Chapter 1
Introduction
“A wise man ought to realize that health is his most valuable possession”. Father of medicine, Hippocrates has quoted these more than thousand years ago. This quote is just as true today as it was long ago. A good health includes complete well-being of a person i.e. social, physical, biological and mental well-being of a person. It has been very rightly said that a healthy body lives in a healthy mind and vice-versa. Social, physical, biological, or mental unfitness of a person reflects the bad health. Without health, the basic activities of life are restricted or prohibited entirely. Each individual exists on a continuum of health ranging from optimum health on one extreme all the way to morbidity and death on the other extreme. In the middle, there are a wide variety of symptoms, health problems and diseases those when ignored or not paid heed to; can become serious health issues both at individual and at social levels. Such bodies become undesirable, unacceptable and even diseased. Ancient history reflects that some diseases were unnamed in those times and ayurveda or the local remedies were the cure for some. Diseases like leprosy were considered undesirable and victim was considered unwanted by the society. But “Science” educated people in later centuries and enlightened their mind and soul. Advent of “genetics” brought wisdom and prudence uprooting reasons for the unhealthy body and formulating interventional strategies which can transform the unhealthy soul back to the healthy one.

With the discovery of the helical structure of deoxyribonucleic acid (DNA) by Watson and Crick in 1953 and the advent of polymerase chain reaction (PCR) techniques in the 1980s along with the use of restriction enzymes for identifying polymorphisms at the DNA level provided scientists with new and more powerful techniques and genetic markers for unraveling underlying unknown causes for various diseases. Moreover, the recent advancements in science and technology determined the sequence of the human genome and identified the genes that contain all of the information needed to build and maintain human body. Human Genome Project (HGP) reported that in human there are
more than 3 billion DNA base pairs comprising approximately 100,000 genes that make up human DNA (IHGSC, 2001). Such databases of DNA sequence (GeneBank) have been made freely accessible without restrictions to all scientists in industry and academia bringing a paradigm shift in studying human genetic variations among different populations. The availability of known genomic variations coupled with easy to type technologies facilitated the use of genomic markers for studying intra and inter population variations. Such studies intensively helped in understanding the human evolution and migrations. The extent of genetic variation and their manifestation are found to vary in different ethnic groups. Some populations are found to be more prone to a particular disease while others are not, as genetic structure of a particular population is shaped by their environmental/geographical position, life style, mating pattern and other genetic factors which are population specific.

Information from the Human Genome Project has caused scientists to re-examine the role of genetics and other risk factors involved in the development of disease. Understanding this complex interplay of genes and environment will lead us to new methods of disease detection and prevention. Since the days of Archibold Garrod, it has been increasingly accepted that the aetiology of most common diseases involves not only discrete genetic and environmental causes, but also interactions between the two. Garrod suggested that “the influences of diet and diseases” might “mask” some of the “inborn errors of metabolism” that he proposed, and that “idiosyncracies as regards drugs” were presumably due to inherited differences. Virtually all-human diseases result from the interaction of genetic susceptibility factors and modifiable environmental factors, broadly defined to include infectious, chemical, physical, nutritional, and behavioral factors. This is perhaps the most important fact in understanding the role of genetics and environment in the development of disease. Many people tend to classify the cause of disease as either genetic or environmental. Indeed, some rare diseases, such as Huntington or Tay Sachs disease, may be the result of a deficiency of a single gene product, but these diseases represent a very small proportion of all human disease. Common diseases, such as diabetes or cancer, are a result of the complex interplay of genetic and environmental factors.
Variations in genetic makeup are associated with almost all disease. Even so-called single-gene disorders actually develop from the interaction of both genetic and environmental factors. For example, phenylketonuria (PKU) results from a genetic variant that leads to deficient metabolism of the amino acid phenylalanine; in the presence of normal protein intake, phenylalanine accumulates and is neurotoxic. PKU occurs only when both the genetic variant (phenylalanine hydroxylase deficiency) and the environmental exposure (dietary phenylalanine) are present.

Restricting analysis of environmental factors in epidemiological studies to individuals who are genetically susceptible to the exposure should increase the magnitude of relative risks, increasing our confidence that the observed associations are not due to chance. The identification of susceptibility and/or resistance alleles provides direct evidence that these genes and their biological pathways are relevant to specific diseases in humans. Understanding these pathways might help to determine which compounds in a complex mixture cause disease. Ultimately, understanding gene–environment interactions might allow us to give individualized preventive advice before disease diagnosis, in addition to offering personalized treatment after a disease, or disease susceptibility, has been diagnosed. Some gene–environment interactions can be identified without any molecular analysis; one example is the much stronger effect of sunlight exposure on skin cancer risk in fair-skinned humans than in individuals with darker skin. Others can be observed as a reproducible effect of an environmental exposure on a susceptible individual; for example, the flushing response seen after alcohol ingestion in individuals with low-activity polymorphisms in the aldehyde dehydrogenase gene (Takeshita et al., 1996). However, rapidly expanding ability, particularly after the completion of the Human Genome Project, to define genetic differences at the DNA-sequence level is opening up a vast new terrain in the search for gene–environment interactions. Genetic variations do not cause disease but rather influence a person’s susceptibility to environmental factors. We do not inherit a disease state per se. Instead, we inherit a set of susceptibility factors to certain effects of environmental factors and therefore inherit a higher risk for certain diseases. This
concept also explains why individuals are differently affected by the same environmental factors. One of the best examples to explain this concept is of the “Cardiovascular diseases”. For example, some health conscious individuals with “acceptable” cholesterol levels suffer myocardial infarction at age 40. Others individuals seem immune to heart disease in spite of smoking, poor diet, and obesity. Genetic variations account, at least in part, for this difference in response to the same environmental factors. Cardiovascular diseases (CVDs), the all-encompassing term for disorders of the heart and blood vessels, is a complex medical condition known to be caused by a complex interaction of multiple environmental and genetic parameters.

1.1. Cardiovascular diseases

Cardiovascular diseases are the diseases of heart and blood vessel. These include numerous problems, many of which are related to a process called *atherosclerosis* known to be caused by a complex interaction of multiple environmental and genetic parameters (Figure 1.1). Atherosclerosis comes from the Greek word ‘athero’ meaning gruel or paste and ‘sclerosis’ meaning hardness. It is named as a process of deposition of fatty substances like cholesterol, cellular waste products, calcium and other substances that build up as plaque in the inner lining of an artery. The atherosclerotic plaque can grow either towards the vessel lumen or into the vessel wall (Fig. 1.1). But most of the damage occurs when they become fragile and rupture which further results in clotting of blood that can block blood flow or break off and travel to another part of the body. If it blocks the blood vessel that feeds the brain, it causes stroke and if it blocks the blood vessel that feeds the heart, it causes a heart attack. Subsequently, if the plaque does not reduce the blood flow and oxygen supply to the surrounding tissue, CVD can be completely asymptomatic whereas a stenotic plaque often results in angina pectoris. Myocardial infarction or ischemic stroke, are consequences of occluding thrombi diminishing the blood flow. Coronary heart disease (CHD) or Coronary artery disease (CAD), ischemic stroke and peripheral arterial disease are the three major manifestations of atherosclerotic CVD (Figure 1.2).
Figure 1.1: Building of atherosclerotic plaque

Figure 1.2: Development of Atherosclerotic CVD and its forms
1.1.1. CVD- A cause of mortality and morbidity in developing countries

Cardiovascular disease (CVD) is the single largest cause for mortality and morbidity in the world (Mather et al., 2006). More than 17 million people died from cardiovascular diseases in 2008 (Norrving, B., 2011). According to WHO, it is estimated that about 7.6 million died due to coronary heart disease and 5.7 million due to stroke in 2005, representing large percent of all global deaths. More than 80 percent of the deaths occurred in low and middle income countries. The World Health Organization (WHO) estimates there will be about 20 million CVD deaths in 2015, accounting for 30 percent of all deaths worldwide (WHO, 2005). The projected trends in CVD mortality and the expected shifts from infectious to chronic diseases over the next few decades are shown in figure 1.3. By 2030, researchers project that non-communicable diseases will account for more than three-quarters of deaths worldwide; CVD alone will be responsible for more deaths in low income countries than infectious diseases (including HIV/AIDS, tuberculosis, and malaria), maternal and perinatal conditions, and nutritional disorders combined (Beaglehole and Bonita, 2008). Thus, CVD is today the largest single contributor to global mortality and will continue to dominate mortality trends in the future.

![Figure 1.3: Projected global deaths by cause. Source: Beaglehole and Bonita, 2008.](image-url)
The overall burden continues to grow in both developed and developing countries, but there are distinct differences in the pattern of growth between the two, as the expected rate of increase in CVD in developing countries in the next two decades is likely to be almost twice that in the developed countries (Gaziano, 2005). CVD is increasing at a faster rate in low and middle income countries and about 42 percent of premature deaths occur due to CVD in these countries.

1.2. Cause of CVD burden in developing countries

Mortality rates generally appear to be most closely linked to a country’s stage of epidemiological transition.

1.2.1. Epidemiological transition

It is a concept first proposed by Abdel Omran in the 1970s (Omran, 1971), refers to the changes in the predominant forms of disease and mortality burdening a population that occur as its economy and health system develops. Prior to epidemiologic transition is the ‘Demographic transition’. Demographic transition is the transition of high to low fertility (and mortality) rates in a country. This was formerly thought to be related to technologic changes and industrialization but probably more directly related to female literacy and status of women than many other factors.

Epidemiologic transition.

1.2.1.1. Stages of epidemiological transition

There are three phases of epidemiological transition which a population goes through (figure 1.4):

i) The age of “pestilence and famine”

ii) The age of “receding pandemics”

iii) The age of “degenerative and manmade diseases” (Increased fat and caloric intake and decreased physical activity-rise of chronic, non communicable diseases)
Transition from first phase to third phase through the second phase is called epidemiologic transition. Every country undergoes this transition. Different countries take different time periods to reach the third phase from the first phase. On an average, developed countries took a shorter course than the developing countries. The developed world is now facing the epidemiological transition, i.e., a transition from infectious diseases like measles, diphtheria, pneumonia etc, to chronic lifestyle-related diseases like heart diseases, cancer, stroke, hypertension and diabetes.

In underdeveloped countries at the early stages of epidemiological transition, infectious diseases predominate, but as the economy, development status, and health systems of these countries improve, the population moves to a later stage of epidemiological transition, and chronic noncommunicable diseases become the predominant causes of death and disease (Gaziano et al., 2006).

*Chronic diseases are now the dominant contributors to the global burden of disease, and CVD is the largest contributor to the chronic disease cluster.*

In terms of economic development, the world can be divided into two broad categories: (1) high-income countries; and (2) low- and middle-income countries, which can be further subdivided into six distinct economic/geographic regions. *Although CVD death rates are declining in most high income countries, trends are increasing in most low and middle income countries.* Currently, 85% of the world's population lives in low-
and middle-income countries, and it is these countries that are driving the rates of change in the global burden of CVD (figure 1.5). Three million CVD deaths occurred in high-income countries in 2001, compared to 13 million in the rest of the world.

Figure 1.5: Burden of disease in developed and developing countries

Approximately 940 million people live in the high-income countries, where CHD is the dominant form of CVD, with rates that tend to be twofold to fivefold higher than stroke rates. The rates of CVD in Canada, New Zealand, Australia, and Western Europe tend to be similar to those in the United States; however, among the countries of Western Europe, the absolute rates vary threefold with a clear north/south gradient. The highest CVD death rates are in the northern countries, such as Finland, Ireland, and Scotland, with the lowest CVD rates in the Mediterranean countries of France, Spain, and Italy. Japan is unique among the high-income countries: stroke rates increased dramatically over the last century, but CHD rates did not rise as sharply. This difference may stem in part from genetic factors, but it is more likely that the fish- and plant-based, low-fat diet and resulting low cholesterol levels have played a larger role. Importantly, Japanese dietary habits are undergoing substantial changes, reflected in an increase in cholesterol levels.
The World Bank groups the low- and middle-income countries (gross national income per capita lower than U.S. $9200) into six geographic regions: East Asia and the Pacific, (Eastern) Europe and Central Asia, Latin America and the Caribbean, Middle East and North Africa, South Asia, and Sub-Saharan Africa. Although communicable diseases continue to be a major cause of death, CVD has emerged as a significant health concern in the low- and middle-income countries (Figure 1.5). In most of these countries, there is an urban/rural gradient for CHD, stroke, and hypertension, with higher rates in urban centers.

While CVD rates are rapidly rising, there are vast differences among the regions and countries, and even within the countries themselves. Many factors contribute to the heterogeneity. First, the regions are at various stages of the epidemiologic transition. Second, vast differences in lifestyle and behavioral risk factors exist. Third, racial and ethnic differences may lead to altered susceptibilities to various forms of CVD. In addition, it should be noted that for most countries in these regions, accurate country-wide data on cause-specific mortality are not precise, as death certificate completion is not routine, and most countries do not have a centralized registry for deaths.

**The East Asia and Pacific region** appears to be straddling the second and third phases of the epidemiologic transition, with China, Indonesia, and Sri Lanka's large combined population driving most of the trends. Overall, CVD is a major cause of death in China, but like Japan, stroke (particularly hemorrhagic) causes more deaths than CHD. China also appears to have a geographic gradient like that of Western Europe, with higher CVD rates in northern China than in southern China. Other countries, such as Vietnam and Cambodia, are just emerging from the pestilence and famine stage.

**The Eastern Europe and Central Asia region** is firmly in the peak of the third phase, with the highest death rates (58%) due to CVD in the world, nearly double the rate of high-income countries. There is, however, also regional variability. In Russia, increased CVD rates have contributed to falling life expectancy, particularly for men, whose life expectancy has dropped from 71.6 in 1986 to 59 years today. In Poland, by contrast, the age-adjusted mortality rate decreased by approximately 30% for men during the 1990s, and slightly more among women.
In general, the *Latin America and Caribbean region* appears to be in the third phase of the epidemiologic transition, although as in other low- and middle-income regions, there is vast regional heterogeneity, with some areas in the second phase of the transition and some in the fourth. Today, approximately 28% of all deaths in this region are attributable to CVD, with CHD rates higher than stroke rates. Approximately one-quarter of the citizens live in poverty, and many are still dealing with infectious diseases and malnutrition as major problems.

The *Middle East and North Africa* region appears to be entering the third phase of the epidemiologic transition, with rates just below high-income nations. In this region, increasing economic wealth has been accompanied characteristically by urbanization but uncharacteristically by increasing fertility rates as infant and childhood mortality rates have declined. The traditional high-fiber diet, low in fat and cholesterol, has changed rapidly. Over the past few decades, daily fat consumption has increased in most of these countries, ranging from a 13.6% increase in Sudan to a 143.3% increase in Saudi Arabia.

Most people in *South Asia* live in rural India, a country that is experiencing an alarming increase in heart disease. CVD accounted for 32% of all deaths in 2000, and the World Health Organization (WHO) estimates that 60% of the world's cardiac patients will be Indian by 2010. The transition appears to be in the Western style, with CHD as the dominant form of CVD. In 1960, CHD represented 4% of all CVD deaths in India, whereas in 1990 the proportion was >50%. This is somewhat unexpected because stroke tends to be a more dominant factor early in the epidemiologic transition. This finding may reflect inaccuracies in cause-specific mortality estimates or possibly an underlying genetic component. It has been suggested that Indians have exaggerated insulin insensitivity in response to the Western lifestyle pattern that may differentially increase rates of CHD over stroke. Certain remote areas, however, are still emerging from the age of pestilence and famine, with CVD accounting for <10% of total deaths. Rheumatic heart disease continues to be a major cause of morbidity and mortality.
Sub-Saharan Africa remains largely in the first phase of the epidemiologic transition, with CVD rates half of those in high-income nations. Life expectancy has decreased by an average of five years since the early 1990s largely because of HIV/AIDS and other chronic diseases, according to the World Bank; life expectancies are the lowest in the world. While HIV/AIDS is the leading overall cause of death in this region, CVD is the third leading killer and is first among those over the age of 30. Hypertension is now a major public health concern and has resulted in stroke being the dominant form of CVD. Rheumatic heart disease remains an important cause of CVD mortality and morbidity.

1.2.2. Epidemiologic transition and India

Currently, developing countries like India are experiencing the double burden of diseases. On the one hand, infectious diseases are still highly prevalent amongst people of the lower socio-economic group due to poverty, poor water supply and sanitation. On the other hand, non-communicable diseases are on the rise amongst the upper class of society, as they adopt similar lifestyles as those of the developed world (Nongkynrih et al., 2004). This means that India is under a dual stage of epidemiologic transition. 80% of the India lives in villages and these rural regions having a low poor economy are straddling between the first and second stages of epidemiologic transition majorly remaining in the first epidemiologic stage. Hence, it is likely that they still have a high prevalence of communicable diseases and a low prevalence of non communicable diseases. On the other hand, urban India having a high economy is then likely to be in the third stage of epidemiologic transition and therefore, there is a rise in the non communicable diseases in them. Therefore, India is facing double burden of diseases. National health survey, 2004 showed that morbidity due to chronic diseases among elderly is higher in demographically more advanced states found to be highest in Kerala, followed by west Bengal, Andhra Pradesh, Punjab, Maharashtra, Jammu & Kashmir, North-East, Gujarat, Karnataka, Tamil Nadu, Uttar Pradesh, Haryana, Himachal Pradesh, Bihar, Rajasthan and lowest being in Orissa. It is noteworthy that there is shift in rate from women to men from demographically more advanced states to less advanced states with Orissa showing equal rates in both the sexes (figure 1.6).
Figure 1.6: Statewise prevalence of chronic disease (majorly CVD) among elderlies (Source: National Sample Survey, 60th round, 2004).

Further, among the various chronic non communicable diseases, CVD shows the largest share in India (figure 1.7). India stands after America, Europe and China in CVD prevalence, compared to Asia and Africa. India has a considerable frequency of the disease which is alarmingly increasing for the last three decades. It is expected that many more people will survive to ages at which the ravages of vascular disease become clinically manifested and that the urbanization and altered lifestyle, concomitants of socioeconomic development, will lead to an increased prevalence of risk factors for such disease and to higher risk attributing to the population. (Shah et al., 2010).

The Global Burden of Diseases (GBD) study reported the estimated mortality from coronary heart disease (CHD) in India at 1.6 million in the year 2000. A total of nearly 64 million cases of CVD are likely in the year 2015, of which nearly 61 million would be CHD cases (the remaining would include stroke, rheumatic heart disease and congenital heart diseases). Deaths from this group of diseases are likely to amount to be a staggering 3.4 million. Epidemiological studies show a sizeable burden of CHD in adult rural and urban populations; of the 30 million patients with CHD in India, there would be 14 million of who are in urban and 16 million in rural areas. In India about 50 per cent of CHD-related deaths occur in people younger than 70 yr compared with only 22 per cent in the West. Extrapolation of these numbers estimates the burden of CHD in India to be more than 32 million patients.
The ICMR-WHO study on Burden of Disease reviewed literature till 2003 on NCDs. The weighted average prevalence for ischaemic heart disease was estimated to be 6.4 per cent in urban areas and 2.5 per cent in rural areas. The meta-analysis of eight studies carried out between 1995 and 2002 in urban areas gives a pooled prevalence rate of hypertension as 164 per thousand, and 157 per thousand in rural areas. The combined urban and rural pooled estimate of prevalence rate of hypertension among adults (>20 yr) was 159 per thousand. An increase of 17.5 per cent in the number of stroke cases in India occurred during the last one and a half decade. Mortality due to strokes has increased by 7.8 per cent from 1998 to 2004. Available evidence yielded that over 9 million stroke cases and about 6.4 million years have been lost due to disability during 2004. This increase in CVDs could be attributable to (i) increase in the population size due to natural growth, (ii) ageing of the population which makes people more vulnerable to chronic diseases at older ages, and (iii) increased vulnerability due to lifestyle changes.

1.3. Individual risks for CVD

Proximal risks for CVD include those associated with consumption patterns (mainly linked to diets, tobacco and alcohol use), activity patterns, and health service use as well as biological risk factors such as increased cholesterol, blood pressure, blood glucose, and clinical disease. The concept of risk factors for CVD was first introduced in an article
from the Framingham Heart Study in 1961 linking the presence of specific antecedent conditions (e.g., elevated cholesterol, hypertension, diabetes mellitus, tobacco use) to future CVD (Kannel et al., 2009) and most recently reported substantial 30-year risk data showing the accumulation of risk over time (Pencina et al., 2009). Importantly, risk factors for the incidence of CVD and those associated with CVD severity or mortality are not synonymous. Risk factors for incidence become important starting very early in life and accumulate with behavioral, social, and economic factors over the life course to culminate in biological risks for CVD such as increased cholesterol, blood pressure, blood glucose, and clinical disease. Over the past few decades, the effectiveness of early screening and long-term treatment for biological risks or early disease has contributed to the sharp declines in CVD mortality seen in many countries.

The recent WHO Global Health Risks Report of 2009 (Lopez et al., 2006) and the earlier World Health Report of 2002 provide comparable and robust estimates of the contribution of risks to total mortality and measures of disability (Mathers et al., 2003; WHO, 2002, 2009b). This kind of data, which was explicitly called for in the 1998 IOM report, allows policy makers to shift their focus upstream from diseases and deaths to risks. Relatively few major behavioral and biological risk factors account for CVD incidence around the world. Tobacco use, diet (including alcohol, total calorie intake, and specific nutrients) and physical inactivity serve as the three major behavioral risks. Between them, they account for a significant proportion of cancer, diabetes, and chronic respiratory disease incidence in addition to CVD (Hu et al., 2008; van Dam et al., 2008; WHO, 2002; Yach et al., 2004, 2005). Concerted action focused on these behavioral risks, along with biological risks such as high blood pressure, high blood lipids, and high blood glucose, would have a wide impact on the global incidence and burden of disease (WHO, 2009b).

The Global Burden of Disease and Risk Factors report provides additional analysis of the relative contribution of individual risk factors specifically to CVD burden. Using 2001 data, the report estimates the percentage decrease in IHD and stroke burden that could be expected if population exposure to a risk factor were reduced to zero by calculating the population attributable fraction for each of the key CVD risk factors. The report found that hypertension, high cholesterol, overweight and obesity, smoking,
low fruit and vegetable intake, and physical inactivity were the leading contributors to IHD and stroke burden worldwide (Lopez et al., 2006).

The INTERHEART study found that abnormal blood lipids are the most important contributors to CVD globally. Tobacco was the second most important risk factor, coequal to lipids in men but lower in women. Other key risk factors included abdominal obesity, psychosocial factors, hypertension, and diabetes (Yusuf et al., 2004).

While the INTERHEART study showed that the top risk factors contributing to CVD are generally consistent globally, the study also found distinct regional differences, much like the data described previously on the rising trends in CVD prevalence over time. For example, while abdominal obesity was the greatest or second-greatest contributor to CVD risk in 8 of the 10 regions studied, it was the smallest contributor in China. In addition, while psychosocial factors were among the top three risk factors by both population attributable risk and odds ratio (measures of risk-factor burden and impact, respectively) in Western Europe, the Middle East, China, and North America, they appeared to be much less influential in Central and Eastern Europe and South Asia (Iqbal et al., 2008; Yusuf et al., 2004).

Few studies have quantified the consequent impact of these risks on the risk of stroke in developing country populations. However, findings from a study in the United Kingdom are informative. A cohort of 20,040 people was followed over 11 years to determine the risk of stroke incidence. Four measures of health behaviors combined—smoking, low physical activity, low plasma vitamin C levels (used as a proxy for fruit and vegetable intake), and not drinking alcohol in moderation (abstaining from alcohol or consuming more than 14 drinks per week)—predicted more than a two-fold increase in stroke incidence (Myint et al., 2009). This is consistent with prior findings in large cohorts of men and women in the United States that a healthful diet and lifestyle—not smoking, regular exercise, moderate alcohol consumption, and not being overweight—was associated with nearly 80 percent lower risk of ischemic stroke compared to having none of these healthy lifestyle components (Chiuve et al., 2008).

The major contributing individual risk factors for CVD are generally consistent across the globe and include abnormal blood lipids, tobacco use and exposure, abdominal
obesity, psychosocial factors, hypertension, and diabetes. However, the detailed underlying risk profile differs across populations and varies over time. Interventions and prevention strategies need to focus on current local risk profiles to ensure they are adapted to the specific settings where they will be applied.

The major risk factors for CVD can be classified into 2 broad categories, traditional and non-traditional. There are many traditional/conventional risk factors associated with cardiovascular diseases as mentioned above. Some risk factors such as family history, ethnicity and age, and sex cannot be changed. Other risk factors that can be treated or changed include tobacco exposure, high blood pressure (hypertension), high cholesterol, obesity, diabetes, unhealthy diets and harmful use of alcohol. Traditional measures of obesity include Body Mass Index (BMI), waist circumference (WC), and waist-hip ratio (WHR), dyslipidemia includes total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL), and fasting blood glucose (FBG) (Figure 1.8). Although these risk factors are insufficient to identify absolute risk, they are strongly associated with the presence of atherosclerotic vascular disease and explain 75% to 90% of events (McGill et al., 2008 and Greenland et al., 2003). With conventional risk prediction models such as the Framingham Risk Score, the absence of these risk factors is associated with a very low likelihood of ever getting CVD (Balagopal et al., 2011).

![Cardiovascular risk factors](image)

**Figure 1.8: Traditional and non-traditional cardiovascular risk factors**
1.3.1. Modifiable traditional risk factors

1.3.1.1. Blood pressure

A recent review of the global burden of high blood pressure found that approximately 54 percent of stroke, 47 percent of IHD, 75 percent of hypertensive disease, and 25 percent of other CVDs were attributable to hypertension. This equates to an annual burden of approximately 7.6 million deaths, or 13.5 percent of the total number of annual global deaths, attributable to high blood pressure (Lawes et al., 2008). Furthermore, Lawes et al. (2008) found that more than 80 percent of the attributable burden of hypertension in 2001 occurred in low and middle income countries, and both another recent review and an analysis commissioned for this report found the prevalence of hypertension to be equally high in developed and developing countries (Gaziano and Kim, 2009; Pereira et al., 2009).

In China alone, it is estimated that the current age-standardized prevalence rate of hypertension is 17.7 percent, which translates into 177 million people, and that approximately 20 percent of deaths in China are attributable to high blood pressure (Yang, 2008). A significant contributor to these levels is the high average daily salt intake in China, which is estimated at 12 g per day—twice the Chinese and WHO recommended levels. Further, only 30 percent of adults with hypertension are aware of their condition, and of those only 6 percent manage their hypertension effectively (Yang et al., 2008). While antihypertensive medications have become more effective, their widespread use remains low and the number of people with uncontrolled blood pressure is increasing (Chobanian, 2009).

In Sub-Saharan Africa, hypertension is a predominant driver of CVD. Hypertensive heart disease and stroke, rather than ischemic heart disease, account for the majority of the CVD burden in the region, especially among black Africans (Mayosi et al., 2009; Mbewu and Mbanya, 2006; Muna, 1993). Prevalence of hypertension is particularly high in urban Sub-Saharan Africa, with between 8 and 25 percent of adults affected, depending on how hypertension is defined (Mbewu and Mbanya, 2006). In
South Africa, the 2003 Demographic and Health Survey found that 12.5 percent of men and 17.9 percent of women were hypertensive (South African Department of Health and Medical Research Council, 2007). Unfortunately, the number of people with uncontrolled hypertension is also high in the region (Mbewu and Mbanya, 2006). Researchers found that more than 70 percent of South African hypertensive patients’ blood pressure remained uncontrolled (South African Department of Health and Medical Research Council, 2007).

Among the major underlying risks for hypertension are sodium, body weight, and access to treatment (Reuser et al., 2009; Steyn, 2006; Yang et al., 2008). Primary prevention focused on sodium reduction, fruit and vegetable intake, weight control, and avoidance of excessive alcohol intake has been shown to make a difference. Finland’s experience (Karppanen and Mervaala, 2006) has potential applications for low and middle income countries where treatment levels remain extremely low and health systems have yet to adapt to managing chronic diseases like hypertension.

1.3.1.2. Overweight and obesity

Another broad trend related to physical activity and nutrition, especially excess calorie intake, is obesity and overweight. This topic was not raised as an important issue at the inception of the Framingham Study, possibly because population levels of overweight in the 1940s were relatively low. It was also only briefly mentioned in the 1998 IOM report. During the past several decades, however, there have been steady increases in levels of overweight and obesity reported from developed and developing countries. Even in low and middle income countries where undernutrition is still highly prevalent, overweight and obesity—especially among women—is a bourgeoning issue (Caballero, 2005). For instance, in South Africa, 59 percent of women and 29 percent of men over age 15 are overweight or obese (South African Department of Health and Medical Research Council, 2007). In China, trend lines for obesity are going up fairly sharply among all geographic groups in communities of all sizes, from rural villages to megacities (Wang et al., 2007).
As mentioned earlier, WHO and FAO reviewed the evidence on the relationship between obesity and the risk of CVD and concluded that overweight and obesity confer a significantly elevated risk of CHD (Joint WHO/FAO Expert Consultation, 2003). Increased body mass index (BMI) is also associated with greater risk of stroke in both Asian and Western populations. The association between obesity and CVD is partly, but not completely, mediated through hypertension, high cholesterol, and diabetes. Abdominal or central obesity measured by waist-to-hip ratio or waist circumference is associated with both CHD and stroke independent of BMI and other cardiovascular risk factors. Moreover, obesity is also an independent risk factor for other cardiovascular outcomes, such as congestive heart failure and sudden cardiac death.

Excess energy intake is one of the key contributors to obesity. As highlighted earlier, the lack of data limits policy makers’ abilities to focus attention on which dietary components lend themselves to effective interventions that would reduce total calorie intake. National surveys of calorie intake from India indicate that in urban areas, cereals account for 56 percent of intake, compared to about 9 percent each for edible oils and dairy, 1 percent for meat and fish, and 0.4 percent for all beverages (Chatterjee et al., 2007). In China, cereals also dominate and account for 58 percent of total calorie intake compared to meat (13 percent) and cooking oils (17 percent). As discussed earlier, trends in consumption indicate very rapid increases in oil use and slow decline in the consumption of cereals as contributors to calories. These trends in developing countries are in contrast to data for the United Kingdom, which could indicate where trends are headed in developing countries. National data from 2003 indicate that cereals and related products account for 31 percent of calories with other major categories including meat (15 percent), milk and related products (19 percent), and beverages (10 percent) (Office of National Statistics et al., 2003).

1.3.1.3. Blood lipids

Researchers have studied the role of blood lipids in the development of atherosclerosis and the increase of CVD risk for decades. The Framingham Study first demonstrated the link between hypercholesterolemia and increased risk of CHD in the 1960s with the finding that lower levels of high-density lipoprotein (HDL) cholesterol as well as
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elevated levels of low-density lipoprotein (LDL) cholesterol were associated with increased CHD risk. Subsequent studies confirmed these results and further established that elevated triglycerides also increase CVD risk (Gotto, 2005). Furthermore, randomized controlled trials have shown that reduction of LDL cholesterol, both in primary and secondary prevention, is associated with reduced coronary event rates (Downs et al., 1998; Sacks et al., 1996). Reductions in LDL cholesterol have also been associated with a lowered incidence of stroke, although the data are not as strong as for CHD (Collins et al., 2004). In addition, lipoprotein(a) (Lp(a)) is an LDL–like particle that was independently associated with CHD and stroke in a recent comprehensive meta-analysis (Erqou et al., 2009).

The INTERHEART study recently confirmed that there was a graded relationship between abnormal lipid levels and risk for CHD in all regions of the world. In fact, the INTERHEART study found that abnormal blood lipids were the most important risk factor for myocardial infarction by odds ratio in all global regions (Yusuf et al., 2004). Further underscoring this, the Global Burden of Disease study estimated that elevated cholesterol was the third leading risk factor for worldwide mortality in general, after hypertension and smoking (Lopez et al., 2006).

While it is clear that dyslipidemia is one of the leading risk factors for CVD, there is significant regional variation in the prevalence of hyperlipidemia. Hypercholesterolemia was found in 22 percent of subjects enrolled in the Heart of Soweto study of patients with newly diagnosed CVD in South Africa (Sliwa et al., 2008). In Mongolia, the Ministry of Health, in collaboration with WHO, performed a STEPS survey across the country and reported 7 percent prevalence of hypercholesterolemia (WHO Regional Office for the Western Pacific, 2007). In contrast, a nationally representative population-based study in Iran found the prevalence of hypercholesterolemia to be more than 45 percent (Alikhani et al., 2009). In accordance with this geographic variability in the prevalence of hypercholesterolemia, the population-attributable risk of dyslipidemia for CHD in the INTERHEART study varied widely by geographic region (Yusuf et al., 2004). Although systematic data specifically regarding Lp(a) and its relationship to CVD among different populations around the world are lacking, levels have been shown to vary among different ethnic groups; in general, Asian Indians have higher Lp(a) levels than ethnic
Chinese and Caucasian populations (Anand et al., 1998, 2000; Low et al., 1996). In addition, African Americans have higher average Lp(a) levels than Caucasians (Marcovina et al., 1996; Srinivasan et al., 1991), and studies in Africa have also shown higher average Lp(a) levels than in Caucasians (Evans et al., 1997).

Successful intervention programs in a number of countries have further supported the causal link between dyslipidemia and CVD by demonstrating that reductions in cholesterol lead to decreased CVD morbidity and mortality. In Finland, a nationwide multisectoral program targeted at multiple cardiovascular risk factors decreased population mean serum cholesterol levels as well as CVD mortality between 1972 and 1992 (Puska et al., 1998; Vartiainen et al., 1994a). These reductions in cholesterol were largely credited to reductions in saturated fat intake as well as more comprehensive cholesterol monitoring and treatment (Puska et al., 2009). Further analysis showed that among men the 13 percent reduction in cholesterol levels was singlehandedly responsible for a 26 percent reduction in CVD mortality (Vartiainen et al., 1994b). The proven success of interventions to reduce cholesterol has shifted thinking on the inevitability of atherosclerosis, with researchers now realizing that it is not an unavoidable byproduct of aging, but rather that it can be prevented and largely reversed through the use of diet modification and secondary prevention with statins.

1.3.1.4. Raised blood glucose/diabetes

Around the world, diabetes is growing increasingly common and is a significant contributor to CVD risk. People with diabetes have a more than two-fold greater risk of fatal and nonfatal CVD compared to non-diabetics, with some indication that diabetes mellitus may confer an equivalent risk of having had a cardiovascular event (Asia Pacific Cohort Studies Collaboration, 2003; Haffner et al., 1998; Stamler et al., 1993). In fact, CVD is the leading cause of morbidity and mortality in people with diabetes (Booth et al., 2006a; Kengne et al., 2007, 2009; Thomas et al., 2003).

The magnitude of the risk of CVD associated with diabetes is even greater in women and younger individuals. Indeed, there is substantial evidence that diabetes mellitus may erase, or substantially attenuate, the “female advantage” in the risk of CVD observed in non-diabetics, and that having diabetes may be equivalent to aging by at least 15 years
Cardiovascular risk associated with blood glucose is continuous; thus, individuals without established clinical diabetes, but who are at increased risk of developing diabetes in the future, also have a higher risk of CVD (Asia Pacific Cohort Studies Collaboration, 2004). Based on this continuous association, higher-than-optimum blood glucose (fasting plasma glucose > 4.9 mmol/l) has been identified as the leading cause of cardiovascular deaths in most regions (Danaei et al., 2006). In 2001, for instance, 1.49 million deaths from IHD (21 percent of all IHD deaths) and 709,000 from stroke (13 percent of all stroke deaths) were attributable to high blood glucose in addition to the 950,000 deaths directly attributed to diabetes mellitus in the world (Danaei et al., 2006). These figures are particularly worrisome given that it is estimated that more than 344 million people around the world will have impaired glucose tolerance in 2010.

Obesity is the single most important risk factor for type 2 diabetes, but unhealthy diet and physical inactivity also independently raise the population risk for diabetes (Schulze and Hu, 2005). According to the International Diabetes Federation’s Diabetes Atlas 2010, the global estimated prevalence of diabetes for 2010 among people aged 20 to 79 years will be approximately 285 million people (6.4 percent of the global population), of which some 70 percent will be living in developing countries (International Diabetes Federation, 2010). By 2030 this figure is expected to increase by more than 50 percent to some 438 million people, or 7.7 of the world’s population if preventive interventions are not put in place. The largest increases will take place in the regions dominated by developing economies. Close to 4 million deaths in the same age group will be attributable to diabetes in 2010, representing 6.8 percent of all-cause global mortality. The highest number of deaths due to diabetes are expected to occur in countries with large populations—1,008,000 deaths in India, 575,000 in China, 231,000 in the United States, and 182,000 in the Russian Federation (Roglic and Unwin, 2010). Currently, 83 percent of all diabetes deaths occur in low and middle income countries (WHO, 2009b).
Diabetes is emerging as a particular concern in Asia, where more than 110 million individuals were living with diabetes in 2007, a large proportion of whom were young and middle aged. Asians tend to develop diabetes at a relatively young age and low BMI, and by 2025 the number of individuals with diabetes in the region is expected to rise to almost 180 million, of which approximately 70 million will be in India and almost 60 million in China (Chan et al., 2009). The reasons for this increased risk are still being fully elucidated; however, “normal weight” Asians often exhibit features of abdominal or central obesity, which is particularly detrimental to insulin resistance and glucose metabolism. Moreover, the increased risk of gestational diabetes combined with exposure to poor nutrition in utero and overnutrition in later life may contribute to increased diabetes, resulting in a situation of “diabetes begetting diabetes” (Chan et al., 2009).

The balance of risks and benefits associated with intensive glucose control has been assessed in recent clinical trials, which have convincingly demonstrated beneficial microvascular outcomes of diabetes. By contrast, these trials have individually failed to show such an effect on cardiovascular outcomes. However, the extension of the follow-up of the Diabetes Control and Complications Trial in type 1 diabetes (Nathan et al., 2005) and the United Kingdom Prospective Diabetes Study in type 2 diabetes (Holman et al., 2008) have shown that intensive glucose control substantially lowered the risk of cardiovascular outcomes, suggesting a legacy effect with still unexplained underlying mechanisms. Recently conducted meta-analyses of relevant trials in people with type 2 diabetes have also consistently shown that intensive glucose control reduces the risk of major cardiovascular events by approximately 10 percent, primarily driven by a 10 to 15 percent reduction in the risk of CHD, compared with standard treatment in people with diabetes. Interestingly, this benefit appeared to be independent of concurring cardiovascular risk factors (Kelly et al., 2009; Ray et al., 2009; Stettler et al., 2006; Turnbull et al., 2009).

As such, prevention and management of diabetes are critical in reducing the global burden of CVD.
1.3.1.5. Tobacco

There are currently more than 1 billion smokers worldwide. Although use of tobacco products is decreasing in high income countries, it is increasing globally, with more than 80 percent of the world’s smokers now living in low and middle income countries (Jha and Chaloupka, 1999). In China alone, there are 303 million adult smokers and 530 million people passively exposed to secondhand smoke (Yang et al., 2008). Tobacco use kills 5.4 million people a year—more than the annual deaths due to tuberculosis (TB), HIV/AIDS, and malaria combined—and accounts for 1 in 10 adult deaths worldwide (Mathers and Loncar, 2006). In the 20th century 100 million deaths were caused by tobacco, and, if current trends continue, there will be up to 1 billion deaths in the 21st century (WHO, 2008c). By 2030, researchers estimate that 80 percent of tobacco-related deaths will occur in low and middle income countries (Mathers and Loncar, 2006).

In the Global Burden of Disease study, Lopez et al. (2006) estimated that in 2000, 880,000 deaths from CHD and 412,000 deaths from stroke were attributable to tobacco. These data are based on updated estimates of the relative risk of death among smokers for CHD, stroke, and hypertensive heart disease. The relative risks are highest in young people (as found by the INTERHEART study and described earlier). However, the most common type of tobacco-related CVD deaths varies around the world. For example, in India, a higher proportion of smokers die from CHD; in China, tobacco kills more through stroke (Ezzati et al., 2005).

India is the third largest producer and consumer of tobacco in the world. (http://tobaccofreecenter.org/resources_country/india). Whereas cigarette consumption is decreasing in developed countries, it is increasing in less-developed countries, such as India. As a result, many Indians face serious tobacco-related health problems that affect both their health and productivity (Reddy et al., 2003). Thus, the need is immediate to institute measures to reduce tobacco use in India. Indians consume tobacco in a myriad of ways. Of the 275 million people in India who use tobacco, only 14% use cigarettes (http://tobaccofreecenter.org/resources_country/india). More commonly, Indians smoke bidis (tobacco rolled inside tendu leaves) and/or chew gutkha (a form of smokeless tobacco) (Yadav et al., 2006). Historically, tobacco use is significantly higher among
men, although tobacco use among women in both rural and urban settings is rising as is evident in the present population. It is also more commonly used among rural versus urban populations (Yadav et al., 2006). Indians typically begin smoking at older ages than those in Europe and North America (Jha et al., 2008). The prevalence of hookah use is high in the villages where it is the second most common form of tobacco used. The use of hookah is almost equally common among rural men and women. Hookah smoking is a habit that has been associated with Indian villages for several centuries (Yadav et al., 2006). Over the years, the use of bidis and hookah has come to be very much ingrained in the rural villages of north India. Bidis are more dangerous than cigarette

1.3.1.6. Dietary patterns

The effect on CVD risk of diets rich in whole grains and low in processed foods that are high in fat, sodium, and sugars has been increasingly investigated in both developed and developing countries. In parallel with economic development, radical dietary shifts toward Westernized diets that are high in animal products and refined carbohydrates and low in whole grains and other plant-based foods have occurred in many developing countries (Hu, 2008). In the INTERHEART study, three major dietary patterns were identified: Oriental (high intake of tofu and soy); Western (high in fried foods, salty snacks, eggs, and meat); and prudent (high in fruits and vegetables). The Western dietary pattern was associated with an increased risk of CHD in all regions of the world, whereas the prudent pattern was associated with a lower risk (Iqbal et al., 2008).

Substantial evidence has accumulated to support the notion that the traditional Mediterranean dietary pattern is protective against CVD (Fung et al., 2009; Martinez-Gonzalez et al., 2009). This pattern is characterized by an abundance of fruits, vegetables, whole grain cereals, nuts, and legumes; olive oil as the principal source of fat; moderate consumption of fish; and lower consumption of red meat.

1.3.1.7. Alcohol

The global burden of diseases attributable to alcohol has recently been summarized, leading to the conclusion that alcohol is one the largest avoidable risk factors in low and
middle income countries (Rehm et al., 2009). Indeed, WHO estimates that the harmful use of alcohol was responsible for 3.8 percent of deaths and 4.5 percent of the global burden of disease in 2004 (WHO, 2009b). In the past few decades, consumption of alcohol has increased dramatically in men in countries undergoing nutrition transition, such as India and China, and has been extremely high in Russia for many decades, where it contributes significantly to overall mortality among men (WHO Expert Committee on Problems Related to Alcohol Consumption and WHO, 2007).

It has long been known that excessive alcohol intake is associated with increased risk for hypertension, stroke, coronary artery disease, and other forms of CVD; however, there is also a robust body of evidence in a range of populations that suggests that light to moderate intake of alcohol may reduce the risk of CHD. Indeed, research suggests that the relationship between alcohol intake and CVD outcomes follows a “J” curve, with the lowest rates being associated with low to moderate intakes of alcohol (Beilin and Puddey, 2006; Lucas et al., 2005). This protective effect of low to moderate intake has been replicated in numerous studies, across populations and gender, and persists even when controlling for potential confounders such as the “sick quitter” effect (Anand et al., 2008; Mukamal and Rimm, 2001; Yusuf et al., 2004). The definition of “low to moderate” continues to be a subject of debate; however, given the totality of the evidence, a prudent recommendation appears to be no more than one drink per day for women and no more than two drinks per day for men (Beilin and Puddey, 2006; Lucas et al., 2005; Mukamal et al., 2006).

1.3.1.8. Physical activity

WHO and FAO highlighted the importance of physical activity as a key determinant of obesity, CVD, and diabetes (Joint WHO/FAO Expert Consultation, 2003). For decades, evidence of the relationship between physical activity and CVD, independent of effects on weight and obesity, has strengthened. Increasing physical activity—including through brisk walking—has been shown to decrease the risk of chronic diseases such as CHD, stroke, some cancers (e.g., colorectal and breast cancer), type 2 diabetes, osteoporosis, high blood pressure, and high cholesterol (Physical Activity Guidelines Advisory Committee, 2008). Physical activity is also important for weight control and
In addition, regular physical activity is associated with a decreased risk of depression and improved cognitive function. Moreover, people who are physically active have improved quality of life and reduced risk of premature death (Physical Activity Guidelines Advisory Committee, 2008). Despite this powerful evidence, measurement weaknesses have contributed to the generally poor quality and availability of data on worldwide physical activity trends and impacts.

Guthold et al. (2008) published new data on levels of physical inactivity in 51 countries, most of which were low or middle income, and observed several trends. Globally, with the exception of several Eastern European countries (Croatia, the Czech Republic, Hungary, Kazakhstan, the Russian Federation, Slovenia, and the Ukraine), women were more likely to be physically inactive than men. Further, adults over 50 years of age were more likely to be inactive than younger adults, and city dwellers were more likely to be inactive than those who lived in rural areas. Physical inactivity levels were, with a few exceptions, similar in Eastern European, South Asian, and Western Pacific countries. In most of these countries, between 5 and 10 percent of men and between 10 and 16 percent of women were found to be physically inactive. By contrast, there was considerable variation in the levels of physical activity in both men and women within and across African, American, and Eastern European countries. For example, while women in 7 of the 18 African countries surveyed had the lowest levels of physical inactivity (fewer than 10 percent classified as physically inactive), Guthold found that more than 40 percent of women in Namibia, Swaziland, and South Africa were physically inactive. Despite the heterogeneity of the data, the study indicated that levels of physical inactivity in a number of low and middle income countries and among certain subgroups, particularly women aged 60–69 years, are disconcertingly high.

Many aspects of improving quality of life (such as better educational and sanitation facilities) are strongly associated with declines in physical activity, suggesting that multisectoral approaches involving workplace, transit, school, and leisure time need to be tackled if the trends are to be reversed. For this to happen, health professionals and policy makers need to fully appreciate the value of physical activity, both as a means to address energy balance and as an important avoidable cause of the global burden of chronic diseases. Currently this is not the case in most countries.
1.3.1.9. Psychosocial risk and mental health

Psychosocial factors have been consistently associated with both the onset and the progression of CVD in large prospective and epidemiologic studies in multiple populations and regions, yet they remain underrecognized when compared with more traditional CVD risk factors. The factors that have been associated with CVD include depression, anxiety, anger, hostility, acute and chronic life stressors, and lack of social support (Everson-Rose and Lewis, 2005; Figueredo, 2009; Shen et al., 2008). Although the causal pathways are not as well elucidated as for other risk factors, a robust body of evidence supports the conclusion that psychosocial factors independently and significantly increase both the risk of developing CVD and CVD morbidity and mortality.

1.3.2. Emerging non-traditional cardiovascular risk factors

Although traditional risk factors are validated for the diagnosis and management of CVD in many populations (McGill et al., 2008, Greenland et al., 2003, Llyod-Jones et al., 2006, Stamler et al., 2006, Steinberger et al., 2009) characterizing these attributes does not fully explain incident CVD. Furthermore, the underlying mechanisms for the association between traditional risk factors and CVD remain elusive. Research in adults suggests that biomarkers of novel pathophysiolgies contributing to CVD have the potential to augment clinical risk stratification by aiding in the prediction, identification, and assessment of atherosclerotic disease (Vasan et al., 2004, Hlatky et al., 2009). There are several novel emerging non-traditional risk factors including various inflammatory markers. Other non-traditional risk factors include the lipoprotein and non lipoprotein risk factors. Lipoprotein risk factors include metabolic syndrome (MS) and non high density lipoprotein cholesterol (non-HDL) and hypertriglyceridemic waist (Enlarged waist combined with elevated triglyceride) and non lipoprotein risk factors include pre-hypertension, homocysteine and emerging measures of obesity viz., body fat%, fat mass and fat free mass (lean body fat).

1.3.2.1. Non high density lipoprotein (non HDL) cholesterol

National Cholesterol Education Programme (NCEP) has recognized emerging novel risk factors for CVD. One of them is non-High Density Lipoprotein cholesterol (non-HDL-C), whose value can be calculated by subtracting HDL-C from total cholesterol (Peters 2008).
Non HDL-Cholesterol: Non-HDL cholesterol is the total cholesterol minus the HDL “good cholesterol i.e. Non-HDL Cholesterol = Total Cholesterol – HDL Cholesterol. So if total cholesterol is 190 and the HDL cholesterol is 40, then non-HDL cholesterol is 150.

It is helpful to know an individual’s non-HDL cholesterol because the level of non-HDL may predict one’s risk of cardiovascular disease even better than the LDL “bad” cholesterol. That’s because the non-HDL number tells about all the bad cholesterol circulating in blood – not just the LDL cholesterol but the VLDL cholesterol levels as well. Both LDL and VLDL particles are artery-clogging “bad” lipids. When we subtract the “good” cholesterol (HDL) from our total cholesterol, we are left with all the “bad” cholesterol. Studies from different populations have reported that non-HDL-C is more strongly associated with CAD than Low Density Lipoprotein cholesterol (LDL-C), (Packard and Saito 2003; Al-Nozha et al., 2004; Garg et al., 2013). Non-HDL-C has been identified as the emerging important lipid parameter found to be associated with cardiovascular diseases; and cardiovascular risk factors such as type 2 diabetes mellitus (T2DM) and metabolic syndrome (MS). Numerous studies have investigated the relationships between LDL-C and non-HDL-C and the risk of coronary heart disease (CHD) (Garg et al., 2013) and other cardiovascular risk factors such as metabolic syndrome, type 2 diabetes mellitus and hypertension (HTN). LDL-C is currently the primary treatment target for dyslipidemia management. However, it has been observed that the risk for future CHD events remains high in patients who have attained the guideline recommended LDL-C goals. Residual risk always remain in currently routine lipid lowering therapy which is almost certainly a reflection of various other comorbid conditions that CHD patients have (for eg. diabetes mellitus, hypertension, smoking etc). Examining non-HDL-C could explain some of this residual risk for future CHD events in such patients. Studies from different populations have reported the association of non-HDL-C with CVD (Garg et al., 2013).

1.3.2.2. Prehypertension

Prehypertension is defined as a systolic blood pressure of 120–139mmHg and/or a diastolic blood pressure of 80–89mmHg (Zhang and Li, 2011). The concept of pre-
hypertension was introduced as the new guideline for the management of blood pressure by the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) (Chobanian et al., 2003). The objectives of defining this classification of blood pressure were to draw the clinical and public healthy attention on the prevention of people in this range. Prehypertension is a precursor of clinical hypertension and is closely related with the increased incidence of cardiovascular disease (Vasan et al., 2001, Liszka et al., 2005, Ferdinand and Pacini, 2007). Patients with Prehypertension (120–139/80–89 mmHg) have an increased risk of cardiovascular morbidity and mortality compared with patients who have normal blood pressure (<120/80 mmHg).

1.3.2.3. Fat distribution

When measures of obesity are studied with respect to CVD risk, upward trends of obesity urge more effective identification of those at cardiovascular risk. Obesity is traditionally defined in terms of BMI—the standard measure of obesity. However, studies now say that BMI does not accurately predict cardiometabolic risk among Indians. Hence, the use of universal BMI cut-off points is not appropriate for comparison of obesity prevalence between various ethnic groups (Rush et al., 2004, Jackson et al., 2009). Obesity as described by American College of Sports Medicine (ACSM, 2006) is described as an excessive amount of adipose tissue, which is defined in young adults as body fat > 25% in males and >32% in females (Sadhu et al., 2010). Positive energy balance resulting in being overweight and obesity is usually calculated by weight and height indices, of which body mass index (BMI in kg/m2) is the most widely used. However, BMI does not differentiate between fat and lean mass tissue (Wellens et al., 1996, Frankfield et al., 2001). At a given BMI, Asians have significantly higher body fat content than whites and blacks do. Measurement of fat mass is needed for more accurate assessment of overweight and obesity (Deurenberg et al., 1998). Many techniques have been developed to assess body composition in humans. The gold standard of body composition in a two compartmental model has been under-water weighing. It measures body density, from which fat and lean mass content are estimated, by assuming standard figures for density of these components (Siri et al., 1963). Other robust methods include, total body potassium method (Boddy et al., 1972),
total body water method (Schoeller et al., 1980) and multicompartmental models of body composition (Harsha et al., 1996). However, for routine clinical and epidemiological approach, simpler and cheaper anthropometric measurements have been used to predict body composition in relation to body density by under-water weighing (Sarria et al., 1998). Equations, to predict body fat from anthropometric measurements allows the estimation of body composition without complex and costly techniques, and can be used easily for on field assessment. Most currently used predictive equations were derived from measurements of persons in affluent, industrialized western population of developed countries and they may be inappropriate for persons in underdeveloped or developing countries, who have different genotypic and phenotypic characteristics (Ramirez-Zea et al., 2006). For example, the equation proposed by Durnin and Womersley 1974, which was developed using under-water weighing method, overestimates body fat and % body fat in population of developing countries (Immik et al., 2003). With increasing obesity at even younger age (North American Association for the study of obesity. 2006) and reports of the metabolic syndrome becoming evident (Mattson et al., 2007), it is relevant to accurately estimate fat mass in Indian adults. In the present study, the predictive equations to quantify body fat and abdominal adipose tissue sub-compartments in healthy Asian Indians are used as given by Goel et al., 2009.

1.3.2.4. Hypertriglyceridemic waist

Seminal works from the Quebec Cardiovascular Study group introduced “hypertriglyceridemic waist” (waist circumference 90 cm and triglycerides 2.0 mmol/L) as a marker of atherogenic metabolic profile in men and demonstrated its utility in estimating the 5-year risk for cardiovascular events (Lamarche et al., 1998, Lemieux et al., 2000, LaMonte et al., 2003). More recently, Kahn and Valdez; 2003 defined normative thresholds for waist circumference (88 cm) and triglycerides (1.45 mmol/L) in both sexes using data from the third National Health and Nutrition Survey (NHANES III). Their work further illustrated the possible advantages of using enlarged waist combined with elevated triglycerides (EWET/HTGW), a simple dichotomous indicator, for identifying individuals with lipid overaccumulation. To date, however, no study has addressed the utility of EWET/HTGW in estimating the risk of the same in
CVD and its related risk phenotypes in Indians. In the present study, EWET/HTGW has been defined as elevated triglyceride (>2mmol/L) combined with elevated waist circumference (>80 cm in women and >85cm in men as per cut-offs for Asian Indians).

Apart from the lipids and obesity markers, homocysteine is another emerging bio-marker for CVD risk. Increased levels of homocysteine are associated with increased cardiovascular risk, independently of other atherosclerotic risk factors and the risk is present even in the upper limits of the “normal” range of 5-15 µmol/L (Stein et al., 1998, Christen et al., 2000). Elevated levels of homocysteine have been found to predict cardiovascular events (Clarke et al., 1991, McCully et al., 1996, Eftychiou et al., 2012). The amount of homocysteine in the blood is regulated by three vitamins: folate, vitamin B_{12}, and vitamin B_{6} (Gerhard et al., 1999). Therefore, deficiencies of these micronutrients lead to increased levels of homocysteine resulting in hyperhomocysteinemia.

1.3.2.5. Metabolic syndrome – a multiplex cardiovascular risk factor

The combination of metabolic disturbances now known as the metabolic syndrome (MS) was described by Kylin in the 1920s as the clustering of hypertension, hyperglycaemia and gout (Kylin, 1923). Two decades later, Vague noted that upper body adiposity (android or male-type obesity) was the type most often associated with the metabolic abnormalities seen with diabetes and cardiovascular disease (CVD) (Vague, 1947). During the 1988 Banting Lecture, Reaven used the term ‘Syndrome X’ and firmly established the clinical importance of this syndrome, although obesity was not included (Reaven, 1988). In 1989, Kaplan renamed it ‘The Deadly Quartet’ and others then coined the term ‘The Insulin Resistance Syndrome’ (Kaplan, 1989, Haffner et al., 1992). It is now agreed that the well established term ‘metabolic syndrome’ remains the most useful and widely accepted description of this cluster of metabolically related cardiovascular risk factors which also predict a high risk of developing diabetes.

Metabolic syndrome (MS) is a cluster of metabolic abnormalities, including centrally distributed obesity, decreased high density lipoprotein cholesterol (HDLC), elevated triglycerides, hypertension and hyperglycaemia. Due to changes in the human environment, behaviour, lifestyle and diagnostic criteria of MS, substantial numbers of
people with MS have been diagnosed over the past two decades. As an important risk factor for heart disease, stroke and diabetes, the MS has become a serious public health problem.

A number of expert groups have attempted to develop a unifying definition for MS. The most widely accepted of these definitions have been produced by the World Health Organization (WHO), The European Group for the Study of Insulin Resistance (EGIR) and the National Cholesterol Education Program- Third Adult Treatment Panel (NCEP ATP III) (WHO, 1999; Balkau et al., 1999; NCEP, 2001). All groups agree on the core components of the MS: obesity, insulin resistance, dyslipidaemia and hypertension. However, they provide different clinical criteria to identify such a cluster. For example, unlike the other two definitions, the ATP III definition does not obligatorily require impaired glucose regulation or insulin resistance as an essential component. In addition, the levels set for each component and the combination of components required to diagnose the MS are slightly different in these three recommendations.

**WHO Definition (1999)**

The WHO definition is based on the assumption that insulin resistance is one of the major underlying contributors to the MS. It therefore requires insulin resistance [or its likely surrogate, impaired glucose regulation, i.e. impaired glucose tolerance (IGT) or diabetes] to be present for the diagnosis to be made. In addition to insulin resistance, at least two other components must also be present for the MS to be diagnosed. The thresholds for systolic and diastolic blood pressures were changed between the provisional publication in 1998 and the definitive publication in 1999 (WHO, 1999; Alberti, 1998).

The working criteria developed by the WHO have been criticised. The inclusion of microalbuminuria as a component is considered by some to be controversial. Moreover, the inclusion of a measurement of insulin resistance has also been open to criticism, since determining whether or not an individual is in the lowest quartile of insulin sensitivity is virtually impossible in clinical practice or in epidemiological studies. Finally, the most appropriate measure of central obesity is also in dispute. Although the
waist-hip ratio (WHR) may carry information relevant to disease endpoints, it is an index of the relative accumulation of abdominal fat.

**EGIR definition (1999)**

Following the publication of the WHO definition in 1999, the EGIR proposed a modified version to be used in non-diabetic subjects only, which is simpler to use in epidemiological studies since it does not require a euglycaemic clamp to measure insulin sensitivity. EGIR proposed the use of fasting insulin levels to estimate insulin resistance and impaired fasting glucose (IFG) as a substitute for IGT. It also had slightly modified cut-points for hypertension, triglycerides (TGs), highdensity lipoprotein (HDL) cholesterol and altered measures and cut-points for central obesity based on waist circumference. Further, if subjects were being treated for dyslipidaemia or hypertension they were considered to have the corresponding abnormalities.

**NCEP ATP III definition (2001)**

The ATP III definition was presented in 2001 as part of an educational programme for the prevention of coronary heart disease (CHD). This definition was designed to facilitate diagnosis in clinical practice and differed in two major ways from the other definitions. First, it did not include a measure of insulin resistance as a component, and second, it was not ‘glucose-centric’, and treated glucose abnormalities as of equal importance with the other components in making the diagnosis. The ATP III guidelines state that the MS may be diagnosed when a person has three or more of five components. These components are: central obesity, an elevated TG level, a reduced HDL-cholesterol level, elevated blood pressure and an elevated fasting glucose concentration. Importantly, the ATP III definition includes waist circumference as the measure of obesity.

**IDF definition (2004)**

There is a strong need for one simple definition/diagnostic tool for clinical practice which could be used relatively easily in any country by any physician to identify patients at considerably increased risk of developing CVD and/or Type 2 diabetes. Such a definition would also allow comparison of the prevalence of the syndrome in different populations and its relationship with various health outcomes. In May 2004 the IDF
held an expert workshop to examine how the currently available definitions of the MS could be improved and developed with the aim of reaching a consensus for the introduction of a new, unifying and working worldwide definition.

The group considered it timely with the growing obesity epidemic to revisit and update levels and cut-points in the diagnosis of this syndrome. It was agreed that the definition should not only reflect the statistical clustering of the various potential components of the MS, but also focus on the prediction of CVD. The consensus group intend that the definition should be easy to use in clinical practice and avoid the need for measurements usually only available in research settings. An additional aim of the workshop was to discuss treatment of those with the MS and the prevention of diabetes and CVD.

The IDF workshop initially discussed the issue of whether the MS is a syndrome in its own right. A syndrome is defined as a recognizable complex of symptoms and physical or biochemical findings for which a direct cause is not understood. With a syndrome, the components coexist more frequently than would be expected by chance alone. When causal mechanisms are identified, the syndrome becomes a disease.

Figure 1.9: Pathogenesis of metabolic syndrome
Metabolic syndrome and cardiovascular risk

There is general agreement in the literature that MS imparts elevated CV risk (Malik et al., 2004; Gami et al., 2007; Galassi et al., 2006; Isomaa et al., 2001; Tong et al., 2005). Using data from NHANES II, Malik et al. 2004 examined the impact of ATPIII-defined MS on coronary heart disease, CV disease, and all cause mortality. They dichotomized MS patients with a diagnosis of diabetes. MS was associated with a hazard ratio (HR) of 2.02 for coronary heart disease. Patients with baseline CV disease had an even higher risk with an HR of 4.19. The HR for overall mortality in MS patients was 1.40; in those with pre-existing CV disease, it was 1.87. The HRs in MS patients without diabetes, MS with diabetes, MS with CV disease but no diabetes, and MS with both T2DM and CV disease, were 1.65, 2.87, 3.89, and 6.45, respectively. The risks of CV mortality paralleled these values and also supported previous studies demonstrating that the higher the number of MS components, the higher the risks of coronary heart disease, CV mortality, and all cause mortality. MS as an entity increasingly predicts the risk for coronary heart disease, CV disease, and overall mortality compared to its individual components (Isomaa et al., 2001).

Tong et al., 2005 examined the incremental risk of coronary heart disease in patients with MS in NHANES III. The authors included patients in the age range of 35–74 years, as the younger age groups would have lower coronary heart disease incidence, and older patients would have an element of survival bias favoring female subjects. The prevalence of MS in female subjects was 21 and 24% in the 35–54 and 55–74 years age groups, respectively. The prevalence in male subjects was 39 and 38%. The odds ratio (OR) of MS for coronary heart disease was 1.05 and 1.95, respectively, in 35- to 54 and 55- to 74-year-old female subjects, and 2.22 and 1.96 in the similar age group in male subjects. These results suggest that in patients over the age of 55 years, there is at least a twofold greater risk of coronary heart disease in both genders. The authors concluded that the lower prevalence of MS in the younger female population may be due to an estrogen protective effect in the premenopausal female. The above data also hold true in an elderly population. Using the Cardiovascular Health Study data, McNeill and co-workers (2006) examined 3,585 community dwelling patients, 65 years and older, to
determine whether MS was predictive of CVD. The HR for CVD in MS patients was 1.30 and 1.35 for female and male subjects, respectively.

1.3.3. Unmodifiable traditional risk factors

1.3.3.1. Age

The older a person is, the more likely it is for the heart and blood vessels to be damaged. The deposition of cholesterol is not an overnight process but is a slow process and deposition takes place in years. So, as the age increases the susceptibility towards the disease also increases (Austin et al, 1991).

1.3.3.2. Sex

Sex or gender also has the effect on CHD. Men are more likely to have heart and vascular disease at an early age than women. Women do not usually have heart and vascular disease before menopause (Regitz-Zagrosek, 2006). In all but the oldest age groups, CVD prevalence, incidence, and mortality rates tend to be higher for men than for women. This finding has remained consistent historically (Lawlor et al., 2001) and across countries and regions (Allen and Szanton, 2005; Pilote et al., 2007). In addition, women experience their first cardiovascular events later in life than men. The INTERHEART study found that, on average, women experience their first MI 9 years later than men (Anand et al., 2008). Similarly, a recent review of stroke epidemiology found that men have their first stroke an average of 4.3 years earlier than women. These findings are supported by WHO Global Burden of Disease data, which show that the average age of MI and first stroke is consistently lower among men across.

The reason most often cited for these gender differences is a protective effect of estrogen on the development of CVD risk factors, most notably hypertension and dyslipidemia (Regitz-Zagrosek, 2006). Estrogen is thought to contribute to premenopausal women’s tendency to have lower systolic blood pressure, higher levels of HDL cholesterol, and lower triglyceride levels than men (Buchanan and Brister, 2001; Pilote et al., 2007; Roeters van Lennep et al., 2002). The specific mechanisms of this protection have not been fully elucidated; however, estrogen is known to affect the
atherosclerotic and blood-lipid control process in a number of different ways (Roeters van Lennep et al., 2002). The erosion of this protection that occurs after menopause provides further evidence of estrogen’s protective role. Indeed, by age 75, women tend to have higher rates of hypertension and CVD than men (Legato, 1998; Narkiewicz et al., 2006).

1.3.3.3. Heredity

There is no question that some people have a significantly greater likelihood of having a heart attack or stroke because they have inherited a tendency from their parents. In some instances, such as familial hypercholesterolemia (very high levels of cholesterol in the blood), the pattern of inheritance is well understood and the specific biochemical defects are well characterized. For most cardiovascular risk factors, however, the specific way in which inheritance plays a role is not at all clear. Unlike diseases or disorders that are inherited from a single genetic defect in a Mendelian fashion, complex disorders are caused by a combination of environmental factors and mutations in multiple genetic loci. Although complex inheritance shows familial aggregation it is rarely in a Mendelian fashion and often shows variable phenotypic expression. This variable expression may occur due to chance, environmental factors or interaction with other gene (Lander and Schork, 1994). Further confounding factors include the possibility of incomplete penetrance, where individuals who inherit predisposing alleles may not manifest the disease; and phenocopy, where individuals who do not inherit predisposing alleles manifest the disease due to other genetic and non-genetic factors. As in almost all situations in medicine, both heredity and environment play a role and it is often difficult to know where one stops and the other begins. Prior generations did not have the level of medical care we now enjoy, nor the general awareness about health; the details of the illness that one’s grandparents or even parents had may not be precise. Prior to the 1960s, many more people smoked and little attention, if any, was paid to diet and fitness. So it is possible that environmental factors, not genes, were thought to be responsible for Grandpa’s heart attack or stroke.

In practical terms, anyone who has a family history of heart disease that occurred at an early age (below 55) should be especially careful to reduce the impact of any risk that
can be controlled. Even if one can successfully control known risk factors, there are, unfortunately, a number of inherited characteristics that we have not yet identified and so cannot favorably affect. Individuals with a history of atherosclerotic cardiovascular disease in the family simply have to be more vigilant if they wish to avoid heart attacks and strokes. One should remember, however, that almost every family has some member who died of a heart or blood vessel disease, since about half of all deaths are attributable to these diseases. If these episodes occurred in relatives who were 75 or 80, it may not be a major cause for concern.

Heredity also includes race. For reasons that are not completely understood, African-Americans have considerably higher rates of diabetes and both moderate and severe high blood pressure, adding to their overall risk of heart disease. Lower rates of coronary heart disease and other cardiovascular disease in the Japanese population compared with the US and other Western populations suggest the possibility of genetic differences that confer some protection from such disease in Japanese people.

There are several well-characterized single-gene disorders that contribute to CVD, such as certain forms of familial hypercholesterolemia linked to mutations of the apolipoprotein B gene, and during the past few years, there have been major advances in the identification of genetic risk factors for CHD, stroke, and CVD risk factors such as blood pressure, blood lipids, obesity, and diabetes (Arking and Chakravarti, 2009; Arnett et al., 2007). The identification of genetic loci associated with CVD, such as 9p21 (Palomaki et al., 2010), has led to major advances in understanding the pathophysiology of CVD, but genetic variants identified to date have explained only a fraction of heritability and do not appear to have substantial added value in predicting CVD beyond traditional CVD risk factors. These genetic risk factors are unlikely to have substantial clinical utility with respect to prediction, diagnosis, and treatment in the near future (Arking and Chakravarti, 2009). The prevailing view within the research community is that the genetic underpinnings of most common forms of CVD involve a complex interplay of many different genes, and much work remains to develop a more thorough understanding of the complex gene–gene and gene–environment interactions involved in the development of CVD (Arnett et al., 2007). Well-known genes implicated in the etiology of the common CVD include apolipoprotein genes, such as
apolipoprotein E (APOE) and APOB genes, thrombosis and hemostasis related genes, such as fibrinogen, factor V, and prothrombin, folate metabolizing gene, such as MTHFR. The susceptibility gene included in the present study is “MTHFR” (C677T).

MTHFR

Methylenetetrahydrofolate reductase (MTHFR) is a vital enzyme catalyzing the NADPH-linked reduction of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which serves as cofactor in methylation of homocysteine to methionine, which is used by the body to make necessary proteins and important compounds. Homocysteine is produced from methionine as a product of a large number of transmethylation reactions dependent on S-adenosylmethionine (Fig.). Three enzymes contribute to homocysteine metabolism, when there is an excess of methionine, homocysteine follows the transulfhydrylization pathway, through which homocysteine is converted automatically to cysteine. The first reaction in this pathway is catalysed by vitamin B6 dependent enzyme cystathionine β synthase (CBS) (Finkelstein, 1990). Under conditions with a negative methionine balance homocysteine follows two pathways. In most tissues, the remethylation of homocysteine is catalyzed by methionine synthase (MS), which uses B12 as coenzyme and methylene-tetrahydrofolate (MTHF) as substrate. The formation of MTHF is catalysed by methylene-tetrahydrofolate reductase (MTHFR) (Engbersen et al., 1995).

Research performed during the past decade has clarified our understanding of MTHFR deficiencies that cause hyperhomocysteinemia with homocystinuria, or mild hyperhomocysteinemia. MTHFR polymorphisms have been linked to hyperhomocysteinemia (high blood homocysteine levels). Various mutations can cause the genetic disorder homocystinuria. Most of these mutations substitute one amino acid for another amino acid in methylenetetrahydrofolate reductase. These substitutions disrupt the function of the enzyme, and may inactivate it completely. Other mutations lead to the production of an abnormally small, nonfunctional version of the enzyme. Without methylenetetrahydrofolate reductase, homocysteine cannot be converted to methionine. As a result, homocysteine builds up in the bloodstream and methionine is depleted. In humans, various derangements of the methioninehomocysteine metabolism
have been associated with reproductive performance, eg, hyperhomocysteinemia, folate deficiency, and vitamin B12 deficiency. (Wouters et al., 1993).

MTHFR gene encodes a protein which is an enzyme that catalyzes the reduction of methylenetetrahydrofolate to methyltetrahydrofolate, “a cofactor for homocysteine methylation to methionine” (Goyette et al., 1994). This enzyme is essential for the homeostasis and normal metabolism of intracellular folate. It is sometimes also denoted as MTR (5 methyltetrahydrofolate-homocysteine methyltransferase). The human MTHFR gene is located at chromosome 1p36.3 and consists of 11 exons with a length of 1980bp. The gene encompasses 19.3 kb DNA, includes 11 exons and is located in the cytosolic region. Expression of this enzyme is more intense in testis, intermediate in brain and kidney, and lower in other tissues. It codes for approximately 656 amino acids and 74.6 kDa protein (Figure 1.10).

![Figure 1.10: Schematic representation of the MTHFR gene, located on chromosome 1p36.3. Exons are shown in black boxes.](image)

The cloning of the MTHFR coding sequence was initially followed by the identification of the first deleterious mutations in MTHFR, in patients with homocystinuria. Shortly thereafter, the 677C>T variant was identified and shown to encode a thermolabile enzyme with reduced activity (Frosst et al., 1995). Currently, a total of 34 rare but deleterious mutations in MTHFR, as well as a total of 9 common variants (polymorphisms) have been reported. The 677C>T (A222V) variant has been particularly noteworthy since it has become recognized as the most common genetic cause of hyperhomocysteinemia. A variant of this enzyme, which is called "thermolabile MTHFR" or C677T /MTHFR is due to a single mutation of the MTHFR
This variant does not metabolize homocysteine as well as the normal MTHFR enzyme, and blood homocysteine levels in individuals with this variant enzyme may therefore be slightly higher than in individuals with the normal enzyme (figure 1.10). Individuals with the 677TT genotype tend to accumulate 5,10-methylenetetrahydrofolate intracellularly at the expense of 5-methyltetrahydrofolate, whereas individuals with the 677CC or 677CT genotypes have predominantly 5-methyltetrahydrofolate intracellularly (Bagley et al., 1998).

The distribution of MTHFR 677T shows heterogeneity among different ethnic and regional groups (Sabbgh et al., 2008). Most studies reported that the 677T polymorphism to be highest in the European and American population while least in African population in comparison to the other countries. The 677T allele frequency is reported to be 24% - 40% in Europeans (Van der put et al., 1997), 26%-37% in Japanese populations (Papapetrou et al., 1996), 30-40% in Mongoloid populations of Asia (Mongolia, China and Japan) (Spiridonova et al., 2004) and ~ 11% in African American population (Stevenson et al., 1997). Studies conducted by Stevenson et al. (1997), Motulsky (1996) and Goldstein and Hirschhorn (2004), predict the fact that the frequency of T allele is ethnic specific and varies from 11% among black to 35% among whites (Table 1.1).
Table 1.1: Worldwide frequency of the C677T allele

<table>
<thead>
<tr>
<th>Population</th>
<th>Sample size</th>
<th>Mutant allele frequency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>United Kingdom</td>
<td>94</td>
<td>0.240</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Spain</td>
<td>33</td>
<td>0.186</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>Italy (Calabria)</td>
<td>48</td>
<td>0.448</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>Italy</td>
<td>258</td>
<td>0.401</td>
<td>De Franchis et al., 1996</td>
</tr>
<tr>
<td>Ireland</td>
<td>99</td>
<td>0.253</td>
<td>Whitehead et al., 1995</td>
</tr>
<tr>
<td>Holland</td>
<td>207</td>
<td>0.256</td>
<td>Van der Put et al., 1997</td>
</tr>
<tr>
<td>Holland</td>
<td>111</td>
<td>0.299</td>
<td>Kluijtman, 1996</td>
</tr>
<tr>
<td>Russia (Russians)</td>
<td>122</td>
<td>0.318</td>
<td>Spiridonova et al., 2002</td>
</tr>
<tr>
<td>Sweden</td>
<td>6644</td>
<td>0.338</td>
<td>Brattstrom et al., 1998</td>
</tr>
<tr>
<td>America</td>
<td>76</td>
<td>0.322</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>South Carolina (blacks)</td>
<td>146</td>
<td>0.106</td>
<td>Stevenson et al., 1997</td>
</tr>
<tr>
<td>South Carolina (whites)</td>
<td>151</td>
<td>0.348</td>
<td>Stevenson et al., 1997</td>
</tr>
<tr>
<td>Georgia</td>
<td>109</td>
<td>0.211</td>
<td>Oiu et al., 1996</td>
</tr>
<tr>
<td>Nu-Chah-Nulth</td>
<td>37</td>
<td>0.189</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Brazil (Indians)</td>
<td>39</td>
<td>0.449</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Cayapa</td>
<td>57</td>
<td>0.430</td>
<td>Frosst et al., 1995</td>
</tr>
<tr>
<td>Canada (Quebec)</td>
<td>57</td>
<td>0.337</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>Educador</td>
<td>41</td>
<td>0.183</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>Africa</td>
<td>234</td>
<td>0.066</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Bantu</td>
<td>44</td>
<td>0.091</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Pygmies</td>
<td>8</td>
<td>0.063</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Gambia</td>
<td>24</td>
<td>0.063</td>
<td>Schneider et al., 1998</td>
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<tr>
<td>Kenya</td>
<td>61</td>
<td>0.049</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Madagascar</td>
<td>97</td>
<td>0.067</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Ghana</td>
<td>348</td>
<td>0.080</td>
<td>Rosenberg et al., 2002</td>
</tr>
<tr>
<td>Dendi</td>
<td>12</td>
<td>0</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>Bariba</td>
<td>13</td>
<td>0.077</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>Bebra</td>
<td>16</td>
<td>0.094</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>Fon</td>
<td>48</td>
<td>0.083</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>54</td>
<td>0.065</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>Asia</td>
<td>279</td>
<td>0.208</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Yemen</td>
<td>46</td>
<td>0.174</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>47</td>
<td>0.330</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Mongolia</td>
<td>36</td>
<td>0.361</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Palembang, Indonesia</td>
<td>61</td>
<td>0.164</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>67</td>
<td>0.045</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Israel</td>
<td>376</td>
<td>0.340</td>
<td>Rosenberg et al., 2002</td>
</tr>
<tr>
<td>Japan</td>
<td>164</td>
<td>0.420</td>
<td>Rosenberg et al., 2002</td>
</tr>
<tr>
<td>Tharu</td>
<td>54</td>
<td>0.194</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>China</td>
<td>12</td>
<td>0.375</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>Indonesia</td>
<td>49</td>
<td>0.020</td>
<td>Pepe et al., 1998</td>
</tr>
<tr>
<td>Australasia PNG Hilanders</td>
<td>85</td>
<td>0.047</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Polynesia (Chinese)</td>
<td>64</td>
<td>0.211</td>
<td>Schneider et al., 1998</td>
</tr>
<tr>
<td>Maewo, Vanuatu</td>
<td>71</td>
<td>0.085</td>
<td>Schneider et al., 1998</td>
</tr>
</tbody>
</table>
In India, a few studies have been reported on the frequency distribution of 677T allele. It is reported to be 9.45% among south Indian population (Devi et al., 2004). In a similar study among Tamilians of south India, 677T was found to be 10.4% (Angeline et al., 2004). Indian genome valley consortium (2008) also included MTHFR C677T as one of the markers and reported an average 677T allele frequency of 14%. Considering the high frequencies of 677T allele observed in Europe and North America, Saraswathy et al., 2011 have hypothesized that this gene might have originated in Europe in the late stage of human evolution. Subsequent migration, settlements and colonization might have helped in spreading the allele to the other parts of the world. C677T MTHFR polymorphic gene may increase the risk of cardiovascular disease and certain kinds of birth defects. This variant is relatively common in many populations worldwide (Table 1.1).

The association studies conducted on CHD patients and MTHFR genotype were found to be associated with 3 fold increased risk of CHD (Kluijtmans et al., 1996). Similar result was observed by Morita et al., 1997 where they reported a stronger association in homozygotes than in heterozygotes. Arruda et al., 1997 in a case control study on the Brazilian population found that the MTHFR- T allele is an important risk factor for the development of arterial disease and for venous thrombosis. Studies conducted by Stevenson (1997), Motulsky (1996) and Goldstein and Hirschhorn (2004), predict the fact that the frequency of T allele is ethnic specific and varies from 11% among black to 35% among whites.

Verhoef et al in 1997 conducted a case control study on Netherland population and found that there was a significant increase in homocystein level in carriers (both homozygous and heterozygous state) of MTHFR mutation. Kadziela et al (2003) in the study conducted on polish population found that homozygotes TT had significantly higher homocystein concentration than heterozygotes and homozygotes (CT or CC). Homocystein plasma concentration was significantly higher in patients with CHD than control and correlated significantly with folic acid levels. In addition, the analysis of serum homocystein levels in carrier patients (MTHFR 677T) showed that homocystein level was significantly higher in the group of male CHD patients with TT genotype.
A case control study on Atherosclerotic patient in Netherlands by Verhoef et al (1998) found a substantial difference between homocystein levels among the different genotypes. Patient with the ‘thermolabile’ MTHFR variant had elevated level of homocystein in plasma. Girelli et al (2003) conducted a study on American population and found that the fasting plasma homocystein level was higher in CHD cases than in controls but there was no significant difference between cases and control with respect to MTHFR genotype. A study conducted on Familial Hypercholesterolemia patients of Italy showed that the serum homocystein level was significantly higher in cases than in controls. The C677T variant of MTHFR gene increases the homocysteine level in the blood hence is one of the important genetic risk factor in vascular disease as reported by Frosst et al (1995). On the other hand, Guttormsen et al (1996) found that the daily supplement of low dose folic acid reduces or normalizes the homocysteine level. Further, Jaques et al. (1996) reported that folate supplement reduces the effect of TT genotype. Reyes – Engel (2002), Weger (2002) and Kumar (2005) reported the effect of the two mutations together in increasing the plasma homocysteine level. Further, the effect was adverse in the individual with 677TT and 1298CC genotypes.

The association studies conducted on CHD patients and MTHFR genotype were found to be associated with 3 fold increased risk of CHD (Kluijtmans et al, 1996). Similar result was observed by Morita et al (1997) where they reported a stronger association in homozygotes than in heterozygotes. Arruda et al (1997) in a case control study on the Brazilian population found that the MTHFR- T allele is an important risk factor for the development of arterial disease and for venous thrombosis. Further, a metanalysis by Klerk et al (2002) concluded that the individuals with 677TT genotype have a significantly higher risk of CHD particularly in individuals with low folate status. In Addition, Fletcher and Kessling (1998) and Ioannidis (2004) reported about the population specificity of allelic association and cause of several error of inference. Since, the genetic marker for gene association varied in frequency across populations, their biologic impact on the risk for common disease may usually be consistent across traditional racial boundaries.
Apart from the association with CHD, studies world over have reported 677C-T mutation to be associated with various other disorders like stroke (Kelly et al, 2002) where they found an association between mild to moderate hyper homocysteinemia and ischemic stroke and reported that TT genotype may have a small influence in determining the susceptibility to ischemic stroke. This report was supported by the meta analysis of 22 cases-control studies by Casas et al, (2004), who found a statistically significant association between ischemic stroke and 677T allele. In another study by Zalavras et al (2002), no association was found association with venous thromboembolism although the homozygosity for the 677T allele was more in patients than control. This report was supported by Lu et al (2002) who also could not find any evidence that the 677 C-T mutation was a risk factor for pulmonary thromboembolism in Chinese population. Further, Nishio et al (1996), in a study on Japanese population could not find any association with hypertension. But, the meta analysis by Qian et al (2007), suggested that C677T is an independent risk factor for hypertension. In a study conducted on pulmonary thromboembolism cases reported that the risk of thrombosis appeared higher for individual with T allele. Muntjewerff et al (2005), in a case-control study on schizophrenia found that the T allele was associated with schizophrenia along with increasing level of homocysteine. This report was supported by the meta analysis conducted by Lewis et al (2005; 2006) where it was reported that T allele is found associated with schizophrenia.

Castor et al (2004), suggested that 677TT genotype might be potential risk factor for diseases associated with DNA hypomethylation. Van der put et al (1998), reported that combined heterozygotes at the two polymorphic site was associated with reduced MTHFR activity and decreased plasma folate levels and two fold increased risk for NTD cases. Further, Isotalo et al (2000), Volcik et al (2001), Zetterberg et al (2002) reported the role of 677CT/1298CC and 677TT/1298CC genotype in fetal viability. They reported that the mutant allele may be detrimental during embryogenesis and was suggested that the carrier should be supplemented with folate during pregnancy.

Kerkeni et al., 2006 conducted a case control study on Tunisian population (100 CAD cases and 120 healthy controls) and found that patients with MTHFR TT genotype had higher plasma tHcy, serum creatinine, cholesterol and triglyceride concentrations than
patients with the MTHFR CC genotype. The C677T genotype of the MTHFR gene is associated with hyperhomocysteinaemia, lipid dysregulation and the presence of CAD in Tunisian population. In the case control study (100 cases and 100 controls) by Aleyasin et al., 2006 among Iranian, higher prevalence of C677T polymorphism as well as elevated level of homocysteine were observed in CAD cases compared to normal controls.

Tripathi et al., 2010 conducted a case control study on North Indian population (329 CAD cases and 331 controls) and their findings showed that MTHFR C677T polymorphism and homocysteine levels were associated with coronary artery disease in the North Indian population.

Sinha et al., 2010 conducted a case control study on Aggarwal population. Their study emphasized that the MTHFR C677T and A1298C polymorphisms are independent risk factors for coronary heart disease in Aggarwal community belonging to a specific geographical area of India. A Summary of the important studies on MTHFR C677T and Cardiovascular diseases has been given in in figure 1.12.

![Figure 1.12: Summarizing important studies on relation between MTHFR C677T and cardiovascular outcomes](image)

Indeed, in addition to the investigation of genes that influence CVD and its risk factors, there has recently been a surge in research examining how environmental factors affect
gene expression. Although research indicates that gene expression is most sensitive to environmental influence from conception to early life, there is also evidence that environmentally related gene expression changes can occur throughout life (Gluckman et al., 2009). This is an important emerging area of research for CVD. Future findings could have implications to help elucidate the physiological processes by which individuals with similar CVD risk profiles have different outcomes. Future research also could conceivably help develop new prevention and treatment strategies aimed at taking advantage of exogenous mechanisms that enhance or suppress the expression of key genes that play a role in mediating the development of CVD.

Looking forward, the explosive growth in molecular genetics techniques, advanced statistical methods, high-throughput technologies, and progress in studying gene–environment interactions should provide researchers with the ability to broaden the scope and applicability of their research. Techniques such as proteomics could lead to potential biomarkers to profile CVD risk more accurately, which could, for example, improve prediction of acute vascular events (Arnett et al., 2007). In addition, there is also significant promise in the emerging field of pharmacogenetics, which could not only help researchers develop more effective medications, but also better understand why certain drugs appear to be more effective in certain people. Since the initial availability of statins in late 1980, few new CVD drugs have emerged. Advances in genomic research could prompt more effective use of existing drugs and new drug development. At this stage, however, research in these fields has only modest potential for influencing population outcomes (Arnett et al., 2007).

1.4. Cardiovascular disease susceptibility: Genes or environment or GXE to play

1.4.1. Disease susceptibility

All cases of CVD have a complex multifactorial etiology. Neither genetic nor environmental agents acting independently cause disease. Full knowledge about an individual’s genetic makeup or exposures to adverse environments cannot predict with certainty the onset, progression, or severity of disease. Disease develops as a consequence of interactions between the “initial” conditions, coded in the genotype, and exposures to environmental agents indexed by time and space that are integrated by dynamic, regulatory networks at levels above the genome. The interaction of an
individual’s environmental experiences with her/his genotype determines the history of her/his multidimensional phenotype, beginning at conception and continuing through adulthood. At a particular point in time, each genotype has a range of possible phenotypes determined by the range of possible environmental histories. To illustrate this relationship, by collapsing an individual’s phenotype into a single dimension, two of the many possible phenotype histories for a genotype are given in Figure 1.13.

![Figure 1.13: Model for gene-environment interaction (Source: Talmund et al., 2007)](image)

This model proposes that each individual occupies a position on the genetic risk spectrum, depending upon how many risk-increasing gene variants have been inherited. Similarly individuals are exposed to a range of environmental challenges depending on life-style choices. This theory proposes that risk of CHD only occurs when an individual at high genetic risk enters a high-risk environment, and genetic risks and environment risks alone will not trigger a CHD event.

The phenotype of an individual in a particular environmental niche, at a particular point in time, is influenced by the phenotype produced by previous genotype-environment interactions and the potential of the genotype-phenotype combination to react to contemporary environments. The potential to react is constantly changing throughout life from conception to death. The consequence of these interactions with exposures to
environmental agents indexed by time and space is that many individuals who have a genotype that predicts an increased risk of disease will remain healthy because of exposures to compensatory environments. The converse will also be true. Individuals who have a genotype that has a low risk of disease might develop disease because of an adverse environmental history. The important role of the individualized history of exposures to environmental agents makes the average reaction of a group of individuals with the same genotype to the wide range of potential environmental agents over a lifetime a poor predictor of the risk of disease for most individuals with that genotype.

CVD research has revealed tens of high-risk environmental agents and hundreds of genes, each with many variations, that influence disease risk. As the number of interacting agents that are involved increases, a smaller number of cases of disease will have the same etiology and be associated with a particular multigene genotype.

1.4.2. GXE model for an individual to develop cardiovascular diseases

A particular multigene genotype is connected to the domain of potential coronary artery phenotypes through the primary biochemical and physiological subsystems (Figure 1.14). The phenotypic measures of health are constantly being shaped, changed, and transposed as a consequence of epigenetic networks of cellular and organismal dimensions that evolve over the lifetime of the individual. At the level of the cell, these networks influence DNA methylation and repair; they also serve to organize coordinated responses to heat-shock, oxygen deprivation, and other environmental changes. The relationships between subsystems influence the trajectory of an individual’s phenotype across the potential reaction surface associated with a particular genotype. The phenotype produced by these subsystems continuously feeds back information to influence the expression of the participating genes and the relationships between the intermediate agents that make up the connecting subsystems. Predicting multifactorial disease outcomes without consideration of epigenetic networks is increasingly seen as naive. Few genetic studies of the CVDs recognize the realities of the dynamic relationships between an individual’s genotype, her/his history of exposures to environmental agents, such as smoking, a high-fat diet, or a statin drug, and the contemporary phenotype in predicting phenotypic outcomes for a future point in time and a particular environmental niche.
1.5. Rationale

India is facing dual phases of epidemiologic transitions. soci-economically deprived groups are still straddling in the first phase where infectious diseases are still on a rise and higher socio-economic and demographically more advanced groups are in the third stage of epidemiologic transition facing a rising turmoil of non-communicable diseases. Thus, India is under double burden of diseases. India still lives in villages with more than 60% of the Indians living in rural areas. However, due to urbanization and westernization many of the rural areas are facing epidemiologic transition, cardiovascular diseases are on a rise and accounts for huge morbidity and mortality among Indians. Therefore, understanding of prevalence of such disease and its associated risk factors in rural India becomes an utmost priority. Moreover, changing lifestyle and environment has a major role in shaping the healthy or unhealthy phenotype in addition to the genes. It is known that alleles are population specific and now there is strong consensus that even environmental factors are culture specific, specific to particular community. Taking a mendelian population to understand the...
disease pattern and the gene-environment interaction in shaping the end phenotype of an individual may yield a better picture. Haryana, basically rural Haryana, is one among the Indian states that follows its culture and tradition and Jats is one of the predominant communities of Haryana who rigidly follow their culture. Not much data is available on Jat community of Haryana. They are residing in rural areas but are not so far from urbanized Delhi. As a result, they are likely to be exposed to drastic changes with respect to environmental factors. Understanding the prevalence of CVD associated risk factors in such population groups who are likely to undergo epidemiological transition may be useful in combating the forthcoming adversities.

1.6. Aim

To understand, estimate and document the distribution of various Cardiovascular diseases and associated risk factors and their interaction with methylenetetrahydrofolate reductase (MTHFR) gene among the Jat community of Haryana.

To achieve this aim the objectives of the present study are:

Objectives

- **Objective 1: To report the distribution of adverse cardiovascular variables (demographic, lifestyle, anthropometric, physiological, and biochemical) among Jats of Haryana (India)**

  It has been achieved by generating data through household survey from different villages of Haryana where Jats are predominantly settled.

- **Objective 2: To understand the extent of distribution of dyslipidemia, hyperhomocystenemia, hypertension, and metabolic syndrome in the present population.**

  It has been achieved by analyzing the collected samples for the presence of these selected parameters.
Objective 3: To estimate the extent of genetic variation in MTHFR gene (C677T) among Jat community and to understand its role in the development of dyslipidemia, hyperhomocystenemia, hypertension, and metabolic syndrome.

It has been achieved by analyzing the collected samples for MTHFR C677T polymorphism and statistically analyzing the relation of the same with the selected parameters using relevant softwares.

Objective 4: To understand the dynamic inter-relationship among environment (nutritional-folate and vitamin B12) and genetics (MTHFR C677T) with special reference to cardiovascular variables

Relevant statistical software like SPSS, Odd ratio calculator, SNP Stats, Graph Pad Prism and MS Excel have been used to achieve the dynamic interrelationship between selected environment and genetic variables.