CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURES

Introduction

"Mental illness" or "mental disorder" normally refers to conditions such as major unipolar depression (or depression or depressive disorder), schizophrenia, and manic depression (Murphy, 2000; Simon et al., 2005). Worldwide, more than one in four people suffer from mental disorder at some point in their life. Based on disability-adjusted life year (DALY), a standard measurement of overall disease burden which expressed as the number of years lost due to ill-health, disability or early death, depressive disorder is predicted to become the second most important cause of DALY lost by 2020. The depressive disorder is characterized by a profound and persistent feeling of sadness and/or loss of interest in things that once preferable. There are 2 main categories classified basing on during of the symptom, acute (or major depressive disorder, MDD) and chronic (or dysthymic disorder) depressions. The causes of depressive disorder are complex. The molecular mechanisms that cause and maintain the symptom are still unknown. Several factors have been hypothesized to be involved, including the decrease in function of monoamines, particularly serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline (NA) in the brain (for review see Porcelli et al., 2010). Several treatment strategies have been explored, however, the use of antidepressant drugs (ADs) for treatment is well established. Up to date, many antidepressants have been developed and most of them act on 5-HT and NA (Nutt et al., 2006). Several selective 5-HT reuptake inhibitors such as fluoxetine (for reviews see Carrasco and Sandner, 2005; Cipriani et al., 2009) and sertraline (for review see Carrasco and Sandner, 2005; Cipriani et al., 2010) have been introduced to reduce the symptom by enhancing activity and increasing levels of 5-HT. However, patients may still on their depressive symptoms after first medication, since this treatment requires 4-8 weeks to determine whether the patients respond to the drug (Kemp et al., 2008). Moreover, an inadequate response following the treatment with antidepressant has been reported occurring among patients and the treatment outcome remains disappointing with remission rates of maximal 37% (Warden et al., 2007).

These problems, led to attentions on seeking for predictors especially those could be matched patients with right medication at early time point of the treatment since the early detection may reduce suffering, prevent the development of negative attitudes as well as increase the chance for successful treatment. Although several indicators including clinical, psychophysiological and neuropsychological were reported could improve response prediction, they could not be used in practice due to poor prognostic sensitivity and specificity (for review see Kemp et al., 2008; Esteve et. al., 2010).

Molecular biomarkers are measurable indicators of a particular biological state that relevant to the risk, the presence or the stage of disease. They can be in many forms including transcriptional profiling and DNA methylation (Ramaswamy and Perou, 2003), metabolomics (Fernie et al., 2004), and protein domain. Till now, many novel and improved proteomic technologies have been developed which resulted in major efforts towards the discovery of new protein biomarkers those can be used in clinical practice to identify risk or diagnose a disease, stratify patients, assess disease severity or progression, predict prognosis, or guide treatment. The peripheral markers for psychiatry disorders have been explored for many years to predict the treatment efficacy, which generally requires knowing in advance whether or not depressive disorder patients are responding to the drugs. However, the specific biomarker(s) for prediction of an individual patient's responsiveness to a particular antidepressant have not been identified. Among the human body sources, plasma is an ultimate source of biomarker discovery since it is the most comprehensive human proteome which represent to all body tissues and to both physiological and pathological processes (Anderson and Anderson, 2002). In this thesis, protein biomarker(s) for prediction of responsiveness to an antidepressant in particular serotonin reuptake inhibitor was identified from plasma by 2DE and mass spectrophotometry (MS).

Review of literatures

1. Mental disorder: characteristics and types

The terms "mental illness" and "mental disorder" normally refer to conditions such as major unipolar depression, schizophrenia, manic depression, and obsessive compulsive disorder (Murphy, 2000; Simon et al., 2005). Principles for

diagnosis and classification of mental disorders are outlined in terms of unit of classification, threshold for illness, clinical significance, syndrome similarity, underpinning biological mechanisms and need for an ontological structure which allows both categorical and dimensional models and expression of co-morbidity (for review see Austin, 2010)

There are many different conditions that are recognized as mental illnesses. The common types include the disorders of anxiety, mood, psychosis, eating, impulse control and addiction, personality, adjustment, dissociation, factitiousness, sex and gender, somatisation and mortor tics (Stengel, 1959)

Anxiety disorders: This type of disorders involves an apprehensive anticipation of future danger or misfortune accompanied by a feeling of dysphoria or somatic symptoms of tension (American Psychiatric Association, 1994). People with anxiety disorders respond to certain objects or situations with fear and dread, as well as with physical signs of anxiety or nervousness, such as a rapid heartbeat and sweating. An anxiety disorder is diagnosed if the person's response is not appropriate for the situation, if the person cannot control the response, or if the anxiety interferes with normal functioning. There are many types of anxiety disorders including generalized anxiety disorder (GAD), post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), panic disorder (PD), social anxiety disorder (SAD), and specific phobias (SP).

Mood disorders (affective disorders): These disorders are mental health problems that can be found in children and adolescents, as well as adults. At any age, mood disorders put individuals at risk for other conditions that may persist long after the initial episodes of depression are resolved (http://www. hopkinsmedicine. org/ healthlibrary/conditions/mental_health_disorders/overview_of_mood_disorders_85,P 00759). The most common mood disorders are depression, mania, and bipolar disorder (Austin, 2010). The symptoms are different depend on age and type of mood disorder present. However, the most common symptoms involve persistent feelings of sadness or periods of feeling overly happy, or fluctuations from extreme happiness to extreme sadness. Mood disorders are multifactorial inherited in which genetic and environment are considered to be involved. Causes of the disease are not well understood until now, however, imbalances in certain neurotransmitters including

serotonin, norepinephrine, and dopamine as well as life events may also contribute to depress mood.

Psychotic disorders: Psychotic disorders involve distorted awareness and thinking. Two of the most common symptoms of psychotic disorders are hallucinations which experiences of images or sounds that are not real, such as hearing voices and delusions false beliefs which the ill person accepts as true, despite evidence to the contrary. Schizophrenia is an example of psychotic disorder.

Eating disorders: Eating disorders involve extreme emotions, attitudes, and behaviors involving weight and food. Anorexia nervosa, bulimia nervosa and binge eating disorder are the most common eating disorders.

Impulse control and addiction disorders: People with impulse control disorders are unable to resist urges, or impulses, to perform acts that could be harmful to themselves or others. Pyromania (starting fires), kleptomania (stealing), and compulsive gambling are examples of impulse control disorders. Alcohol and drugs are common objects of addictions. Often, people with these disorders become so involved with the objects of their addiction that they begin to ignore responsibilities and relationships.

Personality disorders: People with personality disorders have extreme and inflexible personality traits that are distressing to the person and/or cause problems in work, school, or social relationships. In addition, the person's patterns of thinking and behavior significantly differ from the expectations of society and are so rigid that they interfere with the person's normal functioning. Examples include antisocial personality disorder, obsessive-compulsive personality disorder, and paranoid personality disorder. Other, less common types of mental illnesses include.

Adjustment disorder: Adjustment disorder occurs when a person develops emotional or behavioral symptoms in response to a stressful event or situation. The stressors may include natural disasters, such as an earthquake or tornado; events or crises, such as a car accident or the diagnosis of a major illness; or interpersonal problems, such as a divorce, death of a loved one, loss of a job, or a problem with substance abuse. Adjustment disorder usually begins within three months of the event or situation and ends within six months after the stressor stops or is eliminated.

Dissociative disorders: People with these disorders suffer severe disturbances or changes in memory, consciousness, identity, and general awareness of themselves and their surroundings. These disorders usually are associated with overwhelming stress, which may be the result of traumatic events, accidents, or disasters that may be experienced or witnessed by the individual. Dissociative identity disorder, formerly called multiple personality disorder, or "split personality", and depersonalization disorder are examples of dissociative disorders.

Factitious disorders: Factitious disorders are conditions in which physical and/or emotional symptoms are created in order to place the individual in the role of a patient or a person in need of help.

Sexual and gender disorders: These include disorders that affect sexual desire, performance, and behavior. Sexual dysfunction, gender identity disorder, and the paraphilias are examples of sexual and gender disorders.

Somatoform disorders: A person with a somatoform disorder, formerly known as psychosomatic disorder, experiences physical symptoms of an illness even though a doctor can find no medical cause for the symptoms.

Tic disorders: People with tic disorders make sounds or display body movements that are repeated, quick, sudden, and/or uncontrollable. (Sounds that are made involuntarily are called vocal tics.) Tourette's syndrome is an example of a tic disorder.

2. Depressive disorders

Depressive disorders are one type of mood disorder that include major depressive disorder (MDD), the depressed phase of bipolar disorder, and dysthymic disorder. MDD and dysthymia differ primarily in chronicity and severity of symptoms. Amongst, MDD is the most prevalent disorder.

2.1 Major depressive disorder (MDD)

Major depressive disorder (MDD) is a common psychiatric syndrome that can interfere an individual’s thoughts, feelings and behaviors. This disease has been predicted to be the most burdensome disease in the world in the 21st century (World Health Organization, 2004). According to Diagnostic and Statistical Manual of

Mental Disorders (DSM), MDD is characterized by one or more major depressive incidents that are persistent and abnormally depressed mood states that last at least 2 weeks (Diagnostic and Statistical Manual, 4th ed. (DSM-IV)). An incident is generally accompanied by a significant persistent and pervasive depression, irritability and loss of interest or pleasure in almost all activities. It is also associated with other symptoms, such as disruption in appetite, sleep, energy, and ability to concentrate; feelings of worthlessness or excessive guilt; or recurrent thoughts of death, all of which have a negative impact on interpersonal and academic functioning. MDD is often chronic and recurrent (Judd et al., 1998). About 40% of youths who have recovered have a recurrence before 2 years, and 70% before 5 years (Kovacs et al., 1984). Several studies have reported that the clinical presentation of symptoms in women markedly differs from those reported by men. The incidence is higher and more severe in female than in male (Thase et al., 1994), and associated with a greater functional impairment (Angst and Dobler-Mikola, 1984). In addition, the symptom first onset peaks during the childbearing years (Weissman and Olfson, 1995; Kessler et al., 2005). However, although women appear to have a higher risk of new-onset incidence, they have similar recurrence and episode length as men (Eaton et al., 1997).

Patients with MDD have a great deal of heterogeneity in the level of several symptoms such as depression severity, number of earlier episodes, anxiety disorders, chronic medical disorders, co morbidity with personality disorders and maladaptive coping styles (such as high levels of neuroticism), and socioeconomic status (Gaynes et al., 2007; Gopinath et al., 2007). These differences have been found to significantly affect patient outcomes (Trivedi et al., 2006; Gaynes et al., 2007).

Because MDD is a relatively common mental disorder, several investigations have been conducted to identify possible underlying causes. There are several factors i.e. genetic, environment and biology that are identified. The genetic is consider to be involved in early onset, severity and recurrent of the disease (Kendler, Gardner and Prescott, 2000). Although, there is no specific reliable molecular risk factor for occurrence of MDD, however, some chromosomal region i.e. on chromosome 15q25-q26 has been reported can link to recurrent and early onset of depression (Holmans et al., 2007). Besides, environmental factors such as personal losses, economic status

and poor interpersonal relationship are also considered to relate to depression of a person. Other causes of MDD are biological factors. Abnormalities in the neuroendocrine systems, neurotransmitters , and neuroanatomy of the brains are found in both children and adult with MDD (http://www.minddisorders.com/Kau-Nu/Major-depressive-disorder.html). Deficiency of norepinephrine and serotonin due to inhibition of tyrosine hydroxylase, depletion of tryptophan, polymorphism of serotonin reuptake transporter as well as improper functioning of norepinephrine receptors, all have been found in patients with MDD (for review see Belmaker and Agam, 2008). Moreover, abnormalities in the frontal lobe as well as lower levels of electrical activity in the left frontal cortex were suggested to be found in depressed subjects (http://www.minddisorders.com/Kau-Nu/Major-depressive-disorder.html).

2.2 Treatment of depressive disorder

There are various types of treatment for mental disorders, however, the two commons are psychiatric medication and psychotherapy. Pharmacotherapy is often used as first line treatment for depression and there are several medications that have been proven to be effective in treating MDD (Stahl, 2002; Rush et al., 2006). Psychological treatment showed to have significant effects on depression in women with postpartum depression (Lumley, Austin, and Mitchell, 2004), and patients with both depression and general medical disorders (Mohr and Goodkin, 1999; Hackett, Anderson and House, 2004). In comparison, the effects of pharmacotherapy are somewhat superior to those of psychotherapy, especially in case of chronic major depression (Cuijpers et al., 2010). However, studies on success rates of psychotherapy versus medication on treating of depressive disorder revealed that combined treatments are more effective than either use alone especially in case of more severe depressed patients (Friedman et al., 2004; Pampanolla et al, 2004; De Maat et al, 2007; Cuijpers et al., 2010).

The treatment of depressive disorder consists of three phases (for review see American Psychiatric Association, 2000, Karasu et al., 2000; Gartlehner et al., 2007).

1. An acute phase, which is the phase of remission induction. This period takes 6-12 weeks in length. The treatment options i.e. pharmacotherapy, psychotherapy or the combination may be chosen depend on several factors i.e.

severity of symptoms and patient preference. If the antidepressants have been used for initial treatment, the careful monitoring for drug response and side effects are necessary. In case that there is no clinical response after 4-8 weeks of first medication, evaluation for factors that may be cause of non-response as well as adjusting the treatment should be considered.

2. A continuation phase, which remission is preserved. This phase usually takes 16-20 months. The patient should be maintained with antidepressants to prevent relapse.

3. A maintenance phase, this phase takes one or more years to prevent patients against the recurrence of major depressive episodes. Continued medication in this phase may or may not include if patients have single episode of MDD, however, in case of the recurrent MDD patients, maintenance on an antidepressant for years are recommended.

2.3 Antidepressants for treatment of depressive disorder

At the first time, antidepressants including tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) have been approved for the treatment of depression. However, they are no longer used because of their multiple side effects that many patients find intolerable (for review see Gartlehner et al., 2007). Due to drawbacks of these drugs, the second generation of antidepressants with better side effect profile (Williams et al., 2000) were introduced. There are several types of drug in this generation which can be classified by mechanism of action or by chemical structure, however, most of them are in class of selective serotonin reuptake inhibitors (SSRIs) or serotonin norepinephrine reuptake inhibitors (SNRIs) (Liang and Richelson, 2008). These antidepressants act by inhibiting reuptake of serotonin and/or norepinephrine at the presynaptic neuron membrane leading to increasing level of this neurotransmitter and consequently enhance the transmission of neuron signals at the synapse. In general, the clinical responses and remission rates of first- and second-generation antidepressant medications is similar (Liang and Richelson, 2008), however, since SSRI and SNRIs act more specifically, these drugs therefore provide fewer side effects when compare to TCAs and MAOIs (Richelson, 2003).

SSRIs are predominantly prescribed in the treatment of depression, due in part to their limited side-effects particular in adults. They include fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram, and escitalopram. Although these drugs are much more similar, each has a distinct side-effect profile including a predilection to cause gastrointestinal side effects and either daytime sedation or stimulation (for review see Olivier et al., 2010). Furthermore, SSRI are also prescribed to children and adolescents in the treatment of depression and other neurodevelopmental disorders (for reviews see Williams et al., 2010). Potential hazards of SSRIs in the pediatric population involve increases in suicidal ideation and behavior. These adverse outcomes are not observed during adult SSRI treatment, suggesting that SSRIs exert age-dependent effects, with negative outcomes during early life. Amongst, fluoxetine (Prozac) is the preferred SSRI because it is the only SSRI that is registered for treatment of the pediatric population (Bhatia and Bhatia, 2007).

Although large number of antidepressant have been used widely to cure patients who suffered from depression. It had been found that response rate of patient with an initial antidepressant medication are approximately 55% and only 30% of those treated for MDD had achieved remission (Trivedi et al., 2006). Moreover, some residual symptoms are found in patients who achieve remission (Nierenberg et al., 1999). There are two treatment strategies which can be used for the patients who showed inadequate response to the first medication. They include (1) optimization of dosage and duration of antidepressant treatment and (2) switching to another antidepressants (for review see Ministry of Health Malaysia, 2007). Moreover, strategies including drug optimization, augmentation, combination as well as switching were used to treat patient who did not respond to several antidepressant trials (also called difficult to treat patients) (for review see Fleck and Horwath, 2005).

3. Biomarker(s) and proteome of human plasma

In medicine, a biomarker is normally referred to a protein measured in blood (serum or plasma) whose concentration reflects the severity or presence of some disease state, or is anything that can be used as an indicator of a particular disease state or some other physiological state of an organism. They can be classified based

on characteristics to imaging biomarkers and non-imaging biomarkers (or molecular biomarkers). Molecular biomarkers are the biomarkers that have biophysical properties, which allow their measurements in biological samples such as plasma, serum, and cerebrospinal fluid. These include nucleic acids-based biomarkers (such as gene mutations or polymorphisms and quantitative gene expression analysis), peptides, proteins, lipids metabolites, and other small molecules. The biomarker(s) associated with mental disorders have been identified so far involve structural and functional neuroimaging, genomics, neurophysiologic studies, and plasma analysis. For examples, apolipoprotein E (ApoE) gene is found to be a major risk factor for dementia. The presence of both depressive symptoms and APOE-4 allele increased several fold the risk for dementia across subtypes (Irie et al., 2008). The association of the APOE-4 allele with specific genetic variants of other genes such as CYP2D6 negatively modulates the therapeutic response to multi-factorial treatments affecting cognition, mood, and behavior. Besides, a polymorphism in the brain-derived neurotrophic factor (BDNF) gene has been reported in some studies to relate with depression (Sklar et al., 2002; Sen et al., 2003). The finding that single bilateral infusion of BDNF into the brains of animals has been shown to produce an anti-depressant effect in two animal models of depression supported those studies (Siuciak et al., 1997; Shirayama et al., 2002). Moreover, genetic variants in the promoter region of the serotonin transporter gene (SLC6A4) have also focus to be involved in the vulnerability to depression (for review see Kemp et al., 2008).

The proteomic approach is a powerful tool that can provide protein expression profiles, which useful to predict clinical events, therapeutic response, or to probe underlying mechanisms of several diseases such as autoimmune disorders, cardiovascular diseases, and cancers. The urgent need for the proteomic based discovery of novel disease markers and/or evaluating factors associated with specific diseases together with the development of high-throughput techniques that provide highly sensitive analyses of the protein content in cells, tissues, and organisms, as well as of different body fluids, such as plasma and urine, has opened a completely new chapter in biomarker discovery. Biomedical proteome research aimed at biomarker discovery is mainly based on expression proteomics, which analyzes the quantity of certain proteins in different conditions, therefore, the proteomic studies

that aid characterization of proteins and selection of optimal proteomics technologies are key factors in propelling the discovery of novel biomarkers (Bahk et al., 2010). Large numbers of candidate biomarkers are typically identified and have been reported (for review see Kingsmore, 2006). These candidates must be further evaluated to identify those markers that have a statistically significant association with disease (for reviews see Rifai et al., 2006). Once it is identified, the biomarker must be undergo verification/validation phase, and concentrations of candidate proteins must be measured across clinical cohorts (Rifai and Gerszten, 2006).

In plasma, the most abundant protein is albumin which presents at about 40 mg/ml. Besides, other 22 highly abundant proteins exist, including immunoglobulins (IgGs), transferring and fibrinogen. These highly abundant proteins comprise about 98% of the protein content of serum (Anderson and Anderson, 2002). Conversely, it is estimated that over 10,000 different proteins are present in plasma and most of them exist in very low quantities (Adkins et al., 2002). In addition, proteins in serum exhibit large dynamic range concentrations, somewhere between 10 and 15 orders of magnitude. Both the great number of proteins and the extended range of their concentrations result in a great challenge for the identification of potential biomarkers. Human plasma is easily collected and it contains large amounts of proteins including many proteins originating from vascularized tissues. Plasma or serum analyses may thus provide relevant information regarding these tissues. For example, some of the proteins released by tissues could be from the cells that undergo cell death, whereas other proteins could be secreted by tumor cells. Notably, these released proteins could be characterized in terms of their presence or absence or by changes in their concentrations between healthy and diseased states. Consequently, a systematic characterization of proteins in human plasma in health and in diseased states has a considerable potential for identifying possible biomarkers for disease diagnosis, for the development of new therapeutic products and for monitoring responses to drug treatments (Richard et al., 2007). Plasma proteins are useful targets for diagnostic, prognostic, and/or therapeutic development. With proteomic tools available recently, profiling of human plasma proteome becomes more feasible in searching for disease-related protein markers (Anderson and Anderson, 2002). The measurement of proteins expression and modification may lead to the detection and

identification of particular proteins in cellular and may correspond to cellular functions often change during different disease states.

For depressive disorder diseases, no biomarkers have sufficient potential utility to be ready for clinical application at present (Leuchter et al., 2010). However, several promising biomarkers have been investigated and reported. An example was BDNF, the presence of low level of this brain-derived neurotrophic factor in MDD patients 's serum combined with the elevation level of BDNF after treating with antidepressants suggested to potential as a clinically use biomarker in future (Duman and Monteggia 2006; Sen, Duman and Sanacoa, 2008; Leuchter et al., 2010). Furthermore, depression has been associated with the activation of the immune system and acute phase response with changed level of acute phase proteins (Maes, 1995; Erdem et al., 2011). In the depressive cases, results for serum concentration of haptoglobin, one acute-phase proteins showed associate to severity of depressive symptoms (Erdem et al., 2011). Although, this finding could not absolutely conclude the use of haptoglobin as biomarker for depression due to small size of samples, however, the idea of using this protein as one of biomarker for MDD treatment in future is interesting.

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Objectives

To explore and identify protein marker(s) in plasma of Thai patients with major depressive disorder for predict the responsiveness to the treatment with antidepressant by 2-DE and mass spectrometry.