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Finite mixture regression model analysis on antipsychotics induced weight gain: Investigation of the role of the serotonergic genes

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Abstract
Antipsychotics-induced weight gain is a complex phenomenon with a relevant underlying genetic basis. Polymorphisms of serotonin receptors and related proteins were genotyped in 139 schizophrenia patients and incorporated as covariates in a mixture regression model of weight gain in combination with clinical covariates. The HTR1D rs6300 polymorphism was showing a slight significance conferring risk for obesity (heavy weight gain group) under additive model. After correcting for multiple testing all the genetic predictors were non-significant, however the clinical predictors were associated with the risk of heavy weight gain. These findings suggest a role of ethnicity and olanzapine in increasing the risk for obesity in the heavy weight gain group and haloperidol protecting against heavy weight gain. The mixture regression model appears to be a useful strategy to highlight different weight gain subgroups that are affected differently by clinical and genetic predictors.

1. Introduction

Worldwide, obesity has become a prominent health issue (Ogden et al., 2007; Yanovski and Yanovski, 2002). Extreme weight gain as a result of antipsychotic (AP) treatment of

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schizophrenia or schizoaffective disorder has severe ramifications on quality of life, morbidity and mortality (Allison et al., 2003). Weight gain is a frequently observed side-effect with many AP treatments and appears to be underreported and under-recognized in many patients (Wetterling, 2001). Weight gain may add to the schizophrenia stigma, the stigma of obesity and this in turn may lead to poor adherence to the therapy. Weight gain can also increase the risk for type II diabetes and has been linked to metabolic syndrome (Haddad, 2004) and cardiovascular disease (CVD), with related increase in mortality (Newcomer, 2008).

Treatment emergent weight gain varies within the broad class of antipsychotics; however, an individual's propensity to develop weight gain may depend on genetic factors. When looking for possible ethnic differences, clinical studies suggest that Afro-American patients treated with antipsychotics are at higher risk of weight gain (Blin and Micallef, 2001). AP drugs characterize a keystone in the treatment of schizophrenia (Le Hellard et al., 2008). Regretfully, many atypical antipsychotics but also some typical antipsychotics have recently been much criticized because of their tendency to cause a variety of metabolic adverse effects including weight gain. The weight gain induced by AP medications is variable among individuals (Basile et al., 2001).

It is now well established that susceptibility to drug side-effects is shaped by genetic factors and over recent years significant progress has been made toward the identification of specific alleles that confer risk for antipsychotics induced weight gain (Basile et al., 2002). Association studies of functional candidate genes (e.g., involved in drug metabolism and drug mechanism of action) have revealed several promising candidates which might contribute to weight gain liability.

Genetic factors play a significant role in antipsychotics induced weight gain (Le Hellard et al., 2008) and a recent twin study has provided evidence that the initial change in body mass index during treatment with antipsychotics is more strongly correlated between monozygotic twins as compared to same-sex sib-pairs (Theisen et al., 2005).

The purpose of this study is to investigate the influence and possible genetic interactions of representative receptors and modulators within the serotonergic system on antipsychotics induced weight change. We also investigated whether these genetic predictors interact with ancestry (European vs. African-American), specific AP treatment and weight at baseline.

2. Experimental procedures

2.1. Sample

A sample of 139 (101 males and 38 females) unrelated patients affected by schizophrenia was recruited at three different clinical sites in the United States. These sites include: the Case Western Reserve University in Cleveland, Ohio (Dr. HYM, n=68); the Hillside Hospital in Glen Oaks, New York (Dr. JAL, n=12); and the Nathan S. Kline Institute for Psychiatric Research in Orangeburg, New York (Dr. JV, n=59). The subjects were coming from different ethnic backgrounds: there were 72 white European Caucasians, 56 African-Americans, 8 Hispanics, 2 Pacific Islanders and 1 Native American. The diagnosis of schizophrenia was based on fulfilling the DSM-III-R or DSM-IV criteria. The mean age at of the sample was 36.2 ± 9.36. Weight gain was determined as percentage change from baseline to last observation carried forward (mean $\pm$ SD=5.3 ± 7.1%). The endpoint of the weight gain evaluation ranged from 6 to 14 weeks (mean $\pm$ SD=8.3 ± 3.7 weeks). In terms of medications, 91 were treated with clozapine, 22 with olanzapine, 12 with haloperidol, and 14 with risperidone (Table 1). After complete description of the study, written informed consent was obtained from each patient. This study was approved by the Center for Addiction and Mental Health ethical review committee.

2.2. Genotyping

Genomic DNA was extracted using a non-enzymatic high salt procedure (Lahiri and Nurnberger, 1991). We genotyped the following single-nucleotide polymorphisms (SNPs): HTR1A rs6295, HTR1D rs6300, HTR2A rs6313, HTR3A rs1062613, HTR6 rs1805054, SLC6A4 rs1042173, TPH1 rs1800532 using the ABI 7500 Taqman Assay. Lab technicians performing the genotyping were blind to the clinical variables of the samples.

2.3. Clinical covariates

We considered the following potential covariates for the analyses: age, sex, ethnicity (African-American vs. non-African-American), weight at baseline, treatment interval at which weight gain was measured, use of antipsychotics different from clozapine (olanzapine, risperidone, haloperidol) and clinical response to AP defined as 20% reduction of BPRS score.

2.4. Statistical analyses

We used the finite mixture models (FMM) (Deb, 2007) statistical package from STATA version 12.0 in order to analyze the existence of a theoretical model of finite mixture components of weight gain change that considered the possible influence of the polymorphisms of the genes and clinical covariates studied. The finite mixture model offers an intuitively feasible representation of heterogeneity in a finite, usually small, number of finite mixtures latent classes (Deb et al., 2011). Estimates of such finite mixture models may provide good numerical approximations even if the underlying mixing distribution is continuous (Heckman and Singer, 1984; Laird, 1978). Moreover, the finite mixture approach is semi-parametric (it does not require any distributional assumptions for the mixing variable) and under appropriate regularity conditions is the semi-parametric maximum likelihood estimator of the unknown density (Lindsay, 1995).

We postulate that the complexities of the relationship are not accurately addressed by traditional methodology, which may have led investigators to draw erroneous conclusions about the effect of genotype on antipsychotics induced weight-gain. This statistical analysis fits a finite mixture regression model using maximum likelihood estimation, in which the mixing probability may be specified with covariates (genetic and clinical). The selection of the best fitting model was performed using the ''estat'' command implemented in FMM, which displays Akaike's information criteria (AIC) and Schwarz's Bayesian (BIC) information criteria. The lowest value of the two criteria indicated the best fitting model. The influence of each polymorphism on the mixture regression model was assessed testing the genotypes as dummy variables under additive model.

We analyzed one marker at a time to investigate the main effect of each SNP considering the number of markers (n=7) as multiple test correction factor. All significant markers were then combined to the significant clinical covariates to generate a final model.