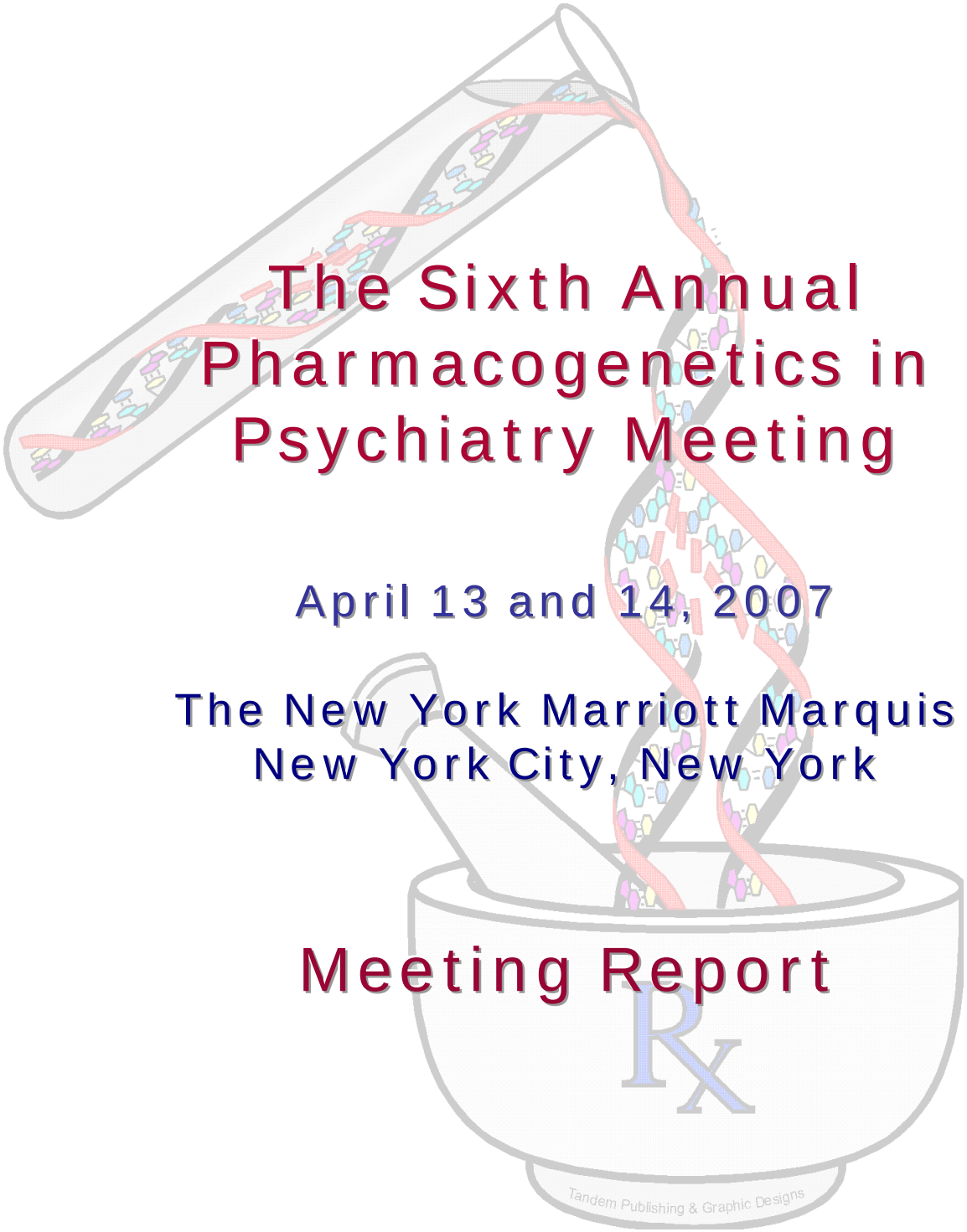

The Sixth Annual Pharmacogenetics in Psychiatry Meeting

April 13 and 14, 2007

The New York Marriott Marquis
New York City, New York

Meeting Report

North Shore LIJ The Zucker Hillside Hospital
North Shore-Long Island Jewish Health System

THE 6TH ANNUAL PHARMACOGENETICS IN PSYCHIATRY MEETING

The Sixth Annual Pharmacogenetics in Psychiatry meeting was held in New York, New York on April 13 and 14, 2007. The meeting was focused on the assessment of genetic factors in the interindividual variation to psychotropic drug response, as well as the evaluation of new phenotypes for examination in pharmacogenetic studies. The meeting was chaired by Anil K. Malhotra, from the Zucker Hillside Hospital in Glen Oaks, New York, and was comprised of one and a half days of oral presentations and an evening poster session. The following is a meeting report of each of the oral sessions contributed by Katherine Aitchison, Pamela DeRosse, Katherine Burdick, Daniel Müller and Anil Malhotra.

Session 1: Pharmacogenetics of Antidepressant Drug Response *(Chair:* Katherine Aitchison (Institute of Psychiatry, King's College, London)

Alessandro Serretti (University of Bologna) presented an overview of the candidate genes studied to date in the field of pharmacogenetics of antidepressant treatment. Interesting points made included that findings may vary by phenotype studied (e.g. remission versus response), by inclusion criteria (e.g. baseline severity of depression), and how for some polymorphisms the direction of effect is the opposite in different studies (Lin, 2007 [ref may need checking]). He stated that in his opinion, the well replicated association of the serotonin transporter promoter (5-HTTLPR) polymorphism with outcome on antidepressant treatment was probably through a complex and indirect effect, owing to the contribution of this polymorphism to phenotypes such as fear and anxiety. New data that he presented included the association between a dysbindin haplotype and response to antidepressant treatment, found in a Korean sample (Pae et al, 2007) and recently replicated by Arias and colleagues (unpublished data).

Katherine Aitchison (Institute of Psychiatry) presented a paper on the effects of antidepressant treatment on the expression of housekeeping genes in a mouse cell line (Sugden et al) which is part of the GENDEP program of work. The expression of twelve "housekeeping genes" were studied using the geNorm probeset and geNorm (Vandesompele et al, 2002) and Normfinder (Anderson et al, 2004) programs in the L929 mouse fibroblast cell line, by qPCR using TaqMan. The data showed that three genes were most invariant under different treatment conditions with two antidepressants (*Atpb5*, *B2M*, and *Cyc1*), whilst other genes were highly unstable, including *GAPDH*, which as previously frequently been used in qPCR studies as a reference gene.

Hans Stassen (Psychiatric University Hospital Zurich) presented data from studies totaling nearly 3000 patients randomized to either placebo or one of 7 different antidepressants, which showed that time to onset of improvement did not differentiate placebo responders from responders to the antidepressants (Stassen et al, in press). The difference between those treated with antidepressants and the placebo responders was seen in the proportion of patients in whom a therapeutic response was induced, especially in the early stages of treatment. He suggested that the best phenotypes for pharmacogenetic studies were severity at baseline, baseline score reduction, and time to a defined degree of reduction in baseline score.

Holly Garriock (UCSF) presented a genetic association study of the μ opioid receptor gene (OPRM1) variants versus antidepressant response in the STAR*D study. Pilot data had shown an association between three single nucleotide polymorphisms (SNPs) in the OPRM1 gene and specific response to citalopram. Further analysis using more SNPs for the gene had revealed an association with four SNPs in the Hispanic subgroup and two SNPs in the non-Hispanic Caucasians. One of the latter results in an amino acid change which is found in an isoform of the

receptor (MOR-1X) which is differentially expressed in the brain compared to the wild-type μ opioid receptor. Further studies to follow-up on this interesting finding were intended.

Session 2: From Pharmacogenetics to Pharmacogenomics: New Data from Large Scale Clinical Trials (Chair: James Kennedy; Center for Addiction and Mental Health, University of Toronto)

Dr. Kennedy began the session by observing the significant contributions of Dr. Robert Kerwin and Dr. Hubert Van Tol, pioneers in the field of pharmacology and pharmacogenetics, who sadly passed away during the past year.

Gonzalo Laje (NIMH) presented data from 1,915 participants from Stage 1 of the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial. Data presented indicated that genetic polymorphisms in the glutamate signaling pathway significantly affect the clinical characteristics of depressed patients. Specifically, data indicated that a single marker (rs4567478) within the gene encoding the ionotropic glutamate receptor KA1 subunit, *GRIK4*, increased the risk for anxious depression in this cohort. Further, these data also indicated associations between treatment emergent suicidal ideation (TESI) and polymorphisms in other genes within the glutamate signaling pathway. Specifically, variation in *GRIK2* and *GRIA3*, which encode subunits for the ionotropic glutamate receptors KA2 and AMPA, respectively, were associated with an increase in suicidal ideas following initiation of antidepressant treatment. This increase in suicidal ideas was also associated with less remission in depressive symptoms and more changes in medications over the course of treatment.

Jeffrey Kraft (UCSF) presented on "Whole Genome Association Study of Response to Citalopram in the STAR*D Sample." Using a two-stage experimental design, the Affymetrix 40K microarray was used to genotype 831 and 835 participants in the discovery and validation samples respectively. Although numerous loci provided suggestive evidence of association to treatment response, the strongest association to citalopram response was identified at the Ser83Asn polymorphism within the *LRP2* gene. This gene, which encodes the protein megalin, is widely accepted to be important during forebrain development but has never before been associated with response to antidepressant treatment. Thus, these data may provide a novel pharmacogenetic target for the treatment of depression and work is underway to replicate these findings in additional samples.

Daniel J. Müller (Charité - University Medicine Berlin) presented data on the genetics of antipsychotic treatment emergent weight gain in schizophrenia. Data were presented indicating the involvement of the C957T polymorphism within *DRD2* in antipsychotic induced weight gain. This association was initially found in a cohort of clozapine treated patients but new data in a mixed medication group, although not significant, are suggestive that variation at this locus may be more generally involved in antipsychotic induced weight gain. In addition, analyses in the mixed medication cohort also found suggestive associations between weight gain and polymorphisms within *DRD3* but no associations between *AKT1* or *DARPP-32* and antipsychotic induced weight gain.

Session 3: Neurocognition as an endophenotype for pharmacogenetic studies (Chair: Todd Lencz, Zucker Hillside Hospital)

Terry Goldberg (Zucker Hillside Hospital) presented data derived from a treatment trial of 104 patients with first-episode schizophrenia and 84 healthy controls. The study, comparing risperidone and olanzapine, included 3 neurocognitive assessments at: baseline, 6 weeks, and 16 weeks. Dr. Goldberg first provided evidence of significant baseline neurocognitive impairment in the first-

episode sample, consistent with the extent and severity noted in more chronic patients with schizophrenia, supporting this as a trait-like feature of the disease. Further, patients demonstrated improved performance over time on nearly all cognitive measures regardless of treatment group; however, the magnitude of improvement in the patient group was nearly identical to that noted in the healthy controls, who were tested at the same three time points. These data suggest that the cognitive gains reported in both patients and controls might be best attributed to practice effects due to repeated exposure to the test materials, as opposed to an actual cognitive enhancing benefit of the second generation antipsychotics. In light of these findings, Dr. Goldberg proposed the possibility that previous trials, which have reported cognitive improvement with SGA treatment but have lacked a healthy control sample, might be in fact measuring practice effects as opposed to true cognitive enhancement. These data are now in press in the Archives of General Psychiatry.

Katherine Burdick (Zucker Hillside Hospital) presented preliminary evidence of epistasis between the Disrupted in Schizophrenia-1 (*DISC1*) gene and the gene coding for a known binding partner of DISC1, Nuclear Distribution Element-Like (*NDEL1*). Dr. Burdick presented new data supporting an association between variation within *NDEL1* and risk for schizophrenia. Further, the associated *NDEL1* haplotype was also related to performance on a measure of verbal working memory (WAIS-R Digits Backward), the same measure that the group has previously linked with *DISC1* genotype. Finally, she presented statistical evidence of an epistatic interaction between *NDEL1* haplotype and *DISC1* Ser704Cys on risk for disease, as the deleterious effect of *NDEL1* on SZ risk was only evident against a background of *DISC1* Ser704 homozygosity. These data support evidence derived from recent work at the protein level, implicating this complex genetic network in the pathophysiology of schizophrenia. DISC1 is of interest in future pharmacogenetic studies as it appears to serve as a hub protein, with multiple binding partners that are critical to a several neurodevelopmental processes that are known to be aberrant in patients with schizophrenia.

Andreas Fallgatter (University of Wurzburg) reported on data from several studies focusing on electrophysiological methods like Event-Related Potentials (ERPs) as measures of abnormalities in brain functions underlying psychiatric diseases like Attention Deficit Hyperactivity Disorder (ADHD) and schizophrenia. Dr. Fallgatter and colleagues employed a multi-channel EEG during performance of a Go-NoGo task to assess the electrophysiological basis of response inhibition, termed NoGo-Anteriorisation (NGA). He reported on results in patients with ADHD and schizophrenia, with diminished NGA in both groups as compared to age- and sex-matched healthy controls. Furthermore, a three-dimensional source location analysis with LORETA localized the dysfunction to the anterior cingulate. Finally, Dr. Fallgatter reviewed recent work demonstrating a significant influence of variants in *COMT* on this measure of prefrontal brain function with the 158 val/val variants being associated with impaired prefrontal brain function. These results exemplify the measurement of disease related disturbances in brain function with ERPs. Future studies will show whether such electrophysiological endophenotypes may contribute to the diagnosis of subgroups of ADHD and schizophrenia and whether they may serve as endophenotypes to further clarify genetic contributions to the disease.

Andreas Papassotiropoulos (University of Basel, Zurich) presented work which identified memory-related genes and gene-clusters in humans. Using a combination of whole-genome association (500K SNP chip), candidate gene approach (gene clustering) and functional MRI (fMRI), the group has shown that variability of human memory performance is related to variation in genes encoding proteins of a signaling cascade, including NMDA receptor, metabotrobic glutamate receptor, adenylyl cyclase, CAMKII, PKA and PKC. The genome-wide screen with >500,000 single nucleotide polymorphisms identified a locus encoding the neuronal protein KIBRA which was strongly associated with memory performance in independent, cognitively normal cohorts from Switzerland and the United States. In addition, gene expression studies revealed high expression

levels of a KIBRA transcript in the human brain, especially in the hippocampus. Finally, he described fMRI results which detected KIBRA allele-dependent differences in hippocampal activations during memory retrieval. Dr. Papassotiropoulos presented these data as an example of the direction that the field is moving, with whole-genome association studies allowing for a substantial increase in our knowledge of the genetic underpinnings of human memory and other neurocognitive endophenotypes.

Session 4: Pharmacogenetics of Antipsychotic Drug Response (Chair: David Goldman, NIAAA)

Vicki Ellingrod (University of Michigan) reported data from a genetic association study between the methyenetetrahydrofolate reductase (MTHFR) 677C/T polymorphism and metabolic syndrome as well as insulin resistance. This variant results in reduced folate metabolism and hyperhomocysteinemia which is linked to cardiovascular disease. Fifty-eight schizophrenic patients treated with atypical antipsychotics were included. Out of these, 23 subjects met metabolic syndrome criteria (MS+), while 25 did not (MS-). Both groups did not vary for age, gender, race or exposure to atypical antipsychotics. Carriers of the T-allele were significantly more likely to meet MS+ criteria (OR = 3.7; 95% CI = 1.24 – 12.66, p = .02). An interaction was found between MTHFR genotype and waist circumference with insulin resistance (F = 8.6, df = 2, p = .0006). Carriers of the TT genotype were found to be at significantly greater risk for insulin resistance with increasing central adiposity (F = 8.35, df = 5, 51, p < .0001). Limitations were the small sample size of TT carriers (n = 5).

Yvon Chagnon (Laval University Robert-Giffard Research Center) addressed the question of biomarkers for antipsychotic induced weight gain. Immortalized lymphocytes were used as a surrogate parameter of post-mortem brain and mRNA expression was evaluated before and after stimulation with olanzapine using the Agilent 18K microchip. Lymphocytes were obtained from obese and non-obese schizophrenia patients treated with various antipsychotics and the corresponding related family-controls from multigenerational families. After stimulation with olanzapine, 4 genes showed a differential expression significant at p <.001, 13 genes at p = .001 and 126 at p = .01. Results of the four groups of 3 patients and four groups of 3 controls were presented for some candidate genes (serotonin receptor 2C (HTR2C), pro-melanin concentrating hormone (PMCH), dystrobrevin binding protein 1 (DTNBP1), corticotropin releasing hormone (CRH) and Neuregulin-1 (NRG1). These genes showed some interesting patterns of differential expression, similar to what was observed in post mortem brains, suggesting that studies on lymphocytes can be used to find biomarkers of the individual response to drugs.

Dan Rujescu (University of Munich) first presented on haloperidol-induced extrapyramidal symptoms (EPS) testing SNPs in the DRD2 gene. In dyskinesia, significant association was found with four SNPs with the haplotype containing these SNPs. Second, a hypothesis free approach was used to search for novel candidate genes including animal models, neuronal cell cultures, and differential gene expression analyses. Immunohistochemical analyses were performed in rats that received an agent mimicking aspects of psychosis (MK-801), haloperidol, a combination of these agents or saline. Parvalbumin-positive interneurons, repeatedly found to be reduced in schizophrenia across different brain regions, were reduced in the hippocampus after administration of MK-801. Interestingly, haloperidol favored a normalization of this condition; converging into the hypothesis that disinhibition of local feedback circuits is ameliorated by haloperidol. Gene expression experiments revealed that a phospholipase was differentially expressed by haloperidol. SNPs were subsequently tested and one haplotype block was associated with akathisia and Parkinsonism. Another differentially expressed gene was a methionine sulfoxide reductase and SNPs in this gene were found to be associated with EPS.

Maria Athanasiou (PGxHealth) focused on a replication study of haplotypes associated with clozapine induced agranulocytosis (CIA; defined as an absolute neutrophil count <500mm^3). Their initial study included 33 cases with CIA and 54 controls using age, race and gender as covariates in a logistic regression model. Seventy-four candidate genes were sequenced including exons, inron-exon boundaries, 5' UTR and promoter region for all individuals. Haplotype analyses revealed permutation test adjusted significant associations with haplotypes in HLA-DQB1, HLA-C, NTSR1, DRD1 and CSF2RB. A recent replication study included 49 cases and 78 controls. All except the HLA-C gene were selected for analyses. The haplotype of the HLA-DQB1 gene that contains three SNPs [+6584 (C/T), +6657 (G/A) and 6672 (G/C)] did replicate in the HLA-DQB1 gene (raw p-value = .002; permutation p-value = .01), while the other findings in the remaining three genes did not replicate. Combined analyses in both samples yielded a highly significant finding (raw p-value = .000001) with a sensitivity of 61% and a specificity of 73%. For the development of a diagnostic test for CIA, the haplotype with the lowest p-value was chosen fro5m the combined sample. This haplotype contains two SNPs [+6657 (G/A) and 6672 (G/C)] and proved to have a sensitivity of 59% and a specificity of 76%. The test is now being assessed in cases of neutropenia (neutrophil count <1000mm^3 but > 500 mm^3) while efforts are being directed to increase sensitivity and specificity by adding new genetic markers and new biomarkers.

Three special lectures were also held during the meeting. **Gail Javitt** (Genetics and Public Policy Center, Johns Hopkins University) presented an update on genetic testing policy. During this talk, the audience was provided with a glimpse into the lack of regulatory control by the Food and Drug Administration (FDA) and the Centers for Medicare and Medicaid Services (CMS) to ensure the safety and validity of the ~1,000 genetic tests that are now commercially available. Data were presented from a survey of laboratory directors conducted in 2006 that indicated that most clinical laboratories conducting genetic testing lack a clear understanding of what constitutes a clinically valid genetic test. Despite this lack of understanding, however, CMS has not issued regulations targeted to laboratories performing genetic testing and the FDA does not regulate laboratories that are using their own "home brew" tests. Further, because the "test kits" developed by the pharmaceutical industry are regulated by the FDA and therefore face greater barriers to market entry, there is an incentive for laboratories to use their own "home brew" tests. The trends in policy development to deal with this, and other related issues, were described and the lecture concluded with a description of a petition recently filed by three national organizations to CMS requesting that the agency create genetic testing specific standards for laboratories performing genetic testing.

David Goldman (NIAAA) presented data suggesting that response to pain is a type of pharmacogenetic phenotype, and reviewed earlier work from his group and others implicating the COMT val158met locus in influencing pain threshold. Moreover, his group has now identified a number of convergent lines of evidence suggesting that the GTP cyclohydrolase (GCH1) gene may mediate pain response, including data from an animal model, pharmacological studies with GCH1 inhibitors, and the identification of a functional haplotype that predicts GCH1 mRNA expression in lymphoblastoid cell lines as well as experimental pain in a population control group.

Todd Lencz (Zucker Hillside Hospital) presented recently completed work using the whole genome association (WGA) approach with the Affymetrix 500K platform. In the first study (Lencz et al. 2007), the WGA approach identified single SNP in pseudoautosomal region 1 that met criteria for genome-wide significance. Follow-up sequencing of a second data set implicating two genes, CSF2RA and ILR3A, with two haplotypes and 7 missense mutations implicated with risk for schizophrenia. A second analysis of the cohort focused on treatment response – using clozapine treatment as a proxy measure for evaluation of clinical responsivity to antipsychotic drug treatment. In a preliminary comparison of treatment-responsive to non-responsive patients, alleles of five

SNPs were found to be overly represented in treatment responders. These SNPs will now be assessed in an independent data set of first episode schizophrenia patients enrolled in a randomized clinical trial of second generation antipsychotic agents.

The 2007 meeting was supported by educational grants from AstraZeneca, Bristol-Myers Squibb, Forest Pharmaceuticals, Merck, Janssen and Pfizer. Next year's meeting will take place in New York on April 4 and 5th, 2008.