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The Potential of Pharmacometabonomics and Pharmacogenomics Approach to Determine Clozapine Response among Schizophrenia Patients

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ABSTRACT

Schizophrenia and related disorders are severe mental illnesses characterized by profound disruptions in emotional processes, speech, behaviour, thinking, and sense of self. It, moreover, has specific antisuicidal and anti-aggressive properties. Clozapine is the most efficacious antipsychotic drug in treatment-resistant schizophrenia, mainly when other antipsychotic medications do not work. It improves negative symptoms (e.g., poverty of speech and withdrawal) and positive symptoms (e.g., hallucinations and delusions). However, it is unclear the most effective dose/response of clozapine with the most negligible side effects. Pharmacogenomics has been recommended to predict clozapine response. However, this might be insufficient to predict the response. Pharmacometabonomics analysis using proton nuclear magnetic resonance (1H-NMR) spectroscopy can help to identify novel biomarkers of clozapine. Many factors could influence the metabolism of clozapine, changing clozapine response, drug dosage standard, and clinical characteristics such as drug-drug interactions, dietary interactions, and age explanation for the critical part of the variability in clozapine dosing/response. Integrating pharmacogenetics and pharmacometabonomics has the advantage of getting more extensive and comprehensive information on variations in drug response.

Keywords: *Schizophrenia, Clozapine, Pharmacogenomics, Pharmacometabonomics, Nuclear Magnetic Resonance*

BACKGROUND

A severe mental illness is characterized by a diagnosis of nonorganic psychosis, prolonged disability, and illness (Schinnar, Rothbard, Kanter, & Jung, 1990). Schizophrenia considered one of the most common severe mental illnesses, is a significant contributor to the global burden of disease (Saha, Chant, Welham, & McGrath, 2005; Whiteford et al., 2013).

Schizophrenia is a psychiatric disorder with complex cognitive and behavioural symptoms. It occurs worldwide and is currently estimated to have a lifetime morbid risk of 0.7% (McGrath, Saha, Chant, & Welham, 2008) and a considerable heritability (Sullivan, Kendler, & Neale, 2003) and is associated with high suicide risk (Hor & Taylor, 2010). Schizophrenia and other severe mental illnesses are well known to be associated with elevated suicide rates, with five per cent dying of suicide (B. A. Palmer, Pankratz, & Bostwick, 2005). Less widely appreciated is that schizophrenia is associated with an increased risk of premature death by many other somatic conditions (Brown, 1997; Talaslahti et al., 2012). Antipsychotic medications have a main character in treating schizophrenia (Alanen, 2018). Between 20% to 30% of the patients have an inadequate response to the treatments (Kennedy, Altar, Taylor, Degtiar, & Hornberger, 2014). Clozapine is the drug of choice for treatment-resistant schizophrenia (TRS) (Kane, Honigfeld, Singer, & Meltzer, 1988; Leucht et al., 2009; Siskind, McCartney, Goldschlager, & Kisely, 2016), where approximately 60% of patients respond to clozapine treatment (Laursen, Mortensen, MacCabe, Cohen, & Gasse, 2014). However, clozapine has several adverse effects that may inhibit the use of the medication and sometimes lead to discontinuation of the clozapine treatment (Legge et al., 2016; Miller, 2000). The leading causes of clozapine discontinuation are sedation, neutropenia, agranulocytosis, tachycardia, dizziness, nausea, vomiting, weight gain, fever, and hypersalivation (sialorrhea) (Legge et al., 2016). Some adverse effects, such as agranulocytosis and gastrointestinal hypomotility, are potentially life-threatening (Idanpaan-Heikkila, Alhava, Olkinuora, & Palva, 1975; S. E. Palmer, McLean, Ellis, & Harrison-Woolrych, 2008). There is individual variation in the adverse effects (N. Seppälä et al., 2015). Monitoring the adverse effects in clinical practice is essential, and proper cautions, such as haematological monitoring, are needed to prevent agranulocytosis. Some of the adverse effects can be managed with symptomatic medications. There is variation between patients in the plasma clozapine levels at a constant dose and notably within patients (Diaz, de Leon, Josiassen, Cooper, & Simpson, 2005; Schaber et al., 2001). Age, sex, body mass index (BMI), caffeine use, and especially smoking contribute towards the variation of concentration/dose ratio (C/D-ratio) of Clozapine (Bowskill, Couchman, MacCabe, & Flanagan, 2012; Carrillo, Herraiz, Ramos, & Benitez, 1998; N. H. Seppälä, Leinonen, Lehtonen, & Kivistö, 1999). Guidelines for therapeutic drug monitoring indicate that clozapine plasma concentrations in the range 0.35–0.60 mg/L are optimal for response (Hiemke et al., 2018), and concentrations higher than 0.60 mg/L have been linked to severe adverse drug reactions. Dose-response relationships between clozapine concentration and weight gain (Simon & De, 2009) or sedation (Perdigués et al., 2016) have been suggested, although this has not been seen for all adverse drug reactions (Nair & MacCabe, 2014). The accurate prediction of clozapine plasma levels, therefore, has important clinical implications. Sophisticated models incorporating lifestyle habits and metabolic indicators can explain up to 48% of the variance in clozapine levels in large patient samples (Couchman, Bowskill, Handley, Patel, & Flanagan, 2013; Rostami-Hodjegan et al., 2004), but no individual factors other than age, smoking habits, and sex have been found to be of clinical value (Flanagan, 2010). Due to the complex nature of clozapine treatment, the decision-making process must be robust and thorough.

Pharmacogenomics and Pharmacogenetics

Pharmacogenetics and pharmacogenomics have been a topic of broad interest recently. Genotyping methods are becoming more cost-effective, and in the future extensive genetic information about patients may be accessible to clinical aid decisions (Dickmann & Ware, 2016; Relling & Evans, 2015). Pharmacogenetics is a field that focuses on the study of genetic variation that interferes with drug response or adverse drug reaction (ADR) (Motulsky & Qi, 2006). Pharmacogenetics involves identifying genes and variations in deoxyribonucleic acid (DNA) sequences related to human drug response (Kelsoe, 2012). Pharmacogenetics aims to

investigate and develop identification tools and tests that help predict unexpected drug responses using genetic assessment techniques (Mroziewicz & Tyndale, 2010; Ventola, 2013). This unexpected response to the drug might be the non-responsiveness to the drug or an ADR, which is idiosyncratic and not due to dose variation (Meyer, 2004). In other words, pharmacogenetics targets the understanding of possible genetic causes of unusual drug responses to discriminate different response groups to individualize drug therapy (Kelsoe, 2012; McLeod & Evans, 2001; Motulsky & Qi, 2006; Ventola, 2013).

Pharmacogenomics appeared latest as an alternative to the classical term pharmacogenetics. Concerning the term "pharmacogenomics", the suffix "Omics" signifies a broad term (CARE, 2013). Pharmacogenomics focuses on the comprehensive study of the interaction between all human genes, their expression and function, disease, drug disposition, and drug response to personalize therapy and develop new drugs (Kelsoe, 2012; McLeod & Evans, 2001; Meyer, 2004; Motulsky & Qi, 2006). Indeed, integrating extensive genetic information gained from individuals' pharmacogenomics data with other clinical information could help develop prescribing models that achieve optimum individualized therapy (Ventola, 2013).

Cytochrome P450 enzymes CYP2C19, CYP3A4, and CYP1A2 are the most functional in the N-demethylation of Clozapine, whereas the role of CYP2C9 and CYP2D6 is less present (Olesen & Linnet, 2001). Uridine diphosphateglycuronosyl transferase (UGT) contributes to the glucuronidation of clozapine metabolites, and flavin-containing monooxygenase 3 (FMO3) participates in the N-oxidation of Clozapine (Erickson-Ridout, Sun, & Lazarus, 2012; Sachse et al., 1999). ATP-binding cassette (ABC) transporters are transmembrane proteins transporting clozapine and other drugs across intra- and extracellular membranes. Genetic variation, such as ATP binding cassette subfamily G member 2 (ABCG2) gene polymorphisms, may also affect the C/D ratio of Clozapine (Akamine, Sugawara-Kikuchi, Uno, Shimizu, & Miura, 2017; Naveen et al., 2020). CYP enzymes, UGT, FMO3, and ABC-transporter genes have been studied regarding clozapine concentrations, but the results are inconsistent (Krivoy, Gaughran, Weizman, Breen, & MacCabe, 2016).

Pharmacogenetic studies on the effectiveness of clozapine and the treatment of clozapine response have also been accomplished. Most of the genes investigated are within the serotonergic and dopaminergic systems. Still, the relations between treatment response and G protein subunit beta 3 (GNB3) and tumour necrosis factor-alpha (TNFA) genes have also been studied. In a meta-analysis, two SNPs in HTR2A (rs6313 and rs6314) and one SNP in HTR3A (rs1062613) were associated with treatment response (Gressier, Porcelli, Calati, & Serretti, 2016). Nevertheless, variants in the TNIK gene were associated previously with a clozapine response among schizophrenia patients in a large candidate gene study of 995 Han Chinese patients (Xu et al., 2016). Studies so far have had moderate sample sizes; the heterogeneous definition of treatment response, clozapine dosage, and compliance were not considered (Gressier et al., 2016). Hitherto, no specific genes could provide insight into the stratification of CLZ efficacy, pharmacokinetics, or agranulocytosis (Li, Solomon, & DeLisi, 2018). Recently, Pardiñas et al. (2019) investigated genome-wide association studies (GWAS) among patients with Schizophrenia on Clozapine. However, this was the first GWAS of clozapine metabolite plasma concentrations. Their identifications indicate the way for the next stage of clinical studies assessing the utility of pharmacogenomics as a sequel to clozapine monitoring procedures, with the potential to influence clinical care through improved titration, dosing, and minimizing of adverse drug reactions (Pardiñas et al., 2019).

Furthermore, Lacaze et al. (2020) used GWAS with clozapine-induced myocarditis among schizophrenia patients to provide the first evidence of SNPs associated with clozapine. Additionally, they provide a novel set of candidate genetic loci for this severe adverse drug reaction and may be of potential clinical

helpfulness by using GWAS. However, GWAS did not reach the conventional statistical threshold used in human genetics and required replication in more extensive studies (Lacaze et al., 2020). Consequently, even with the updated studies of clozapine, there are still no pure findings of using pharmacogenomics to identify clozapine response/dose, especially in Malaysia (Albitar, Harun, Zainal, Ibrahim, & Sheikh Ghadzi, 2020; Leon et al., 2020).

Pharmacometabonomics

In many drug therapies, it is challenging to measure drug response. It may take time for the response to be noticeable, halting achieving effective treatment by choosing the optimum therapy early. Therefore, pharmacometabonomics was proposed (Clayton et al., 2006). Pharmacometabonomics or pharmacometabolomics in some literature is a metabonomic analysis that aims to discover novel biomarkers in the metabolome which associated with a drug's response or toxicity (Corona, Rizzolio, Giordano, & Toffoli, 2012; Nicholson, Wilson, & Lindon, 2011). These novel biomarkers can be used as a classifying tool to classify patients as responsive and nonresponsive to drugs or develop and may not build drug toxicity (Yang & Marotta, 2012). Drug response metabotype can predict a patient's response; besides that, it could explain pathways and monitor the patient's outcome during disease management, which will improve the personalization of therapy (Clayton, Baker, Lindon, Everett, & Nicholson, 2009; Holmes et al., 2006; Yang & Marotta, 2012).

Like metabolomics, pharmacometabonomics reflects the variation in genes, gene expression, and protein expression and their environmental interaction (Guţiu et al., 2010). It is an economical and less invasive approach to predicting drug response (Yang & Marotta, 2012).

Pharmacometabonomics is an emerging field that could predict the effectiveness and drug-induced of clozapine more accurately. Pharmacometabonomics includes both genetic and environmental (lifestyle). The term pharmacometabonomics started to appear in 2006 as a new knowledge within medical sciences. The principle of pharmacometabonomics was defined as identifying a mixture of predose metabolite profiling and chemo-metrics to model and forecast the response of drugs (Clayton et al., 2006). Pharmacometabonomics shows a clear connection between an individual's metabolic phenotype in the form of a predose urinary metabolite profile and the metabolic destiny of a standard dose (Clayton et al., 2009). A few studies describe pharmacometabonomics, one of which concerns the antipyretic drug acetaminophen (Paracetamol) conducted by the pioneers of pharmacometabonomics (Clayton et al., 2009). Pharmacometabonomics profile reveals new drug targets and explores new treatment strategies. The alterations of cellular metabolic stages describe the combination of genome, transcriptome, and proteome changes. Therefore, pharmacometabonomics is a complementary tool for drug target identification and validation. Several studies have demonstrated pharmacometabonomics to guide the selection the right drug for the right metabotype (Abdulkader A Bawadikji, Teh, Kader, Sulaiman, & Ibrahim, 2017).

The primary metabolite of Clozapine is N-Desmethylclozapine (norclozapine), but its effects on metabolic function remain unknown (Yuen et al., 2019). However, the most reliable predictors of a good clozapine response are higher activity, prefrontal cortical structural integrity, and a lower ratio of serotonin and dopamine metabolites, homovanillic acid (HVA): 5-hydroxyindoleacetic acid (5-HIAA) in CSF. Therefore, Samanaite and her colleagues recommended that future studies ensure adequate clozapine plasma concentrations and clozapine trial length, including multivariate models, to raise predictive accuracy (Samanaite et al., 2018). However, drug metabolites might be different or the same as the metabolites of pharmacometabonomics. The central concept of pharmacometabonomics is to know the response of the drug before taking the treatment. For instance, the primary metabolite of warfarin is 3'-hydroxywarfarin. Gemmati et al. (2016)

suggested that the monitoring of 3'-hydroxywarfarin could be of significant advantage in monitoring INR.

Consequently, additional active metabolites should be recognized and investigated as novel, valuable indicators (Gemmati et al., 2016). Meanwhile, A. A. Bawadikji et al. (2019) indicated that alpha and beta glucose could be used as biomarkers of unstable INR in plasma. Thus, there is no study on clozapine using the pharmacometabonomics technique; therefore, this technique might allow identifying the metabolites that can be used as biomarkers of clozapine response.

The integration of pharmacogenetics and pharmacometabonomics

The "Pharmacometabonomics inform pharmacogenomics/pharmacogenetics" approach reveals that pharmacometabonomics can be used to identify genetic variation associated with the variation of drug toxicity/response (Kaddurah-Daouk, Weinshilboum, & Network, 2014). Merely this concept is based on the fact that gene expression or variation in genes may lead to protein variations and, eventually, metabolite levels associated with these pathways that will change (Raamsdonk et al., 2001). Consequently, a metabotype associated with drug response may have some metabolites related to the gene expression or gene variations implicated in the variable response.

Several studies have recently used the integration of genetics and metabolomics (Hartiala et al., 2016; Shah et al., 2011; Shahin et al., 2015). The approach's primary purpose was to better understand particular traits on different systems' biology levels. However, none has evaluated the diagnostic accuracy of integrating pharmacogenetics and pharmacometabonomics biomarkers. Integrating pharmacogenetics and pharmacometabonomics has the advantage of getting more extensive and comprehensive information on variations in drug response. For instance, combining these two methods has revealed more knowledge on aspirin response variation, which is also antiplatelet (Lewis et al., 2013).

Biomarkers

The NIH Biomarkers Definitions Working Group has defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Freeman & Vrana, 2010; Group et al., 2001).

The biomarker is a biological indicator of the disease, physiological state, clinical status, response to drug therapy, or pathogenic process, which can be estimated and appraised for its indicative accuracy (Group et al., 2001). Accordingly, genetic variability associated with a biological status can be an indicative biomarker. The value of pharmacogenetics and pharmacogenomics biomarkers stems from their role in personalized medicine using identified genetic variabilities to predict drug response and avoid ADR prior to drug use. This value increases when the drug of concern has a narrow therapeutic index or if the therapeutic failure of the drug is associated with significant events (Ventola, 2013). In 2008, the FDA issued a table of valid pharmacogenetics biomarkers, which contains a list of drugs with FDA warnings warning of pharmacogenetic testing prior to drug use, and this list is frequently updated (Genomics, 2015).

Pharmacometabonomics has been used to identify novel biomarkers of drugs such as paracetamol, simvastatin, cisplatin, and warfarin (Abdulkader A Bawadikji et al., 2017; A. A. Bawadikji et al., 2019). Pharmacometabonomics is a scientific field that measures and evaluates metabolites found in body fluids and tissues. The main objectives of pharmacometabonomics are to examine and understand the mechanisms of changes in metabolite levels in cells and tissues and the relation between these changes with diseases and medications (George G. Harrigan, Maguire, & Boros, 2008). Many studies have used metabolomic analysis to identify biomarkers of diseases such as asthma, COPD, cancer, and metabolic disorders (Hocquette, 2005; Hunt, 2007; Montuschi, Paris, & Melck, 2009; Robroeks et al., 2010).

The primary aim of using pharmacometabonomic analysis is to produce biochemically based

fingerprints of diagnostic or other classification values. A second stage, crucial in such studies, is identifying the substances causing the diagnosis or classification, and these will become the combination of biomarkers that define the biological or clinical context (Lindon, Nicholson, & Holmes, 2007). According to Ekström, Godoy, and Riva (2010), in rats, N-desmethylclozapine was the major active metabolite of clozapine, which displayed a more significant excitatory effect on the submandibular and parotid glands than clozapine, mediated by the M1-muscarinic receptor.

Authorship

Abdulkader Ahmad Bawadikji, were involved in the study design and had input into and approved the final manuscript.

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Conflict of interest

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