THE PRESENCE OF SINGLE-NUCLEOTIDE POLYMORPHISMS IN
*HPS4* GENE IN A SUBSET OF SCHIZOPHRENIC PATIENTS IN
PAHANG: A PRELIMINARY STUDY

NORAFIZA BINTI ZAINUDDIN, PhD (CORRESPONDING AUTHOR)
DEPARTMENT OF BIOMEDICAL SCIENCE, KULLIYYAH OF ALLIED HEALTH
SCIENCES, INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA, JALAN
SULTAN AHMAD SHAH, BANDAR INDERA MAHKOTA, 25200 KUANTAN,
PAHANG, MALAYSIA.
znorafiza@iium.edu.my

MAIZATUL AKMA BINTI MAMAT
DEPARTMENT OF BIOMEDICAL SCIENCE, KULLIYYAH OF ALLIED HEALTH
SCIENCES, INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA, JALAN
SULTAN AHMAD SHAH, BANDAR INDERA MAHKOTA, 25200 KUANTAN,
PAHANG, MALAYSIA.
maizatul269@gmail.com

NORLELAWATI BINTI A. TALIB, MD, PhD
DEPARTMENT OF PATHOLOGY & LABORATORY MEDICINE, KULLIYYAH OF
MEDICINE, INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA, JALAN
SULTAN AHMAD SHAH, BANDAR INDERA MAHKOTA, 25200 KUANTAN,
PAHANG, MALAYSIA.
noleata@iium.edu.my
ABSTRACT

Schizophrenia is a devastating mental disorder that affects people’s normal life with heterogeneous features of its clinical presentation, as well as its molecular attribute. In order to identify the potential molecular aberration, particularly single nucleotide polymorphism (SNP) which could be important in the aetiology of schizophrenia, polymerase chain reaction (PCR)-DNA sequencing approach was utilized for targeting the exon (and intron) 9 of the Hermansky-Pudlak syndrome type 4 (HPS4) gene. DNAs were extracted from peripheral blood of nine schizophrenic patients and one normal individual prior to PCR-DNA sequencing. Following DNA sequencing, a SNP (A>G) which is rs713998 at nucleotide position 22618 of exon 9 of the HPS4 gene was observed in eight schizophrenia samples. Moreover, DNA sequencing results also revealed an intronic aberration/SNP which is rs3747129 (C>T) at nucleotide position 22789 of intron 9 of the HPS4 gene in four schizophrenia samples. A SNP which is rs739289 (G>T) at nucleotide position 22677 of the intron was also found in eight schizophrenia samples. The importance of both the exonic and intronic aberrations is yet to be confirmed with further research involving larger population and other relevant clinical parameters. That notwithstanding, these preliminary results suggested that single nucleotide aberrations, particularly SNPs might have a role in the development of schizophrenia.

KEYWORDS: Schizophrenia, HPS4, SNP, sequencing
INTRODUCTION
Schizophrenia is a psychotic disorder which involves disabilities in thinking, perception, affecting language and social behavior. Schizophrenia affects more than 21 million people worldwide (World Health Organization, 2016) and the incidence rate was 15.2 per 100,000 in range of 7.7 to 43.0 per 100,000 (Bakar, Jali, & Yusof, 2011; Ministry of Health Malaysia, 2009). The symptoms of schizophrenia were grouped into three categories: positive symptoms such as delusions and hallucinations, negative symptoms such as reduced activity to carry out activities and social withdrawal and cognitive symptoms such as difficulties with concentration and inability to remember something. (Lang, Puls, Müller, Strutz-, & Gallinat, 2007). There were 10% to 15% of the schizophrenic patients that cannot control themselves committed suicide especially younger adult males (Malaysian Psychiatric Association, 2006). According to the National Institute of Mental Health (2009), there is no specific cause of schizophrenia. Schizophrenia results from interaction between genetic and environmental factors with an estimated heritability of up to 80% (Hall et al., 2007; Saito et al., 2013). In addition, it is higher risk in having schizophrenia among first-degree family history of schizophrenia with an odds ratio of almost ten (Sullivan, 2005).

In Malaysia, there were 7351 cases of schizophrenia registered under the National Mental Health Registry from 2003 until to 2005 in which 3714 were new cases (Ministry of Health Malaysia, 2009). Malay constituted the highest percentage of schizophrenia cases which was 54%, followed by 28% Chinese, 9% Indians and lastly others which were 9% of the cases in Malaysia (Ministry of Health Malaysia, 2009). It is an urge to conduct this study due to the high schizophrenia occurrence as no society or culture is free from schizophrenia and this illness is a serious public health problem (Jablensky, 2000). Among the many candidate genes studied, HPS4 gene is a gene that has shown encouraging positive association towards schizophrenia (Saito et al., 2013).

The HPS4 gene is one of the potential genes that can be used in schizophrenia studies. Review of the functional studies of HPS4 gene revealed that this gene encodes HPS4 protein, a component of biogenesis of lysosome-related organelles complexes that is important in intracellular trafficking for cellular function (Carmona-Rivera, Simeonov, Cardillo, Gahl, & Cadilla, 2013; Merideth et al., 2010). According to Martina and his colleagues (2003), two cytosolic proteins which were HPS1 and HPS4 were interacting with one another to make a complex as known as biogenesis of lysosome-related organelles complex 3 (BLOC-3). The HPS4 gene is one of the Hermansky-Pudlak syndrome group which was an autosomal recessive disorder characterized by defective lysosome-related organelles with different genetic mutations (Wei et al., 2013; Nazarian, Falcón-Pérez, & Dell’Angelica, 2003).

The HPS4 gene is located at the human chromosome 22q12.1 known as a psychosis-critical region, which is involved in the cognitive dysfunction in schizophrenia (Karayiorgou, Simon, & Gogos, 2010). According to Takahashi and his colleagues (2003), the gene in this region (22q12.1) is able to control the exploratory eye movement (EEM) phenotype and it was shown that EEM dysfunction affected the
The presence of single-nucleotide polymorphisms in HPS4 gene

Schizophrenic patients. The mutation in HPS4 gene can cause executive function deficits in schizophrenia (Kuratomi et al., 2013). The study of HPS4 gene had been demonstrated to play a certain part in the development of schizophrenia due to the discovery of mutations in HPS4 gene in two Japanese siblings with Hermansky-Pudlak syndrome and they had a comorbid psychotic phenotype (Saito et al., 2013).

In the present study, one of the research objectives was to compare between HPS4 normal sample sequence with wild type reference sequence [NCBI Reference Sequence: NG_009763.2]. The second research objective was to identify the molecular aberration of exon 9 of the HPS4 gene in DNA samples from peripheral blood of schizophrenic patients. The genetic variations was analysed using the polymerase chain reaction-DNA sequencing analysis to identify any aberrations, particularly SNPs in the exon and intron 9 of the HPS4 gene that might have a role as a screening purpose in the development of schizophrenia among Malaysian population. This is preliminary findings, so it would not suffice to be extrapolated to the general population. For screening purposes, more information should be gathered, including data on aberration on other exons or pathways, and from previously analysed genes or biomarkers involved in schizophrenia.

MATERIALS & METHODS

Sample Collection

The peripheral blood from nine schizophrenic patients and one normal subject were obtained from the Molecular Research Laboratory of Kulliyyah of Medicine, International Islamic University Malaysia (IIUM) Kuantan Campus. The ethical approval was obtained from the Ethical Committee of Kulliyyah of Medicine, IIUM and Medical Research and Ethics Committee, Ministry of Health, Malaysia. The samples were stored in cold temperature (-20°C).

All subjects were legally identified as Malay and aged between 18 to 60 years old. The patients must understand the study procedures and also must sign the informed consent. The patients had a consensus best-estimate DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) diagnosis of schizophrenia of at least six months duration. The symptoms of psychosis were not deemed to be secondary to substance use or neurological disorder.

Genomic DNA Extraction

The extraction of DNA from peripheral blood was performed using a Gentra® Puregene® Purification kit (Qiagen, Germany) according to the manufacturer’s protocol. The extracted DNA was stored at -20°C.
DNA Quantification

DNA quantification was done to measure the yield (concentration) of DNA extracted and to determine the purity of the samples. The instrument used was BioPhotometer plus (Eppendorf, Germany) that gives the reading of absorbance at the ratio of A260/A280 and A260/A230 DNA purity. All samples were kept at stock concentration that ranged between 100 ng/µL and 300 ng/µL. Working DNA of 6 ng/µL was prepared from each of the samples from the stock solution.

Primer Selection

The primers for exon 9 of HPS4 gene were taken from a previous study by Saito and colleagues (2013). In this study, the targeted gene was exon 9 of HPS4 gene, located on chromosome 22 and produced a 407 length amplicon. The targeted DNA sequence of the exon 9 in HPS4 gene was amplified using a forward primer, 5'-TTATGAACAGATGGCAGACG-3' and a reverse primer, 5'-CCCAATCACAATCTCCTGG-3' (Saito et al., 2013).

Polymerase Chain Reaction

PCR was performed with a total reaction of 15 µL consisting 0.75 µL of each primers, 3.0 µL of PCR buffer, MgCl₂ at 2mM concentration, 0.75 µL of dNTPs, 0.5 µL of Taq DNA polymerase and 5.0 µL of DNA. The cycle conditions included initial denaturation at 94 °C for 1 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 63 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 5 min.

Agarose Gel Electrophoresis

The PCR products were separated on a 1.5% agarose gel. The electrophoresis was run in 1X TBE buffer for 45 minutes at 110 volts. Then, the DNA was visualized under ultraviolet light using Alpha Innotech™ gel-doc viewing system.

DNA Purification

The PCR products were purified from any proteins, excess salts or ethanol contamination using Wizard® SV Gel and PCR Clean-Up System kit, following the manufacturer’s protocol. DNA was stored at 4°C, or -20°C for longer use.

DNA Sequencing

Nine selected schizophrenia samples and one normal sample were sent for DNA sequencing to First Base Asia (Malaysia) using a specific sequencing primer, 5'-TTATGAACAGATGGCAGACG-3'. The expected size of the sequencing product was 407 bp.

Data Analysis

The DNA sequences of schizophrenia and normal samples were obtained in a 5’ to 3’ direction and were interpreted using DNA Baser Assembler software. The DNA
sequence of the normal sample was aligned to the wild type reference sequence [NCBI Reference Sequence: NG_009763.2]. Then, the DNA sequences of all schizophrenia samples were compared to the normal sequence in order to identify any molecular aberrations of exon 9 of the HPS4 gene.

RESULTS
The DNA sequence of the normal sample was 99% similar to the wild type sequences retrieved from GenBank database [Homo sapiens HPS4, biogenesis of lysosomal organelles complex 3 subunit 2 (HPS4) transcripts; NCBI Reference Sequence: NG_009763.2]. This was achieved using the Basic Local Alignment Search Tool (BLAST).

The Figure 1 shows partial electropherograms representing SNP (A>G) which was rs713998 on exon 9 of the HPS4 gene at nucleotide position 22618 (g.22618A>G). The normal sample, CD8 was confirmed to be of wild type homozygous A/A genotype (Figure 1A). DNA sequences of all schizophrenia samples were homozygous mutant G/G genotype [g.22618 A>G (rs713998)], except one which was PS16, with a mutation detected in the heterozygous state (A/G) (Figure 1J). Intronic aberrations were also observed, as shown in detail in Figures 2 and 3.
Figure 1(A-J) Partial electropherograms representing SNP A>G (rs713998) on exon 9 of the HPS4 gene at nucleotide position 22618 (g.22618A>G). The normal sample was in homozygous A/A state, which was similar to the wildtype, whereas the schizophrenia samples were in homozygous G/G state, except PS16.
THE PRESENCE OF SINGLE-NUCLEOTIDE POLYMORPHISMS IN HPS4 GENE .........

(A) Normal sample, CD8 with homozygous C/C

(B) Schizophrenia sample, PS3 with heterozygous C/T

(C) Schizophrenia sample, PS2 with heterozygous C/T

(D) Schizophrenia sample, PS4 with homozygous C/C

(E) Schizophrenia sample, PS5 with homozygous C/C

(F) Schizophrenia sample, PS14 with homozygous C/C

(G) Schizophrenia sample, PS6 with heterozygous C/T

(H) Schizophrenia sample, PS16 with homozygous C/C
THE PRESENCE OF SINGLE-NUCLEOTIDE POLYMORPHISMS IN \textit{HPS4} GENE ...........

\textbf{Figure 2(A-J)} Partial electropherograms representing SNP, rs3747129 (C>T) on intron 9 of the \textit{HPS4} gene at nucleotide position 22789 (g.22789C>T). PS3, PS2, PS6 and PS17 were in heterozygous C/T states, whereas the rest were similar to the wildtype.
The presence of single-nucleotide polymorphisms in HPS4 gene

Figure 3(A-J) Partial electropherograms representing SNP, rs739289 (G>T) on intron 9 of the HPS4 gene at nucleotide position 22677. All schizophrenia samples were in the homozygous T/T states, except PS16, which was heterozygous.
DISCUSSION

Based on previous studies, there were reports on few mutations in the HPS4 gene (OMIM: 606682). One of them was mutation on the human light ear (le) homolog, HPS4 gene in a number of non-Puerto Rican individuals with HPS (Suzuki et al., 2002). There was a finding regarding the association of HPS4 gene with schizophrenia that showed an encouraging association between HPS4 gene and schizophrenia among Japanese population (Saito et al., 2013). That study reported on SNPs, rs119471022 which is a nonsense mutation causing p.Gln181X in exon 7 that was associated with schizophrenia.

In the present study, which was based on previous reports indicating an association of HPS4 gene with schizophrenia, the PCR-DNA sequencing was used to enable us identifying the molecular aberrations in exon 9 of HPS4 gene of schizophrenic patients and its application helps us to better understand the disease at the molecular level.

Sequencing results have shown that the DNA sequence of the normal sample (CD8) is similar to the sequence of exon 9 of HPS4 by comparing with Homo sapiens HPS4, biogenesis of lysosomal organelles complex 3 subunit 2 (HPS4) transcript [NCBI Reference Sequence: NG_009763.2]. Percentage of the normal sample was 99% similar to the wild type database sequence by NCBI BLAST (http://www.ncbi.nlm.nih.gov/blast/). It is postulated that the normal sample was acceptable as a positive marker to compare with the schizophrenic samples to detect any aberration in the sequences.

The first aberration found in exon 9 of the HPS4 gene was at nucleotide position 22618 (g.22618 A>G). Based on the DNA sequencing result, eight of nine schizophrenia samples showed mutant G/G homozygous for the g.22618 A>G and one of the samples, PS16 was in the heterozygous state (A/G). According to the NCBI and Ensemble genome database, this aberration is a single nucleotide polymorphism (SNP) which is rs713998 on human chromosome 22. Studies were focused on identifying the genetic cause of schizophrenia as deletion in the chromosome 22 region is one of the cause that contribute to the symptoms of schizophrenia (DeLisi et al., 2002). The schizophrenic patients had deficits of executive function which rely heavily on the frontal lobe structures of the brain (Eisenberg & Berman, 2010). Interestingly, a study by Kuratomi and his team (2013) claimed that rs713998 polymorphism exhibits a significant association with the executive function under the dominant genetic model in Japanese patients with schizophrenia. These similar findings between Japanese and Malaysian population may suggest a SNP, rs713998 as a susceptibility marker for schizophrenia. We, however, have yet to confirm the previous claim with our preliminary findings.

Apart from exonic aberration, we have also managed to identify intronic aberrations, of which the importance is yet to be confirmed. Our study found SNP, rs3747129 (C>T) at nucleotide position 22789 (g.22789C>T) in four out of nine schizophrenia samples and SNP, rs739289 (G>T) at nucleotide position 22677 (g.22677G>T) in eight out of nine schizophrenia samples. Both aberrations were located on intron 9 of the HPS4
gene. Rs3747129 is a single-nucleotide variation on human chromosome 22. According to Yngvadottir et al. (2008), rs3747129 is reported as a nonsense SNP that introduces a premature termination codon in the gene. It is claimed that this SNP was likely to trigger nonsense-mediated mRNA decay (NMD) so the protein cannot be produced, which will then lead to gene lost. Besides, this SNP was also likely to become truncated protein if the NMD did not occur. Thus, this SNP may be associated with the development of disease.

Another SNP located on intron 9 of the HPS4 gene was rs739289 (G>T) at nucleotide position 22677. Rs739289 is a single-nucleotide variation on human chromosome 22. However, no association of this SNP with the development of schizophrenia has been reported yet. In theory, SNPs can occur within a coding region in the gene (exon) as well as in non-coding region (introns) of the human genome. Polymorphism in the coding region may alter protein sequence and function during translation process, whereas polymorphism or variation in the intron region do not code for any protein. Although intronic SNPs do not alter the protein sequence but the resulting sequence might be crucial in the splicing process and may affect the gene expression and the risk of getting the disease.

Thus, this preliminary study has successfully identified molecular aberrations in the exon, as well as in the intron of the gene of interest, within limited number of samples. In addition, a comprehensive view on the presence of SNP in HPS4 gene and its relationship to the development of schizophrenia may be attained in future studies with a bigger population and inclusion of relevant clinical characteristics.

CONCLUSIONS

This study has successfully identified SNPs in exon 9 of the HPS4 gene, as well as in intron 9 in schizophrenia samples. Accordingly, HPS4 gene could be one of the candidate genes in the development of schizophrenia. Nonetheless, the significance of our findings is yet to be confirmed in a larger setting.

ACKNOWLEDGEMENT

Staff of Biomedical Science Department, Kulliyyah of Allied Health Sciences, IIUM & staff of Molecular Research Laboratory, Kulliyyah of Medicine, IIUM.

REFERENCES


Carmona-Rivera, C., Simeonov, D. R., Cardillo, N. D., Gahl, W. A., & Cadilla, C. L. (2013). A divalent interaction between HPS1 and HPS4 is required for the
formation of the biogenesis of lysosome-related organelle complex-3 (BLOC-3).

Biochimica et Biophysica Acta - Molecular Cell Research.


