FKB5 in an animal model, a conventional knockout mouse has been constructed and made available to the scientific community (Tranguch et al. 2005; Touma et al. 2011). These Fkbp5-deficient mice show no overt phenotype unless they are older than 10 months of age (O’Leary et al. 2011) or exposed to stress (Touma et al. 2011; Hartmann et al. 2012).

To elucidate the effects of Fkbp5-deletion on molecular pathways, we compared the expression profile of Fkbp5+/+ and Fkbp5−/− litter mates. A marked difference in glyoxalase-1 (Glo1) mRNA was observed with Fkbp5−/− mice expressing greater levels (not shown). Consistent with this observation, about 2-fold more Glo1 protein was found in Fkbp5−/− mice (Figure 1A). For more detailed molecular analyses, we sought to establish a cellular model. Therefore, we overexpressed FKB5 by transient transfection in either primary rat astrocytes or HEK293 cells. However, overexpression of FKB5 did not change Glo1 mRNA (not shown) and also not alter protein levels of Glo1 (Figure 1B).

We noted that the genes Fkbp5 and Glo1 are only approximately 2 Mb apart from each other on chromosome 17 of the mouse (Figure 1C). In addition, gene duplication around Glo1 was observed in several mouse strains (Egan et al. 2007; Williams et al. 2009). The Fkbp5 deletion was constructed in 129Sv J ES cells, and the resulting mice were then crossed with C57BL/6 animals; 129Sv J mice carry the Glo1 gene duplication but C57BL/6 mice do not (Williams et al. 2009).

Therefore, it appeared likely that through selection of Fkbp5+/+ and Fkbp5−/− alleles in the subsequent crossings the Glo1 gene duplication originating from 129Sv J mice was coselected with the Fkbp5−/− allele, whereas the unduplicated Glo1 cosegregated with the Fkbp5+/+ allele. To test this hypothesis, we used polymerase chain reaction (PCR) primers designed for monitoring the Glo1 gene duplication (Williams et al. 2009). DNA samples from Fkbp5+/+, Fkbp5−/+ and Fkbp5−/− mice were probed. No Glo1 gene duplication was detectable in Fkbp5−/− mice, whereas the PCR signal in Fkbp5−/− mice was clearly detectable and twice as high as in Fkbp5+/− mice (Figure 1D). Therefore, the greater levels of mRNA and protein of Glo1 in Fkbp5−/− mice compared with wild-type mice are likely due to the double Glo1 gene dose in these mice. In general, this so-called “flanking allele” problem is a well-known and likely common phenomenon in gene knockout via homologous recombination (Gerlai 1996; Crusio et al. 2009). It could be avoided, for example, by genome editing with engineered nucleases or by using inducible gene knock out techniques (Sauer 1998; Carbery et al. 2010).

GLO1 is a ubiquitously expressed enzyme involved in the detoxification of methylglyoxal (Thornalley 2008). Methylglyoxal is a toxic byproduct of glycolysis that leads to protein modification and apoptosis (Thornalley 2008) and influences behavior when acting as GABA A receptor agonist (Distler et al. 2012). GLO1 has been linked to diabetic complications, anxiety disorders, schizophrenia, seizure susceptibility, pain, cancer, and aging (Thornalley 2008; Distler and Palmer 2012). At least some of these diseases and phenotypes also
have been associated with Fkbp5, making Fkbp5+/− mice potentially very useful genetic model for further investigation. Our observation of Glo1 gene duplication in Fkbp5+/− mice suggests that the Glo1 status should be taken into consideration when interpreting data. Studies on neuroendocrine and stress effects of Fkbp5 gene deletion published so far are likely not biased by the Glo1 gene duplication, in particular because no differences between Fkbp5+/+ and Fkbp5+/− mice have been observed under basal conditions when neuroendocrine parameters or behavior, including anxiety-like behavior, is assessed (Touma et al. 2011; Hartmann et al. 2012).

### LITERATURE CITED


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