

phrenia associated Icelandic haplotype (HapICE) region may harbor single nucleotide polymorphisms (SNPs) that could assist in differentiating high-risk individuals who will or will not transition to psychosis. Two such SNPs (rs4281084 and rs12155594) were recently shown to independently predict psychosis transition in a large ultra-high risk cohort in Australia and for every additional rs4281084-A allele and/or rs12155594-T allele (allelic load range 0 - 4) the relative risk of psychosis onset increased 1.56 (95% CI = 1.20 - 2.04) (Bousman et al., *Translational Psychiatry*, 2013, 3, e251). Although functional annotation of several SNPs within the HapICE region has been previously undertaken, the functional effects of rs4281084 and rs12155594 are not known. The aim of this study was to utilize online bioinformatics tools as well as post-mortem brain and neuroimaging data from individuals with and without a psychotic disorder to elucidate what effect, if any, these SNPs have on NRG1 function, regulation, gene expression, and brain structure.

Methods: To functionally annotate the NRG1 rs4281084 and rs12155594 loci a three-pronged approach was employed. First, the two SNPs were analyzed in silico via a pipeline that utilized a variety of online bioinformatics resources to provide detailed information of each SNP's genomic location, linkage disequilibrium (LD) profile, presence of regulatory elements (e.g. transcription factor binding sites, CpG islands), and/or overlap with copy number variations. In addition, using the Sanger Institute's gene expression variation (Genvar) software each SNP's association with in vitro gene expression was examined in lymphoblastoid cell lines (LCLs) derived from umbilical cords of 75 Geneva GenCord individuals. Second, we examined postmortem prefrontal cortex from the Victorian Brain Bank Network (BA 9) from 40 (12 non-psychiatric controls, 28 schizophrenia) via DNA and mRNA analysis conducted using the Affymetrix Exon 1.0 ST Array, to determine if the two NRG1 SNP's or their combined allelic load had cis-regulatory effects on pan-NRG1 gene expression. Finally, structural magnetic resonance imaging (sMRI) and genotype data from 156 non-psychiatric controls and 177 individuals with a psychotic disorder (e.g. schizophrenia) from the Australian Schizophrenia Research Bank were examined to determine if the two SNPs or their combined allelic load were associated with brain volumes in regions of interest (i.e. lateral ventricles, superior frontal, middle frontal, superior temporal, or anterior cingulate) defined based on the Desikan-Killiany atlas.

Results: In silico analysis revealed that rs4281084 is located 207 base pairs upstream of the putatively functional rs6994922 (SNP8NRG243177) SNP. The rs4281084 locus is in strong LD ($D' = 1.0$, $r^2 = 0.95-0.02$) with four of the five HapICE loci as well as a putative cis-regulatory locus (rs7014762) within the HapICE region. rs12155594 is located between two microsatellites (478B14-848 and 420M9-1395) that are part of the 7-marker HapICE haplotype and sit between the Type II and Type I promoters. The mapped location of rs4281084 sits within a v-maf musculoaponeurotic fibrosarcoma oncogene homolog K (MAFK) transcription factor binding site whereas rs12155594 was not found to overlap with any known regulatory elements. However, three previously reported copy number variants as well as a long

interspersed nuclear element 1 (L1) retrotransposon overlap the rs12155594 locus. Analysis of human brain mRNA showed that neither rs4281084 nor rs12155594 were associated with NRG1 gene expression individually, but combined to impact mRNA level. Individuals (both controls and people with schizophrenia) with a greater allelic load were significantly more likely to have higher pan-NRG1 expression ($F_{3,33} = 3.85$, $p_{adj} = 0.018$). Similarly, structural MRI analysis revealed no associations between each SNP and brain volumes when examined individually. However, significantly larger right (37%-50%, $F_{2,170} = 6.95$, $p_{adj} = 0.01$) and left (33% - 45%, $F_{2,170} = 5.21$, $p_{adj} = 0.03$) lateral ventricle volumes were observed for cases with a psychotic disorder who had greater allelic load compared to those with no to low allelic load but no associations were observed in controls.

Conclusions: These results represent the first attempt to functionally annotate and biologically characterize the impact of two recently identified NRG1 loci associated with psychosis onset. They build on a growing body of research supporting the functional importance of genetic variation within the HapICE region of the NRG1 gene. In concordance with previous functional studies of other loci located in the HapICE region, our results suggest that the combined allelic load of rs4281084 and rs12155594 have potential cis-regulatory effects on NRG1 expression and are associated with increases in bilateral ventricle volume. Collectively, these results and those of others suggest that the functional effects conveyed by sequence variation within NRG1 are likely not driven by one SNP but rather, a diverse accumulation of nucleotide changes particularly in upstream regions capable of regulating gene expression.

Keywords: gene association, gene expression, imaging genetics.

Disclosure: Nothing to Disclose.

T178. Galanin-System Genes and 5-HTTLPR Are Differentially Involved in Stress Induced Anxiety and Depression and Interact with Each Other in Anxiety but Not in Lifetime or Current Depression

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Background: The gap between the progress in fundamental neuroscience and the slow advance in the treatment of neuropsychiatric disorders has led to the re-evaluation of approaches, with special focus on novel mechanisms that have preliminary clinical validation. Results of a recent study indicated that the galanin neuropeptide and its receptors play a significant role in the pathogenesis of major depression, but this effect is present only in subjects with high exposure to stress (Juhasz G et al., *Brain galanin system genes interact with life stresses in depression-related phenotypes*. PNAS 111:16 pp. E1666-1673, 2014). Furthermore, chronic pain induced loss of motivation requires galanin receptor-1 triggered depression of excitatory synaptic transmission in indirect pathway nucleus accumbens medium spiny neurons in mice (Schwartz N et al., *Decreased motivation during chronic pain requires*

long-term depression in the nucleus accumbens. *Science* 345:6196 pp.535-542, 2014). These results are in agreement with the fact that galanin expression is strongly stimulated by stress in animals, and validate the galanin system as a target for antidepressant drug development. The similar gene-stress interaction effects of the galanin system genes (GAL, GALR1, GALR2 and GALR3) and the previously described 5-HTTLPR polymorphism in the SERT gene raised the question as to whether these genetic effects could be separated according to different depression-related phenotypes. Other questions included possible interactions between the galanin system genes and 5-HTTLPR, and interaction of these genetic effects with gender on the different phenotypes.

Methods: We studied a population cohort (NewMood) of >2,300 individuals from Manchester and Budapest. 5-HTTLPR of the serotonin transporter gene and twelve SNPs of the galanin-system genes were genotyped. Phenotypic outcome measures included self-reported lifetime depression, current depression and current anxiety using the Brief Symptoms Inventory. The list of Threatening Life Experiences (TLE) was used to measure life stress-related environmental factors in the last 12 months. Statistical analyses were performed by multivariate system-based Bayesian analysis of relevance in highly TLE exposed persons. Analysis of interactions between the galanin system genes and 5-HTTLPR, and the genes and gender were performed by using the predictor variables jointly.

Results: For lifetime depression the order of relevance for the analysed genes was GALR2 > GALR3 > GAL > GALR1 > 5-HTTLPR with a similar pattern observed for current depression. In contrast, the contribution of genes to anxiety was very different, 5-HTTLPR > GAL > GALR1 > GALR2 > GALR3. For lifetime and current depression there were no interactions between the 5-HTTLPR polymorphism and the galanin system genes. For current anxiety, epistasis between 5-HTTLPR-GALR1 and GAL-GALR1 were found. In addition, interactions with gender were found for GAL and GALR3 in depression phenotypes, and for GAL and 5-HTTLPR in anxiety. After omitting TLE from the model none of these genes were relevant for depression-related phenotypes.

Conclusions: These results show that in subjects exposed to high life stress GAL system gene variations and combinations are associated predominantly with depression, while 5-HTTLPR s allele is predominantly associated with anxiety. In addition, interactions between galanin system genes and 5-HTTLPR are present for anxiety but not for lifetime or current depression. Our results underline the importance of assessing gene-environment interactions via different depression-related phenotypes. Furthermore, non-conventional methods of analysis will help us to better understand disease aetiology and to identify putative drug targets for anxiety and depression.

Keywords: galanin, life stress, depression, anxiety.

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T179. Abnormal X Chromosome Inactivation in Females with Major Psychiatric Disorders

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Background: Mania, major depression and schizophrenia are severe brain disorders. No biological hallmark has been identified for any of these disorders. Our previous studies found that the DISC1-Boymaw fusion gene from the Scottish family inhibits protein translation and thereby may contribute to pathogenesis of major psychiatric disorders. Excessive protein translation has been suggested as core pathology for Fragile X Syndrome and autism. We speculate that abnormal protein translation may contribute to a wide range of mental disorders not only in rare families, but also in the general population of patients. To investigate our hypothesis, we analyzed protein translation activity in the lymphoblastoid cells of psychiatric patients. Indeed, we observed abnormally high or low protein translation activities in the patients' cells. Surprisingly, all of the abnormal protein translation activities come from female patients. These findings prompted us to investigate functions of the X chromosome in the female patients.

Methods: Patients with either mania and psychosis or recurrent major depression were randomly collected from the general population. All patients and healthy controls are European Caucasians with age matched. Protein translation activities and RNA expression were measured in the lymphoblastoid cells. Postmortem brain RNA samples were provided by Stanley Medical Research Institute. RNA expression analysis was conducted using Q-RT-PCR. β -actin RNA expression was used as an internal control for normalization.

Results: Since we found that all variation of protein translation activities in the lymphoblastoid cells comes from female patients, we examined whether X chromosome inactivation (XCI) may be dysregulated in female patients'