be of substantial interest to the psychiatric genetics research community.

Disclosure

Nothing to Disclose.

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HIPPOCAMPAL MICRORNA ALTERATIONS IN RESPONSE TO CHRONIC STRESS IN YOUNG AND OLD FEMALE MICE

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Background: In recent years endogenous, small, non coding RNAs (microRNAs) that have important regulatory roles in animals and plants by targeting mRNAs for cleavage or translational repression, are being studied. miRNA function in animals, including their involvement in cell proliferation, apoptotic events, differentiation, fat and lipid metabolism, CNS, major depression, bipolar disorder and other diseases, have been researched and established. Chronic stress (CS) has a deleterious effect on several physiological systems. Moreover stress is an important factor that can trigger many pathophysiological conditions including neuropsychiatric disorders such as depression. The response to CS and the biological pathways involved, including the role of miRNAs, is still poorly understood.
Methods: To model effects of age we studied 3 vs. 21 month old female mice. To model the effects of CS we exposed mice to usual environment or to repeated, unpredictable daily stressors for a total of six weeks. Then, behavioral tests were performed over a three-week period of, during which exposure to the abovementioned stressors continued, after which the mice were sacrificed. Altogether 80 mice were studied. RNA from 12 hippocampi was analyzed on microarray chip.
Results: Using a two-way ANOVA, we observed a significant ($p<0.05$) interaction: 1) weight loss, 2) locomotion in an open field arena, 3) anxiety, and 4) cognition. CS had differential effects depending on age, so that the old female mice were much more susceptible to stress than the young ones. Specifically, while in old mice exposure to CS resulted in significant ($p<0.05$) weight loss, marked hyperlocomotion and impaired cognition compared to controls, in young mice CS was associated with modest improvement on several measures such as spatial memory and anxiety. Calculation of granular-cell layer surface area, yielded a significant ($p<0.05$) age by exposure interaction with a larger area in young mice exposed to CS as compared to older mice exposed to CS. Using DESeq2, 580 microRNAs were analyzed. A significantly higher expression profile was observed for microRNA-375 in the young CS group compared to the young control group ($padj<0.05$) and the old CS group ($padj<0.005$). Statistically significant higher expression profile was also observed for microRNA-7a in the young CS group compared to the old CS group ($padj<0.05$). qPCR validation and replication step is in progress.
Discussion: The present data suggest that CS, a common precipitating factor for neuropsychiatric disorders, has differential effects depending on age. Notably, while old mice were susceptible in terms of developing a plethora of behavioral and cognitive features and cognitive decline, young mice displayed striking resilience. These data are presented together with relevant mechanistic insights, such as differential effects of stress on key brain regions such as the dentate gyrus. Given recent compelling evidence implicating these two MiRs and their immediate target genes in neuroprotective processes secondary to a wide range of insults, including emotional stress, oxidative stress, ischemia-reperfusion injury, apoptosis and more, as well as in protective processes outside of the CNS, the hypothesis that selective expression of these MiRs only in young mice that were exposed to CS could help them protect against other, potentially deleterious, effects of stress, while 'enjoying' some of the enhancing effects that chronic stress exposure has been shown to exert could be supported.

Disclosure

Nothing to Disclose.

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TRANSGENIC MOUSE OVEREXPRESSING EAAT3 (NEURONAL GLUTAMATE TRANSPORTER): A NOVEL GENETIC MODEL OF OBSESSIVE-COMPULSIVE DISORDER

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Background: SLC1A1 (neuronal glutamate transporter EAAT3) is a strong candidate gene implicated in Obsessive-Compulsive Disorder (OCD). EAAT3 regulates -among other transporters- extracellular levels of glutamate in the cortico-striato-thalamo-cortical (CSTC) circuit implicated in OCD. SLC1A1 is the most evident 'brain-related' gene of interest located in the chromosomal region 9p24, the region

identified in the first genome-wide linkage study of mixed large and small families with OCD. In the first case-control study of this gene in OCD we found that SLC1A1 was associated with OCD. The strongest evidence from this study indicated that a haplotype was almost two times more frequent in OCD patients than controls (OR = 1.89); two of three SNPs of this haplotype were found to be expression Quantitative-Trait Loci (eQTLs). Here, we present the generation of a Cre-controllable EAAT3 overexpressing mouse for in vivo functional studies

Methods: A Cre-dependent, transgenic mouse overexpressing EAAT3 (OE) was generated by standard procedures. A pCLE-EAAT3 transgenic vector was generated, containing 1.7 kb beta-actin promoter combined with a CMV-IE enhancer (CAG promoter) for ubiquitous expression, and a eGFP gene flanked by loxP sites. After eGFGP, EAAT3 mouse cDNA was directionally subcloned. PCLE-EAAT3 was extensively characterized to generate a Cre-dependent, functional EAAT3 expression in vitro. To generate EAAT3 condtional OE EAAT3 mice, pCLE-EAAT3 vector was injected into zygotes of FVB/N mice. Founder lines were identified throug GFP visualization, and genotyped by Southern blot and PCR with specific GFP primers. Conditional EAAT3 OE (cOE) mice was generated by breeding EAAT3 OE mice with CamKIIa-Cre mice to drive overexpression in principal forebrain neurons, and PCR was performed for gentotyping. Molecular and immunohistochemical determinations were performed to validate the increased EAAT3 expression under CamKIIa promoter. CamKII- EAAT3 cOE mice were assesed for anxiety-like (open field test, light-dark) and OCD-like (marble-burying, grooming) behaviors.

Results: Compared to littermates lacking CamKII-Cre gene, CamKII- EAAT3 cOE mice showed no gross neurological alterations and exhibited normal body weith progression and locomotor activity. EAAT3 overexpression was found both by quantitative PCR and immunodetecion in several forebrain areas. CamKII- EAAT3 cOE mice were found to have increased anxiety-like behavior in the open field and light-dark tests. In the marble-burying test, CamKII- EAAT3 cOE mice buried higher number of marbles compared to their control littermates. Both chronic (21 days) clomipramine (40mg/kg) and fluoxetine (30 mg/kg) restored the OCD-like behaviors.

Discussion: We show here that increased EAAT3 expression in forebrain driven by CamKIIa promoter elicited OCD-like behaviors in mice. Remarkably, chronic administration of serotonin reuptake inhibitors restored the observed phenotypes, addind predictive validity to our novel OCD genetic mouse model.

We expect this overexpressing EAAT3 mouse to provide critical information on the role of EAAT3 dysfuntion at the gene regulatory, neurochemical, and anatomical levels during various stages of development. In addition, mice with conditional altered EAAT3 expression may offer exciting possibilities for generating new animal models of psychiatric and/or neurodegenerative disorders and also help in the development of drugs that target glutamate neurotransmitter system for effective OCD treatment.

Disclosure

Nothing to Disclose.

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Concurrent Oral Session 3: Schizophrenia - The Functional Significance of Genetic Findings

ULTRA-RARE PROTEIN-ALTERING VARIANTS AMONG 4,877 SWEDISH INDIVIDUALS WITH SCHIZOPHRENIA

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Background: Because individuals affected with schizophrenia have fewer offspring, purifying selection is expected to prevent high-risk alleles from reaching even modest allele frequencies. Similarly to rare copy number variants implicated in schizophrenia risk, variants with a large effect on schizophrenia risk are likely to be rare in populations, requiring sequencing to find them.

Methods: We generated whole exome sequencing data for 4,877 schizophrenia unrelated cases and 6,203 unrelated controls from Sweden. We define variants present uniquely in a single individual and not present in the ExAC database as ultra-rare variants (URVs). We further defined URVs as gene-disruptive and putatively protein-damaging (dURVs) those variants that are predicted to truncate or abrogate an encoded protein or that compromise protein function as assessed by 7 separate algorithms (SIFT, PolyPhen-2 HDIV, PolyPhen-2 HVAR, LRT, Mutation Taster, Mutation Assessor, and PROVEAN). We then measure the excess of synonymous URVs and dURVs in cases and controls and we measure the relative excess of dURVs across several gene sets with respect to the exome-wide excess. We further measured the excess of dURVs and more general sets of rare exome variants across each gene.

Results: We observed, and for the first time we estimated, an excess of 0.25 (95% CI=0.17-0.32) dURVs in schizophrenia cases on a background of about 4 dURVs per individuals. We found that the excess of dURVs in schizophrenia cases