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Insulin Receptor Substrate-1 Gene (G972R) Polymorphism and Metabolic Syndrome in Type-2 Diabetes Mellitus Patients

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Metabolic syndrome (MS) is one of the fastest growing health problems worldwide. It is major risk factor for both diabetes and cardiovascular disease. Many candidate gene studies have identified the linkage between MS and a number of genes. The insulin receptor substrate-1 (IRS-1) gene especially at codon 972 (G972R) is also a candidate for insulin resistance in type 2 diabetes and MS. The aim of the present study was to determine the insulin receptor substrate-1 gene (G972R) polymorphism and MS in type 2 diabetes mellitus (T2DM) patients. The genomic DNA of the 100 subjects from Diabetes Out-patients Department of YGH was amplified by polymerase chain reaction (PCR) and digested by restriction fragment length polymorphism (RFLP) with BstN1 used for codon 972. Metabolic syndrome was defined as in IDF (International Diabetes Federation) criteria. Metabolic syndrome was found in 84% (16/19) of carrier patients and 82% (67/81) of non-carrier patients. It was not significantly associated with presence or absence of IRS-1 (G972R) mutation. There was a significant difference (p=0.005) in proportion of MS between IRS-1 (G972R) non-carriers with family history of diabetes mellitus and those without. It, therefore, seems reasonable to argue that family history of T2DM does not play an important role in the development of MS in the IRS-1 (G972R) non-carrier.

Key words: Insulin receptor substrate-1, Metabolic syndrome

INTRODUCTION

Metabolic syndrome (MS) is a very common, multi-component condition characterized by insulin resistance, dyslipidaemia, abdominal obesity and hypertension, that is associated with an increased risk of T2DM, cardiovascular disease and artherosclerosis.[1]

Insulin resistance is present in the majority of people with MS. Thus, metabolic syndrome is strongly associated with risk for incident diabetes, likely because some of its components are more strongly associated with diabetes risk.[2] Hong Kong has one of the highest prevalence of diabetes (8.6%) and various components of MS (11.6-23.6%) in Asia.[3] Metabolic syndrome was detected in 39.6% of patients with acute ischaemic stroke and 50% of patients with acute myocardial infarct in Myanmar subjects.[4, 5]

The etiology is complex, determined by the interplay of both genetic and environmental factors.[6] Candidate gene studies have identified linkage between MS and a number of genes such as PPAR gamma, adiponectin, and beta-adrenergic receptors.[7] Moreover, De la Cruz-Mosso, *et al.*[8] suggested that the -844 G/A PAI-1 polymorphism was related with the risk of

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developing metabolic syndrome, obesity and atherogenic dyslipidemia, and the HindIII C/G PAI-1 polymorphism was associated with the increase of total cholesterol levels in Mexican children.

In addition to these genes, in a previous insulin receptor substrate-1 (IRS-1) proteins have been identified and IRS-1 functions as one of the key downstream signaling molecules pathway.[9, 10]

The binding of insulin to its receptor triggers a complex signaling cascade of protein phosphorylation and dephosphorylation culminating in the metabolic and mitogenic effects of insulin. Elucidation of insulin signaling pathway has been central to understanding the mechanisms underlying insulin resistance in diabetes. The insulin receptor is a tetrameric protein composed of two α- and two β-subunits. The β-subunit cytosolic domain possesses tyrosine kinase activity. Insulin binding to the α-subunit extracellular domain activates the β-subunit tyrosine kinase, resulting in both autophosphorylation of the receptor and phosphorylation of downstream signal transduction elements. The autophosphorylation of the insulin receptor tyrosine residues starts up a protein phosphorylation cascade. First out are a set of proteins known as insulin-receptor substrates (IRS-1-4). These are coupled to several additional protein kinase signal systems: Pathways signaling through PI3-kinase and phosphatidylinositol (3, 4, 5) P3 (PI-3 kinase and protein kinase B/Akt), Mitogen-activated protein kinases (MAPKinases).[11] Thus, genetic changes in IRS-1 may potentially contribute toward the development of insulin resistance, the most common of these being a glycine to arginine change at codon 972 (G972R).[9]

And also, the G972R polymorphism of IRS-1 gene was associated with insulin resistance, salt sensitivity and non-dipper hypertension.[12] However, not many studies exist concerned with relationships between IRS-1 gene polymorphism and MS. Therefore, the aims of the study were to determine IRS-1 gene (G972R) polymerphism and proportion of MS in T2DM patients and also examine the relationships between gene polymorphism and MS.

MATERIALS AND METHODS

After getting informed consent, history taking and physical examinations including anthropometric measurement were done and 5 ml of blood samples were collected from diabetic patients attending Diabetes Outpatients Department of YGH. Fasting blood sugar, lipid profile, polymerase chain reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) for IRS-1 gene were measured at Pathology Research Division, Department of Medical Research. Data were analyzed by SPSS (version 16.0) statistical software. Metabolic syndrome was defined as in IDF (International Diabetes Federation) criteria. The disease association with proportions of sample variables was tested by ˈChiˈ square test with 95% confidence interval.

Ethical consideration

The study including proforma and written informed consent form was submitted to the Ethical Review Committee, UM (I), Yangon to obtain ethical approval study.

RESULTS

Among 100 T2DM patients, 81 patients were of homozygous (G/G) genotype, 18 patients were of heterozygous (G/A) and only one patient of homozygous (A/A) genotype. The allele frequencies of ˈGˈ was 90% and that of "A" was 10% (Fig. 1).

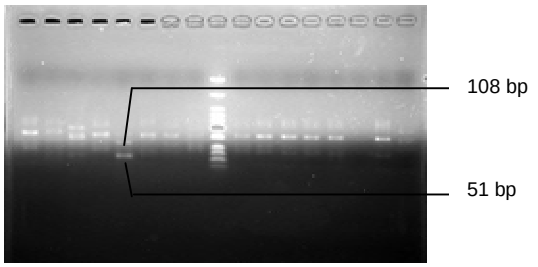

Fig. 1. Homozygous A/A genotype of codon 972 IRS-1 gene at lane 5

Table 1. Distribution of metabolic syndrome in the IRS-1 (G972R) carrier and non-carrier groups

Metabolic syndrome	Carrier (n=19) (%)	Non-carrier (n=81) (%)	Remark
Presence	16(84)	67(82)	NS
Absence	3(16)	14(17)	

NS=not significant, P value=0.876

Metabolic syndrome distribution in the IRS-1 (G972R) carrier and non-carrier groups

Metabolic syndrome was found in 84% (16/19) of carrier patients, 82% (67/81) of non-carrier patients. Metabolic syndrome was not significantly associated with presence or absence of IRS-1 (G972R) mutation (Table 1).

Table 2. Metabolic syndrome between IRS-1 (G972R) non-carriers with and without family history of diabetes mellitus (DM)

Metabolic syndrome	IRS-1(G972R) non-carrier		Total	Remark
	With family history of DM	Without family history of DM		
Presence	15	52	67	S
Absence	4	10	14	
	19	62	81	

S=Significant, P value=0.005

Metabolic syndrome between IRS-1 (G972R) non-carrier with and without family history of diabetes mellitus

There was significant difference (p=0.005) in proportion of MS between IRS-1 (G972R) non-carriers with family history of diabetes mellitus and those without (Table 2).

DISCUSSION

Prevalence of IRS-1 (G972R) polymorphism

The prevalence of G972R polymorphism in the present study was much higher than other studies in Asia region. The BMI of the present study was 28.35±4.36 which was much higher than 24±3.0 (Japan),[13] 25.69±5.27 (Northern Indian)[14] and 25.1±4.3 (South Indian).[15] But the prevalence of G972R does not seem to be related to BMI since the prevalence was quite high 15.8% in the Orkunoglu, *et al.*[16] study in which BMI is 22.14±3.98. Polymorphism plus other environmental and metabolic risk factors could increase one's chance of developing T2DM.

Metabolic syndrome distribution in the IRS-1 (G972R) carrier and non-carrier groups

The prevalence of MS seems to vary among different study populations based on the presence of risk factors including BMI, life styles, ethnicity, race, age and sex. It was 83% in the present study, 85% in Scott, *et al.* study,[17] 59.5% in Ranjith, *et al.*,[18] 54.2% in Rojas, *et al.*,[19] 64.6% in Chung-Hua.[20]

The prevalence of MS was varied when the criteria used for the diagnosis of MS were different. Using the clinical definitions, namely the original NCEP-ATP III, the prevalence of MS in the Philippines in 2003 was 11.9%. It became 18.6% when the modified AHA/NHLBI criteria were used.[21] Similarly, the prevalence of MS as defined by the NCEP ATP III criteria was 60.4% whereas it was close to it, but not exactly the same, 59.5% in young Indian patients when the IDF criteria were used.[18]

In the present study, MS was not significantly associated with IRS-1 (G972R) polymorphism (84% in carrier *vs.* 82% in non-carrier). Such absence of association between MS and insulin resistance was reported also by Ranjith, *et al.*[18]

But Sarac, *et al.* study showed there was statistically significant association of IRS-1 and IRS-2 polymorphisms with MS.[22] One reason for absence of association between MS and polymorphism is the present study included 100 overweight and obese subjects with T2DM and Sarac, *et al.*[22] study had 100 MS subjects and 30 normal control subjects. In addition, the present study determine the relationship between MS and G972R carrier and non-carrier group and that of Sarac, *et al.*[22] study showed relationship between gene and 100 MS and control groups.

Metabolic syndrome between IRS-1 (G972R) non-carrier with and without family history of diabetes mellitus

In the present study, the proportion of IRS-1 (G972R) non-carrier without family history of diabetes mellitus was higher than that with family history of diabetes mellitus in the MS patients, so there was a significant association between MS and without family history of diabetes mellitus in IRS-1 (G972R) non-carrier. In a nationally representative sample of United State adults without diabetes, family history of diabetes shows a significant, independent association with metabolic syndrome.[23]

Although family history of diabetes associated with MS,[24] the prevalence of chronic diabetic complications and metabolic syndrome is not associated with maternal type 2 diabetes in Scheffel, *et al.* study.[25] It, therefore, seems reasonable to argue that family history of T2DM does not play an important role in the development of MS in the IRS-1 (G972R) non-carrier.

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