1. Introduction

1.1. Overview

Diabetes is a serious metabolic disorder, which literally can affect whole body. Diabetes is a common, chronic disease that profoundly affects health and longevity. Susceptibility is influenced by inheritance, and there has been substantial progress in identifying genes which, when mutated, influence individual risk of disease. Through study of common and rare forms, both polygenic and monogenic, diabetes genetics encompasses many pressing issues in human genetic research (Florez et al., 2003).

1.2. Diabetes mellitus

The term "diabetes mellitus" describes a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs (Vaziri et al., 2003). Diabetes mellitus is defined by hyperglycemia: fasting (blood glucose >126 milligrams per deciliter [mg/dl] or seven mill moles per liter), non-fasting (random blood glucose >200 mg/dl on two occasions), or after an oral glucose tolerance test. Hyperglycemia was recognized as early as 1798 as a unifying biochemical manifestation of the disorder. The discovery of insulin eighty years ago furnished a therapeutic intervention and a miracle cure for young patients for whom insulin deficiency is absolute. Prior to this discovery, the disease was a death sentence (Florez et al., 2003).

1.3. Types of diabetes mellitus

Diabetes is classified into different types generally based on the mechanism it disturbs blood glucose homeostasis. Among them the two are principle: One is called insulin dependent (type 1) and another one is called non-insulin dependent (type 2) diabetes.

1.3.1. Type 1 diabetes: Type 1 diabetes (T1D) usually develops in childhood and adolescence and patients require lifelong insulin injections for survival. It accounts for only 5–10% of those with diabetes, In type 1 diabetes there is no insulin or not enough of it.

This type of diabetes basically presents in childhood or early adult life. It can be diagnosed distinguished from type 2 diabetes by the presence of immune and genetic markers of immune-mediated disease and it can turn into diabetic ketoacidosis if it is delayed to diagnosis. Type 1 diabetes is increasing rapidly worldwide but most common in people of European country. In case of type 1 diabetes most of the insulin-secreting beta cells in the pancreas are lost therefore this type of patient requires insulin treatment. Supplied insulin uptake the excess amount of blood glucose level and reduce the complications due to high level of glucose in blood. To prevent the type 1 diabetes we can take different type of early preventive actions, surviving beta cells regeneration, transplantation of islet or stem cell transplantation and gene therapy. (Linder *et al.*, 2015)

1.3.2. Type 2 diabetes:

This type of diabetes is the most prevalent in world population that accounts for ~90–95% of those with diabetes. Type 2 diabetes mellitus is a complex blood glucose–homeostasis disorder characterized by both insulin resistance and pancreatic b-cell dysfunction (Duggirala et al., 1999) i.e., there is generally enough insulin but the cells upon which insulin should act are not normally sensitive to its activity. A combination of genetic susceptibility and lifestyle like increased calorie intake, unhealthy meal planning choices, less exercise, Obesity are the major causes for type 2 diabetes. Having a family history is also a major cause of type 2 diabetes.

Type 2 diabetes (T2D) usually develops in adulthood and is related to obesity, lack of physical activity, and unhealthy diets. It shows some symptoms like tiredness, excessive thirst, needing to pee a lot particularly at night, losing weight, and blurred vision etc. Long time suffering from type 2 diabetes is associated with serious health complexity with eyes, heart and nerves and kidney. Some risk factors are-

- **Weight.** The more fatty tissue one has, the more resistant their cells become to insulin.
- **Inactivity**. The less active the person is, the greater his/her risk. Physical activity helps to control your weight, uses up glucose as energy and makes cells more sensitive to insulin.
- **Family history**. Risk increases if a parent or sibling has type 2 diabetes.

- **Race.** People of certain races - including black people, Hispanics, American Indians and Asian-Americans - are at higher risk.
- **Age.** Risk of T2D increases as you get older. But type 2 diabetes is also increasing among children, adolescents and younger adults.
- **Gestational diabetes.** Risk of T2D increase if individuals was previously diagnosed with gestational diabetes.
- **Polycystic ovary syndrome.** For women, having polycystic ovary syndrome — a common condition characterized by irregular menstrual periods, excess hair growth and obesity — increases the risk of diabetes.
- **High blood pressure.** Having blood pressure over 140/90 millimeters of mercury (mm Hg) is linked to an increased risk of type 2 diabetes.
- **Abnormal cholesterol and triglyceride levels**. If the person has low levels of high-density lipoprotein (HDL), or "good," cholesterol, risk of type 2 diabetes is higher. Triglycerides are another type of fat carried in the blood. People with high levels of triglycerides have an increased risk of type 2 diabetes. Your doctor can let you know what your cholesterol and triglyceride levels are

To prevent the type 2 diabetes one should follow a balanced diet chart because if anyone has the family history of Types 2 diabetes but she/he follow a proper meal chart, can avoid this type of diabetes in her/his life time. (Linder *et al.*, 2015)

1.3.3. Gestational diabetes: Gestational diabetes is a form of diabetes that causes high blood glucose during pregnancy. In all pregnancies, hormones secreted by the placenta interfere with the body's use of insulin causing what's called insulin resistance. If insulin production is not high enough to counter the insulin resistance, blood glucose rises. High blood glucose is known to cause adverse outcomes in pregnancy. In the first trimester, high blood glucose can cause pregnancy loss or malformations in the baby. Later in pregnancy, as a mother's elevated blood glucose crosses the placenta, it will cause the baby to produce insulin in response. Excessive insulin leads to excessive growth of the baby. This can cause issues during labor and delivery,

increasing cesarean section rate as well as neonatal hypoglycemia (baby's low blood glucose at birth). In addition, babies who have grown excessively are at risk for childhood obesity and Type 2 diabetes. As rates of Type 2 diabetes increase worldwide, so do rates of gestational diabetes. About 1 in 10 pregnant women is diagnosed with gestational diabetes. GDM is commonly diagnosed as high blood glucose starting between 24 and 28 weeks of pregnancy. Occasionally it is diagnosed earlier in pregnancy, which may indicate high blood glucose prior to pregnancy. (Donovan *et al.*, 2010)

1.3.4. Latent autoimmuno diabetes in adults: LADA, (Latent Autoimmune Diabetes in Adults) diabetes is rare and known as "late-onset" diabetes. Most adults diagnosed with LADA are older than 30 years of age. It's progression is slow; sometimes causing a misdiagnosis of Type 2 diabetes. LADA patients, (like Type 2), may initially use oral medication, exercise and diet to manage their diabetes, but eventually, the pancreas will altogether stop producing insulin, (like Type 1), thus necessitating insulin injections. This can take anywhere from a few months after diagnosis to several years. It's believed that more than 50% of non-obesity-related Type 2 diabetes diagnoses may actually be LADA. In terms of the diabetes population, LADA is estimated to account for 5-10% of cases. Symptoms are similar to Type 1 and Type 2 diabetes – excessive thirst, drinking and urination, in addition to blurry vision. (Gottsa, Bakhtadze and Berger, 2005)

1.3.5. Monogenic diabetes: Monogenic diabetes is a rare type of diabetes that's caused by a single gene mutation. It accounts for about 1-2% of all diabetes cases, though its prevalence may actually be up to 5%. It has characteristics of both Type 1 and Type 2, and is often misdiagnosed as one of those more common types. There are two main forms of Monogenic diabetes- neonatal diabetes and Maturity onset diabetes of the young (MODY). Neonatal diabetes is usually diagnosed in infants from birth to 6 months, though diagnosis may occur later in some cases. MODY (Maturity Onset Diabetes of the Young) is usually diagnosed in late childhood to adulthood. Monogenic diabetes is usually passed on in an autosomal dominant gene, (a sex independent gene that's inherited from one of the parents). This means only one copy of the mutation is needed to develop diabetes. There is usually a strong family history of diabetes and in multiple generations, (although it's possible for someone to have a spontaneous mutation).

Diagnosis, therefore, involves genetic testing for these diabetes-causing gene mutations that disrupt insulin production. One type of MODY involves a baseline fasting blood sugar that's 40 mg/dl higher than "normal," as well as hypoglycemia that occurs at a higher level. Another type of MODY involves kidney and reproductive organ abnormalities. Research thus far is limited, and much of it is somewhat outdated. (Chambers *et al.*, 2016)

1.3.6. Brittle diabetes: Brittle diabetes is a rare form of insulin-dependent diabetes and is marked by frequent and severe episodes of hypoglycemia and/or hyperglycemia (DKA). This instability of blood sugar levels often leads to hospitalization and necessitates frequent self-monitoring of blood glucose, the use of an insulin pump and a continuous glucose monitoring device (CGM). In rare cases, a pancreas transplant may be necessary. Emotional stress, hormonal imbalance, malabsorption, hypothyroidism, adrenal insufficiency, Impaired glucose counter-regulation (the patient's body doesn't react predictably when blood glucose levels drop), drugs or alcohol etc. can cause brittle diabetes. Brittle diabetes primarily affects those with Type 1 diabetes and is most common in women in their 20s and 30s, but can occur in men as well and at any age. It affects 3/1000 insulin-dependent individuals. (Tattersall *et al.*, 1991; Rgn and Frcp, 2001)

1.3.7. Cystic Fibrosis-related Diabetes (CFRD): People who have Cystic Fibrosis develop excessive mucus, which in turn can scar the pancreas. If scarring occurs, the pancreas stops producing normal amounts of insulin, causing the person to become "insulin deficient" like someone with Type 1 diabetes. Sometimes, a person with CFRD may not be able to absorb the insulin like someone with Type 2 diabetes, making them, (like Type 2), "insulin resistant." The later may occur when the person is sick, on steroid medication or when pregnant. (Moran *et al.*, 2010)

1.4. Prevalence in world population

Diabetes mellitus is one of the most common chronic diseases in nearly all countries, and continues to increase in numbers and significance, as economic development and urbanization lead to changing lifestyles characterised by reduced physical activity, and increased obesity. Estimates of the current and future burden of diabetes are important in order to allocate community and health resources, to emphasise the role of lifestyle, and encourage measures to

counteract trends for increasing prevalence (Whiting et al., 2011). According to the data published in 1993, in European populations, age-standardized prevalence of diabetes varied from 3% to 10%. Some Arab, migrant Asian Indian, Chinese, and Hispanic American populations were at higher risk with prevalence of 14–20%. The highest prevalence of diabetes were found in the Nauruans (41%) and the Pima/Papago Indians (50%). The highest estimates for prevalence of impaired glucose tolerance were seen in female Muslim Asian Indians in Tanzania (32%) and in urban male Micronesians in Kiribati (28%). Prevalence of diabetes rose with age in all populations in which age-specific data were examined. This trend was most pronounced in those at moderate to high risk. The ratio of prevalence of diabetes in men versus women varied markedly between populations with little discernible trend, although Impaired glucose tolerance (IGT) was generally more common in women. In most communities, at least 20% of diabetes cases were unknown before the survey, and in many communities, >50% were previously undiagnosed. In both Chinese and Indian migrant populations, relative prevalence was high when compared with indigenous communities (King and Rewers, 1993). Certainly, the recent data support the constant rising of diabetes case around globally. The rise of type 2 diabetes in South Asia is estimated to be more than 150% between 2000 and 2035. Although aging, urbanization, and associated lifestyle changes are the major determinants for the rapid increase, an adverse intrauterine environment and the resulting epigenetic changes could also contribute in many developing countries. The International Diabetes Federation estimated that there were 382 million people with diabetes in 2013, a number surpassing its earlier predictions. More than 60% of the people with diabetes live in Asia, with almost one-half in China and India combined. The Western Pacific, the world's most populous region, has more than 138.2 million people with diabetes, and the number may rise to 201.8 million by 2035 (Nanditha et al., 2016).

1.5. Prevalence in Bangladesh

In Bangladesh, which had a population of 149.8 million in 2011, a recent meta-analysis showed that the prevalence of diabetes among adults had increased substantially, from 4% in 1995 to 2000 and 5% in 2001 to 2005 to 9% in 2006 to 2010 (Saquib et al., 2012). According to the International Diabetes Federation, 415 million people have diabetes in the world and 78 million people in the South East Asia region; by 2040, this will rise to 140 million. There were 7.1 million cases of diabetes in Bangladesh in 2015 (International Diabetes Federation, 2017).

1.6. Diagnosis of diabetes

Symptoms of diabetes include tiredness, polyuria, polydipsia, and unexplained weight loss should be identified first. The particular patient should be go through some blood tests such as

- **Random Blood Glucose test:** This is also called casual plasma glucose. This is very simple test. No need any fasting condition. This is defined as any time of day without regard to time since last meal. The concentration for diabetes plus random blood glucose ≥11.1 mmol/l (200 mg/dl).
- **Fasting Blood Glucose:** Fasting is defined as no food or caloric intake for a minimum of 8 hours. The concentration for diabetes plus fasting ≥ 7.0 mmol/l (126 mg/dl) (Table 1.1).
- **2 hours Post hold Glucose:** This is also called Oral glucose tolerance test (OGTT). This test is done according to the guideline of WHO, using a intake of 75 g anhydrous glucose dissolved in water . The concentration for diabetes plus is ≥11.1 mmol/l (200 mg/dl) (Table 1.1).
- **Glycated hemoglobin:** The life span of RBC in human body is 90 to120 days. The glycated hemoglobin A fraction HbA1c is a demonstration indicator of the average glucose Concentration. To control blood glucose level HbA1c is recommended as an essential indicator. HbA1c ≥ 6.5% is considered as diabetes mellitus (Table 1.1)**.**

Table 1.1: Tests to diagnose Diabetes.

	HbA1c (%)	Fasting plasma glucose (mg/dL)	Oral glucose tolerance test (mg/dl)
Diabetes	≥ 6.5	≥ 126	≥200
Prediabetes	5.7-6.4	100-125	140-199
Normal	≤ 5.7	≤ 99	≤139

1.7. Pathophysiology of diabetes

Both genetic and environmental factors influence the development of diabetes. Commonly type 1 diabetes results in autoimmune destruction of pancreatic β cells. Environmental factors such as dietary factors, enteroviruses may trigger development autoimmunity to lead type 1 diabetes. Insulitis with gradual β-cell destruction leads to pre-diabetes and finally to overt DM. These patients are susceptible to other autoimmune diseases, such as Hashimoto's thyroiditis, celiac disease, Addison's disease, and myasthenia gravis. Forty genetic loci have been associated with T1DM by a genome-wide association study and meta-analysis. A number of genetic loci in the major histocompatibility (HLA) region are associated with increased susceptibility to developing T1DM, including the alleles DR3/4, DQ 0201/0302, DR 4/4, and DQ 0300/0302. The risk of T1DM is approximately 5% if there is an affected first-degree relative and slightly higher if the affected parent is the father rather than the mother. (Barrett *et al.*, 2010; Daneman, 2010). Understanding the pathogenesis of type 2 diabetes is complicated by several factors. Patients present with a combination of varying degrees of insulin resistance and relative insulin deficiency, and it is likely that both contribute to type 2 diabetes. the clinical features can arise through genetic or environmental influences. Moreover, hyperglycemia itself can impair pancreatic beta-cell function and thus cause insulin resistance, leading to a cycle of hyperglycemia causing a worsening metabolic state. Type 2 diabetes is often accompanied by other conditions, including hypertension, high serum low-density lipoprotein (LDL) cholesterol concentrations, and low serum high-density lipoprotein (HDL) cholesterol concentrations that, like type 2 diabetes, increase cardiovascular risk. This clinical conditions is referred to as the metabolic syndrome. Hyperinsulinemia occurring in response to insulin resistance may play an important role in causing these abnormalities. Increased free fatty acid levels, inflammatory cytokines, and oxidative factors have all been implicated in the pathogenesis of metabolic syndrome, type 2 diabetes, and their cardiovascular complications. (Yanbing Li, Xiahua Chen, 2004)

Hyperglycemia is direct linked to physiological & behavioral responses. In case of hyperglycemia, the brain recognizes it and sends a message to pancreas and other organs to decrease its effect through nerve impulses. Genetic susceptibility, environmental factors,

autoimmune factors, lifestyle- altogether drive towards type-1 and type-2 diabetes. Pathogenesis of two main type of diabetes has been shown below (Figure 1.1).

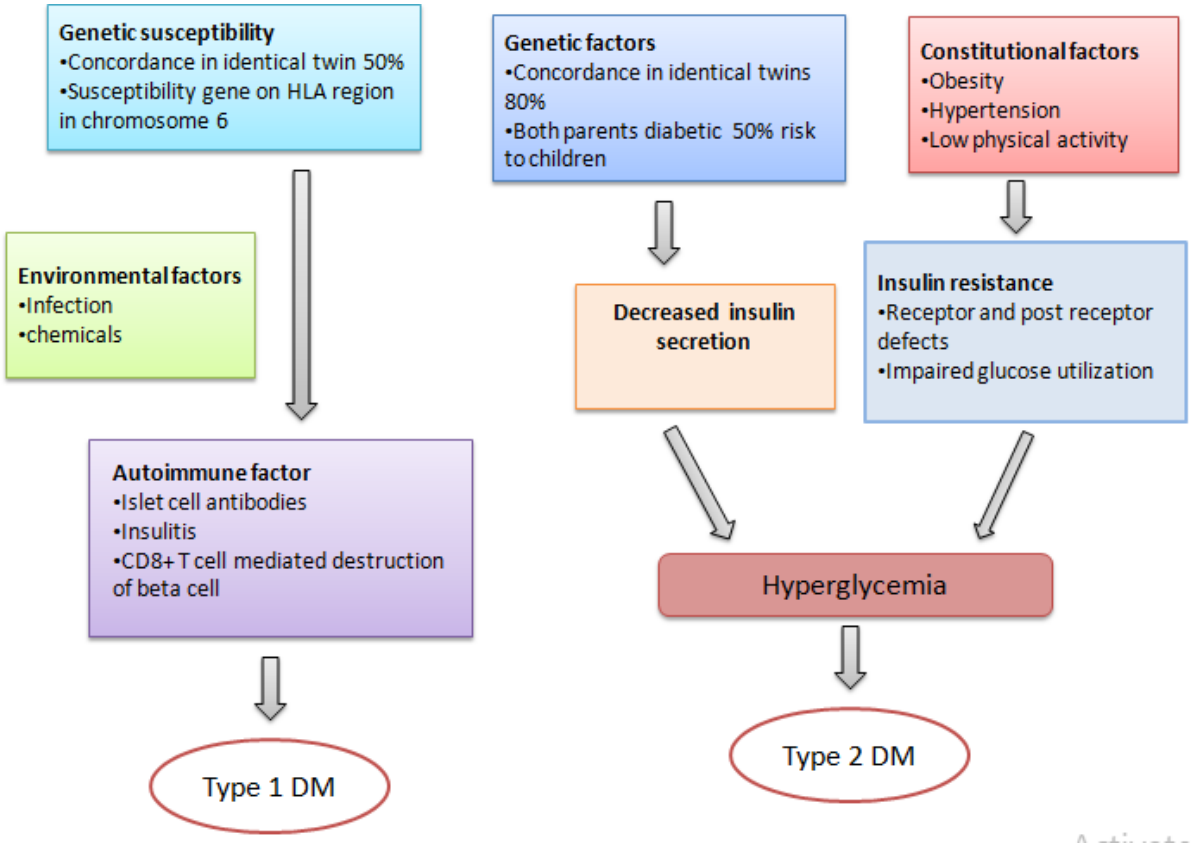

Figure 1.1: Schematic mechanisms involved in pathogenesis of two main types of diabetes mellitus.

1.8. Diabetes related complexities

Diabetes is recognized as an emerging global epidemic which represents one of the leading causes of morbidity and mortality all over the world. Hyperglycemia is most the common characteristic of both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). It has the potential to cause serious complications due to its sinister and continual nature. Complications due to diabetic can be divided into two major phase. One is acute phase another one is chronic phase.

1.8.1. Acute phase complications

Acute phase is defined as rapid onset of disease and symptoms revealed within a short period. Several examples of various acute phase diabetic complications are given below which can cause death of patient within a short time.

Diabetic ketoacidosis: ketone bodies are intermediate substrates in that metabolic sequence of ketosis. Ketosis happens when low insulin level metabolized fatty acid to ketone for fuel. Elevated levels of ketone bodies in the blood decrease the blood's pH which leads to Diabetic ketoacidosis .

Hyperglycemia hyperosmolar state: A person with above 16 mmol/L blood glucose levels, sometimes water is osmotically drawn out of cells into the blood and the kidneys ultimately begin to excreat glucose into the urine. The result is high blood osmolarity . If enough fluid is not replaced this will turn into dehydration. Electrolyte imbalances are most common and dangerous in this case.

Hypoglycemia: It is defined as abnormally low blood glucose which can cause even death in some cases. In hypoglycemic condition patient may become agitated, sweaty, weak, and sometimes can be senseless for a short time even patient can died in severe condition.

Diabetic coma: In this a case a person with diabetes mellitus become unconscious due to one of the acute complications of diabetes like severe diabetic hypoglycemic condition and diabetic ketoacidosis.

1.8.2 Chronic Phase complications

It means long time or life time suffering from a disease. Chronic phase complications in diabetes as follows:

Microangiopathy or microvascular disease: It is characterized by the damage of very small blood vessels of different organ in human body which can cause one or more of the following:

Diabetic Nephropathy: It is the chronic loss of kidney function occurred in diabetes mellitus patient. In this case the nephrone of glomeruli become damaged as a result of protein loss in the urine cause a low serum albumin of resulting body swelling which reflects the nephrotic syndrome**.**

Diabetic neuropathy: It is the nerve damaging disorders related with diabetes. In this condition the vasa nervorum become damaged due to high blood glucose level as a result of blood flow become hampered than the usual and develop diabetic neuropathy include mononeuropathy, mononeuropathy multiplex, autonomic neuropathy and thoracoabdominal neuropathy.

Diabetic retinopathy: It is a medical condition in which the retina becomes damaged due to diabetes mellitus. It is a primary cause to develop blindness. Diabetic retinopathy affects up to 80 percent of those who have had diabetes for 20 years or more. Some growth factors such as vascular endothelial growth factor (VEGF), growth hormone, and transforming growth factor β play important roles in the development of diabetic retinopathy.

Diabetic cardiomyopathy: This is a disease of the heart muscle in diabetic patient. In this case the heart develops inability to circulate blood through the body effectively resulting of heart failure.There are four main causes to develop heart failure in diabetic cardiomyopathy:

- microangiopathy and related endothelial dysfunction, autonomic neuropathy
- metabolic alterations that include abnormal glucose use and increased fatty acid oxidation
- generation and accumulation of free radicals and
- alterations in ion homeostasis, especially calcium transients.

Macrovascular disease: The term “Macro” refers large therefore macrovascular disease reflects of any disease related to large blood vessels including the coronary arteries, the aorta, and the sizable arteries in the brain and in the limbs. Diabetes is a strong risk factor for stroke and cerebrovascular disease and coronary artery disease. . Patients with type 2 diabetes have a high risk of stroke, with an increased risk of 150–400%.Stroke-related mortality rate are, elevated with diabetes patients. type 1 diabetes patients bear a unequal burden of coronary heart disease.

Ischemic heart disease and cerebrovascular mortality rate at all ages of type 1 diabetes has higher from compared to the general population.
The pathological mechanism to develop macrovascular disease is formation of atherosclerosis as a result of narrowing the arterial walls throughout the whole body. It is assumed that chronic inflammation and injury to the arterial wall leads to atherosclerosis.

Moreover to form atheroma there is indication of increased platelet adhesion and hypercoagulability in type 2 diabetes. Impaired nitric oxide generation and increased free radical formation in platelets and altered calcium regulation, may promote platelet aggregation. In case of diabetic patients high levels of plasminogen activator inhibitor type 1 may also impair fibrinolysis. In type 2 diabetes the combination of increased coagulability and impaired fibrinolysis may further increases the risk of vascular occlusion and cardiovascular disease.

Diabetic nephropathy

Diabetic nephropathy (DN) is one type of kidney disease caused by diabetes and this is the leading cause of chronic and end-stage kidney disease in the United States and worldwide. About 1 out of 4 adults with diabetes has kidney disease. When uncontrolled blood glucose level stands for long time the pathophygiological abnormalities of DN reflects which is followed by various changes in the nephrons resulting in excretion of increased amount microalbumin through urine. Diabetic nephropathy is directly influenced by high blood pressure. Other risk factors for diabetic nephropathy include: Smoking, age, sex, obesity, high blood cholesterol with diabetes and family history of DN.

1.9. Prevalence of diabetic nephropathy in world population and in Bangladesh

Diabetic nephropathy (DN) is a solicitous complication that takes place in 20% to 40% of all diabetic patients. In the Western country, Diabetic nephropathy is the primary single cause of end-stage kidney disease (ESKD). Diabetic nephropathy (DN) is more common in African-Americans, Asian-Americans, and Native Americans. In Caucasians patients with type 1 than type 2 diabetes mellitus progressive kidney disease is more frequent. The predominance of diabetes all over the world has reached epidemic proportions. It is estimated that more than 8%

of the global population (nearly more than 350 million people) are diabetic. Therefore this is predictable to grow diabetic over 550 million people by the year 2035. It has been estimated that more than 40% of people with diabetes will develop chronic kidney disease (CKD) with a significant number who will develop ESKD requiring renal replacement therapies like dialysis and or transplantation. (Gheith and Al-otaibi, 2016)

Bangladesh is a small but over Populated County. According to IDF there were 6.926.300 cases of diabetes in Bangladesh in 2017 (International Diabetes Federation, 2017). Prevalence of diabetes in adults: 6.9%. It is a middle income country. There is very few research work on diabetic nephropathy. The overall prevalence of nephropathy was 24.0%; male (27.1%), female (21.8%). (Rahim *et al.*, no date)

1.10. Pathogenesis of diabetic nephropathy

The pathogenesis and progression of diabetic nephropathy are a result of interactions between metabolic and hemodynamic pathways. The mechanism may be like that the metabolic and hemodynamic abnormalities seen in diabetes which are interact with each other which are linked to reactive oxygen species (ROS) generation. Gene regulation and activation of transcription factors are influenced by interactions among metabolic stimuli, hemodynamic factors and various ROS in diabetes. The consequences of molecular activation and inhibition of the various pathways lead to functional and structural changes that clinically demonstrated as diabetic nephropathy, which characterized by increasing albuminuria and declining renal function (Figure 1.2).

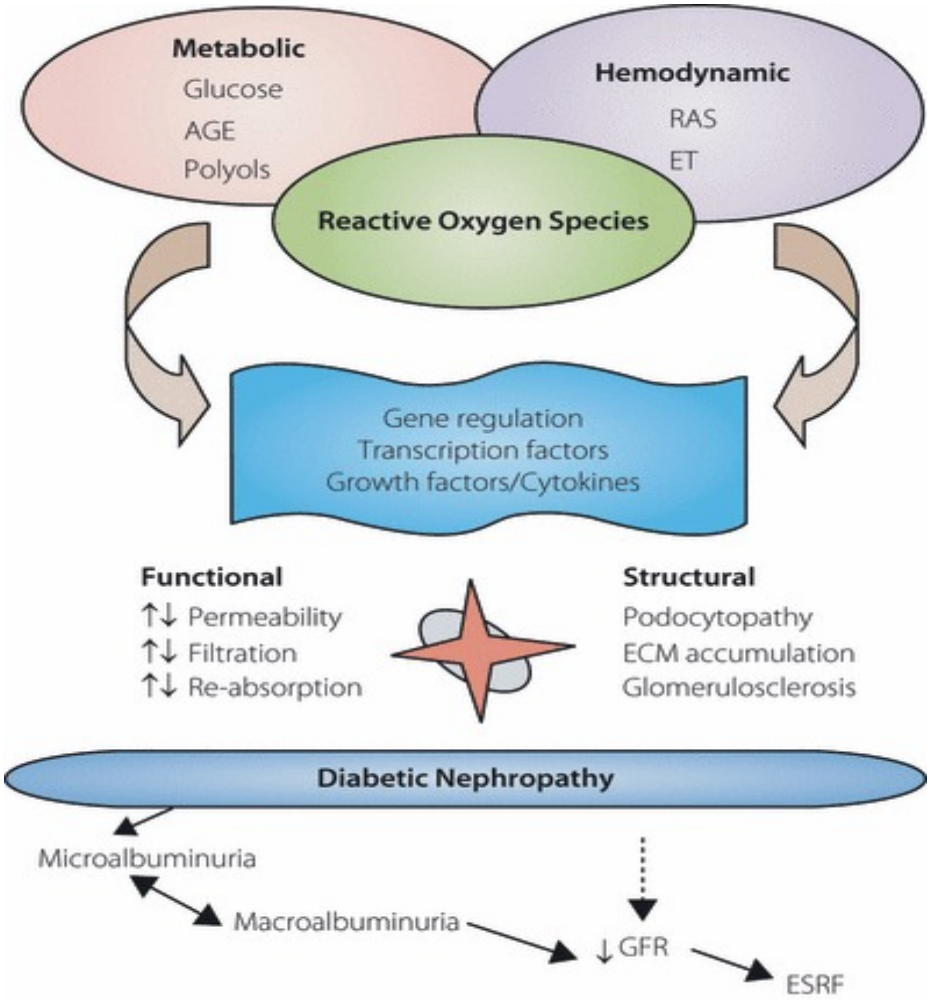

Figure 1.2: Pathogenesis of diabetic nephropathy. The pathogenesis of diabetic nephropathy is likely to be as a result of metabolic and hemodynamic abnormalities, as seen in diabetes, interacting with each other and with various reactive oxygen species-dependent pathways. Both gene regulation and activation of transcription factors are influenced by the interactions between metabolic stimuli, hemodynamic factors and reactive oxygen species generation in diabetes. The consequences of this molecular activation or inhibition are functional and structural changes leading to the classical hallmarks of diabetic nephropathy. AGE, advanced glycation end-

products; ECM, extracellular protein production; ESRF, end-stage renal failure; ET, endothelin; GFR, glomerular filtration rate. (Cao and Cooper, 2011)

1.11. Causes of diabetic nephropathy

Diabetic nephropathy is called when nephrones ofthe kidneys are damaged, allowing micro albumin to pass into the urine. The condition becomes critical as the level of albumin increases. When uncontrolled blood glucose level stands for long time the pathophygiological abnormalities of DN reflects which is followed by various changes in the nephrons resulting in excretion of increased amount microalbumin through urine. Diabetic nephropathy is directly influenced by high blood pressure. Other risk factors for diabetic nephropathy include: Smoking, age, sex, obesity, high blood cholesterol with diabetes and family history of DN. (Gheith and Al-otaibi, 2016)

1.12. Genetic association of diabetic nephropathy

Genetic linkage analysis consists mainly four steps: identifying linked loci, confirming linked loci, fine mapping of confirmed loci and then testing genes in the linked region in functional studies. Genetic association studies are more sensitive. The most important genetic marker for genetic association analysis is single nucleotide polymorphisms (SNPs). Because large quantity of SNPs covering the entire human genome at a high density. Through linkage based studies, candidate gene studies meta-analysis, and genome wide association studies, multiple genes have been identified which can play role in diabetic nephropathy. (Tang, Zeng and Zhang, 2015)

From various genetic association studies of diabetic nephropathy 34 replicated genetic variants were identified. Among these, 21 were significantly associated with diabetic nephropathy in a random-effects meta analysis. These variants were in or near the following genes: ACE, AKR1B1 (two variants), ApoC1, ApoE, EPO, NOS3 (two variants), HSPG2, VEGFA, FRMD3 (two variants), CARS (two variants), UNC13B, CPVL and CHN2, and GREM1, plus four variants not near genes. The odds ratios of associated genetic variants ranged from 0.48 to 1.70. Additional variants were detected in subgroup analyses: ELMO1 (Asians), CCR5 (Asians) and CNDP1 (type 2 diabetes). (Mooyaart *et al.*, 2011)

The virtual part and significance of the identified genes in the pathogenesis of diabetic nephropathy will be helpful in future study.

1.13. Apolipoprotein E and its isoforms

Apolipoprotein E (ApoE) is a plasma glycoprotein consists of 299 amino acids. ApoE is a small (34 kD) circulating protein associated primarily with VLDL and HDL, and is the primary ligand for several lipoprotein receptors, making it a crucial component in the clearance of lipid from the circulation. Its importance as a major determinant of plasma cholesterol and cardiovascular disease risk is underscored by the spontaneous hyperlipidemia and atherosclerosis in mice lacking ApoE (Mahley and Rall, 2000). Mice lacking ApoE (ApoE-/-) accumulate cholesterol-rich remnant particles with total plasma cholesterol levels exceeding 400 mg/dl (Zhang *et al.*, 1992). Although diets high in fat and cholesterol accelerate plaque development, ApoE-/- mice develop complex fully-formed atherosclerotic lesions even when fed a low fat, low cholesterol diet (Pendse *et al.*, 2009). In humans the APOE gene is polymorphic, resulting in production of three common isoforms: ApoE2, E3, and E4. The ApoE isoforms differ from one another by only one or two amino acids at position 112 and 158, with E2 having cysteines at both positions, E3 a cysteine at 112 and arginine at 158, and E4 arginines at both positions (Mahley and Rall, 2000). ApoE consists of two main structural domains that are connected by a hinge region (Figure 1.3). The N-terminal domain consists of a four alpha helix bundle containing the receptor binding region (residues 136-150). The C-terminal domain is predicted as a series of alpha helices and contains the major lipid/lipoprotein binding region (residues 244-272). Despite independently folding, the two domains can influence the properties of one another. The cysteine /arginine amino acid residues at positions 112 and 158 influence 7 interactions between the two domains. For instance, when the cysteine at 112 is experimentally replaced by an arginine in an apoE3 molecule, the positive charge effectively pushes the arginine at position 61 into a position where it forms a salt bridge with glutamate 255 (Dong and Weisgraber, 1996). Consequently, the N-terminal domain is pulled close together with the C-terminal domain (Figure 1.3, top). The modified ApoE3 molecule presents a lipid binding preference for VLDL similar to that of apoE4. In the absence of this interaction the preference is for high density lipoproteins, as with ApoE3

(Dong and Weisgraber, 1996). The compact form of apoE4 also leads to a much lower stability than apoE3. Likewise, ApoE3 has a lower stability than ApoE2 (Zhong and Weisgraber, 2010). The interaction of the N- and C-terminal domains in ApoE2 resembles that of apoE3. The difference between these two molecules instead arises from the additional cysteine residue at position 158 in ApoE2, which affects the LDL receptor binding region by indirectly generating an additional salt bridge with arginine 150, thereby lowering the overall positive charge and thus the receptor binding potential of the region (Zhong and Weisgraber, 2010). Upon binding to lipid, ApoE undergoes a major conformational change (Dong and Weisgraber, 1996; Zhong and Weisgraber, 2010). The ApoE molecule is thought to form a molecular envelope (horseshoe shape) around the surface of the phospholipid outer shell of a nascent lipoprotein particle. Interestingly, it has been demonstrated that artificial lipid complexes created in vitro with apoE4 bind to the LDLR with a similar to slightly higher affinity than lipid complexes made with apoE3. In contrast, particles made with apoE2 demonstrate a dramatically lower LDLR binding affinity (VLDL-3/4 (93%) > VLDL-3/3 (82%) > VLDL-4/2 (53%) > VLDL-3/2 (36%) > VLDL-2/2 (30%) (Gaulton *et al.*, 2015). In addition, apoE4 has been shown to have a preference to bind to triglyceride-rich VLDL, while apoE3 prefers to bind smaller, denser HDL particles.

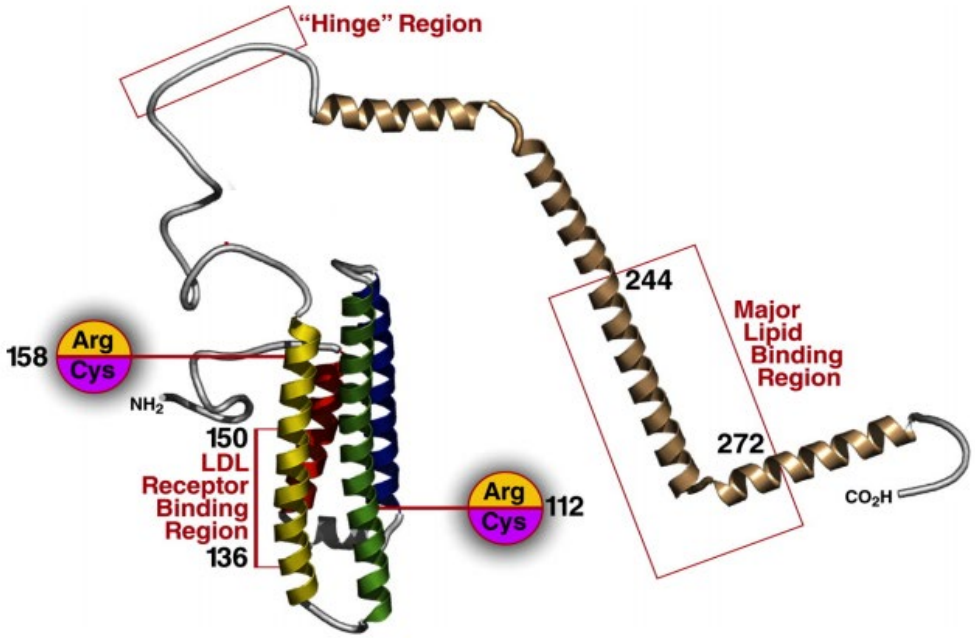

Figure 1.3: Structure and functional domains of ApoE. The N-terminal domain of apoE consists of a four α helix bundle (α helix 1 = red, α helix 2 = blue, α helix 3 = green, α helix 4 = yellow). The LDL receptor binding domain is located in a region of α helix 4. The C-terminal domain of apoE is predicted as a series of α-helices and contains the major lipid/lipoprotein binding region.

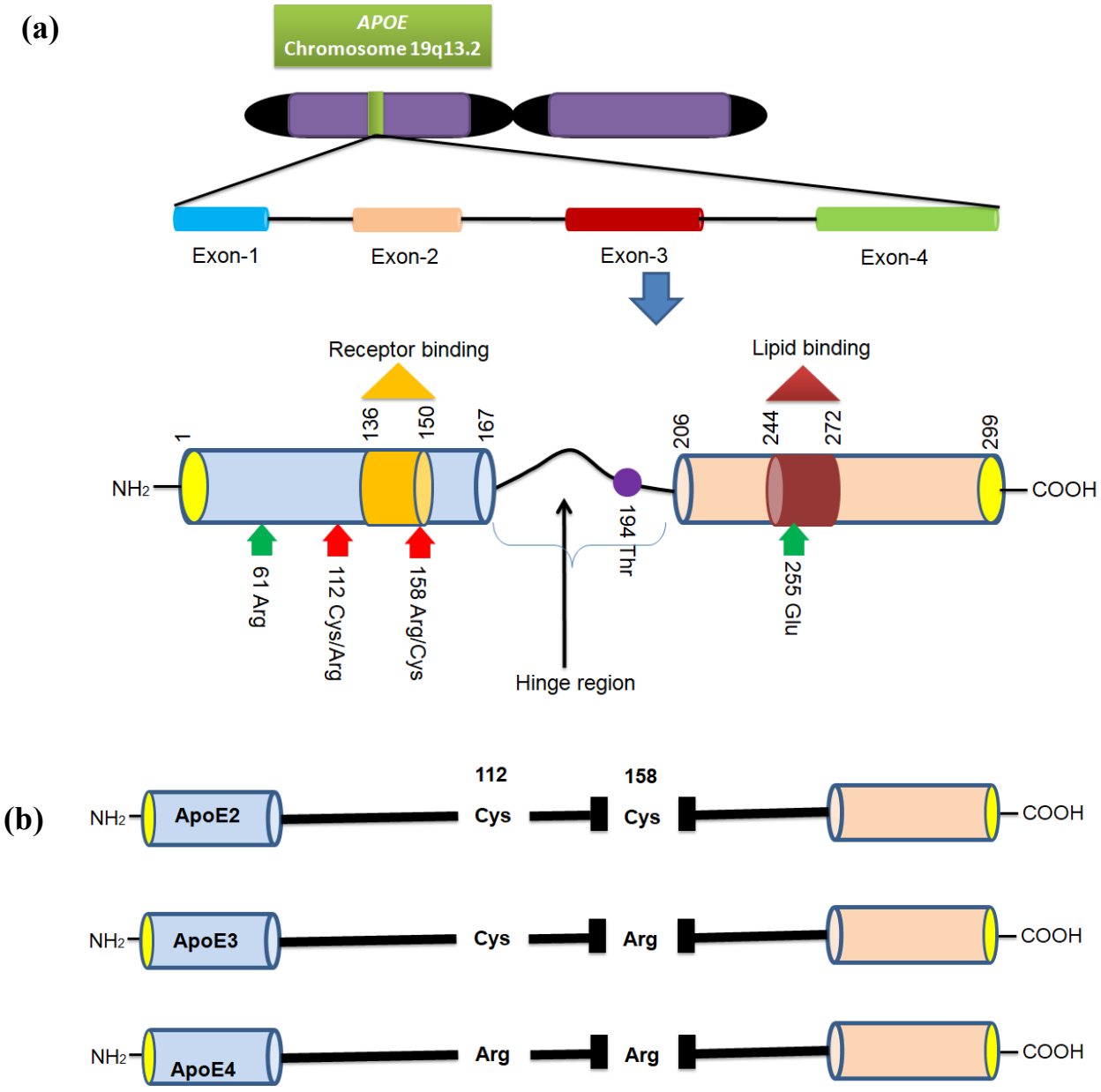

Figure 1.4: Schematic representation of structure and function of ApoE. (a) ApoE gene is located on chromosome 19. ApoE protein is a polypeptide with 299 amino acid residues. Receptor binding region (residues 136-150) is in the N-terminal domain and a lipid binding region (244-272) is in the C-terminal domain. (b) Three major ApoE isoforms are located at residues 112 and 158 (red circles), where ApoE2 has Cys residues at both position. ApoE3 has Cys residue at 112 and an Arg residue 158 and ApoE4 has Arg residues at both position.

ApoE gene located on the chromosome at position 19q13.2. It contains four exons and three introns. Several individual SNPs have been identified in the human ApoE gene. In particular, two SNPs, rs7412 (CGC → TGC, Arg158Cys) and rs429358 (TGC → CGC, Cys112Arg), are responsible for the three major alleles: epsilon-2 (E2), epsilon-3 (E3), and epsilon-4 (E4). They are responsible for three homozygous (E2/E2, E3/E3, and E4/E4) and three heterozygous (E2/E3, E3/E4, and E2/E4) genotypes and such that, six different phenotypes accordingly- three homozygous (E2/E2, E3/E3, and E4/E4) and three heterozygous (E2/E3, E3/E4, and E2/E4) (Zannis *et al.*, 1982). The three major protein isoforms, ApoE2 (Cys112-158Cys), ApoE3 (Cys112-158Arg), and ApoE4 (Arg112-158Arg), differ from each other by only one or two amino acids at positions 112 and 158 (Figure 1.4). These differences alter APOE structure and function, respectively (Utermann G, Hees M, 1977; Rall *et al.*, 1982).

The predominant isoform, ApoE3, contains cysteine at 112 and arginine at 158; ApoE2 has cysteine at both positions and is associated with higher ApoE plasma concentrations, and ApoE4 has arginine at both sites and is associated with lower concentrations compared to ApoE3 (Siest et al., 1995). ApoE4 isoform (*E*4* allele) is a well-known marker associated with increased risk of coronary artery disease (CAD) (Chaudhary et al., 2012; Zhang et al., 2014) and late-onset of Alzheimer's disease (Farrer et al., 1997; Kamboh et al, 1995). A large body of data, including several initial findings from our own group, suggests that E2 carriers have a propensity for higher levels of total plasma cholesterol along with increased risk of heart disease and T2D when compared with people having the commonest E3, while E2 carriers are protective (Bennet et al., 2007; Burman et al., 2009).

1.13.1. Association of ApoE with diabetes

ApoE has a major role in lipid and lipoprotein metabolism. Variation in ApoE is known to have a significant impact on various inflammatory and metabolic diseases, in addition to its well-known regulatory role in lipoprotein metabolism and lipid transport within tissues by enhancing lipoprotein uptake of ApoE-bearing receptors (E-specific remnant receptor and low–density lipoprotein receptor, (LDLR) (Mahley, 1988). ApoE, an essential mediator of lipid metabolism in normolipidemic patients, plays a major role in diabetic dyslipidemia as well. Increases in VLDL triglycerides, decreases in HDL, the accumulation of smaller/denser LDL, slower clearance of postprandial chylomicrons, a decrease in lipoprotein lipase (LPL) activity, and a decrease in LDLR expression, are all commonly noted phenotypes associated with both type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes (Ark *et al.*, no date)

A study demonstrated that ApoE4 allele as an independent risk factor for T2DM (Lebedy, Raslan and Mohammed, 2016). Genotypes E4/E4 and E3/E4 associated with increased risk for CHD in NIDDM patients and the prevalence of CHD disease among diabetic patients with genotypes E4/E4 or E3/E4 was 81 vs. 58 % among patients with genotype E3/E3, and 53 % among those with genotypes E2/E2 or E3/E3 (Laakso *et al.*, 1991). Also, E4-bearing genotypes associated with increased risk for macro and micro vascular complications in NIDDM patients both in men and women, in contrast to E2 phenotype which somehow protected from macroangiopathy and associated with lower plasma TC and LDL-C concentrations and lower plasma lipoprotein (a) levels (Ukkolaa *et al.*, 1993)

1.13.2. Genetic association of ApoE with diabetes and diabetes caused nephropathy

Apolipoprotein E (ApoE) is a candidate gene for the development of T2DM due to its critical role in the lipid metabolism. ApoE is important for the development of several plasma-lipoprotein lipid particles like very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL), high density lipoproteins (HDL) and chylomicrons. Besides its role to the formation of the different kind of lipoproteins, ApoE acts also as a ligand for the binding of lipoproteins to plasma lipoproteins receptors. Low-density lipoprotein receptor (LDLR) is a membrane protein that mediates the endocytosis of cholesterol-rich LDL and specifically

recognizes Apolipoprotein E. Thus, it plays an important role in the regulation of plasma and cellular lipid concentrations (Rall and Mahley, 1992; Kumar *et al.*, 2016). Insulin resistance is known to be strongly associated with metabolic dyslipidemia and the correlation of lipid profiles with diabetic phenotypes is important, since T2D patients have an atherogenic lipid profile, which greatly increases their risk of coronary heart disease compared to people without diabetes (Taskinen, 2002; Anthopoulos, Hamodrakas and Bagos, 2010).

ApoE polymorphisms may change both the structure and the function of the protein. E3 is the wild-type isoform with its normal function but those are defective for binding to the APOE receptor, accumulate with triglyceride (TG)-rich lipoproteins and remains particles derived from TG rich lipoproteins in plasma. However, the E4 isoprotein increased binding to the APOE receptor, causes reduced TG levels and increased total cholesterol and low-density lipoprotein (LDL) cholesterol levels in plasma. Some recent work shows the frequency of the E2 allele was significantly higher in diabetic nephropathy patient than in those without nephropathy. These raised the possibility that the E2 allele increased the risk of DN in Chinese, Japanese and Korean patients with T2DM However, additional few studies in East Asian populations conflicted with this result. In addition, it had been reported that the E4 allele was a protective factor for DN except for the little-mentioned study concerning the association between E4 allele and risk for DN. These difficulties in estimating the potentially true and modest effects of APOE genotypes on DN risk in East Asian populations may be due to ethnic distinctions or limited sample sizes in the individual studies.

1.14. Lipoproteins and its isoforms

Lipid is one of the major biomolecules in terms of important role they play, but since it is hydrophobic and thus, insoluble in aqueous environment, transportation of lipid around the body via blood is a problem. Apolipoproteins are some specialized proteins that bind with these lipids as well as able to interact with aqueous environment, forms lipoproteins and thus transport lipids. There are several types of Apolipoproteins- Apolipoprotein A (ApoA), Apolipoprotein B (ApoB), Apolipoprotein C (ApoC), Apolipoprotein D (ApoD), Apolipoprotein E (ApoE), Apolipoprotein H (ApoH) etc. These apolipoproteins may also have subtypes like, ApoA1, ApoA2, ApoB-48, ApoB-100 etc. Different types of apolipoproteins binds with different lipids

to form different types of lipoproteins, eg; ApoA1 forms HDL (High density lipoprotein) which removes harmful cholesterol from body (Guerra *et al.*, 2005). Lipoprotein A (LpA) is a cholesteryl ester–rich lipoprotein distinguished from other lipoproteins by the presence of a unique apolipoprotein (apo), apo(a) (Figure 1.5). Plasma concentrations of LpA vary over a 1000-fold range, and the distribution of levels is highly skewed (Albers, Adolphson and Hazzard, 1977).

The Apo A1 gene encodes apolipoprotein A1, which is the key protein component of high density lipoprotein (HDL) in human blood. Defects in this gene are associated with HDL deficiencies (Figure 1.5 b). Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein. Decreased levels of apo A1 are associated with vascular disease.

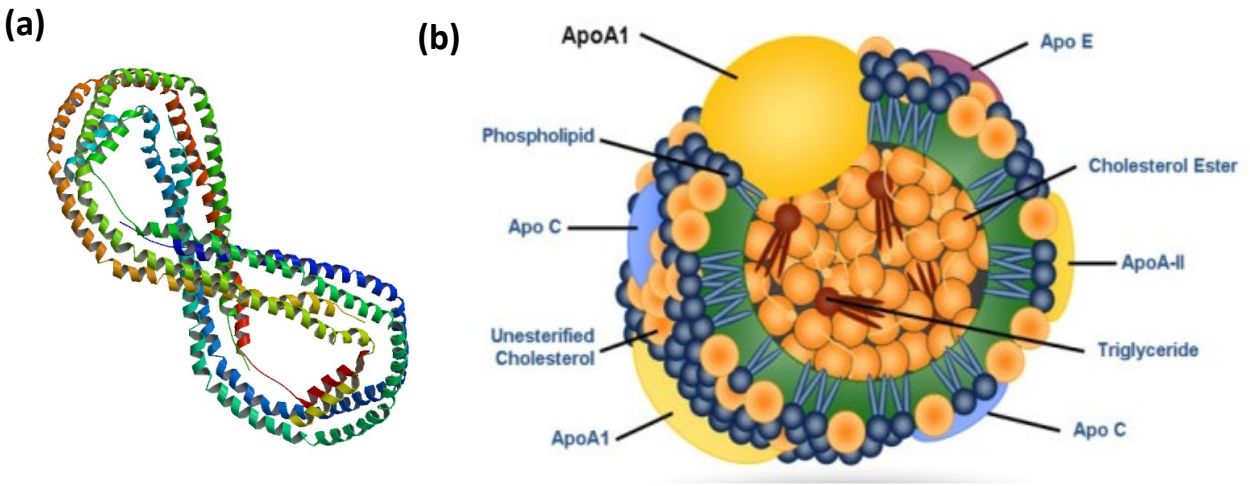

Figure 1.5: (a) Structure ApoA1 protein. (b) Structure of HDL. The outer shell is made of apoproteins, phospholipids and free cholesterol and the inner core is made of cholesteryl esters and triglycerides.

Genetic Association of Apolipprotein A Genetic variation in the APOA1 gene affect levels of HDL cholesterol and apoA-I resulting the risk of IHD and myocardial infarction (MI) in the general population. The APOA1 gene is located on the 11th chromosome and the exact location

is 11q23.3 (Figure 1.6). The gene contains 4 exons. APOA1 encodes a 45.4 kDa protein which is composed of 396 amino acids with 21 peptides observed by mass spectrometry data.

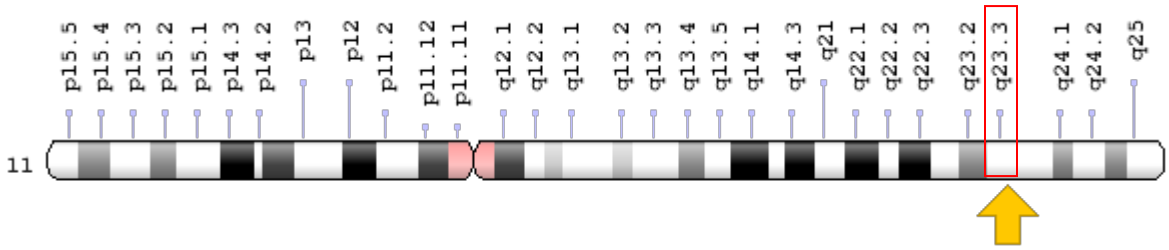

Figure 1.6: Chromosomal location of ApoA1.

1.14.1. Genetic association of ApoA1 with diabetes and chronic kidney disease.

Some commonly identified risk predictors of diabetes are age, family history of diabetes, body mass index, abdominal obesity, hypertension, sex, fasting glucose level, physical inactivity and high density lipoprotein-cholesterol. Apolipoprotein A1(ApoA1) and apolipoprotein B (ApoB) are the major parts of the protein moieties of HDL and low density lipoprotein-cholesterol (LDL-C), respectively (Gotto, 1990). High concentrations of ApoA1 were found to be predictive of type-2 diabetes in Turkish population (Onat *et al.*, 2010). A study among Korean population stated that the ratios of lipid and lipoprotein profiles, total cholesterol/HDL-C and ApoB/HDL-C, can be independently associated with later development of type 2 diabetes (Seo *et al.*, 2011). The reduction of HDL-C levels was associated with beta-cell dysfunction in subjects with impaired glucose tolerance (Maria and Stefano, 2013). rs670 of APOA1 gene may be associated with low HDL-C disease in chinese population; obesity was the risk factor for low HDL-C disease; the low HDL-C disease is influenced by APOA1, obesity, and their interactions. (Wang *et al.*, 2017) Dyslipidemia may affect the kidney directly by causing deleterious renal lipid disturbances, as well as indirectly through systemic inflammation and oxidative stress, vascular injury, and other signaling molecules with renal action. lipid abnormalities are associated with the development of renal disease in individuals who were free of kidney disease (Ruan, Varghese and Moorhead, 2009; Onat *et al.*, 2010; Bobulescua, 2011). In recent past, the G-75A and C+83T polymorphisms within ApoA1 gene were described in the native population of Assam, where significant differences were noted in their distribution as compared to populations of neighboring regions (Bora et al, 2016). The *apolipoprotein A1* gene (*APOA1*), which is a part of the *APOA1-*

CIII-AIV gene cluster on chromosome 11, is a major site controlling the expression of lipids and lipoproteins (Dallinga-Thie et al, 1996; Wojciechowski et al, 1991; de Franca et al, 2005; Ding et al, 2012). Thus, allelic variants of *APOA1* that influence cardiovascular disease risk are of interest.

1.15. Rationale of the study

Diabetic nephropathy (DN) is one of the major chronic complications of diabetes. ApoE is a high affinity ligand of several lipoprotein receptors in liver like low-density lipoprotein receptor and LDL-related protein which plays important roles in triglyceride lipoprotein and cholesterol metabolism. ApoE gene polymorphism is one of the important factors affecting body's lipid levels, in particular serum cholesterol levels. The ApoE affinity to LDL receptor and lipoprotein particles was changed because of its different allele. Several studies reported that the association of ApoE gene polymorphisms with type 2 diabetic nephropathy (T2DN) with incompatible findings. Lipoproteins are core full of fat and cholesterol along with a lipid membrane that contains protein known as apolipoproteins. Apolipoprotein A1 (ApoA1) is the most abundant component of HDL-C. As a result it has a potential effect in offering protection against atherosclerosis or coronary artery diseases. ApoA1 gene polymorphism has been found to be associated with coronary artery disease in different population. However, to our knowledge no study has demonstrated its association with the risk of type 2 diabetes with or without nephropathy in Bangladeshi population.

1.16. Objectives of the study

The objective of the present study was to find out the genotypic and functional association of apolipoprotein E and lipoprotein(a) gene polymorphisms with diabetic nephropathy in Bangladeshi population and thus, to evaluate the possibility of these genes for their involvement as the independent risk factor for the development of diabetic nephropathy.

The specific objectives of this study were to analyze

- Allelic and genotypic frequencies of ApoE in diabetic nephropathy patients.
- Association of genotypes and alleles regarding ApoE gene polymorphisms with the risk of developing type 2 diabetes with nephropathy
- Allelic and genotypic frequencies of lipoprotein(a) i.e., ApoA1 in diabetic nephropathy patients.
- Association of genotypes and alleles regarding lipoprotein ApoA1 gene polymorphisms with the risk of developing type 2 diabetes with nephropathy.
- Distribution pattern of clinical parameters in different genotypes with respect to ApoE gene in the study participants.
    - Distribution pattern of clinical parameters in different genotypes with respect to lipoprotein ApoA1 gene in the study participants.