A PRELIMINARY STUDY OF NEUREGULIN 1 GENE EXPRESSION IN SCHIZOPHRENIA PATIENTS ON PALIPERIDONE IN A MALAYSIAN POPULATION IN UNIVERSITY MALAYA MEDICAL CENTRE (UMMC)

By

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MASTER OF PSYCHOLOGICAL MEDICINE

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By

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CERTIFICATION

This is to certify that the candidate, Dr. Nazariah Aiza Binti Harun , had carried out this research project , and to the best of my knowledge ,this dissertation is entirely her work.

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I would like to say Alhamdullilah that this study managed to be completed.

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LIST OF ABBREVIATIONS

APA	: American Psychiatric Association
BARS	: Barnes Akathisia Rating Scale
CNS	: Central Nervous System
DALY	Disability Adjusted Life Years
DNA	: Deoxyribonucleic Acid
DSM IV TR	: Diagnostic And Statistical Manual Of Mental Disorders, Fourth Edition, Text Revision
FGA	: First Generation Antipsychotic
GABA	: Gamma-Aminobutyric Acid
GAD67	: Glutamate decarboxylase67
GAT-1	: GABA membrane transporter 1
HPA	: Hypothalamic Pituitary Adrenal
M.I.N.I	: MINI International Neuropsychiatric Interview
MOH	: Ministry Of Health
NMDA	: N-methyl-D-aspartate
NRG 1	: Neuregulin 1
PANSS	: Positive And Negative Symptoms Of Schizophrenia Scale
PCR	: Polymerase chain reaction

PFC : Prefrontal Cortex

PBL : Peripheral Blood Lymphocytes

RNA : Ribonucleic acid

SNP : Single Nucleotide Polymorphism

SGA : Second Generation Antipsychotic

UMMC : University Malaya Medical Center

WHO : World Health Organization

YLD : Years Lived With Disability

ABSTRAK

KAJIAN AWAL EKPRESI GEN NEUREGULIN 1 DALAM PESAKIT SKIZOFRENIA YANG MENGAMBIL PALIPERIDONE DI KALANGAN RAKYAT MALAYSIA DI PUSAT PERUBATAN UNIVERSITI MALAYA (UMMC)

LATAR BELAKANG: Polimorfisme gen Neuregulin 1(NRG 1) telah dipertimbangkan sebagai gen yang menyebabkan penyakit Skizofrenia . Ini terbukti dalam pelbagai kajian yang dijalankan dalam beberapa populasi Eropah dan Cina yang menunjukkan kaitan yang kuat diantara gen NRG 1 dan Skizofrenia. Terdapat banyak kajian yang berkaitan di seluruh dunia dan di rantau Asia tetapi tidak di Malaysia yang terdiri daripada 3 kaum utama. Oleh itu, adalah penting untuk memahami kelebihan mengukur ekpresi gen NRG 1 yang memainkan peranan penting sebagai faktor risiko untuk penyakit Skizofrenia dan ini menyarankan kemungkinan gen NRG 1 mempunyai potensi sebagai 'biomarker' untuk penyakit Skizofrenia.

OBJEKTIF: Pesakit Skizofrenia yang menerima rawatan paliperidone akan diukur untuk ekspresi gen NRG 1. Tujuan kajian ini adalah untuk menyiasat ekpresi gen NRG 1 dalam pesakit Skizofrenia dan kaitan diantara ekspresi gen NRG 1 dengan simtomatologi.

METODOLOGI: Kajian keratan rentas ini dijalankan di Pusat Perubatan Universiti Malaya (PPUM). Subjek terdiri daripada mereka yang menghadiri perkhidmatan psikiatri dan perubatan di UMMC dari Februari 2011 hingga November 2011 (10 bulan). Tahap ekpresi gen NRG 1 diukur bagi semua indidvidu yang mengambil rawatan Paliperidone dan kawalan sihat . Maklumat mengenai faktor-faktor sosio-

demografi (umur,status perkahwinan ,status pekerjaan) dan klinikal yang berkaitan ,(sejarah penyakit mental dalam keluarga,tempoh berpenyakit mental dan Psikopatologi dinilai menggunakan Mini International Neuropsychiatric Interview (M.I.N.I) Positive And Negative Syndrome Scale (PANSS) dan kesan-kesan sampingan dinilai menggunakan Barnes Akathisia Rating Scale (BARS) dikumpulkan.

KEPUTUSAN: Sejumlah 20 pesakit Skizofrenia dan 15 kawalan sihat telah dimasukkan ke dalam kajian ini. Di kalangan pesakit skizofrenia majoriti adalah bujang (80%) , tidak bekerja (70%) dan mempunyai sejarah keluarga penyakit skizofrenia (55%). Purata ukuran ekspresi gen NRG 1 dalam 20 pesakit Skizofrenia kami ialah 0.405 (SD= 0.491). Tiada faktor yang signifikan berkaitan dengan ekspresi gen NRG 1. Tiada faktor signifikan yang berkaitan dengan ekspresi gen NRG 1 dengan psikopatologi dan kawalan sihat.

KESIMPULAN: Purata ukuran ekspressi gen NRG 1 di kalangan rakyat Malaysia adalah 0.405 (SD= 0.491). Pesakit yang mempunyai markah PANSS yang rendah mempunyai ukuran ekspresi gen NRG 1 yang rendah (mean =0.304) berbanding dengan pesakit yang mempunyai markah PANSS yang tinggi (mean = 0.524) dan kawalan sihat.Keputusan ini menyarankan bahawa ada perubahan ekspresi gen NRG 1 dalam pesakit skizofrenia yang mengambil paliperidone di kalangan rakyat Malaysia. Pada masa akan datang , satu kajian prospektif dengan saiz sampel yang lebih besar diperlukan untuk kajian yang lebih mendalam untuk topik ini.

ABSTRACT

A PRELIMINARY STUDY OF NEUREGULIN 1 GENE EXPRESSION IN SCHIZOPHRENIA PATIENTS ON PALIPERIDONE IN A MALAYSIAN POPULATION IN UNIVERSITY MALAYA MEDICAL CENTRE (UMMC)

BACKGROUND: The Neuregulin 1gene (NRG 1) polymorphism has been considered as a susceptibility gene for Schizophrenia. This is evident from studies done in several European and Chinese populations that suggesting a strong association between NRG 1 gene and Schizophrenia. There are numerous related studies worldwide and in Asian region but not in Malaysia that consists of 3 major races. Therefore, it is important for us to understand the reason for measuring NRG 1 gene expression given the importance of the gene as a risk factor for Schizophrenia, suggesting that NRG1 may have the potential as a biomarker for schizophrenia.

OBJECTIVE: Schizophrenic patients on paliperidone treatment to be measured for NRG 1 gene expression. The aim of this study is to investigate NRG 1 gene expression in Schizophrenia subjects and the association of NRG 1 expression level with symptomatology.

METHODOLOGY: This was a cross sectional study conducted in University Malaya Medical Centre (UMMC). Subjects were recruited from those attending the psychiatry and medical services in UMMC from February 2011 until November 2011 (10 months). The NRG 1 gene expression was measured for subjects on paliperidone treatment and in the control group. The information on socio-demographic (age, marital status, employment status) and clinical factors (family history of mental illness, duration of

illness and psychopathology using Mini International Neuropsychiatric Interview (M.I.N.I) Positive And Negative Syndrome Scale (PANSS) and side effects using Barnes Akathisia Rating Scale (BARS) were collected.

RESULT: A total of 20 Schizophrenia subjects and 15 healthy controls were recruited for this study. Among the 20 schizophrenia subjects majority were single (80%), unemployed (70%) and have family history of schizophrenia (55%). The average NRG 1 gene expression level in our 20 schizophrenia patients was 0.405 (SD= 0.491) and in the control group was 0.435 (SD= 0.273). No factors were found to be significantly associated with NRG 1 gene expression level. There was no significant association between NRG 1 gene expression level and psychopathology.

CONCLUSION: The average NRG 1 gene expression level among Malaysians was 0.405 (SD= 0.491).Those with low PANSS score had lower NRG 1 levels (mean =0.304) compared to those with high PANSS score (mean = 0.524) and control group. This suggests that there is altered NRG 1 expression in Schizophrenic patients on Paliperidone in Malaysian population. In the future, a prospective study with larger sample size is needed to look further into this topic.

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND AND HISTORICAL PROSPECTIVE

Each individual responds differently to treatment regimes; this is also true of mental illness sufferers. In schizophrenia, antipsychotics are the mainstay of treatment for psychotic symptoms (Arranz and de Leon 2007). Individuals respond in numerous ways to treatment; be it a favourable outcome, an adverse event or no response. There are multiple factors involved in determining a patient's response to treatment : environmental factors, adherence , severity of illness, co-morbid medical conditions , type of drug prescribed (atypical or conventional, generic or original compound), drug – drug interaction , age and of course, the individual's genetic profile itself plays a role (Aitchison 2002). In order to improve patient care one of the emerging fields in psychiatry is pharmacogenomics and pharmacogenetics.

Why are these fields important? Pharmacogenomics and pharmacogenetic profiling will enable psychiatrists to individualize treatment and minimize exposure to multiple drugs. Pharmacogenetics was coined by Vogel in 1950s to define *inherited variability in response to drug treatment*. Pharmacogenetics is concerned with finding identifying or investigating candidate genes that can cause individuals to have differences in drug effect (Arranz and de Leon 2007). The genes are selected based on their drug targets or coding for metabolic enzymes.(Arranz 2003).These differences would affect therapeutic efficacy, effectiveness and adverse reactions. In 1995, the Human Genome Project was initiated; this mega project later on helped pharmacogenetics to evolve into pharmacogenomics . Pharmacogenomics uses a hypothesis based approach that studies genome factors and drug response at cellular,

tissue , individual , group level (Aitchison 2002). Information regarding functional activity and differential gene expression in areas related to the aetiology of the disease is another approach use in the field of pharmacogenomics (Arranz 2003).

The search includes genes determining disease susceptibility and those causing individual variations in drug response, based on the knowledge derived from the Human Genome Project (Aitchison 2002).Both of these fields' aim to guide pharmacotherapy and improve outcome by providing individualized treatment. In the future both fields will help discover novel drugs and accelerate clinical improvement. Pharmacogenomics and pharmacogenetics have great promise particularly in psychiatry as there is lack of biological based treatment guidelines. As of now, there are no diagnostic biomarkers in psychiatry .Individual variability in drug response can often be understood as a combination of factors affecting the pharmacokinetic and pharmacodynamic effects of drugs. This is where pharmacogenomics studies are helpful and candidate genes include polymorphic drug-metabolizing enzymes, drug transporters and polymorphic drug targets that affect disease-related pathway (Tsapakis, Basu et al. 2004).

1.2 .GLOBAL BURDEN OF MENTAL ILLNESS

Mental illness is the 'plague' of the 21st century population that is fast becoming a growing concern globally. Schizophrenia is a chronic illness that debilitates an individual and remains with them throughout their life. Schizophrenia is the most frequently encountered psychotic illness. The lifetime prevalence of schizophrenia is approximately 1% and the point prevalence is around 0.5%. In the Global Burden of Disease 2000 study it was found that schizophrenia accounted for 2.8% of the years lost to death (YLD) and 1.1% of the Disability Adjusted Life Year DALYs (Michaud, Murray et al. 2001). The Global Disease Burden of Disease Study developed new ways to measure health status that accounted for disability along with the number of deaths and the impact of premature death. To quantify the burden of disease the researchers came up with DALY (disability adjusted life year). The DALY is a summary measure of population health that combines in a single indicator years of life lost from premature death and years of life lived with disabilities. One DALY can be thought of as one lost year of 'healthy' life. In the Version 1 estimates for the Global Burden of Disease 2000 study, published in the World Health Report 2001 (2)(World Health Organisation), schizophrenia is the 7th leading cause of YLDs at global level, accounting for 2.8% of total global YLDs. Hence, this shows that mental illness has a significant and detrimental impact on social health, on economy incurring direct and indirect costs to individual, families and government.

1.3. LOCAL BURDEN OF MENTAL ILLNESS – MALAYSIAN PERSPECTIVE

The third national health and morbidity survey was conducted in 2006 on 36,519 respondents aged 16 years and above using General Health Questionnaire (GHQ 28) and found overall unadjusted prevalence for mental disorders was 11.2 % (Health 2006) . Mental illness is considered to be a non communicable and chronic disease, in Malaysia, chronic diseases accounted for 71% of all deaths in 2002 (WHO 2002).In 2005 , the Malaysia burden of Disease and Injury Study was conducted and found that the total burden of disability in Malaysia population in year 2000 amounts to 1.1 million years (Yusoff AF 2005). The National Mental Health Registry (NMHR) reported that 70% of patients were never employed or unemployed at the time of registration. Furthermore, the demographic profile shows that the majority of patients with schizophrenia in Malaysia are in the productive age of 20-40 years(Aziz, Salina et al. 2008). In view of all the findings it reflected the impact of mental illness in developing countries such as Malaysia and the burden would continue to increase with the increase in migration an urbanization of its population.

1.4 LITERATURE REVIEW

1.4.1 SCHIZOPHRENIA SYPTOMATOLOGY

There was much debate regarding naming this illness, initially it was known as demence precoce by Morel, Hebephrenia by Hecker and Catatonia by Kahlbaum. The term schizophrenia came about at the end of 19th century and it was coined by a Swiss psychiatrist Eugene Bleuler which is still in use until now (Michael 2000). Schizophrenia is a mental illness that is distressful to both patient and physician. For the physician the diagnosis can be made difficult at times, as the presentation may not be as straight forward as it seems. To diagnose the symptoms must be present for a significant portion of time during a 1-month period; during which the patient experiences: Two (or more) of the following DSM IV TR criteria for Schizophrenia (Association. 2000):

- Delusions
- Hallucinations
- Disorganized speech
- Grossly disorganized or catatonic behavior or
- Negative symptoms, such as affective flattening, alogia, or avolition .

These symptoms burden the patients until they are unable to function socially and occupationally (Association. 2000) . Affective symptoms can also manifest in schizophrenia either as depression or anxiety. The more common symptom is depression whereby it can be the core feature of schizophrenia , occur in prodromal phase of illness , it

is the prominent symptom in acute or chronic episodes or post psychotic depression (early or late onset) (Mulholland and Cooper 2000) . The other symptoms that are seen in schizophrenia are motor symptoms, cognitive symptoms , lack of insight , minor physical anomalies and neurological signs (soft or hard) (Tandon, Nasrallah et al. 2009).

People afflicted with schizophrenia are at higher risk of having intellectual impairment approximately 3-5% are affected (Morgan, Leonard et al. 2008) , substance abuse estimated at 47 % , affective disorder most commonly depression estimated at 50 % and anxiety disorders. The symptoms for anxiety and depression are relatively common throughout the course of illness, with an estimated prevalence of 15% for panic disorder, 29% for post traumatic stress disorder, and 23% for obsessive-compulsive disorder (Buckley, Miller et al. 2009).

The mortality rate in individual with schizophrenia is increased and this can be due to several factors (Capasso, Lineberry et al. 2008) .Two thirds of the increments in mortality can be explained by natural causes . The use of certain atypical antipsychotics has lead to the rise of metabolic syndromes resulting in increased incidence of type 2 diabetes and ending in cardiovascular events that takes lives (Auquier, Lancon et al. 2007). However, the increased prevalence of medical conditions can also be due to other factors such as sedentary lifestyle, smoking, hereditary and unhealthy diets. Under recognition and inadequate treatment of co morbid medical conditions and an increased likelihood of adverse outcomes of some treatments for co morbid medical conditions also contribute to the rise of mortality in schizophrenia (Tandon, Nasrallah et al. 2009). Suicide remains one of the major cause of death in schizophrenia and claims 9-13% life of schizophrenic

patients (Meltzer 1999). Suicide can be prevented with early detection and commencement of treatment in schizophrenia patients.

1.4.2 NEUROBIOLOGY OF SCHIZOPHRENIA

1.4.3. NEUROPATHOLOGICAL AND NEURANATOMICAL

In contrast to Alzheimer's disease, that has diagnostic neuropathology, which is quantifiable and correlates with the clinical severity of the disorder, the same cannot be said for schizophrenia (PJ Harrison 2005). Based on the success of understanding the neuropathological and neurodegenerative basis for Alzheimer disease, Parkinson disease Huntington's disease and related polyglutamine diseases suggests some potential lessons for schizophrenia (Ross, Margolis et al. 2006) .

There is no "pathognomonic" neuropathological changes identified in schizophrenia, the changes are more subtle. Despite this there are several important observations are made over time, using neuroimaging studies , advance histochemicals study , receptor autoradiography, in situ hybridization and gene array techniques (Keshavan, Tandon et al. 2008). There is evidence of structural brain abnormalities in schizophrenia which is , reduced brain weight , enlarged third and lateral ventricle and reduced cortical gray matter weight and related structures (Lewis and Lieberman 2000).Studies have consistently shown absence of glial proliferation (Arnold, Trojanowski et al. 1998) and Golgi studies showed reduction in the synapse rich neuropil (Selemon and Goldman-Rakic 1999). Synaptic connectivity in schizophrenia may be impaired ranging from the dendritic tree to cell body, axon terminal, synaptic terminal and associated glial elements (PJ Harrison 2005).

The neurochemical phenotypes involved in schizophrenia are unclear; as such several are implicated including glutamate deficits in the hippocampus and cerebellum. There are alterations of GABAergic as well as glumatergic synaptic populations in dorsolateral prefrontal cortex (DFPC), changes in cortical dopaminergic innervations and signaling (Lewis 2000). Many of these structural abnormalities are present in first episode psychosis, treatment naïve individuals with schizophrenia. They may be present prior to onset of illness which suggests they are primary disease process and not secondary to illness or consequence of treatment(Lewis and Lieberman 2000).

Neuroanatomical findings have evolved with advanced neuroimaging techniques, MRI findings confirms structural brain abnormalities in schizophrenia. Among the changes seen using MRI is ventricular enlargement, medial temporal lobe involvement (includes hippocampus and parahippocampal gyrus, amygdala , superior temporal gyrus , parietal lobe involvement as well as subcortical brain region involvement. The subcortical region includes cerebellum, basal ganglia, corpus callosum , thalamus and Cavum septi pellucidim (CSP) (Shenton, Dickey et al. 2001) . Meta analysis of first episode schizophrenia have shown whole brain and hippocampal volume reductions (Steen, Mull et al. 2006). It has been suggested that development of cerebral asymmetry and anomalies in cerebral dominance is critical in the pathogenesis of schizophrenia and could be related to susceptibility genes (Crow, Ball et al. 1989).

1.4.4. NEUROENDOCRINE IN SCHZOPHRENIA

The stress diathesis model has long been discussed for Schizophrenia, Rosenthal, 1970 postulated that the behavioral expression of the biological vulnerability for schizophrenia is influenced by exposure to stress. Stress is commonly associated with the index psychotic episode and the subsequent relapses (Norman and Malla 1993). As we know when an individual is in 'stress' they will go into the flight or fight mode that activates the Autonomic Nervous System (ANS) and Hypothalamic Pituitary Adrenal (HPA) system. These reactions constitute the biological stress response that is typically associated with behavioral change (Elaine F. Walker 1997) .

There are 3 chemical substances released by the HPA axis which are corticotropin releasing hormone, adrenocorticotropic hormone and glucocorticoid. During a stressful period, there is increased release of these chemical substances and most studies looks at cortisol levels and post dexamethasone levels. Cortisol is the major glucocorticoid hormone in humans. Glucocorticoids have effects throughout the body and they are critical to the physiological changes glucocorticoid receptors (GRs) located in various regions throughout the brain serve to regulate the activity of the HPA axis. The hippocampus contains a particularly high density of GRs, and it is believed to play an important role in the feedback system that serves to modulate the activation of the HPA axis (Keshavan, Tandon et al. 2008). If an individual is continuously in a stressful state it will cause permanent changes to the HPA axis and subsequent damage to the hippocampus. Hippocampus damage has been linked to the neurotoxic effects of excessive glutamate release, which is potentiated by

glucocorticoids (Elaine F. Walker 1997). Animal studies showed that chronic stress and/or high glucocorticoid levels induces deleterious effects on neuroplasticity (McEwen 2008). A large number of studies reported that baseline cortisol levels are higher in schizophrenic individuals than controls, the cortisol levels are higher in prefrontal cortex and Cerebrospinal fluids of schizophrenics (Issa, Zhan et al. 2010) . The dexamethasone suppression test (DST) is typically used with psychiatric patients as a challenge to the HPA axis, with the goal of assessing the integrity of HPA regulation by means of feedback mechanisms (Elaine F. Walker 1997).A systematic review revealed that incidence of dexamethasone non suppression, a measure of HPA axis overactivation is significantly higher in schizophrenia than controls (Yergani 1990) .The post dexamethasone cortisol levels were dependent on phase of illness and medication status. Elevated cortisol post DST levels is seen preceding a psychotic episode compared to during recovery period (Sachar 1970) and in individual with severe negative symptoms (Walker et al,1997). Furthermore, elevated cortisol secretion in psychotic individuals has been linked with greater symptoms severity, impaired cognition and ventricular enlargement (Tandon, Mazzara et al. 1991) .

1.4.5. NEUROTRANSMITTER IN SCHIZOPHRENIA

There are few neurotransmitters implicated in schizophrenia and the oldest and widely held for schizophrenia is dopamine. In 1950s, the accidental finding of phenothiazine ability to treat the positive symptom of schizophrenia put more emphasis on the dopamine hypothesis of schizophrenia. This theory postulates that dopamine dysfunction leads to manifestation of schizophrenia symptoms (Arvid Carlsson 1999) .In the early years there was no direct evidence to support this theory and with evolution and progression of research there is some evidence to lend support to this theory . As classically described by Von Rossum, 1967, the over activity of dopeminergic pathways causes the elevation of dopamine and symptoms of schizophrenia. Furthermore elevation of dopamine release in basal ganglia is seen after amphetamine challenge and this correlates to the induction of psychotic symptoms (Laruelle, D'Souza et al. 1997). Baseline elevation of dopamine in schizophrenic patients who are treatment naïve very tightly correlates with amphetamine induced dopamine release. In contrast to the original theory of dopamine hyperactivity, there is a new theory of dopamine hyper function. The theory suggested that unknown development or for biochemical reasons, causing a primary defect and disrupts the efficient, tight dopaminergic transmission, triggering feedback activation and receptor upregulation resulting in increase dopaminergic tone (Arvid Carlsson 2006).

In recent years, other aberrations of neurotransmitters are looked into and whether their dysfunction would lead to schizophrenia. Lately, there is more interest in the role of Glutamate in schizophrenia. Glutamate is an excitatory neurotransmitter and widely found in the Central Nervous System (CNS). It is not only involved in fast synaptic transmission but also plays a role in neuroplasticity and cognitive function (Tsapakis and Travis 2002).

The reduced glutamate level in the cerebrospinal fluid of patients with schizophrenia was reported in 1980 by Kim et al, that initially lead to glutamate hypothesis of schizophrenia (Kim, Kornhuber et al. 1980). The deficiency of glutamate function at N-methyl-D-aspartate (NMDA) receptors can produce psychotic symptoms. Findings that provide supports this hypothesis came from studies using neuroimaging such as positron emission tomography (PET) scan. It was shown that glutamate receptor function seem to be abnormal or function abnormally in patients with positive symptoms of schizophrenia (Tamminga 1998) . Other evidence is the clinical observations of psychotic symptoms induced by Phencyclidine (PCP) a NMDA-receptor antagonist and ketamine (Javitt 1991). Post mortem studies also detected a large number of abnormalities in the expression of GLU-related proteins especially NMDA receptor subunits most significantly the hippocampus and prefrontal cortex (Harrison, Law et al. 2003) , but few of these observations have been independently replicated .

GABA dysfunction has long been postulated as a theory for schizophrenia .Postmortem brain studies of schizophrenic patients have shown that glutamic acid decarboxylase (GAD), the enzyme responsible for the synthesis of GABA as well as the uptake and release of GABA is reduced. GABA play an important role in the circuitry of the PFC which we know is also affected in schizophrenia patients. A postmortem study has found that the density of GABA membrane transporter 1 (GAT-1) immunoreactive chandelier neuron axon cartridges was decreased by 40% in schizophrenic subjects compared to both normal control and those with other mental illness (Woo, Whitehead et al. 1998). GAT-1 is the principal neuronal transporter for GABA.

Thus if any alteration in GAT-1 protein would also affect GABA concentrations, decreased GAT-1 levels is associated with elevated GABA concentrations which causes increased inhibitory effects and a reciprocal decrease in the excitatory output of chandelier neuron axon (David A 2000). Other studies have identified that treatment with NMDA antagonist for a period of days can cause reduction of cortical glutamate decarboxylase67 (GAD67) and parvalbumin mRNA which would lead to expected reduction in GABA levels and therefore decrease inhibition. How can this occur? Pyramidal cells activity is monitored by NMDA channel which acts as a sensor. If the channel malfunction suggesting low pyramidal cell activity, interneuron may synthesize less GABA and parvalbumin, in order to restore pyramidal cell activity to the normal level. However if the homeostatic loop malfunctions too , it could lead to produce over activity of pyramidal cell activity and eventually the symptoms of schizophrenia will occur (Lisman, Coyle et al. 2008). The importance of malfunction of the PFC circuitry is that it alters GABA level causing GABAergic hypofunction. This leads to clinical manifestation of Schizophrenia such as poor memory, poor affect regulation and altered working memory (Shulman 2005). It is hypothesized that there is a feedback loop that helps regulates dopamine, GABA and glutamate neurotransmitters. For example hypofunction of NMDA receptor removes the excitatory drive to inhibitory GABAergic neurons. The GABAergic neurons in return regulates non –NMDA excitatory neurons. These neurons acts on the frontal cortex and the limbic regions, leading to reduced inhibitory control, increasing firing resulting in psychotic symptoms being produced (Farber 1998). This suggests that interactions between GABA and glutamate can influence their function and subsequently lead to manifestation of schizophrenia symptoms.

1.4.6. GENETICS OF SCHIZOPHRENIA

It was suggested by Kallman, 1946 that there is a genetic basis for schizophrenia however due to lack of research tools in the early years this illness was mainly thought due to dysfunctional family dynamics as described by Bateson , 1965 and Lidz , 1965 (Benjamin.J.Sadock 2007). However as time progresses it has become more evident there is a genetic basis to this illness as schizophrenia seems to aggregates in families (Tandon R 2008) . If a family member is affected the risk of developing schizophrenia increases. The higher the genetic affinity of the affected family member the likelihood of having schizophrenia increases. Data from family, twin and adoption further substantiates this genetic basis. Twin studies have shown incidence of schizophrenia in dizygotic twins is about 17% of affected individuals and in monozygotic twins incidence up to 50% (Gottesman 1991). Furthermore, adoption studies demonstrated that the risk of schizophrenia is related to the presence of the disorder in biological parents and not in the adoptive parents (Gottesman 1982).

Finally, family studies have reported the incidence of schizophrenia is 2% in third degree relatives (e.g., first cousins) of an individual with schizophrenia, 2%–6% for second degree relatives (e.g., nieces/nephews), and 6%–17% in first degree relatives (e.g., parents, siblings or children) (Gottesman 1991).These family studies have shown that simple major genes affected is unlikely instead polygenic models more plausible that is multiple genes are affected leading to this illness. Hence like diabetes, cancer and heart disease schizophrenia is a complex genetic disorder, it is not caused by defect of a single gene and neither does it have simple patterns of inheritance. In fact there is multiple interacting risk alleles, each accounting for only a small increment in risk (PJ Harrison 2005). What we know is

heritability is high in families with affected family members and genetic factors contribute 80% liability for schizophrenia. At the moment there is no diagnostic biomarker for schizophrenia and further studies need to be carried out. Biomarkers' in schizophrenia can be a tremendous help to chart the phenotypic variation in the course, outcome and response to treatment (Keshavan, Tandon et al. 2008).

1.4.7. NEUREGULIN 1 (NRG 1) AND SCHIZOPHRENIA

The neuregulins consists of 4 genes and neuregulin 1(NRG1) is the most well characterized member of the family, it is important in many organs including heart, breast and nervous system (Harrison and Law 2006). The human NRG1 gene is located on chromosome 8p13, past few years new discoveries has been made , until recently there were only 3 types of NRG 1 that is known (types I-III) however recent transcripts containing additional 5' exons were found in the human brain , the novel types of NRG 1 proteins are called types IV–VI(Steinthorsdottir, Stefansson et al. 2004). NRG 1 is a pleiotropic growth factor that is important in CNS development and function (Li, Collier et al. 2006). NRG 1 has a multitude of function , it is involved in the modulation of neuronal migration, synaptogenesis, gliogenesis, neuron –glia communication, myelination and neurotransmission in the brain and other tissues(Stefansson, Steinthorsdottir et al. 2004). How is it involved in schizophrenia? Based on the glutamate hypothesis of schizophrenia whereby psychotic symptoms can be produced by decreased glutamatergic function at NMDA receptors or increased glutamatergic function at AMPA and kainate receptors (Tsapakis and Travis 2002). NRG 1 is reported to play a role in regulation of NMDA receptors expression and glutamate signaling pathways (Ozaki, Sasner et al. 1997) and it

also modulates neurotransmitter release from GABAergic interneurons. Dysfunction of NRG1 in schizophrenia, could explain in part, the apparent deficiency in glutamate-receptor expression and binding described within in some parts of brains of schizophrenia patients(Huang, Won et al. 2000). In a landmark study in 2002 , Stefansson et al reported an association between NRG 1 and schizophrenia following extensive fine-mapping of the 8p locus and haplotype-association analysis, supplemented by a transmission/disequilibrium test, identifies neuregulin 1 (NRG1) as a candidate gene for schizophrenia. Researchers also found 16% fewer functional NMDA receptors in the NRG 1 mutant mice which is in keeping with reports suggesting a role for NRG1 in regulation of NMDA subunit expression(Ozaki, Sasner et al. 1997).

The chromosome 8p has been highlighted as a susceptibility locus for schizophrenia based from genome wide association studies and meta analysis linkage scans (Stefansson, Sigurdsson et al. 2002). Studies have been conducted in both Caucasian and Asian population to see the association between NRG 1 and schizophrenia. It is found that these studies support NRG 1 as a schizophrenia susceptibility gene despite having differing results. The Icelandic study identified a ‘‘core at-risk haplotype’’ consisting of five SNPs (SNP8NRG221132, SNP8NRG221533, SNP8NRG241930, SNP8NRG243177, and SNP8NRG433E1006) and two microsatellites D8S181029 and H12-121L21(deCODE haplotype). Whereas family studies done in Han Chinese population, identified different SNP’s at risk SNP8NRG221533, SNP8NRG221132 and D8S1770. In a later study in 2006, it found up-regulation of type I expression in the hippocampus in schizophrenia, (ii) association of type I expression with a single SNP residing in the original deCODE risk

haplotype, and (iii) association of type IV expression with a single SNP and a four-marker haplotype representing the 5_upstream region of the original at-risk haplotype associated with schizophrenia. The evidence of altered NRG 1 isoform expression in the brain and disease linked variation in NRG 1 , suggest that alteration of transcript regulation is a potential molecular mechanism behind the genetic association of NRG1 with schizophrenia (Amanda J. Law and Ryota Hashimoto 2006).

1.4.8. NEUREGULIN 1 (NRG 1) POLYMORPHISM AND ASSOCIATION WITH ANTIPSYCHOTICS

Genome wide scans supported the association between schizophrenia and NRG 1, significant evidence for linkage has been reported in three chromosomal regions. These three regions are located on chromosome 6p24-22, chromosome 13q32 and of course chromosome 8p21-22. It is chromosome (8p21-p12; Neuregulin 1) that presents evidence for NRG1 as a Schizophrenia candidate gene (Lewis, Levinson et al. 2003). The increased risk for schizophrenia has been postulated due to abnormal signaling of glutamatergic and dopaminergic pathways in the brain. One of the identified functions of NRG 1 is to help upregulate N-methyl-D-aspartate glutamate (NMDA) receptors. It is also likely responsible for regulating synaptic connectivity and plasticity (Ozaki M and . 2000). Even though there are discrepancies or inconsistencies, bulk of data have shown that either an increase of NRG 1 isoform (protein or mRNA) expression or increased ErbB4 receptors (protein or mRNA) expression. This may highly indicate that, NRG1–ErbB4 signaling and expression to be increased in schizophrenic subjects, although some studies have shown decreased expression of NRG1/ErbB4. Thus, the enhanced NRG1 signaling may contribute to N-

methyl-D-aspartate (NMDA) receptor hypofunction in schizophrenia (Hahn, Wang et al. 2006). Finnish study use one SNP (SNP8NRG221533) as a genetic marker to compare the allele frequencies of Neuregulin 1 in patients with schizophrenia and control subjects. The schizophrenic group was divided into responders and non responders, the responders were treated with conventional antipsychotics whereas those non responders were given clozapine. The study found NRG1 genotype or allele frequencies showed similar distributions between patient and control groups. However, TT genotype was overrepresented in the non-responders group compared with the responders. (Olli Kampman and Esa Leinonen 2004). In another study conducted in Han Chinese, NRG 1 mRNA was compared between two groups. One group was treated with risperidone and the other with quetiapine, the NRG 1 expression after 4 weeks on treatment showed no significant difference between the two. Interestingly, the antipsychotics did have an effect on NRG 1 mRNA expression. After 4 weeks on treatment , the NRG 1 mRNA increased which suggest antipsychotics regulates positively the expression of NRG 1 (Hong-Xing Zhang and Xuan Ouyanga 2008).

Further studies lend support to this theory , haloperidol, clozapine and risperidone have all been found to increase NRG1 and ErbB4 expressions but not in the prefrontal cortex of animal tissue after 4 weeks on treatment (Wang X-D 2008). In a cross sectional study using peripheral blood, the NRG 1 expression was evaluated using immortalized lymphocytes. Twelve schizophrenic and twelve controls lymphocytes were grown individually with or without the presence of the antipsychotic olanzapine. Findings showed that before and after

olanzepine stimulation there were no alteration of NRG 1 RNA expressions in immortalized lymphocytes and in either of the isofroms studied (Chagnon, Roy et al. 2008).
There are still inconsistencies among studies in regards to antipsychotic effect on NRG1 expression, in general short term treatment with antipsychotics (up to 4 weeks) increases or upregulates the expression (mRNA or protein) of NRG1 isoforms and ErbB4 receptors. Whereas, long term treatment or continuous treatment with antipsychotics (at least 12 weeks) decreases or downregulate their expression (at least at protein level). These effects may be due to multiple binding profiles with various G-coupled protein receptors (e.g. dopamine, and serotonin receptors) of antipsychotics. Unfortunately, why does this phenomenon occur is still unclear.

There are several reasons why this may occur; NRG1 and ErbB4 expression and signaling are affected differently by the multiple and varied antipsychotics and is also dependent on treatment duration. Apart from that, the studies conducted used different various types of tissue such human tissue (for example postmortem studies using brain tissue from different regions (such as Prefrontal Cortex) or human blood (PBL's), some studies used animal tissue (for example rats PFC) . As such this can affect the outcome of the studies due to the differing mediums used. Studies are needed to investigate the interactions between NRG1–ErbB4 and the other signaling pathways (such as glutamatergic, GABAergic and dopaminergic). Furthermore, the interactions between NRG1/ErbB4 and other schizophrenia susceptibility genes under antipsychotic treatment also require investigation(Bo Pan 2011).

1.5. ANTIPSYCHOTIC PHARMACOLOGY

The treatment of schizophrenia has come a long way starting with the incidental discovery of chlorpromazine (CPZ) by Laborit which open up the field of psychopharmacology (Tost, Alam et al. 2010).The accidental discovery of antipsychotic spurred interest in the neurobiological basis of psychosis , among which give rise to the dopamine hypothesis . This discovery leads to further development of antipsychotics, from conventional antipsychotics to atypical group of antipsychotics. Atypical antipsychotics are considered to be standard treatment of care and this is evident in most countries guidelines. This group of antipsychotics have demonstrated efficacy for a broader spectrum of symptoms than conventional antipsychotics, and have a lower propensity to cause side effects such as movement disorders (Jones 2010).

1.5.1.PALIPERIDONE

Description

Paliperidone is an orally administered antipsychotic and the principal active metabolite of risperidone (9-hyroxyrisperidone). In Malaysia it has been approved for the acute treatment of Schizophrenia as well as for recurrence prevention. In UMMC the available form is paliperidone ER or also known as Invega and it is this form used for this study. It is available as prolonged-release capsules (orange-brown: 1.5 mg; white: 3 mg; beige: 6 mg; pink: 9 mg; yellow: 12 mg), prolong release means that drug release gradually occurs. paliperidone ER is formulated using OROS®(osmotic controlled-release system, ALZA corporation,CA, USA) technology, which reduces peak to-trough variations in plasma concentrations and eliminates the need for initial dose titration (Eerdekens M 2006).

Paliperidone resembles a capsule shaped tablet in appearance, comprises of an osmotically active trilayer core surrounded by a subcoat and semipermeable membrane. The trilayer core is composed of two drug layers containing the drug and excipients and a push layer containing osmotically active component. Each strength is identified by a unique colour overcoat and print markings. Paliperidone also contain the following inactive ingredients carnauba wax, cellulose acetate, hydroxyethyl cellulose, propylene glycol , povidone, sodium chloride, stearic acid , butylated hydroxytoluene, hypromellose, titanium dioxide and iron oxides. The 3mg tablets also contain lactose monohydrate and glycerol triacetate.

Clinical pharmacology

Pharmacodynamics

Paliperidone is a centrally active dopamine D2 antagonist with predominant serotonergic 5-HT2A antagonistic activity paliperidone also blocks alpha1-adrenergic receptors and blocks, to a lesser extent, H1-histaminergic and alfa2-adrenergic receptors. Paliperidone is not bound to cholinergic receptors beta 1 and beta 2 adrenergic receptors. Even though paliperidone is a strong D2-antagonist, which is believed to relieve the positive symptoms of schizophrenia, it causes less catalepsy and decreases motor functions to a lesser extent than traditional neuroleptics. Dominating central serotonin antagonism may reduce the tendency of paliperidone to cause extrapyramidal side effects. Positron emission tomography (PET) studies, suggest that paliperidone ER dosages between 6 and 9 mg/day result in a D2 receptor occupancy of 70–80% (a range which is associated with optimal efficacy. Paliperidone ER dosages above 19.6 ng/mL were associated with >80% D2 receptor occupancy and are therefore more likely to be associated with extrapyramidal adverse events (Keating 2010).

Pharmacokinetics

Absorption

Following a single dose paliperidone exhibits a gradual ascending release rate, allowing the plasma concentrations of paliperidone to steadily rise to reach peak plasma concentration (Cmax) approximately 24 hours after dosing. With once-daily dosing of INVEGA, steady-state concentrations of paliperidone are attained within 4-5 days of dosing in most subjects. The absolute oral bioavailability of paliperidone following INVEGA administration is 28% (90% CI of 23%-33%). Administration of paliperidone prolonged-release tablets with a standard high-fat/high-caloric meal increases Cmax and AUC of paliperidone by up to 50-60% compared with administration in the fasting state.

Distribution

Paliperidone is rapidly distributed the apparent volume of distribution is 487 L. the plasma binding of Paliperidone is 74%.It binds primarily to alpha 1 acid glycoprotein and albumin.

Metabolism & Elimination

Paliperidone ER is only minimally metabolized in the liver and is primarily eliminated via renal clearance. Thus it has a lower potential for clinically significant pharmacokinetic drug interactions with drugs that are metabolized by the cytochrome P450 (CYP450) enzyme system. One week following administration of a single oral dose of 1 mg immediate-release ^{14}C-paliperidone, 59% of the dose was excreted unchanged into urine, indicating that paliperidone is not extensively metabolised by the liver. Approximately 80% of the administered radioactivity was recovered in urine and 11% in the feaces. Although in vitro studies suggested a role for CYP2D6 and CYP3A4 in the metabolism of paliperidone, there

is no evidence in vivo that these isozymes play a significant role in the metabolism of paliperidone. Population pharmacokinetics analyses indicated no discernable difference on the apparent clearance of paliperidone after administration of paliperidone between extensive metabolisers and poor metabolisers of CYP2D6 substrates. In vitro studies in human liver microsomes showed that paliperidone does not substantially inhibit the metabolism of medicines metabolised by cytochrome P450 isozymes, including CYP1A2, CYP2A6, CYP2C8/9/10, CYP2D6, CYP2E1, CYP3A4, and CYP3A5. Four metabolic pathways were identified as being involved in the elimination of 9-OHR, each of which accounted for up to a maximum of 6.5% of the biotransformation of the total dose. Biotransformation of the drug occurred through oxidative N-dealkylation, monohydroxylation of the alicyclic ring, probably by CYP2D6, alcohol dehydrogenation, and benzisoxazole scission, the latter incombination with glucuronidation or alicyclic hydroxylation. Once ingested, paliperidone undergoes directly phase 2 metabolism (conjugation reactions) or is excreted unchanged in the urine, which makes it the antipsychotic with the lowest potential of inducing pharmacokinetic drug-drug interactions (Meyer 2007).Paliperidone had a terminal elimination half life (t½b) of approximately 23 hours in patients with normal renal function (http://www.ema.europa.eu).

1.5.2 Drug-drug interaction

Limited data are available regarding drug interactions with paliperidone ER. However, paliperidone is not a substrate of CYP1A2, CYP2A6, CYP2C9 and CYP2C19; thus, clinically important interactions between paliperidone and other drugs metabolized by CYP isoenzymes are not expected. Interaction between paliperidone and organic transport inhibitors is not expected.(Agency.). There is data that shows co administration of paliperidone with carbamazepine will lower the plasma concentration of 9-hydroxyrisperidone probably by inducing CYP3A4 mediate metabolism. In contrast co administration with sodium valproate does not cause any change(Spina E 2000).

1.5.3 Clinical Efficacy Trials in Schizophrenia

The efficacy of antipsychotics has long been debated whether it the typical or atypical antipsychotics works better. Pragmatic trials such as Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) (Lieberman JA and Keefe RSE 2005) and Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS 1) (Jones PB and Markwick A 2006) demonstrated high discontinuation rates across all antipsychotics . In CATIE the lowest discontinuation rate was for olanzepine phase 1 and clozapine in phase 2. The outcome in CUtLASS whereby in phase 1 patients on FGA and SGA showed no significant differences in life measures or schizophrenia symptoms and in phase 2 , clozapine was found to be more effective than any SGA in reducing symptoms but not quality of life (Constantine 2007). The reason for discontinuation cited is lack of efficacy , switching, side effects or patients choice (Lewis 2008). This reflects the need for an antipsychotic that is not only tolerable but also efficacious. The efficacy of paliperidone in

the treatment of Schizophrenia was evaluated in 1,690 adult subjects with schizophrenia who participated in 3 double blind 6-week, multicenter, randomized, placebo controlled study of whom 1,394 received paliperidone ER at fixed doses, the outcome was consistent in each trial. In a clinical trial whereby 444 adult subjects with schizophrenia who participated in a placebo controlled 6 week trials , of which 432 subjects received paliperidone ER at fixed doses ranging from 6mg to 12mg once daily. Those on paliperidone ER showed significant improvement in mean total PANSS score compared to placebo. The 3mg paliperidone ER group showed improvement in mean PANSS total score from day 4 and day 15 from 12mg group respectively (Marder, Kramer et al. 2007).

Long term efficacy studies has been conducted for paliperidone ER , in a analysis of pooled data from three separate 52-week, international, multicenter, open-label studies with flexible dosing of paliperidone ranging from 3mg to1 5mg showed improvement in PANSS total, PANSS Marder factor, and CGI-S scores were observed for those patients completing 52 weeks of paliperidone ER therapy. Long term treatment with paliperidone ER was generally safe and well tolerated, with no unexpected AEs emerging over the study period, and was associated with a favorable metabolic profile (Emsley, Berwaerts et al. 2008). In another long term study which assessed the efficacy of paliperidone ER in delaying symptom recurrence in adults with Schizophrenia data demonstrated significant efficacy in delaying recurrence of symptoms in stabilized patients. (Kramer, Simpson et al. 2007).

1.5.4. Side effects

Adverse events are defined as *untoward occurrence including undesirable sign & symptoms, disease or accidents or abnormal findings* (leading to dose reduction / discontinuation / intervention). Treatment emergent adverse events is defined as any event not present prior to treatment or worsened in either intensity or frequency while undergoing therapy after baseline evaluation. Adverse events that occur during clinical trials are obtained by investigators and recorded using tools and terminology of their own choosing. Meta analysis by Jones et al, whereby 31 studies evaluated with total of 5313 subjects of which 851 received paliperidone ER. For atypical antipsychotics as a group, the odds of withdrawal due to AEs were similar to placebo (OR 1.02; 95% CI 0.83, 1.25). The OR for withdrawal due to AEs was lower with paliperidone ER (OR 0.88; 95% CI 0.67, 1.15) than risperidone (OR 2.09; 95% CI 0.8, 5.41). In 3 acute efficacy trials with paliperidone ER treatment emergent adverse events was reported in 66-77% of patients in the paliperidone group compared with 66% in the placebo group (Meltzer H 2006).

1.5.5 Incidence of adverse events in placebo controlled clinical trials for schizophrenia

In 3 acute efficacy trials with Paliperidone ER, among the commonly reported adverse events are headache, agitation, anxiety and insomnia at 12%, 8% and 4% respectively. serious adverse events in paliperidone ER group (5-6%) is comparable to placebo group (6%).Incidence of extrapyramidal symptoms (EPS) was EPS was comparable between the paliperidone ER 3mg and 6mg groups (13% and 10% respectively) and placebo 11% (Meltzer H 2006). Dose relatedness for EPS was seen with the 2 higher doses of paliperidone ER 9mg (25%) and 12mg (26%) respectively. Pooled data from 3 placebo

controlled, 6 week fixed dose studies showed comparable weight gain between paliperidone group on 3mg and 6mg (7% and 6%) compared with placebo (5%). For the 2 higher doses 9mg and 12 mg weight incidence is 9% for both dose. In both genders that received paliperidone e ER serum prolactin levels noted to increase after commencing treatment from 3 6 week double blind placebo controlled, fixed dose studies. paliperidone ER causes modest increase in the corrected QT interval (QTc), the incidence of QTc prolongation on paliperidone ER ranged from 3% to 5% compared with 3% on placebo. paliperidone ER should also be avoided in patients with congenital long QT syndrome or a history of cardiac arrhythmias and avoided in combination with drugs that are known to prolong QTc interval (http://www.ema.europa.eu).

1.6 RATIONALE OF STUDY

Studies conducted previously on NRG 1 as a susceptibility gene for Schizophrenia were done in the Western population (Stefansson, Sigurdsson et al. 2002) which mainly consists of Caucasians and studies done in Asian population was carried out in China and the subjects were Chinese of Han descent only (Li, Collier et al. 2006). Hence it will be interesting to see what result will come out of a multiracial population such as ours. Pharmacogenomics is a relatively new area in Malaysia across all fields of medicine including Psychiatry; as such there is no published local data at the time study was conducted. Hence, this *preliminary study* was designed to determine whether there is alteration of NRG 1 gene expression in schizophrenic patients in our local population.

1.6.1 GENERAL OBJECTIVE

To investigate gene expression of Neuregulin 1(NRG 1) in Schizophrenia patients on paliperidone in a Malaysian population.

1.6.2 SPECIFIC OBJECTIVES

1. To investigate the expression of Neuregulin 1 (NRG 1) in Schizophrenic patients on paliperidone in a Malaysian population from peripheral blood.
2. To determine the association between NRG 1 level and symptomatology of schizophrenia.
3. To assess motor related adverse events in study subjects

1.7 RESEARCH HYPOTHESIS

There is altered gene expression of NRG 1 in Malaysian population with Schizophrenia patients which would be influenced by paliperidone treatment.

CHAPTER 2: METHODOLOGY

2.1 STUDY SETTING

The study was conducted at the University Malaya Medical Centre (UMMC). UMMC is located on the border of Kuala Lumpur and Petaling Jaya (PJ) cities. Its catchment area is the population of PJ. The majority of residents are of Chinese descent, middle income group, educated and urbanized. Chinese form 40 percent of PJ residents 37 percent are Malays, 16 percent are Indians and 7 percent are other races. UMMC is the oldest teaching hospital in the country. It was established in 1965 and continues to provide services to Klang Valley residents. The faculty of Medicine helps to provide the manpower needed to run this great establishment. The faculty prides itself for its outstanding undergraduate and postgraduate course that places great emphasis on research. Hopefully the research carried out will benefit the nation.

In UMMC there are several services available to the community and one of those is psychiatry services which includes clinics. The psychiatry clinic is open daily, includes walk in new case clinic, walk in old case clinic and clinic for scheduled appointments. There are also various sub specialty clinics available on allocated days such as adult liaison, psycho-oncology, psychogeriatric, memory, addiction, compliance and child clinic.

2.2. STUDY DESIGN

This is a cross sectional study looking at the NRG 1 gene expression level in schizophrenic patients on paliperidone and comparing it with a control group. The control group NRG 1 gene expression and socio-demographic data will also be assessed.

2.3. SAMPLE SIZE

In Malaysia there is no previous data in regards to data on gene polymorphism of NRG 1, in Asian population such studies has been carried out in Japan, China and Korea. Hence we determined our same size based on previous studies conducted in Asian population. We thought the gene expression findings of Asian population would be closer to our population than the Caucasian population. This study is largely based on a study conducted by group of researches (Hong-Xing Zhang and Xuan Ouyanga 2008) that recruited 31 first onset schizophrenic patients (15 male and 16 female) ,Chinese of Han descent. In this particular study they looked at the expression of neuregulin-1 gene in peripheral blood and results showed that NRG-1 mRNA expression in PBLs of schizophrenic were lower than the control group. However after treatment with antipsychotic the level of NRG-1 gradually increased. In a study done in a Caucasian population setting in Australia (Nikola A. Bowden and Ulrich Schall 2006), the study of gene expression profiles in peripheral blood lymphocytes in schizophrenia only 14 schizophrenic patients were recruited and 14 non psychiatric control subjects . Hence, the decision was made to recruit at least 15 schizophrenic subjects and 15 healthy controls. The subjects were recruited via the psychiatry services in UMMC based on convenience sampling. In the end a total of 20 schizophrenic subjects were recruited for the study with 15 healthy controls.

2.3.1 INCLUSION CRITERIA

1. Ages between 18 to 65 years old
2. Able to give written informed consent
3. Fulfils DSM-IV TR diagnosis of schizophrenia and confirmed by MINI
4. Schizophrenic patients on paliperidone treatment for at least 4 weeks.

2.3.2. EXCLUSION CRITERIA

1. DSM-IV Axis I diagnosis other than schizophrenia
2. DSM-IV diagnosis of substance dependence within 6 months prior to screening (nicotine and caffeine dependence are not exclusionary)
3. significant risk of suicidal or violent behaviour

2.3.3. Control sample

Control group would be healthy volunteers with NO history of mental illness or family history of mental illness. The control group participants were attending UMMC. The control group is of multiracial origin who matched for age and gender for the schizophrenic patients as much as possible. The need for control without psychiatric illness is necessary to compare NRG 1 expression in this group to the schizophrenics.

2.4 DATA COLLECTION

The study subjects were recruited from individuals who came into contact with psychiatric services at University Malaya Medical Center (UMMC) from February 2011 until November 2011. Those that fulfilled the inclusion and exclusion criteria were enrolled into the study .The diagnosis was confirmed using Mini International Neuropsychiatric Interview (M.I.N.I)., based on the diagnostic criteria for schizophrenia in Diagnostic and Statistical Manual of Mental Disorders ,Fourth Edition , Text Revision (DSM IV TR). The patient as well as control was given a Patient Information Sheet and explained regarding the study. Once the person agreed to participate, he/she was asked to sign an Informed Consent Form. The demographic data will be collected by clinical interview and from the patient's medical records, following a pro-forma.

The symptoms were assessed using Positive and Negative Syndrome Scale (PANSS) (Kay, Fiszbein et al. 1987). Adverse effects enquired from patients and reported by patients were documented. The compliance to treatment was determined by pill counting and verification from family. Venous blood was taken for each patient during their visits for gene analysis. Clinical side effects assessed using Barnes Akathisia Rating Scale (BARS) .

2.5. STUDY MEASUREMENT

2.5.1 IDENTIFICATION DATA

Information collected includes socio-demographic data (age, gender, ethnicity, education level, marital status and employment status) and clinical characteristics.

2.5.2 CLINICAL DATA

Among the clinical data collected were duration on treatment, the current dose of paliperidone and reported side effects by patients.

2.5.3. MINI INTERNATIONAL NEUROPSYCHIATRIC INTERVIEW (M.I.N.I)

The MINI INTERNATIONAL NEUROPSYCHIATRIC INTERVIEW (M.I.N.I) is a short and structured diagnostic interview, developed jointly by psychiatrists and clinicians in the United States and Europe , compatible with DSM-IV and ICD-10 psychiatric disorders. It inexpensive and easy to administer. Administering the MINI should not take more than 15 minutes, as such it is designed to meet the need for short but accurate structured psychiatric interview that can be used internationally for clinical trials and epidemiology studies. The M.I.N.I has been compared to the Composite International Diagnostic Interview (CIDI) and the Structured Clinical Interview for DSM-IH-R patients (SCID). In respect to comparison with SCID – P were characterized by good or very good Kappa values (0.50-0.90), with only a single value for current drug dependence 0.43. In comparison to CIDI, Kappa values were also good or very good diagnoses with the exception of generalized anxiety disorder (GAD) (kappa = 0.36), agoraphobia (sensitivity = 0.59) and bulimia (kappa = 0.53). Inter-rater and test-retest reliability were good, 0.79 to 1.00 as reported by the authors.

2.5.4. Positive and Negative Syndrome Scale (PANSS)

The PANSS or the Positive and Negative Syndrome Scale is a medical scale developed in order to provide a well-defined instrument to specifically assess both positive and negative symptoms of schizophrenia as well as general psychopathology. It was developed in 1987 by Stanley R. Kay, Abraham Fiszbein, and Lewis A Opler and widely used for research purposes. It combines 2 scales, 18 items of the Brief Psychiatric Rating Scale develop by Overall and Gorham, 1962) and 12 items of the Psychopathology Rating Schedule by Singh and Kay, 1975 and all items were given a complete definition as well as detailed anchoring criteria for all rating points. The scale consists of 30-item as an instrument for measuring the prevalence of positive and negative syndromes in schizophrenia. The patient is rated from 1-7 on 30 different symptoms based on interview with the patient as well as reports of family members or health workers. The scoring can be divided into 3 categories based on symptomatology : high if scores equal or more than > 95 , medium if in between > 75 and < 95 , and low if score less than < 75 (Chris M Kozma 2010).

2.5.5. Barnes Akathisia Rating scale (BARS)

Akathisia is one of the side effects of antipsychotic that is relatively common and distressing to a patient. The Barnes Akathisia Scale (commonly known as BARS) is a rating scale that is administered by physicians to assess the severity of drug-induced akathisia and is derived to incorporate diagnostic criteria for pseudoakathisia. The severity of mild, moderate, and severe is based on objective and subjective assessment .It comprises items for rating the observable, restless movements which characterise the condition, the

subjective awareness of restlessness, and any distress associated with the akathisia. In addition, there is an item for rating global severity. The scoring for BARS as follows: Objective Akathisia, Subjective Awareness of Restlessness and Subjective Distress Related to Restlessness are rated on a 4-point scale from 0 – 3 and are summed yielding a total score ranging from 0 to 9. The Global Clinical Assessment of Akathisia uses a 5-point scale ranging from 0 – 4. The inter-rater reliability for the scale items (Cohen's x) ranged from 0.738 to 0.955 (TR 1989).

2.6. GENE EXPRESSION

2.6.1. DNA extraction

This was done using the Spin Protocol which is used to purify DNA from blood or body fluids. After recruiting a patient or control, blood was drawn and collected in ehtylenediamine tetraacetic acid (EDTA) tubes. After that EDTA blood tube was kept at a temperature of about – 80^0C . Next, 20 µl QIAGEN Protease (or proteinase K) was pipette into the bottom of a 1.5 ml microcentrifuge tube and 200 µl of the sample was added to the microcentrifuge tube. 20 µl Buffer AL was added to the sample which was mixed by pulse-vortexing for 15 seconds. The sample then was incubated at 56^0C for 10 minutes. The 1.5ml microcentrifuge tube was briefly centrifuged to remove drops from inside of the lid. 200 µl of ethanol (96-100%) was added to the sample and was mixed again by pulse-vortexing for 15 seconds. After mixing, the 1.5ml microcentrifuge tube was briefly centrifuged again to remove drops from the inside of the lid. The above mixture was carefully applied to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. The tap was closed and centrifuged at 8000 rpm for 1 minute. The QIAamp Mini spin column was

placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded. The QIAamp Mini spin column was carefully opened and 500 µl Buffer AW1 was added without wetting the rim. The cap was closed and centrifuged at 8000 rpm for 1 minute. The QIAamp Mini spin column was placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded. The QIAamp Mini spin column was carefully opened and 500 µl Buffer AW2 was added without wetting the rim. The cap was closed and centrifuged at 14000 rpm for 3 minutes. The QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube and the tube containing the filtrate was discarded. The QIAamp Mini spin column was carefully opened and 200 µl Buffer AE or distilled water was added. This was incubated at room temperature for 1 minute and then was centrifuged at 8000 rpm for 1 minute

2.6.2. RNA Extraction

Manual Purification of Total RNA from human whole blood collected into PAXgene blood RNA tubes (BRT)

The PAXgene Blood RNA Tube (BRT) was centrifuged for 10 minutes at 3000-5000 x g using a swing-out rotor. The supernatant is removed by decanting or pipetting. 4ml RNase free water (RNFW) was added to the pellet and the tube was closed using a fresh secondary BD Hemogard closure. The mixture was vortexed until the pellet was visibly dissolved and was centrifuged for 10 minutes at 3000-5000 x g using a swing-out rotor. The entire supernatant was removed and discarded. 350 µl resuspension buffer (BR1) was added and vortexed until the pellet was visibly dissolved. The sample was pipette into a 1.5ml miccrocentrifuge (MCT). 300 µl binding buffer (BR2) and 40 µl proteinase K (PK) were

added and mixed by vortexing for 5 seconds and incubated for 10 minutes at 55° using a shaker-incubator at 400-1400 rpm. After incubation, the temperature of the shaker-incubator was set to 65°. The lysate was pipette directly into a PAXgene Shredder spin column placed in a 2ml processing tube and centrifuged for 3 minutes at maximum speed (but not exceeding 20 000 x g). The entire supernatant of the flow- through fraction was carefully transferred to a fresh 1.5 ml microcentrifuge without disturbing the pellet in the processing tube. 350 µl ethanol was added and mixed by vortexing and centrifuged briefly to remove drops from the inside of the tube lid. 700 µl of sample was pipetted into the PAXgene RNA spin column placed in a 2ml processing tube and centrifuged for 1 minute at 8000-20000 x g. The spin column was placed in a new 2 ml processing tube and the old processing tube (PT) containing flow through was discarded. The remaining sample was pipette into the PAXgene RNA Spin column (PRC) and centrifuged for 1 minute at 8000-20000 x g. The spin column was placed in a new 2 ml processing tube and the old PT containing flow-through was discarded. 350 µl wash buffer was pipetted into the PRC and it was centrifuged for 1 minute at 8000-20000 x g. The PRC was placed in a new 2 ml PT and the old PT containing flow-through was discarded. 10 µl DNase (RNFD) stock solution was added to 70 µl digestion buffer (RDD) in a 1.5ml microcentrifuge (MCT). It was mixed by gently flicking the tube and centrifuged briefly to collect residual liquid from the sides of the tube. The RNFD incubation mix was pipetted directly onto the PAXgene PRC membrane and placed on the bench top for 15 minutes. 350 µl wash buffer 1 was pipetted into the PAXgene PRC and centrifuged for 1 minute at 8000-20000 x g. The spin column was placed in a new 2 ml processing tube and the old PT containing flow-through was discarded. Another 500 µl wash buffer 2 was added to the PRC and centrifuged for 3

minutes at 8000-20000 x g. The PT containing flow-through was discarded and the PAXgene PRC was placed in a new 2 ml PT and centrifuged for 1 minute at 8000-20000 x g. The PT containing flow-through was discarded and the PAXgene PRC was placed in a 1.5ml MCT and 40 µl elution buffer was pipette directly onto the PAXgene PRC membrane and was centrifuged for 1 minute at 8000-20000 x g to elute the RNA. The elution step above was repeated using 40 µl elution buffer and the same MCT. The elute was incubated for 5 minutes at 65° in the shaker incubator without shaking. After incubation, chill immediately on ice. If the RNA samples will not be used immediately, they should be stored at – 20° or -70° C.

2.7. STATISTICAL ANALYSIS

This data for this study was analyzed using Statistical Package for Social Sciences (SPSS) Version 17.0. Descriptive statistics were used to summarize the data.. To test for statistical significance, Mann Whitney U Test for categorical variable and spearman rho correlation. Exploratory data were carried out to check whether the data was normally distributed. Non-parametric test were for non-normality distributed data. All test were two-tailed with results considered significant at $p<0.05$.

2.8. ETHICAL CONSIDERATION

Ethical approval was obtained from the Medical Ethics Committee, UMMC. The confidentiality of the participants was assured and the purpose of the study was explained to the participants. Written informed consent was obtained from all participants.

CHAPTER 3: RESULTS

3.1 SAMPLE DESCRIPTION

A total of 50 unrelated patients were approached but only 20 consented to join this study along with 15 healthy controls. 15 refused to participate, 10 were switched to other antipsychotic and 5 patients their diagnosis was changed to bipolar, schizoaffective disorder.

3.2. SOCIO-DEMOGRAPHIC CHARACTERISTICS OF SCHIZOPHRENIA SUBJECTS

3.2.1.AGE

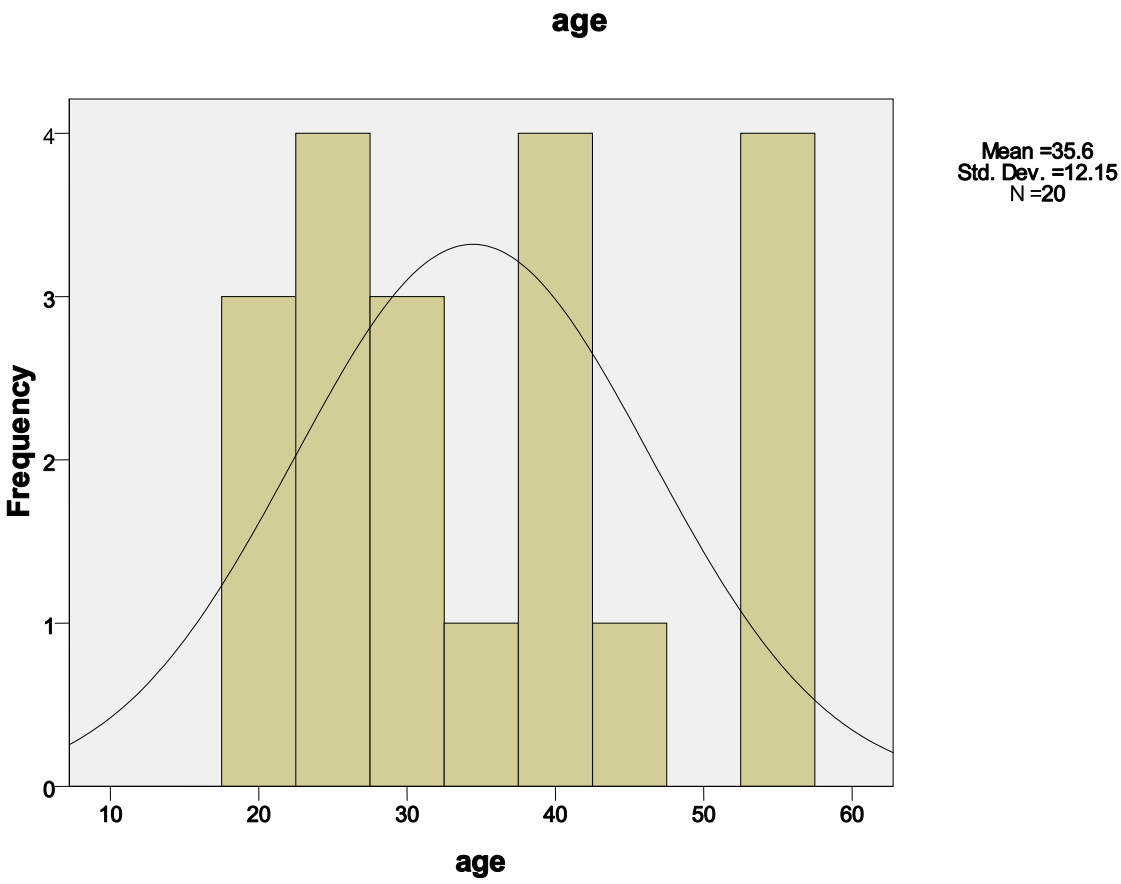

Figure 1 Age Distribution of Schizophrenia Patients

Tests of Normality

	Kolmogorov-Smirnov[a]		
	Statistic	df	Sig.
Age	.178	20	.098

a. Lilliefors Significance Correction

Table 1: Test of Normality for Age Distribution

Figure 1 shows the distribution of age among 20 samples. The average age was 35.60 years (range = 18 - 65 years). The figure shows a normally distributed population and based on Kolmogorov-Smirnov test on Table 1, the p value was found to be more than 0.05.

3.2.2 GENDER

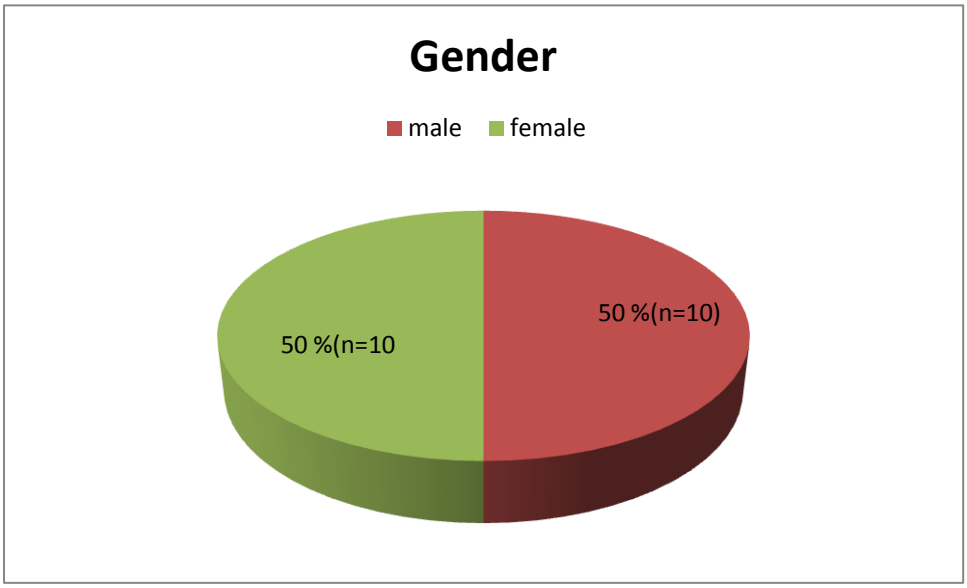

Figure 2 Gender Distribution of subjects

The figure above shows the gender distribution for this study, male accounted for 50% (n=10) and female amounted the same 50% (n=10).

3.2.3 ETHNICITY

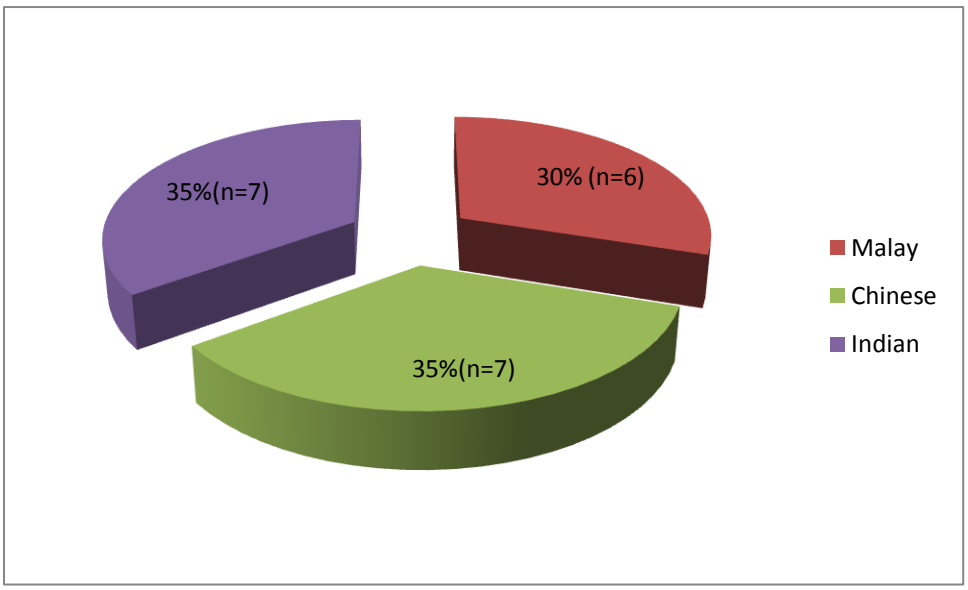

Figure 3 Distribution of Ethnicity among schizophrenia Subjects

There was equal distribution between Chinese and Indian 35% (n=7) respectively and Malays constitute the smallest number, 30% (n=6).

3.2.4 MARITAL STATUS

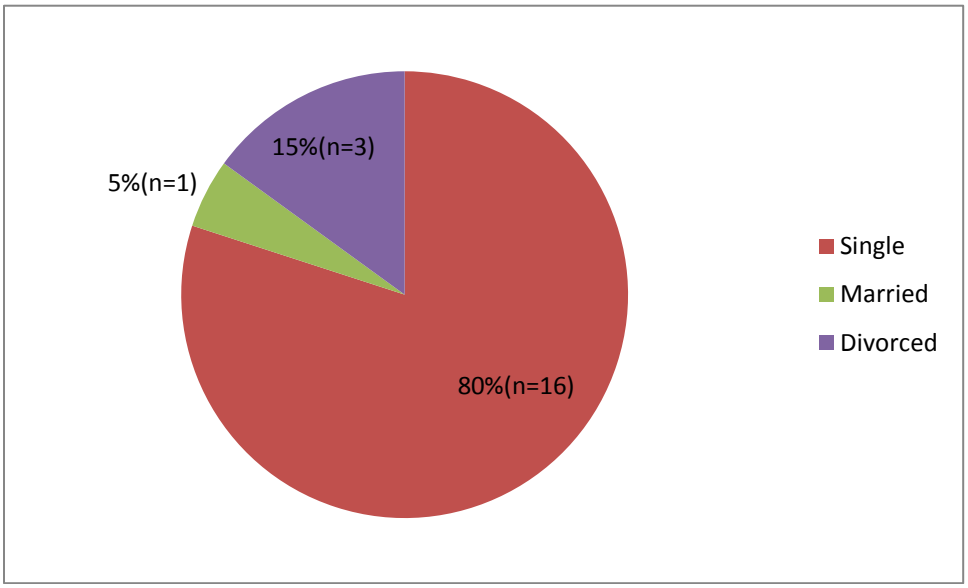

Figure 4 Marital Status of schizophrenia Subjects

Majority of patients were single and accounted at 80% (n=16), followed by divorced 15% (n=3) and only 5% (n=1) is married.

3.2.5 EDUCATIONAL LEVEL

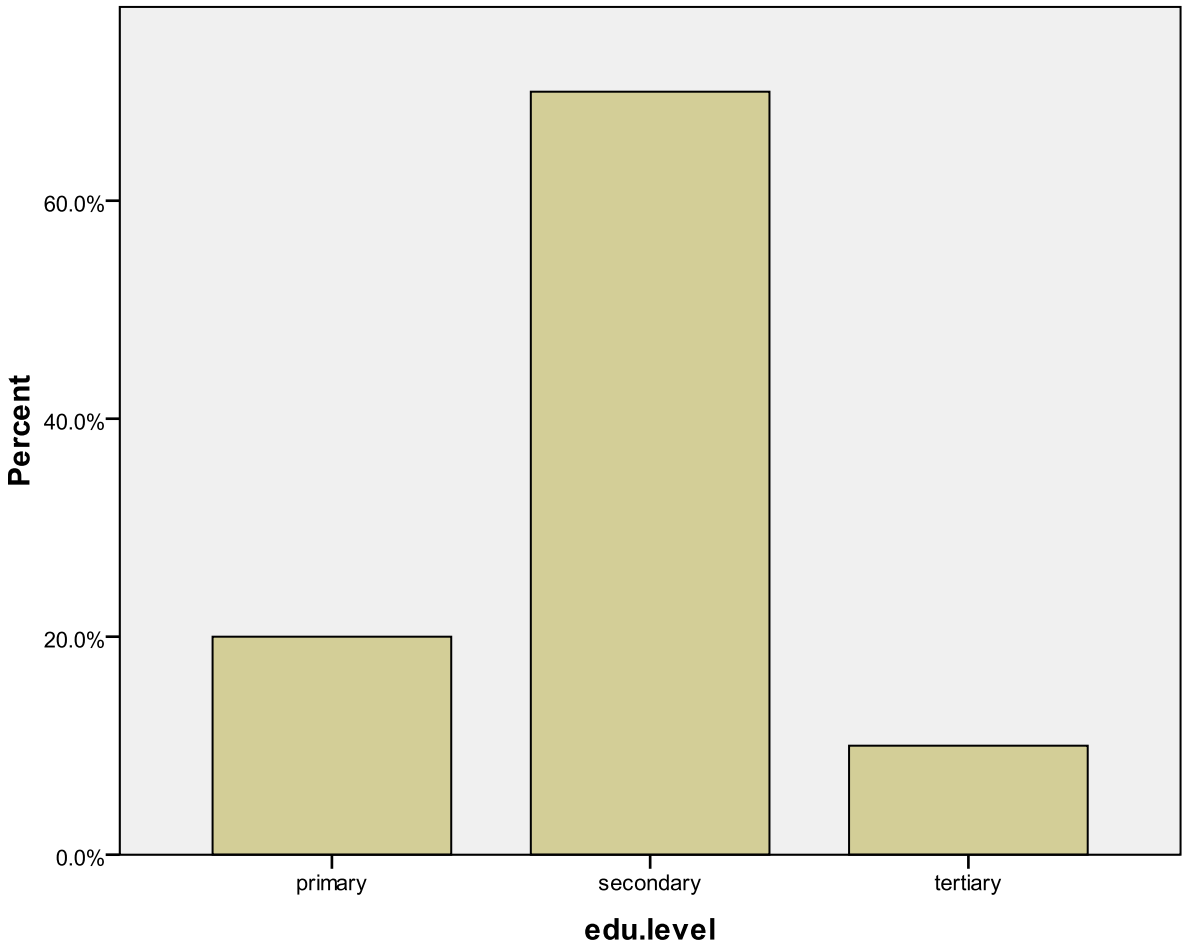

Figure 5 Education Status of schizophrenia subjects

Based on the bar chart 70 %(14) of subjects had secondary school education, 20 %(n=4) only went to primary school and a small percentage 10 %(2) had tertiary education.

3.2.6. EMPLOYMENT STATUS

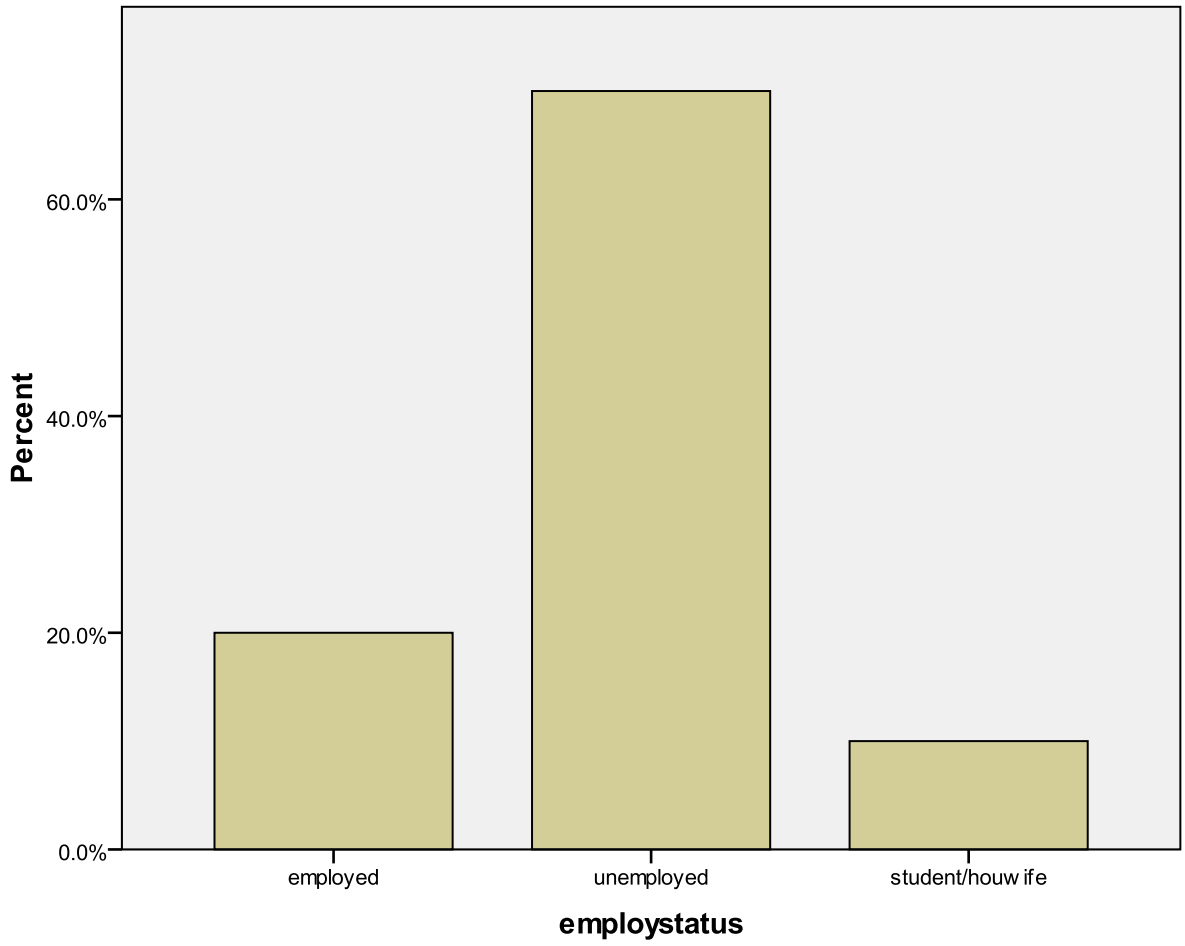

Figure 6 Employment status of schizophrenia subjects

A large number of study subjects are unemployed 70% (n=14), 20% (n=4) are employed and 10 % (n=2) is either student or housewife.

3.2.7 FAMILY HISTORY

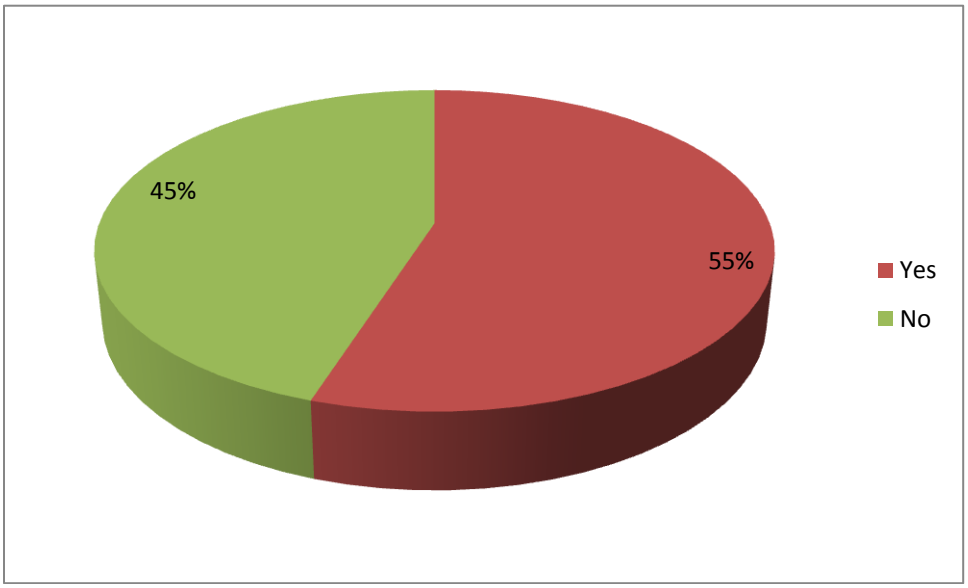

Figure 7: Family History of mental illness among patients

The figure above show that 11 patients (55%) has family history of mental illness whereas 9 (45%) of them do not have any family history of mental illness.

3.2.8 DURATION OF ILLNESS

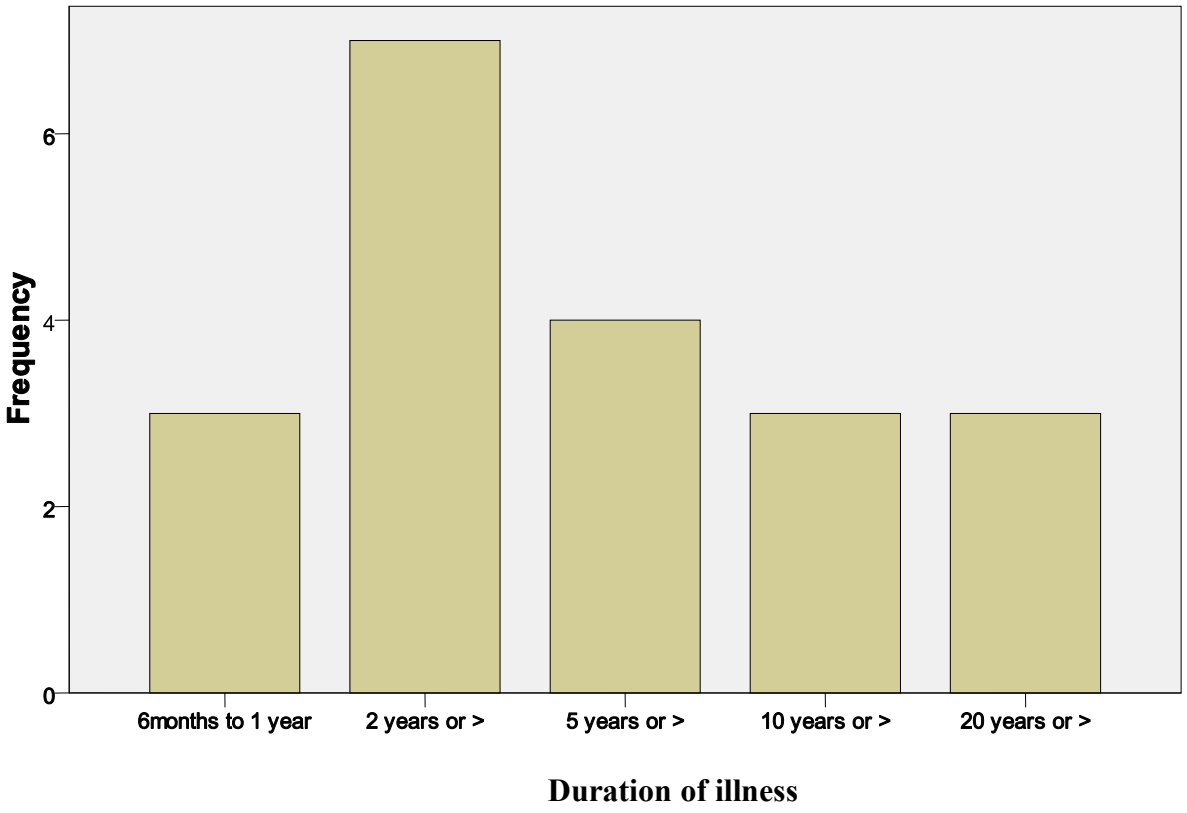

Figure 8: Duration of Illness of Study Subjects

Based on the figure, majority of subjects 35% (n=7) had the illness > 2 years, 20% (n=4) had the illness more than 5 years, 15% (n=3) had the illness for 6months to 1 year. 15% (n=3) had the illness more than 20 years and more than 10 years respectively. The total mean duration of illness is 2.8 years, SD =1.32.

TABLE 2: SUMMARY OF SOCIO-DEMOGRAPHIC CHARACTERISTICS OF SCHIZOPHRENIA SUBJECTS

SOCIO-DEMOGRAHIC CHARACTERISTCS	MEAN	SD	n	%
Age	35.60	12.15		
Gender				
Male			10	50
Female			10	50
Ethnicity				
Malay			6	30
Chinese			7	35
Indian			7	35
Marital status				
Single			16	80
Divorced			1	15
Married			3	5
Educational level				
Primary			4	20
Secondary			14	70
Tertiary			2	10
Employment			4	20
Employed			14	70
Unemployed			2	10
Student /housewife				

TABLE 3: SUMMARY OF SOCIO-DEMOGRAPHIC CHARACTERISTICS OF CONTROL SUBJECTS

SOCIO-DEMOGRAHIC CHARACTERISTCS	MEAN	SD	n	%
Age	41.27	8.92		
Gender				
Male			8	53.3
Female			7	46.7
Ethnicity				
Malay			7	46.7
Chinese			6	40
Indian			2	13.3
Marital status				
Single			7	46.7
Divorced			6	40
Married			2	13.3
Educational level				
Primary			2	13.3
Secondary			11	73.3
Tertiary			2	13.3
Employment				
Employed			5	33.33
Unemployed			4	26.67
Student /housewife			6	40

3.3 DISTRIBUTION OF CLINICAL DESCRPITIVES

3.3.1 POSITIVE AND NEGATIVE SYNDROME SCALES (PANSS)

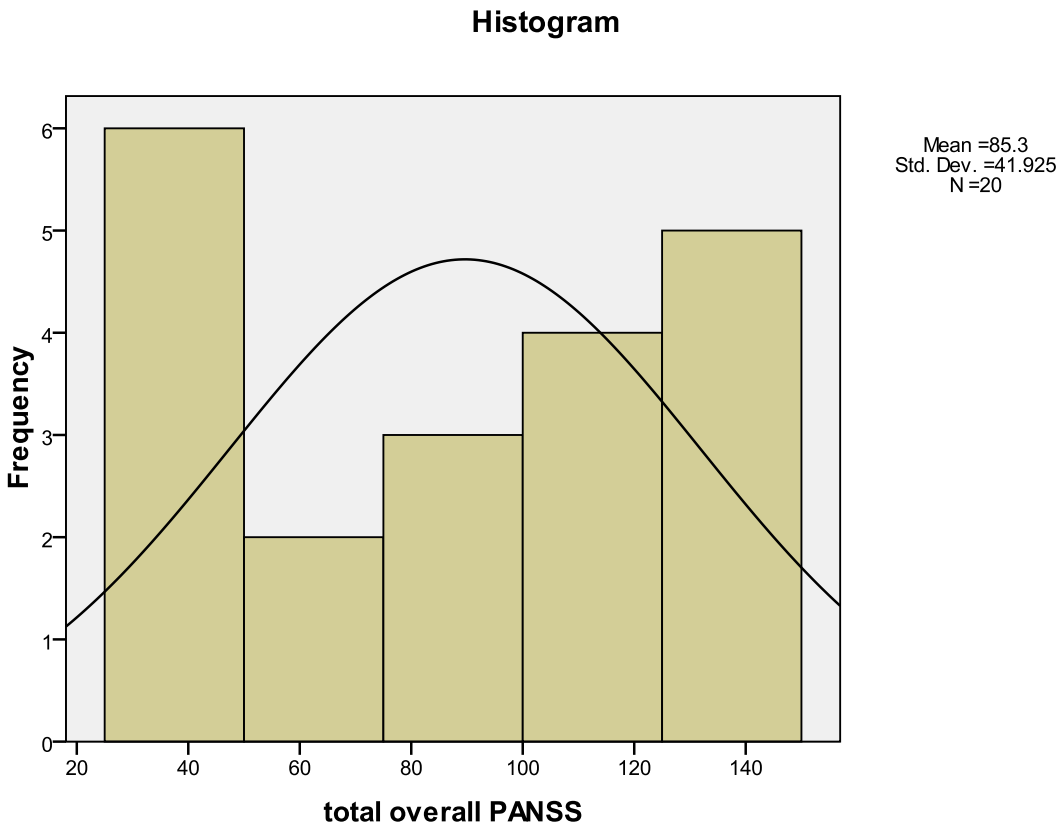

Figure 9: Distribution of total overall PANSS score in study subjects

Based on the figure, the total overall mean PANSS score is 85.30, SD =41.92.

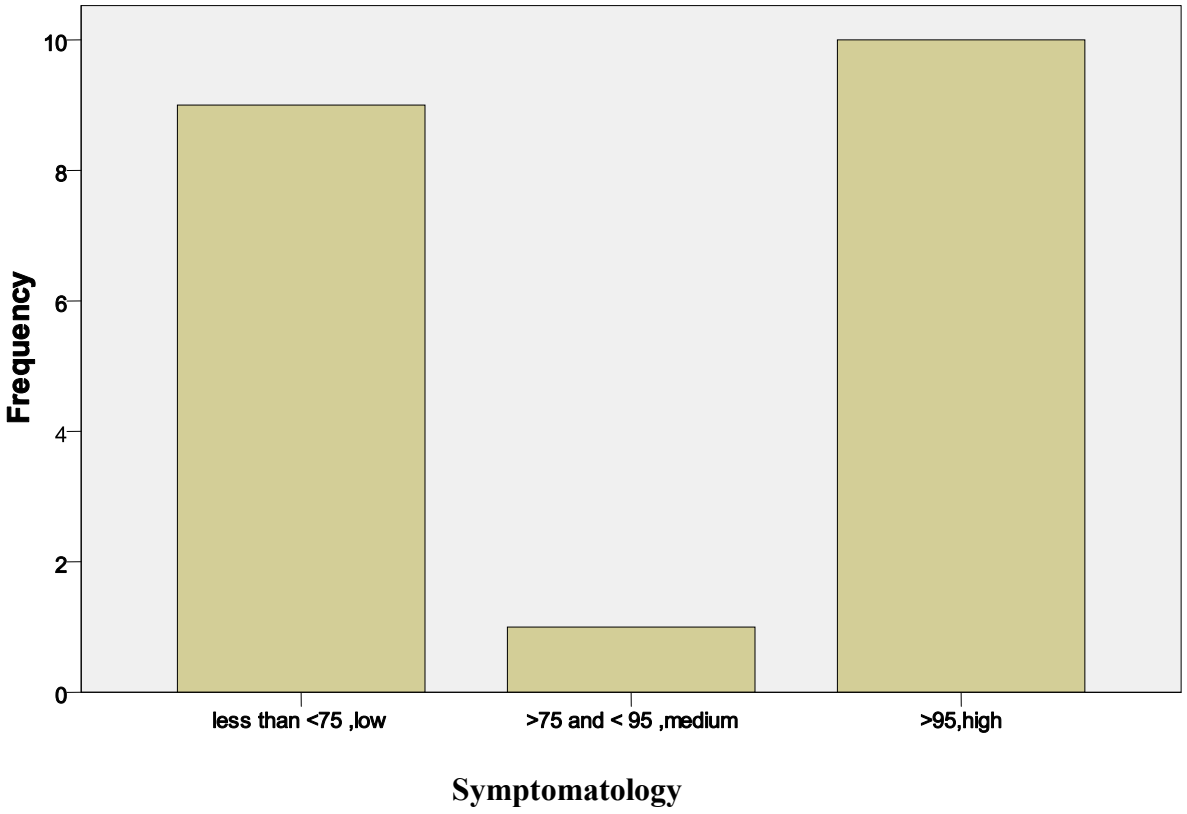

Figure 10: Distribution of PANSS symptomatology

PANSS score has been divided into 3 categories low if score less than <75, medium if in between ≥75 and < 95 and high if scores equal or more than ≥ 95 (Chris M Kozma 2010). From the figure, our study showed that 50% (n=10) had high symptomatology, followed by low symptomatology 45% (n=9) and medium symptomatology 5% (n=1).

3.3.2 BARNES AKATHISIA RATING SCALE

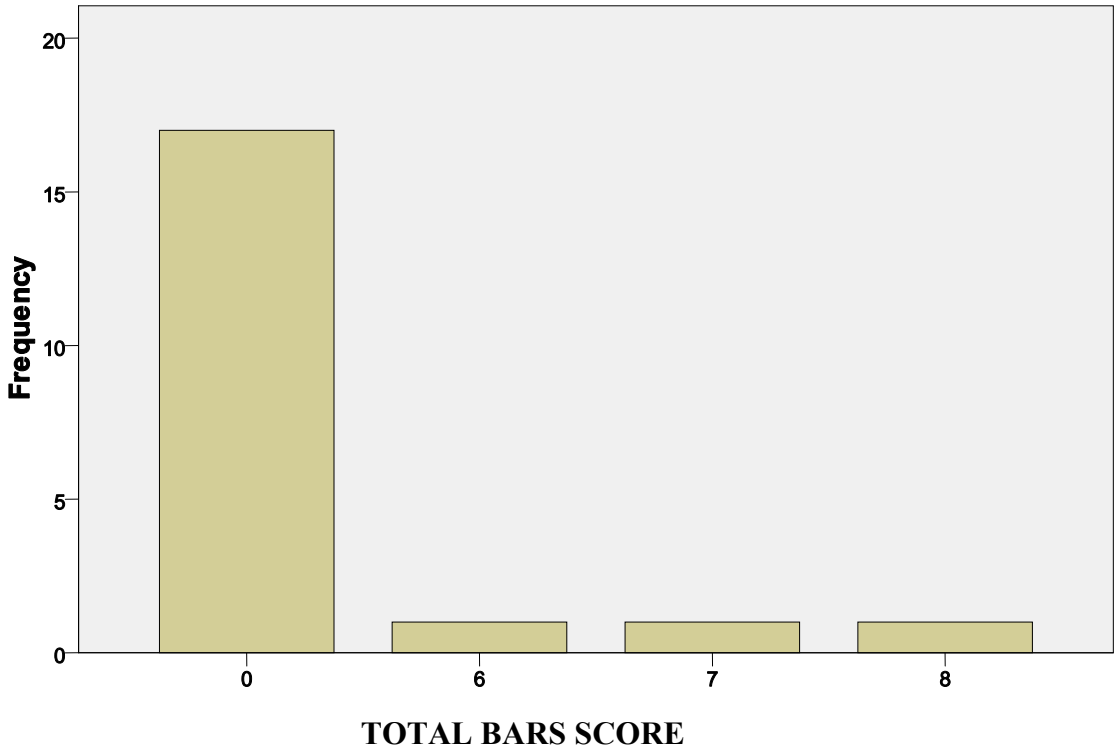

Figure 11: Distribution of BARS score among study subjects

The majority of subjects have the total score of 0 which is 85% (n=17) of subjects, 5% have the total score of 6 (n=1) and 7 (n=1) and 8 (n=1) respectively.

3.3.3. NRG 1 GENE EXPRESSION LEVEL IN SCHIZOPHRENIA

TABLE 4: UNIVARIATE ANALYSIS ASSOCIATION OF DEMOGRAPHIC AND CLINICAL FACTORS WITH NRG 1 GENE EXPRESSION LEVEL

Variable	Mean rank	P value	Z
Age			
≤35 years	9.40		
≥35 years	11.60	.406	-.832
Gender			
Male	9.70		
Female	11.30	.545	-.605
Ethnicity			
Malay	8.83		
Non-Malay	11.21	.409	-.825
Education			
Primary	12.25		
Secondary/Tertiary	10.06	.508	-.661
Total PANSS			
≤87	9.40		
≥87	11.60	.406	-.832
Total BARS			
≤5	10.06		
≥5	13.00	.427	-.794
Paliperidone dosage			

≤ 6mg **≥6mg**	8.50 11.36	3.22	-.990
Duration on Paliperidone **≤ 6 months** **≥6 months**	10.50 10.50	1.000	.000

*Mann- Whitney Test

Based on Table 4, NRG 1 gene expression level was compared between above variables as to find out any associated factors. There was no significant difference between NRG 1 gene expression level with age, gender, ethnicity, education, duration on paliperidone, paliperidone dosage, total PANSS and total BARS.

TABLE 5: NRG 1 EXPRESSION LEVEL IN SCHIZOPHRENIA PATIENTS AND CONTROLS

Subjects	NRG 1 gene expression level Mean	P value
PANSS		
Low PANSS score subjects	0.304	
Medium PANSS score subjects	0.118	0.45
High PANSS score subjects	0.524	
Schizophrenic subjects	0.405	
Control subjects	0.435	0.268

*Mann- Whitney Test

Based on table 5 , the NRG 1 gene expression level in those with low PANSS score is low, mean =0.304 whereas in the high PANSS score group is higher , mean = 0.524. The NRG 1 level in the high PANSS score group is higher than that of control subject, mean =0.435. There is no significant association between NRG 1 level in subjects and NRG 1 level in controls. There is no difference between NRG 1 levels in Low PANSS score subjects, Medium PANSS score subjects and High PANSS score subjects.

TABLE 6: UNIVARIATE ANALYSIS OFASSOCIATED FACTORS WITH GENERAL PSYCHOPATHOLOGY (PANSS) OF THE STUDY SUBJECTS

Variable	Mean rank	P Value	Z
Age			
≤ 35	10.95	.734	-.340
≥35	10.05		
Gender		.406	-.832
Male	11.60		
Female	9.40		
Ethnicity			
Malay	9.33	.564	-.578
Non-Malay	11.00		
Education level			
Primary	11.50	.705	-.378
Secondary/Tertiary	10.25		
Paliperidone dosage			
≤6mg	10.75	.902	-.124
≥6mg	10.39		
Duration on			
Paliperidone	13.38	.277	-1.087
≤6 month	9.78		
≥6month			

*Mann- Whitney Test

Based on Table 6, total PANSS score was compared between above variables as to find out any associated factors. There was no significant difference between total PANSS score with age, gender, ethnicity, education level, duration on paliperidone and paliperidone dosage.

TABLE 7: UNIVARIATE ANALYSIS OF ASSOCIATED FACTORS WITH POSITIVE SCALE (PANSS) OF THE STUDY SUBJECTS

Variable	Mean rank	P Value	Z
Age			
< 35	10.90	.761	-.304
≥35	10.10		
Gender		.287	-1.065
Male	11.90		
Female	9.10		
Ethnicity			
Malay	8.92	.431	-.788
Non-Malay	11.18		
Education level			
Primary	11.68	.601	-.523
Secondary/Tertiary	10.16		
Paliperidone dosage			
≤6mg	10.50	1.000	.000
≥6mg	10.50		
Duration on Paliperidone			
≤6 month	12.63	.419	-.808
≥6month	9.97		

*Mann- Whitney Test

Based on Table 7, positive symptoms PANSS score was compared between above variables as to find out any associated factors. There was no significant difference between positive symptoms PANSS score with age, gender, ethnicity, education level, duration on paliperidone and paliperidone dosage.

TABLE 8: UNIVARIATE ANALYSIS OF ASSOCIATED FACTORS WITH NEGATIVE SCALE (PANSS) OF STUDY SUBJECTS

Variable	Mean rank	P Value	Z
Age			
≤35	11.05	.677	-.417
≥35	9.95		
Gender			
Male	11.60	.404	-.834
Female	9.40		
Ethnicity			
Malay	9.42	.591	-.538
Non-Malay	10.96		
Education level			
Primary	11.38	.740	-.332
Secondary/Tertiary	10.28		
Paliperidone dosage			
≤6mg	10.42	.967	-.041
≥6mg	10.54		
Duration on Paliperidone			
≤6 month	13.13	.320	-.995
≥6month	9.84		

*Mann- Whitney Test

Based on Table 8, negative symptoms PANSS score was compared between above variables as to find out any associated factors. There was no significant difference between negative symptoms PANSS score with age, gender, ethnicity, education level, duration on paliperidone and paliperidone dosage.

TABLE 9: UNIVARIATE ANALYSIS OF ASSOCIATED FACTORS WITH BARNES AKATHISIA RATING SCALE SCORE OF STUDY SUBJECTS

Variable	Mean rank	P Value	Z
Age			
≤35	10.90	.627	-.486
≥35	10.10		
Gender			
Male	12.00	.068	-1.824
Female	9.00		
Paliperidone dosage			
≤6mg	10.67		
≥6mg	10.43	.894	-.133
Duration on Paliperidone			
≤6 month	9.00	.362	-.912
≥6month	10.88		

*Mann- Whitney Test

Based on Table 8, total BARS score was compared between above variables as to find out any associated factors. There was no significant difference between total BARS score with age, gender, duration on paliperidone and paliperidone dosage.

TABLE 10: SUMMARY OF CLINICAL DESCRIPTIVES

PANSS	MEAN	SD	n	%
Positive symptoms	21.95	11.05		
Negative symptoms	22.15	10.36		
General psychopathology	41.20	20.86		
Total PANSS	85.30	41.93		
BARS				
Objective Assessment	.30	.733		
Subjective				
Awareness of restlessness	0.25	.639		
Distress related to restlessness	.20	.523		
Clinical assessment of akathisia	.30	.733		
Total BARS	1.05	2.59		
Paliperidone dose			6	30

3mg **6mg** **9mg**			10 4	50 20
Duration on treatment **≥ 4 weeks** **≥ 8 weeks** **≥24 weeks**			4 4 12	20 20 60
NRG 1 levels **NRG 1 levels in subjects** **NRG 1 levels in control**	.405 .435	.491 .273		

CHAPTER 4: DISCUSSION

4.1 METHODOLOGY ISSUES

While doing this study several hurdles were encountered. This study started out as a prospective study as this design is most suited for genetic research. Initiating treatment and assessing their baseline symptoms and later on at end of study will give a more accurate picture of efficacy of treatment and response to treatment. The gene analysis would also be more accurate if able to recruit antipsychotic naïve patients as the sample would not have been tainted by previous antipsychotics treatment.

4.2 SAMPLE DESCRIPTION

4.2.1 AGE AND GENDER

There were 10 males patients (50%) and 10 females patients (50%). The mean age for our study subjects was 35.60 and subjects age ranged from 18 to 65 years old. For categorization of age based on gender, the mean age for female subjects were 40.20 years (SD= 12.56) and for male subjects mean age is 31.0 years (SD=10.22). This finding is in keeping with other studies that showed mean age of onset is younger age of males 49.4 years (SD=13.1)) and older in female subjects 50.0 years (SD=12.7).Furthermore , those with family history the age of onset is younger (Jimmy Lee 2011).

4.2.2 MARITAL STATUS

Our study found that majority of the patients were single 80% (n=16) and they were staying with their family or at the nursing home. The relationship between marital status and schizophrenia works both ways. That is those who are single are prone to developing schizophrenia and those diagnosed to have Schizophrenia are more likely to remain ingle (Agerbo, Byrne et al. 2004). This is also found in earlier studies that showed marital status as one of the strongest correlate of mental disorder risk. Individuals who were separated or divorced had twice the risk compared to those married (Regier, Farmer et al. 1993).

4.2.3 EMPLOYMENT STATUS

Schizophrenia impairs an individuals' cognitive functioning, this may hinder a person ability and chance of being employed. In this study 70% (n=14) were unemployed and this findings replicates other study findings. There are barriers to gaining employment in those with mental illness, among the factors that lessens their chances are stigma from employers, negative attitude of employers and community, patients own attitude towards work and limited job openings that suits patients ability (unskilled and undemanding job). In a study comparing the employment rate between 3 countries United Kingdom (UK), France and Germany, the employment rate was lowest in France 11.5%(n=288) compared to UK 12.5% (n=302) and 30.3%(n=618) Germany .However , the difference in employment rate is also attributed to social and service factors that varies between countries (Marwaha, Johnson et al. 2007).

4.2.4 FAMILY HISTORY

From the 20 subjects, 55% of the patients have family history of mental illness and 45% of them did not have any family history. In the field of genetics family history plays an important role as evident in several studies. A study done in Han Chinese schizophrenic family trios, suggested that NRG 1 polymorphism could possibly be a pathogenic mutation for schizophrenia in Chinese as the transmission disequilibrium test analysis for an NRG1 variant (rs3924999) showed a significant difference between the two transmitted alleles (Yang, Si et al. 2003). In contrast, a study done in Han Chinese in Taiwan gave conflicting results. The family-based association study (15 schizophrenic bios and 221 schizophrenic trios) , 38Gln was transmitted in excess by the parent to the affected offspring whereas the case –control association study showed no significant difference in genotype frequency between the schizophrenia and normal control (Hong, Huo et al. 2004).

4.3 DISTRIBUTION OF CLINICAL DESCRIPTIVES OF THE STUDY

4.3.1 Distribution mean dose of paliperidone and duration of treatment

The study showed that the dosage of paliperidone varied between 3mg to 9mg per day and the majority of patients dosage was 6mg ,50% (n=10) and 60% (n=12) of the patients had been on treatment for 6 months or more. Randomized placebo controlled study using fixed doses of oral Paliperidone at 6mg, 9mg and 12 mg respectively showed that all 3 doses of Paliperidone ER had broader spectrum of efficacy than placebo. Its capability of rapidly improving the acute symptoms of Schizophrenia is due to its rapid onset of action Day 4 from the first observation point for those on 12 mg dose and from Day 8 for those on Paliperidone ER 6 mg and 9 mg(J. Kane and P. Lim 2007).

In another 6-week, randomized,placebo-controlled study demonstrate that patients' presenting with acute episode of schizophrenia on Paliperidone ER (3 mg, 9 mg and 15 mg improved significantly than placebo group. The improvement in total PANSS score is statistically significant (M. Davidson and P. Lim 2007).

Schizophrenia is an illness that plagues an individual through his/her life and in this study, 60% (n=12) of the patients had been on treatment for 6 months or more and majority had the illness for more than 2 years 35% (n=7). 15 %(n=3) had the illness more than 10 years and 20 years respectively. Paliperidone has also been found to be effective to treat chronic schizophrenia , in 3 open label 52 weeks extension studies , long term treatment with paliperidone ER is found to be effective in improving patient functioning , improving and maintaining symptom control . The dose range for paliperidone ER is between 3 mg ,6mg ,9mg ,12mg or 15mg administered once daily with favourable metabolic profile and no unexpected treatment emergent adverse effects.(Emsley, Berwaerts et al. 2008). Based on

the mentioned studies there is no definite dose range of paliperidone ER for schizophrenia treatment.

4.3.2 POSITIVE AND NEGATIVE SYNDROME SCALES (PANSS).

A study done in UMMC , 2009 among 150 patients attending outpatient psychiatry clinic found no significant differences in positive, negative and general psychopathology in both genders. The mean total score of PANSS was 46.5 in the females and 48.2 in the males (Zuraida 2009). Among our study subjects 50% (n=10) had high symptomatology, followed by low symptomatology 45% (n=9) and medium symptomatology 5% (n=1). This was based on a study done by Kozma et al, the PANSS score was divided into 3 categories low if score less than <75, medium if in between ≥75 and < 95 and high if scores equal or more than ≥ 95 (Chris M Kozma 2010). Since this was a cross sectional study unable to assess if the patients had any response, a PANSS score reduction of 20%-50% from baseline would suggest response and patient is seen at least 4 times for assessment (Stefan Leucht and Eva Etschel 2005).

The total mean positive symptoms score is 21.95, total mean negative symptoms score is 22.95,total mean general psychopathology is 41.20 and total overall means PANSS is 85.30. The total PANSS score, total positive symptoms score and total negative symptoms score were compared between selected variables as to find out any associated factors. There were no significant difference between total PANSS score, total positive symptoms score and total negative symptoms score with age, gender, ethnicity, education level, duration on paliperidone and paliperidone dosage.

4.3.3 BARNES AKATHISIA RATING SCALE (BARS)

Antipsychotics are effective in treating psychosis inpatients however be it typical or atypical they have side effects that influences tolerability of the drug. paliperidone ER documented side effects includes the commonly reported adverse events are headache, agitation, anxiety and insomnia at 12%, 8% and 4% respectively. Incidence of extrapyramidal symptoms (EPS) due to paliperidone ER includes akathisia,parkinsonism and dystonia, The EPS rate for paliperidone 6 mg (10.2%) appear to be slightly lower than for usually therapeutic dosages of risperidone. In a 6 week placebo-controlled trial with patients' on paliperidone ER treatment, BARS was used to assess akathisia. The findings showed that akathisia was rated as absent in 92%–93% of patients in the paliperidone ER 6 mg and placebo groups, in 90% of the paliperidone ER 9 mg group, and in 87% of the paliperidone ER 12 mg group (J. Kane and P. Lim 2007). In this study BARS was used to assess drug induced akathisia, the total score of 0 which is 85% of subjects, 5 % have the total score of 6 (n=1) and 7 (n=1) and 8 (n=1) respectively. This indicates that paliperidone has a good safety profile and tolerable to patients.

4.3.4 NRG 1 GENE EXPRESSION LEVELS IN SCHIZOPHRENIA AND ASSOCIATED FACTORS

From this study it showed there was no significant difference between NRG 1 gene expression level with age, gender, ethnicity, education, paliperidone dosage and duration on paliperidone. What are the factors that influence the gene expression of NRG 1 expression in individuals? Based on the stress diathesis model , interaction between psychosocial and preponderance of biological vulnerability can lead to manifestation Schizophrenia (Elaine F. Walker 1997). Studies have shown that schizophrenia is heavily influenced by a genetic component, unlike RNA and protein, the DNA primary genomic sequence is unaffected by external factors. However, we do have to remember that schizophrenia is a multifactorial and complex illness.

Next what is the function of this gene and the role it plays in Schizophrenia? NRG 1 is held responsible for regulation of NMDA , GABA and nicotinic receptors, it regulates myelination ,neural –glial signaling ,glial development and differentiation ,synapse formation and modulate synaptic transmission, neuronal migration and specification and transcriptional regulation (Harrison and Law 2006). The fact that it can regulate the NMDA, GABA receptors enhances their role as susceptibility gene for Schizophrenia. Besides the classic Dopamine hypothesis for schizophrenia other hypothesis such as glutamate hypo function at NMDA receptors can produce psychotic symptoms or hyper function at non-NMDA receptors (AMPA and kainate) can also produce psychotic symptoms (Tsapakis and Travis 2002).

There is evidence that NRG 1 regulates NMDA receptor function in the mature brain , NRG 1 is able to decreased NMDA receptor-mediated currents and channel activity in cortical pyramidal neurons by increasing receptor internalization (Gu, Jiang et al. 2005).Furthermore, the core $HAPI_{CE}$ risk haplotype identified in the Icelandic population is associated with Bipolar Disorder based on a case-control study and exerts an effect on subjects causing cases of psychosis with manic or mood incongruent features. In another population, there is association of NRG1 haplotypes in cases of psychosis with preservation of affect and a fairly good prognosis (Green EK, Grozeva D et al. 2005)

Other factors that could have affected NRG 1 gene expression is its interaction with other genes or the involvement of multiple genes in Schizophrenia. There is preliminary evidence that interactions between several susceptibility genes could alter their individual effect to the risk of schizophrenia. There is direct evidence of gene-gene interaction; this was detected for NRG1-NRG2, NRG1-NRG3 and EGFR-NRG2, and for ERBB4-NRG1, ERBB4-NRG2, ERBB4-NRG3 and ERBB4-ERBB2 suggestive evidence was seen. Genetic interaction among these loci may increase susceptibility to schizophrenia (Isabel Benzel, Peter R Maycox et al. 2007).

There is no significant difference between NRG 1 gene expression and the duration on paliperidone or with paliperidone dosage. The study of NRG 1 expression in tissue or peripheral system has been inconsistent. There are significantly increased expression levels of NRG1 transcript variants in type I and type III isoforms in peripheral blood lymphocytes in patients with Schizophrenia. However , it was reported that decreased NRG 1 expression levels in Schizophrenia based on another study conducted in Han Chinese .The NRG 1 expression levels significantly increased after treatment with risperidone and quetiapine for

2 weeks compared to levels prior to treatment (Hong-Xing Zhang and Xuan Ouyanga 2008). Despite this inconsistency, there is evidence supporting that N-methyl-D-aspartate (NMDA) receptor hypofunction can be associated with increased signaling and expression of NRG1–ErbB4(Hahn, Wang et al. 2006). One of the replicated findings is the effect clozapine has on NRG 1 level. In a study using in vitro human fetal brain showed that after exposure to clozapine for 3 weeks there was increased expression for NRG 1 (Gursharan Chana 2009). In a study using animal tissue clozapine reversed the increased activity of the NRG1 TMc-mutant mice in the novel open-field test and T-maze test (Stefansson, Sigurdsson et al. 2002). In a Finnish study, patients' were divided into responders and non responders whereby non responders were given clozapine as treatment. SNP8NRG221533 was used as the genetic marker and showed that TT genotype was overrepresented in the non-responders group compared with the responders. All of this support that NRG 1 expression levels can be affected by antipsychotic regardless of duration, however it is still inconclusive the importance of NRG 1 genotype to treatment of Schizophrenia (Olli Kampman and Esa Leinonen 2004).

4.3.5. LIMITATIONS AND RECOMMENDATIONS

This was a preliminary study with several limitations:

1. This study was under powered because of the small sample size. They are several reasons the sample size is small: the initial study started out as a prospective study however had to be converted to a cross sectional study. It was unfortunate that several blood samples the RNA was damaged hence could not be analyzed. Another factor is that, this study involved only one centre which was University Malaya Medical Centre which contributed to small sample size.

2. In this cross sectional study, clinical effect or response of paliperidone could not be assessed. In addition, to recruit antipsychotics naive patients that fulfill the inclusion criteria were difficult as this is a tertiary centre and most of them were referred here. Ideally, further prospective study should be done so that potential influences such as clinical response and effect can be found in the future. It would be best that a prospective study is carried out to get a more accurate picture of gene expression and genotyping.

3. This study only investigates the gene expression and not genotyping it would be good to strengthen findings by doing genotyping.

4. Furthermore, it was difficult to obtain antipsychotic naïve patients and finding those on paliperidone. There were some studies that also look at clozapine effect on NRG 1, since in UMMC we have a specific clozapine clinic. It will be more feasible to recruit patients for a study from this group as there is a ready sample. It would be a good measure to recruit patients who is on other types of antipsychotics, perhaps

comparing NRG1 expression between 2 groups of patients on different antipsychotics.

5. The lack of serum drug monitoring to monitor patients compliance to treatment was another limitation. Compliance to medication could affect the clinical response and effect on the genes hence affecting the study outcome. In the future study, the drug level monitoring should be made available in order to detect non compliant patients and exclude them from the study.

CHAPTER 5: CONCLUSION

1. The mean NRG 1 gene expression level in our 20 schizophrenic patients were: mean = 0.405 and SD = 0.491 and the mean NRG 1 gene expression level in our 15 control patients were: mean =0.435 and SD = 0.273.

2. There was no significant association between the age, gender, ethnicity, education, dosage of paliperidone, duration on paliperidone and NRG 1 gene expression level.

3. There was no significant correlation between NRG 1 gene expression level and psychopathology.

4. Those with low PANSS score had lower NRG 1 levels (mean =0.304) compared to high PANSS score (mean = 0.524).

IMPLICATIONS

This study would help to highlight the necessity for more research in this field especially in a Malaysian setting. This research would help personalized treatment and spare patients from going through multiple antipsychotics and expose them to unnecessary side effects. This study would hopefully help us to come up with an exclusive Malaysian database that caters to our multiracial population.

This was a cross-sectional study whereby clinical effect or response cannot be assessed. In addition to that, a prospective study assessing clinical response would be of beneficial help in relation to NRG 1 expression and to investigate NRG 1 genotyping in a multiracial population. Hopefully in the future, by determining the levels of NRG1 expression in the different races, we can determine which antipsychotics treatment to dispense to our patients.

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APPENDICES

APPENDIX A

Patient Information Form

Patient's details

1. Patient's name:_____
2. R/N :_____
3. IC number: ……
4. Age :_____ years
5. Sex : ( ) Male ( ) Female
6. Race : ( ) Malay ( ) Chinese ( ) Indian
7. Marital Status : ( ) Single ( ) Married ( ) divorced
8. Employment Status: ( ) Employed ( ) Unemployed ( ) student/housewife
9. Duration of illness ……..
10. Family history of mental illness ( ) Yes ( ) No
11. Duration on Paliperidone treatment ……..
12. Current paliperidone dosage ……
13. Side effects due to current treatment ……..

Parent/guardian detail

Parent/guardian name :……

Age:…….

IC number :……

Occupation :……..

APPENDIX B *UNIVERSITY MALAYA MEDICAL CENTRE*

CONSENT BY PATIENT FOR CLINICAL RESEARCH

I, ……Identity Card No……..……

(Name of Patient)

of ……...

(Address)

hereby agree to take part in the clinical research (questionnaire study) specified below:

Title of Study: A PRELIMINARY STUDY OF NRG 1 GENE EXPRESSION IN SCHIZOPHRNEIA PATIENTS ON PALIPERIDONE IN UMMC

the nature and purpose of which has been explained to me by Dr. Nazariah Aiza binti Harun, Pegawai Perubatan Sarjana Perubatan Psikologi *(Name & Designation of Doctor)*

and interpreted by ……..……..…..…… *(Name & Designation of Interpreter)*

to the best of his/her ability in ……..…… language/dialect.

I have been told about the nature of the clinical research in terms of methodology, possible adverse effects and complications (as per patient information sheet). After knowing and understanding all the possible advantages and disadvantages of this clinical research, I voluntarily consent of my own free will to participate in the clinical research specified above.

I understand that I can withdraw from this clinical research at any time without assigning any reason whatsoever and in such a situation shall not be denied the benefits of usual treatment by the attending doctors.

Date: ……...…….. Signature or Thumbprint ……..……

(Patient)

IN THE PRESENCE OF

Name ……..…..……..…)

Identity Card No. ……..……..……) Signature ……

) (*Witness for Signature of Patient)*

Designation ……..……)

I confirm that I have explained to the patient the nature and purpose of the above–mentioned clinical research.

Date ……. Signature ……

(Attending Doctor)

CONSENT BY PATIENT FOR CLINICAL RESEARCH	R.N. Name Unit	Age ……

FPU-DOF-BK-012-05-R01

CONSENT BY RESPONSIBLE RELATIVE FOR CLINICAL RESEARCH

I, ……..IdentityCard No……...……

(Name)

of

……

(Address)

Hereby agree that my relative ……I.C. No……..……

(Name)

participate in the clinical research (questionnaire study) specified below:–

Title of Study:

the nature and purpose of which has been explained to me by Dr. Nazariah Aiza Binti Harun, Pegawai Perubatan Sarjana Perubatan Psikologi
and interpreted by *(Name & Designation of Interpreter)*

to the best of his/her ability in ……..…… language/dialect.

I have been informed of the nature of this clinical research in terms of procedure, possible adverse effects and complications (as per patient information sheet). I understand the possible advantages and disadvantages of participating in this research. I voluntarily give my consent for my relative to participate in this research specified above.

I understand that I can withdraw my relative from this clinical research at any time without assigning any reason whatsoever and in such situation, my relative shall not be denied the benefits of usual treatment by the attending doctors. Should my relative regains his/her ability to consent, he/she will have the right to remain in this research or may choose to withdraw.

Signature or Thumbprint

Relationship to Patient

Date:

IN THE PRESENCE OF

Name ……..…… Identity Card No.

(*Witness)* Signature

Designation ……

I confirm that I have explained to the patient's relative the nature and purpose of the above–mentioned clinical research.

Date …….. Signature ……
(Attending Doctor)

APPENDIX C

Source Document:			
Rater Initials	Time Performed	Has the Rater changed from the previous rating?	
	__ __ : __ __ (24 hours)	Yes ❑ No ❑ N/A 1st assessment ❑	
Patient Initials	Patient ID No.	Date of Interview	Visit No.
		__ __ / __ __ __ / __ __ __ __ (D D M M M Y Y Y Y)	

- ☐ P1. Delusions
- ☐ P2. Conceptual disorganization
- ☐ P3. Hallucinatory behavior
- ☐ P4. Excitement
- ☐ P5. Grandiosity
- ☐ P6. Suspiciousness/persecution
- ☐ P7. Hostility
- ☐ N1. Blunted affect
- ☐ N2. Emotional withdrawal
- ☐ N3. Poor rapport
- ☐ N4. Passive/apathetic social withdrawal
- ☐ N5. Difficulty in abstract thinking
- ☐ N6. Lack of spontaneity and flow of conversation
- ☐ N7. Stereotyped thinking
- ☐ G1. Somatic concerns
- ☐ G2. Anxiety
- ☐ G3. Guilt feelings
- ☐ G4. Tension
- ☐ G5. Mannerisms and posturing
- ☐ G6. Depression
- ☐ G7. Motor retardation
- ☐ G8. Uncooperativeness
- ☐ G9. Unusual thought content
- ☐ G10. Disorientation
- ☐ G11. Poor attention
- ☐ G12. Lack of judgment and insight
- ☐ G13. Disturbance of volition
- ☐ G14. Poor impulse control
- ☐ G15. Preoccupation
- ☐ G16. Active social avoidance

PANSS QuikScore™ Form

Use this scale for all items:

1 = Absent
2 = Minimal
3 = Mild
4 = Moderate
5 = Moderate/ Severe
6 = Severe
7 = Extreme

MHS

In the U.S.A., P.O. Box 950, North Tonawanda, NY 14120-0950, (800) 456-3003.
In Canada, 3770 Victoria Park Avenue, Toronto, ON M2H 3M6, (800) 268-6011.
Internationally, +1-416-492-2627.
Fax, +1-416-492-3343 or (888) 540-4484.

APPENDIX D

Name:_____

Date:_____

Barnes Akathisia Rating Scale (BARS)

Instructions: Patient should be observed while they are seated, and then standing while engaged in neutral conversation (for a minimum of two minutes in each position). Symptoms observed in other situations, for example while engaged in activity on the ward, may also be rated. Subsequently, the subjective phenomena should be elicited by direct questioning.

Objective

0 Normal, occasional fidgety movements of the limbs

1 Presence of characteristic restless movements: shuffling or tramping movements of the legs/feet, or swinging of one leg while sitting, *and/or* rocking from foot to foot or "walking on the spot" when standing, but movements present for less than half the time observed

2 Observed phenomena, as described in (1) above, which are present for at least half the observation period

3 Patient is constantly engaged in characteristic restless movements, *and/or* has the inability to remain seated or standing without walking or pacing, during the time observed

Subjective

Awareness of restlessness

0 Absence of inner restlessness

1 Non-specific sense of inner restlessness

2 The patient is aware of an inability to keep the legs still, or a desire to move the legs, *and/or* complains of inner restlessness aggravated specifically by being required to stand still

3 Awareness of intense compulsion to move most of the time *and/or* reports strong desire to walk or pace most of the time

Distress related to restlessness
0 No distress
1 Mild
2 Moderate
3 Severe

Global Clinical Assessment of Akathisia
0 *Absent.* No evidence of awareness of restlessness. Observation of characteristic movements of akathisia in the absence of a subjective report of inner restlessness or compulsive desire to move the legs should be classified as pseudoakathisia
1 *Questionable.* Non-specific inner tension and fidgety movements
2 *Mild akathisia.* Awareness of restlessness in the legs *and/or* inner restlessness worse when required to stand still. Fidgety movements present, but characteristic restless movements of akathisia not necessarily observed. Condition causes little or no distress.
3 *Moderate akathisia.* Awareness of restlessness as described for mild akathisia above, combined with characteristic restless movements such as rocking from foot to foot when standing. Patient finds the condition distressing
4 *Marked akathisia.* Subjective experience of restlessness includes a compulsive desire to walk or pace.However, the patient is able to remain seated for at least five minutes. The condition is obviously distressing.
5 *Severe akathisia.* The patient reports a strong compulsion to pace up and down most of the time. Unable to sit or lie down for more than a few minutes. Constant restlessness which is associated with intense distress and insomnia.

Scoring the Barnes Akathisia Rating Scale (BARS)

The Barnes Akathisia Rating Scale is scored as follows:

Objective Akathisia, Subjective Awareness of Restlessness and Subjective Distress Related to Restlessness are rated on a 4-point scale from 0 – 3 and are summed yielding a total score ranging from 0 to 9. The Global Clinical Assessment of Akathisia uses a 5-point scale ranging from 0 – 4.