Prevalence of Genetic and Non Genetic risk factors for Diabetes Among Obese Individual

By

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MASTER OF SCIENCE IN BIO SCIENCES

DEPARTMENT OF BIOINFORMATIC & BIOSCIENCES
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This is to certify that,this thesis submitted by Miss Sidra Azam is accepted in its present form without any change by the Department of Bioinformatic & BioSciences, Capital University of Science & Technology, Islamabad, Pakistam; as satisfying the thesis requirements for the degree of Master of Science In BioScience. The title of Thesis is: Prevalence of Genetic and Non Genetic Risk Factors for Diabetes Amonge Obese Individuals.

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Declaration

The material and information contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Sidra Azam

Dedication

This thesis is dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time. I also dedicate this work to my husband;Sabir Shabir who has encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish that which I have started.

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LIST OF ABBREVIATIONS

Melanocortin -4 receptor gene	(MC4R)
Pro-opiomelanocortin	(POMC)
Prohormone Convertase 1	(PCSK1)
Genome wide association	(GWA)
Type 2-diabetes	(T2D)
Cardiovascular diseases	(CVD)
Socioeconomic status	(SES)
Non communicable diseases	(NCDs)
World Health Organization	(WHO)
Fat Mass and Obesity associated gene	(FTO)
Catechol-O-Methyl Transferase	(COMT)
Angiotensin converting enzyme	(ACE)

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Abstract

The incident of obesity and its co morbidities such as (diabetes, heart diseases) keeps on expanding at a disturbing rate. Obesity has negative impacts on health of individual, leading to increased chances of diabetes type II in obese individual. Both prevalence and incidence of diabetes in obese individual are increasing. This increase in the prevalence and incidence of diabetes in obese individual are due to of environmental and genetic factors. Along these lines,identification and comprehention of key responsible element of obesity and diabetes and its related complexities in Pakistan is foremost to controlling the diabetes pandemic. With this understanding, the suitable screening, protection and remedial techniques can be executed and additionally created. Pakistan comes to the ninth highest ranking country in terms of Global burden of obesity and its Co morbidities. In Pakistan different gene are reported to be involve in diabetes such as COMT, FTO,ACE and IL-6 but ACE gene is more frequently reported. This study was designed to identify genetic and life style factors which are responsible for diabetes in obese individuals.The linkage between diabetes and obesity with reported genes was checked through STR markers.Fourteen different families from various region of Pakistan were analysed band on anthopometeric, Physiological and molecular finding. None of the sampled families showed linkage to the reported genes which suggested that there is need to do genome wide search for diabetes in obese individual in future. In environmental factor life style and diet also plays very important role in causing diabetes in obese individual.

CHAPTER 1
INTRODUCTION

1 INTRODUCTION

Obesity represents the excess body fats that affect the health negatively and causes various diseases such as diabetes, cardiovascular diseases and many other related diseases as well (Javed et al., 2015)It has been estimated that obesity causes 3.4 million deaths every year in the world and 4 percent life long, disability (Ng et al., 2014). As it is heterogenous disorder therefore many factors are involved to cause the obesity such as environmental, genetic, hormonal as well as behavioral factors. (Cheung & Mao, 2012).

Obesity occurs as a result of correlation of environmental and genetic factors. It is reported that environmental factor plays important role in causing obesity. In the environmental factor availability of food, physical activity, place of living as well as eating habits are included. Eating and physical activity makes the complex interaction in the body and affect metabolic, hormonal and neuronal signals in the brain that changes the body and ultimately cause the obesity (Farooqi, 2015).

Obesity tends to keep running in families, implying that it might have a hereditary cause. As well, relatives share qualities as well as eating routine and way of life propensities that may add to fatness. Isolating these life style elements from an inheritance is difficult. Although, developing confirmation focuses to heredity as a solid deciding element of fatness. It is investigated that adults who were embraced as kids, scientists found that in patient' adults weights were nearer to their natural guardians' weight than their new parents.

Obesity is categorized into subgroups, depending upon suspected etiology such as monophonic obesity, Syndromic obesity, Polygenic Obesity. In mono generic obesity single gene causes severe obesity in an individual. While in case of syndromic obesity clinically fat patients have other diseases such as mental disorder, dysmorphic components, and organ-particular formative variations from the normal. Polygenic obesity is the type of obesity multiples genes are involve and it is most common form of obesity. Polygenic or regular weight, which influences the all inclusive community and however may have related to certain health risk factors(Herrera & Lindgren, 2010).First monogenic obesity was reported in 1997 and till date 20 different genes are reported to be involved in obesity(O'Rahilly, 2009).

Different genes are responsible for causing obesity.FTO was the first gene discovered in 2007 to

be associated with obesity (Rankinen et al., 2006).Other genes are also involve in obesity such as Leptin gene,Melanocortin -4 receptor gene(MC4R),Pro-opiomelanocortin (POMC) and Prohormone Convertase 1(PCSK1)("Genetic Factors Contribute to Obesity," n.d.).

Genes can cause obesity due to improper function, as a result of mutation. It was noted that a mutation in the leptin gene causes the obesity. In the leptin gene single gene mutation leads toward the obesity. So, obesity can be monogenic("WHO | Obesity: preventing and managing the global epidemic," 2015)(Gibson et al., 2004). Some other different genes are involved in obesity. In Polygenic obesity, multiple genetic factors are involved that responsible for obesity.In human it is estimated that about 200 different genes or loci are linked to obesity (Lau, 1999). So the pattern of obesity suggests that the effect is due to of polygenic. In each variant different genes making the small effect(Sørensen & Echwald, 2001).

Obesity occur because of communications amongst environmental and genetic factors.In nonsyndromic obesity 40 to 70% inheritance variations are exist.Genome wide association (GWA) studies have drastically changed the pace of recognition of regular hereditary meaning less variations. To date, more than 40 genetic variations have been related with obesity and fat circulation. These variations do not completely clarify the heritability of weight, different types of changes, for example, epigenetics effect, must be considered(Herrera, Keildson, & Lindgren, 2011).

It is a fact that genes are important to considered in many cases of obesity; an individual's nature's domain additionally assumes a significant part. Surroundings incorporate lifestyle practices, for example, the thing that an individual consumes. Individuals can't change their hereditary makeup; however they can change what they eat and how dynamic they are. A few people have overcome the capacity to shed pounds and keep it off. Psychological factor additionally may impact dietary patterns. There is certainly says that obesity is firmly affected by environmental factors(Sørensen & Echwald, 2001).

Now it is noticed that the epidemic of obesity is majorly because of environmental factor. High consumption of energy such as usage of fat foods or fast food that gives high calories resulting obesity occur. In environmental factors television watching, improper ways of eating, physical inactivity all things are considered. Demand of our society has been changed, resulting

imbalance of energy intake and energy expenditure occurs. Imbalance of energy in the body is responsible for fat accumulation in the body as a result individual become obese.
Overweight and obesity are ending up plainly more far reaching with worldwide projections of more than 2.16 billion overweight and 1.12 billion fat people by 2030 (Herrera et al., 2011). This clearly displays an overall clinical and general doctors problem, related with social and individual feedback. It is likewise corresponded with an expanded danger of type 2-diabetes (T2D), cardiovascular infection, cancer and mortality(Flegal, Graubard, Williamson, & Gail, 2007).

Obesity is also associated with adverse health outcomes such as cardiovascular disease (CVD) and Type 2 diabetes mellitus (DM) and in the presence of other risk factors for noncommunicable diseases (NCDs) such as smoking, hypertension, elevated blood cholesterol, has a multiplicative effect (Ashraf Chaudhry, Ahmad, & Zeeshan Ashraf, 2012). Body weight is controlled by regulatory mechanism that's depend on several factors such as environmental, societal, physiological, behavioral and genetic and it is difficult to understand all of them. In the body energy is maintained by metabolic, nutritional, hormonal and neuronal signals that are integrated with the brain to produce changes in the behavior that affects the eating habits, physical activities.

The Global prevalence of obesity has been increased. The world health organization has warned that the epidemic of obesity is increasing in many countries. Though the previous study it is noticed that Eastern Mediterranean countries the level of obesity reached at the alarming situation among the children and adults (Friedman & Halaas, 1998) (Musaiger & O., 2011a). In the United States more than 66% of adults are presently overweight and 33% are obese ("Bastard JP, " n.d.). By 2002, about 500 million individuals were overweight around the world. In the United States rates of obesity have multiplied since 1970 to more than 30%, with more than 66% of Americans now overweight (Harms & Seale, 2013).

In South Asia, Pakistan comes to the ninth highest ranking country in terms of Global burden of obesity and its Co morbidities (Ng et al., 2014). As many other countries the epidemic of obesity in South Asian countries due to the adoption of Western lifestyle.World widly, changing patterns

for nourishment, increment in inactive way of life, fast industrialization and urbanization are the key elements which add to expanding weight of obesity as a rising scourge. Countries sharing the most astounding weight of obesity likewise share the most astounding weight of diabetes mellitus and metabolic disorder (Flegal, Carroll, Ogden, & Curtin, 2010).This relationship of obesity with non-transferable infections makes this issue as a need general medical problem of Pakistan. It is evaluated in 2008, that 1.46 billion adults of world are overweight and 502 million are obese, where 170 million of the youngsters around the globe are heavy and overweight (Flegal et al., 2010)(Campos, Saguy, Ernsberger, Oliver, & Gaesser, 2006).

1.1 AIM of Studies

This research is designed to understand the role of genes reported to be involved in type 2 diabetes in Pakistani kindred and also to calculate the impact of environmental and well as socio-economic factors on the onset of diabetes associated with obesity.

1.2 Objectives

- To determine prevalence of diabetes in obese Pakistani kindred.
- To determine correlation of non genetic factor with obesity.
- To determine correlation of environmental factors with diabetes in obese individual.

CHAPTER 2
REVIEW OF LITERATURE

2. Literature Review

In the last few years, some advances have made on the non syndromic obesity. For measuring the obesity World Health Organization (WHO) recommended the BMI in 1997.These attributes of BMI were defined by Europeans and North Americans, and classified overweight as BMI >25 and obesity as BMI>30 (Ashraf Chaudhry et al., 2012). In February 2000 it is recommended by, the WFIO Regional Office for the Western Pacific for adults for the Asia-Pacific region: overweight at BMI >23 and obesity at BMI>25.

2.1 Prevalence oF Obesity

Obesity is emerging in Pakistan as a public health problem now it becomes difficult to cope with this Problem. Pakistan is similar to other countries, where obesity affects first urban middle-aged women; with economic development, increasing urbanization and lifestyle changes (including diet and physical activity), obesity then occurs in men and younger women (Samanic, Chow, Gridley, Jarvholm, & Fraumeni, 2006). Consequently, obesity is influencing a vast extent of the world`s population (Campos et al., 2006).Nations bearing the significant weight of obesity pandemic incorporate Papua New Guinea; 79–80 percent obese general population, Qatar 34–45 percent large populace, Lebanon 36–38 percent obese populace and United States 32–35 percent of obese population generally (Phd et al., 2011).

Obesityt was later considered as a noteworthy general medical problem of created nations, yet for most recent two decades because of quick social and ecological moves it is recognized that obesity have expanded three circumstances in center and low wage nations and influencing individuals of all age bunches including kids while these nations are as of now battling with serious issues identified with unhealthiness particularly among childrens (Hossain, Kawar, & El Nahas, 2007).

Obesity is the worldwide issue that is highly risen in South Asian countries including Pakistan, where change in lifestyle effect the gen environment imbalance. In the greater part of the Asian nations the predominance of overweight and obesity has expanded many overlap in the previous couple of decades and the size shifts between countries(de Onis & Blössner, 2000). South East Asia and Western Pacific region are currently facing an epidemic of diseases associated with obesity such as diabetes and CVD. India has the highest number of people with diabetes in the

world and China occupies the second position. The countries and regions in Asia are at different phases of development. Some like Vietnam and Indonesia are in the early stages of development while others like Japan, Singapore, Malaysia and Hong Kong are at more advanced stages. The highest rate of obesity in Asia is in Thailand and the lowest is in India followed by Philippines. China which once had the leanest of population is now rapidly catching up with the West in terms of prevalence of overweight and obesity(Griffiths & Bentley, 2001).

2.2 Prevalence oF Obesity in Pakistan

Like different nations, obesity in Pakistan is additionally ascending at a disturbing rate. The greater part of the worldwide deaths are brought about by obesity so rising obesity in Pakistan involves extraordinary concern nowadays. According to an examination, WHO expresses that roughly 26 % of ladies in Pakistan experience the ill effects of the issue of obesity while just 19 % of the men are obse. However, the current 2013 measurements have uncovered that in 2013, the rates were 28 % for men and 38 % for ladies, which is a huge gap between the two sexes. Obesity is higher in urban territories (56% in men and 67% in ladies when contrasted with country zones.In fact, even adolescence obesity is likewise developing at a quick pace. According to 2013 measurements it was 10 % which is a terrific figure. As indicated by express Tribune, Pakistan has been positioned ninth out of 188 nations with respect to obesity. This clearly indicates how enormous of an issue it is currently. As a result of this issue, around 3.4 million individuals deaths occurs in 2010(Ng et al., 2014).

2.3 Genetic Factor

Obesity is influenced by several factors,its include environmental factors and genetic factor are also responsible for obesity.Genes may also affect the amount of fats store in the body and where the fats are distributed. Abundance in fat tissue mass can be viewed as a disturbance to be decided between energy intake and consumption. In current circumstances, this overabundance in fat tissue fuel storage is viewed as a disease(Zhao & Grant, 2011).

2.3.1 Monogenic Obesity

Obesity might be monogenic, if one gene is involved in it. Monogenic obesity is the obesity connected with a single gene mutation. In these cases single gene variants are sufficient by themselves to cause obesity in food rich societies. Patients with monogenic obesity generally show greatly extreme phenotypes characterized by an early childhood obesity establishment frequently all the interfaced for extra behavioral, developmental alternately endocrine issue similar to hyperphagia furthermore hypogonadism. Monogenic obesity commonly brings about extreme obesity similarly as the principle manifestation.

2.3.2 Polygenic Obesity

Poly means many so in polygenic obesity many genes are involved. It is most common type of obesity. Polygenic or general weight, which impacts the comprehensive group and however may have identified with certain diseases(Herrera & Lindgren, 2010).

Early studies show that different genes are involved in obesity such as FTO,MC4R,POMC , PCSK1 and many more. With Pakistani population FTO gene is associated with obesity. There are many other genes that are responsible for obesity in the families, but FTO is most reported (Rankinen et al., 2006). Pakistan is the sixth most crowded nation of the world with an aggregate population of 184.35 millions in 2012 to 2013(Jafar et al., 2013). In Pakistani population, few genes are reported that are associated with obesity.

Table 2.1 List of Genes reported to be Associated with Obesity

Gene Name	**Symbol Of Gene**	**Chromosome Location**	**Role in Obesity**
Fat Mass and Obesity associated gene	FTO	16p12.2	Promote Food intake
Melanocortin -4 receptor	MC4R	18q22	MC4R bounded by alpha –melanocyte stimulate hormones and those hormone stimulate appitite.
Pro-opiomelanocortin	POMC	2p23.3	POMC is an important effector in the regulation of Appetite. POMC single gene mutations cause an obese phenotype.
Prohormone Convertase 1	PCSK1	5q15-q21	Regulate insuline biosynthesis
Leptin	LEP	7q31.3	organic parts of leptin incorporate the control of food intake, body weight direction and homeostasis of energy

2.3.3 FTO gene

Gene has impact each part of human physiology, improvement, and adjustment.In 2007, researchers recognized the principal obesity related gene variations in the supposed "fat mass and obesity related" (FTO) gene on chromosome 16.These gene variations are genuinely normal, and individuals who convey one have a 20 to 30 % higher danger of obesity than individuals who do not(Dina et al., 2007).The relationship of the two important contributors of polygenic obesity SNP rs17782313 near MC4R and SNP rs1421085/rs9939609 in FTO with nutrition consumption related endophenotypes has been all around documented in the literature.

2.3.4 MC4R

The second obesity related gene variation that analysts recognized lies on chromosome 18, near the melanocortin-4 receptor gene this gene is responsible for an uncommon type of monogenic obesity (Loos et al., 2008).The obesity predisposing SNP variation close MC4R was related with expanded sentiment hunger expanded nibbling , diminished satiety and expanded aggregate fat and protein energy intake ,the impacts of the variation on nourishment related parameters being watched both in kids and adults(Stutzmann et al., 2009).

2.3.5 Leptin

Leptin is a hormone and it function is to keep up energy in a body, adjust by directing food intake and calorie consume rate. As the quantity of fat store in cells, leptin is discharged into the blood circulation system and signs that make you eat pretty much. Really leptin is a 16-kDa protein. It is emitted by adipocytes and has real part in the body weight control by keeping up a harmony between food intake and consumption ofenergy.

Activity of leptin is subject to its binding with its receptor, known as leptin receptor (LEPR). LEPR have a place with gp130 group of cytokine receptor. After the alternative splicing of LEPR, six distinctive isoforms were shaped. These isoforms are LEPRa, LEPRb, LEPRc, LEPRd, LEPRe and LEPRf. Out of these six, LEPRb is the critical and longest isoform that has the limit of solid signaling when contrasted with others.Defect in leptin signaling cause severe obesity. First missense mutations which were present in the leptin receptor (LEPR) were reported. These mutations disrupt LEPR signaling. Mutations associated to human obesity were involved in structural as well as functional relationships within the LEPR.Deformity in leptin signaling cause extreme obesity. In the first place missense mutation which were available in the leptin receptor (LEPR) were reported. These transformations disturb LEPR signaling. Mutation in LEPR causes obesity in human, and LERP is involve in structure and function relationship in human(Nanjappa, Raju, & Muthusamy, 2011).

2.3.6 POMC

Proopiomelanocortin (POMC) shortfall causes serious obesity that starts at an early age.As well as obesity, individuals with this condition have low levels of a hormone known as

adrenocorticotropic hormone (ACTH) and have a tendency to have red hair and pale skin. Affected newborn children are generally a typical weight during childbirth, however they are always eager, which prompts excessive nourishing . The children consistently put on weight and are seriously fat by age 1. Affected people encounter unnecessary hunger and stay large forever. Bioactive peptides drive from the prohormone, expert opiomelanocortin (POMC), are produced in neurons of the hypothalamus and go about as endogenous ligands for the melanocortin-4 receptor (MC4R), a key particle under- lying hunger control and energy homeostasis.It is therefore vital to comprehend numerous parts of POMC quality direction in the mind, as pharmacological control of POMC expression/preparing could be a potential technique to encounter obesity. Single gene mutation causes sever obesity in human(Conditions, n.d.).

2.4 Environmental Factor

Environmental factors play a very important role an increasing the obesity. Metabolic disease is also responsible for obesity. The increased caloric intake and physical inactivity are responsible for the obesity. There are several evidences that show environmental factors play major role in causing obesity.

2.4.1 Family Lifestyle

Globally Pakistan comes to the ninth highest country in term of obesity burden (Ng et al., 2014). In South Asian countries epidemic of obesity is due to of adopting the western life style. The risk of obesity is increased in children if parents are obese, not because of genetic, also because of same eating and physical habits.

2.4.2 Unhealthy diet

Another reason of obesity in Pakistan is because of eating fast food and consumption of high energy(“WHO | Obesity: preventing and managing the global epidemic,” 2015). Globally the intake of sweet beverages and juices has risen. Epidemiologic studies have shown that regular consumption of sweet beverages, not only contribute to weight gain also increase the risk factor of type 2 diabetes (Shabana & Hasnain, 2015) (Musaiger & O., 2011b).Consumption of canned food and canned drinks are also responsible for obesity (Hassan et al., 2016).

2.4.3 Physical inactivity

Urbanization plays both positive and negative roles in population. Urbanization and modernization bring change in traditional values. Due to of globalization, many changes occur in the work places, now workplaces are more mechanized and computerized and it affects the physical activities and economic status as well ("Silent Victories: The History and Practice of Public Health in Twentieth ... - Google Books," n.d.). Physical inactivity is a cause of excessive weight gain. Physical inactivity and obesity are associated with each other. Through Physical inactivity energy expenditure can be estimated by simple observation (Pitta et al., 2009). When an individual is inactive then expedition of energy will be less as compared to energy intake so some calories are remained in the body resulting person become obese with the passage of time.

The study conducted in Western countries indicates that there is an association between television watching and obesity in such a way eating during television viewing is one factor of obesity. While viewing the TV individual may be overeating and consumed more food (Musaiger et al., 2012). Often sitting while playing video games, using the internet and viewing TV decline physical activity and contribute towards obesity. In the Arab Countries, it was reported that while watching television children eat more sweets, chocolates, potato chips, and nuts and these are the things that contribute in obesity (Yahia, Achkar, Abdallah, & Rizk, 2008).

2.4.4 Hormonal imbalance

Hormonal disturbance is also responsible for obesity. In case of stress, stress hormone release such as cortisol that trigger the release of triglycerides and store the fat cells deep inside the abdomen. Cortisol increases the appetite and the individual who is in stress condition make the worst food choice and eat too much in this way excessive fat stored in the body.Leptin gene is responsible for the secretion of leptin horomone which is also involve in body fat distribution and regulation of hunger and digestive system in mind. leptin is fundamentally a gathering of 167 diverse amino acids that are created by the leptin quality appropriate from the fat tissue. The protein encoded by the leptin gene shows various natural parts related with obesity by official with a characterized receptor situated in the hypothalamus. These organic parts of leptin incorporate the control of food intake, body weight direction and homeostasis of energy("What Genetic Factors Contribute to Obesity?," n.d.).

The decrease rates of leptin generation among obse people by the adipocytes have been fascinated for the reason for obesity.Mutations in the leptin quality or the leptin receptor quality have been found to bring about expanded hunger, overeating, enormous weight increase, disabled thermoregulation, insulin resistance and diabetes, immune dysfunction, sexual development disappointments and various neuroendocrine disturbances in both human subjects and rats(Ioffe, Moon, Connolly, & Friedman, 1998).

2.4.5 Age

Obese children, adults are also seen in many societies, so obesity can occur at any time. But in the age of puberty hormonal changes occur in the body and with the passage of time hormonal changes less active lifestyle and increase the chances of obesity in an adult. with the passage of time in an individual amount of muscles in the body start decreases. Lower level of muscle mass decreases the rate of metabolism in the body. These changes also reduce the requirements of calories and in this way extra calories, which is present inside the body causes the weight gain.

2.4.6 Pregnancy

During pregnancy, normally a woman's unavoidable weight gain. After the birth of baby some women find difficulty in reducing the weight. Sometime this weight increases contributes towards the obesity. The occurrence of maternal obesity and its specialist co morbid conditions (diabetes, cardiovascular illness) keeps on expanding at a disturbing rate, with significant general wellbeing suggestions. Maternal obesity not only influence the woman, as well as effects the health of the child, and prompting expanded childhood obesity and diabetes(Leddy, Power, & Schulkin, 2008).

2.4.7 Sleep

Improper sleep also contributes towards the obesity, in such a way sleep causes changes in hormones if enough sleep is not getting or sleep too much that causes hormonal change that changes increase the appetite. Then individual start eating too much and gain weight.

2.5 Association of obesity with other diseases

Obesity is one of the most significant public health disease in the United States and other westernized societies. Its commonness is expanding worldwide and it is related with concerning medicinal comorbidities, most outstandingly the metabolic disorder and type 2 diabetes(Pi-Sunyer, 1991). Obesity is a significant risk figure for heart diseases and Type 2 diabetes mellitus (DM) and within the sight of other hazard variables for noncommunicable sicknesses, for example smoking, hypertension has a multiplicative effect(Sciences, n.d.).

In South Asia noncommunicable diseases (NCDs) such as smoking, hypertension, elevated blood cholesterol and coronary artery disease (CAD) are growing epidemically and it has multiplicative effects (Murray, Lopez, Harvard School of Public Health., World Health Organization., & World Bank., 1996).Obesity causes so many diseases such as pulmonary diseases, cardiovascular diseases, metabolic diseases, and also responsible for several cancers(Coleman, Gill, & Wilkinson, 1998). In Pakistan, higher prevalence of the CVD risk factors high blood pressure, obesity and elevated blood cholesterol are found in the higher socioeconomic status (SES) groups. This is in direct contrast to developed countries where there has been a reversal of the SES gradient with respect to CVD risk factors in the last century and prevalence in now higher in the lower SES groups.

Noncommunicable diseases (NCDs) now become a public health challenge for developing countries, because of unhealthy lifestyle, in which physical inactivity, unhealthy diet, and smoking and of obesity. WHO estimated that 40% of all deaths in developing countries are because of NCDs (James & Ralph, 1999). Noncommunicable diseases that are most problematic are hypertensions, heart diseases and diabetes ("Risk Factors for Cardiovascular Disease in School Children - a Pilot Study," n.d.).

Body weight is co-related to a health condition. Though the previous study it is noticed that there is the negative association between health and obesity. Obesity is a major risk factor for certain chronic diseases such as hypertension, diabetes and heart diseases. The prevalence of heart diseases and diabetes is higher in obese individuals as compared to non obese individuals.

Cardiovascular diseases are increasing because of overweight and obesity in many countries as well as in Pakistan. Rates of coronary artery disease (CAD) increase with the age. Because of their greater age, CAD is more likely to have co-morbidities such as diabetes, obesity and

hypertension. Throughout the world, obesity is increasing and badly affect the health and associate with many diseases such as diabetes mellitus type 2. In South Asia there is an extreme increase in the rate of NCD like diabetes and coronary heart disease. Through the early study it is noticed that Pakistan ranked 8th worldwide with diabetes and this is a very alarming situation in Pakistan (Sciences, n.d.). Type 2 diabetes ultimately develops in patients is the result of an insulin resistant state (McGarry, 2002). Obesity is the result of a chronic inflammatory state of the increase in plasma levels of C-reactive protein, inflammatory cytokines like TNFα, IL6, MCP-1 and IL8 and multifunctional protein like leptin and Osteopontin (Kiefer et al., 2008).

2.5.1 Role of COMT gene in Diabetes

The Catechol-O-Methyl Transferase (COMT)is one of the critical gene of the dopaminergenic pathway with a primary capacity of COMT is to expel harmful metabolites from the body.The COMT has been already related with the brain disorders and inflammatory responses. up till now a few reviews demonstrated that COMT is also involve in type 2 diabetes (T2D) and kidney disorders(Zain, Awan, & Baig, 2016). COMT gene is an essential part of the dopaminergenic pathway. In spite of the fact that it is related with the mental working and anomalies particularly parkinson disease(Lelli-Chiesa et al., 2011). This pathway has been examined in a few reviews for its relationship with diabetes and kidney diseases on the premise of hypertension (Yeh et al., 2010).

Genetic studies shows that the dopamine receptor gene and COMT were connected to basic hypertension (Jose, Eisner, & Felder, 2000). Besides, dopamine likewise controls the action of Angiotensin II which is the key part of Renin Angiotensin Aldosterone System (RAAS) pathway. In this way, the relationship of dopamine and Angiotensin II could be found in controlling the vascular tone, sodium particle balance and renal damage. Some experiments in mice shows that If this enzyme is blocked it can give defense from the progression of diabetic nephropathy(Lal et al., 2000).

2.5.2 Role of ACE gene in Diabetes

Type 2 diabetes mellitus is a main source of morbidity and mortality. Cardiovascular disorder (CVD) is the most common confusion and principally represents the overabundance morbidity

and mortality in diabetic patients, yet microvascular Complication, for example, kidney disease and retinopathy, are succeeding and add to the aggregate disease load. Lipid irregularities in patients with sort 2 diabetes are a noteworthy issue and connected with the expanded danger of CVD(Battisti, Palmisano, & Keane, 2003).

ACE gene is the most reported gene that is involve in the pathogenesis of diabetic nephropathy including smaller scale and large scale albuminuria and movement from miniaturized scale to full scale albuminuria. The ACE gene is situated on the long arm of chromosome 17 (17q23) and has 21 kilo bases long containing 26 exons and 25 introns. More than 160 ACE gene polymorphisms are realized that the vast majority of them are single nucleotide polymorphisms(Rigat et al., 1990). In diabetic patients hyperglycaemia builds tissue angiotensin II which actuates oxidative anxiety, glomerular hyperfilteration, endothelial harm, thrombosis, irritation and vascular remodling(Ruggenenti, Bettinaglio, Pinares, & Remuzzi, 2008).

Angiotensin converting enzyme (ACE), assume imperative parts in controlling various physiological procedures. ACE play a key function in the renin-angiotensin framework.ACE play a vital role in blood pressure homeostasis by creating the vasoconstrictor peptide angiotensin II(Rahimi, 2012).

2.5.3 Role of ACE gene in Obesity

Angiotensin II could have an imperative part in the development and separation of human adipose tissue(Jones, Standridge, & Moustaid, 1997).It has been shown in vitro that angiotensin II animates the union of prostacyclin in the adipocytes, which empowers the separation of the precursors of adipocytes (Darimont, Vassaux, Ailhaud, & Negrel, 1994). In addition, homozygotes for DD genotype of the ACEgene show angiotensin II higher plasma values,7 demonstrating a plausible higher inclination to obesity.

Angiotensin converting enzyme (ACE) is an essential segment of RAS that may impact metabolic results in fat tissue. The deletion "D allele", of ACE quality I/D (inclusion/erasure) polymorphism has been appeared to be related with rise in the serum level of ACE. ACE, is an important enzyme in the renin angiotensin framework (RAS), can change over angiotensin I (Ang I) into vasoconstrictor particle angiotensin II (Ang II). Body fat and body weight could be

raised and brought down as needs be by stimulating and limiting the generation of Ang II, proposing a possible connection amongst ACE and obesity(Pan et al., 2016).

CHAPTER 3

MATERIALS AND METHODS

3 MATERIAL AND METHODS

3.1 Selection of Genes

Bioinformatics tool Polysearch was used to search genes that are associated with Diabetes. Genes were selected on the basis of manual search and literature research in Pakistani population. . To find out the set of obesity-associated genes in Pakistani population, a biomedical text mining tool i.e. PolySearch2 has been used. The tool provides two types of search options quick search and advanced search, from that advanced search was chosen. The search category was set as "Given Disease, Find all associated genes/proteins". Input to the tool consisted of "Diabetes" provided in the search keyword field. The term "Pakistani population" was used as custom filter words. Databases were selected according to research requirements such as Pubmed, PubmedCentral, NCBI books, OMIM, and MedlinePlus etc. List of genes was generated as a result with reference to articles including the query words.

3.2 Study Area:

The current research work was conducted at the wet lab, Department of Bioinformatic & Biosciences, Capital University of Science & Technology Islamabad.

3.3 Primer Desiging

Primer design was done with the help bioinformatic tool " Prime 3" against STR marker of two genes which are Angiotensin converting enzyme (ACE) and Catechol-O-Methyl Transferase (COMT). STR markers of genes were taken from the UCSC Genome Browser

Table 3.1.List of Diabetes-associated genes reported fromPakistani population

Gene name	Gene symbol	Gene ID	Gene role	Co-morbidity	Sampling area	Reference	Year
ACE, Angiotensin converting enzyme	ACE	1636	ACE play a key function in the renin-angiotensin framework and regulation of blood pressure homeostastis .	Cardiovascular disease, type 2 diabetes, kidney disease and diabetes.	Lahore, Sheikhupura, Bhakkar, and Burewala	(Rahimi) Ruggenenti et al.,	2012 2008
COMT, Catechol-O-Methyl Transferase	COMT	1312	To expel harmful metabolites from the body.	Hypertention. Diabetes, Kidney disease and obesity	Lahore,Central Punjab Pakistan	Yeh et al.,	2010

3.4 Sampling

In this study, we collected the sample of families with at least 3 obese individuals and perform the anthropomatric measurements, bio physical analysis, pedigree analysis and biochemical analysis. Informed consent was taken from the families after explaining the purpose and expected benefits of this research project. After having informed consents from all members taking part in the study, pedigrees were drawn during field sample trips with information collected from elders of family, relatives and friends.

Blood samples were taken from obese and non obese individuals using sterile syringes or multiple sampling needles and immediately transferred to 5 to 10 ml sodium EDTA vacutainers. After collecting the blood samples IDs were assigned to sample and were stored at 4C till DNA extraction.

Table 3.2 List of STR Marker of ACE gene

GENE	MARKERS	Forward Primer	Reverse Primer
ACE	PMC310924P2	GCCAGGAAGTTTGATGTGAAC	GATTCCCCTCTCCCTGTACCT
	ACE 2	GCCCAGGAGGATGTTTAAGGA	CTTGCCGTTGTAGAAGTCCCA
	SHGC-57821	ATGCAGGGAGTGAGGAGGTG	GCCTCAGACGCTGGAGTGTA
COMT	D22S1662	TGTGATGATGGCATAGGCAT	CTCAGGTGGTCTTGTAGCTGC
	PMC20764P1	TCACCATCGAGATCAACCCC	ACAACGGGTCAGGCATGCA
	RH27791	GACAACGTGATCTGCCCAGG	AGGTGTGCTTTGCATTTAGG

3.5 Extraction of Genomic DNA

The chemical which is used in DNA extraction are TKM 1 buffer, triton-x, TKM 2 buffer,SDS, 6M Nacl, TE buffer.For DNA extraction, these reagents are used.

Table 3.3 Composition of Preparation of Regents

Reagents	Preparation Of Reagents
TKM 1 buffer	0.605g tricHCL(10mM), 0.372g KCL(10mM),1.016g of Mgcl2(10mM),0.372g of EDTA(2mM) dissolved in 500ml of distilled water.
Triton-x	0.1ml of 100% triton-x added to 9.9ml of distilled water.
TKM 2 buffer	0.121g of tricHCL(10mM), 0.074g KCL(10mM),1.203g of Mgcl2(10mM),0.074g of EDTA(2mM),0.467g of Nacl(0.4M) was dissolved in 100ml of distilled water.
SDS	One gram of sodium dodecyl sulphate was dissolved in 10ml of distilled water.
6M Nacl	8.765g of Nacl was dissolved in 25ml of distilled water.
TE buffer	0.030g of trisHCL(10mM)pH 8, 0.009g of EDTA(1mM) was dissolved in 100ml of distilled water.

We used non enzymatic salting out method to extract the genomic DNA.First of all we label the tubes ,then 900µlof TKM 1 buffer is added in it and 50µl of 1x triton-x were added to 300µl of blood in an autoclaved 1.5ml eppendrof tubes. Then incubated at 37ºC for 5 minutes to lyse the RBCs Cells were centrifuged at 8000 rpm for 3 mintues and supernatant was discarded.This step was repeated 3 times with decreasing amount of 1x triton-x till RBC lysis was complete and white pellet of WBCs was obtained. For cell lysis, 300µl of TKM 2 and 40µl of 10% SDS were added to cell pellet.Mixed thoroughly and incubated at 37ºC for 5 mintues.After incubation 100µl of 6M NaCl was added and vortexed to precipitate the protein.Cell were centrifuged at 8000rpm for 5 minutes.Supernatant was transferred into a new eppendorf tube containg 300µl of isopropanol.DNA was precipated by inverting the eppendrof slowly.Eppendrof tubes were centrifuged again at 8000rpm for 10 minutes to pellet down the DNA.Supernatant was discarded , 200 µl of 70% ethanol was added and mixed slowly to remove any excess salt.At the end the tubes were centrifuged at 8000rpm for 5 minutes to pellet down the DNA. Supernatant was discarded and resultant DNA was air dried for 30 minutes.After through drying, 50µl of TE buffer was added to dissolve the DNA.Transfer all samples to the labeled Eppendorf tube. Then stock DNA and label it(Loni & Lecturer, n.d.).

3.6 Genotyping

After DNA extraction, the quality and quantity of extracted genomic DNA was analyzed through Agrose Gel electrophrosis and spectrophotometAfter getting the result of DNA, genotyping performed by using STR markers. PAGE analysis will be performed to interpret the PCR products.

3.7 Mechanism used for amplification

PCR was used to amplify the DNA. All the ingredients of the PCR were added to PCR tubes. 1.5 µL DNA sample was added to each PCR tube. PCR is an automated Biosystems machine. It takes 90 to 120 minutes to run PCR. Recipe of PCR is, add 1 µL of 10X buffer,then add 1 µL of 2mM dNTPs, after this add 0.3 µL of taq polymerase in it, then add 1 µL of Mgcl2, after this add 0.3 µL of forword primer and then add 0.3 µL of reverse primer , after adding the primers add 1.5 µL of template DNA and in the last add 4.6 µL of double distill water.

3.8 Visualization of bands

In polyacrylamide gel electrophoresis analysis first the PAGE solution is prepared. In PAGE solution preparation take 100 ml beaker, then add 10 X TBX buffers that will be 2ml, and then add 40% polyacryamide solution which is 8.48ml and then add dH20. After then adding APS 20ul and then add TEMED (tetra methyl ethylene diamine) which is 100ul.After making the solution put it in the gel assembly and place for polymerization and load the DNA in it.Gel is then shifted to the gel assembly and run the gel. After running the gel for 30 mintues.Then gel is shifted in the buffer and then adds the ethidium bromide to stain the gel and put it in the shaker for 10 minutes so solution of ethidium bromide stain the DNA, which help in the DNA visualization.

CHAPTER 4

RESULTS & DISCUSSION

4 RESULT AND DISCUSSION

4.1 Data Collection

In this study, we take the blood sample from 12to14 different obese families of various region of Pakistan include Kashmir, Khyber pakhtunkhwa and Central Punjab. We collected patient information by filling the questionnaire. After having informed consents from all members taking part in the study, pedigrees were drawn during field sample trips with information collected from elders of family, relatives and friends.

4.1.1 Family A

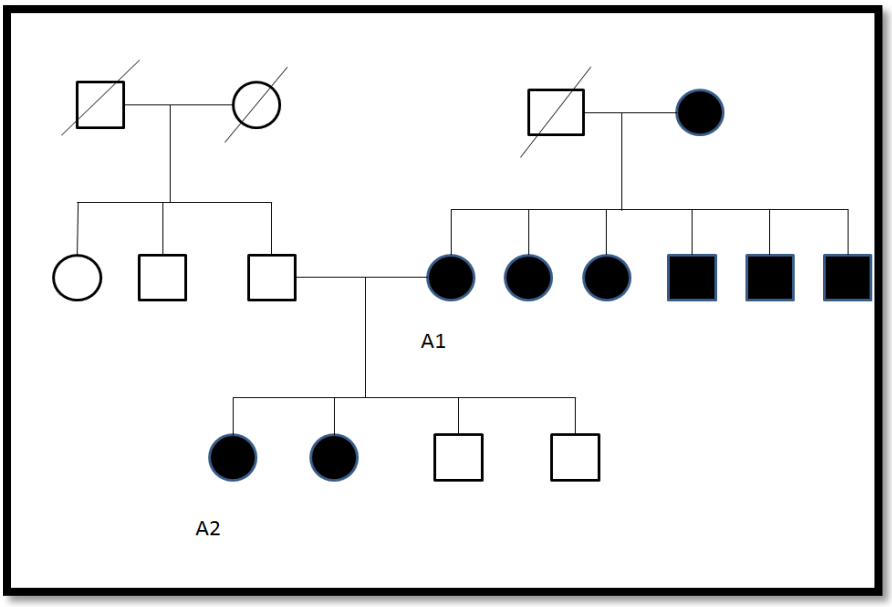

Figure 1: Pedigree of Family A

Family chart indicating Obese and normal individual of family A. Solid block indicate obese while hollow block show non obese individuals. Square indicates man, circle indicate female and diagonal line show dead individual.

Family A was sampled from urban area of Kashmir. This family was identified by a proband that was obese and had BMI 39.48.This family has total 9 obese individuals out of which 6 membersd were sample out, two we obtained with BMI 39.48 and 29.94.This family shows no

other Comorbidites. The Pedigree of the family shown in Figure 1 and other information is summarized in table 4.1.

Table 4.1: Table Of information of BMI and Other related disease with obesity

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	A1	39.48	Yes	Yes	No
2	A2	29.94	No	No	No

4.1.2 Family B

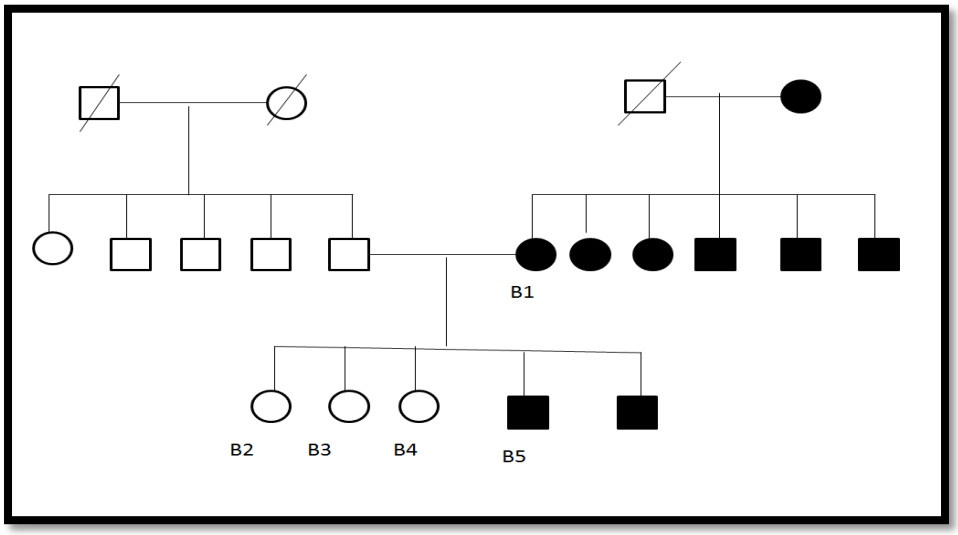

Figure 2: Pedigree of Family B

Family chart indicating Obese and normal individual of family B. Solid block indicate obese while hollow block show non obese individuals. Square indicates man, circle indicate female.Diagonal line show dead individual.

Family B was sampled from the urban area of Kashmir. This family was identified by a proband who was obese and had BMI 32.44. This family has total 9 obese individuals out of which we were able to sample 6 members, out of these 6, 5 we obtained with BMI 39.48, 29.94,20.19,24.14,16.10 and 27.91. This family shows no other Comorbidites. The Pedigree of the family shown in Figure 2 and other information is summarized in table 4.2.

Table 4.2: Information about BMI and Other related disease with obesity

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	B1	32.44	Yes	No	Yes
2	B2	20.19	Yes	No	No
3	B3	24.14	No	No	No
4	B4	16.10	No	No	No
5	B5	27.91	No	No	Yes

4.1.3 Family C

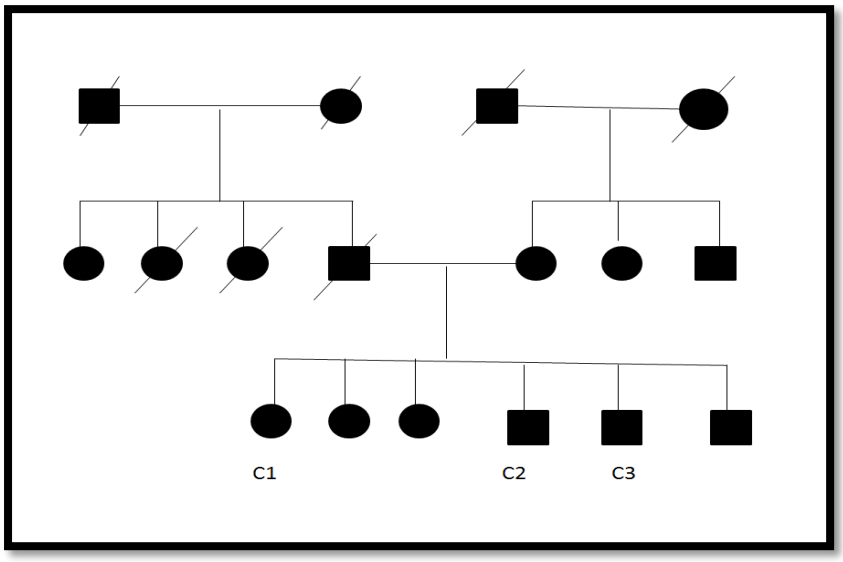

Figure 3: Pedigree of Family C

Family chart indicating Obese and normal individual of family C. Solid block indicate obese while hollow block show non obese individuals. Square indicates man, circle indicate female.Diagonal line show dead individual.

Family C was sampled from urban area of Kashmir. This family was identified by a proband who was obese and had BMI 49.26. This family has total 17 obese individuals out of which we were able to sample 4 members, out of these 4, three we obtained with a BMI 49.26,32.73 and 37.28. This family shows no other comorbidities.Pedigree of the family shown in Figure 3 and other information is summarized in table 4.3.

Table 4.3: Information about BMI and Other related disease with obesity

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	C1	49.26	Yes	No	Yes
2	C2	32.73	Yes	No	No
3	C3	37.28	Yes	No	No

4.1.4 Family D

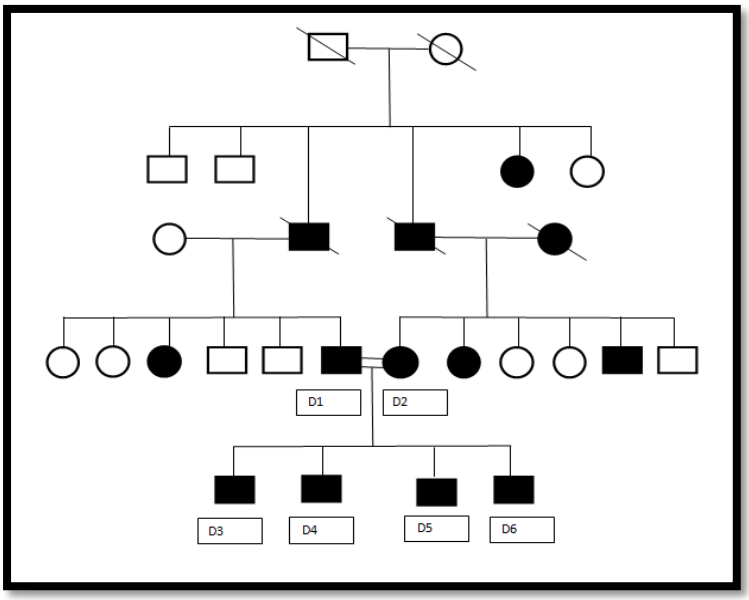

Figure 4: Pedigree of Family D

Family chart indicating Obese and normal individual of family D. Solid block indicate obese while hollow block show non obese individuals. Square indicates man, circle indicate female and diagonal line show dead individual.

Family D sampled from rural area of district Malakand of KP. This family was identified by a proband that was obese and had BMI 36.1. This family had total 21 individuals out of which 10 were affected and we were able to sample 6 members. All these 6 members born of consanguineous marriages were obese with BMI 35.1, 36.3, 36.1, 46.2, 43, and 31. The Pedigree of the family shown in Figure 4 and other information is summarized in table 4.4.

Table 4.4: Information about BMI and other related diseases with obesity

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	D1	35.1	Yes	Yes	No
2	D2	36.3	Yes	Yes	No
3	D3	36.1	No	No	No
4	D4	46.2	Yes	No	No
5	D5	43	No	No	No
6	D6	31	No	No	No

4.1.5 Family E

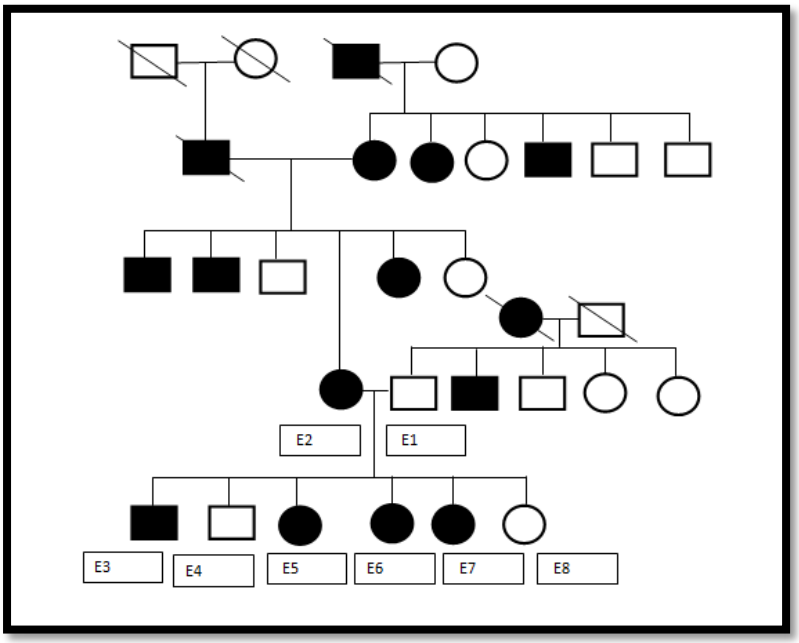

Figure 5: Pedigree of Family E

Family chart indicating Obese and normal individual of family E. Solid block indicate obese while hollow block show non obese individuals. Square indicates man, circle indicate female and diagonal line show dead individual.

Family E was sampled from rural area of district Malakand of KP. The family consists total of 30 members out of which 9 were obese and 6 were overweight. We were able to sample 8 individuals of this family whose mother E2 was obese with BMI 39.2 her two daughters were also obese with BMI E5 36.9 and E6 34.1. One son and one daughter were overweight with a

BMI 25.6, 27.2. One son and one daughter were normal. Other information related to this family shown in tables 4.5.

Table 4.5: Information about BMI and other related diseases with obesity

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	E1	25.4	Yes	No	No
2	E2	39.2	Yes	No	No
3	E3	25.6	No	No	No
4	E4	19.4	No	No	Yes
5	E5	36.9	No	No	No
6	E6	34.1	No	No	No
7	E7	27.2	No	No	No
8	E8	18	No	No	No

4.1.6 Family F

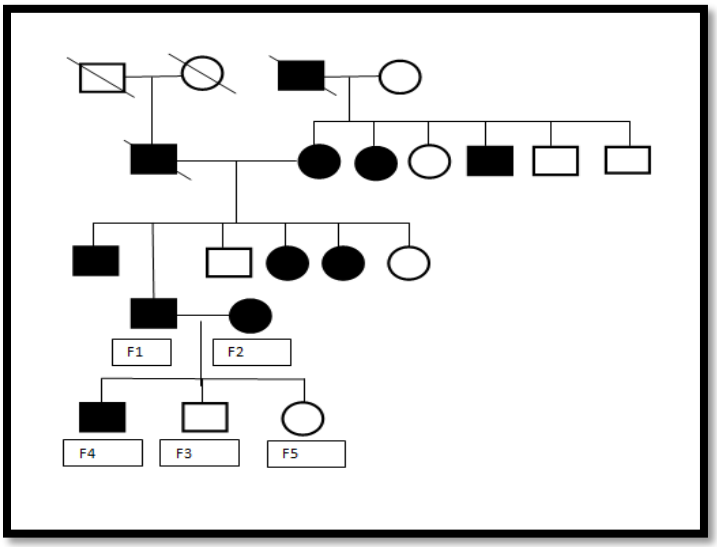

Figure 6: Pedigree of Family F

Family chart indicating Obese and normal individual of family F. Solid block indicate obese while hollow block show non obese individuals. Square indicates man, circle indicate female and diagonal line show dead individual.

Family F was sampled from the Dargai area of KP which is a rural area of district Malakand. Family F had total 21 members out of which 6 were obese and 4 were overweight. Only five members of this family were sampled in which one member was obese and two were overweight. One overweight member was 13 year old boy with BMI 26.9, his father had BMI 35.1 and his

mother had a BMI 26. Other 2 family members were normal.

Table 4.6: Information about other obesity related diseases of family F.

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	F1	35.1	No	No	No
2	F2	26	No	No	No
3	F3	14.6	No	No	No
4	F4	26.9	No	No	No
5	F5	24.2	No	No	No

4.1.7 Family, G

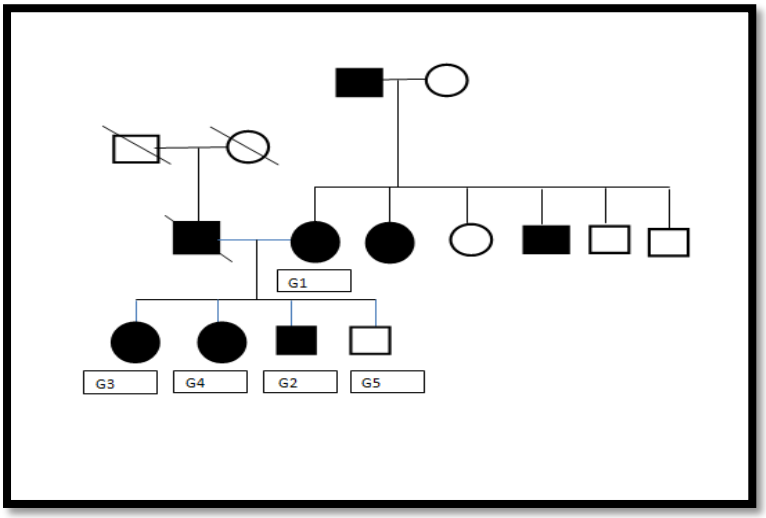

Figure 7: Pedigree of Family, G

Family chart indicating Obese and normal individual of family G. Solid block indicate obese while hollow block show non obese individuals. Square indicates man, circle indicate female and diagonal line show dead individual.

Family G was Sampled from Malakand district of KP which is a rural area. Family G had 4 obese individuals and 4 overweight individuals out of 15 members. Five individuals were able to sample in which two were obese with BMI G2 34.9, G3 30.2 and three were overweight with BMI G1 28.4, G4 29.7, and G5 25.8.

Table 4.7: information of obesity related diseases about Family G.

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	G1	28.4	Yes	No	No
2	G2	34.9	No	No	No
3	G3	30.2	No	No	No
4	G4	29.7	No	No	No
5	G5	25.8	No	No	No

4.1.8 Family H

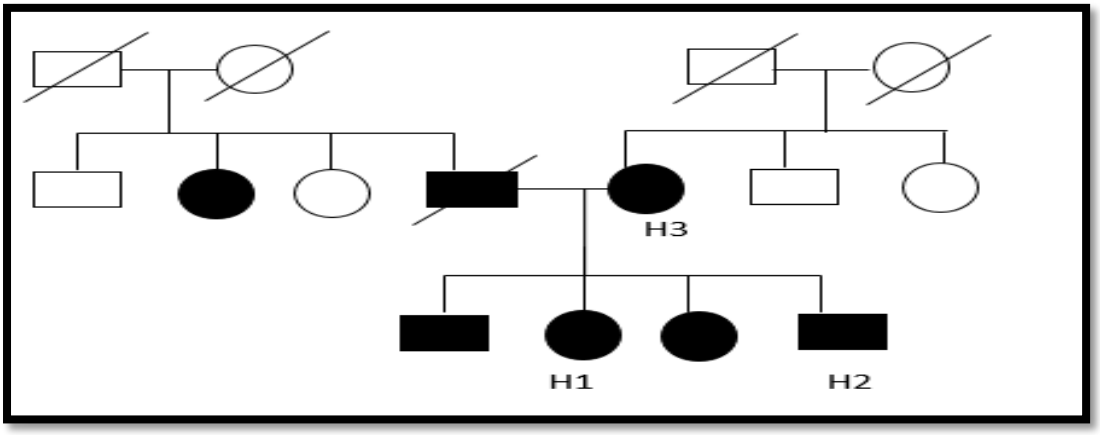

Figure 8: Pedigree of Family H

Family chart indicating Obese and normal individual of family H. Solid block indicate obese while hollow block show non obese individuals. Square indicates man, circle indicate female and diagonal line show dead individual.

Family H was sampled from the District of Azad Kashmir. This family was identified by a profane that was obese and had a BMI 39.48. This family has total 15 obese individuals out of which we were able to sample 7 members, out of these 3, two we obtained with BMI 49.1and 29.8. This family shows no other Comorbidites.The Pedigree of the family shown in Figure 1 and other information are summarized in table 4.8.

Table 4.8: BMI and Other related disease of family H

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	H1	40.2	No	No	No
2	H2	29.8	No	No	No
3	H3	49.1	Yes	No	Yes

4.1.9 Family I

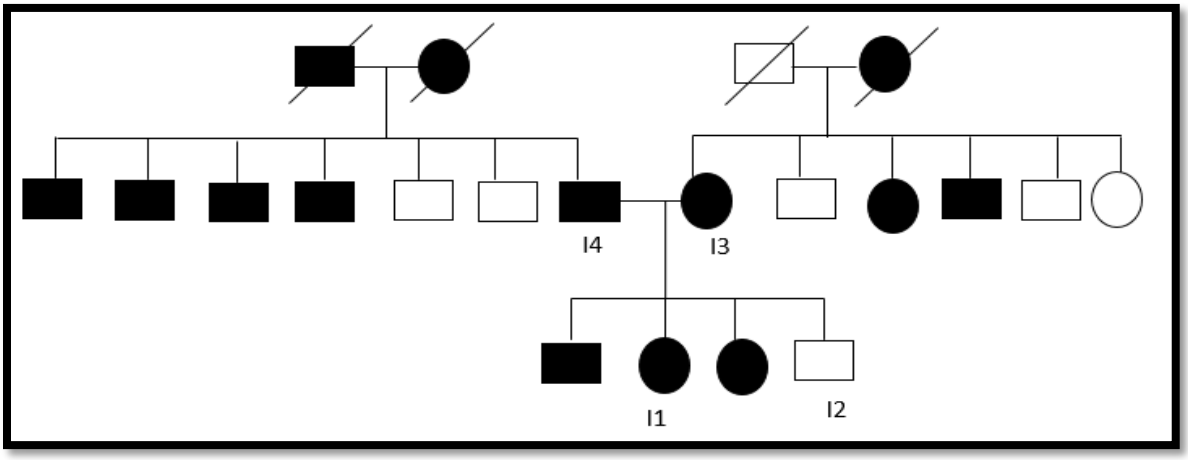

Figure 9: Pedigree of Family I

Family chart indicating Obese and normal individual of family I. Solid block indicate obese while hollow block show non obese individuals. Square indicate a man, circle indicate female and diagonal line show dead individual.

Family I originated from District of Azad Kashmir. This family consists of 21 individuals. Out of 21 individuals, 15 were obese and other was healthy. Out of 14 individuals, 4 individuals were sampled and given ID. Highest BMI calculated from this family was 60.1 and lowest BMI calculated was 20.5. Pedigree of this family shown in Figure 9. Information related to these individuals is summarized in table 4.9.

Table 4.9: BMI and Other related disease of family I

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	I1	40.2	No	No	No
2	I2	20.5	No	No	No
3	I3	60.1	No	Yes	Yes
4	I4	41.8	No	No	Yes

4.1.10 Family J

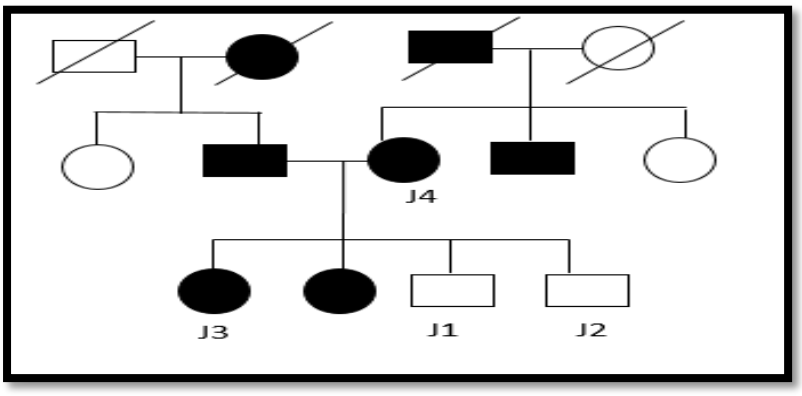

Figure 10: Pedigree of Family J

Family chart indicating Obese and normal individual of family J. Solid block indicate obese while hollow block show non obese individuals. Square indicates man, circle indicate female and diagonal line show dead individual.

Family J originated from the District of Azad Kashmir. This family consists of 13 individuals. Out of 13 individuals, 9 were obese and another was healthy. Out of 13 individuals, 4 individuals were sampled and given ID. Highest BMI calculated from this family was 51.6 and lowest BMI calculated was 17.9. The Pedigree of this family shown in Figure 10. Information related to these individuals is summarized in table 4.10.

Table 4.10: BMI and Other related disease of family J

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	J1	17.9	No	No	No
2	J2	17.9	No	No	No
3	J3	43	No	No	Yes
4	J4	51.6	No	No	Yes

4.1.11 Family K

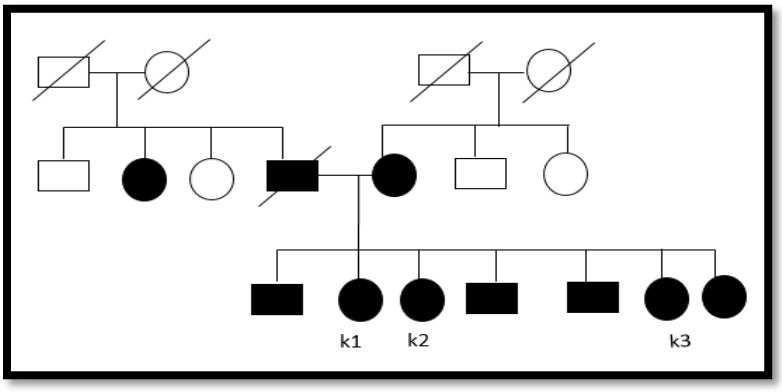

Figure 11: Pedigree of Family K

Family chart indicating Obese and normal individual of family K. Solid block indicate obese while hollow block show non obese individuals. Square indicate a man, circle indicate female and diagonal line show dead individual.

Family k originated from the District of Azad Kashmir. This family consists of 18 individuals. Out of 18 individuals, 10 were obese and another was healthy. Out of 10 individuals, 3 individuals were sampled and given ID. Highest BMI calculated from this family was 55.8 and lowest BMI calculated was 51.6. The Pedigree of this family shown in Figure 11. Information related to these individuals is summarized in table 4.11.

Table 4.11: BMI and Other related disease of family K

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	K1	55.8	Yes	No	No
2	K2	55.8	No	No	No
3	K3	51.6	Yes	Yes	No

4.1.12 Family L

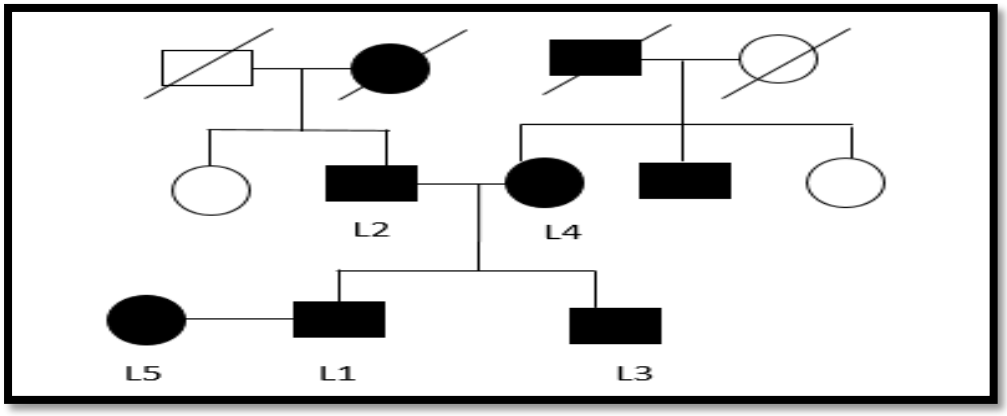

Figure 12: Pedigree of Family L

Family chart indicating Obese and normal individual of family L. Solid block indicate obese while hollow block show non obese individuals. Square indicates a man, circle indicate female and diagonal line show dead individual.

Family L originated from the District of Gujrat. This family consists of 12 individuals. Out of 12 individuals, 8 were obese and other were healthy. Out of 8 individuals, 5 individuals were sampled and given ID. Highest BMI calculated from this family was 62.3 and lowest BMI calculated was 43.0. The Pedigree of this family shown in Figure 12. Information related to these individuals is summarized in table 4.12.

Table 4.12: BMI and Other related disease of family L

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	L1	62.3	No	No	Yes
2	L2	43.0	Yes	No	No
3	L3	61.9	No	No	Yes
4	L4	51.6	No	No	Yes
5	L5	51.6	No	No	No

4.1.13 Family M

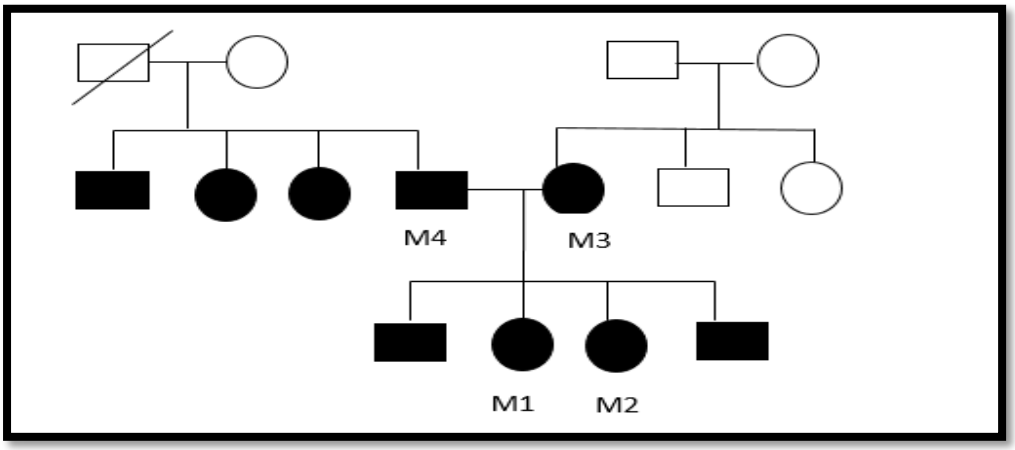

Figure 13: Pedigree of Family M

Family chart indicating Obese and normal individual of family M. Solid block indicate obese while hollow block show non obese individuals. Square indicates a man, circle indicate female and diagonal line show dead individual.

Family M originated from the District of Gujrat. This family consists of 15 individuals. Out of 15 individuals, 9 were obese and other were healthy. Out of 9 individuals, 4 individuals were sampled and given ID. Highest BMI calculated from this family was 43 and lowest BMI calculated was 25. The Pedigree of this family shown in Figure 13. Information related to these individuals is summarized in table 4.13.

Table 4.13: BMI and Other related disease of family M

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	M1	25	No	No	No
2	M2	19.5	No	No	Yes
3	M3	43	Yes	No	No
4	M4	43	Yes	No	No

4.1.14 Family N

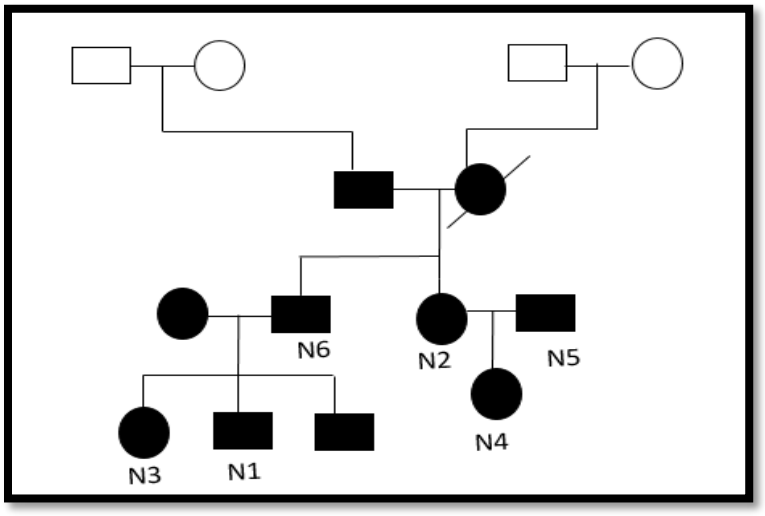

Figure 14: Pedigree of Family N

Family chart indicating Obese and normal individual of family N. Solid block indicate obese while hollow block show non obese individuals. Square indicates man, circle indicate female and diagonal line show dead individual.

Family N originated from the District of Gujrat. This family consists of 14 individuals. Out of 14 individuals, 10 were obese and another was healthy. Out of 10 individuals, 6 individuals were sampled and given ID. Highest BMI calculated from this family was 46.7 and lowest BMI calculated was 30.3. The Pedigree of this family shown in Figure 14. Information related to these individuals is summarized in table 4.14.

Table 4.14: BMI and Other related disease of family N

Serial No	Sample ID	BMI	BP	Diabetes	Eating Disorder
1	N1	35.2	No	No	Yes
2	N2	46.7	No	Yes	Yes
3	N3	30.3	No	No	Yes
4	N4	31.6	No	No	Yes
5	N5	39	Yes	Yes	Yes
6	N6	44.3	Yes	Yes	Yes

4.2 Selection of genes

To find out the set of obesity-associated genes in Pakistani population, a biomedical text mining tool i.e. PolySearch2 has been used (http://wishart.biology.ualberta.ca/polysearch). The tool provides two types of search options quick search and advanced search, from that advanced search were chosen. The search category was set as "Given Disease, Find all associated genes". Input to the tool consisted of "Obesity and Diabetes" provided in the search keyword field. The term "Pakistani population" was used as custom filter words. Databases were selected according to research requirements such as Pubmed, PubmedCentral, NCBI books, OMIM, and MedlinePlus etc.

4.3 Genotyping

After DNA extraction, we perform the PCR so to get the PCR product. Genotyping will be performed by using STR markers. PAGE analysis performed to interpret the PCR products. List of STR primers of ACE and COMT gene is shown in table 4.33.

Table 4.15: Information OF Different STR Markers

Name Of STR marker	Gene Name	Size OF Marker	Temperature	Chromosomal Location
PMC310924P2	ACE	143bp	62	17q23.3
STS Marker ACE	ACE	123bp	61	17q23.3
SHGC-57821	ACE	102bp	62	17q23.3
D22S1662	COMT	251bp	60	22q11.21
PMC20764P1	COMT	273bp	60	22q11.21
RH27791	COMT	176bp	60	22q11.21

These STR marker PMC310924P2, STSmarker ACE, SHGC-57821 are the repetitive sequence of ACE gene, which is responsible for diabetes. I have checked it for 14 obese families either it linked with obesity or not. These STR marker PMC310924P2, STSmarker ACE, SHGC-57821 are located on chromosome 17q23.3.

These three STR marker D22S1662, PMC20764P1 and RH27791 are the repetitive sequence of the COMT gene, which is responsible for diabetes. I have checked it for 14 obese families either it linked with obesity or not. These STR markers D22S1662, PMC20764P1 and RH27791 are located on chromosome 22q11.21.

Genotyping results of Family A

Family A is autosomal recessive.The results of this family against STR marker ACE shows that this family is not linked with these STR marker PMC310924P2, STS Marker ACE and SHGC-57821, because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 showing homozygosity among all affected (A) and in normal (N) individuals.

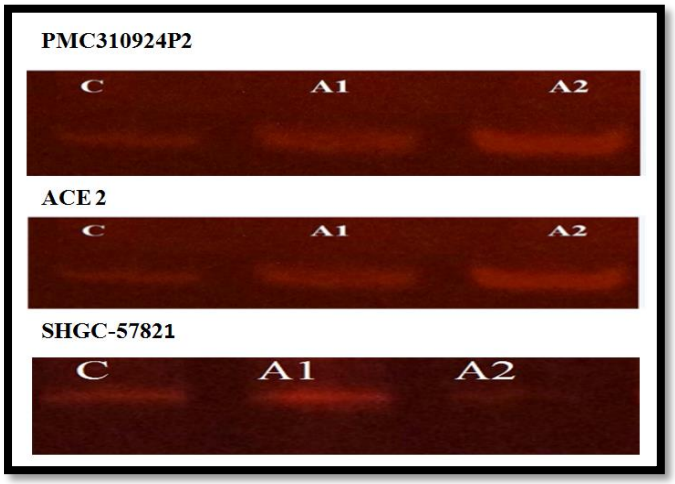

Figure 15: Genotyping results of selected marker of Family A

Genotyping Results of Family B

Family B is autosomal recessive. The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, STS Marker ACE and SHGC-57821, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

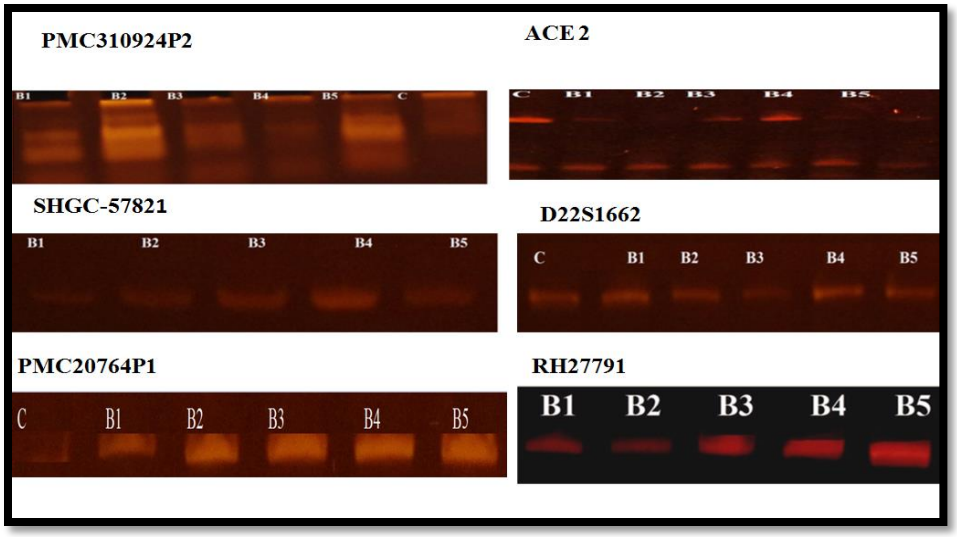

Figure 16: Genotyping results of selected marker of Family B

Genotyping Results of Family C

Family C is autosomal dominant. The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, STS Marker ACE and SHGC-57821, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

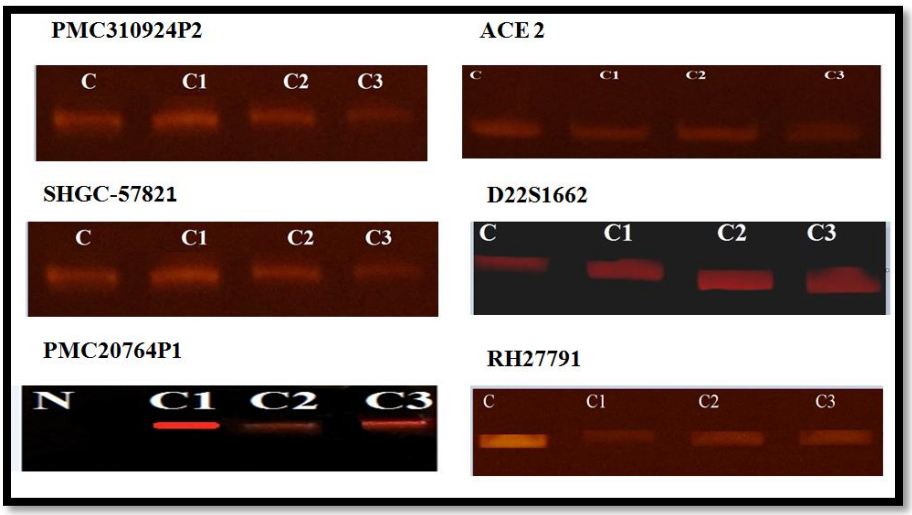

Figure 17: Genotyping results of selected marker of Family C

Genotyping Results of Family D

Family D is autosomal dominant.The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR markers PMC310924P2, STS Marker ACE, D22S1662 and PMC20764P1 because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

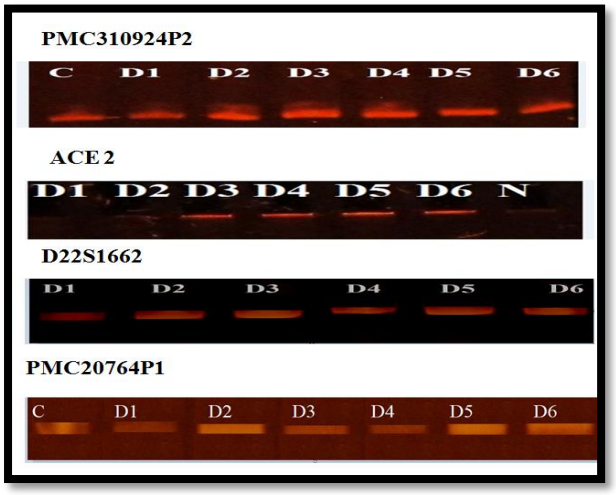

Figure 18: Genotyping results of selected marker of Family D

Genotyping Results of Family E

Family E is autosomal recessive.The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, STS Marker ACE and SHGC-57821, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

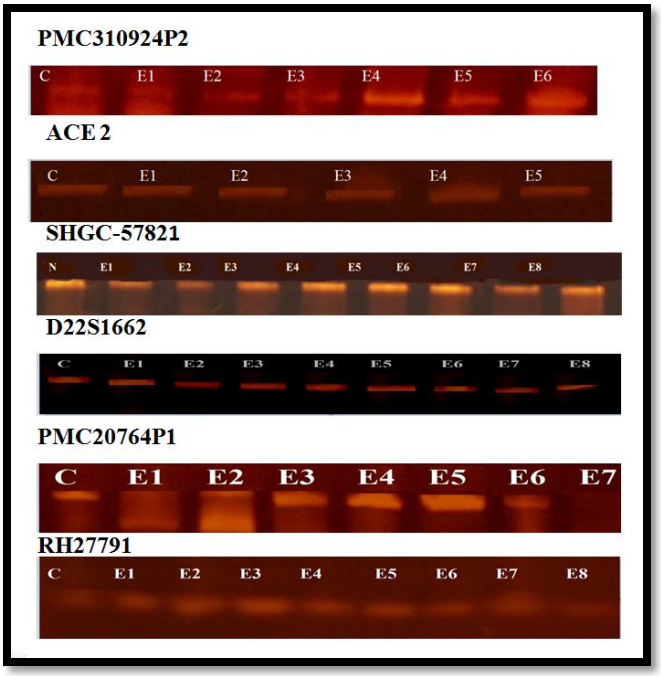

Figure 19: Genotyping results of selected marker of Family E

Genotyping Results of Family F

Family F is autosomal dominant.The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, STS Marker ACE and SHGC-57821, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

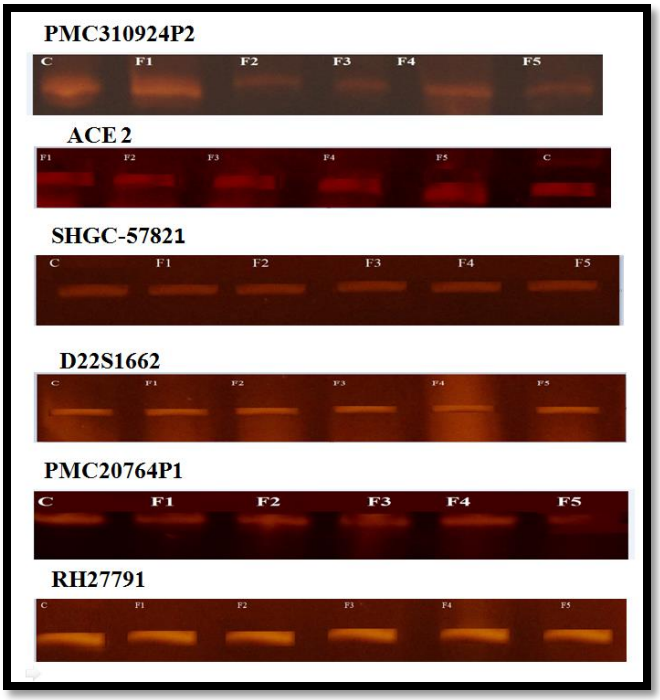

Figure 20: Genotyping results of selected marker of Family F

Genotyping Results of Family G

Family G is autosomal dominant.The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, STS Marker ACE and SHGC-57821, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

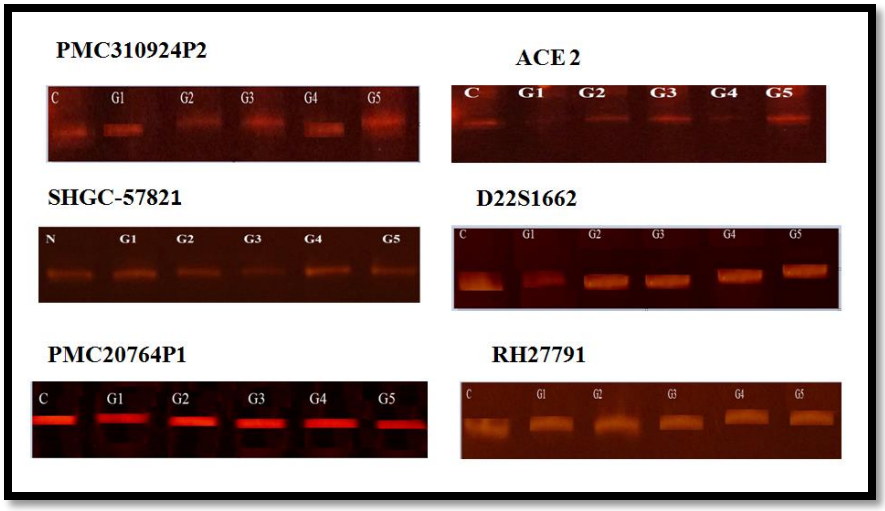

Figure 21: Genotyping results of selected marker of Family G

Genotyping Results of Family H

Family H is autosomal dominant.The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, STS Marker ACE and SHGC-57821, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

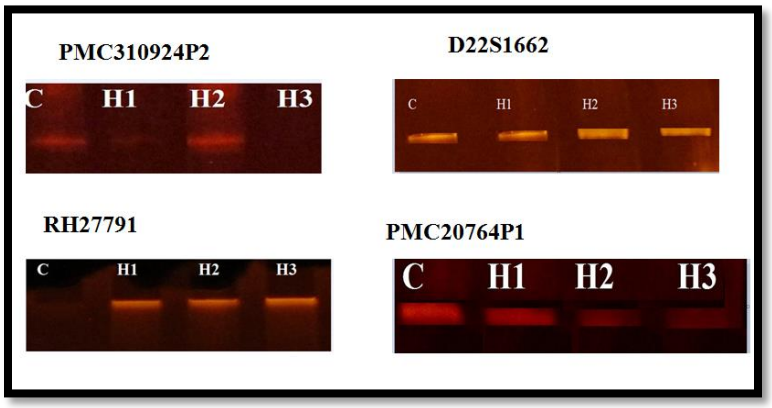

Figure 22: Genotyping results of selected marker of Family H

Genotyping Results of Family I

Family I is autosomal dominant. The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, STS Marker ACE and SHGC-57821, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

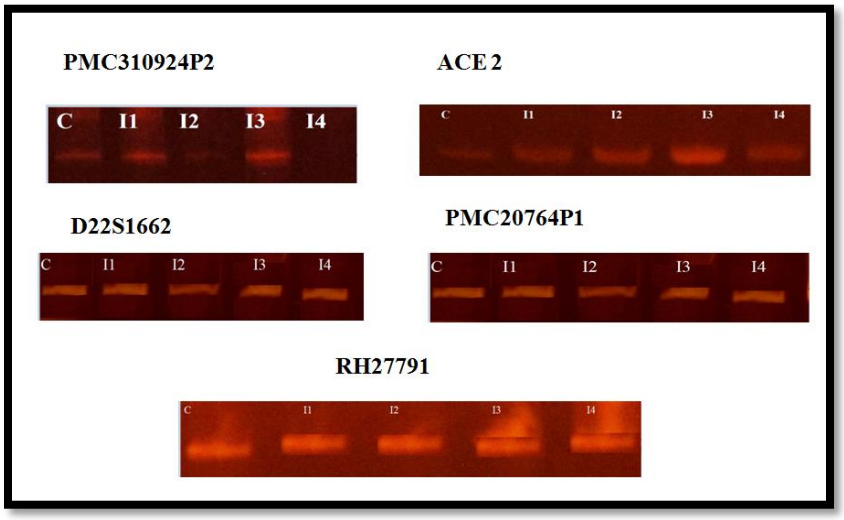

Figure 23: Genotyping results of selected marker of Family I

Genotyping Results of Family J

Family J is autosomal dominant. The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, STS Marker ACE and SHGC-57821, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

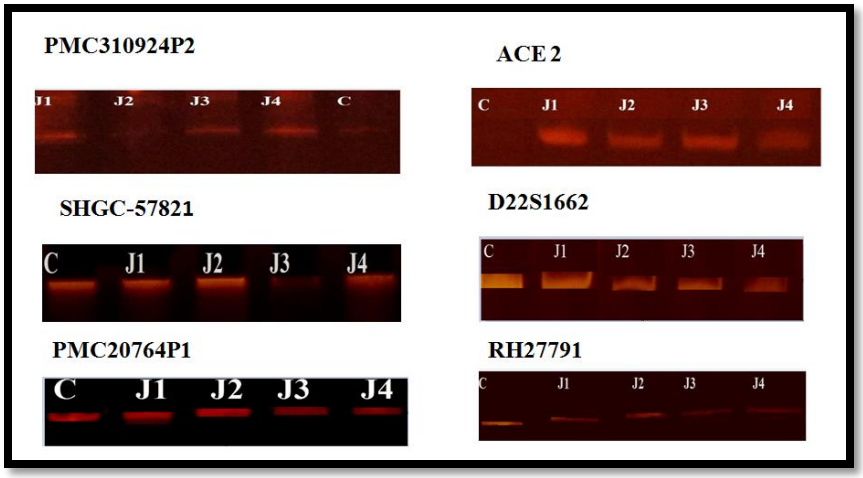

Figure 24: Genotyping results of selected marker of Family J

Genotyping Results of Family K

Family K is autosomal dominant. The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

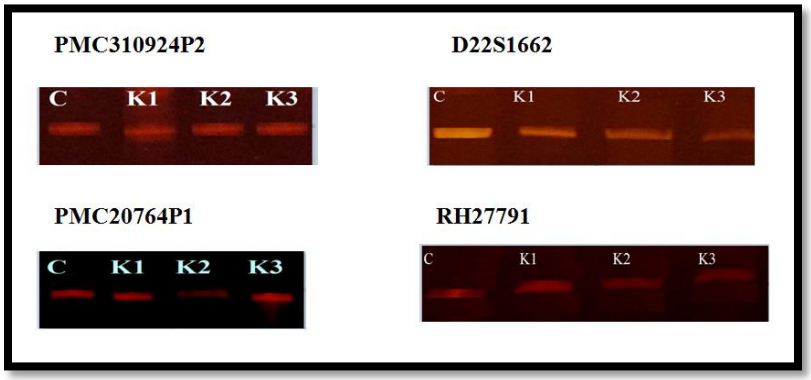

Figure 25: Genotyping results of selected marker of Family K

Family K is autosomal dominant. The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is

not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

Genotyping Results of Family L

Family L is autosomal dominant.The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, STS Marker ACE and SHGC-57821, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

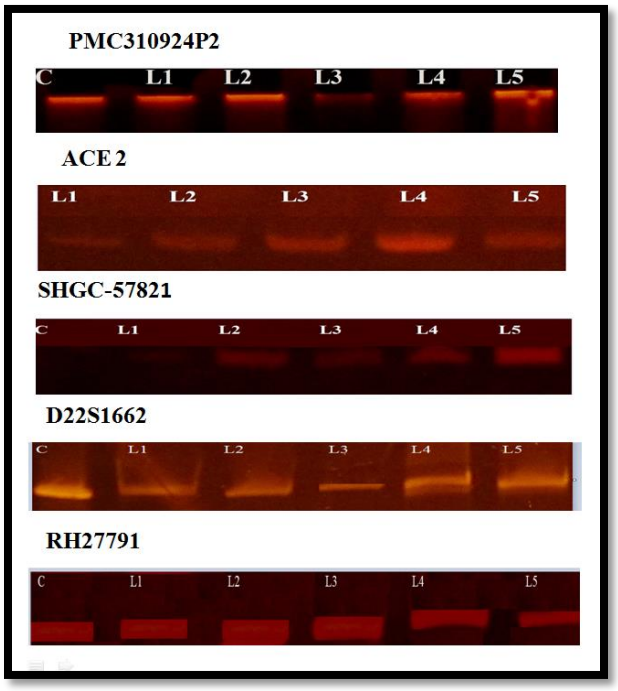

Figure 26: Genotyping results of selected marker of Family L

Genotyping Results of Family M

Family M is autosomal dominant. The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR markers PMC310924P2, STS Marker ACE and SHGC-57821, D22S1662, PMC20764P1 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

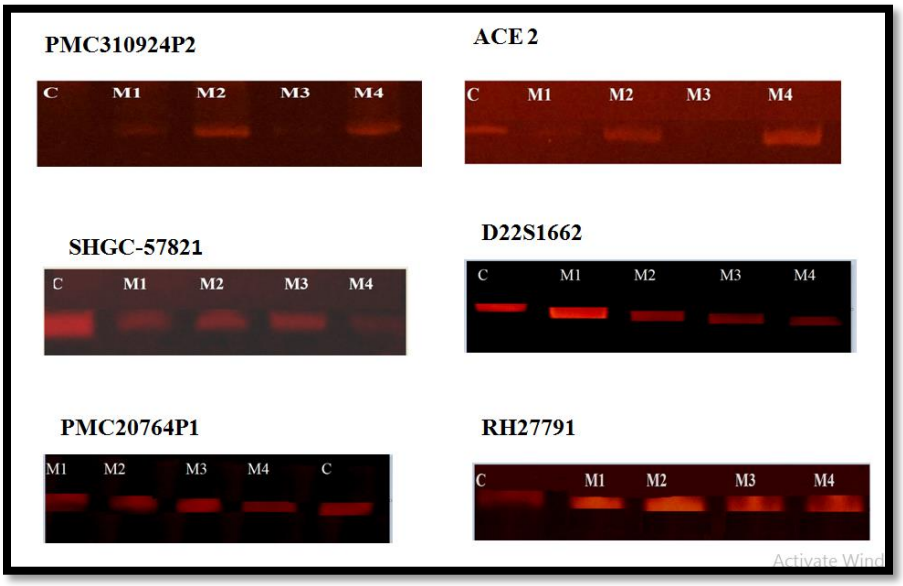

Figure 27: Genotyping results of selected marker of Family M

Genotyping Results of Family N

Family N is autosomal dominant. The results of this family against STR marker ACE and COMT shows that this family is not linked with these STR marker PMC310924P2, STS Marker ACE, D22S1662 and RH27791because the pattern of banding in Obese and normal individuals is not distinguishable. Electropherogram of ethidium bromide stained non- denaturing polyacrylamide gel of family A on chromosome 17q23.3 and 22q11.21 showing homozygosity among all affected (A) and in normal (N) individuals.

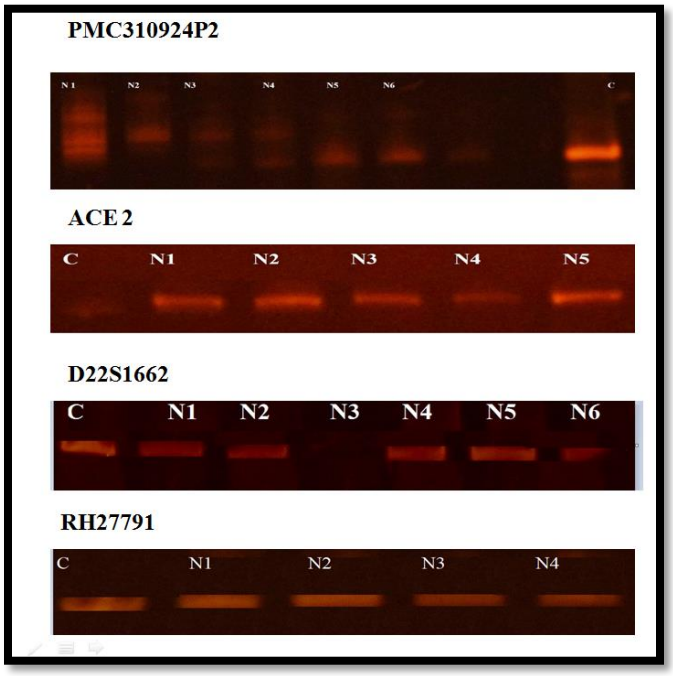

Figure 28: Genotyping results of selected marker of Family N

In our research, peripheral blood samples from fourteen families were taken, in which three families having an autosomal recessive mode of inheritance and eleven have an autosomal dominant mode of inheritance and these families belong to the different area of Pakistan such as district of Gujrat from Punjab, Bagh district of AJK and Malakand district of KPK.

Genomic DNA of all fourteen family members was amplified with microsatellite markers of PMC310924P2, ACE 2, SHGC-57821, D22S1662, PMC20764P1 and RH27791. All families show not linkage with these PMC310924P2, ACE 2, SHGC-57821, D22S1662, PMC20764P1 and RH27791 microsatellite markers because the pattern of banding is not distinguishable.

PMC310924P2, ACE 2, and SHGC-57821 these three are microsatellite of ACE gene and these are located on chromosome 17q23.3. These three are microsatellite markers D22S1662, PMC20764P1 and RH27791 of COMT gene that is located on chromosome 22q11.21.

A few reviews have proposed that level of angiotensin-converting enzyme is higher in large individual when contrasted with non obese individual and angiotensin- converting enzyme II empowers the generation of prostaglandins in segregated epididymal adipocytes, these perceptions recommend that a paracrine angiotensin framework may exist to direct a few parts of white fat tissue work so along these lines it is include in obesity(Cooper et al., 1997).

In 2012 the study conducted in Islamabad shows that ACE gene have a significant role in type 2 diabetes. In this research 276 Patients were studied (Mansoor et al., 2012).But in present study we did not find the association of ACE gene in diabetic obese individuals. The result might be differ because of difference in geographical area.It might be possible in these individual other genes are involve in diabetes.

COMT gene was also found to have relationship with diabetes (Prasad et al., 2008).COMT to be found related with diabetes and nephropathy in Asian Indian population(Prasad et al., 2008).

In the present study, we do not find the association of COMT gene in diabetes among obese individuals; it might be of geographical difference results can be differing. There is another reason of different result that is genetic heterogeneity of obesity.

The catechol-o-methyl transferase gene is an important member of the dopaminergenic pathway. dopamine likewise controls the action of Angiotensin II which is the key part of Renin Angiotensin Aldosterone System (RAAS) pathway. In this way, the relationship of dopamine and Angiotensin II could be found in controlling the vascular tone, sodium particle adjust and renal damage.In 2016 study was conducted in fasilabad to check the role of COMT (900 I/D C) polymorphism in diabetes, and the fundamental finding of this review shows the positive correlation of family history of diabetes with COMT(900 I/D C) polymorphism which has not been already reporte(Zain et al., 2016).

Human and animal studies have involved dopamine in hunger regulation. Dopamine accessibility is controlled to a great extent by three enzymes: COMT, MAOA and MAOB. The COMT and

SLC6A3 polymorphisms demonstrated no relationship with weight, BMI or obesity. They found that the COMT and SLC6A3 polymorphisms demonstrated no relationship with weight or BMI, and the genotype frequencies did not contrast between the obese and non obese individual. COMT and SLC6A3, which were not related with obesity in this companion(Need, Ahmadi, Spector, & Goldstein, 2006).

In 2009, study was conducted in Denmark to check the role of COMT gene in type 2 diabetes and BMI, and which was observed that's COMT was examined in relation to fat BMI and did not associate with glucose homeostasis or type 2 diabetes(Kring et al., 2009). In present research COMT gene also reported no association with diabetes among obese individual of different areas of Pakistani population such as district of Gujrat from Punjab, Bagh district of Azad jammu Kashmir and Malakand district of Khyber pakhtunkhwa.So it is suggested that there is need to explour the futher genes that involve in the diabetes among Pakistani population.

It is likely that numerous genes add to general obesity, each having a little impact. However if obesity can be better define, and further sorted with more physical and psychological measures, it is conceivable that inside each of these classifications the phenotype could be credited to few genes with expansive impacts.

4.4 Statistical Analysis

This Graph explain that in our data set 32% obese individual have blood pressure and 14% obese individual have diabetes and 29 % obese individual has eating disorder and reaming 25% has no co morbidities. Its mean that total 75% obese individual are associated with different disorders. Out of 64 obese individuals 14% individuals have diabetes along with obesity.

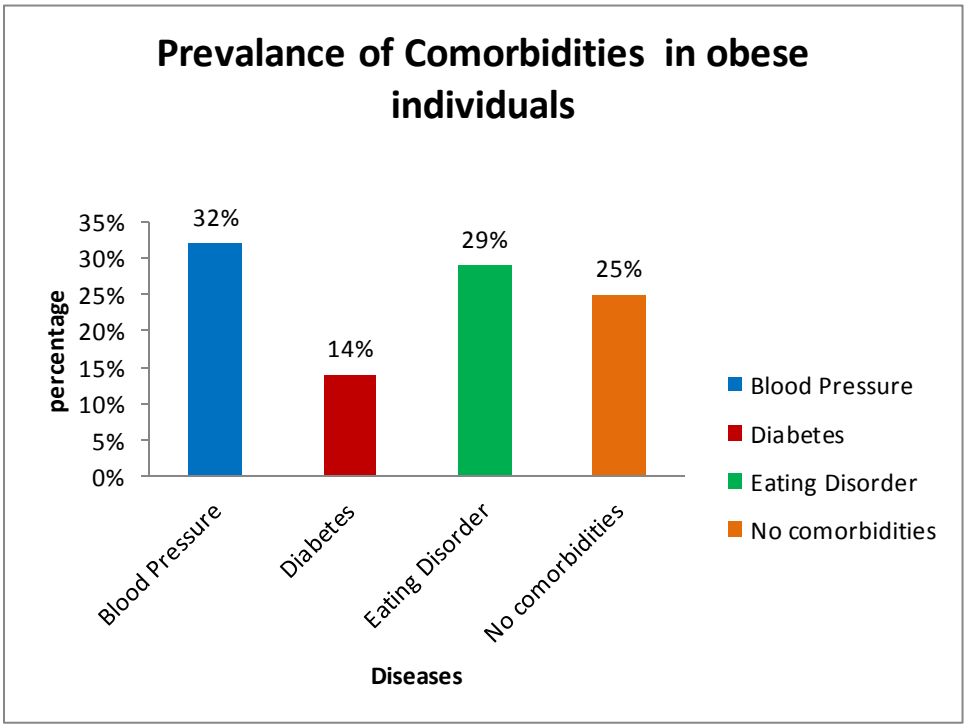

Figure 29: Prevalence of Comorbidites in obese individuals

This Graph explains the percentage of different obese individuals. In our data set only 8% are under weight and 8% are normal weight and 19% are overweight and 14% belongs to obesity class I, 16% belongs to obesity class II and 35% belongs to obesity Class III that is very dangerous and problematic.

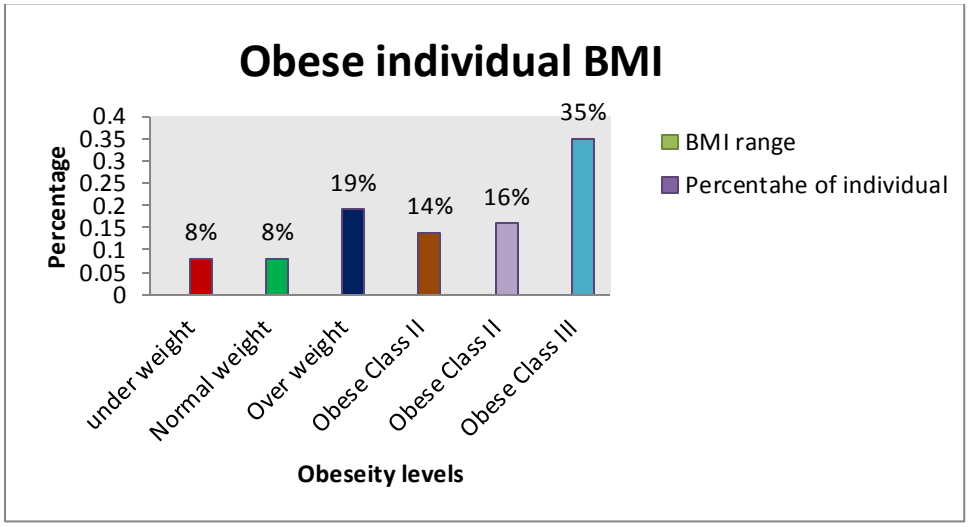

Figure 30: BMI ratio of obese individuals

This Graph explains that those individuals that have diabetes belonge to seviour Class of obesity.50% diabetic patients belonge to Obesity Class II and 50% belong to obesity Class III.It shows that diabetes some how related with severity of obesity.

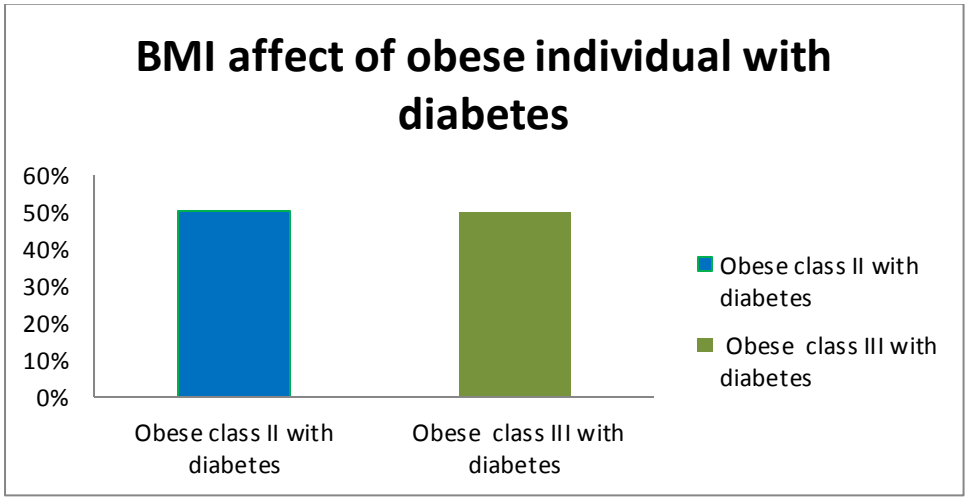

Figure 31 BMI ratio of diabetic individuals

This graph explains the weight gain pattern in obese individuals. 76% of obese individual have a steady weight gain pattern, 7% have a variable weight gain pattern, 4% have sudden weight gain because of pregnancy and remaining 7% have no weight gain pattern.

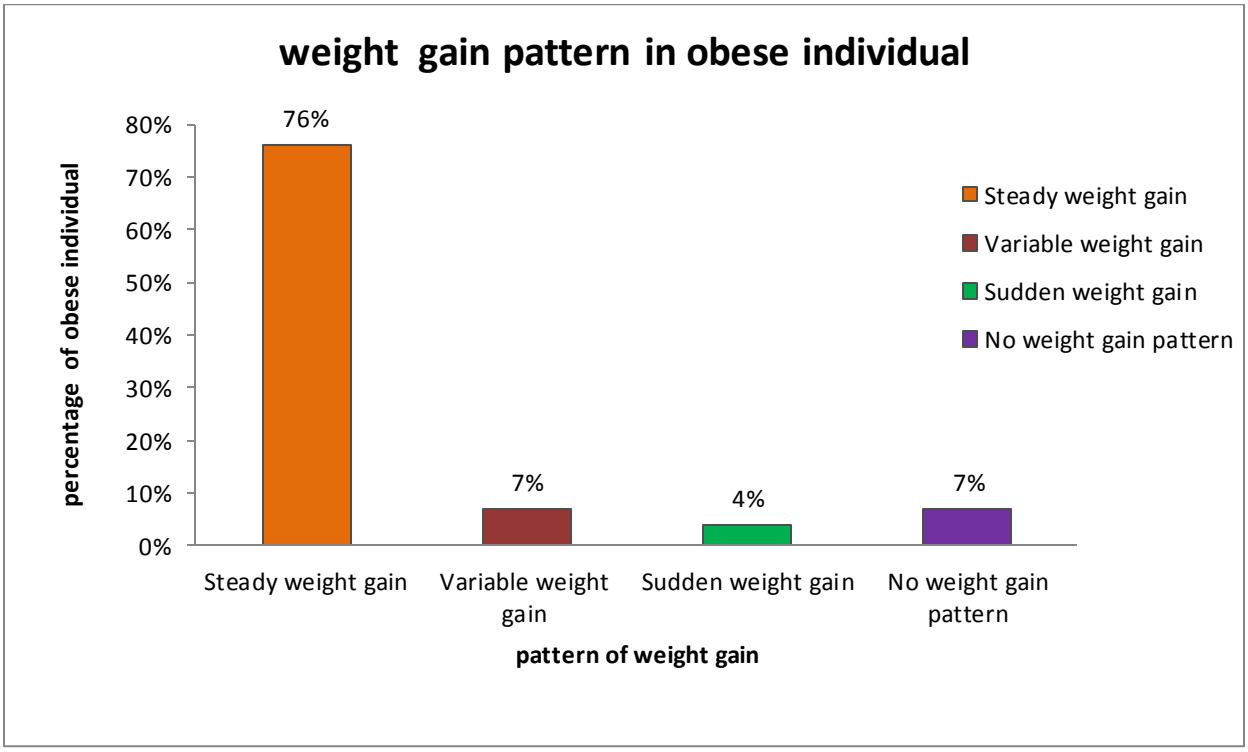

Figure 32: weight gain pattern of obese individuals

This graph explains the weight gain pattern in diabetic individuals are steady.

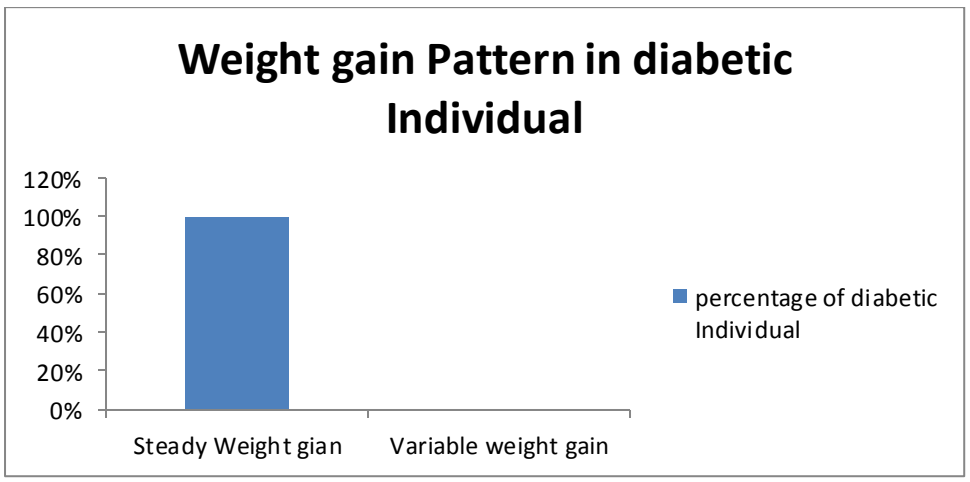

Figure 33: weight gain pattern in diabetic individuals

This graph explains the dietary history of obese individual. This graph shows that 75% of obese individual have normal eating habits and not eat so much and 25% of obese individual have over eating habits and these people eat so much. So over eating also cause obesity.

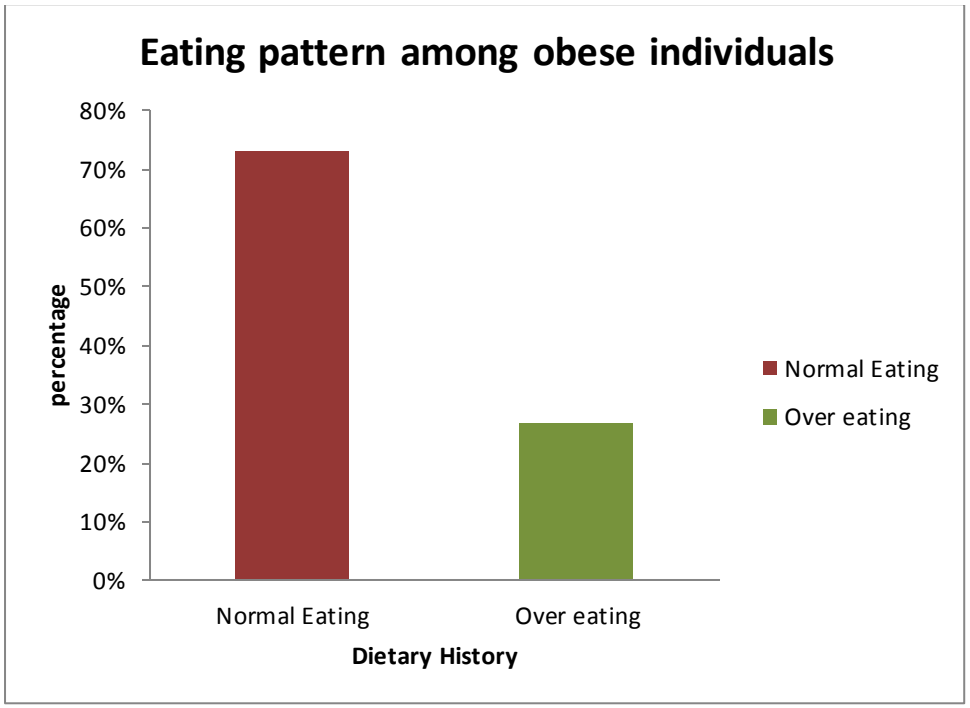

Figure 34: Eating Pattern among obese individuals

This graph explains the life style of diabetic individual. This graph shows that 37% of diabetic individuals are physically active and 63% are not physically active.So large ratio shows that mostly diabetic individuals are mostly inactive so that's why mostly diabetic individuals become obese.

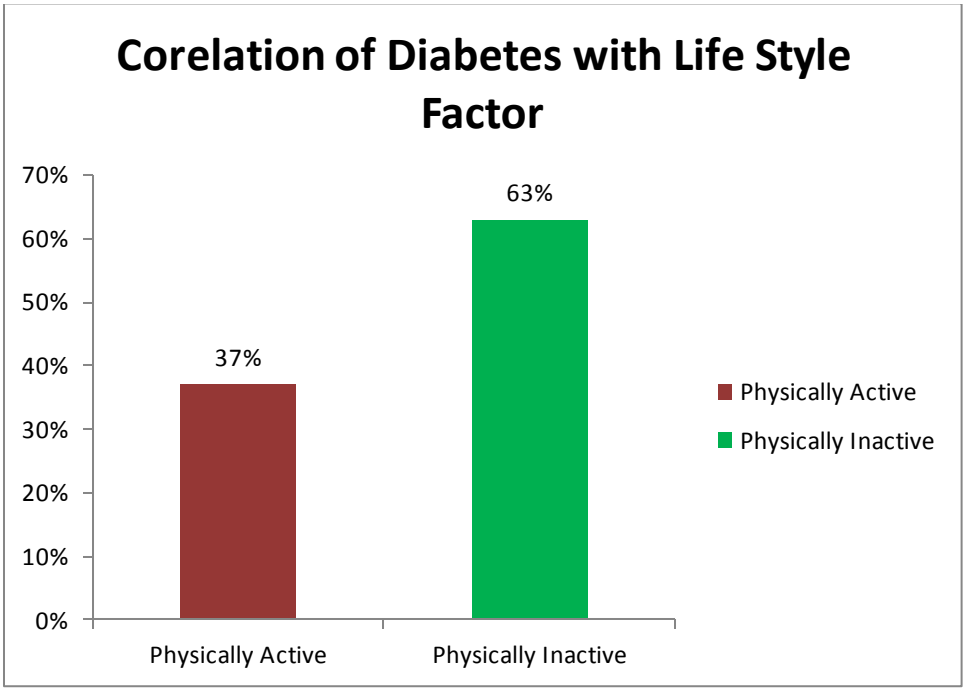

Figure 35: Eating Pattern among diabetic individuals

This graph explains the lifestyle of obese individuals. This graph shows that 49% of obese individual are physically active and 51% individual are not physically active and only 7% obese individual do exercise and 93% obese individuals not do exercise, it means that lifestyle plays very important role in causing the obesity. Physical inactivity and lack of exercise are the main cause of obesity in individuals. Environmental factor plays very significant role in causing obesity. So it is concluded that the lifestyle has very great impact on obesity. If people are physically inactive then the calories not burns and remain inside the body resulting obesity occurs.

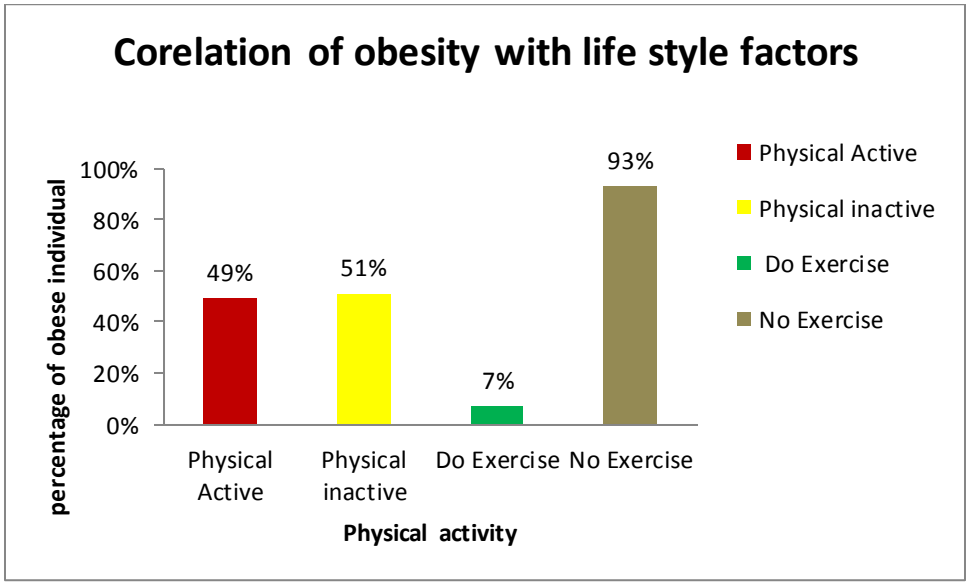

Figure 36: Correlation of obesity with life style

This graph explains the lifestyle of diabetic individuals. This graph shows that 37% of diabetic individual are physically active and 63% individual are not physically active and only 12% diabetic individual do exercise and 88% diabetic individuals not do exercise, it means that lifestyle plays very important role in causing the obesity in diabetic individuals. Physical inactivity and lack of exercise are the main cause of obesity in individuals. Environmental factor plays very significant role in causing obesity. So it is concluded that the lifestyle has very great impact on obesity. If people are physically inactive then the calories not burns and remain inside the body resulting obesity occurs.

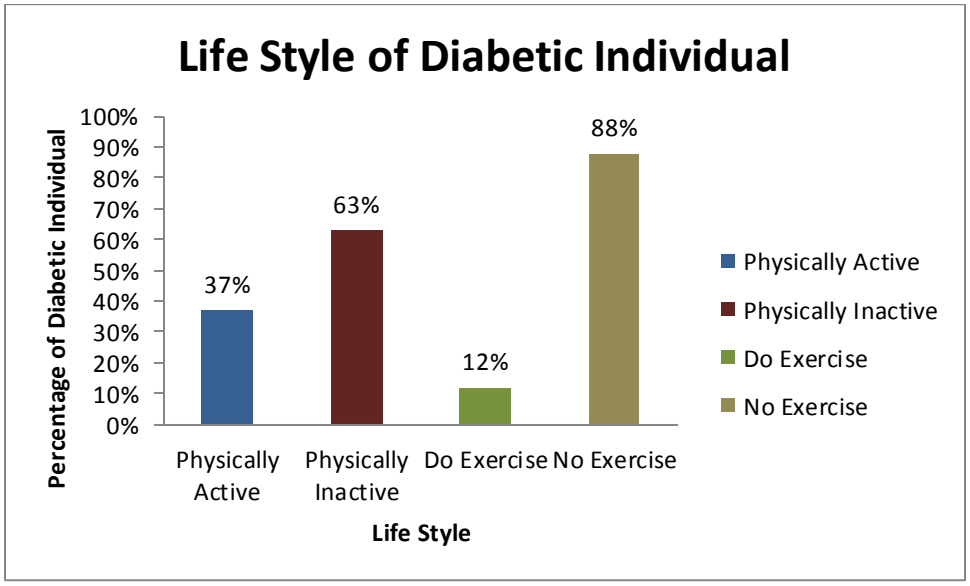

Figure 37: Life style of diabetic Individuals

CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

5 Conclusion

The increasing prevalence of diabetes in obese individual in Pakistan has significant health and economic implications. Both prevalence and occurrence of diabetes in obese individual are expanding. In Present study ACE and COMT genes are checked to find the correlation of diabetes in obese individual. Linkage with these STR markers PMC310924P2, ACE 2, SHGC-57821, D22S1662, PMC20764P1 and RH27791 was not shown in these 14 family, thus the genome wide search for the identification of a novel region that is involve in diabetes in obese individual in Pakistani population would be suggested further. The study showed that both ACE and COMT genes are not involved in diabetes among obese individual, because the pattern of banding is not distinguishable in the affected and normal individuals in collected samples. It might be of difference in result of greophical area difference or may be of genetic heterogenity.We have need to further explore the other gene that is involved in diabetes in obese individual. In environmental factor life style and diet plays very important role in causing obesity. In obese individual diabetes might be of eating more sugary food and consumption of soft drinks and juices. Moreover further investigations are recommended for more molecular elucidations of other genes that are involve in diabetes for obese individual.

5.1 Future Directions

Despite improvements in our understanding, there are as yet numerous obstructions to look the cause of diabetes in obese individual.Many factors might be associated with the high prevelance of diabetes among obese individual, futher studies can be conducted on it.Also we can do futher detail analysis of different genes which are associated in diabetes among obese individuals.Microarray analysis can also be used to reveal futher genetics of Obesity and its association with diabetes.Genome wide analysis can be performed for futher studies of diabetes in obese individuals.

CHAPTER 6
REFERENCES

References

Ashraf Chaudhry, M., Ahmad, F., & Zeeshan Ashraf, M. (2012). Frequency of overweight and obesity in students of medical college of Lahore. *Inst. Med. Sci*, *8*(2), 137–140. Retrieved from http://apims.net/Volumes/Vol8-2/FREQUENCY OF OVERWEIGHT AND OBESITY IN STUDENTS OF MEDICAL COLLEGE OF LAHORE.pdf

Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve BRecent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 17:4-12 (PDF Download Available). (n.d.). Retrieved May 2, 2017, from https://www.researchgate.net/publication/7166013_Bastard_JP_Maachi_M_Lagathu_C_Kim_MJ_Caron_M_Vidal_H_Capeau_J_Feve_BRecent_advances_in_the_relationship_between_obesity_inflammation_and_insulin_resistance_Eur_Cytokine_Netw_174-12

Battisti, W. P., Palmisano, J., & Keane, W. F. (2003). Dyslipidemia in Patients with Type 2 Diabetes. Relationships between Lipids, Kidney Disease and Cardiovascular Disease. *Clinical Chemistry and Laboratory Medicine*, *41*(9), 1174–81. https://doi.org/10.1515/CCLM.2003.181

Campos, P., Saguy, A., Ernsberger, P., Oliver, E., & Gaesser, G. (2006). The epidemiology of overweight and obesity: public health crisis or moral panic? *Published by Oxford University Press on Behalf of the International Epidemiological Association International Journal of Epidemiology*, *35*, 55–60. https://doi.org/10.1093/ije/dyi254

Cheung, W. W., & Mao, P. (2012). Recent Advances in Obesity: Genetics and Beyond. *ISRN Endocrinology*, *2012*, 1–11. https://doi.org/10.5402/2012/536905

Coleman, R., Gill, G., & Wilkinson, D. (1998). Noncommunicable disease management in resource-poor settings: a primary care model from rural South Africa. *Bulletin of the World Health Organization*, *76*(6), 633–40. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10191559

Conditions, G. (n.d.). proopiomelanocortin deficiency - Genetics Home Reference. Retrieved from https://ghr.nlm.nih.gov/condition/proopiomelanocortin-deficiency

Cooper, R., McFarlane-Anderson, N., Bennett, F. I., Wilks, R., Puras, a, Tewksbury, D., … Forrester, T. (1997). ACE, angiotensinogen and obesity: a potential pathway leading to hypertension. *J Hum Hypertens*, *11*(2), 107–111. https://doi.org/10.1038/sj.jhh.1000391

Darimont, C., Vassaux, G., Ailhaud, G., & Negrel, R. (1994). Differentiation of preadipose cells: paracrine role of prostacyclin upon stimulation of adipose cells by angiotensin-II. *Endocrinology*, *135*(5), 2030–2036. https://doi.org/10.1210/endo.135.5.7956925

De Onis, M., & Blössner, M. (2000). Prevalence and trends of overweight among preschool children in developing countries. *The American Journal of Clinical Nutrition*, *72*(4), 1032–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11010948

Dina, C., Meyre, D., Gallina, S., Durand, E., Körner, A., Jacobson, P., … Froguel, P. (2007). Variation in FTO contributes to childhood obesity and severe adult obesity. *Nature Genetics*, *39*(6), 724–726. https://doi.org/10.1038/ng2048

Farooqi, S. I. (2015, January). Genetic, molecular and physiological mechanisms involved in human obesity: Society for Endocrinology Medal Lecture 2012. *Clinical Endocrinology*. https://doi.org/10.1111/cen.12588

Flegal, K. M., Carroll, M. D., Ogden, C. L., & Curtin, L. R. (2010). Prevalence and Trends in Obesity Among US Adults, 1999-2008. *JAMA*, *303*(3), 235. https://doi.org/10.1001/jama.2009.2014

Flegal, K. M., Graubard, B. I., Williamson, D. F., & Gail, M. H. (2007). Cause-Specific Excess Deaths Associated With Underweight, Overweight, and Obesity. *JAMA*, *298*(17), 2028. https://doi.org/10.1001/jama.298.17.2028

Friedman, J. M., & Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature*, *395*(6704), 763–770. https://doi.org/10.1038/27376

Gibson, W. T., Farooqi, I. S., Moreau, M., DePaoli, A. M., Lawrence, E., O'Rahilly, S., & Trussell, R. A. (2004). Congenital Leptin Deficiency Due to Homozygosity for the Δ133G Mutation: Report of Another Case and Evaluation of Response to Four Years of Leptin Therapy. *The Journal of Clinical Endocrinology & Metabolism*, *89*(10), 4821–4826. https://doi.org/10.1210/jc.2004-0376

Griffiths, P. L., & Bentley, M. E. (2001). The nutrition transition is underway in India. *The Journal of Nutrition*, *131*(10), 2692–700.

Halford, J. C. G., Harrold, J. A., Lawton, C. L., & Blundell, J. E. (2005). Serotonin (5-HT) drugs: effects on appetite expression and use for the treatment of obesity. *Current Drug Targets*, *6*(2), 201–13. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15777190

Harms, M., & Seale, P. (2013). Brown and beige fat: development, function and therapeutic potential. *Nature Medicine*, *19*(10), 1252–1263. https://doi.org/10.1038/nm.3361

Hassan, N. E., Wahba, S., El-Alameey, I. R., El-Masry, S. A., AbuShady, M. M., Abdel Hameed, E. R., … Boseila, S. (2016). Dietary Behaviour Pattern and Physical Activity in Overweight and Obese Egyptian Mothers: Relationships with Their Children's Body Mass Index. *Open Access Macedonian Journal of Medical Sciences*, *4*(3), 353. https://doi.org/10.3889/oamjms.2016.095

Herrera, B. M., Keildson, S., & Lindgren, C. M. (2011). Genetics and epigenetics of obesity. *Maturitas*, *69*(1), 41–9. https://doi.org/10.1016/j.maturitas.2011.02.018

Herrera, B. M., & Lindgren, C. M. (2010). The genetics of obesity. *Current Diabetes Reports*, *10*(6), 498–505. https://doi.org/10.1007/s11892-010-0153-z

Hossain, P., Kawar, B., & El Nahas, M. (2007). Obesity and Diabetes in the Developing World — A Growing Challenge. *New England Journal of Medicine*, *356*(3), 213–215. https://doi.org/10.1056/NEJMp068177

Ioffe, E., Moon, B., Connolly, E., & Friedman, J. M. (1998). Abnormal regulation of the leptin gene in the pathogenesis of obesity, *95*, 11852–11857. Retrieved from http://www.pnas.org/content/95/20/11852.full.pdf

Jafar, T. H., Haaland, B. A., Rahman, A., Razzak, J. A., Bilger, M., Naghavi, M., … Hyder, A. A. (2013). Non-communicable diseases and injuries in Pakistan: strategic priorities. *Lancet (London, England)*, *381*(9885), 2281–90. https://doi.org/10.1016/S0140-6736(13)60646-7

James, W. P., & Ralph, A. (1999). New understanding in obesity research. *The Proceedings of the Nutrition Society*, *58*(2), 385–93. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10466181

Javed, A., Jumean, M., Murad, M. H., Okorodudu, D., Kumar, S., Somers, V. K., … Lopez-Jimenez, F. (2015). Diagnostic performance of body mass index to identify obesity as defined by body adiposity in children and adolescents: a systematic review and meta-analysis. *Pediatric Obesity*, *10*(3), 234–244. https://doi.org/10.1111/ijpo.242

Jones, B. H., Standridge, M. K., & Moustaid, N. (1997). Angiotensin II Increases Lipogenesis in 3T3-L1 and Human Adipose Cells [1]. *Endocrinology*, *138*(4), 1512–1519. https://doi.org/10.1210/endo.138.4.5038

Jose, P. A., Eisner, G. M., & Felder, R. A. (2000). Renal dopamine and sodium homeostasis. *Current Hypertension Reports*, *2*(2), 174–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10981146

Kiefer, F. W., Zeyda, M., Todoric, J., Huber, J., Geyeregger, R., Weichhart, T., … Stulnig, T. M. (2008). Osteopontin Expression in Human and Murine Obesity: Extensive Local Up-Regulation in Adipose Tissue but Minimal Systemic Alterations. *Endocrinology*, *149*(3), 1350–1357. https://doi.org/10.1210/en.2007-1312

Kring, S. I. I., Werge, T., Holst, C., Toubro, S., Astrup, A., Hansen, T., … Søorensen, T. I. a. (2009). Polymorphisms of serotonin receptor 2A and 2C genes and COMT in relation to obesity and type 2 diabetes. *PLoS ONE*, *4*(8), 2–9. https://doi.org/10.1371/journal.pone.0006696

Lal, M. A., Körner, A., Matsuo, Y., Zelenin, S., Cheng, S. X., Jaremko, G., … Aperia, A. (2000). Combined antioxidant and COMT inhibitor treatment reverses renal abnormalities in diabetic rats. *Diabetes*, *49*(8), 1381–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10923641

Lau, D. C. (1999). Call for action: preventing and managing the expansive and expensive obesity epidemic. *CMAJ : Canadian Medical Association Journal = Journal de l'Association*

Medicale Canadienne, *160*(4), 503–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10081466

Leddy, M. A., Power, M. L., & Schulkin, J. (2008). The impact of maternal obesity on maternal and fetal health. *Reviews in Obstetrics & Gynecology*, *1*(4), 170–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19173021

Lelli-Chiesa, G., Kempton, M. J., Jogia, J., Tatarelli, R., Girardi, P., Powell, J., … Frangou, S. (2011). The impact of the Val158Met catechol- O-methyltransferase genotype on neural correlates of sad facial affect processing in patients with bipolar disorder and their relatives. *Psychological Medicine*, *41*(04), 779–788. https://doi.org/10.1017/S0033291710001431

Loni, & Lecturer. (n.d.). GENOMIC DNA ISOLATION FROM HUMAN WHOLE BLOOD SAMPLES BY NON ENZYMATIC SALTING OUT METHOD SAJJA SUGUNA 1* , NANDAL D H 2 , SURESH KAMBLE 3 , AMBADASU BHARATHA 4 , RAHUL KUNKULOL 5 1* Tutor, Professor & Head 2 , Professor 5 , Dept. of Pharmacology, Rural Medical College, PIMS(DU) Loni, 3 Incharge Director, Center for bio technology, PIMS (DU). Retrieved from http://www.ijppsjournal.com/Vol6Issue6/9478.pdf

Loos, R. J. F., Lindgren, C. M., Li, S., Wheeler, E., Zhao, J. H., Prokopenko, I., … Mohlke, K. L. (2008). Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nature Genetics*, *40*(6), 768–775. https://doi.org/10.1038/ng.140

McGarry, J. D. (2002). Banting Lecture 2001. *Diabetes*, *51*(1). Retrieved from http://diabetes.diabetesjournals.org/content/51/1/7

Murray, C. J. L., Lopez, A. D., Harvard School of Public Health., World Health Organization., & World Bank. (1996). *The global burden of disease : a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020*. Published by the Harvard School of Public Health on behalf of the World Health Organization and the World Bank. Retrieved from http://www.hup.harvard.edu/catalog.php?isbn=9780674354487

Musaiger, A. O., Al-Mannai, M., Tayyem, R., Al-Lalla, O., Ali, E. Y. H., Kalam, F., … Chirane, M. (2012). Prevalence of Overweight and Obesity among Adolescents in Seven Arab Countries: A Cross-Cultural Study. *Journal of Obesity*, *2012*, 981390. https://doi.org/10.1155/2012/981390

Musaiger, A. O., & O., A. (2011a). Overweight and obesity in eastern mediterranean region: prevalence and possible causes. *Journal of Obesity*, *2011*, 407237. https://doi.org/10.1155/2011/407237

Musaiger, A. O., & O., A. (2011b). Overweight and obesity in eastern mediterranean region: prevalence and possible causes. *Journal of Obesity*, *2011*, 407237. https://doi.org/10.1155/2011/407237

Nanjappa, V., Raju, R., & Muthusamy, B. (2011). A Comprehensive Curated Reaction Map of Leptin Signaling Pathway. *Journal of Proteomics & Bioinformatics*, *04*(9), 184–189. https://doi.org/10.4172/jpb.1000188

Need, a. C., Ahmadi, K. R., Spector, T. D., & Goldstein, D. B. (2006). Obesity is associated with genetic variants that alter dopamine availability. *Annals of Human Genetics*, *70*(3), 293–303. https://doi.org/10.1111/j.1529-8817.2005.00228.x

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., … Gakidou, E. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*, *384*(9945), 766–781. https://doi.org/10.1016/S0140-6736(14)60460-8

O'Rahilly, S. (2009). Human genetics illuminates the paths to metabolic disease. *Nature*, *462*(7271), 307–314. https://doi.org/10.1038/nature08532

Pan, Y.-H., Wang, M., Huang, Y.-M., Wang, Y.-H., Chen, Y.-L., Geng, L.-J., … Zhao, H.-L. (2016). ACE Gene I/D Polymorphism and Obesity in 1,574 Patients with Type 2 Diabetes Mellitus. *Disease Markers*, *2016*, 1–6. https://doi.org/10.1155/2016/7420540

Phd, H., Finegood, D. T., Swinburn, B. A., Sacks, G., Hall, K. D., Mcpherson, K., … Gortmaker, S. L. (2011). Obesity 1 The global obesity pandemic: shaped by global drivers and local environments. *Series 804 Www.thelancet.com Lancet*, *378*, 804–14. https://doi.org/10.1016/S0140-6736(11)60813-1

Pi-Sunyer, F. X. (1991). Health implications of obesity. *The American Journal of Clinical Nutrition*, *53*(6 Suppl), 1595S–1603S. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2031492

Pitta, F., Breyer, M.-K., Hernandes, N. A., Teixeira, D., Sant'Anna, T. J. P., Fontana, A. D., … Hartl, S. (2009). Comparison of daily physical activity between COPD patients from Central Europe and South America. *Respiratory Medicine*, *103*(3), 421–6. https://doi.org/10.1016/j.rmed.2008.09.019

Prasad, P., Kumar, K. P., Ammini, A., Gupta, A., Gupta, R., & Thelma, B. (2008). Association of dopaminergic pathway gene polymorphisms with chronic renal insufficiency among Asian Indians with type-2 diabetes. *BMC Genetics*, *9*(1), 26. https://doi.org/10.1186/1471-2156-9-26

Rahimi, Z. (2012). ACE insertion/deletion (I/D) polymorphism and diabetic nephropathy. *Journal of Nephropathology*, *1*(3), 143–51. https://doi.org/10.5812/nephropathol.8109

Rankinen, T., Zuberi, A., Chagnon, Y. C., Weisnagel, S. J., Argyropoulos, G., Walts, B., … Bouchard, C. (2006). The human obesity gene map: the 2005 update. *Obesity (Silver Spring, Md.)*, *14*(4), 529–644. https://doi.org/10.1038/oby.2006.71

Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P., & Soubrier, F. (1990). An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *Journal of Clinical Investigation*, *86*(4), 1343–1346. https://doi.org/10.1172/JCI114844

Risk Factors for Cardiovascular Disease in School Children - a Pilot Study. (n.d.). Retrieved March 30, 2017, from http://www.jpma.org.pk/full_article_text.php?article_id=258

Ruggenenti, P., Bettinaglio, P., Pinares, F., & Remuzzi, G. (2008). Angiotensin Converting Enzyme Insertion/Deletion Polymorphism and Renoprotection in Diabetic and Nondiabetic Nephropathies. *Clinical Journal of the American Society of Nephrology*, *3*(5), 1511–1525. https://doi.org/10.2215/CJN.04140907

Samanic, C., Chow, W.-H., Gridley, G., Jarvholm, B., & Fraumeni, J. F. (2006). Relation of body mass index to cancer risk in 362,552 Swedish men. *Cancer Causes & Control*, *17*(7), 901–909. https://doi.org/10.1007/s10552-006-0023-9

Sciences, C. H. (n.d.). The Obesity Pandemic - Implications for Pakistan, (February 2000).

Shabana, & Hasnain, S. (2015). Effect of the Common Fat Mass and Obesity Associated Gene Variants on Obesity in Pakistani Population: A Case-Control Study. *BioMed Research International*, *2015*, 1–8. https://doi.org/10.1155/2015/852920

Silent Victories: The History and Practice of Public Health in Twentieth ... - Google Books. (n.d.). Retrieved April 13, 2017, from https://books.google.com.pk/books?id=YPYRDAAAQBAJ&pg=PA397&lpg=PA397&dq=Report+of+the+Conference+on+Socioeconomic+Status+and+Cardiovascular+Health+and+Disease..+Bethesda,+Maryland:+US+Department+of+Health+and+Human+Services,+National+institutes+of+Health,

Sørensen, T. I., & Echwald, S. M. (2001). Obesity genes. *BMJ (Clinical Research Ed.)*, *322*(7287), 630–1. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11250836

Stutzmann, F., Cauchi, S., Durand, E., Calvacanti-Proença, C., Pigeyre, M., Hartikainen, a-L., … Froguel, P. (2009). Common genetic variation near MC4R is associated with eating behaviour patterns in European populations. *International Journal of Obesity (2005)*, *33*(3), 373–8. https://doi.org/10.1038/ijo.2008.279

Wang, S. S., Morton, L. M., Bergen, A. W., Lan, E. Z., Chatterjee, N., Kvale, P., … Caporaso, N. E. (2007). Genetic variation in catechol-O-methyltransferase (COMT) and obesity in the prostate, lung, colorectal, and ovarian (PLCO) cancer screening trial. *Human Genetics*, *122*(1), 41–49. https://doi.org/10.1007/s00439-007-0374-7

What Genetic Factors Contribute to Obesity? (n.d.). Retrieved May 17, 2017, from https://www.ukessays.com/essays/biology/what-genetic-factors-contribute-to-obesity-biology-essay.php

WHO | Obesity: preventing and managing the global epidemic. (2015). *WHO*. Retrieved from http://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/

Yahia, N., Achkar, A., Abdallah, A., & Rizk, S. (2008). Eating habits and obesity among Lebanese university students. *Nutrition Journal*, *7*(1), 32. https://doi.org/10.1186/1475-2891-7-32

Yeh, T.-K., Yeh, T.-C., Weng, C.-F., Shih, B.-F., Tsao, H.-J., Hsiao, C.-H., … Chang, C.-Y. (2010). Association of polymorphisms in genes involved in the dopaminergic pathway with blood pressure and uric acid levels in Chinese females. *Journal of Neural Transmission*, *117*(12), 1371–1376. https://doi.org/10.1007/s00702-010-0492-6

Zain, M., Awan, F. R., & Baig, S. M. (2016). Association of Family History of Type 2 Diabetes with COMT Gene Polymorphism (I/D) in Pakistani Population. *Journal of Down Syndrome & Chromosome Abnormalities*, *2*(1), 1–3. https://doi.org/10.4172/2472-1115.1000108

Zhao, J., & Grant, S. F. A. (2011). Genetics of childhood obesity. *Journal of Obesity*, *2011*, 845148. https://doi.org/10.1155/2011/845148

APPENDICES

PID No:_____

QUESTIONNAIRE FOR RESEARCH PROJECT

GMEResearch

Project Title: **Association of obesity and commonly reported SNPs in Pakistani families**

Sponsor: Pakistan Science Foundation (PSF)

Investigator(s): Capital University of Science & Technology, Expressway, Kahuta Road, Zone-V, Islamabad. PHONES: +92-51-2512800-1, +92-51-4486700-4, FAX NUMBER: +92-51-4486705 UAN: +92-51-111-555-666 Extensions: 123,280,0

Instructions:

1. This survey form may contain words that are new to you. If you read any words that are not clear to you, please ask the person who gave you this form to explain them to you.
2. Your records will be kept confidential and will not be released without your consent except as required by law.
3. Your identity will be kept private.
4. If the results of this study are written in a scientific journal or presented at a scientific meeting, your name will not be used.
5. Your initials _____ indicate your permission to be identified by name in any publications or presentations.
6. If you do not want to be acknowledged by name in any publications or presentations, please initial here _____.
7. The data will be stored in a locked file cabinet.
8. Your signed consent form will be stored in a cabinet separate from the data.
9. Your decision to take part in this research study is entirely voluntary.
10. You may refuse to take part in or you may withdraw from the study at any time without penalty or loss of benefits to which you are normally entitled.
11. You may be asked to leave the study for any of the following reasons:
12. Failure to follow the Project Director's instructions;
13. A serious adverse reaction which may require evaluation;
14. The Project Director thinks it is in the best interest of your health and welfare; or
15. The study is terminated.
16. You may wish to discuss this with others before you agree to take part in this study.
17. If you have any questions about the research now or during the study, please contact: _____

PID No:_____

QUESTIONNAIRE FOR RESEARCH PROJECT

1. BIODATA: (This information provided by Patient will be confidencial)

First Name: _____ **Middle Name:** _____

Last Name/Surname: _____ **Date of Birth**_____

Age:_____**Gender:**_____

Contact No: (Office)_____**Home:**_____

Cell:_____**Email:**_____

Permenant Address:

Address: _____

City:_____ **Province:**_____

Temporary Address:

Address:_____

City: _____ **Province:**

_____

PID No:_____

QUESTIONNAIRE FOR RESEARCH PROJECT

1. ANTHROPOMETRIC MEASUREMENT:

Weight (kg)	
Height (m)	
BMI (kg/m^2)	
HC (cm)	
WHR (WC/HC)	
Total cholesterol (TC)	
Triglycerides (TG) (mmol/l)	
HDL-C (mmol/l)	
LDL-C (mmol/l)	

WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

2. OBESITY RELATED COMPLAINTS

- High blood pressure Yes / No _____
- Diabetes Yes / No _____
- Heart disease Yes / No _____
- Eating disorder Yes / No _____

PID No:_____

QUESTIONNAIRE FOR RESEARCH PROJECT

3. FAMILY HISTORY

	Severe Obesity	Heavy	Normal Weight	Diabetes	Heart Problem	Eating disorder
Father						
Paternal GrandFather						
Paternal GrandMother						
Father's Brothers						
Father's Sisters						
Mother						
Maternal GrandFather						
Maternal GrandMother						
Mother's brothers						
Mother's Sisters						
Your brothers						
Your sisters						
Yours sons						
Yours daughters						

4. PROGRESSION OF WEIGHT GAIN PATTERN

☐ No pattern

☐ Steady and gradual increase of weight over the years

☐ Sudden increases of weight with pregnancies

☐ Variable weight gain/loss due to intermittent diet and exercise

PID No:_____

QUESTIONNAIRE FOR RESEARCH PROJECT

GMEResearch

5. PHYSICAL ACTIVITY:

What is your exercise program?

- I am unable to exercise due to ☐ Severe joint pain
  ☐ Shortness of breath
  ☐ Wheelchair/bed
- I am able to exercise but I do not have a regular routine
- I walk / run _____ times per week for ____ minutes
- I swim _____ times per week for _____ minutes
- I lift weights ____ times per week for ____ minutes
- Other

_____

6. DIETARY HISTORY

What do you consider to be your daily eating pattern?

☐ than normal ☐ Normal ☐ Overeat

☐ Binge ☐ Serious eating disorder ☐ Excessive snacking

- Do you eat/snack just before bedtime? Yes/No
- Which meals do you eat each day?

☐ Breakfast ☐ Lunch ☐ Supper ☐ Snacks

- What and how much do you usually eat for breakfast?

_____

PID No:_____

QUESTIONNAIRE FOR RESEARCH PROJECT

GMEResearch

- What and how much do you usually eat for lunch?

  _____

- What and how much do you usually eat for supper?

  _____

- What are your favorite snacks?

  _____

- How much of them do you eat per sitting?

  _____

7. SOCIAL AND PERSONAL HISTORY

- Highest level of education:

  _____

- Occupation: _____

  Part time /Full time

- Do you have children? No / Yes - How many? _____
- Marital status:

  Single / Married /Separated / Divorced

8. MEDICAL/CLINICAL HISTORY

- Medication to control obesity_____
- Diet plan to control obesity_____
- Any surgery if yes when or for what_____
- Medicins using for any other diesease_____

PID No:_____

QUESTIONNAIRE FOR RESEARCH PROJECT

GMEResearch

9. SAMPLES:

- **Blood Sample:** ☐
- **Stool Sample:** ☐

Thank you for completing the questionnaire please return it to_____ **Faculty of Computing, Department of Bioinformatics and Biosciences, Capital University of Science and Technology, Islamabad.** If you have any concerns regarding this research please contact me or my supervisor in the first instance.

Consent

I have read the above description of this research study. I have been informed of the risks and benefits involved, and all my questions have been answered to my satisfaction. Furthermore, I have been assured that any future questions I may have will also be answered by a member of the research team. I voluntarily agree to take part in this study. I understand I will receive a copy of this consent form.

_____ _____

Subject's Signature Date