**Analysis of three genetic polymorphisms in Malaysian essential hypertensive and type 2 diabetic subjects**

R. Vasudevan¹, Patimah Ismail¹*, Johnson Stanslas² and Norashikin Shamsudin²

¹Genetic Research Group, Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia.
²Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia

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Genetic polymorphisms were associated with an increase in the risk of developing disease and they are integral to the development of genetic marker to identify the individuals at risk. The genotypic distribution of various genetic polymorphisms involved in essential hypertension (EHT) and type 2 diabetes mellitus (T2DM) in Malaysian subjects has not been well characterized. The main objective of this study was to determine the association of S477X polymorphism of LPL gene, A6244G polymorphism of IRS-1 gene and C825T polymorphism of GNβ3 gene with EHT and T2DM in Malaysian subjects. This study includes 70 EHT, 60 T2DM, 65 EHT with T2DM and 75 control subjects. Genotyping of all the three polymorphisms was performed by PCR-RFLP method with the respective primers and restriction enzymes. The genotypic and allelic frequencies of the respective polymorphisms of the genes did not differ significantly (p>0.05) with EHT and T2DM in Malaysian subjects. The results of this study suggested that, S477X genotypes of LPL gene, A6244G genotypes of IRS-1 gene and C825T genotypes of GNβ3 gene was not associated with EHT and T2DM in Malaysian subjects.

**Key words:** Essential hypertension, Type 2 diabetes mellitus, genetic polymorphism.

**INTRODUCTION**

Genetic polymorphisms are integral to the development of genetic markers to identify individuals at risk of developing diseases. Several research strategies such as investigations of specific candidate genes, genome-wide searches, the use of intermediate phenotypes, comparative genomics, and the combination of these methods have been utilized to dissect the genetic variants of both essential hypertension (EHT) and type 2 diabetes mellitus (T2DM) (Timberlake et al., 2001; Tanira et al., 2005). One such research strategy is the ‘candidate gene’ approach and one of the most extensively studied in EHT and T2DM pathogenesis (Agarwal et al., 2005; Radha et al., 2007). EHT and T2DM are polygenic or multifactorial disease is a result of the interaction between the environment and multiple genes. The susceptibility was associated with frequent polymorphisms that create variation in exons or influence the expression of genes in the regulatory parts (McCarthy et al., 2002). These sequence variants were associated with just a limited increase in the risk of developing the disease. They can be considered as susceptibility variants, but they are not causative factors that unambiguous to determine the disease.

Lipoprotein lipase (LPL) a hydrolytic enzyme is predominantly found in capillaries, adipose tissues and bound to the vascular endothelium. Since it plays a pivotal role in lipid metabolism, LPL is considered as a strong candidate gene for atherogenic lipid profiles and coronary heart disease (Bockmeer et al., 2001). Because of increased hypertension and lipid abnormalities in diabetics, LPL gene and neighboring markers were included among the candidate loci in a study of non-diabetic members of Taiwanese families (Wu et al., 1996).

The human LPL gene consists of ten exons, spans about 30 kb on chromosome 8p22 (Fisher et al., 1997). Three common coding polymorphisms (D9N, N291S and S447X) in the LPL gene have been described. Recently, GOLD study (Izar et al., 2008) shows that, D9N polymorphism was associated with myocardial infarction in...
T2DM. N291S polymorphism was a major predisposing factor to hypertriglyceridaemic patients in the Northern Irish population (Wright et al., 2008). However S447X (C-G 1595) polymorphism of LPL gene creates a premature stop codon, truncating the last two amino acids, serine and glycine residues from the carboxyl end of the protein associated with the opposite LPL and plasma lipid effects as compared to other polymorphism D9N and N291S (Fisher et al., 1997; Wittrup et al., 1999).

Linkage of a quantitative trait locus for systolic blood pressure to genetic markers within the LPL gene has previously been identified (Wu et al., 1996). However, a linkage study in Caucasians failed to link LPL locus and the nearby regions with hypertension (Hunt et al., 1999). LPL variants in the promoter of the LPL gene may be associated with changes in lipid metabolism leading to obesity and T2DM. However, a study in Asian Indians, the –T93G SNP of the LPL gene was associated with obesity but not with T2DM, whereas the –G53C SNP appears to be protective against both obesity and T2DM (Radha et al., 2007).

Insulin is an essential peptide hormone that regulates metabolism, growth, and differentiation. Biological actions of insulin are initiated when insulin binds to its cell surface receptor. Insulin receptor substrate-1 (IRS-1) is the first substrate of the insulin receptor in the insulin signaling pathway (Gupta, 1997). Due to this central role, the IRS-1 function could be related to the development of T2DM. Insulin resistance has been described as a possible underlying link for the development of the clustering of T2DM, hypertension, obesity, and dyslipidemia (Reaven et al., 1996; Modan et al., 1985). Almind et al. (1993) found the association of IRS-1 gene polymorphisms with T2DM, following that, several studies have been investigated the role of the insulin receptor in the pathogenesis of EHT and T2DM. In Australian population, the prevalence of the Rsal restriction fragment-length polymorphism (RFLP) major allele (7.0 kb) has been reported in white hypertensive subjects (Ying et al., 1991; Schrader et al., 1996). A quantitative change in systolic blood pressure levels was noticed between the genotypes but there was no significant difference in blood pressure between the alleles. An increased prevalence of the N1 allele of the Nsfl RFLP located in exon 8 was observed in white hypertensive subjects (Schrader et al., 1996). However, Munroe et al. (1995) failed to show an association between the insulin receptor and hyper-tension in whites. In addition, a large study of 933 Hong Kong Chinese with various aspects of the metabolic syndrome, the insulin receptor gene Nsfl RFLP shows no differences either in the genotype or allele frequency dist-ributions between the control group and the cohorts with glucose intolerance, hypertension, or dyslipidemia alone or in combination.

G-Proteins are heterotrimers composed of α, β and γ subunits, they are key mediators of intracellular signal transduction processes initiated by several receptors. Hence, alterations that affect G-protein function or expres-

**MATERIALS AND METHODS**

All the patients were recruited from Universiti Putra Malaysia (UPM) Physician Clinic, Hospital Kuala Lumpur. Unrelated healthy individuals were collected randomly from UPM staff members and volunteers. Informed written consent was obtained from all the subjects before enrollment. Study protocol was approved by the Ethical Committee of the Faculty of Medical and Health Sciences, Universiti Putra Malaysia (UPM/FPSK/PADS/T-TAD/T7-MJKEtikaP-Per/F01). The subjects were divided as group 1 (EHT without T2DM), group 2 (T2DM), group 3 (EHT with T2DM) and group 4 as control subjects. Hypertension was diagnosed, if patients were receiving antihypertensive treatment, or systolic/diastolic blood pressure was ≥ 140/90 mmHg. The fasting blood glucose levels have been obtained from the medical records of all the known diabetic subjects. For control subjects, hypertension was excluded on the basis of blood pressure measurements according to the above-mentioned procedure and the fasting blood glucose level was measured using MediSense Precision-G Blood Glucose moni-tor. Socio-demographic factors were assessed by both Malay and English language questionnaires. 4 – 5 ml venous blood drawn in EDTA tube as anticoagulant was collected from each individual. Genomic DNA extraction was carried out using the DNA isolation kit (BioBasic.Inc, Canada). The purity of extracted DNA was quantified using Eppendorf UVette™ in Biophotometer (Eppendorf, Hamburg, Germany). Standard PCR was carried out to amplify the products. A
Table 1. Oligonucleotides for amplification and screening for three polymorphisms using PCR-RFLP method

<table>
<thead>
<tr>
<th>Gene polymorphism</th>
<th>Forward Primer (FP)</th>
<th>Reverse Primer (RP)</th>
<th>PCR cycling conditions/ temperature (°C) / (m) or (s)</th>
<th>Restriction endonuclease</th>
<th>PCR product (bp)</th>
<th>Restriction fragment size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A6224G</td>
<td>FP-5’-CGGTCT TGT AAG GGT AAG TG-3’</td>
<td>RP- 5’-GAA TTCACA TTC CCA AGA CA-3’</td>
<td>ID - 94°C- 3 m D- 94°C- 45 s A- 58°C- 45 s Ext- 72°C-1 m F. Ext- 72°C-5 m No. of cycles -35</td>
<td>NsII</td>
<td>324</td>
<td>GG-239+85, AG-324+239+85. (Thomas et al., 2000)</td>
</tr>
<tr>
<td>S477X</td>
<td>FP- 5’-TAC ACT AGC AAT GTC TAG GTG A-3’</td>
<td>RP- 5’-TCA GCT TTA GCC CAG AAT GC-3’</td>
<td>ID - 94°C- 5 m D- 94°C- 1 m A- 61.8°C- 30 s Ext- 72°C 30 s F. Ext- 72°C-5 m No. of Cycles- 30</td>
<td>MnlII</td>
<td>488</td>
<td>SX-285,247, 203,38 SX- 85,203,247, 203,38 (Groenemeijer et al., 1997)</td>
</tr>
<tr>
<td>C825T</td>
<td>FP- 5’- TGA CCC ACT TGC CAC CCG TGC-3’</td>
<td>RP-5’-GCA GCA GCC CAG GGC TGG C-3’</td>
<td>ID - 94°C- 5 m D- 94°C- 50 s A- 61.3°C- 30 s Ext- 72°C-50 s F. Ext- 72°C-5 m No. of Cycles- 35</td>
<td>BseDI</td>
<td>268</td>
<td>TT-268, CC – 152,116, TC – 268, 152,116 (Benjafield et al., 1998)</td>
</tr>
</tbody>
</table>


RESULTS AND DISCUSSION

To the best of our knowledge, there was no previous data published on the basis of three polymorphisms in relation to EHT and T2DM in Malaysian population. The current study is the first report in determining the genotype and allele frequencies of S447X polymorphism of LPL gene, NsII RFLP of IRS-1 gene and C825T polymorphism of GNβ3 gene in relation to EHT and T2DM in Malaysian subjects. However, our previous data (Vasudevan et al., 2008 a, b, c) shows that BglII polymorphism of renin gene was not associated with Malaysian hypertensive subjects, C511T polymorphism of interleukin gene failed to show an association with Malaysian type 2 diabetic subjects while G2350A polymorphism of angiotensin converting enzyme shows a positive association with EHT and T2DM in Malaysian subjects. A total of 270 subjects consisting of 70 EHT, 65 EHT with T2DM, 60 T2DM and 75 unrelated healthy individuals as controls were recruited for this study. The subjects were also stratified to 163 males and 107 female subjects. Table 2 show the distribution of the genotype and allele frequencies of LPL, IRS-1 and GNβ3 gene polymorphisms among the subjects.

LPL gene has been investigated extensively in linkage studies and in studies of its association with lipid profiles and coronary artery disease, EHT and T2DM. Nearly 80% of variants in LPL gene have been identified in coding regions while the rest are in non-coding regions (Murthy et al., 1996; Lalouel et al., 1992). Among that, S477X polymorphism of LPL gene may regulate hypertension and it plays a key role in the transportation of serum lipoprotein and energy metabolism. The results of this study shows that there was no significant difference found between S477X polymorphism and Malaysian hypertensive and type 2 diabetic subjects (p>0.05).

LPL Ser447-Stop mutation was detected by cutting the...
PCR product with the restriction endonuclease *MnlI*. The PCR product of 488 bp contains two *MnlI* restriction sites, of which one is polymorphic which reveals the Ser447-Stop mutation. PCR products were digested with 10 U *MnlI* results in three fragments of 290, 250, and 200 bp, respectively (Figure 1a). The homozygous X allele of S477X polymorphism was found very less in T2DM group (11.67%) as compared to other groups. The frequencies of the wild-type and the Ser447-Stop alleles were found high in all the four groups (>75%) as compared to the variant allele. This study is in well accordance to Turkish population study (Komurcu-Bayrak et al., 2007), however their reports suggested that S477X polymorphism was associated with metabolic syndrome. Recent studies suggested that the relationship between the S447X variant and its differential effect on lipid levels may be due to differences in the prevalence of both genetic and environmental factors (Corella et al., 2002; Lee et al., 2004). LPL gene plays a role in determining insulin resistance in Mexican-American group (Goodarzi et al., 2004) with a high prevalence of the insulin resistance syndrome. However, in this study, there was no significant difference found in gender (Table 3). Although the S447X variant of the LPL gene has been shown to result in premature termination of the LPL gene translation (Fisher et al., 1997) and several studies had revealed a positive association between this variant and BP levels, but we failed to show an association in the present study.

Insulin receptor substrate-1 gene encodes proteins and involved in the signal transduction that characterizes insulin biological activity. Several polymorphisms have been described in this gene, among that a silent mutation in exon 8 of the insulin receptor gene at alanine 523 results in an A-to-G transition at nucleotide 6224 introducing an *Nsi*I restriction site has been associated with arterial hypertension (Schrader et al., 1996), blood pressure (Thomas et al., 2000). The 324-base pair (bp) amplification product of A6224G polymorphism of IRS1 gene digested with *Nsi*I restriction enzyme was shown in the Figure 1b.
The insulin receptor gene Nsil RFLP allele and genotype distribution in the subjects was described in Table 2. The prevalence of the mutant G allele and GG genotype in hypertensive group were 27.14 and 10.00%, respectively, compared to 28.00 and 2.67% of control group. No differences were observed in the genotype and allele frequency distributions between the patient group and the control groups. The result of this study was well in accordance to the reports of Thomas et al. (2000). However in their studies the insulin receptor gene Nsil RFLP was associated with DBP in the Chinese subjects. In contrast to the current study, Schrader et al. (1996) also described that the G allele was more prevalent in white hypertensive than in normotensives, particularly those who were not obese or dyslipidemic (Schrader et al., 1996). Table 3 shows the genotypic and allelic distribution of A6244G polymorphism of IRS1 gene between male and female subjects. There was no significant difference found in gender, however G allele was found higher in male subjects (58.27%) than females (41.73%) which is similar to the studies done in the Chinese subjects (Thomas et al., 2000). In some studies more common polymorphisms in insulin receptor genes have been associated with T2DM (Morgan et al., 1990) and hypertension (Thomas et al., 2000), but such associations have not been found in other populations (Magre et al., 1995; Lepretre et al., 1998).

The common C825T polymorphism of the gene encoding the 8 3 subunit of heterotrimeric G proteins associated with enhanced G protein activity has been shown to influence lipolysis in human adipocytes and increased intracellular signal transduction. The variants found in the GNB3 gene seem to be associated with hypertension, insulin sensitivity and obesity (Stefan et al., 2004; Kopf et al., 2008). In this study, C825T polymorphism of GNB3 gene was not associated either in EHT or T2DM. The PCR products was digested with the restriction enzyme BseDI (Figure 1c) shows homozygous subjects for CC (152 and 116 bp), homozygous for TT (268 bp) and CT for heterozygote genotypes (268, 152, and 116 bp). The frequency of the T allele of C25T polymorphism in group 1 (50%) and group 2 (49.20%) was more or less identical to control group (44%) and there was no significant difference found between the groups (p>0.05). Lack of association between the 825T allele with hypertension was also reported in a French and Irish sample (Brand et al., 1999). Snapir et al. (2001) showed that the 825T allele was not associated with an increased risk for developing hypertension in 903 Finnish men during a 4.2-year follow-up period. No association of C825T polymorphism of GNB3 gene with hypertension or blood pressure levels was reported in Japanese (Kato et al., 1998) and Taiwan Chinese subjects (Tsai et al., 2000). However, the results of this polymorphism was in contrast to the results of Emirati population study ($\chi^2 = 22.5, P<0.001$) comprising 254 controls and 256 patients with clinical diagnoses of T2DM (Jawad et al., 2005). No relationship was found between 825T and diabetes in Polish T2DM subjects (Dzida et al., 2002). However, association of 825T allele was found in Polish patients with T2DM in the development of diabetic nephropathy and the similar results were also observed in German populations (Blöthner et al., 1999; Zychma et al., 2000). The data was stratified according to the gender and there was no association of C825T polymorphism of GNB3 gene observed (Table 3).

Figure 1. Agarose gel electrophoresis showing the genotypes of three gene polymorphisms. 1a) S477X genotypes of LPL gene; 1b) A6244G genotypes of IRS-1 gene; and 1c) C825T genotypes of GNB3 gene. M represents 100 bp ladder and the B represents the blank contains negative control without the template DNA.
gene polymorphisms (Siffert et al., 1999) or may be due to the environmental factors which may contribute to the conflicting results (Persu, 2006).

Conclusion

The present study shows that the S477X genotypes of LPL gene, A6244G genotypes of IRS-1 gene and C825T genotypes of GNB3 gene were not associated with EHT and T2DM and failed to be a genetic marker in predicting the development of EHT or T2DM in Malaysian Subjects. However, we cannot exclude that the variants might not influence the risk for EHT and T2DM. To find out the susceptibility genes predisposing to EHT and T2DM in Malaysian population, replicate studies with larger sample size are needed to confirm the associations of three genetic polymorphisms or others such as D9N and N291S of LPL gene, G927R of IRS-1 gene and C1429T polymorphism of GNB3 gene with EHT or T2DM in Malaysian subjects are recommended.

Competing interests

The authors declare that they have no competing interests.

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