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Analysis of differential gene expression mediated by clozapine in human postmortem brains

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ABSTRACT

Clozapine is the only medication indicated for treating refractory schizophrenia, due to its superior efficacy among all antipsychotic agents, but its mechanism of action is poorly understood. To date, no studies of human postmortem brain have characterized the gene expression response to clozapine. Therefore, we addressed this question by analyzing expression data extracted from published microarray studies involving brains of patients on antipsychotic therapy. We first performed a systematic review and identified four microarray studies of postmortem brains from antipsychotic-treated patients, then extracted the expression data. We then performed generalized linear model analysis on each study separately, and identified the genes differentially expressed in response to clozapine compared to other atypical antipsychotic medications, as well as their associated canonical pathways. We also found a number of genes common to all four studies that we analyzed: *GCLM*, *ZNF652*, and *GYPC*. In addition, pathway analysis highlighted the following processes in all four studies: clathrin-mediated endocytosis, SAPK/JNK signaling, 3-phosphoinositide synthesis, and paxillin signaling. Our analysis yielded the first comprehensive compendium of genes and pathways differentially expressed upon clozapine treatment in the human brain, which may provide insight into the mechanism and unique efficacy of clozapine, as well as the pathophysiology of schizophrenia.

1. Introduction

Schizophrenia is a chronic, debilitating psychiatric disorder with a lifetime prevalence of about 1% of the general population (Perala et al., 2007; Saha et al., 2005). Up to one third of schizophrenia patients fail to respond to standard antipsychotic therapy and present with a treatment-refractory form of the disease, which causes a significant loss in quality of life (Conley and Kelly, 2001; Kennedy et al., 2014; Miyamoto et al., 2014). Clozapine, the first atypical (or 2nd-generation) antipsychotic agent, is the only medication indicated for refractory schizophrenia, and has the greatest efficacy despite the development of newer atypical agents (Conley and Kelly, 2001; Leucht et al., 2013; McEvoy et al., 2006; Meltzer, 2012; Siskind et al., 2016). However, it has a number of unique and serious side effects, most notably the rare but life-threatening risk of agranulocytosis. Given these side effects and the difficulty of implementing care (as patients must be enrolled in a centralized registry and followed up with regular blood counts), clozapine is underutilized and currently recommended for patients who have failed therapy with two other antipsychotic medications (Conley and Kelly, 2001; Hasan et al., 2012; Siskind et al., 2016).

Despite a long history of utilization, clozapine's mechanism of action and the basis of its superior efficacy over other antipsychotics are still poorly understood. Clozapine is known to bind to a broad array of receptors, including dopamine, serotonin, histamine, muscarinic, and adrenergic receptors (Ereshefsky et al., 1989), but it is unclear which of these are most relevant to its efficacy. It is likely that the regulation of downstream gene expression is critical, since clozapine and other antipsychotics usually require several weeks to evoke a stable therapeutic response.

Abbreviations: CC, cerebellar cortex; PFC, prefrontal cortex; PMC, premotor cortex; STG, superior temporal gyrus.

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Several groups have applied microarray or RNA-sequencing to postmortem brains of patients treated with antipsychotics including clozapine. However, they do not differentiate between antipsychotic medications, and address questions other than the specific impact of clozapine on gene expression (Aston et al., 2004; Chen et al., 2013; Iwamoto et al., 2005; Mudge et al., 2008; Pietersen et al., 2014a,b; Schmitt et al., 2011; Wu et al., 2012). At present, we are not aware of any studies that have characterized the differential gene expression profile of clozapine in the human brain.

Therefore, we performed a systematic review to identify gene expression studies of brains from patients on antipsychotic therapy, and analyzed their expression data to determine which genes are modulated specifically by clozapine, instead of performing a conventional meta-analysis. As a result, we found four such studies in the literature, and our novel analysis of their expression data identified the genes and pathways in each study that are differentially expressed in response to clozapine compared to other atypical antipsychotics. We also determined which of these genes and pathways are common to all four studies, in order to formulate a consensus of the literature regarding the gene expression profile of clozapine in the human brain. Our analysis highlighted three genes (*GCLM*, *ZNF652*, and *GYPC*) that are modulated by clozapine in all four datasets, as well as four pathways (clathrin-mediated endocytosis, SAPK/JNK signaling, 3-phosphoinositide signaling, and paxillin signaling).

2. Experimental/materials and methods

2.1. Systematic review

We reviewed the literature to identify studies that used microarray or RNA-seq to characterize differential gene expression in the postmortem brains of patients treated with antipsychotic agents including clozapine. We searched three reference databases, PubMed, Embase, and BIOSIS (on May 14, 2015), and the gene expression data repositories GEO and ArrayExpress (on July 17, 2015). Detailed search strategies are listed in Table S1.

2.2. Eligibility criteria for study selection

Studies were selected in accordance with the following eligibility criteria: a) gene expression studies that used microarray or RNA-seq; b) studies that analyzed human postmortem brain; c) studies whose cohorts included at least one patient exposed to clozapine, and at least one patient exposed to an atypical antipsychotic other than clozapine. We excluded abstracts without full articles, review articles, editorials, case reports, and studies not written in English. If results were duplicated in multiple publications, only one was selected. We also excluded studies if we were unable to obtain adequate expression data for analysis; for example, we excluded studies without data available in the public domain when we could not contact the authors after three attempts at correspondence. Two reviewers screened studies independently in two rounds, first with abstracts alone and second with the full text articles retrieved from eligible abstracts; any discrepancy between reviewers was resolved by consensus. Furthermore, to ensure a more comprehensive review, we manually searched for any additional eligible studies in the reference lists of all full-text articles that we screened.

2.3. Data extraction

Information was extracted from eligible studies in a predetermined form. We noted the first author and year of publication, diagnoses and treatments of study participants who had received clozapine or other atypical antipsychotic medications, source of tissue (brain region and brain bank), and microarray platform. Normalized gene expression datasets for eligible studies were retrieved from the NCBI Gene Expression Omnibus (GEO) database or directly from the authors of the original studies. All available phenotypic information for the subjects analyzed in the studies were extracted by two reviewers independently, and any discrepancy was resolved by consensus.

2.4. Data analysis

We analyzed the gene expression data of each study separately using a uniform approach. We used a generalized linear model approach coupled with empirical Bayes standard errors shrinkage (Smyth, 2004), including coefficients for data heterogeneity as derived from surrogate variable analysis (SVA) (Leek and Storey, 2007), to identify differentially expressed genes between groups of patients treated with clozapine or other atypical antipsychotics. This design-contrast parametrization approach allowed us to capture data heterogeneity, and control (in an unbiased way) for unknown sources of variation using all samples in each dataset. Correction for multiple testing was performed with the Benjamini-Hochberg method. Differentially expressed genes were selected based on false discovery rate (FDR) and ordered according to the moderated *t*-statistics obtained from our linear model analysis. We then used Ingenuity® Pathway Analysis (IPA®, build version 389077M and content version 27821452, release date 2016-06-14, Qiagen, Redwood City) to identify pathways and biological processes associated with the differentially expressed genes.

3. Results

3.1. Systematic review

We first performed a systematic review of the literature to identify gene expression studies of postmortem brains from patients on antipsychotic therapy (Fig. 1). Our search of PubMed, Embase, and BIOSIS yielded 5238 publications, of which 1300 were duplicated between the three databases and were excluded. We then screened 3938 abstracts, excluded 3849 based on our eligibility criteria (see Experimental/Materials and methods), and reviewed the remaining 89 publications in full text, of which 83 were excluded. When we reviewed the reference lists of these 89 articles, we identified one additional study that satisfied our eligibility criteria. We also screened 72 studies yielded by a direct search of two gene expression data repositories, GEO and ArrayExpress, but excluded all of them due to our eligibility criteria. Of the remaining studies, two publications by Pietersen et al. (2014a,b) analyzed specific cell types (parvalbumin interneurons and pyramidal neurons) in the superior temporal gyrus from the same cohort of patients, so we considered them as one study and combined the gene expression data into a single dataset for analysis.

As a result, we identified seven studies that analyzed gene expression in brains of patients treated with antipsychotics including clozapine (Aston et al., 2004; Chen et al., 2013; Iwamoto et al., 2005; Mudge et al., 2008; Pietersen et al., 2014a,b; Schmitt et al., 2011; Wu et al., 2012). We were able to obtain the expression data from four of these seven studies, either from GEO or directly from the authors. The details of the search and review process, including the number of studies excluded for each eligibility criterion, are described in Fig. 1.

3.2. Characteristics of studies

We extracted the following information from the four studies identified in our review, summarized in Table 1. Each study included two or three patients diagnosed with schizophrenia or bipolar affective disorder who had been treated with clozapine, and varying numbers of patients treated with other atypical antipsychotics (risperidone, olanzapine, or quetiapine); however, none had information on duration of disease or treatment. None of the studies had the gene expression profile of clozapine as their objective. Therefore we queried their expression data to address our own question: which genes have

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