PHARMACOGENETIC VARIATION IN DRUG METABOLISM ALTERS THE EFFICACY OF SMOKING CESSATION

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Genetically variable CYP2A6 metabolically inactivates ≈90% of nicotine to cotinine and ≈100% of cotinine to 3'-hydroxycotinine. The ratio of 3'-hydroxycotinine to cotinine provides a reliable measure of nicotine metabolism rate. We found the odds of quitting with nicotine patch were reduced by 30% with each increasing quartile of the ratio (Lerman 2006); faster metabolizers were less successful in quitting than slow metabolizers. The ratio did not predict cessation in the nicotine spray group, presumably because participants titrated their dosing to accommodate their different rates of metabolism (Maliayandi 2006). We have replicated and extended this in a study of longer duration patch treatment (Schnoll 2009; Lerman 2010). We have also examined pharmacokinetic variation in a placebo controlled trial of bupropion (Zyban). Fastest metabolizers (4th quartile of the ratio) benefited significantly from bupropion compared to placebo (OR=4.5) while little/no benefit was seen for slow metabolizers (Patterson, 2008). This suggests that CYP2A6 slow metabolizers do well on the patch, particularly if they stay on the patch for an extended duration, but gain little therapeutic benefit from bupropion, while CYP2A6 fast metabolizers respond well to bupropion. Bupropion is metabolized by CYP2B6. We found a significant *CYP2B6* genotype by treatment interaction (OR=2.9) where bupropion produced higher abstinence rates than placebo for smokers in the *CYP2B6*6* group (45%) (Lee 2007). In contrast, the *CYP2B6*1* normal metabolizers (55%) did well on placebo, receiving no additional benefit from bupropion. Thus a pretreatment test to determine smokers' nicotine metabolism rate or genotype may be useful for treatment optimization.

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PHARMACOGENETIC TAILORING FOR SMOKING CESSATION: TESTING KEY ASSUMPTIONS

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Despite growing interest in pharmacogenetics to tailor smoking cessation treatment, two key assumptions of this approach remain largely untested. These are that: 1) pharmacogenetic tailoring will be cost-effective relative to the current “one-size-fits-all” approach, and 2) there will be no negative psychological of behavioural consequences of providing genetic feedback. Here we present the results of two studies which tested these assumptions.

In the first, we took the results of preliminary pharmacogenetic studies of *DRD2* genotype and smoking cessation and subjected these to a cost-effectiveness analysis. We used multiparameter evidence synthesis methods to combine evidence on cessation by genotype with evidence on cessation irrespective of genotype. We considered four types of treatment: nicotine replacement therapy (NRT) pharmacotherapy, bupropion SR pharmacotherapy, combination NRT and bupropion, and standard care as the control. Our results indicated that the most cost-effective strategy is to prescribe both NRT and bupropion regardless of genotype, as a first-line treatment for smoking cessation. We conclude that it should not be assumed that genetic tailoring will necessarily be more cost-effective than applying the current “one-size-fits-all” model of pharmacotherapy for smoking cessation. In addition, single-gene tests are unlikely to be cost-effective, partly because the predictive value of these tests is likely to be modest.

In the second, we conducted an open label, parallel group randomised trial (ISRCTN: 14352545) in primary care. Adult smokers (n = 633) were prescribed NRT patch, randomised to a top-up dose of NRT based explicitly either on *OPRM1* genotype or heaviness of smoking and followed for 6-months. Outcomes measures were: 1) proportion of prescribed NRT consumed in the first 28 days, and 2) motivation to make another quit attempt among those not abstinent at 6-month follow-up. There was a significant effect of genetic feedback compared to phenotypic feedback on adherence at 7-day follow-up (75% vs 69%, p = 0.040), and a marginally non-significant effect at 28-day follow-up (69% vs 64%, p = 0.098). There were similar effects on abstinence at six-month follow-up (14% vs 8%, p = 0.018). Amongst those not abstinent at 6-month follow-up, there was no significant difference in motivation to make another quit attempt between trial arms (p = 0.23). We conclude that genetic feedback may motivate adherence to medication, and does not appear to reduce motivation to attempt another quit attempt following initial failure.

Taken together, these results indicate that two key assumptions which underpin a pharmacogenetic approach to smoking cessation treatment may be valid, but only under certain conditions. For instance, it may not always be the case that such an approach is cost-effective, given the additional clinician time which may be required. Future pharmacogenetic research programmes should also explore the translational implications of pharmacogenetics.

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FREQUENCY OF COMT POLYMORPHISMS IN METHAMPHETAMINE DEPENDENT HISPANICS AND CAUCASIANS

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Background: Polymorphisms in catechol-o-methyltransferase (COMT) have been associated with a variety of psychiatric disorders including addiction. Yet few studies have examined COMT polymorphisms in methamphetamine dependence outside of Japan. We performed a pilot study to determine genotype frequencies for COMT single nucleotide polymorphisms (SNPs) among Caucasian and Hispanic methamphetamine users entering a methamphetamine treatment study in Los Angeles.

Methods: The functional Val158Met SNP in COMT (rs4680), along with four other SNPs in a haplotype that influences COMT function (rs165656, rs4633, rs4818, rs6269) were genotyped in self-reported Caucasian (N=32) and Hispanic (N=28) methamphetamine dependent participants in a methamphetamine clinical trial in Los Angeles. Genotype frequencies and clinical characteristics between Caucasians and Hispanics were compared.

Results: All SNPs examined were in Hardy-Weinberg equilibrium in both populations. Genotype frequencies for Hispanics differed from those in Caucasians ($P<0.05$) for rs4680, rs165656, and rs4633 but not rs4818 or rs6269. Genotypes frequencies in Hispanics for COMT Val158Met (rs4680) were Val/Val 53%, Val/Met 36%, Met/Met 11% versus Val/Val 26%, Val/Met 61%, Met/Met 13% in Caucasians. Hispanics were younger and provided fewer methamphetamine-free urine drug screens during treatment relative to Caucasians but did not differ in baseline or lifetime methamphetamine use.

Discussion: Frequency of COMT polymorphisms differs in Caucasians and Hispanics with methamphetamine dependence. Pharmacogenetic studies of methamphetamine dependence in samples of Caucasian and Hispanic methamphetamine users stratified by ethnicity are needed to determine if COMT polymorphisms are associated with response to treatment for methamphetamine dependence.

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INTRAVENOUS NICOTINE SENSITIVITY AND CHRNA5 (RS16969968) GENE VARIATION IN CIGARETTE SMOKERS

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Cigarette smoking is the single most important source of preventable morbidity and premature mortality in the United States, with an estimated 19.8% of adults classified as current smokers. Nicotine effects are primarily mediated by neuronal nicotinic acetylcholine receptors (nAChRs), a group of ligand-gated ion channels distributed throughout the brain. Several genome-wide and candidate gene-based association studies have reported evidence for a relationship between of variants mapped to the *CHRNA5/A3/B4* gene cluster with various nicotine-related behaviors. One of the most biologically compelling single nucleotide polymorphisms (SNPs) in this cluster is rs16969968, a nonsynonymous SNP in the *CHRNA5* receptor subunit gene. To our knowledge, researchers have not tested whether rs16969968 moderates the subjective effects of intravenous (IV) nicotine. We are conducted a placebo-controlled study, where rs16969968 genotype (A/A vs. A/G or G/G) is the between-subject factor. So far, 40 smokers (20 from each rs16969968 group) participated in one experimental session following overnight abstinence, where they received saline, 0.5 or 1.0 mg/70 kg in order. The Drug Effects Questionnaire (DEQ) was administered at baseline as well as before and after each of the three IV injections. Preliminary analysis of the data suggests that rs16969968 moderates several subjective drug effects from IV nicotine. Results from this study help clarify relevance of rs16969968 in nicotine sensitivity in smokers.

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PHARMACOGENETICS RELATED TO SPECIFIC ADDICTIONS: FUNCTIONAL VARIANTS AND EPIGENETICS

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Studies of pharmacogenetics related to specific addictions may increase our understanding of the mechanisms involved in the addictive diseases by elucidating differential responses to both drugs of abuse and also pharmacologic therapeutic agents. Variants that change the pharmacodynamics or pharmacokinetics of a drug of abuse or medication are of interest. In our Laboratory, we have conducted and are conducting such studies and, to date, have identified three genes where functional variants make a significant difference in the pharmacodynamics and/or the pharmacokinetics of a drug of abuse, as well as epigenetic changes, which may be of importance to the mechanisms of an addictive disease or to the response to treatment by potential altering of gene expression. The genes which we have identified to date to have such functional variants or epigenetic changes include the mu opioid receptor gene, the dynorphin opioid peptide gene, and the ABCB1 gene involved in drug disposition. Recent finding related to each of these suggest that a focus on pharmacogenetic studies may be of immediate importance and applicability to the diagnosis and treatment of addiction.

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SYSTEMATIC DISSECTION OF DOPAMINE RECEPTOR GENES (DRD1 – DRD5) IN ANTIPSYCHOTIC INDUCED WEIGHT GAIN

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Antipsychotic induced weight gain has emerged as a serious complication in the treatment of patients with atypical antipsychotics, especially with clozapine and olanzapine. The dopaminergic system may be implicated as it activates motivation in reward circuits related to increased intake of palatable food.

Methods: A total of 206 patients (139 European Americans and 56 African Americans) who underwent treatment for chronic schizophrenia or schizoaffective disorder were evaluated for antipsychotic induced weight gain for at least six weeks. A total of 36 tag-SNPs and one variable number tandem repeat (VNTR), spanning the five dopamine receptor genes (DRD1 – DRD5), were evaluated for association with 1) percent weight change and 2) the occurrence of at least 7% weight gain.

Results: We found DRD2_rs1079598 to be significantly associated with weight gain in the entire sample (7% increase, $p > .05$). This finding hold true in patients of European origin receiving clozapine or olanzapine ($p=0.009$). Interesting trends (at least 7% increase) were observed for DRD2_rs6277 (C957T, a functional polymorphism; $p=0.092$), and DRD2_rs1800497 (TaqIA) ($p=0.077$) and for two SNPS in the DRD3 gene. The C957T polymorphism was also associated with percent weight change ($p=0.093$). Haplotypes of C957T and rs2234689 were associated with weight gain (7% yes/no; window $p=0.033$) as well as percent weight change (window $p=0.028$). Specifically, the C-C haplotype was associated with the highest percentage weight gain ($p=0.009$), and the same haplotype was over-represented in the group with at least 7% weight gain ($p=0.006$). The results for the other tested dopamine receptor polymorphisms and haplotypes were not significant.

Conclusions: In this study we provide evidence that the dopamine receptor DRD2 gene, and particularly the C957T polymorphism, might be associated with antipsychotic-induced weight gain in chronic schizophrenia patients.

METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) 677C/T AND CATECHOL-O-METHYLTRANSFERASE (COMT) VAL158MET VARIANTS AND ENDOTHELIAL FUNCTIONING IN SCHIZOPRENIA SUBJECTS TREATED WITH ANTIPSYCHOTICS

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Introduction: The risk of cardiovascular disease (CVD) with atypical antipsychotic use (AAPs) is well known. Previously we reported a greater metabolic syndrome risk in MTHFR 677T allele carriers. The COMT Val158Met variant may also increase this risk.

Purpose: To examine the relationship between the MTHFR 677C/T and COMT Val158Met variants, metabolic syndrome and endothelial dysfunction in schizophrenia and bipolar subjects.

Methods: 173 subjects are currently included in this cross-sectional analysis. Subjects are screened for the metabolic syndrome (NCEP ATP-III criteria), and MTHFR 677C/T and Val158Met genotype. Additionally serum folate, vitamin B12, and homocysteine are measured. The Endo-PAT 2000 was used to measure endothelial functioning in some subjects.

Results: Overall, 81subjects (47%) meet metabolic syndrome criteria. The subject's mean age (± s.d.) is 41.86 ± 11.62 years, 79% are Caucasian, 52% are male, 69% are receiving clozapine, olanzapine, risperidone or quetiapine, and the mean Body Mass Index (BMI) is 31.2 ± 7.6 kg/m^2. 30% had a diagnosis of bipolar disorder. 60% were current smokers. There are no differences in age, gender, race, AAP exposure, or BMI between genotype groups. The mean PAT index (endothelial functioning measure) is 1.72 ± 0.49. A PAT index of <1.35 has 80% sensitivity and 85% specificity to identify endothelial dysfunction.
Homocysteine was highest in MTHFR T/COMT Val carriers (mean = 22.7 ± 17.9) after controlling for folate concentrations ($F = 4.77$, $df = 4, 84$, $p < 0.0016$). A significant relationship was found for the MTHFR T/COMT Val carriers and metabolic syndrome controlled for folic acid concentration ($\chi^2 = 14.4$, $p = 0.0061$). The MTHFR TT/COMT Val allele carriers also had the worst endothelial functioning at similar folate concentrations ($F = 3.88$, $df = 4, 36$, $p = 0.01$).

Conclusion: Overall, our results show a relationship between homocysteine, MTHFR and COMT and endothelial dysfunction. These data add more clues into folate's role in mental illness and may lead to new treatment options as we work to eliminate the metabolic risks associated with AAP treatment. Due to the small sample size, these results should be taken cautiously and need to be confirmed as we recruit additional study subjects.

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CANNABINOID RECEPTOR 1 GENE AND ANTIPSYCHOTIC INDUCED SIDE EFFECTS: INSIGHTS FROM ANTIPSYCHOTIC INDUCED WEIGHT GAIN AND TARDIVE DYSKINESIA

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Background: The Cannabinoid receptor 1 (CNR1) is one of the most commonly expressed G-coupled receptors. It is expressed pre-synaptically in the basal ganglia and the hypothalamus, brain regions associated with movement and feeding, among others. Generally, CNR1 receptor activators tend to inhibit movement, and activate feeding, an effect prevented by rimonabant and other selective CNR1 receptor antagonists. Similarly, CNR1 receptor knockout mice exhibit reduced food intake and increased locomotor activity. Thus we investigated the role of the CNR1 gene in antipsychotic induced weight gain (APWG) and Tardive Dyskinesia (TD). The former caused in general by atypical antipsychotics (e.g. clozapine) the latter by typical antipsychotics (e.g. haloperidol).

Methods: Twenty tagSNPs spanning the CNR1 gene were analysed in schizophrenia patients (APWG, n=183; TD n= 192) of European ethnic origin. TD was assessed using the Abnormal Involuntary Movement Scale (AIMS) or the modified Hillside Simpson Dyskinesia Scale. In a prospective fashion, change in weight from baseline was assessed in patients who underwent treatment (with either clozapine, olanzapine, haloperidol or risperidone) for up to 14 weeks.

Results: All the polymorphisms were in Hardy-Weinberg equilibrium ($p>0.05$). Significant genotypic ($p=0.007$), allelic ($p=0.02$) and haplotypic ($p=0.017$) association was observed between TD and rs806374 (T>C). Another polymorphism, rs806378, was significantly associated with APWG in Europeans treated with clozapine or olanzapine. 'T' allele carriers (CT+TT) gained more weight (5.96%), than the CC carriers (2.76%, $p=0.008$). This was reflected in the allelic as well as haplotypic analysis (C vs. T allele, 3.84% vs. 5.83%, $p=0.035$). No significant association was observed with other SNPs for either phenotype.

Conclusions: These results indicate a possible role of CNR1 in the development of TD and APWG in our patient population. Association of different SNPs with the different phenotypes may indicate a role of tissue specific factors (accumbens & hypothalamus for weight gain, dorsal striatum for TD) in regulating the expression of CNR1 gene.

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ANTIPSYCHOTIC-INDUCED WEIGHT GAIN: PHARMACOGENETIC STUDIES IN DRUG-NAÏVE PATIENTS

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Clinically significant weight gain is a commonly reported adverse event associated with antipsychotic use, and represents a major public health concern in the clinical management of schizophrenia and other serious mental illnesses. Weight gain has been noted across most currently available antipsychotic agents, including first-generation “typical” antipsychotics, but large inter-individual differences are observed and mechanisms remain poorly understood. Antipsychotic-induced weight gain is particularly pronounced in the first episode of schizophrenia; effects of prior treatment lead to an under-estimation of the extent of this potentially serious outcome in studies of chronic patients. Because long-term treatment with antipsychotics causes substantial physiological changes, including dopamine receptor upregulation, pharmacogenetic studies of patients in the first episode (FE) of schizophrenia may be methodologically advantageous.

In this presentation, we will present data from two studies of antipsychotic-induced weight gain in drug-naïve populations. First, we present a hypothesis-driven candidate gene study of the D_2 receptor gene (*DRD2*) in a randomized, prospective 16-week trial of olanzapine and risperidone in 58 patients with first episode (FE) schizophrenia. Carriers of the deletion allele at -141C Ins/Del (rs1799732), a functional promoter region polymorphism in *DRD2*, demonstrated significantly more weight gain over the course of treatment regardless of assigned medication, and independent of dose. Second, we will present novel data from a genomewide association study (GWAS) in a naturalistic 12-week trial of more than 200 treatment-naïve children and adolescents receiving their first prescription of antipsychotics. These subjects gained an average of 10-20 pounds in 12 weeks, depending on drug assignment.

GENETIC VARIATION IN INDOL1 IS ASSOCIATED WITH ANTIDEPRESSANT TREATMENT OUTCOME IN MAJOR DEPRESSIVE DISORDER

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Background: The essential amino acid tryptophan is the precursor to serotonin (5-HT), but it can also be metabolized into kynurenine (KYN) through indoleamine-2,3-dioxygenase (IDO). Increased immune activation has long been associated with symptoms of depression and has also been shown to upregulate the expression of IDO. The presence of additional IDO directs more tryptophan down the KYN pathway, leaving less available for synthesis of 5-HT and its metabolites. Acute tryptophan depletion has been associated with depressed mood and other psychiatric symptoms. Recent research suggests that KYN and its metabolites may play a bigger role in the pathophysiology of MDD. KYN is metabolized through a series of enzymes to quinolinic acid, a potent *N*-methyl-D-aspartate (NMDA) receptor agonist with demonstrated neurotoxic effects.

Methods: We searched for genetic predictors of citalopram treatment outcome in 1,953 patients enrolled in the Sequenced Treatment Alternatives for Depression (STAR*D) study. Genotypes corresponding to 23 single-nucleotide-polymorphisms in the genes INDO and INDOL1 were extracted from a larger genome-wide set and analyzed using single marker and haplotypes tests to look for association with previously defined response, remission and QIDS-C change phenotypes with adequate correction for racial stratification.

Results: Of the 23 markers examined, none were significantly associated with all three phenotypes. However, 2 two-marker haplotypes, rs2340952/rs16888478 (TC) and rs3808606/rs3739319 (GC) were associated with response, remission and QIDS-C change.

Conclusion: We conclude that genetic variation in *INDOL1* may play a role in antidepressant treatment outcome; however, these results need to be replicated in an independent sample.

REPLICATION OF ASSOCIATION OF THE NTRK2 GENE WITH LITHIUM RESPONSE IN BIPOLAR DISORDER IN A PROSPECTIVE SAMPLE

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Lithium is the oldest mood stabilizer medication and the gold standard for treatment of bipolar disorder. Lithium responders comprise a clinically distinct subset of bipolar disorder patients many of whom have a excellent response to the drug. The identification of genes that predict response would be invaluable in guiding clinicians in treatment selection. 144 lithium responders and 102 non-responders were idenitfied by retrospective review of research interviews and medical records. A SNP (rs1387923) 3' of the gene for the trkb neurotrophin receptor (NTRK2) was associated to response (p=0.005). This association was observed only in patients who had predominantly euphoric rather than dysphoric mania. Though retrospective assessment of response is easier, a prospective trial is more definitive. We now report initial results from a prospective trial of lithium response. 77 subjects were entered into a clinical trial the goal of which was to stabilize the patients on lithium monotherapy over 3 months and then follow them for 2 years. In this initial analysis, total time in the study was examined using Cox proportional hazard survival analysis. After incorporating several clinical co-variates, the same SNP in NTRK2 also showed association in the prospective sample ($X^2=14.1$, p=0.028). The same allele was associated with response as in the retrospective sample. These data provide further support for the role of NTRK2 in lithium response.

GENDEP: TRANSLATIONAL MEDICINE OUTPUT

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Background: GENDEP is a European multicentre Integrated Project (http://gendep.iop.kcl.ac.uk/results.php), which has to date generated many findings of clinical relevance.

Methods: For design of the clinical trial and genotyping methodolgy, see references on the above url. Samples for transcriptomics were taken with PAXgene tubes.

Results (since data last presented at PIP). The genetic underpinning (Perroud and Aitchison et al, 2009) of a relative increase in suicidal ideation in men prescribed NOR would appear to include a variant in *ADRA2A*. Variants in *BDNF* were also associated with increasing suicidal ideation, and in interaction with variants in the BDNF receptor (*NTRK2*), and this was in part replicated by GWAS. Retirement and history of suicide attempts predicted treatment-worsening suicidal ideation (Perroud et al, 2009). There was a significant gene-environment interaction between life events measured using the LTE-Q and the serotonin transporter for the effect on response to ESC (Keers et al, in press). In analysis of *GNB3*, the TT genotype of the C825T polymorphism was associated with better somatic symptom response to NOR, and less treatment-emergent insomnia and greater weight gain (Keers et al, submitted). Q-PCR and serum analysis in samples from the Brescia GENDEP centre vs. controls revealed reduced BDNF leukocyte mRNA levels and BDNF serum levels in medication-free depressed subjects at baseline, which were normalized by 12 weeks of treatment with ESC (Cattaneo et al, 2010).

Conclusion: Comprehensive prospective data collection and novel analytical approaches are yielding findings of research interest and clinical relevance.

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A GENOME-WIDE ASSOCIATION STUDY OF CITALOPRAM-ASSOCIATED SIDE-EFFECTS IN THE STAR*D SAMPLE

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Background: Intolerance to an antidepressant medication is a common reason for drug discontinuation. Limited candidate gene studies of small samples have provided equivocal findings to date. Here we report results from a genome-wide association study of several phenotypes addressing tolerance to citalopram.

Methods: 1,953 individuals from the Sequenced Treatment Alternatives for Depression (STAR*D) study were treated with citalopram and genotyped using Affymetrix 5.0 and 500K mapping arrays. Multi-dimensional scaling was used to correct for population stratification in our multiethnic sample. Our outcomes were general side effect frequency, intensity, and burden, as well as specific side effects such as anxiety and headache. We tested for association of each SNP using logistic regression and controlled for gender and other clinical and demographic variables in the analyses.

Results: We found no associations at genome-wide significant levels. The strongest findings for summary side effect phenotypes (FISER/GRSEB) were for burden ($p = 8.6 \times 10\text{-}7$, near DNAJC1, OR = 3.22), frequency ($p = 6.5 \times 10\text{-}6$, within SUMF1, OR = 1.89), and intensity ($p = 6.6 \times 10\text{-}6$, near KIAA0574, OR = 1.49). SUMF1 appeared in among the strongest findings in both intensity and frequency phenotypes. Specific treatment-emergent side effect findings included anxiety ($p = 4.2 \times 10\text{-}7$, in CALN1, OR = 2.68) and headache ($p = 9.5 \times 10\text{-}7$, intergenic, OR = 2.29). Genes of functional interest in the top 25 SNPs of individual phenotypes include NPAS3, NRG1, NKX2-1, and SLC6A1.

Conclusions: These results make progress towards the ultimate goal of predicting, based on one's genetic constitution, if a patient will be able to tolerate citalopram without adverse effects. The pathways suggested by these results are novel for citalopram tolerance and for the specific phenotypes tested. Replication in an independent sample will be a critical next step for validation of our findings.

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A ROLE OF SEROTONIN TRANSPORTER GENE IN PTSD DEVELOPMENT AND TREATMENT OUTCOME

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Background: Posttraumatic stress disorder (PTSD) is a debilitating disorder often associated with a significant degree of comorbidity. Many PTSD patients do not or only partially respond to various therapeutic interventions. Evidence indicates that genetic factors are likely to play an important role in stress vulnerability and individual treatment outcomes. We investigated a functional serotonin transporter gene promoter region polymorphism (*5-HTTLPR*) among combat veterans with and without PTSD. The polymorphism was further examined in patients participated an escitalopram, a selective serotonin reuptake inhibitor (SSRI) treatment trial.

Methods: *5-HTTLPR* functional polymorphisms were genotyped in 199 individual with and without DSM-IV diagnosed PTSD, and in 20 PTSD patients participated in a 12-week escitalopram trial. Clinician Administrated PTSD Scale (CAPS) were assessed both before and after treatment.

Result: The homozygote short (S/S) was presented in 29% of the individuals diagnosed with PTSD vs in 11% of controls, which indicated the *S/S* genotype is significantly associated with PTSD condition (X^2 = 9.67, p<0.002). Among the 20 PTSD participated escitalopram trial, the five individuals with S/S showed poorest treatment response (lowest reduction in CAPS score of 19) after 12 weeks of escitalopram therapy, followed by eight S/L individuals (CAPS reduction of 50). L/L individuals demonstrated best response (CAPS reduction of 57) ($F_{2,17} = 4.73$, $p = 0.023$).

Conclusion: Our preliminary data indicate that the presence of two copies of short alleles (*S/S*) of *5-HTTLPR* may constitute a risk factor for PTSD development following exposure to trauma, and the same genotype render individual to a less favorable treatment outcome.

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ASSOCIATION ANALYSIS OF THE 5-HTTLPR POLYMORPHISM IN THE SLC6A4 GENE AND TREATMENT RESPONSE TO VENLAFAXINE XR IN GENERALIZED ANXIETY DISORDER

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Background: Generalized Anxiety Disorder (GAD) is a highly prevalent chronic psychiatric disorder with significant morbidity and mortality. Currently, antidepressant drugs are the preferred choice for acute and chronic treatment. Treatment response is often variable, with some patients responding well to medications while others fail treatment. Growing evidence suggests that genetic factors might be involved explaining treatment response and tolerability to antidepressant medication. Several studies in major depression have implicated a role of the serotonin transporter gene (SLC6A4) in treatment response to antidepressants. In this study we tested the hypothesis that the genetic variant in the promoter region of the SLC6A4 gene predicts treatment outcome in GAD patients treated with venlafaxine XR.

Methods: Treatment response was assessed in 118 patients (European-Americans n=84; African-Americans n=31; others n=3) that participated in a 6-month open label clinical trial of venlafaxine XR for GAD. Primary analysis included HAM-A reduction at 6 months, response was defined as HAM-A reduction of >50% and remission was defined as HAM-A <7. Genotypes were obtained using standard procedures. Genotype and allele frequencies were compared between groups using chi-square contingency analysis.

Results: We observed a trend for an association of the 5-HTTLPR-L-allele and response at 6 months in our European-American sample (n=84), with responders having an L-allele frequency of 60% and non-responders having an L-allele frequency of 42% (p=0.08). Similar trends were observed for remission (remitters L-allele 60% vs. non-remitters 52%) although not statistically significant. There was no statistically significant difference for response or remission at 6 months in the combined sample.

Conclusion: Our results suggest that the L-allele of 5-HTTLPR at the SLC6A4 locus is associated with a better outcome after 6 months of treatment with venlafaxine XR in GAD patients. Future studies with larger sample sizes are necessary to further characterize the effect of the 5-HTTLPR in treatment response to antidepressants in GAD.

DOES BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) GENE VARIANT PREDICT ANTIDEPRESSANT RESPONSE IN OBSESSIVE-COMPULSIVE DISORDER (OCD)?

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Background: Brain-derived neurotrophic factor (BDNF) has extensive effects on the nervous system including cell survival, differentiation, neuronal growth and maintenance, as well as cell death. Moreover, it promotes synaptic plasticity and interacts with dopaminergic and serotonergic neurons, suggesting an important role on the alteration of brain function with antidepressant medications. The differential effects of BDNF gene variants could lead to changes in brain circuitry that would in turn cause variable response to antidepressants.

Hypothesis: Therefore, we hypothesized that genetic variation in this candidate gene may predict response to a specific selective serotonin reuptake inhibitor (SSRI) in patients with obsessive-compulsive disorder (OCD).

Method: Retrospective response data on multiple SRI trials was collected in 107 patients with OCD. Individuals were grouped into those who improved following an adequate trial of one or more SRI(s) as compared with those who reported “minimal”, “no change”, or “worsening” in response to SRI(s) trialed. We examined the functional Val-66-Met polymorphism in addition to 4 other single nucleotide polymorphisms across the BDNF gene. This was followed by exploratory analyses on a drug-by-drug basis. In total, 68 patients had complete data set for adequate trial of one or more SRI(s) and genotypings.

Results: Genotype 1-1 and allele 1 from C270T were associated with significant response to fluvoxamine treatment (genotype χ^2=5.000, *P*=0.025; allele: χ^2=4.444, *P*=0.035). A trend in paroxetine response was also observed with genotype 1-1 and allele 1 of C270T (genotype χ^2=3.529, *P*=0.060; allele: χ^2=3.277, *P*=0.070) in addition to allele 1 of rs11030104 (genotype χ^2=3.656, *P*=0.161; allele: χ^2=3.695, *P*=0.055) and allele 2 of rs2049045 (genotype χ^2=3.697, *P*=0.157; allele: χ^2=3.848, *P*=0.050). Moreover, C270T genotype 1-1 and allele 1 were found to be associated with any SRI response (genotype χ^2=4.832, *P*=0.028; allele: χ^2=4.478, *P*=0.034) as well as any SSRI response (genotype χ^2=4.832, *P*=0.028; allele: χ^2=4.478, *P*=0.034).

Conclusion: BDNF genetic variant might play an important role in predicting SSRI response in OCD. However, replication in larger and independent samples is required.

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MYELIN SYSTEM GENES AND WHITE MATTER TRACT INTEGRITY IN SCHIZOPHRENIA

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Introduction: Gene association and postmortem gene expression studies have implicated oligodendrocyte and myelin related (OMR) genes in schizophrenia, particularly in frontal and temporal cortex. Magnetic resonance diffusion tensor imaging (DTI) studies have implicated fronto-temporal white matter tracts in schizophrenia. No study, however, has linked these genetics and imaging findings *in vivo*. Here, we examine the relationship of the myelin associated glycoprotein gene and other myelin system genes with white matter tracts connecting between and within frontal and temporal cortex.

Methods: Ninety-eight subjects (49 controls and 49 schizophrenia patients) (age range 20-65) completed all imaging and genetics protocols. Groups were matched on age, gender, and ethnicity. Both DTI and conventional MRI imaging protocols were completed. For DTI, 23 directions and 2 b=0 images were obtained and 3 repetitions of the entire sequence was performed and then averaged. Whole brain tractography, followed by segmentation and measurement (fractional anisotropy) of the left and right uncinate, arcuate, and cingulate fasciculi as well as the genu and splenium of the corpus callosum was performed. For genetics, each individual was genotyped at the MAG rs 2301600, rs720309, and rs756796 single nucleotide polymorphisms using the ABI-7500 system. These SNPs comprise a previously identified MAG gene risk haplotype in schizophrenia.

Results: Following separate repeated measures ANCOVA with diagnosis and genotype as between group factors and the eight white matter tracts as within group factors MAG genotype by diagnosis interactions were shown at both MAG rs720309 ($F_{1,91}$ = 4.4, p =0.038) and MAG rs756796 ($F_{1,91}$ = 8.5, p = 0.004), but not at MAG rs2301600.

Discussion: These results demonstrate that the MAG gene influences white matter phenotypes in schizophrenia. Work is currently underway investigating the relationship of genetic variation at other myelin system gene SNPs (with previously demonstrated association with schizophrenia) in order to more comprehensively investigate the genetic underpinnings of white matter disruption in schizophrenia. Future techniques will include multivariate statistical modeling to explore relationships between genotypes and phenotypes.

MAPPING GENE REGULATORY ELEMENTS FOR BRAIN EXPRESSED GENES USING NEXT GENERATION SEQUENCING

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Current evidence indicates that psychiatric disorders are more likely to result from genetic variation that changes gene expression rather than variations in the coding region. However, the role of variation in gene regulation as a contributor to psychiatric disorders has been largely ignored because of the difficulty in identifying the location of gene regulatory elements outside of the proximal promoter. We used a genome-wide approach to identify brain-relevant gene regulatory elements, in two cell lines, a neuroblastoma and a glioblastoma. We used chromatin immunoprecipitation (ChIP) to modified histones and transcription factors combined with high throughput sequencing (ChIP-sequencing) to identify the position of brain-relevant regulatory elements across the genome. To maximize the identification of regulatory elements, we performed ChIP using antibodies to acetylated histone H3, (a marker of active chromatin), the enhancer-associated protein p300 (a marker of enhancers), and RNA polymerase II (a marker of regions of active transcription). This approach was very successful, and we now have genomic maps of putative regulatory elements for genes expressed in these cell lines including genes associated with psychiatric disorders (e.g. DISC1, NTRK3, DTNBP1, SNAP25). We also mapped putative gene regulatory elements in gene “deserts” in the region of positive markers from the GWAS studies. We are currently screening the putative regulatory regions in associated genes for genetic variation and testing the relationship to the respective psychiatric disorder in DNA from families. The use of ChIP combined with high throughput sequencing is a powerful approach to map gene regulatory elements allowing gene findings for psychiatric disorders to move forward to functional studies. We are following on the success of this approach by identifying regulatory regions in brain tissue, specifically the DLPFC and the hippocampus.

NEXT GENERATION RE-SEQUENCING OF THE DTNBP1 GENE: RELATIONSHIP WITH SCHIZOPHRENIA AND RELATED PHENOTYPES

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Background: Diagnostic heterogeneity in schizophrenia (SZ) has hampered treatment strategies, with current medications effectively targeting only certain aspects of the illness. Elucidating the underlying genetic etiology of dysfunction within core symptom domains may allow for treatment tailored to diverse clinical presentations. The gene encoding dysbindin (DTNBP1) may be a prime treatment target as several studies have linked *DTNBP1* with phenotypic variation in SZ including variation in cognitive function and negative symptoms as well as brain structure and function. The risk alleles that have been identified, however, vary by study and at present none have been found to be causal. Thus, it has been suggested that there is considerable allelic heterogeneity in which multiple rare variants within the gene contribute to disease risk.

Method: Using Illumina Next Generation Sequencing we sought to comprehensively assess allelic variation within the *DTNBP1* gene in a sample of 35 SZ cases and 35 healthy controls. Samples were selected for sequencing based on prior identification of the presence or absence of a previously identified 6-locus risk haplotype.

Results: We identified over 4,000 novel variants in the DTNBP1 gene. Of these, 86 variants had a minor allele frequency (MAF) > 0.05. Although several functional variants were identified, none of them demonstrated a causal relationship to SZ. Several variants, however, were nominally associated with the diagnosis of SZ as well as its associated phenotypes.

Conclusions: Although limited, these data suggest that variants within DTNBP1 confer risk for SZ and its related phenotypes.