# Single Nucleotide Polymorphisms of Histamine H4 Receptor Gene are Associated with Drug Treatment in Thai Schizophrenia Patients

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***Background:*** *Schizophrenia is a chronic mental disorder characterized by abnormal thoughts, cognition, and social behavior. Genetic and environmental factors contribute to the cause of schizophrenia. The choice of antipsychotics (typical or atypical) used is based on benefits, risks, and costs.*

***Objective:*** *The present study investigated two single nucleotide polymorphisms (SNPs) of the histamine H4 receptor (HH4R) gene in schizophrenia patients who have been treated with typical and atypical drugs.*

***Material and Method:*** *This study was conducted in 60 schizophrenia patients treated with typical antipsychotic (30) and atypical antipsychotic (30) drugs. The DNAs were extracted from whole blood using Flexigene DNA kit (Qiagen, Germany) and genotyped by polymerase chain reaction (PCR) followed by high-resolution melting (HRM) analysis. The difference in genotypic and allelic distribution between patients treated with typical and atypical antipsychotic drugs were assessed by Chi-square test using SPSS software version 11.5.*

***Results:*** *The result revealed that two SNPs (rs8088140 and rs657132) in intronic region of HH4R gene are significant associated with appropriate treatment of schizophrenia patient with typical or atypical antipsychotic drugs. The rs8088140 SNP showed significant at p-value = 0.034, with odd ratio value and 95% CI [2.82 (1.34 to 5.95)], whereas the rs657132 SNP displayed significant at p-value = 0.039, with odd ratio value and 95% CI [2.25 (1.08 to 4.67)].*

***Conclusion:*** *Two SNPs in HH4R gene (rs8088140 and rs657132) may be used to indicate the proper antipsychotic drugs for treatment of Thai schizophrenia patients.*

***Keywords:*** *Schizophrenia, Single nucleotide polymorphism, Histamine H4 receptor, Antipsychotic drug, High-resolution melting analysis*

Schizophrenia is a chronic disease characterized by abnormalities in thoughts, cognition and behavior. Incidence of this disease is approximately 1% of the worldwide population[1]. The disease can be divided into two types of symptoms: positive and negative symptoms[2]. Positive symptom is the disturbances that are added to the person's personality, including hallucinations hearing something that does not really exist and speak alone, delusions, individuals may believe that someone is spying on them, disordered thinking and speech repeat words and ideas, and disorganized behavior; this can range from having problems with routine behaviors like hygiene. Negative symptoms are capabilities that are lost from the person's personality, including social withdrawal, emotional flatness, lack of enthusiasm and free all day without tasks[3]. Treatments for schizophrenia include drug and psychosocial therapy[4,5]. Drug therapy is very important for the schizophrenia patients. Medications are often used to help in controlling the symptoms of schizophrenia. They help to reduce the biochemical imbalances that cause schizophrenia and decrease the likelihood of relapse. However, antipsychotic medications should be taken only under the supervision of a mental help professional. There are major types of antipsychotic medication: typical antipsychotics (i.e. chlorpromazine, haloperidol, thioridazine), and atypical antipsychotics (i.e. risperidone, clozapine). The problem

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of continued non-treatment with drugs, a factor that makes patients unequal, is reaction of patient differences: first factor is genetic differences for each patient, especially the single nucleotide polymorphisms (SNPs) that attributes to each person with reaction differences i.e. reaction for chemicals, drugs and vaccine.

Drug treatment for schizophrenia patients are classified into two groups: first group is typical antipsychotics drug such as perphenazine which blocks the dopamine receptor D2, while second group is atypical antipsychotics drug as serotonin-dopamine antagonist such as risperidone that binds to D2 receptor and actives serotonin receptor of many subtype in 5-$HT_{2C}$, 5-$HT_{2A}$ and histamine receptor etc. In a previous study, it was found that the histamine H4 receptor is the common variant rs4483927 and is significantly associated with risperidone efficacy in Chinese Han patients with schizophrenia[6].

To investigate the association of SNPs and drug treatment in schizophrenia patients, this study examined genotype of histamine H4 receptor gene in schizophrenia patients that responds well when treated with typical (perphenazine) or atypical (risperidone) antipsychotic drugs.

**Material and Method**
*Subjects*

Blood samples from schizophrenia patients treating with typical (30) and atypical (30) antipsychotics drugs were collected. All patients participating in this study have signed informed consent which was approved by the ethical committees of Srinakharinwirot University (SWUEC-036/56E). Antipsychotic medications by typical and atypical drugs are shown in Table 1.

*Genotyping*

Genomic DNAs of patients were extracted from whole blood (5 ml) using Flexigene DNA kit (QIAGEN, German). The DNA concentration was measured by Nanodrop 2000 (Scientific). Then, the DNA concentration was adjusted to 25 ng/µl for PCR amplification. Genotyping was performed using polymerase chain reaction and high-resolution melting analysis (HRM). The SNP primers were designed from the website http://www.ncbi.nlm.nih.gov/projects/SNP. Two SNPs in histamine H4 receptor (HH4R) gene (rs8088140 and rs657132) were analyzed. The primer sequences and position of the SNPs in the HH4R gene are shown in Table 2 and Fig. 1, respectively.

The real-time PCR reaction was performed in a total volume of 20 µl composing of 2 µl of DNA (25 ng), 10 µl of supermix, 0.6 µl of 10 µM of each forward and reverse primers and 6.8 µl of sterile-distilled water. PCR thermal profiles consisted a pre-denaturing at 98°C for 3 min, followed by 40 cycles of denaturation at 98°C for 10 s, annealing and extension at 58.7°C for rs8088140 or 60.3°C for rs657132 for 15 s. PCR products were genotyped by using HRM analysis at temperature between 65°C to 95°C using a CFX 96 Real-Time PCR Detection System (Bio-RAD) and by sequencing using ABI 3730 xl (Applied Biosystems) to confirm genotype of patients. The differences in genotype distribution between schizophrenia patients treated with typical and atypical antipsychotics drugs were analyzed by Pearson's Chi-square test implemented in SPSS program for windows. Significant level was set at $p < 0.05$.

**Table 1.** Typical drugs and atypical antipsychotic drugs used for treatment of schizophrenia patients

| Subject | Antipsychotic drugs for treatment schizophrenia patients |
|---|---|
| Atypical | Risperidone, Clozapine |
| Typical | Perphenazine, Chlorpromazine, Fluphenazine, Haloperidol, Thioridazine |

**Table 2.** Primers used in real-time PCR and HRM of histamine H4 receptor (*HH4R*) gene

| Gene | dbSNPsID | Location | Primer sequence 5' to 3' | Size of product (bp) |
|---|---|---|---|---|
| HH4R | rs8088140 | Intron | F1: CTTGGATAATTCACTCACCTATGC<br>R1: AGACTTAGTATACCAGTTGGGACAA | 90 |
| HH4R | rs657132 | Intron | F2: ATCTCATGGCTGGGAGGAC<br>R2: TTCCTGTTGTTTTCAGCAACC | 94 |

*HH4R* 8088140 position 3557970 (mRNA)
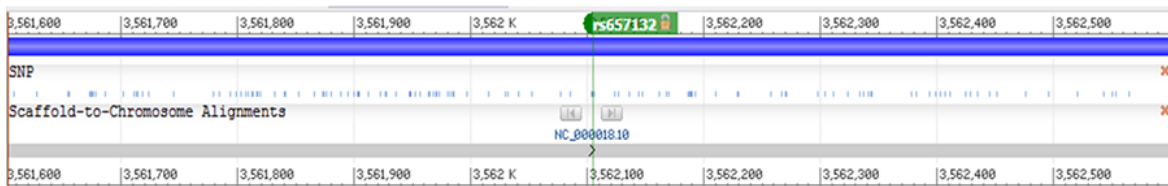
*HH4R* 657132 position 3562104 (mRNA)

**Fig. 1** The positions of selected SNPs (rs8088140 and rs657132) in HH4R gene.

**Table 3.** Allele and genotypic distribution frequencies of *HH4R* gene in schizophrenia patients treated with typical drugs and atypical drugs

| SNP ID/Drug | Genotype | | | Allele | | *p*-value | OR (95% CI) |
|---|---|---|---|---|---|---|---|
| **rs8088140** | A/C | A/A | C/C | A | C | | |
| Typical drugs | 11 (37%) | 4 (13%) | 15 (50%) | 19 (32%) | 41 (68%) | 0.034 | 2.82 (1.34 to 5.95) |
| Atypical drugs | 8 (27%) | 13 (43%) | 9 (30%) | 34 (57%) | 26 (43%) | | |
| **rs657132** | A/G | A/A | G/G | A | G | | |
| Typical drugs | 10 (33%) | 7 (24%) | 13 (43%) | 24 (40%) | 36 (60%) | 0.039 | 2.25 (1.08 to 4.67) |
| Atypical drugs | 4 (13%) | 16 (54%) | 10 (33%) | 36 (60%) | 24( 40%) | | |

Odds ratio (OR) and 95% confidence interval (95% CI) used to inform the relative risk (RR) of schizophrenia patients in rs8088140, rs657132 polymorphisms[7].

**Results**

We analyzed two SNPs (rs8088140 and rs657132) that are located in the intronic region of histamine H4 receptor (*HH4R*) gene in the two groups of schizophrenia patients who treated well with typical (perphenazine) or atypical (risperidone) antipsychotic drugs. The result is shown in Table 3. The two SNPs, rs8088140 and rs657132 showed significant differences in genotype distributions between schizophrenia patients treatment with typical and atypical antipsychotic drugs ($p<0.05$). The SNPs, rs8088140 was significant at $p = 0.034$, OR and 95% CI were 2.82 (1.34-5.95), whereas rs657132 was significant at $p = 0.039$, OR and 95% CI were 2.25 (1.08 to 4.67), respectively. The melting curve pattern (positive control) of the two SNPs,

rs8088140 and rs657132, from schizophrenia patient treatments with typical and atypical antipsychotic drugs are shown in Fig. 2 and 3.

**Discussion**

In the present study, we found significant associations between polymorphisms in *HH4R* gene with schizophrenia patients treated with typical and atypical antipsychotic drugs.

The *HH4R* gene was used in this study because polymorphism of this gene has been reported to be associated with schizophrenia patients. Histamine interacts with histamine H4 receptor to impact antipsychotic response. Information on pharmacogenetic studies of this receptor would be useful in developing personalized medication. The *HH4R* polymorphism may be a molecular marker for the prediction of risperidone efficacy and suggests a novel pharmacologic link between *HH4R* gene and treatment
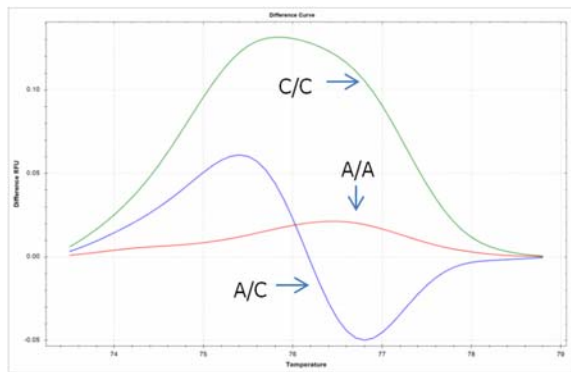
**Fig. 2** Melting curve pattern of SNP rs8088140 of schizophrenia patients treatment with typical and atypical antipsychotic drugs.
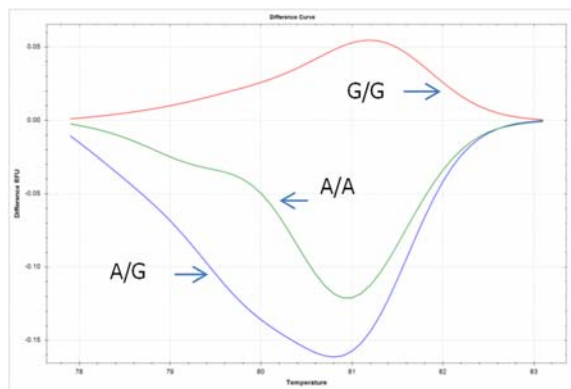


**Fig. 3** Melting curve pattern of the SNP rs657132 of schizophrenia patients treatment with typical and atypical antipsychotic drugs.

of schizophrenia[6]. Schizophrenia is a complex disorder and had a clear absence of a target gene or a biological marker. Various attempts have been made in identifying polymorphisms of *HHR* in patients diagnosed with schizophrenia[8].

Histamine is a bioactive amine widespread in the body, and exerts various biological functions through the regulation by specific histamine receptor. There are four members in histamine receptor family, namely histamine H1, H2, H3 and H4 receptors[9,10]. Histamine H4 receptor is important for the human body as it plays an extensive and physiological role in the pathophysiological process of many diseases[11]. It is a membrane protein receptor that belongs to the G-protein coupled receptor family. The gene is located in chromosome 18q[12].

Two SNPs in the intronic region of *HH4R* gene-rs8088140 and rs657132, respectively, appear to be associated with schizophrenia patient treatments with typical and atypical drugs ($p<0.05$). The rs8088140 showed significant at $p$-value = 0.034, odd ratio value and 95% CI [2.82 (1.34 to 5.95)], whereas the rs657132 showed significant at $p$-value = 0.039, odd ratio value and 95% CI [2.25 (1.08 to 4.67)]. In the future the physician can check the SNP pattern using DNA from blood of schizophrenia patients and the data can be informed which drug is appropriate for treatment of each patient, which will improve medicine effectiveness and useful in developing personalized medicine.

**What is already known on this topic?**

Histamine H4 receptor common variant rs4483927 is significantly associated with risperidone efficacy in Chinese Han patients with schizophrenia.

**What this study adds?**

This study analyzed two SNPs, rs8088140 and rs657142, in *HH4R* gene and found that both SNPs are significant associated with schizophrenia patients treated with typical drugs and atypical drugs. The *HH4R* polymorphism may be used as a molecular marker for selecting antipsychotic drugs for treatment of schizophrenia patients and useful for developing personalized medicine.

**Potential conflict of interests**

None.

**References**

1. Cardno AG, Marshall EJ, Coid B, Macdonald AM, Ribchester TR, Davies NJ, et al. Heritability estimates for psychotic disorders: the Maudsley twin psychosis series. Arch Gen Psychiatry 1999; 56: 162-8.

2. Andreasen NC, Flaum M. Schizophrenia: the characteristic symptoms. Schizophr Bull 1991; 17: 27-49.

3. Tandon R, Nasrallah HA, Keshavan MS. Schizophrenia, "just the facts" 4. Clinical features and conceptualization. Schizophr Res 2009; 110: 1-23.

4. Kasper S. Optimisation of long-term treatment in schizophrenia: treating the true spectrum of

symptoms. Eur Neuropsychopharmacol 2006; 16 (Suppl 3): S135-141.

5. van Os J, Kapur S. Schizophrenia. Lancet 2009; 374: 635-45.

6. Wei Z, Wang L, Yu T, Wang Y, Sun L, Wang T, et al. Histamine H4 receptor polymorphism: a potential predictor of risperidone efficacy. J Clin Psychopharmacol 2013; 33: 221-5.

7. Bland JM, Altman DG. Statistics notes. The odds ratio. BMJ 2000; 320: 1468.

8. Micallef S, Stark H, Sasse A. Polymorphisms and genetic linkage of histamine receptors. Life Sci 2013; 93: 487-94.

9. Repka-Ramirez MS. New concepts of histamine receptors and actions. Curr Allergy Asthma Rep 2003; 3: 227-31.

10. Li MK, Luo XX, Xie JJ. A new member of histamine receptor family: H4 receptor. Sheng Li Ke Xue Jin Zhan 2003; 34: 53-4.

11. Oda T, Morikawa N, Saito Y, Masuho Y, Matsumoto S. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. J Biol Chem 2000; 275: 36781-6.

12. Hashimoto T, Harusawa S, Araki L, Zuiderveld OP, Smit MJ, Imazu T, et al. A selective human H(4)-receptor agonist: (-)-2-cyano-1-methyl-3-[(2R,5R)-5- [1H-imidazol-4(5)-yl]tetrahydrofuran-2-y] methylguanidine. J Med Chem 2003; 46: 3162-5.

## การแปรผันของเบสหนึ่งเบสในจีนตัวรับฮิสตามีนเอช 4 มีความสัมพันธ์กับการรักษาด้วยยาต้านโรคจิตเภทกลุ่มใหม่และกลุ่มเก่า

พลเพชร ทองเกตุ, กันตก์นิษฐ รัตนธนาวรรณ, วันเพ็ญ ธุรกิตต์วัณณการ, วาสนา สุขุมศิริชาติ

*ภูมิหลัง:* โรคจิตเภทเป็นโรคเรื้อรังที่เกิดจากความผิดปกติทางจิต ซึ่งทำให้เกิดการเปลี่ยนแปลงด้านความคิด ความรู้สึกและพฤติกรรมทางสังคม สาเหตุเกิดจากปัจจัยทางพันธุกรรมและสิ่งแวดล้อม การเลือกยาต้านโรคจิตเภท กลุ่มเก่าและกลุ่มใหม่ขึ้นอยู่กับประโยชน์ ความเสี่ยงและราคาเป็นหลัก

*จุดประสงค์:* เพื่อหารูปแบบของสนิปส์ในจีนตัวรับฮิสตามีนเอช 4 (HH4R) ในคนไข้โรคจิตเภทที่ตอบสนองต่อยาต้านโรคจิตเภทกลุ่มเก่าและกลุ่มใหม่

*วัสดุและวิธีการ:* ตัวอย่างเลือด 60 ตัวอย่าง ประกอบด้วยคนไข้จิตเภทที่รักษาด้วยยากลุ่มเก่าจำนวน 30 คน และคนไข้จิตเภทที่รักษาด้วยยากลุ่มใหม่จำนวน 30 คน ทำการสกัดดีเอ็นเอและหาสนิปส์โดยเทคนิคพีซีอาร์และตามด้วยไฮเรสโซลูชั่นเมลติ้ง (เอชอาร์เอ็ม) จากนั้นวิเคราะห์หาความถี่ของจีโนไทป์ในคนไข้โรคจิตเภทที่ตอบสนอง ต่อยาต้านโรคจิตเภทแต่ละชนิดโดยใช้ Chi-square test โปรแกรม SPSS รุ่น 11.5

*ผลการศึกษา:* พบความสัมพันธ์ของสนิปส์ 2 ตำแหน่ง (rs8088140 และ rs657132) ในจีนตัวรับฮิสตามีนเอช 4 กับการตอบสนองต่อยาต้านโรคจิตเภทกลุ่มเก่าและกลุ่มใหม่อย่างมีนัยสำคัญทางสถิติโดยที่ rs8088140 มีค่า p เท่ากับ 0.034, odd ratio และ 95% CI เท่ากับ 2.82 (1.34 ถึง 5.95) ส่วน rs657142 มีค่า p เท่ากับ 0.039, odd ratio และ 95% CI เท่ากับ 2.25 (1.08 ถึง 4.67)

*สรุป:* สนิปส์ rs8088140 และ rs657132 ในจีนตัวรับฮิสตามีนเอช 4 สามารถบอกความสัมพันธ์กับการรักษาด้วยยาได้ ซึ่งจะช่วยให้ทราบว่าควรรักษาด้วยยาต้านโรคจิตเภทกลุ่มเก่าหรือกลุ่มใหม่จึงจะได้ผลการรักษาดีไม่ต้องเสียเวลาในการทดลองยา