

Does Genetic Polymorphisms Affect Health?

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Professor Dr. Patimah Ismail



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Does Genetic Polymorphisms Affect Health?



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Does **Genetic**
Polymorphisms
Affect
Health?



PROFESSOR DR.PATIMAH ISMAIL

Does **Genetic** **Polymorphisms** Affect Health?

PROFESSOR DR.PATIMAH ISMAIL

B.Sc Hons (Malaya), Ph.D (Wales, UK)

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ABSTRACT

Genetic polymorphisms are variations found in DNA sequences and they are integral to the development of genetic markers to identify individuals at risk and it requires certain techniques and strategies to detect the mutations. Most of the variations found in DNA sequences are stable and occur in the form of single nucleotide polymorphism (SNPs), insertion/deletion (I/D) and variable tandem repeats. Among the genetic polymorphisms, SNPs are the most abundant form in the human genome, accounting for more than 90% of all differences among individuals. Variation occurs when a single nucleotide (A, T, C, or G) alters in the genome sequence. SNPs are stable, di-allelic and the two alleles represent the “wild-type” and the “mutant type” forms. SNPs are frequently studied in relation to various cancers for their known biochemical or physiological function. Several studies have been done in understanding the possible role of genetic variation in the human genome. Identifying the association between the ancestral variants in genes are common in polygenic diseases which become increasingly reasonable with improved methods for detecting genetic variants on a genome-wide scale. Over the years, several researches have been conducted to detect the cause of various genetic disorders. Genetic polymorphisms play an important role in human health especially in the development of essential hypertension and type 2 diabetes mellitus. Our genetic research group works toward understanding the interaction between genes and environmental exposures in various human diseases such as congenital heart disease, hypertension, metabolic syndrome, end stage renal disease, prostate cancer, polycystic kidney disease etc. Our group research has two main elements: investigating the role of environmental exposure in critical target gene mutation and the

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role of genetic susceptibility and environmental exposure in those disorders. In addition, more fundamental researches are going on in order to know the genetic and allelic frequency of certain susceptibility genes for the development of various disorders.

INTRODUCTION

Genetic Polymorphism

Genetic polymorphism is defined as the inheritance of a trait controlled by a single genetic locus with two alleles, in which the least common allele has a frequency of about 1% or greater whereas the term mutation is usually reserved for DNA sequence variants that cause diseases and are present in the population at a frequency of less than 1% (Gelehrler and Collins, 1990). Genetic polymorphisms are different forms of DNA sequence. “Poly” means many, and “morph” means form. Polymorphisms are a type of genetic diversity within a population’s gene pool.

DNA sequences are different among individuals in groups and populations. Most of the variations found in DNA sequence are stable and occur in the form of single nucleotide polymorphism (SNPs), insertion/deletion (I/D) and variable tandem repeats. SNPs are stable, they are di-allelic, the two alleles represent the “wild-type” and the “mutant type” forms (Doris *et al.*, 2002). Variation occurs when a single nucleotide (A, T, C, or G) alters in the genome sequence. Common sequence variants occur in about 1 in every 1000 bases of coding/ regulatory sequence. In every gene there is an average of 4-8 SNPs occurring either in the exons or coding regions or in the nearby exons and introns boundaries in the upstream regulatory regions. Each person would be heterozygous for 24,000-40,000 non-synonymous (amino acid altering) substitutions (Lele *et al.*, 2003).

How Do Mutations Occur?

Genetic polymorphisms may be the result of chance processes, or may have been induced by external agents that could be such as viruses or radiation causes. If there are differences in DNA

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sequence among individuals, it has been shown to be associated with diseases and it is usually called a genetic mutation. Changes in DNA sequence which have been confirmed to be caused by external agents are also generally called “mutations” rather than “polymorphisms” (Smith, 2002).

Mutation can occur when there is a mistake which can be called copying errors when DNA replicates itself. Other changes are introduced as a result of DNA damage which is due to environmental agents including sunlight, cigarette smoke and radiation in which these mutations are not passed on to children. Germline mutation, which can be passed from a parent to a child when a mutation occurs in the DNA of cells that produce eggs and sperms and every cell in their body, will have this error in their DNA and cause diseases to run in families, and are responsible for the hereditary diseases.

Types of Mutations

Human DNA polymorphisms can be split into two groups: single nucleotide polymorphisms and insertion/ deletion (INDELS) of one or more nucleotides. Insertion/Deletion can be further divided into those with multiple alleles (multiallelic) and those with only two alleles (diallelic). Nearly all of the multiallelic indels are based on tandem repeats, mostly short tandem repeats. More recently, millions of SNPs candidates have been identified and are beginning to apply (Sachidanandam *et al.*, 2001)

Single Base Substitutions

A SNP (“snip”) is a single base mutation in DNA and it is a source variance in a genome. SNPs are the most simple form and most common source of genetic polymorphism in the human genome (90% of all human DNA polymorphisms).

There are two types of nucleotide base substitutions resulting in SNPs.

1. A transition substitution occurs between purines (A, G) or between pyrimidines (C, T). This type of substitution constitutes two thirds of all SNPs.
2. A transversion substitution occurs between a purine and a pyrimidine.

Mis-sense Mutations

Mis-sense mutation is a point mutation in which a single nucleotide is changed which results in a codon that codes for a different amino acid. For example in sickle cell anaemia the 17th nucleotide of the gene for the beta chain of haemoglobin is changed from 'A' to 'T'. This changes the codon from 'GAG' to 'GTG' resulting in the 6th amino acid of the chain being changed from glutamic acid to valine (Figure 1). This apparently trivial alteration to the beta globin gene alters the quaternary structure of haemoglobin, which has a profound influence on the physiology and well being of an individual.

	Thr	Pro	Glu	Glu
Normal	ACT	CCT	GAG	GAG
			↓	
Mutated	ACT	CCT	GTG	GAG
	Thr	Pro	Val	Glu

Figure 1 An example of mis-sense mutation in which a substitution of 'A' in the second codon to a 'G' leads to an amino acid substitution of Glutamine to Valine.

Nonsense Mutations

In a nonsense mutation, the new base changes a codon that specified an amino acid into one of the STOP codons (TAA, TAG, TGA). This will cause translation of the mRNA to stop prematurely and a truncated protein to be produced (Figure 2). This truncated protein will not function correctly. Nonsense mutations occur in between 15% to 30% of all inherited diseases including cystic fibrosis, haemophilia, retinitis pigmentosa and duchenne muscular dystrophy.

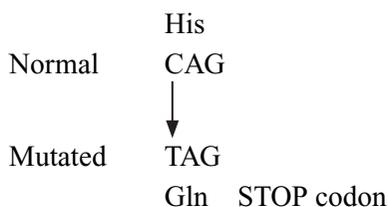


Figure 2 An example of nonsense mutation (Histidine to Glutamine) which can occur in Cystic fibrosis patients.

Silent Mutations

Silent mutations are mutations that will not change the final protein product and can only be detected by sequencing the gene. Most amino acids that make up a protein are encoded by several different codons. As an example, the third base in the 'CAG' codon is changed to an 'A' to give 'CAA', which is a glutamine which would still be incorporated into the protein product. This is because the mutated codon still codes for the same amino acid. These types of mutations are 'silent' and have no detrimental effect.

Frameshift Mutations

A frameshift mutation is caused by insertions or deletions of a number of nucleotides that is not evenly divisible by three from a DNA sequence. This mutation in general causes the reading of the codons to code for different amino acids, but there may be exceptions resulting from the redundancy in the genetic code. If the stop codon (“UAA”, “UGA” or “UAG”) in the original sequence would not be read, then a stop codon could result at an earlier or later site (Figure 3). Thus the protein resulting from this could either be abnormally short or abnormally long, and will most likely not be functional.

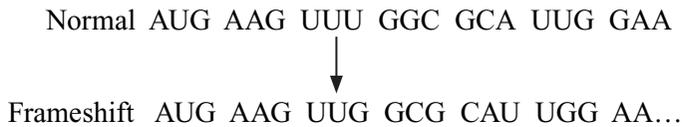


Figure 3 Deletion of U occurs in Tay-Sachs disease.

Single Nucleotide Polymorphisms

Single nucleotide polymorphisms are the most common type of genetic variation among people. Findings from the Human Genome Project show that the DNA of any two people is more than 99.9 percent identical, but 0.1 percent accounts for all the genetic differences among people. In literal terms, that means that one person might have blue eyes rather than green, or a susceptibility to lung cancer, or perfect pitch, because the sequence of their DNA - a long chain of adenine (A), guanine (G), cytosine (C) and thymine (T) molecules – differs from another person. Rather than having an A-T pair of molecules at a certain spot on the DNA chain, a person might have a G-C pair. On the other hand, that difference might

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not have any effect at all on a person's health or appearance. SNPs in humans are unknown, but there are probably between 10 and 30 million SNPs, about one every 100 to 300 bases. Of these SNPs, perhaps four million are common SNPs, with both alleles of each SNP having a frequency above 20 percent. Most SNPs have no effect on health or development, but some of these genetic differences have proven to be very important in the study of human health.

Polymorphisms usually affect on gene structure, function and regulation and can identify the individuals who are predisposed to disease (Collins *et al.*, 1997). The functional polymorphisms affect gene regulation or protein sequence whereas non-functional polymorphisms do not. Some SNP alleles are the actual functional variants that contribute to the risk of getting a disease (Ralston *et al.*, 2002). Individuals with such a SNP allele have a higher risk for that disease than do individuals without that SNP allele. Most SNPs are not these functional variants, but are useful as markers for finding them. To find the regions with genes that contribute to a disease, the frequencies of many SNP alleles are compared in individuals with and without the disease. SNP alleles in a particular region are more frequent in individuals with the disease than in individuals without the disease, those SNPs and their alleles are associated with the disease. These associations between a SNP and a disease indicate that there may be genes in that region that contribute to the disease.

For most SNPs, any population has individuals of all possible genotypes for a SNP, but populations differ in the frequencies of individuals with each of the different genotype. About 85 percent of human SNPs variation are within all populations, and about 15 percent are among populations. Thus two random individuals within a village are almost as different in their SNP alleles as any two random individuals from anywhere in the world. Although a

small proportion of SNPs have alleles that are common in some groups but rare in others, most SNP alleles that are common in one group will be common in other groups. Under the Common-Disease/Common-Variant theory, common variants that contribute to a disease in one group will also contribute to the disease in other groups, although the amount of the contribution may vary (<http://www.genome.gov>, Haplotype Map Development Report).

RISK FACTORS

Essential hypertension (EHT) and Type 2 Diabetes Mellitus (T2DM) are the two most common polygenic complex disorders caused from interaction of several genes with each other and with other factors such as obesity, dietary salt intake, smoking and alcohol consumption. Framingham study reported the prevalence of hypertension increases significantly with age and the age-related rise in blood pressure and it was observed consistently in most populations (Ihab *et al.*, 2001). People at age 35 and older are at risk of developing hypertension. But the prevalence of hypertension increases to 10.7% and exceeds 45% in those over 60 years of age. This may be due to an exposure to environmental factors and lifestyle. The normal age-related rise in blood pressure is exaggerated in population with T2DM which may be due to premature vascular aging or vascular inflammation leading to increase arterial stiffening.

Diabetes tends to run in families. A person with a family history of diabetes is 2-4 times as likely to develop diabetes as someone without a family history. Familial and genetic factors may also play an important role in the determination of some major risk factors, especially hypertension, lipid abnormalities and glucose intolerance. The genetic link for T2DM is stronger than the genetic link for type 1. Having a blood relative with T2DM increases the risk in both

men and women. Experimental models of genetic hypertension have shown that the inherited tendency to hypertension resides primarily in the kidney. For example, animal and human studies show that a transplanted kidney from a hypertensive donor raises the blood pressure and increases the need for antihypertensive drugs in recipients coming from “normotensive” families. Conversely a kidney from a normotensive donor does not raise the blood pressure in the recipient.

Cigarette smoking is another major risk factor for developing hypertension. Cigarette smoking can repeatedly produce a transient rise in BP of approximately 5 to 10 mmHg (Freestone *et al.*, 1982). Habitual smokers generally have lower blood pressures than non-smokers as observed in most (Mikkelsen *et al.*, 1997) but not all studies (Primatesta *et al.*, 2001). Smoking in any hypertensive patient markedly increases the risk of secondary cardiovascular complications and appears to enhance the progression of renal insufficiency (Regalado *et al.*, 2000).

The incidence and mortality of coronary heart disease (CHD) is twice as high in male cigarette smokers compared to non-smokers (Fodor and Tzerovska, 2004). The risk of developing CHD is directly related to the number of cigarettes smoked. Individuals who discontinue smoking may reduce this risk within a year or two, although it still remains slightly higher compared to non-smokers. Even though dietary habits can have a direct effect on blood pressure, lifestyle choices such as alcohol intake, cigarette smoking, physical activity, and obesity can profoundly impact blood pressure regulation (Tam *et al.*, 2005).

When a person smokes, the body resists insulin and there is an increase in glucose level lead to diabetes. Adding smoking to diabetes drastically raises the risk of other diabetic complications. Smoking is also related to the premature development of

microvascular complications of diabetes and may even have a role in the development of T2DM (Haire-Joshu *et al.*, 1999). Kawakami *et al.* (1997), examined the effects of smoking on the 8-year incidence of T2DM on 2,312 Japanese men and concluded that age of smoking initiation and number of cigarettes smoked are major risk factors for developing diabetes. In another study, the effects of cigarette smoking are evaluated on insulin sensitivity of 40 patients with T2DM and found that insulin resistance is markedly aggravated among those who smoked (Targher *et al.*, 1997).

Obesity

The prevalence of obesity is rapidly increasing in developing as well as in industrialised countries resulting in a prohibitive health and economic burden on society (Hall *et al.*, 2002). Dietary intake and physical activity are important contributing factors in the development of obesity. If calorie intake is in excess of requirement and is not utilised overtime, it will be stored mainly as body fat and later it will lead to overweight or obesity. Obesity people are more likely to develop high blood pressure than others. The relationship between obesity and blood pressure appears to be linear and exists throughout the non-obese range. But the strength of the association of obesity with hypertension varies among different racial and ethnic groups (Shai *et al.*, 2006). The overall scheme for the mechanism of obesity which can cause several disorders is shown in Figure 4.

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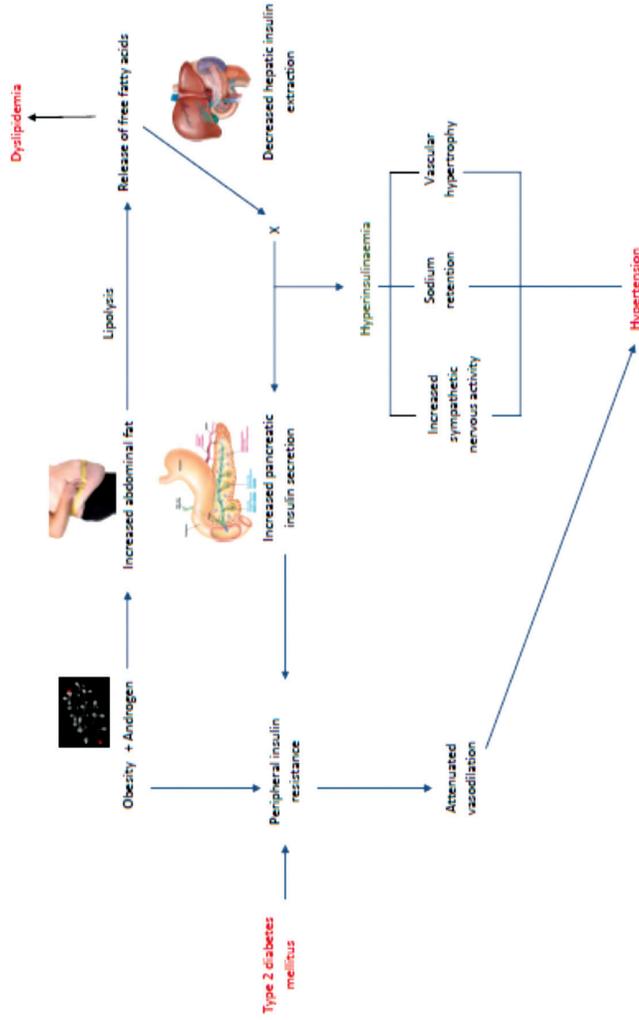


Figure 4 Overall scheme for the mechanisms by which obesity, if predominantly upper body or visceral in location, could promote diabetes, dyslipidaemia and hypertension via hyperinsulinaemia (Adapted from Vikrant and Tiwary, 2001).

Body weight of an individual is usually determined by an interaction between genetic, environmental and psychosocial factors acting through the physiological mediators of energy intake and expenditure. A strong positive correlation between body mass index and blood pressure has been noted consistently in cross-sectional and longitudinal studies (Calderon *et al.*, 2005). Obese individuals have higher cardiac output, stroke volume, and central and total blood volume and lower peripheral resistance than non-obese individuals with similar blood pressure (Oren *et al.*, 1996). Increased body mass index (BMI) and/or waist/hip ratio (WHR) increases the risk of hypertension, and it has been found that waist circumference (WC) is positively associated with high blood pressure (Feldstein *et al.*, 2005).

Adolescent obesity is also associated with the insulin resistance syndrome which includes hypertension and T2DM. Obesity is thought to be susceptible to type-2 diabetes primarily by causing insulin resistance (Dewan *et al.*, 2003). Insulin resistance places a greater demand on the pancreatic capacity to produce insulin, which also declines with age, leading to the development of clinical diabetes. Physical inactivity, both a cause and consequence of weight gain, also contributes to insulin resistance.

Obesity is a major underlying cause of metabolic syndrome which includes abdominal obesity, hypertension, insulin resistance with or without glucose intolerance, or diabetes (Sarti & Gallagher, 2006). The RAAS seems to be activated in obesity, despite a state of volume expansion and sodium retention. Elevated serum aldosterone levels have been reported in the obese (Engeli *et al.*, 2002) and it is postulated by a fatty acid derived from adipose tissue causes the release of a hepatic factor that, in turn, steps up aldosterone synthesis (Goodfriend *et al.*, 1999).

Cholesterol

Cholesterol is a fat-like substance presents in cell membranes that circulates in the blood and it is a precursor of bile acids and steroid hormones. Cholesterol travels in the blood in distinct particles containing both lipid and proteins (lipoproteins). It circulates through Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL). Hypertension and hypercholesterimia (high intake of dietary fats) often co-exist as risk to CHD. Several studies have reported the relation between blood pressure and cholesterol level (Lewington and Clarke, 2005). More than 40 percent of people with diabetes have abnormal levels of cholesterol and similar fatty substances that circulate in the blood. These abnormalities appear to be associated with an increased risk of cardiovascular disease among persons with diabetes. The strong association between hyperlipidemia, hypertension and T2DM lead to an extensive list of long-term complications, including a high rate of cardiovascular death and even amputation due to accelerated atherosclerosis, and also the typical complications of diabetes such as retinopathy, nephropathy, and neuropathy (Khan *et al.*, 1996).

Low-density lipoprotein accounts for the majority of cholesterol in the blood. It is sometimes referred to as “bad” cholesterol because it can build up in the arteries and cause heart disease and stroke. On the other hand High-density lipoprotein help to remove cholesterol from the body and referred to as “good” Cholesterol. Higher levels of HDL more than 60mg/dl are better in which it can protect the heart disease.

Triglycerides are a type of fat in which majority of them are circulating in the blood. They are carried throughout the body by a special lipoprotein called a chylomicron. Triglycerides can come from the fat we eat, or fat which is made in the body from carbohydrates. Too many triglycerides in the blood are called

hypertriglyceridemia, which is a risk factor for cardiovascular disease (heart disease). People on low carbohydrate diets rarely have triglycerides above normal (150 mg/dL). High triglyceride levels are associated with low HDL, obesity, diabetes and high pressure (Fruchart *et al.*, 2005). The presence of increased triglycerides and decreased HDL levels are the best predictor of cardiovascular disease in patients with T2DM (Laakso *et al.*, 1993). Plasma triglyceride level is frequently increased in T2DM, mainly due to an increased number of VLDL particles (Howard *et al.*, 1995). In type 2 diabetic patients, the major features of dyslipidemia are increased triglyceride levels, decreased HDL-C levels, and changes in the composition and level of LDL-C (Hobbs *et al.*, 2006). Epidemiologic data indicate that risk factors for coronary disease in T2DM include elevated LDL-C levels, HDL-C levels, elevated blood pressure, hyperglycaemia and cigarette smoking (Turner *et al.*, 1998). Although concentrations of total and LDL cholesterol in diabetic individuals are reportedly comparable with levels found in people without diabetes, low levels of HDL cholesterol and elevated triglyceride levels, both are probable contributors to cardiovascular diseases (CVD) (Laakso *et al.*, 1996). The prevalence of these abnormalities appears to be approximately twice as high as in patients with T2DM.

Serum Electrolytes

Serum electrolytes such as sodium, potassium and chloride play a central role in the normal regulation of blood pressure (Giasuddin *et al.*, 1991). Especially sodium and potassium regulate the fluid balance of the body and influence the cardiac output. Several studies suggested that the present levels of intake of mineral elements are not optimum for maintaining normal blood pressure but predispose to the development of hypertension.

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Sodium plays a central role in many physiologic functions including the regulation of intravascular and extravascular volume status and blood pressure levels (Ajani *et al.*, 2005). Sodium retention occurs as a characteristic alteration in Type I or Type II diabetes; exchangeable body Na⁺ is increased by 10% on average. This abnormality develops in the uncomplicated stage of diabetes and differentiates diabetic from non-diabetic essential hypertensive subjects. The pathogenetic role of sodium retention in diabetes-associated hypertension is supported by positive correlations between systolic or mean blood pressure and Na⁺ and by normalisation of blood pressure after removal of excess sodium by diuretic treatment in hypertensive diabetic subjects. The latter may also have an enhanced sensitivity of blood pressure to sodium. Plasma levels of active renin, angiotensin II, aldosterone, and catecholamine are usually normal or low in metabolically stable type I or type II diabetes. However, an exaggerated vascular reactivity to nor epinephrine and angiotensin II commonly occurs already at uncomplicated stages of Type I or Type II diabetes. This may be a manifestation of functional (i.e., intracellular electrolytes) and/or morphological (proliferation, narrowing, and stiffening) vasculopathy. Diabetes-associated sodium retention, vasculopathy, and a presumably inherited predisposition for both diabetes and EHT may represent important complementary factors favoring the frequent occurrence of hypertension in the diabetic population (Weidmann and Ferrari, 1993). In a large prospective study of nutritional factors and development of hypertension among American men, a low potassium intake is a predictor for the development of hypertension with a relative risk of 1.54 for subjects in the lowest quintile of potassium intake (Acherio *et al.*, 1992).

Genetic Factors

Hypertension is an important public health challenge worldwide because of its prevalence and its role as a risk factor for cardiovascular disease. Hypertension is a complex genetic disorder caused by interplay between several ‘risk’ genes and environmental factors. Genetic elements contribute to 30–50% of the blood pressure variability in human essential hypertension (Garcia *et al.*, 2003). In 2005, Kearney *et al.* reported that an estimated total number of adults with hypertension in 2000 was 972 million. Of these, 333 million were estimated to be in economically developed countries and 639 million in economically developing countries. By 2025, the number of people with hypertension will increase by about 60% to a total of 1.56 billion as the proportion of elderly people will increase significantly. Other reasons are the continuing population increase and changes in lifestyle, which include a diet rich in sugar and high-fat processed foods and sedentary behavior, mediated by television, computers and cars. Since the proportion of hypertensive people will increase dramatically worldwide, the prevention, detection, treatment and control of this condition should be a top priority.

Hypertension is about twice as common in subjects who have one or two hypertensive parents. Many epidemiological studies suggested that genetic factors account for approximately 30% of the variation in blood pressure in various populations (Izawa *et al.*, 2003). Although separate genes and genetic factors have been linked to the development of EHT, multiple genes are most likely to contribute the development of the disorder in a particular individual (Figure 5). The role of these genes is extremely difficult to determine the relative contribution to the disease. It has been well supported from comparisons of parents with their monozygotic and dizygotic twin studies (Koskenvuo *et al.*, 1992).

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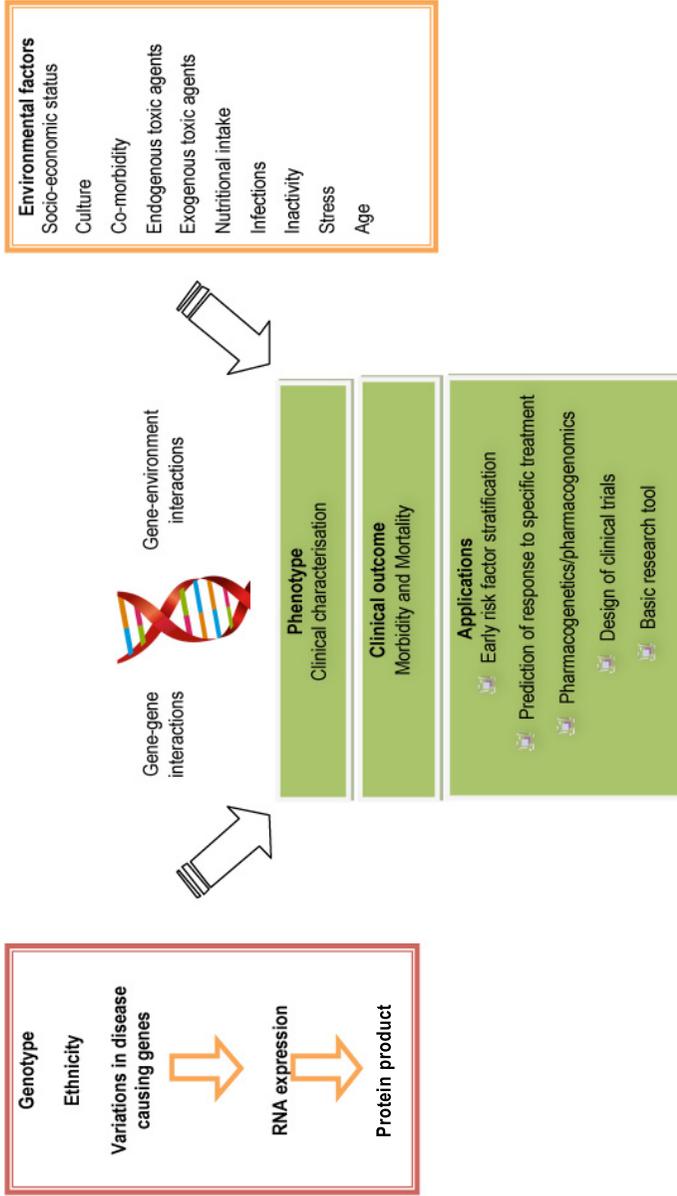


Figure 5 Schematic diagram shows the interaction among genetic and environmental factors in the development of hypertension (Adapted from Nordfors *et al.*, 2005)

By acquiring detailed knowledge about an individual's genetic background, through the analysis of polymorphisms and their impact on gene expression and protein levels, as well as the influence of environmental exposure, a big step is taken towards an early prediction of a patient's clinical outcome, thereby making it possible to identify 'high-risk patients' at an early stage of the disease. However, the mechanisms that resulted in a specific phenotype are complex and represent a great challenge for clinicians and researchers, considering the great number of existing both known and unknown gene-gene and gene-environment interactions. The genetic and clinical data collected may be used for several applications, such as early risk factor stratification, for prediction of response to a specific treatment, in pharmacogenetics and pharmacogenomics, for the selection of participants in clinical trials and finally as a basic research tool (Nordfors *et al.*, 2005)

The identification of genetic variants that contribute to the development of hypertension is complicated, for example cardiac output and total peripheral resistance phenotypes determine the development of hypertension but also controlled by intermediary phenotypes, including the autonomic nervous system, vasopressor/vasodepressor hormones, the structure of the cardiovascular system, body fluid volume and renal function, and many others. Moreover, these intermediary phenotypes are also controlled by complex mechanisms including blood pressure itself (Williams *et al.*, 1994). Thus there are many genes that could participate in the development of hypertension. Genetic factors also influence behavioral patterns, which might lead to BP elevation. Tendencies towards obesity or alcoholism are influenced by both genetic and environmental factors; thus the proportion of blood pressure variability caused by inheritance is difficult to determine and may vary in different populations (Adamo and Tesson, 2008).

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Genes play an important role in the development of T2DM. T2DM is a polygenic disorder with multiple genes located on different chromosomes contributing to its susceptibility. Analysis of a particular genetic factor is further complicated by numerous environmental factors interrelated with genes to influence the disorder.

The prevalence of T2DM varies widely among different populations. Part of the observed ethnic variability can be attributed to non genetic environmental factors; however, the fact that the prevalence of the disease varies substantially among ethnic groups that share a similar environment suggests that genetic factors also contribute to predisposition to the disease (Diamond, 2003). Another source of evidence for genetic contribution to the disease is familial aggregation also share common environmental traits. The odd ratio for offspring of a single affected parent is 3.5 and 6.1 if both parents are affected compared to those with no parental diabetes history (Meigs *et al.*, 2000). The report from several studies supported a genetic basis for measures of both insulin sensitivity and insulin secretion (Gerich *et al.*, 1998).

Understanding the genetic variation that contributes to the pathogenesis of T2DM will require the use of a wide variety of in vitro and in vivo tools for functional analysis. Understanding the contribution of genetic variation to disease within populations will require a simultaneous acquisition of detailed genetic and environmental (life-style) data from very large population cohorts and case-control studies.

GENETIC POLYMORPHIC STUDIES

Study Strategies

There are two main strategies for identifying genes involved in human disease. In monogenic disorders linkage analysis is performed using panels of genetic markers (i.e. microsatellite markers) to identify a particular locus that co-segregates with disease in affected families. However in multifactorial disorders, where a phenotype depends on complex interactions between several genes, as well as with environmental factors, it is more difficult to identify a particular gene variant contributing to a specific phenotypic trait. In this case, the most common approach is the association studies using sets of SNPs in candidate genes for genotyping of clinically well-characterised patient materials, which need to be large enough to detect also small effects of SNPs. A critical step in such association studies is to identify candidate genes for investigation. In contrast to linkage studies, candidate gene association studies therefore require previous understanding of the pathophysiologic pathways involved in a particular trait.

Basic research strategies that may be applied in studies of patients with end-stage renal disease are;

- Linkage studies in families
- Monogenic disorders
- Microsatellite markers
- Association studies in populations
- Polygenic disorders
- Well-characterised clinical material
- High throughput SNP-analysis
- Microarray technology (DNA chips)
- Hypothesis generation

The genetic polymorphisms are integral to the development of genetic markers to identify the individuals at risk of developing the diseases. To identify the common disease susceptibility loci, many studies encompassing genome-wide association mapping, linkage (Krushkal *et al.*, 1999), candidate gene association (Yamada *et al.*, 2002) have been utilised. It has been realised that genetic susceptibility to the common complex disorders probably involves many genes, most of which have small effects (Cordell and Clayton, 2005)

Candidate Gene Approach

The search for susceptibility genes in predisposes to hypertension and T2DM centers on two major techniques, linkage mapping and the candidate gene approach. Linkage mapping also known as positional cloning, is the process of systematically scanning the entire genome of various members of families affected by the disorder using regularly spaced, genetic variation those exact position is known (i.e., genetic markers). Using those families, the genetic regions associated or “in linkage” with the disease can be identified by observing that affected family members which share certain alleles located in those regions more frequently than would be expected by chance. These regions can then be isolated, or cloned, for further analysis and characterization of the responsible genes. Linkage mapping techniques have already resulted in the identification of several potential DNA regions that may contain susceptibility genes for hypertension (Caulfield *et al.*, 2003) and T2DM (Huang *et al.*, 2006). The primary advantage of linkage mapping is that investigators need no prior knowledge of the physiology or biology underlying the disorder being studied, which is important for complex disorders. Whereas the linkage mapping approach is an unbiased search of the entire genome without any

preconceptions about the role of a certain gene, the candidate gene approach allows researchers to investigate the validity of an “educated guess” about the genetic basis of a disorder.

The candidate gene approach involves assessing the association between a particular allele or a set of alleles of a gene that may be involved in the disease. In other words, this type of association study tries to answer the question, “Is one allele of a candidate gene more frequently seen in subjects with the disease than in subjects without the disease?” The candidate genes are selected on the basis of known biochemical or physiologic components related to disease (Kohara *et al.*, 2008). In contrast with linkage mapping studies, however, studies of candidate genes do not require large families with both affected and unaffected members, but can be performed with unrelated cases and control subjects or with small families. Moreover, candidate gene studies are better suited for detecting genes underlying common and more complex diseases where the risk associated with any given candidate gene is relatively small (Kwon *et al.*, 2000, Collins *et al.*, 1997).

Case-Control Study

Genetic studies can be categorised into candidate gene approaches and genome-wide screens. Association studies are adequate for testing candidate genes, or narrowing down to a particular gene once a region of linkage has been detected. The association or case-control study tests the different allele or genotype frequencies between the case and control populations which allow the use of large set of unrelated individuals using this paradigm rather than using a pedigree-based linkage analysis. In general, a case-control study has greater statistical power than a linkage analysis, but it is also more liable to show false positive results.

Does Genetic Polymorphisms Affect Health?

This strategy is to search for the association between one or more markers within the critical interval and the disease under investigation. This is based on the concept that the susceptibility allele is more prevalent in the Hypertensive, T2DM group or any other disease, than in the controls (Lander and Schork, 1994). In recent years the population based case–control studies of unrelated individuals earned the status of gold standard in the search for the association of the sequence differences with complex diseases. The important advantage is a large analytical power and the disadvantages are frequent results of borderline significance and lack of confirmatory reports from other populations or ethnic groups (Ardile *et al.*, 2002; Morton and Collins, 1998). These studies have greater power to detect the effects of common variants (Risch & Merikangas, 1996).

Control subjects are a valid comparison group for these association studies, though it could not be predicted whether some of them would develop various diseases in future. Statistical evidence for an association between an allele and a phenotype comes from one of three situations. (i) The allele itself might be functional and directly affect expression of the phenotype. (ii) The allele might be correlated with, or be in linkage disequilibrium with, a causative allele located nearby. (iii) The association could be attributable to chance, artifact, or selection bias (Agarwal *et al.*, 2005). Usually, association studies are performed using a case-control cohort format with all the difficulties of ensuring that the controls are true controls (Cardon *et al.*, 2003). Population stratification (namely the separation of a study population into subgroups) is often a confounding factor. Association and linkage studies are complementary methods that, together, provide the means to probe the genome and describe the genetic etiology of complex human traits, potentially elucidating the mechanism leading to some of

the most important contemporary health problems (Williams *et al.*, 1997).

Some questions to be answered when reading a research paper on association between DNA polymorphisms and phenotype (Adapted from Nordfors *et al.*, 2005);

- Is the material large enough (i.e >200-300 patients) to be sufficiently powered
- Have controls been evaluated?
- Have the positions of SNPs been clearly defined?
- Are data about Hardy-Weinberg calculations presented and are the criteria fulfilled in controls?
- Has linkage disequilibrium among different SNPs on the same chromosome been evaluated?
- If the material has sufficient power – has a haplotype analysis been performed?
- Has the protein products been analysed?
- Does the SNP affect transcription of the gene or protein expression?
- Has a multiple regression analysis been performed accounting for the impact of age, race, gender and co-morbidity?
- Are the results confirming the results in other patient groups?

Cross Sectional Study

A cross-sectional study is an observational study in which disease and exposure status is measured simultaneously in a given population (Kelsey, 1996). It is also called as ‘Synchronic study’ which provide a snapshot of the frequencies and characteristics

of exposure/disease in a population at a particular point in time and, as a result, valuable insights for delineating the multi-step association between exposure and disease occurrence (Kang *et al.*, 2005). This strategy is to search for the association between one or more markers within the critical interval and the disease under investigation. This is based on the concept that the susceptibility allele is more prevalent in EHT, T2DM group or any other disease, than in the controls (Lander and Schork, 1994). The cross-sectional studies take place at a single point in time and cannot identify cause-and-effect relationships, though they do identify the existence of health problems. The cross-sectional studies are considered to be “hypothesis generating”, such that clues to exposure/disease relationships can often be seen than case-control, cohort studies (Kelsey, 1996)

CANDIDATE GENES OF HYPERTENSION AND TYPE 2 DIABETES MELLITUS

Several studies have been suggested that, several genes were hypothesised to be involved in both EHT and T2DM. Some of the candidate genes showed no consistent definition for hypertension, other than those used clinically and also there are no differences between the ratios of positive to negative studies whether a rigorous or loose definition is used. Moreover there is no gene consistently associated with or linked to EHT and T2DM (Agarwal *et al.*, 2005, Radha *et al.*, 2007; Van Ittersum *et al.*, 2000).

The screening of candidate genes for nucleotide variants that are associated with EHT and T2DM is a core component of much diabetes and hypertension genetics research. Historically, several studies of the genetics of EHT and T2DM have analysed to select the candidate genes because of known or presumed biological function, with an understandable focus on the biochemical

pathways. The choice of candidates are inevitably limited by our incomplete understanding of the regulation of the processes and the pathophysiology of EHT and T2DM.

Genetic Polymorphisms in Hypertension

The World Health Organization (WHO) definition of High blood pressure or hypertension is a systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 95 mmHg. Hypertension is a major chronic disease in the world and it is present in 20-30% of adult population. Essential hypertension which is a heterogeneous disorder, with different patients having different causal factors that lead to high BP, account for 98% of hypertensive patients, whereas secondary hypertension is due to underlying diseases such as renal artery disease, adrenal hormone excess or drugs (Beevers and MacGregor., 1999). It has been approximated that there are nearly 972 million people with hypertension world-wide (Vasan, 2009). Epidemiological studies demonstrated that hypertension is a great risk of cardiovascular diseases such as stroke and heart attack. (kannel, 2004). The environmental factors that contribute to the development of hypertension include smoking, alcohol consumption, salt intake and inactivity.

Polymorphisms of Renin Angiotensin-Aldosterone System

Renin Angiotensin-Aldosterone System (RAAS) is familiarly involved in the regulation of blood pressure; a genetic variability in the degree of expression of one of its enzymes may account for variability in the blood pressure or may play a role in elevated high blood pressure. The genes coding for each protein involved in the RAAS have been identified and studied for any relation with hypertension and other cardiovascular disorders (Malik *et al.*, 1997).

Does Genetic Polymorphisms Affect Health?

Genetic polymorphisms involved in RAAS genes susceptibility to hypertension and T2DM have been intensively scrutinised by linkage and association studies in various populations. Insertion/Deletion polymorphism of angiotensin-1 converting enzyme (ACE) gene, M235T, T174M, A20C and A6G variants in angiotensinogen (AGT) gene, *BgII*, *MboI* dimorphism in Renin (REN) gene, A1166C polymorphism of angiotensin II type1 receptor (AT1R), T344C, Lys173Arg variant in aldosterone synthase (CYP11B2) and the Gly460Trp polymorphism of adducin (ADD) gene have been implicated in the pathogenesis of hypertension, renal and cardiovascular complications of diabetes (Fabris *et al.*, 2005). Genetic variants of RAAS genes are well studied in various populations with conflicting results.

Angiotensin Converting Enzyme Gene Polymorphism

Angiotensin Converting Enzyme gene is a major component in RAAS which has been determined as a candidate gene for the development of EHT (Agachan *et al.*, 2003). The Framingham Heart Study found strong evidence for a QTL on chromosome 17, located close to the ACE gene and linked to blood pressure (Levy *et al.*, 2000). The ACE gene encodes somatic and germinal isoenzymes. The somatic ACE isoenzyme is expressed in many tissues including vascular endothelial cells, renal epithelial cells and testicular leydig cells, whereas the testicular or germinal ACE isoenzyme is expressed only in sperm. Tissue-bound ACE is essential for the control of BP and the structure and function of the kidneys (Lele *et al.*, 2003).

The ACE polymorphisms appear to have a significant impact on the progression of Hypertension and Diabetes (Figure 6). The ACE (dipeptidyl carboxypeptidase) is a zinc metallopeptidase, which catalyses the conversion of angiotensin I to angiotensin II, a potent

vasoconstrictor and through protease activity it also inactivates bradykinin, a potent vasodilator (Erdos and Skidgel, 1987). ACE gene has been mapped to chromosome 17q23 and encoded by a 21 Kb gene that consists of 26 exons and 25 introns (Mattei, *et al.*, 1989).

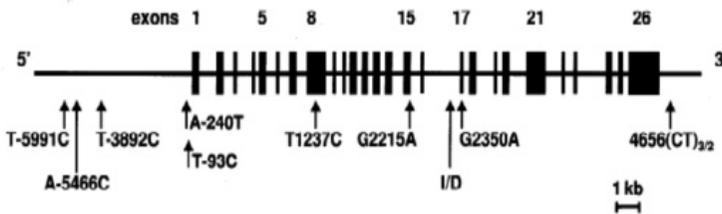


Figure 6 Schematic diagram showing the biallelic polymorphisms numbered in base pairs relative to the start of the transcription sites between the exons 1-26 of ACE gene (Adapted from Keavney *et al.*, 1998).

Insertion/ Deletion Polymorphism of ACE Gene

Insertion/Deletion (I/D) polymorphism of a 287 bp Alu repeat sequence in intron 16 of ACE gene is strongly associated with plasma and cellular ACE levels (Rigat *et al.*, 1990) and it indicates that, the polymorphism may modulate the expression of ACE gene (Suehiro *et al.*, 2004). Plasma ACE levels are affected mainly by this polymorphism and subjects that are homozygous for the deletion (DD), heterozygous (I/D) and those that are homozygous for the insertion (I/I) have the highest, intermediate and lowest ACE plasma levels, respectively (Rigat *et al.*, 1990).

A relationship between the D-allele dose and enzymatic levels is established for both circulating and cellular ACE (Tiret *et al.*, 1992). The DD genotypes and D alleles of ACE gene have been reported in various populations in which it has a strong association

with increased risk of hypertension (Zee *et al.*, 1992) diabetes and its complications (Marre *et al.*, 1994) compared to II genotypes and I allele of ACE gene. However, some studies have failed to show the association of D allele (Chiang *et al.*, 1997) and diabetes (Daimon *et al.*, 2003). A strong association of I allele of ACE gene is found in Australian population with familial hypertension (Zee *et al.*, 1992). The conflicting results of I/D polymorphism of ACE gene in hypertension and other diseases might be due to different ethnic groups and genders (Barley *et al.*, 1994).

Angiotensinogen Gene Polymorphisms

Angiotensinogen (AGT) is the precursor of angiotensin II, a polypeptide primarily produced by the liver which acts as a physiologically important regulator of blood pressure, exerting physiologic effects on the sodium homeostasis, as well as a growth factor of cardiac myocytes. Gaillard *et al.*, (1989) found that the human angiotensinogen gene contains 5 exons (Figure 7). In 1992, Jeunemaitre *et al.*, reported significant evidence for linkage and association of the (AGT) gene to hypertension.

The AGT polymorphisms might not only be associated with plasma concentrations of AGT, but might also increase the eventual risk of developing hypertension. Several lines of evidence have supported the etiological link between AGT and hypertension; for example, in transgenic mice, the increasing number of AGT alleles was shown to be correlated with blood pressure elevation (Kim *et al.*, 1995). An angiotensinogen M235T polymorphism has been found to be associated with high plasma AGT levels in subjects that are homozygous for the T variant and hence associated with hypertension in European and Asian populations (Danser *et al.*, 1998)

The TT or allele T of the M235T variant of the AGT is significantly associated with EHT in which it is a possible genetic marker or risk factor for hypertension in Malaysian subjects (Say *et al.*,2005). Rather than focusing on the M235T polymorphism in Malaysian population and to surpass the limitation of previous association studies, it looks for other susceptible polymorphism of the AGT gene for EHT and T2DM. The promoter polymorphisms A6G and A20C, which may play regulatory roles in the AGT gene expression, are suggested to be located in the same haplotype block with the M235T polymorphism (Hilgers, *et al.*, 2001). Inoue *et al.*, 1997 revealed that the elevation of plasma angiotensinogen is not due to the M235T substitution itself but a linked A for G substitution at position -6 in the core promoter of the AGT gene. The A6G polymorphism affects the basal rate of AGT transcription with the -6A variant causing an increase in transcription and elevated plasma angiotensinogen levels is associated with hypertension (Hunt *et al.*, 1998).

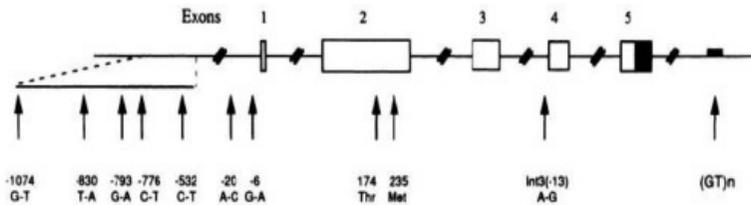


Figure 7 Schematic diagram of the human Angiotensinogen gene and location of identified variants. The positions of the variants in the 5' region are numbered by reference to the transcription-initiation site as defined by Gaillard *et al.*, (1989).

A6G, a common variant in the proximal promoter of the ATG gene, an adenine instead of a guanine 6 bp upstream from the site of transcription initiation, is in very tight linkage disequilibrium with

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T235 and marks the original form of the gene. These observations suggested a biologic mechanism by which individual differences in the AGT gene may predispose carriers to the development of EHT. In addition, C for A substitution at position 20 polymorphism of the angiotensinogen gene core promoter that may cause altered gene transcription have been described: and elevated plasma angiotensinogen leads to increased angiotensinogen transcription in vitro. Evidence has been provided that this polymorphism affects transcription factor binding and the gene transcription rate (Zhao *et al.*, 1999). The -20 C allele is associated with hypertension and elevated plasma angiotensinogen in a Japanese population (Ishigami *et al.*, 1997). An adenine-to-cytosine transition at nucleotide -20 of the 5' upstream core promoter region of the human AGT gene (A-20C) mutation transcribes a reporter gene at a greater level than the wild type (A-20A). Therefore, the A-20C mutation may also affect the transcription activity of AGT mRNA in humans and thereby alter the plasma AGT level (Ishigami *et al.*, 1997).

Aldosterone Gene Polymorphism

Aldosterone Synthase Gene Polymorphism

Aldosterone controls sodium balance and intravascular volume and plays an important role in regulating the blood pressure (White, 1994). Aldosterone is synthesized from deoxycorticosterone in the zona glomerulosa of adrenal cortex by a mitochondrial cytochrome P450 enzyme, aldosterone synthase (CYP11B2) (Curnow *et al.*, 1991). The CYP11B2 gene encoding aldosterone synthase is mapped to chromosome 8q24.3 which is adjacent to a closely related gene that encodes steroid 11 β -hydroxylase (CYP11B1), an enzyme required for cortisol biosynthesis (Mornet *et al.*, 1989).

Conversely, an inherited form of hypertension, glucocorticoid-suppressible hyperaldosteronism, is caused by genetic recombination

between the CYP11B1 and CYP11B2 that increase expression of the CYP11B2 and lead to inappropriate secretion of aldosterone (Lifton *et al.*, 1992). Therefore, it is plausible that polymorphisms in CYP11B2 might affect aldosterone biosynthesis and thus perhaps influence hypertension.

Mutations in the CYP11B2 lead to various forms of aldosterone deficiency, characterised by salt wasting and hypotension (Mitsuuchi *et al.*, 1993) suggesting that abnormalities in CYP11B2 can lead to important changes to arterial pressure and could be responsible for hypertension. White and Slutsker, (1995) determined the haplotypes in CYP11B2 has strongly associated with hypertension or plasma aldosterone concentrations.

Davies, *et al.*, (1999) have indicated the role of the T344C polymorphisms in aldosterone synthase gene in hypertension. But studies in Japanese and German populations showed the negative association of the T344C polymorphisms in association with hypertension (Kato *et al.*, 2002). However, it is impossible to conclude which polymorphism is responsible for high blood pressure or high aldosterone secretion. On the other hand, Fardella *et al.*, 1996 indicated that Arg173 alleles are found higher in hypertensive than the normotensive group. They also found amino acid polymorphism showing Lys173 rather than Arg173 in CYP11B2 among a large number of Chilean patients with low-renin hypertension. The decreased frequencies of Arg173 and 2344C variants in the CYP11B2 gene are genetically linked to low-renin hypertension in the Japanese population.

Adducin Gene Polymorphism

Adducin, the rate-limiting factor in RAAS, plays a crucial role in the regulation of blood pressure. Adducin is a heteromeric cytoskeleton protein composed of α and β -subunits. Variation in the α -adducin

(ADD1) protein may affect ion transport through modification of actin cytoskeleton assembly and modulation of sodium pump activity (Staessen and Bianchi, 2005). Transport of sodium by the renal proximal tubule determines whole-body volume and fluid homeostasis and blood pressure and it has been suggested that defects in transport mechanisms may be the cause of hypertension (Woolfson *et al.*, 1996).

Studies using Millan hypertensive and normotensive strains of rats suggested that genetic alterations in tubular reabsorption may be a cause of hypertension (Bianchi *et al.*, 1994). A case-control study in Milan of 190 white hypertensive patients using several microsatellite markers on chromosome 4 found a significant association with hypertension for one located closest (20 kb) to the α -adducin gene (Casari *et al.*, 1995). Cusi *et al.*, (1997) found a significant association between a Gly460Trp polymorphism (G460W) in the ADD1 gene and salt sensitivity in patients with EHT. Several studies have suggested that the association or linkage of G- to-T substitution polymorphism of amino acid residue 460 at nucleotide position 614 of exon 10 of the α -Adducin gene in EHT with controversial results in different populations.

In Italian, French and Japanese population studies of the Gly460Trp polymorphism, a significant association of the mutated allele (Trp) with hypertension is shown (Castellano *et al.*, 1998). All these data strongly supported the notion that Adducin polymorphism is associated to high blood pressure. Follow-up studies of different populations do not replicate the initial report whereby no association is found between the Gly460Trp allele and hypertension in Chinese (He *et al.*, 2001), Scottish (Clark *et al.*, 2000) or Japanese population (Ranade *et al.*, 2000).

Adrenoceptors

All adrenergic receptors have seven membrane-spanning domain, extra cellular amino terminal and intra cellular carboxy terminals. They are also linked to guanine-nucleotide- binding proteins (G proteins) which are composed of α , β and γ subunits (Garland & Biaggioni, 2001). The α -adrenoceptors mediate smooth muscle contraction, vasoconstriction, ejaculation and sodium excretion. The β -adrenoceptors mediate smooth muscle relaxation, vasodilatation, bronchial relaxation and lipolysis.

Sympathetic nervous system plays an important role in the regulation of blood pressure and energy expenditure via coupling of catecholamine with adrenoceptors. (Masuo, 2010)

Polymorphisms of β 2-adrenoceptor

The β 2-adrenergic receptor has been well studied. Nine single nucleotide polymorphisms are found in the coding region of the DNA sequence. Four of these result in amino acid change: the glycine for arginine substitution at codon 16 (Arg16Gly), the glutamic acid for glutamine substitution at codon 27 (Gln27Glu), the threonine for isoleucine substitution at codon 164 (Thr164Ile) and the methionine for valine substitution at codon 34 (Val34Met). All these polymorphisms encode the receptor with functional differences except the Val34Met polymorphism. (Garland and Biaggioni, 2001). The most common polymorphisms are Arg16Gly with an allele frequency of 0.4/0.6 and Gln27Glu with an allele frequency of 0.55/0.45 in the Caucasian population. The Thre164Ile polymorphism is rare occurring in only 3 to 5 % of the general population. (Masuo, 2010).

Beside the polymorphisms of the β 2 adrenergic receptor in the coding region, there is also another polymorphism in the 5' leader

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citron of the receptor resulting in an arginine or cysteine of amino acid 19. This polymorphism with a frequency of approximately 0.47 in whites, it can affect receptor expression. (Garland and Biaggioni, 2001). Other studies showed that Gly16 receptors have greater reduction in number or enhanced down-regulation compared with Arg16 (wild type). On the other hand, Glu27 receptor is resistant to down-regulation when compared with Gln27 (wild type).

The finding of the polymorphisms in the β 2adrenergic receptor allows the researchers to correlate the polymorphism with diseases that involve of β 2-adrenergic receptor activity such as hypertension. Studies have shown that the Gly16 homozygous subjects had higher mean blood pressure than Arg16 homozygous. They also found that Arg16 is associated with greater vasodilator response to β 2 agonist salbutamal compared with the Gly16 allele. (Garland and Biaggioni, 2001).

Other studies with similar findings demonstrated that young normotensive white men homozygous for the Gly16 allele have higher blood pressure and lower peripheral vasodilatation after the administration of β 2 agonist salbutamal (Masuo, 2010).

Another longitudinal study done by Masuo *et al.*, showed that Gly16 allele is related greater weight and blood pressure elevation over 5 years period. In this study 160 young, nonobese nomotensive individuals were recruited. Change in blood pressure, body weight, plasma noepinephrine and β -adrenergic receptor polymorphisms were measured over a period of 5 years. The result showed increase in body weight, blood pressure in Gly16 allele carriers. Subjects with Glu27 allele have higher blood pressure.

From the results obtained in these studies, it can be said that the functional molecular variations of β 2-adrenergic receptor might cause attenuated vasodilatation leading to increase in total peripheral resistance and consequently resulting in hypertension. (Kato *et al.*, 2002)

Polymorphisms of β 3-adrenoceptor

The β 3 adrenergic receptor is mainly located in adipose tissue and it is involved in the regulation of lipolysis and thermogenesis. Due to the role of the receptor in the lipolysis, studies have been extensively done to correlate the receptor variant and obesity. A polymorphism in causing the substitution arginine for tryptophan at codon 64 (Try64Arg) has been well studied. This polymorphism has been shown to lower receptor affinity for catecholamine and decrease receptor function. (Masuo, 2010). This slow lipolysis may lead to visceral obesity and obesity related diseases such as hypertension and diabetes mellitus. Other epidemiological studies have shown strong association between β 3 adrenergic receptor polymorphism (Try64Arg), hypertension, metabolic syndrome and obesity. (Masuo, 2010)

Alpha-Adrenoreceptor Gene Polymorphism

Catecholamines play an important role in the regulation of blood pressure and metabolism in humans (Gavras *et al.*, 2001). There are three known α 2-adrenoceptor subtypes, α 2A, α 2B, and α 2C, which are encoded by distinct genes at different chromosomes. Knockout animal studies have suggested that sympathetic outflow from the central nervous system is inhibited by stimulation of the α 2A-adrenergic receptor, thus mediating a hypotensive effect, whereas stimulation of the α 2B -adrenergic receptor mediates a hypertensive effect by opposing the sympatho-inhibitory action of the α 2A-adrenergic receptor in the central nervous system. The α 2C-adrenergic receptor does not seem to mediate any effect in blood pressure regulation (Gavras *et al.*, 2001).

A polymorphism in the non-coding region of the α 2A-adrenoceptor gene has been reported to be associated with salt-

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sensitive hypertension in an African-American population (Lockette *et al.*, 1995). But a common variant (12Glu9) of the human $\alpha 2B$ -adrenergic gene encodes a receptor protein leading to the deletion/insertion of three consecutive glutamate residues at amino acid positions 301 to 303 has been associated with EHT (Wowern *et al.*, 2004) and acute coronary events (Snapir *et al.*, 2001). Several studies suggested that I/D polymorphism of $\alpha 2B$ -adrenergic receptor gene control the sympathetic nervous system activity and possibility to develop EHT (Wowern *et al.*, 2004).

In a population-based, prospective study on 912 middle-aged men, the DD genotype is associated with an increased incidence of acute myocardial infarction (AMI) in comparison to the other two genotypes (Snapir *et al.*, 2003). Several reports in Caucasians revealed the association of various cardiovascular and metabolic phenotypes with I/D polymorphism of $\alpha 2B$ -adrenergic receptor gene (Heinonen *et al.*, 1999). However some reports do not support a role for I/D polymorphism of $\alpha 2B$ -adrenergic receptor gene in late complications in type 1 diabetic patients (Heinonen *et al.*, 2005) and EHT (Snapir *et al.*, 2001).

CANDIDATE GENES FOR CONGENITAL HEART DISEASE

Homocysteine Metabolism Enzyme Gene Polymorphisms and Congenital Heart Disease

Congenital heart disease (CHD) is known as a serious birth defect where there is an abnormal development of the structure and function of the heart that are present at birth. It is among the most common human congenital defects occurring in 6-8 out of 1000 live births (Hoffman *et al.*, 2002). There are many factors causing the CHD etiology whereas both genetic and environmental factors also play an important role in the etiology, but the specific

cause still remains unclear (Song *et al.*, 2006). Homocysteine is a naturally occurring amino acid that is present in the humans. It is involved in the metabolism of the essential amino acid methionine which is a product used in transmethylation reactions that utilise S-adenosylmethionine (AdoMet) as a methyl donor. After being formed, homocysteine can be catabolised to amino acid cysteine or it can be used to regenerate AdoMet (Kluijtmans *et al.*, 2003).

Hyperhomocysteinemia is an elevated level of homocysteine in the blood and some of the factors that cause this could be due to genetic disorders, vitamin B12 and vitamin B6 deficiency, folic acid deficiency, renal failure, increasing age, hypothyroidism and smoking (Pereira *et al.*, 2005). This condition has also been associated with the development of CHD and many genetic association studies have been done to investigate this association.

A few types of CHD are induced by single gene mutation and chromosome aberration but most of CHDs are polygenic diseases caused by both genetic and environmental factors (Zhu *et al.*, 2004). The common enzymes involved in homocysteine or folate metabolism are the Methylenetetrahydrofolate Reductase (MTHFR), Methionine Synthase (MTRR), Methionine Synthase Reductase (MTR) and Cystathionine-b-synthase (CBS) enzymes. Deficiencies or polymorphism in any of these enzymes are suspected to lead to an inborn error in the metabolism of homocysteine and also causing homocystinuria (Michael *et al.*, 2000). There is evidence that this deficiency could alter the susceptibility to CHD (Garcia-Fragoso *et al.*, 2010).

Various studies have been done in various populations on the association of these enzyme gene polymorphisms and development of CHD but with conflicting results. This may be due to the differences between ethnic or geographic populations. Population stratification which is the separation of a study population into

subgroups may also occur if the case and control populations are not well matched for ethnicity or geographic origin.

For the MTHFR enzyme, it is one of the main regulatory enzymes of the homocysteine metabolic pathway and it involves the two most common gene polymorphism which are the C677T and the A1298C (Frosst *et al.*, 1995). These polymorphisms may result in decreased enzyme activity and causes increased level of homocysteine in individuals especially those with low folate status (Castro *et al.*, 2003). A study done in Germany by Junker *et al.*, 2001 was the first study that showed an association between C677T gene polymorphism and the development of CHD. Other studies done for the MTHFR gene polymorphism can also be found in Puerto Rico and India (Elavazhagan *et al.*, 2009, Garcia-Fragoso *et al.*, 2010).

A study done in Beijing, China showed a nuclear family study on the association of the MTR enzyme of the A2756G gene polymorphism and CHD. They have found that mothers having the polymorphism may have an increase risk of having a child born with CHD (Zhu *et al.*, 2004). For the MTRR A66G enzyme gene polymorphism, a study done in The Netherlands investigated the relation between this polymorphism and CHD risk (Van Beynum *et al.*, 2006).

Michael *et al.*, 2006 reported a high prevalence of the 844ins68 gene polymorphism of the CBS enzyme. Since CBS enzyme is also involve in the homocysteine metabolic pathway, researchers suggested that it may also be related to development or risk of CHD. Various studies have also been done to investigate this relationship. For example a study done by Zhu *et al.*, 2008 found an association between the CBS gene variation and serum homocysteine level associated with CHD and a Chinese nuclear family study by Song *et al.*, 2006 showed that there is also a relationship between the CBS polymorphism and CHD.

A genetic association analysis involving a study of the SNPs genotype frequency in a group of cases and controls could be used to examine the statistical correlation between a person's genotype with his phenotype or disease. So, by this, the genetic polymorphisms of the homocysteine metabolism genes can provide a basic for studying the relationship between genetic variants and the development or progression of CHD.

Cardiac Transcription Factors Gene Polymorphism

Members of cardiac transcription factors genes family codes for proteins (transcription factors) that regulate the expression of genes responsible for embryonic cardiac development which include the formation of the heart chambers (atria and ventricles), valves and the conducting system. Because of this critical role, mutations within these genes will affect the gene expression which in turn will contribute to the pathogenesis of the malformed heart (Clark *et al.*, 2006). Among the transcription factors gene family there is four genes: GATA4, TBX5, TBX20 and NKX2-5 that had been implicated more in the cause of CHD.

GATA4 Gene Polymorphism

GATA4 gene is a one of the transcription factors genes that localised at chromosome 8p23.1-p22. The gene consists of 7 exons and contains two zinc finger domains that are highly conserved in mammals. It expresses in the heart during embryogenesis and plays a major role in the process of the heart development (Clark *et al.*, 2006). The GAtA4 gene regulates the expression of MYH6 gene which play an important function in the development of the cardiac atria (Molkentin and Kalvakolanu 1994). It has 2 zinc finger domains: C-terminal and N-terminal domains, the C-terminal

domain encoded by exon 2, mediates DNA binding and interaction with other proteins including Nkx2-5 and TBX5 (other transcription factors) while the N-terminal domain encoded by exon 3, help in stabilising the binding to DNA and interacting with a subclass of zinc finger protein multi-type (ZFPM) previously named friend of GATA4 (FOG) (Clark *et al.*,2006).

TBX20 Gene Polymorphism

TBX20 is an ancient member of the Tbox superfamily related to TBX1, located on chromosome 7p15-p14, it contains 6 exons and its 22 kb size, code for 297-amino acid protein (Meins *et al.*,2000) and interact with GATA4, GATA5, TBX5 and NKX2-5 genes (Stennard *et al.*,2003).

The first report of the association between TBX20 and CHD done by (Kirk *et al.*,2007) by screen the coding region of TBX20 by direct sequencing in 352 patients with different forms of CHD and 11% of those patients have a family history of CHD, two unique mutations are reported missense I152M (456C>G) and nonsense Q195X (583C>T) mutations within the T-box DNA-binding domain TBX20 in two Caucasian families with history of various forms of CHD (septation defects, valve abnormalities (mitral valve stenosis and prolapse) in addition to cardiomyopathy. Analysis of these mutated protein showed direct effects of this mutation on the protein structure, its thermal stability, and DNA binding.

A study conducted by (Liu *et al.*,2008) to determine the TBX20 mutations in Han Chinese population and whether there is an association of these mutations with CHD, involved 203 patients with various forms of CHD (62 of them are TOF patients) and 300 control were also involved in this study in which only 6 patients had family history of CHD, the exons 2-6 which coded

for T-box domain and their flanking introns were sequenced after PCR amplification, Three non-synonymous sequence alternation (A63T, I121F, and T262M) were identified in 3 patients (two ASD patients one of them had an additional Total anomalous pulmonary venous connection and one TOF patient) which were not seen in 300 controls, I121F and T262M mutations occurred within the highly conserved T-box DNA-binding domain and the change in the amino acid was expected to affect protein function according to SIFT program (Sorting Intolerant From Tolerant), in addition to two synonymous sequence variants(c.666C>T and c.786G>C) detected only in two patients and not detected in the control and eight SNPs detected both on the patients and controls but the difference in the allele frequency between the patients and controls was statistically insignificant, four of them reported for the first time (S167S, P177P, A181A, and I219I) and the other four were known SNPs in dbSNP (c.766T>C as rs3999941, c.808C>T as rs3864455, c.859C>T as rs2723753 and c.883A>C as rs2723754). The frequency of TBX20 mutations in the CHD patients was about 1.5% and in TOF patients was about 1.6% (one of 62) so TBx20 mutations in Chinese population were low and contributed to ASD, TOF and Total anomalous pulmonary venous connection (Liu *et al.*,2008)

Endothelial Nitric Oxide Synthase Gene Polymorphism

Nitric oxide, which plays important role in the regulation of cell growth and apoptosis, is produced by the enzyme Endothelial nitric oxide synthase (eNOS) Common 894G>T in the gene code for eNOS associated with birth defects such as spina bifida, and its association with CHD have been investigated both in the affected children and their mothers, an association of the sum of the eNOS

894 GT and TT genotypes versus GG genotypes in the children is associated with increased CHD risk, while in the mothers the sum of eNOS 894 GT or TT genotypes versus GG genotypes is not associated with increased CHD risk (Van Beynum *et al.*, 2008).

TECHNIQUE TO DETECT SINGLE NUCLEOTIDE POLYMORPHISM

PCR- Restriction Fragment Length Polymorphism (PCR-RFLP)

A variation in the DNA sequence result either in polymorphism or mutation which leads to chances of causing different diseases. Some of these polymorphisms that occur with a high frequency within the population can be a useful tool for gene tracking for a given disease. Such investigations have initially been done by Southern blot techniques, but now it has been replaced by PCR based methodology. By using restriction enzyme analysis or Restriction Fragment Length Polymorphisms (RFLPs), the nucleotide substitutions can be identified (Figure 8). Currently, SNP typing is becoming more and more popular in genetic research and some typing methods have been developed with many other methods, such as Taqman assay, the ligase detection reaction and D-HPLC (Kirk *et al.*, 2002), developed in recent years but still RFLP-PCR is relatively simple and economical (Haliassos *et al.*, 1989). A restriction fragment length polymorphism is defined by the existence of alternative alleles associated with restriction fragments that differ in size from each other. The basic principle of RFLP is the DNA restriction enzymes recognise specific sequences in DNA and catalyse endonucleolytic cleavages, yielding fragments of defined lengths. Differences among individuals in the lengths of a particular restriction fragment may be displayed by electrophoresis in agarose

gels, separating the fragments according to their molecular size. The results from many kinds of genotypic differences shows one or more individual bases could differ, resulting in loss of a cleavage site or formation of a new one; alternatively, insertion or deletion of blocks of DNA within a fragment could alter its size. These genotypic changes can be recognised by the altered mobility of restriction fragments on agarose gel electrophoresis (Botstein *et al.*, 1980).

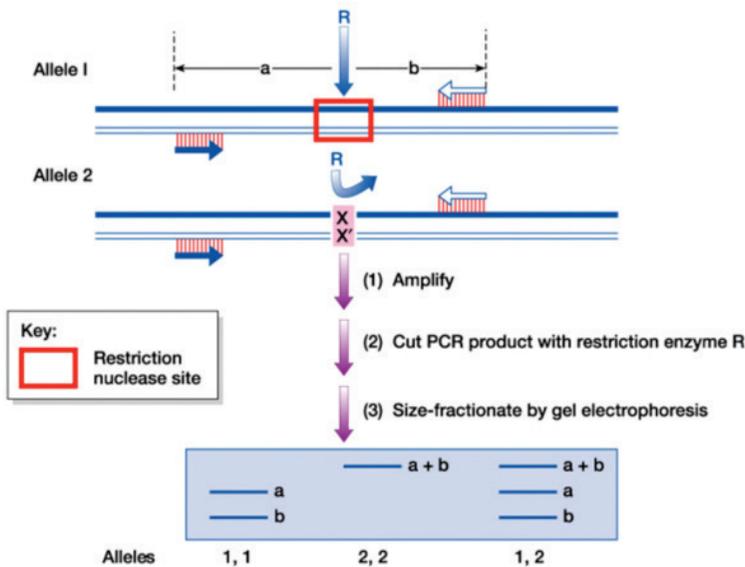


Figure 8 Schematic diagram shows the principle involved in PCR-RFLP method (Adapted from Human Molecular Genetics, 2004)

RFLPs were first used as a tool for genetic analysis in 1974. Linkage of temperature-sensitive mutations of adenovirus to specific restriction fragment length differences was used to locate the mutations on a physical map of the restriction fragments (Grodzicker *et al.*, 1974). Although RFLP is a useful technique in finding out the known mutations, it has got several limitations,

including the fact that the flanking regions of most SNP loci do not have appropriate restriction endonuclease recognizing site for typing and cannot able to detect the unknown mutations. The main advantage of this approach is that it does not require the use of radioactive isotopes and it is more acquiescent to analyses in clinical settings.

Restriction Enzymes

Restriction endonucleases (REs) are present in bacteria presumably to destroy DNA from foreign sources (e.g., infecting bacteriophage) by cleaving the foreign DNA at specific restriction sites. The host bacteria DNA is protected from cleavage because specific recognition sites are modified, usually by methylation at one of the bases in the site, making the site no longer a substrate for RE cleavage. Host bacteria used to propagate cloned DNA in the laboratory are usually mutant in the host restriction genes; thus their intracellular enzyme activities will not destroy the foreign recombinant sequences. The cleavage site specificities for many REs have been defined. They cut DNA within or near to their particular recognition sequences, which typically are four to six nucleotides in length with a twofold axis of symmetry. These ends are complementary (“sticky”) and can be enzymatically reattached to any other EcoRI generated termini using T4 DNA ligase. Many restriction enzymes, like EcoRI, generate fragments with protruding 5’ tails; others (e.g., PstI) generate fragments with 3’ protruding, cohesive termini, whereas still others (e.g., Ball) cleave at the axis of symmetry to produce blunt-ended fragments. Each restriction endonuclease has a specific sequence and number of nucleotides required to create the recognition site. Some REs do not require a specific nucleotide in every position of the recognition site.

These enzymes allow cloning and purification as well as for RFLP technique. REs are typically isolated from a variety of bacterial strains and available commercially (Roberts, 1982).

Agarose Gel Electrophoresis

Electrophoresis is a technique used to separate and sometimes purify macromolecules - especially proteins and nucleic acids - that differ in size, charge or conformation. Amplified PCR products, restriction fragments and other products can be identified or separated by using the standard method through agarose gel electrophoresis. Molecules in a mixture can be separated according to size by electrophoresis, a technique dependent on the fact that dissolved molecules in an electric field move at a speed determined by their charge-mass ratio. The basic principle behind in this technique is the nucleic acids in solution generally have a negative charge because of the presence of phosphate groups are ionized; thus they migrate toward a positive electrode. Molecules separated into the electrophoresis in an agarose gel move through the pores, the longer length nucleic acids moves slowly compared to the shorter length sequences. Agarose gel electrophoresis technique is simple, rapid to perform, and capable of resolving mixtures of DNA fragment that cannot be separated adequately by other sizing procedures (Sambrook, 2001). Agarose, which is extracted from seaweed, is a linear polymer is commercially available in the market. Agarose is usually contaminated with other polysaccharides, salts, and proteins which can affect both the migration of the DNA and the ability of the DNA recovered from the gel to serve as a substrate to enzymatic reactions. Agarose gels are cast by melting the agarose in the presence of the desired buffer until a clear, transparent solution is achieved. The melted solution is then poured into a mold and allowed to harden. Upon hardening, the agarose forms a matrix, the density of which is determined by

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its concentration. When an electric field is applied across the gel, DNA, which is negatively charged at neutral pH, migrates toward the anode. Furthermore, the location of DNA within the gel can be determined directly by staining the gel with the intercalating dye ethidium bromide and can be detected by direct examination of the gel in ultraviolet light (Sharp *et al.*, 1973).

GENETIC POLYMORPHIC STUDIES IN MALAYSIA- RESEARCH PAPERS FROM GENETIC RESEARCH GROUP

Hypertension and Type 2 Diabetes Mellitus

Analysis of renin-angiotensin aldosterone system gene polymorphisms in Malaysian essential hypertensive and type 2 diabetic subjects

Vasudevan Ramachandran, Patimah Ismail, Johnson Stanslas and Norashikin Shamsudin

Cardiovascular Diabetology 2009, 8:11

The renin-angiotensin aldosterone system (RAAS) plays an important role in regulating the blood pressure and the genetic polymorphisms of RAAS genes has been extensively studied in relation to the cardiovascular diseases in various populations with conflicting results. The aim of this study was to determine the association of five genetic polymorphisms (A6G and A20C of angiotensinogen (AGT), *MboI* of renin, Gly460Trp of aldosterone synthase and Lys173Arg of adducin) of RAAS genes in Malaysian essential hypertensive and type 2 diabetic subjects. RAAS gene polymorphisms were determined using mutagenically separated PCR and PCR-RFLP method in a total of 270 subjects consisting of 70 hypertensive subjects without type 2 diabetes mellitus (T2DM), 60 T2DM, 65 hypertensive subjects with T2DM and 75 control subjects. There was significant difference found in age,

body mass index, systolic/diastolic blood pressure, fasting plasma glucose and high density lipoprotein cholesterol levels between the hypertensive subjects with or without T2DM and control subjects. No statistically significant differences between groups were found in the allele frequency and genotype distribution for A20C variant of AGT gene, *MboI* of renin, Gly460Trp of aldosterone and Lys173Arg of adducin ($p > 0.05$). However, the results for A6G of AGT gene revealed significant differences in allele and genotype frequencies in essential hypertension with or without T2DM ($p < 0.001$). Among the five polymorphisms of RAAS genes only A6G variant of AGT gene was significantly associated in Malaysian essential hypertensive and type 2 diabetic subjects. Therefore, A6G polymorphism of the AGT gene could be a potential genetic marker for increased susceptibility to essential hypertension with or without T2DM in Malaysian subjects

Association of insertion/deletion polymorphism of angiotensin-converting enzyme gene with essential hypertension and type 2 diabetes mellitus in Malaysian subjects

Vasudevan Ramachandran, Patimah Ismail, Johnson Stanslas, Norashikin Shamsudin, Saidi Moin, Rusni Mohd Jas
J Renin Angiotensin Aldosterone Syst 2008; 9; 208

The deletion (D) allele of the angiotensin-converting enzyme (ACE) gene has been studied in various populations in relation to hypertension (HTN) and type 2 diabetes mellitus (T2DM) with contradictory results. This study sought to determine the association of insertion (I)/D polymorphism of the ACE gene in hypertensive and T2DM subjects in a Malaysian population. A total of 260 subjects consisting of 65 HTN, 60 T2DM, 65 T2DM with HTN and 70 controls were recruited. Genotyping was performed by

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polymerase chain reaction initially and mistyping of DD genotypes was performed with an insertion-specific primer. The frequency for II, ID and DD genotypes of the ACE gene was 36.92%, 52.31% and 10.77% in HTN, 40.00%, 41.67% and 18.33% in T2DM, 30.77%, 53.85% and 15.38% in T2DM with HTN and 57.14%, 40.00% and 2.86% in controls, respectively. The frequency for the D allele was 36.92% in HTN, 39.17% in T2DM and 42.31% in T2DM with HTN compared to 22.86% in controls. The genotype and allele frequency of the ACE gene polymorphism differed significantly in patients when compared to controls ($p < 0.05$). The D allele of the ACE gene is associated with essential HTN and T2DM in Malaysian subjects.

Association of Insertion/Deletion Polymorphism of Alpha-Adrenoceptor Gene in Essential Hypertension with or without Type 2 Diabetes Mellitus in Malaysian Subjects

R. Vasudevan, Patimah Ismail, Johnson Stanlas, Norashikin Shamsudin, Aisyah binti Ali

Int. J. Biol. Sci. 2008, 4

An insertion/deletion (I/D) polymorphism of Alpha2B-Adrenoceptor (ADRA2B) gene located on chromosome 2 has been studied extensively in related to cardiovascular diseases. The main aim of the present study was to examine the potential association of D allele frequency of I/D polymorphism of ADRA2B gene in Malaysian essential hypertensive subjects with or without type 2 diabetes mellitus (T2DM). This study included 70 hypertensive subjects without T2DM, 65 hypertensive subjects with T2DM and 75 healthy volunteers as control subjects. Genotyping of I/D polymorphism was performed by conventional PCR method. There was significant difference found in age, body mass index, systolic/diastolic blood pressure and high density lipoprotein cholesterol level between the case and control subjects. DD genotypic frequency of I/D

polymorphism was significantly higher in hypertensive subjects (42.84% vs. 29.33%; $P=0.029$) and in hypertensive with T2DM subjects (46.15% vs. 29.33%; $P=0.046$) than control group. D allele frequency was higher in hypertensive group (67.41%) than control subjects (52.67%). However, no significant difference was found between the three genotypes of I/D polymorphism of ADRA2B gene and the clinical characteristics of the subjects. The result obtained in this study showed that D allele of ADRA2B gene was associated with essential hypertension with or without T2DM in Malaysian subjects.

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

R. Vasudevan, Patimah Ismail, Johnson Stanslas and Norashikin Shamsudin

African Journal of Biotechnology Vol. 8 (10), pp. 2069-2075, 18 May, 2009

Genetic polymorphisms are 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 characterised. The main objective of this study is to determine the association of S477X polymorphism of LPL gene, A6244G polymorphism of IRS-1 gene and C825T polymorphism of GNB3 gene with EHT and T2DM in Malaysian subjects. This study included 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 GNB3 gene was not associated with EHT and T2DM in Malaysian subjects.

End Stage Renal Disease

Analysis of insertion/deletion polymorphisms of the angiotensin converting enzyme gene in Malaysian end-stage renal disease patients

Aisyah Ali, Ramachandran Vasudevan, Patimah Ismail, Christopher Lim Thiam Seong and Srikumar Chakravarthi

Journal of the Renin-Angiotensin-Aldosterone System Vol. 12, Issue 3, Sept 2011 pp 224-230

Insertion/deletion (I/D) polymorphisms found in the angiotensin converting enzyme (ACE) gene have been associated with hypertension, diabetes and renal disease. The present study sought to determine the association of I/D polymorphisms of the ACE gene with end-stage renal disease (ESRD) patients in Malaysia. A total of 380 subjects were recruited to determine the genotypes of I/D polymorphisms of the ACE gene. Genotyping was performed using a PCR method. Statistical analyses were carried out using statistical software, and a level of $p < 0.05$ was considered statistically significant. The frequencies for II, ID and DD genotypes of the ACE gene were 24.7%, 65.80% and 9.47%, respectively, in ESRD patients, and in control subjects were 45.26%, 47.37% and 7.37% respectively. The frequency for the D allele was found to be higher (42.40%) in ESRD patients compared to control subjects (31.05%). The genotypic and allelic frequencies of I/D polymorphisms of the ACE gene differed significantly ($p < 0.05$) between ESRD patients and control subjects in the Malaysian population. The findings of

this study indicated that I/D polymorphisms of the ACE gene are a useful marker and are likely to play a major role in determining genetic susceptibility to ESRD in the Malaysian population.

Association of variable number of tandem repeats polymorphism in the IL-4 gene with end-stage renal disease in Malaysian patients

*R. Vasudevan, M.N. Norhasniza and I. Patimah
Genet. Mol. Res. 10 (2): 943-947 (2011)*

Variable number of tandem repeats (VNTR) polymorphism in the interleukin 4 (IL-4) genes has been associated with end-stage renal disease (ESRD) subjects in many different populations, although with conflicting results. We determined the 70 bp of VNTR polymorphism at intron 3 of the IL-4 gene in Malaysian ESRD subjects. Buccal cells were collected from 160 case and 160 control subjects; genomic DNA was amplified using PCR, followed by agarose gel electrophoresis. There were significant differences in genotypes and alleles of the IL-4 gene. We concluded that VNTR polymorphism of the IL-4 gene is a risk factor for the development of ESRD among Malaysians.

Malaysia is a multi racial country consisting of Malays, Chinese, Indians and the indigenous people of Sabah and Sarawak. Malays and other indigenous are found to have a higher prevalence of hypertension compared to the Chinese and Indians, but the Indians have more prevalence in T2DM compared to Malays and Chinese whereby the difference is not significant among the different ethnic groups (Lim *et al.*, 2004). Based on the candidate gene approach, many case-control and the cross-sectional studies have been done in Malaysia in predisposition to various diseases. Say *et al.*, 2004 showed a significant difference between the M235T variant of angiotensinogen gene and hypertension. But the same variant in a

recent study showed a lack of association in Malay hypertensive subjects (Ghazali *et al.*, 2008). This proves that, the genetic variation differs within the same ethnic or different groups due to various environmental factors (Persu *et al.*, 2006).

A cross-sectional comparative study showed no association in A1166C polymorphism of angiotensin II type I receptor gene with BP and aortic pulse wave velocity among the Malays (Rehman *et al.*, 2007). A possible involvement of genetic polymorphism of CYP2D6 in patients with cardiovascular disease can be observed in a cohort study in Malaysia.

FUTURE DIRECTIONS

The clinical potential of the SNP revolution has yet to be realised. It remains to be determined whether the current approaches, namely candidate gene studies and whole-genome scans, will be effective in dissecting the genetic basis of common as well as rare complex diseases. So far, the majority of published studies have identified a handful of genetic markers (i.e. SNPs) that alter the risk for disease outcome. It is difficult to predict how many genetic markers are validated in well-designed confirmation studies. If the current approaches are successful, the next step will be to understand the relationship among informative SNPs and haplotypes and begin to build profiles comprising genetic markers present in different regions of the genome. If this can be accomplished, an additional, difficult step will be to determine the significance of each contributing SNP or haplotype. In the near future, it will be necessary to develop more sophisticated analytical tools to investigate gene–gene interactions, but on a scale larger than currently available. Similarly, it will also be critical to account for gene–environment interactions, many of which will require parallel investigation in animal models or in vitro laboratory studies.

Deciphering the contribution of genetic variation to disease outcome carries important implications for the theory and practice of medicine. Based upon the initial findings of such investigations, it is anticipated that the most significant observations derived from SNP-based studies will be to identify genetic markers that alter the risk for a disease outcome. The intercalation of these data into clinical medicine will be a daunting task, because it will require substantial shifts in the public's perceptions of genetic risk factors and the establishment of a new set of paradigms for clinical medicine. Currently, genetic testing is restricted mainly to a set of informative, highly penetrant disease conditions, in which the presence of a genetic mutation is predictive of a defined outcome. The exact manner in which the findings of SNP-based studies will be integrated into clinical practice remains to be defined. Most likely, it will impact preventive and early intervention strategies, encouraging individuals to make smart choices with respect to exposures or participation in high-risk activities.

The promise of pharmacogenomics could substantially shift current algorithms for drug therapy in clinical medicine. Selection of a particular drug could take into account an individual's genetic profile to predict the likelihood for response. Determination of genetic profiles associated with severe or life-threatening toxicity will permit health care providers to choose alternative prescriptions. An informative example is the altered metabolism of a commonly used drug for treatment of childhood acute lymphoblastic leukemia, 6-mercaptopurine, which has a frequency of slightly less than 1%. Previously, it has been noted that a small subset of patients developed potentially fatal hematopoietic toxicity. This low-prevalence thiopurine intolerance occurs in individuals who are homozygous for one of several rare variants in the TPMT (thiopurine S-methyltransferase) gene²⁷. These data suggested that screening

of newly diagnosed patients could avoid deleterious complications in all and thus, therapy could be modified for the at-risk group.

CONCLUSIONS

Thus, genetic polymorphisms do affect the health of an individual and do not directly cause the diseases but merely alter the risk of developing a disease. By analyzing the disease associated SNPs we may predict the likelihood that an individual will develop a specific disease or physiological condition and can be used to develop clinical prevention. Knowledge about the relationships between polymorphisms and disease outcomes and conducting the molecular epidemiological studies to detect the genetic polymorphisms can also be used for reinforcing healthy lifestyles, drug development, animal toxicity studies, improvement of human clinical trials, post market monitoring surveillance for drug efficacy and toxicity, motivating positive behavior changes, helping to target medical therapy, and aiding in better focusing surveillance activities.

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BIOGRAPHY

Professor Dr. Patimah Ismail was born on 21st May 1954 in Jerteh, Terengganu. She received her formal education in Sekolah Kebangsaan Alor Keladi and later transferred to Sekolah Pusat Jerteh, Terengganu. When she passed her Standard Six Examination, she was offered to study in a boarding school in Johor. She continued her secondary school in Sekolah Tun Fatimah, Johor Bharu, Johore until the Higher School Certificate (HSC). She was awarded the Malaysia Rubber Research Development Board (MRRDB) scholarship to complete her degree at The University of Malaya, Kuala Lumpur. Upon completion of her degree, she joined Universiti Pertanian Malaysia as a tutor at the Faculty of Fisheries and Marine Science. In 1981 she was offered to do her Ph.D under the Commonwealth Academic Staff Scholarship (AACU) to study at the University of Swansea, Wales, United Kingdom.

Upon completion of her Ph.D in 1984, Professor Dr. Patimah began her teaching career in which she was appointed as a lecturer in the Department of Fisheries Biology, Faculty of Fisheries and Marine Science, UPM. In 1996, when the Faculty of Fisheries and Marine Science got dissolved and moved to Terengganu, she was offered to join the Faculty of Biomedical Science which later changed its name to The Faculty of Medicine and Health Sciences. In 1998 she was promoted as an Associate Professor and Professor in 2009 at the Department of Biomedical Science in the Faculty of Medicine and Health Sciences, UPM.

After a few years of experience in research on toxicology and biotechnology, Professor Patimah managed to secure a Top Down research project an Animal Biotechnology. Since then she is actively involved in research on genetic polymorphism, phylogenetic studies, mutagenicity and also on the development of novel genes for the improvement of indigenous aquarium fish. Her research projects

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started in 1986 when the R & D for Malaysia Plan was commenced and she was involved in the Recreational Fisheries Project. Later she was deeply involved into research projects when she was granted few other grants from IRPA and SAGA to carry out research especially on genetic diseases such as hypertension, diabetes and cancers.

The results of her research are published in national and international journals. Professor Patimah managed to disseminate her research results mainly on areas particularly pertaining to biotechnology, genetics and toxicology at both national and international conferences and seminars. She has published over 200 research and technical papers in national and international referred journals and supervised over 15 PhDs, 20 Masters and 80 undergraduate students in which her interest to be an academician is still deep in her heart. She also presented papers at numerous conferences and seminars both at national and international levels

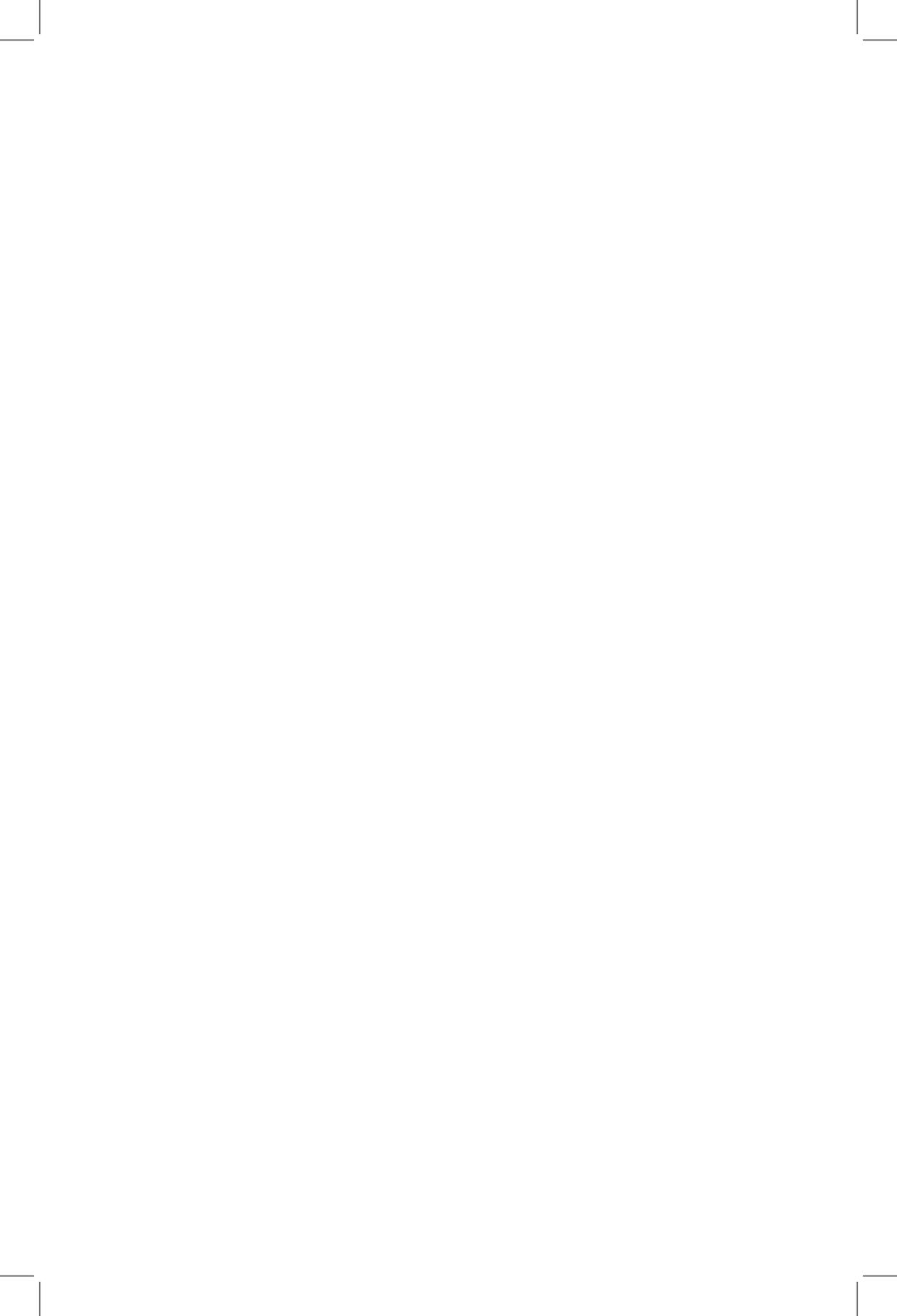
She has served in numerous committees in particular those related to her administrative work. She was Head of the Biomedical Department, Deputy Director of the Centre of External Programme and Deputy Director of the Centre of Foundation Studies for Agricultural Science. At the same time she is an expert panel in Biotechnology for the Ministry of Human Resources.

For her effort in teaching, research and administrative work, she received Awards for Outstanding performance for 4 consecutive years and currently she is still actively involved in teaching and research. Her teaching commitment is not only in the Department of Biomedical Science but also in the Department of Community Health and at the Centre of Foundation Studies for Agricultural Science. She received the SETIA PUTRA award in 2005 for her commitment to the Faculty of Fisheries and Marine Science and the Faculty of Medicine and Health Sciences for 25 years of service,

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As an expert in genetics and biotechnology, she is known at the international level through her collaboration and paper published in prestigious journals and also attended international conferences and seminars. She has collaborated with the known writer of **THE EVE**, Professor Steven Oppenheimer formally from Oxford University, Dr. Martin Richard of University of Leeds, United Kingdom. In her work with them, a documentary **THE REAL EVE** has been shown in World Premier on Discovery Channel. The other collaborators are Prof. Howard Bradbury of Australia (cyanogens in cassava), Perundurai S. Dhandapany of CCMB, Hyderabad, Indian Deletion Project, Dr. Massoud Housmand of National Institute for Genetic Engineering and Biotechnology (NIGEB), Iran, Dr. Zivar of Iran and Dr. Parvin Pasalar of the Department of Biochemistry, Faculty of Medicine Tehran University of Medical Sciences Enghelab Ave and Professor Dr. Cyrus Azimi of the University of Tehran.

Today she encourages young lecturers to work hard. Her life is an example which teaches us to follow our dreams, no matter how big those dreams are.



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