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Assessment of *OPRM1* and *HRH2* gene variants in Vietnamese using RFLP-PCR

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Abstract. The gene encoding the mu-opioid receptor (*OPRM1*) has been reported to associate with a range of substance dependence and *HRH2* is the gene coding histamine H2 receptor, the site of action for various compounds, e.g. amitriptiline and mianserin, used in the treatment of psychiatric disorders. Both *OPRM1*and *HRH2* genes contain some single nucleotide polymorphisms (SNPs), including +118A/G in *OPRM1* and +398T/C in *HRH2*, which were reported to change in protein sequences and result in affecting the therapeutic response. Some previous studies revealed linkage between these rare alleles and propensity towards drug dependence. This paper reports the data of an investigation on frequencies of several SNPs at *OPRM1* and *HRH2* loci in a Vietnamese population by using RFLP-PCR technique. For *OPRM1*, the IVS2+691G allele appeared with a frequency of 25%. The SNP +543G/A were found at the *HRH2* locus with frequency of 5%. These frequencies seemed to concur with those found in previous studies in some other Asian populations, such as Japanese, Indian or Chinese but notably the frequencies of these alleles found in Vietnamese appeared to differ significantly from those found in European and African-American populations as revealed by previous investigations.

Keywords: mu-opioid receptor, histamine H2 receptor, single nucleotide polymorphism (SNP), PCR-RFLP.

1. Introduction

Pharmacogenetic approaches provide a method for identifying mechanisms underlying interindividual response variation and, in particular, identifying patients who have a higher probability of benefiting from a particular medication [1]. Because medications appear to exert their therapeutic effects through distinct mechanisms, knowledge of specific genetic predictors of response could make it possible for treatment to be matched to patients so as to optimize therapeutic response and minimize adverse effects.

Compounds used to treat many diseases work by activating a receptor or inhibiting the action if it is natural ligand. Variation in receptors amongst the population is known to be caused by allelic variation and this variation can alter the response of a disease to a drug amongst patients.

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The mu-opioid receptor is implicated in the reward, tolerance and withdrawal effects of alcohol and other drugs of abuse. Thus, the genetic loci that code for these opioid receptors, OPRM1, OPRD1, and OPRK1, respectively, are potential targets for pharmacogenetic studies of naltrexone (NTX) treatment effects. The mu-opioid receptor plays a central role in mediating the effects of morphine and related opioid agonists. Detection of genetic variation affecting OPRM1 expression or mu-opioid receptor function would be an important step towards understanding the origins of inter-individual variation in response to mu-opioid receptor ligands and in diseases of substance dependence. The *OPRM1* gene encodes the mu-opioid receptor, *OPRM1* maps to chromosome 6q24–q25 [2].

Four nucleotide variants (+17C/T, +118A/G, +440C/G (relative to the ATG) and IVS2+691C/G) were observed [3]. One obvious candidate variant for such an effect is the A118G single nucleotide polymorphism (SNP) in exon 1 of OPRM1. This polymorphism encodes an Asn40Asp amino acid substitution and is reported to be functionally important, but while there are several reports showing that this variant is functional, they are not easily placed in a consistent framework regarding the nature of the functional effects [4]. Additionally, the C17T which encodes a variant receptor with a Val at position 40 instead of Ala, have been found with varied frequencies across populations. These findings demonstrated that the opioid system is involved in the reinforcing properties of alcohol and that allelic variation at OPRM1 is associated with differential response to medications active at the mu-receptor. Therefore, genetic variation at loci coding for opiate system proteins, including OPRM1 might be expected to affect risk for drug dependence and alcoholism, and possibly other addictive behaviors as well.

Histamine is natural constituent of many organs and tissues including the gastrointestinal tract, the immune system and the brain [5]. It is a central neurotransmitter in the brain and is formed in the posterior hypothalamus from exogeneous histidine by histidine decarboxylase (HDC). There are three known histamine receptors: H1, H2 and H3, the latter functioning as an autoreceptor. The H2 receptor is a site of action of various compounds used in the treatment of psychiatric disorder, e.g. amitriptiline and mianserin. Health *et al.* (2002) have reported the successful response of patients with chronic, predominantly negative type schizophrenia, to the highly specific H2 receptor antagonist famotidine [6]. *HRH2* is gene encoding the human histamine H2 receptor. The A649G SNP in this gene, leading to amino acid substitution of an Asn at position 217 by Asp was found to be associated with schizophrenia in British Caucasians [6].

In the present study, we applied PCR-RFLP in investigating the frequency of such SNPs in Vietnamese subjects as our preliminary assessment [7]. For *OPRM1*, the 118G allele appeared with a relatively high frequency of 38%. Neither the 17T nor 649G allele of *HRH2* were found in the study, suggesting that these variants, if present, is uncommon in the Vietnamese population. In this publication, we extended our assessment by analyzing the frequencies of IVS2 +C691G locus in intron 2 of *OPRM1* gene and G543A SNP in *HRH2* gene in Vietnamese, using PCR-RFLP method. These results suggest that natural variations that might affect function and/or be associated with psychiatric phenotypes related to therapeutic response in medication.

2. Materials and methods

2.1. Materials

The Vietnamese population sample is composed of 100 Vietnamese individuals and were collected randomly from patients in the Hanoi Huu Nghi Hospital and National Institute of Hematology and Blood Transfusion, Vietnam. Venous blood samples were collected in vials containing EDTA and stored in -20°C for a week to a month.

2.1. Methods

DNA extraction. Genomic DNA was extracted from blood samples by using standard precipitation described by Sambrook *et al.* (2001) [8] with some minor modifications. The extracted DNA products were analyzed on a 0.8% agarose gel and measured at OD_{280} and OD_{260}. OD_{260}/OD_{280} was calculated to identify the extraction efficacy and intactness of the genomic DNA.

PCR-RFLP genotyping. For *OPRM1*, fragment 235bp was amplified by using the primers designed incorporating mismatched base (capitalized below) to produce artificial restriction sites (bold) in order to analyze SNP IVS2+691C/G: forward primer: 5'–gct ctg gtc aag gct aa**G** aat–3' (where a G was substituted for an A at position -4 to create a *Hinf*I site); reverse primer: 5'–gat cat cag tcc ata gca cac gg–3'). PCR cycling parameters consist of a 5min hold at 95°C, followed by 30 cycles of 95°C (1min), 61°C (1min) and 72°C (1min). Reaction mixture consists of 0.4μM each primer (Bioneer), 0.2mM dNTP, 1u Taq polymerase/25μL of reaction and 4mM $MgCl_2$. Following amplification, the reaction mixture was digested with HinfI restriction endonuclease (Fermentas) which correspondingly cuts the 691C allele resulting in 20bp and 215bp fragments. Fragments were resolved by 3% agarose gel electrophoresis.

For *HRH2*, the primers included: forward 5'-cca atg gca cag cct ctt–3' and reversed 5'–gca gca gaa gag ctg ttg–3' was used to amplify a 909 bp fragment. Optimized conditions for this PCR reaction are 0.4μM of each primers, 0.4mM dNTP, 1u Taq polymerase/25μL reaction and 3mM $MgCl_2$. PCR cycling parameters consist of a 5-min hold at 96°C, followed by 30 cycles of 96°C (1min), 57°C (1min) and 72°C (1min 20s). PCR products were subsequently added to *XmiI* restriction endonuclease (Fermentas) which yield fragments of 536bp and 373bp upon *XmiI* digestion of the variant PCR product containing allele (rare) 543A. The common allele 543G has no *XmiI* restriction site. Reaction products were analyzed by electrophoresis on the 1.5% agarose gel.

The χ^2 test was applied for verifying the allele frequency distribution of the SNPs.

3. Results and discussion

DNA extraction. Genomic DNA was extracted from blood samples anticoagulated with either EDTA by using the methods described by Sambrook *et al* (2001) [8].

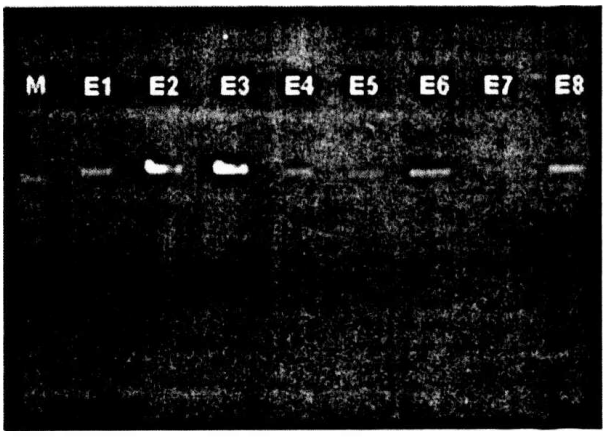

Fig 1. Electrophoresis on 1% agarose gel of genomic DNA extracted from blood samples. M: Marker λ *Hin*dIII; E1 – E8: Genomic DNA products.

In our study, the results showed that genomic DNA was extracted successfully with this method (Fig.1). Whole genomic DNA appears as a sharp, bright band in agarose gel of electrophoresis. Optical density assay showed relatively purified products of $OD_{260/280}$ values ranging from 1.6 to 2 and the concentration of DNA were 30 - 400μg/mL. The DNA samples subsequently were diluted to concentration of 50 μg/mL for further PCR experiments.

Polymerase chain reactions. The optimization of primer annealing was performed on purified DNA samples. The annealing temperature was identified as 61°C for the best result. For amplification of 235bp fragment to analyze IVS2+691C/G SNP in *OPRM1* gene, PCRs were operated and we have obtained specific DNA bands at the size of 235bp as expected according to its theoretical calculation (Fig. 2).

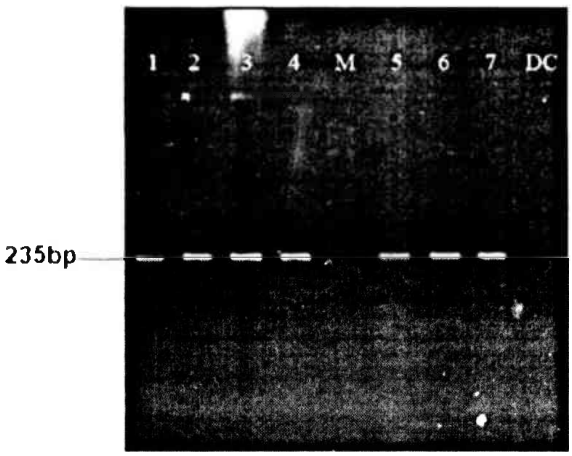

Fig 2. Electrophoresis of PCR products *OPRM1* gene on agarose 1.5%, 60V. DC: negative control; lane 1-7: PCR products; M: DNA marker 1kb.

For genotyping of *HRH2*, the amplifying also obtained the 909bp fragment (nucleotide +8 to nucleotide +916), which appeared as one sharp and specific band in the electrophoresis. These results were illustrated in Figure 3.

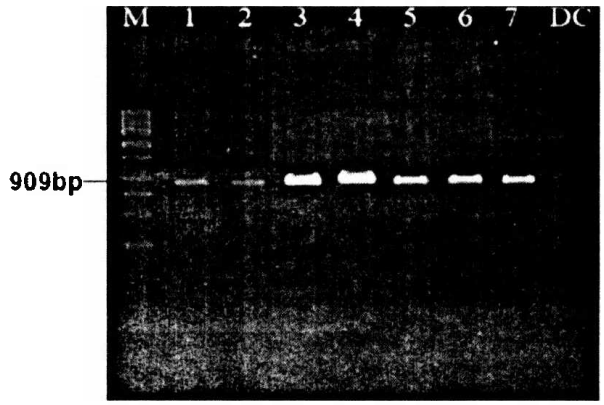

Fig 3. Electrophoresis of PCR products *HRH2* gene on agarose 1.5%, 60V. DC: negative control; lane 1-7: PCR products; M: DNA marker 1kb.

Genotyping and data analysis. The PCR products from both of *OPRM1* and *HRH2* genes were used for digestion reaction with *Hinf*I and *XmiI* respectively. Upon *Hinf*I digestion, in 100 samples in this study, frequency of individuals with homozygotic genotype IVS2+691C/C was 0.56, heterozygotic genotype +691C/G was 0.37 and +691G/G was 0.07. The figure 4 below is illustration of this result.

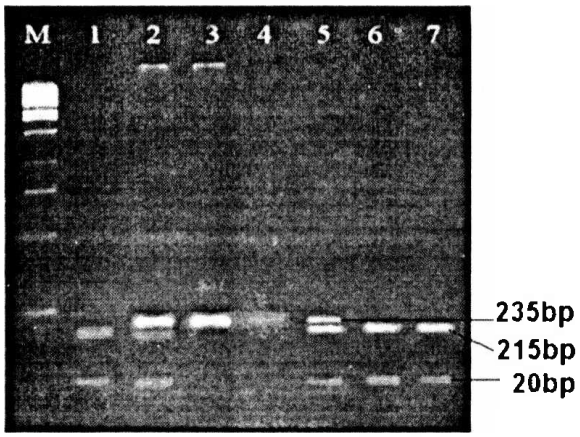

Fig 4. Digestion of PCR product IVS2-*OPRM1* gene with *Hinf*I for analysis of IVS2 +691C/G SNP. M: marker 1kb; Lane 1, 6 and 7: homozygote CC; lane 3 and 4: GG; lane 2 and 5: heterozygote C/G.

From that, we calculated allele frequencies of +691C and +691G as 0.75 and 0.25.

respectively. These results confirmed by χ^2 test ($\chi^2_{(2)}$ = 0.098 lower than the χ^2 value of statistical significant at p = 0.05 which is 5.99), showing that the frequencies of these alleles reached to balanced state and there were no deviations from Hardy-Weinberg expectations in the population. The comparisons of +691G allele frequencies between Vietnamese and other population indicated that, the frequency of this rare allele Vietnamese is of average to other Asian populations and relatively low compared to other populations world-wide.

Genotyping of *HRH2* gene for analysis of allele frequencies of +543G/A SNP was performed by digestion reaction of *Xmn*I. Because of the common allele +543G without restriction site and the rare (mutated) allele +543A with one restriction site of this enzyme and result in two bands 536 and 373 bp, we can identify the genotype of individuals in study.

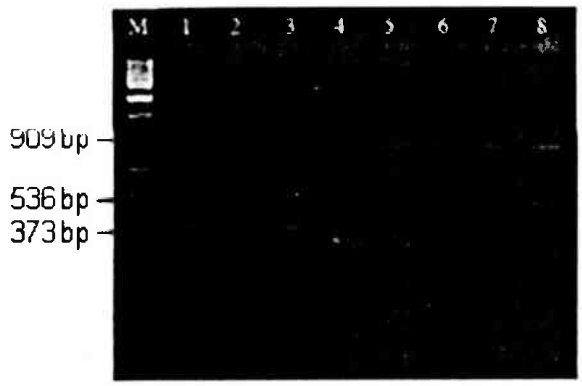

Fig 5. Digestion of PCR product *HRH2* gene with *Xmn*I for analysis of +543G/A SNP. M: DNA marker 1kb; Lane 1and 3: heterozygote G/A; lane 2, 4 – 8: homozygote GG.

Amongst of 100 samples, 90% are GG homozygous, 10% are GA heterozygous and there is no AA homozygote in our study. The frequencies of these alleles are 0.95 (G) and 0.05 (A). These frequencies were tested using χ^2 test ($\chi^2_{(2)}$ = 0.277, lower than the χ^2 value of statistical significant at p = 0.05 which is 5.99). This result also indicated that there were no deviations from Hardy-Weinberg expectations for distribution of alleles +543G/A in this Vietnamese population.

4. Conclusion

The assessment of the frequencies of single nucleotide polymorphisms in *OPRM1* gene (IVS2+691C/G) and gene coding histamine H2 receptor *HRH2* (+543G/A) with 100 Vietnamese individuals showed that, both of these SNPS were found in our subjects with identified frequencies. The +691G variant in the *OPRM1* gene was at the frequency of 0.25 and +543A variant of *HRH2* was 0.05. In the analyzed loci, the frequencies of genotypes are followed Hardy – Weinberg expectation. This means that, the genetic compositions of these alleles are quite balanced, at least in our 100 individuals of this study.

The comparisons of the frequencies of common SNPs in *OPRM1* and *HRH2* genes in Vietnamese appeared to be similar to those previously found in Asians, e.g. Japanese, Indian, Chinese etc. but significantly differed from those of Europeans and African-Americans, like our judgment in previous publication [7].

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Đánh giá đa hình đơn nucleotit trong gen *OPRM1* và *HRH2* ở người Việt Nam sử dụng RFLP-PCR

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Gen mã hóa thụ thể mu-opioid (*OPRM1*) được cho là có liên quan tới sự phụ thuộc thuốc và các chất gây nghiện. *HRH2* là gen mã hóa thụ thể histamine H_2, vị trí hoạt động của nhiều hợp chất được sử dụng trong điều trị các bệnh liên quan đến tâm thần (amitriptiline và mianserin). Cả hai gen này đều chứa những vị trí đa hình đơn nucleotit - SNP (chẳng hạn, +118A/G ở gen *OPRM1*, +398T/C ở gen *HRH2*...) dẫn đến những thay đổi về trình tự axit amin của protein và từ đó làm ảnh hưởng tới đáp ứng thuốc trong điều trị. Nhiều nghiên cứu trước đây đã đưa ra mối liên kết giữa các alen hiếm này và xu hướng phụ thuộc thuốc. Trong nghiên cứu này, chúng tôi khảo sát các SNP IVS2+691C/G và +543G/A ở hai gen *OPRM1* và *HRH2* trong nhóm 100 cá thể người Việt Nam được thu thập ngẫu nhiên từ Bệnh viện Hữu Nghị, Hà Nội và Viện Huyết học và Truyền máu Trung ương, Việt Nam, bằng kỹ thuật RFLP-PCR. Kết quả cho thấy, với gen *OPRM1*, alen IVS2+691G xuất hiện với tần số 25%. Alen +543A ở HRH2 được tìm thấy với tần số 5%. Các tần số này khá gần với tần số các alen tương ứng trong các quần thể người ở Châu Á, như Nhật Bản, Ấn Độ, Trung Quốc... nhưng khác biệt so với tần số của các alen này ở người Châu Âu và người Mỹ gốc Phi.