

Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale



**94th National Congress of the
Italian Society for Experimental Biology**

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ABSTRACT BOOK

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Xia-Gibbs syndrome (XIGIS; OMIM #615829) is a very rare neurodevelopmental disorder caused by *de novo* heterozygous truncating mutations in the AT-hook DNA-Binding Motif-Containing 1 (*AHDC1*) gene (OMIM# 615790)¹⁻³. XIGIS is a phenotypically heterogeneous disorder in which patients usually present poor muscle tone and severe developmental delays with symptoms of autism spectrum disorders^{2,3}. The genetic basis of XIGIS were discovered in 2014 by Fan Xia and Richard Gibbs and more than 270 cases have been reported so far. Human *AHDC1* gene is a protein-coding gene located on chromosome 1p36, it contains 5 noncoding 5-prime exons, a single 4.9-kb coding exon and a noncoding 3-prime exon¹. The single coding exon encodes for a protein of 1,603 amino acids, containing two AT-hook DNA binding motifs. All XIGIS-associated mutations described to date are located in the single coding exon and likely lead to the translation of truncated forms of *AHDC1* protein¹⁻³ which could be involved in defective neural development, causing the neurological features of Xia-Gibbs syndrome. However, the functions of *AHDC1* are unknown and indeed the gene belongs to the group of T-dark genes. Thus, our research focuses on the characterization of *AHDC1* functions. Since the presence of AT-hook DNA binding motifs suggests that *AHDC1* might bind DNA, we started investigating its possible role in gene expression regulation by identifying genes responding to its perturbation in *in vitro* cellular models (SH-SY5Y and U-87MG cells, of neuronal and glial origin, respectively). By performing RNA-seq analysis upon *AHDC1* silencing, we found that the biological pathways related to intellectual disabilities, neural differentiation and nervous system functioning and development are enriched among differentially expressed genes in *AHDC1* knocked-down cells. In addition, studying *AHDC1* protein, we confirmed the nuclear localization of the endogenous *AHDC1* by immunofluorescence. Finally, by coupling overexpression of *AHDC1* with mass spectrometry analysis, we are investigating its structure and post-translational modifications as well as its interactors. Our preliminary results support the hypothesis that *AHDC1* could be crucial in regulating development and differentiation in the nervous system and therefore could help in the identification of the mechanism at the basis of pathogenesis for XIGIS which is still poorly understood.

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A NEW ANIMAL MODEL OF INTERNET GAMING DISORDER: SEXUAL DIFFERENCES IN BEHAVIOR AND BRAIN ACTIVITY

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In 2013 the American Psychiatric Association included Internet Gaming Disorder (IGD) as a mental disorder in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). IGD mainly occurs among adolescents, who after developing addiction, show psychopathological traits such as social anxiety, depressive disorder, attention deficit.

However, the different studies conducted so far show several limitations, such as exposure period, duration, and gender. Trying to address the lack of experimental model for such disorder, in the present work we proposed an IGD rat model to investigate some peculiar tracts of the disorder, such as sexual dimorphism, and to better understand the alleged role of hormones or the brain areas involved in IGD. In fact, previous studies in adolescents showed sexual differences in both play behavior and activation of related brain areas, such as mesocorticolimbic reward system (Nucleus Accumbens, NAc; Orbitofrontal Cortex, OFC; Prefrontal Cortex, PRL; Ventral Tegmental Area, VTA), the visual processing and cognitive control areas (Inferior Parietal Lobule and Middle Occipital Gyrus), the Thalamus and the Insula. We developed, for the first time, using a new apparatus provided with a touchscreen platform, an IGD rat model that resembles the fundamental features of the disorder (e.g., addiction, hyperactivity) and also sexually dimorphic activation of related brain areas. After five weeks of training, male and female Wistar Kyoto (WKY) rats were assessed for: a) their attachment to the game, as time spent in front of the screen, under different conditions (alone, together with a new object, and in a social and sexual context), b) their compulsiveness during gaming (duration and number of touches on apparatus), and c) the maintenance of these conditions after a period of game pause and a reward interruption. According to multicriteria described in the literature, it was possible to identify IGD-rats in 16/18 males and in 21/21 females, which obtained scores between 66 and 99%. IGD-rats showed a significant increase in frequency and duration of play, and time spent in front of the screen compared to both controls and rats which have been trained but did not develop addiction. Moreover, IGD-females showed greater interaction and duration of play, which was maintained even in the presence of sexual or social stimuli, compared to IGD-males. Last, with immunohistochemical techniques, we analyzed the *c-fos* (a neuronal activity marker) immunoreactivity to investigate different neural areas correlated to addiction disorder: cortex (PRL, M1, Orbital cortex), Amygdala, NAc, and paraventricular nucleus of the thalamus (PVT). Quantitative analysis of *c-fos* immunoreactivity showed a significant increase in all areas of the cortex and the amygdala of IGD-rats compared to controls. Sexually dimorphic immunoreactivity in PVT and NAc was reduced in IGD-females and increased in IGD-males compared to control rats. In conclusion, we developed a first animal model of IGD in rats, with great translational potential. In fact, it presents characteristics also found in IGD patients: the development of addiction-like behavior, the sex difference in susceptibility, and the changes in brain activity. The use of our animal model of IGD will allow us to further investigate the neurological basis of the disorder taking into account also the sex differences.

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