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Fkbp5-humanized mice shed light on female higher vulnerability to stress

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For the last twenty years, we have known that vulnerability or resilience to early life stress is modulated by genetics; individuals carrying certain alleles of certain genes are more or less likely to present stress-associated pathologies than others[1,2]. In a new study, Nold and colleagues[3] used humanized mice to investigate the effects of genetic and environmental factors on stress responses.

Such a stress vulnerability/resilience gene is the one encoding FK506-binding protein 51 (FKBP5). FKBP5 acts as a co-chaperone that changes folding and activity of the glucocorticoid receptor (GR) reducing locally glucocorticoid signaling, the major biological pathway involved in the stress response. Since *FKBP5* is itself a target of GR-mediated gene transcription, FKBP5 is a key element of a rapid intracellular feedback loop that decreases GR signaling when glucocorticoid levels are elevated. Single nucleotide polymorphisms (SNP) in the human *FKBP5* gene, such as rs1360780, are associated with differential induction of the FKBP5 protein upon glucocorticoid stimulation. The rs1360780-AT, or AT allele, is considered as the high induction allele of *FKBP5* conferring increased risk of developing a psychopathology after being exposed to adversity during childhood compared to the resiliency-associated rs1360780-CG (cytosine/guanine) allele. This occurs because the AT-containing sequence is associated with a chromatin 3D conformation that promotes direct contact of a glucocorticoid response element with the transcription start site of FKBP5 gene through TATA-box protein binding compared to the CG allele, thus leading to an enhanced mRNA transcription of *FKBP5*. Consequently, the AT allele leads to a more dynamic FKBP5-mediated

intracellular inhibition of GR that interferes with GR-dependent feedback of the hypothalamic-pituitary-adrenal (HPA) axis on the systemic level, thereby contributing to HPA axis dysregulation and stress-related phenotypes. This has been supported by studies showing prolonged cortisol responses, non-suppression of the HPA axis by dexamethasone, alteration in hippocampal shape and amygdala volume and increased anxiety, depressive or cognitive symptoms in AT-allele carriers[4].

No SNPs at the same location or with the same functional impact as the human rs1360780 SNP are found in rodent models. This fact prompted Nold and colleagues to develop *Fkbp5*-humanized C57BL/6 mouse lines carrying either the AT or CG allele to study the causal relationship and mechanistic impact of the human *FKBP5* SNPs on stress physiology and the development of stress-related behavioral disorders. After confirming previously that in these mice the AT allele leads to increased expression of *Fkbp5* in brain cells upon stimulation by GR[5], here[3] the authors exposed mice of the two lines and of both sexes to maternal separation as an early life adversity (ELA) model to analyze the interaction of *Fkbp5* genotype x ELA x sex on HPA functioning, stress-related behaviors and gene expression profiling in stress-related brain regions. Gene expression was qualitatively validated in human induced pluripotent stem cells (hiPSCs) from AT or CG carriers that had been differentiated in neurons and astrocytes.

Regarding the effects on the HPA axis, female mice showed differences in serum glucocorticoid levels between AT versus CG-allele carriers across 3 time points of the day in controls and ELA groups, with less rhythmicity and higher rest levels in AT females confirmed by elevated adrenal weight. Clear disruption of circadian rhythmicity is however difficult to ascertain with only 3 ill-defined time points (morning, noon, evening). Following a 5 min restraint stress or after dexamethasone suppression, no significant differences were found between genotypes neither in controls nor in ELA groups. These results differ from previous human studies where prolonged cortisol exposure after stress and non-suppression after dexamethasone were observed only in AT-allele carriers[6–8]. Regarding males the present

study detected no effect of genotype in male mice for HPA axis functioning whatever the early life condition.

Behaviorally, males seem also less impacted than females by the genotype and ELA treatment. As a first behavioral response to stress, habituation to a novel environment was assessed by measuring locomotor activity in a brightly illuminated open-field. Activity decreased over 15 min in both AT and CG females but to a lesser extent under ELA condition. AT-allele female carriers tended to display a slower habituation in both control and ELA condition. ELA induced a higher total nocturnal distance run in female CG-allele carriers only, while in AT genotype both control and ELA group display intermediary levels. In males, the activity measured in the open field arena was similar among groups. Unfortunately, time spent in the center of the arena is not reported which could have provided information on anxiety levels. The latter were evaluated in the light/dark box test where ELA groups spent less time in the dark compartment for both male and female compared to control groups with a tendency for lesser time for the CG-allele carriers. More time in the light compartment is usually interpreted as less anxiety. This is expected for CG versus AT genotype but not for ELA versus control groups, which makes the interpretation of these data quite difficult. More expected results were obtained in the social preference test where CG control females showed social preference which was found decreased by ELA, whereas no social preference was detected with the AT genotype independently from the early life condition. The opposite was found in males with AT-allele carriers in which ELA had a negative effect on social preference, while CG-allele carriers were not affected.

Differential gene expression analyses following RNA sequencing were carried out in the hypothalamus, ventral and dorsal hippocampus of females from the four experimental groups (AT and CG females exposed to control or ELA conditions) to identify transcriptional signatures that could explain differences in HPA functioning and behavior, according to *Fkbp5* genotype x ELA interaction. Interestingly, ELA modified a much higher number of genes in CG (903 genes) versus AT (114 genes) mice, mirroring behavioral and HPA data, which showed that ELA affects less AT-allele carriers. Bioinformatic analyses revealed significantly altered

pathways in the hypothalamus and the ventral hippocampus with a decrease in pathways related to circadian entrainment, neurotransmitter signaling and regulation of synaptic plasticity in AT-allele carriers and in ELA groups of both genotypes, and an increase in pathways related to metabolism (protein digestion and absorption, ribosome activity, oxidative phosphorylation) in mice with AT genotypes. Similar data were obtained from the mRNA of cells derived from hiPSCs from AT- versus CG-allele carriers, especially in astrocytes.

Overall, this *Fkbp5*-humanized mouse model is a new and interesting tool that revealed a strong sexual dimorphism in the *Fkbp5* genotype x ELA interaction as in humans[9], partially altered HPA axis regulation, some impact on stress-related behavior and modification in signaling pathways in the hypothalamus and the hippocampus. All these alterations associated with *Fkbp5* AT allele may contribute to the risk of developing psychopathologies in adult female individuals exposed to early life adversity and cumulating chronic stress over their lifetime. A large amount of work still remains to demonstrate the causal relationship between the various changes observed in the *Fkbp5*-humanized mouse model and the development of such psychopathologies.

Figure

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