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Molecular genetic response to oxidative stress differs in hypothalamus of hypertensive ISIAH and normotensive WAG rats

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Motivation and Aim: It is known that an important link in the pathogenesis of hypertension is oxidative stress, which occurs under the influence of various environmental or endogenous factors, including psycho-emotional stress. The response to psycho-emotional stress may depend on the genotype and physiological state of the organism as a whole. However, the features of the molecular genetic mechanisms of the response to oxidative stress depending on the genotype have not been fully studied. The purpose of this work was to establish the inter-strain hypothalamic differences in the expression of genes associated with the response to oxidative stress in hypertensive ISIAH and normotensive WAG rats after exposure to a single two-hour restraint (emotional) stress.

Methods and Algorithms: Male (3 months old) hypertensive ISIAH rats and normotensive WAG rats were used in the study. The animals were divided into 4 groups: ISIAH_control, WAG_control, ISIAH_stress, WAG_stress (n=7 in each group). The basal systolic blood pressure was measured in all animals by the tail-cuff method using ether anesthesia [1]. After 7 days, the rats from the experimental groups were subjected to restraint stress, for which the rats were placed in a tight wire-mesh cage for 2 hours. Upon completion of the stress procedure, blood pressure was measured in these rats without anesthesia, after which immediate decapitation and isolation of the hypothalamus were performed. RNA-Seq analysis of hypothalamic samples was carried out on the DNBSEQ platform (DNBSEQ Technology) at BGI Hongkong Tech Solution NGS Lab. Pair-end sequencing of cDNA libraries was performed with a read length of 150 base pairs and sequencing depth of more than 30 million uniquely mapped reads. Bioinformatics analysis included reads mapping to the reference rat genome mRatBN7.2/rn7 using Star package [2], calculation of significant surrogate variables using the SVA package [3] to account for undesirable systematic variation in the sample preparation process, which were further included as factors in the differential expression analysis performed in DESeq2 [4] with the threshold significance set to FDR adjusted p-value<5 %. For functional annotation of differentially expressed genes (DEGs), the Rat Genome Database and DAVID (The Database for Annotation, Visualization and Integrated Discovery) databases were used. The gene set enrichment analysis for promoter binding sites for transcription factors was carried out using the web-based tool Enrichr [5]. Validation of differential expression of target genes was carried out using

real-time PCR (q-PCR). The study protocol was approved by the Bioethical Council of the Federal Research Center The Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia), protocol No. 115 of December 20, 2021.

Results: Functional annotation of DEGs revealed two groups of genes representing (1) a response to oxidative stress common to the two strains (59 DEGs) and (2) oxidative stress response genes specific to ISIAH rats (111 DEGs). DEGs from the common group change the transcription level unidirectionally in rats of both strains. However, in the hypothalamus of normotensive WAG rats, the response to oxidative stress is much less pronounced. Among the common DEGs, the genes Nos1, Ppargc1a, Abcc1, Srxn1, Cryab, Hspb1 and Fosl1 changed the transcription level most significantly. The gene set enrichment analysis for promoter binding sites for transcription factors revealed two terms associated with binding sites for the transcription factor CREB1 and the glucocorticoid receptor (NR3C1) in the hypothalamus of both rat strains, suggesting that these transcription factors may play a key role in regulation of the response to oxidative stress regardless of the genotype/strain differences and hypertensive state of the rats. A specific response to oxidative stress in hypertensive ISIAH rats is associated with induction of Fos gene expression, which was confirmed by q-PCR.

Conclusion: This study demonstrates both common and strain specific features in the molecular genetic mechanisms of the hypothalamic response to oxidative stress in hypertensive ISIAH rats being a model of stress-sensitive form of hypertension and normotensive WAG rats, complementing existing understanding of this process depending on the genotype and physiological state of the body.

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