CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Association of G196A (rs6265) Gene Variant of BDNF With Obesity Associated Depression

by

Sehrish Imtiaz

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

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Dedicated to Almighty ALLAH and the Holy Prophet Muhammad (P.B.U.H)and
My Loving Family

CERTIFICATE OF APPROVAL

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Abstract

Brain Derived NeurotrophinFactor (BDNF) is small secretary protein, which belongs to neurotrophin family. BDNF is plays a significant role in nervous system development and proper functioning. It also gives anorexigenic function i.e. appetite control to brain and it is also evident that nuclear modification in BDNF leads to dysregulation of appetite. As an important regulator of food intake BDNF can play an important role in onset of obesity. Obesity, which has now become a health hazard for the public, is also contributing in disease burden and onset of other comorbidities. BDNF has also been reported to play an important part in regulation of cognitive behaviors. It is considered as an important genetic component in onset of Major Depressive Disorders. Single nucleotide polymorphisms are most common genetic variations and contribute significantly in changes related to gene expression. Their inheritance pattern can give significant information about disease gene associations. The study is designed to determine the relationship between obesity and obesity associated depression with G196A gene variant of BDNF, a major stimulator of changes in regulation of food intake. In order to determine the association of G196A gene variant with obesity and obesity induced depression, PCR-RFLP assay was designed. PCR amplified product was cleaved using Afl III restriction enzyme. Out of 400 collected samples, 357 were further analyzed owing to the reliance and completeness of collected information. Prevalence of obesity and depression was determined, and it was found that out of total sampled population, 26% are obese, 28% are overweight. Depression was determined based on DSM IV standards and it was found that 40% population shows signs of depression. For obesity associated depression, 19% individuals had both obesity and depression. Association of gene variant rs6265 of BDNF was calculated, p value of 0.278 was found between obesity and rs6265 indicating no significant association, while p value of 0.138 was found between depression and rs6265.

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Abbreviations

BDNF	Brain Derived Neurotrophin Factor
BMI	Body Mass Index
GWAS	Genome Wide Association Studies
SNP	Single Nucleotide Polymorphism

Chapter 1

Introduction

1.1 Background

Obesity is one of the major emerging health problems all around the globe. According to WHO, obesity and overweight refers to abnormal accumulation of fats which confers health risks. This abnormal accumulation of fats could be the outcome of metabolic disbalance between calories obtained and calories utilized. It can be classified on the basis of BMI that is defined as weight of person in kilograms divided by height of a person in meters square [1]. Obesity has become one of the fastest growing health issues worldwide owing to the rapid changes in lifestyle and exponential increase in processes food intake. Etiology of obesity is considered complex and multifactorial as the outcome is amalgam of multiple genetic and environmental factors.

Prevalence of obesity among gender, age and various societal groups varies between populations. Women generally have higher obesity risk than males; which is often due to biological reasons including hormonal disbalances, pregnancies etc. In connection to age, adults between 18 to 50 have higher rates of weight gains in comparison to children and old age individuals. Further, certain ethnic groups within populations such as African-Americans of United States have higher rates of

obesity than other racial or ethnic groups [2]. Prevalence also varies among different income groups, although population with higher income groups are considered to utilize more processed and junk food, this factor along with lazy life style adds to the onset of obesity, whereas on the other hand high prevalence in lower income group could be because of various reasons including lack of exercise opportunity, unavailability of healthy and hygienic food and few hormonal disbalances [3].

Rate of onset of obesity has doubled since 1930 all over the world. This exponential increase is fairly distributed among rich and poor countries. Pakistan stands at 9th position in WHO list of obese countries [1]. This significant proportion of obesity adds to the disease burden because obesity owing to insulin resistance and inflammation pathways leads to many other disease conditions. Based on category of obesity and its duration, obesity can lead to various other metabolic diseases such as Type 1 Diabetes and cardiovascular diseases including hypertension, stroke and coronary heart disease as shown in Figure 1.1.

Obesity is considered as mother of all diseases and act through disruptions in insulin resistance or inflammatory pathways. As insulin plays quite an important role in metabolisms therefore disruption can lead to imbalance metabolisms and exclusively causes diabetes type II and ultimately cardiovascular diseases. On the other hand, inflammation especially, chronic inflammation triggers the production to inflammatory cytokines including Tumor Necrosis Factors and Interleukins. These cytokines in association with fatty tissues become adipokines and trigger the onset of Diabetes type II again through insulin resistance pathways, cardiovascular diseases through triggering atherosclerosis and so on. In addition to this, several clinical conditions such as dyslipidemia, liver dysfunction, respiratory dysfunction and musculoskeletal diseases could be outcome of obesity through various pathways. The distortion in body image due to obesity is also reported to cause significant effects on mental health. These impacts were initially considered as outcome of societal prejudice against fitness. However, recent studies have explained the molecular mechanism responsible for obesity induced mental health issues [4].

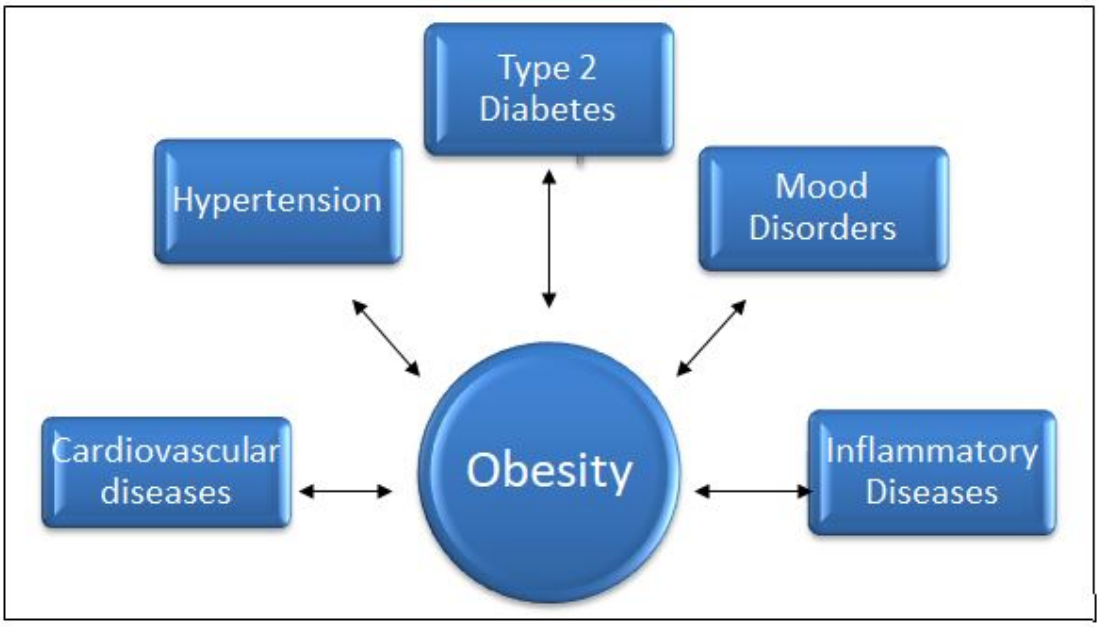

FIGURE 1.1: Disease Conditions Which are Associated with Obesity and Considered as Both Cause and Outcomes of Obesity.

1.2 Obesity and Depression

Depression and obesity both have very high prevalence and are becoming public health issue. It is also quite common to observe both as comorbidity in same individual. Low grade anxiety and depression is always present in obese individual because of societal pressure regarding body shaming and fitness. However, it is observed that a high degree of depressive disorders are also very common among obese and overweight population [5]. Although the association varies among different populations but this association of obesity and depression become more significant in females then males.

The distribution of depression and obesity also varies among different ethical and societal subsets of population [6]. The variation among population and ethnic groups lead to an increased interest in subject. Population geneticists have tried to figure out genetic susceptibility of obesity induced depression. So far, a single gene or mutation has not been found but various pathways and genomic networks have been reported to be involved. Similarly, the biological link between obesity and depression is still vague and unclear but several hypotheses are there.

Obesity is considered as inflammatory disorder which triggers various inflammatory pathways. Obesity also causes diabetes through insulin resistance pathway, which induce alterations in brain and cause depression. The whole story can go other way round as depression due to neuroendocrine disruptions can result in obesity. This bi directional association is matter of focus among scientist in recent decades. As obesity is characterized by a disbalance in energy intake and energy utilization, therefore, gene involved in hunger elevation or satiety has always been the first target to be explored. Brain Derived Neurotrophic Factor (BDNF) is reported to associated with body weight and dietary issues [7] and is focus of interest.

1.3 Association of BDNF with Obesity and Depression

Among major contributing factors of obesity, genetic predisposition is key factor. Any environmental or socioeconomic factor can have its impact only if the host is susceptible to develop a disease. Various genes have their role in onset of obesity and depression. In this regard BDNF gene is an important example. BDNF is a small secretary protein from neurotrophin family. It has a major role in the development of central nervous system (CNS). BDNF is mainly expressed in CNS, especially hippocampus, amygdale and cerebral cortex.

External and internal stimuli trigger changes in gene expression of BDNF which in turn effects hippocampus and mood. This emotional regulation is outcome of environmental factors along with biological, genetic and pharmacological factors.

BDNF gene is present on p arm of chromosome 11 (chr:11p14.1) and 67 kb in size. Through alternative splicing this gene can express 34 transcripts based on temporal and physiological needs of the cell.This gene has nine exons, whereas coding sequence is present only in exon IX. Rest of the eight exons encode promoter and regulate cell specific expression. Among which exon IV is considered as an

important site for regulation of activity dependent expression of BDNF (Figure 1.3). BDNF is initially produced as pro-BDNF; a precursor molecule. Pro BDNF is converted into mature BDNF by cleaving off N terminal pro domain [8].

BDNF plays a significant role in nervous system development and proper functioning. It exclusively gives brain anorexigenic capacity i.e. regulation of appetite [9]. It is also evident that nuclear modification in BDNF can result in dysregulation of appetite resulting in weight changes and dietary issues. Gene variant is referred as mutation in case it is rare, while a common genetic variant can be referred as polymorphism. Various gene variants of BDNF are reported to be associated with metabolism and appetite, some of the variations and their locations in gene are summarized in Figure 1.2. Most reported gene variants include C270T gene variant in exon 1, G196 A in exon 5, G 11757C variant in 3' flanking region of exon 5. Among all gene variantsG196A is reported to have a significant influence on intracellular property, movement, distribution and secretion of BDNF. It has also been reported to be associated in mental health issues including seizures, OCD (Obsessive Compulsive Disorder) and bipolar disorder [11].

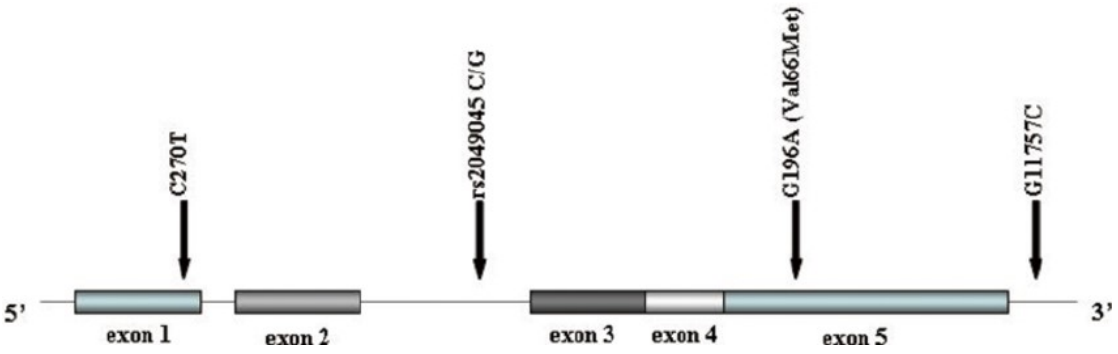

FIGURE 1.2: Location of Various Polymorphisms Reported in BDNF Gene and Their Location on Exons.

1.4 Association of G196A Gene Variant of BDNF with Obesity and Depression

Although a gene variant arises in population as a result of random mutational process, however its persistence and inheritance among population is subjected to

various genetic factors. These genetic factors such as impact of variation, pattern of variability is also influenced by demographic factors [7,10]. Gene variant usually refers to alteration in DNA sequence, these alterations could be benign, pathogenic or without any significance. Single nucleotide polymorphisms especially substitutions are among the most common among these variations.

The genetic variant with population frequency of more than one percent is referred as polymorphism. These are extensively used by population geneticists to determine genetic variability among population. Although these variants do not cause any disease or harm to the protein but their population frequency leads to determination of disease susceptibility. The distribution frequency of these variant among various ethnic groups within population is usually more informative than their presence or absence. Similarly, in case of BDNF G196A gene variant, allele A is often reported to be associated with increase in obesity. However, these reports vary among different populations and ethnicities. Polymorphism rs6265 also referred as Val66Met is present near the middle of prodomain (Figure 1.3) and considered to be involved in impairment in release of mature BDNF [12].

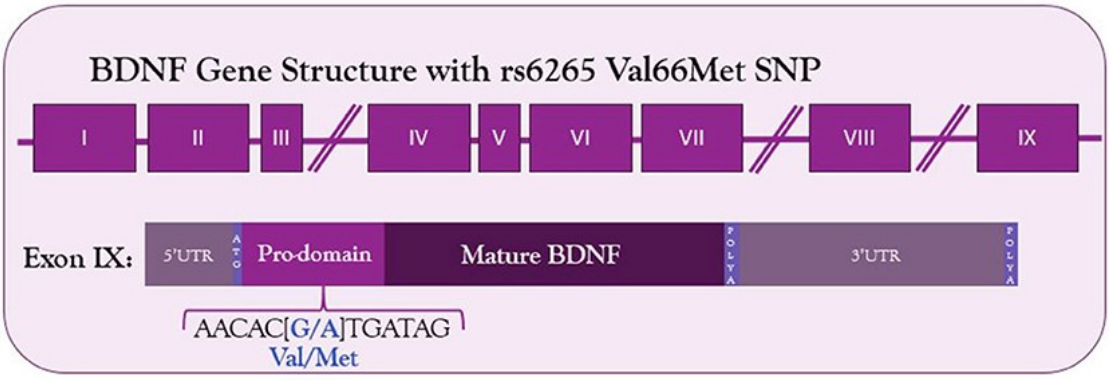

FIGURE 1.3: Structure of BDNF Gene and Location of rs6265 Polymorphism.

1.5 Problem Statement

It is established from literature review that the association between G196A gene variant of BDNF and obesity exists. Association is also evident between depression and the said gene variant. However, there is no data available for association of

BDNF with obesity induced depression. This aspect has exclusively not reported for Pakistani population.

1.6 Scope

The findings of this study will be significantly useful for the geneticists aiming to perform genetic screening to determine genetic predisposition of obesity induced depression. Similarly, it will be helpful in designing a successful and efficient policy to control depression and obesity in Pakistan.

1.7 Aims and Objectives

BDNF gene is well documented to be associated with obesity and depressive disorders. The study is designed to determine the association of obesity and obesity associated depression with G196Agene variant of BDNF.

The study is designed with following objectives:

1. Prevalence of obesity associated depression.
2. Association of obesity associated depression with G196A gene variant of BDNF.

Chapter 2

Literature Review

This chapter covers major areas of thesis topic and provides detailed literature review.

2.1 Obesity

Obesity is a complex, multifactorial but largely preventable and manageable disease [4]. Along with obesity, overweight individuals contribute as over one third of world's population [6, 7]. If the same situation continues it might possible that by 2030 probably 38% of the population above 18 years will be overweight and 20% will be obese [9]. The factors contributing towards this upward trend varies among various populations. Changes in food habits, life styles, physical activities have not only contributed in onset of obesity in adults but also in childhood obesity [11].

Obesity is simply defined as excess body weight with reference to height. This definition is not aligned with this etiologically complex phenotype where we can observe that adiposity and fatness of body is linked with metabolically dysfunctions in addition to increase in body size [13].

Obesity increases the risk of fatal chronic diseases such as diabetes, cardiac diseases, muscular diseases, psychological diseases and many more. Childhood obesity is also a major concern these days due to its association with general wellbeing of child and also with the susceptibility to various diseases in adulthood [14]. In this way, financial and psychosocial expenses of obesity separately and in combination with associated diseases are quite high.

In addition to environmental factors, genetic factors have a significant role. Leptin and melanocortin pathways and intersection of these pathways with brain signaling pathway controls the appetite and metabolism. Any disturbance in these pathways, a consequence of dysfunction in single gene could result in hyperphagia. The disturbances in sensory system, satiety and appetite could lead to disbalance in energy metabolism and could result in increased body weight [14].

Two well reported polygenic contributors i.e SNP rs17782313 near MC4R and SNP rs1421085 and rs9939609 in FTO [14,15] have high association with the intake of food and food behavior. FTO variant that predispose obesity was linked with the increased consumption of dietary fats in children and adults. This risk variant is also associated with more hunger feeling or diminished the feeling of satiety in children and adults. The obesity predisposing SNP variant near MC4R lead to increased feeling of hunger [16], less satiety resulting in more fats, protein and energy intake.

Variants have effects on other risk factors as well. A recent study based on Genome Wide Association studies of SH2B1, KCTD15, MTCH2, NEGR1 and BDNF also shows additional obesity genes and their association with dietary intake and nutrient specific food preference [17].

These genetic basis reveal that in most cases obesity is an outcome of issues related to food intake. In this regard, it could be concluded that increase in body weight is linked with disbalances in energy intake and energy expenditure resulting in metabolic issues [18].

2.1.1 Risk Factors Associated with Obesity

Genetics, both monogenic and multigenic, even polymorphisms in regulatory regions of certain genes and gene variants of various genes show high association with onset of obesity. In addition to these eating habits and lifestyle have a significant contribution towards development of obesity. Metabolic disorders, eating disorders, variation in sleep cycles, more intake of processed and high caloric food are major contributors in addition to lack of physical activity and exercise. Age, gender, marital status, education, economic status also show association with obesity.Although obesity has well reported genetic determinants, but the role of environmental factors cannot be undermined as depicted in figure 2.1.

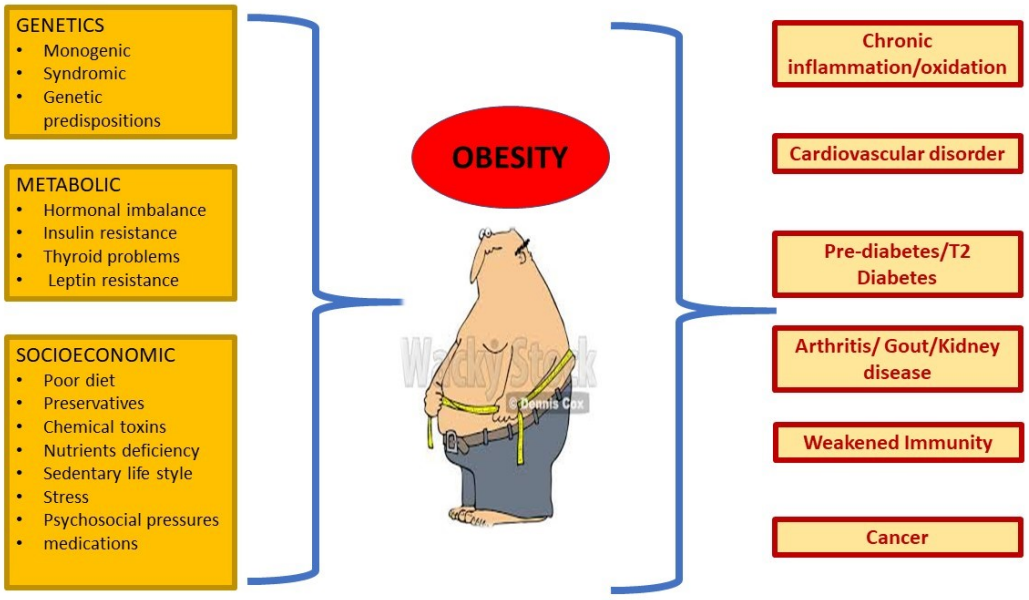

FIGURE 2.1: Genetics, Environment and Socioeconomic Status All Act as Risk Factors of Obesity

2.1.1.1 Genetic Risk Factors

More than 60 genes are reported to be associated with weight gain in both monogenic andmulti genic forms [11,12] out of which 32 are more frequent and are reported to account for more than 1.5% weight gain variations among individuals [11] some of the frequently reported are mentioned in Table 2.1. These 32

candidate genes are been reported to just increase the susceptibility of obesity, and could result in disease only in case where environmental factors exists [13]. Presence of a genetic risk allele of a gene can contribute to small extend in weight gain but the exponential increase in the obesity is outcome of various other factors and their interactions [14]. Association studies have been performed to determine the interaction between genetic risk predispositions and environmental exposure including diet, exercise, life style. A term " Thrifty genes" is used for the one which increase the overall probability of disease onset [14].

In many ways, these gene-environment interactions are gambling out in populations: as an example, a variation in FTO (rs9939609), the most reported and validated obesity associated polymorphism, increases the chances of weight problems in risk allele carriers. This contribution is estimated to be 23% the rest is obviously contributed by life style and environment [15]. Although, these modes of interactions have thus far been investigated in exceedingly few genetic risk loci out of tens of millions, and with just a handful of environmental elements, raising vital questions of how to combine this complexity for public fitness and in the end personalized medicine. The major genes linked to obesity are given in Table 2.1.

Table 2.1: List of Candidate Genes Associated with Obesity

Gene	**Impact**	**Author**
LEP (Leptin)	Involved in dietary intake	Lanas et al. (2015)
MC4R (Melanocortin 4 Receptor)	Alter food behavior related endophenotypes	Taylor et al. 2011; Bauer et al. 2009
NPY	Involved in dietary intake	Lanas et al. (2015)
POMC (pro-opiomelanocortin)	Involved in dietary intake	Baudrand et al. (2005)

Table 2.1 continued from previous page

Gene	Impact	Author
AGRP	Involved in dietary intake	Lanas et al. (2015)
CARTPT	Involved in dietary intake	Lanas et al. (2015)
FTO	Affect food intake and energy balance of body	Church et al. 2010, Choquet and Meyre 2011; Bauer et al. 2009;
LEPR (Leptin Receptor)	Involved in dietary intake	Baudrand et al. (2005)
INS (Insulin Gene)	Involved in dietary intake	Lanas et al. (2015)
PCSK1 (pro-convertase I)	Affect food intake and energy balance of body	Bauer et al. 2009;
ADBR2	Involved in energy expenditure	Matsuura et al. (2008)
ADBR3	Involved in energy expenditure	Al-Safar et al. (2015)
UCP1	Weight loss in obese patients undergoing laparoscopic adjustable gastric banding	Baudrand et al. (2005)

Table 2.1 continued from previous page

Gene	Impact	Author
UCP2	Weight loss in obese patients undergoing laparoscopic adjustable gastric banding	Al-Safar et al. (2015)
UCP3	Weight loss in obese patients undergoing laparoscopic adjustable gastric banding	Baudrand et al. (2005)
CLOCK	Involved in energy expenditure	Matsuura et al. (2008)
PPARG2	Adipose tissue growth	Al-Safar et al. (2015)
CEBPA	Adipose tissue growth	Chen et al. (2015)
IL6	Adipose tissue growth	Al-Safar et al. (2015)
FAB94	Adipose tissue growth	Traurig et al. (2016)
PNPLA3	Adipose tissue growth	Chen et al. (2015)
PLPIN5	Adipose tissue growth	Traurig et al. (2016)

Table 2.1 continued from previous page

Gene	Impact	Author
SREBF1	Responsible for increased risk of coronary heart disease in patients with obstructive sleep apnea	Bielicki et al. (2014)
Deletion of the long arm of Chromosome 15	Prader-Willi syndrome-increased obstructive sleep apnea risk	Matsuura et al. (2008)
Caveolin-1	Increased cardiovascular risk in obesity	Baudrand et al. (2005)
TCF7L2 receptor	Occurrence of type 2 diabetes mellitus in the obese	Al-Safar et al. (2015)
BDNF(Brain derived Neurotrophic factor)	Neurotrophic factor that has significant association with BMI and macronutrient in takee.g. rs6265 SNP	Traurig et al. (2016)
Amyloid A	The size of adipocytes increased in obese people	Clement and Langin (2007)
NTKR2 (neurotrophictyrosin kinase)	The size of adipocytes increased in obese people	Clement and Langin (2007)
UCP2	Weight loss in obese patients undergoing laparoscopic adjustable gastric banding	Al-Safar et al. (2015)

Table 2.1 continued from previous page

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UCP3	Weight loss in obese patients undergoing laparoscopic adjustable gastric banding	Baudrand et al. (2005)
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Amyloid A	The size of adipocytes increased in obese people	Clement and Langin (2007)
NTKR2 (neurotrophictyrosin kinase)	The size of adipocytes increased in obese people	Clement and Langin (2007)

Furthermore, parental eating routine, way of life and different exposures are likewise a hazard for obesity in onset including starvation, nutrient deficient diet, [16] and weight gain during pregnancy and gestational periods [17]. Various studies have focused on impacts of fetal programming and weight gain in various population with contrasting results [17].

2.1.1.2 Socioeconomic Risk Factors

During last century, due to economic and industrial boost, income class is considered as fundamental in exponential increase of obesity. From the mid twentieth century, in USA and Europe riches get legitimately connected with obesity, as indicated by them the wealthier the individual the more overweight it will be. In contrary to this during last few decades, because of the greater accessibility of cheap and unhealthy food, combined with changing sociocultural standards, now

riches are conversely related with obesity in the USA, it is among the individuals who are beneath or at the dimension of poverty which contribute to higher rate of obesity [19]. In US, study was led for the homeless people and its pervasiveness was seen with that of non-homeless population, results were in opposition to our average expectations that poverty and sustenance weakness leas to slimness [20].

Similar to income groups, gender and its association with obesity has reported varying results, women with lots of household work and access to less food show high chances of developing obesity [21]. In men, also, those in low-pay strata would, as per observations, have a higher trend to get obese, although the trend varies a lot among various countries and ethnic groups. That is, in specific countries, tendency of obesity was connected with more higher income groups, yet in others, lower pay was identified with progressively positive weight status. The differences between genders with respect to economic stability and earnings are quite evident, men from low income group are mostly in underweight category in contradiction to women of same income group although both men and women are subjected to higher degree of labour and physical work [22].

With economic development and stability in low income countries, it is typical to increase chances of obesity and sometimes even higher in comparison to the higher income countries. Valid reasons for this trend are yet unclear but various hypothesis exist. In examinations of the activity of preparing and wealth in women and weight status in four focus pay countries (Colombia, Peru, Jordan, and Egypt), makers viewed a vital collaboration among instruction and riches: in women with alongside zero preparing, more significant salary was connected with 9–40% higher odds of forcefulness, while in those with increasingly raised measures of preparing, the association with pay was either not present or associated with 14–16% lower odds of heftiness. This suggests in right presently evolving economies, preparing may adjust the clearly negative effects of growing acquiring power in creating obesogenic conditions.

Regardless, the cautious effect of instruction still can't be found in the more deplorable countries, for instance, India, Nigeria, and Benin, where both preparing

and wealth were clearly associated with extended odds of weight [23 This is possibly self-evident, as stoutness was decently phenomenal at <6.0% of women in these countries, and >50% of women had for all intents and purposes no training.

Promising for better opportunities, at that point, is that with regards to a worldview of illnesses due to luxurious lifestyle, in which the progress to wealth appears to be more leading to gain of weight and along with this more noteworthy interminable chronic load, higher level of education may yet counterbalance some of the alarming difficulties that lay before us [24].

2.1.1.3 Environmental Risk Factors

Study on the present environment will generally spotlight on different quantifiable qualities that help to identify weight status while holding sociodemographic and other individual dimension attributes steady. Such type of qualities extends from increasingly solid components (e.g. stores, parks, transportation etc.) to progressively factor scored factors (e.g., walkability, neighborhood fitness). Most study of the assembled condition have been cross-sectional, that tends to concentrate on a different attribute; so, discoveries on the impacts of given qualities on obesity still have conflicts [16-22], revealing the essential test of teasing out whether neighborhood qualities assume a causal work in obesity or whether healthy disapproved of people occupy health cordial regions in any case (private choice predisposition) [24].

Research demonstrates that the creating picture centers to the expansion of eating routine related manufactured conditions when contrasted with those related with physical development. While the proximity of neighborhood physical development or recreational spaces has been connected with extended physical activity levels or imperativeness use [24,25], strong sustenance circumstances, depicted by the availability of produce or closeness of business sectors over settlement stores or drive-through restaurants, play a possibly dynamically noteworthy activity [26].

Research on the causality of the assembled condition as obesity inducing or health promoting is basic for districts and general wellbeing specialists to legitimize possibly expensive upgrades to open spaces and additionally zoning guidelines. There is a neglected requirement for institutionalized measures, definitions, and criteria set up private and word related geographic radii important to wellbeing, and research methodologies that can consider the multifaceted nature of something as apparently basic as an area [27].

2.1.2 Comorbidities of Obesity

Obesity is related with corresponding or expanded danger of almost every chronic condition, from diabetes, to dyslipidemia, to poor emotional wellness as delineated in figure 2.2. Its effects on danger of stroke and cardiovascular ailment, certain malignancies, and osteoarthritis are critical [28]. Obesity is considered as the mother of all diseases as obesity leads to many other chronic disorders. Obese patients are at risk of developing various diseases. These diseases increase the cost of medication by 77% and it is assumed that overall health care cost increases to 36%.

Obesity influences thyroid function and causes hyperthyroidism; water retention is a major outcome of hyperthyroidism. Increase in weight loss is always associated with increased androgen level, and metabolic disorders. These diseases lead to the cause of cardiovascular disorder that shows the effect causing chronic disabilities. Most prominent of which is metabolic syndrome. Obesity is also associated with dyslipidemia, which in turn results in gall bladder disease, strokes and cardiac diseases. Obesity is also associated with hypertension, sleep apnea, and ultimately depression and anxiety. Nonalcoholic fatty liver disease (NAFLD) diabetes and osteoarthritis are also outcome of obesity. Gall bladder, liver and intestinal cancers are also considered as outcome of obesity.

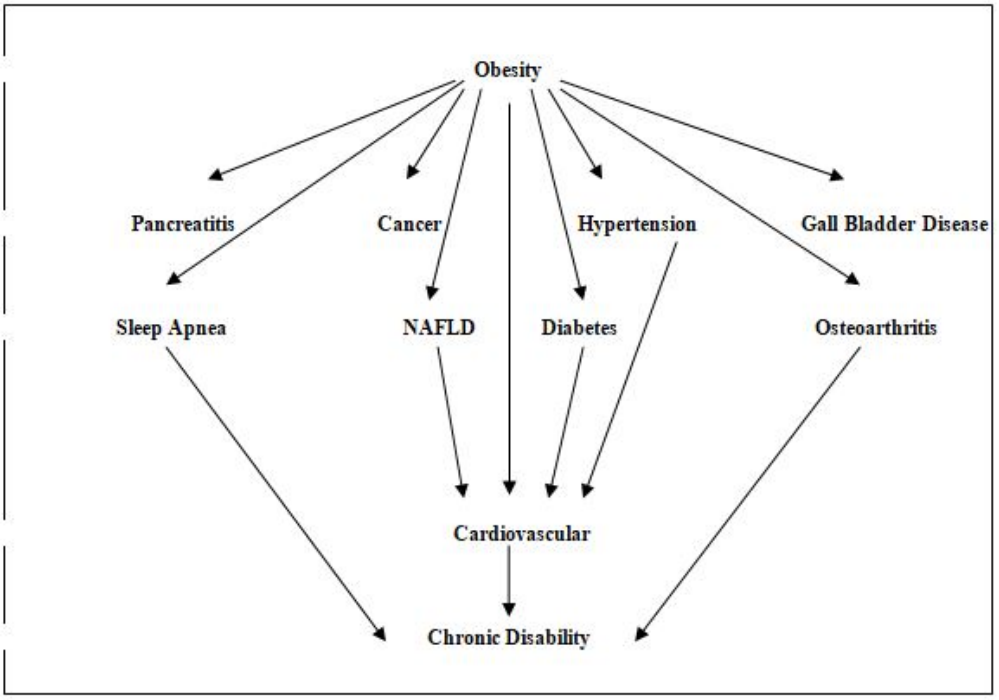

FIGURE 2.2: Obesity and Important Comorbidities as Outcome of Obesity Through Disruption of Insulin Resistance and Inflammatory Pathways [39].

2.2 Depression

The principal formal meaning of mental issue was introduced in DSM-IV originating from a profound theoretical review completed by APA's Committee on Nomenclature, headed by Spitzer. This definition was intended to address different needs psychiatry had at the time, eminently to fill in as a beginning stage for an a hypothetical and proof-based grouping of mental issue, to legitimize the expulsion of homosexuality from orders, and to counter the contentions of antipsychiatry [29].

Depression is very often confusing with anxiety. Symptoms of anxiety and depression are overlapping such as irritability, apprehension, panic, negative rumination, worry, social withdrawal, distress, dysfunction, agitation, insomnia, etc. Anxiety is characterized by worries about future, agoraphobia (fear of entering open or crowded places), muscular tensions, hypervigilance and startle response. On the other hand, depression is characterized by depressed mood, anhedonia (inability to feel pleasure), guilt, ruminations about past, lack of interest, retardation,

hypersomnia, weight gain or loss.

Depression can be appearing in any age but mostly it starts in early adulthood or late adolescence. If it remains untreated it can last for long time and can go and come back after sometime affecting the health and mental conditions. Depression may also lead tochanging in sleeping and eating habit, energy loss and difficulty in concentrating.

Depression is a very common disease in United States; more than 19 million people are affected. In a recent study, 6.7 percent adults and 12.8percent adolescents are reported with depression (NIH).In Pakistan, the risk of depression development in general population is 10-25 percent in females while 5-12 percent in males. In case of chronic patients, the risk increases up to 25-33 percent [30].

2.2.1 Risk Factors Associated with Depression

Mental issues are progressively perceived to be chronic and disabling, having a place with a gathering of genuine medicinal ailments including coronary illness, malignant growth and diabetes [31]. The Global Burden of Disease report has uncovered that neuropsychiatric conditions represent a fourth of all incapacity balanced life years. Moreover, the weight of mental issue is probably going to be thought little of as a result of lacking energy about the connection between psychological sickness and other health conditions. A few wellbeing conditions increment the hazard for mental issue and co-horribleness confounds help-chasing, conclusion and treatment and can influence guess. A regularly thought little of hazard factor for psychological well-being has to do with presentation to maternal or dietary perinatal conditions, just as introduction to early pressure [32].

For the most common ailments, we currently have medical and psychosocial mediations of demonstrated viability in randomized, controlled preliminaries. Numerous patients with state of mind issue will react to treatment and some will recoup totally [34]. Nonetheless, the accessible medicines seem still inadequate, along these lines asking an increasingly compelling extension between fundamental research

revelations and the improvement of novel remedial procedures for the fix and counteractive action of psychological maladjustments. One the restricting components for creating fitting remedial methodologies could get from the idea that scientists have so far moved toward psychological maladjustments as in a general sense not quite the same as all other restorative illnesses [33].

These contemplations have provoked us to manage these issues by requesting various commitments from specialists associated with essential research in emotional wellness. The point of this Special Issue of Neuroscience and Biobehavioral Reviews is to give a diagram of how various methodologies can be utilized in translational neuroscience so as to encourage the recognizable proof of powerlessness factors, novel symptomatic markers just as pharmacological focuses to be abused by the pharmaceutical business for the advancement of creative preventive methodologies and treatment procedures [35]. The major risk factors associated with depression are shown in Figure 2.3.

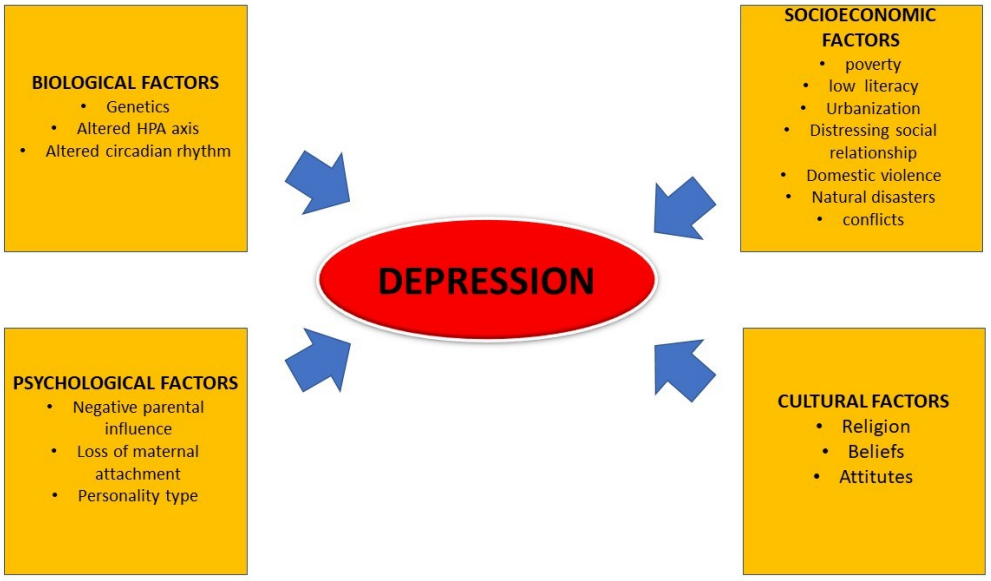

FIGURE 2.3: Risk Factors Associated with Depression [106]

2.2.1.1 Environmental Risk Factors

Inside this issue studies performed in people give a reasonable proof that presentation to unfriendly occasions over the span of advancement inclines a person to

substance misuse. Understanding the job that advancement plays in the outflow of these hazard components is regularly disregarded, yet conclusively needs more thoughtfulness regarding completely comprehend the effect of early life occasions on the complex neurobiological disturbance, including HPA pivot and dopamine framework dysfunctions, assuming an essential job in addictive and emotional issue - and perhaps metabolic – powerlessness [36].

Notwithstanding the significant data given by human investigations, these have a constrained ability to clarify fundamental components of sickness. In this issue we incorporated various papers showing how, as of late, there has been a developing accentuation on creating complex models that consolidate various factors which can be controlled by the experimenter subsequently giving new chances to interpretation from essential to clinical research [37]. We will furnish instances of studies performed with the basic point of understanding the job of natural/healthful factors in forming mental health and working.

While studies performed utilizing rat offers an extraordinary chance to pose inquiries that can be replied in a brief timeframe scale and take into consideration the examination of neurobiological substrates, those utilizing non-human primates offer an incredible chance to pose inquiries that can be replied in a brief span scale and take into account the investigation of neurobiological substrates, those utilizing non-human primates give the nearest match to people as far as hereditary, conduct, organic and social comparability [38]. What's more, non-human primates' generally long-life expectancy, broadened earliest stages, and socio-full of feeling conduct parallel numerous parts of human improvement. The test that essential science needs to meet is to utilize a relative way to deal with advantage the most from what each model, despite its limitations, can enlighten us regarding the components basic an expanded hazard for emotional well-being [45].

2.2.1.2 Socioeconomic Risk Factors

Adversity of childhood, coming from maltreatment, parental misfortune, seeing of aggressive behavior at home or family unit brokenness is a noteworthy reason for

poor mental and physical health [40]. One noteworthy result of early difficulty is an extraordinarily expanded hazard for substance use, misuse, and reliance. Childhood abuse delivers a course of physiological and neuro-humoral occasions that adjust directions of mental health and the neurobiological results of introduction to youth misuse parallel the impacts of presentation to formative worry in preclinical examinations.

The point of the survey by Anderson and Teicheris to abridge a portion of the as of late detailed impacts of early weight on mental health in creatures and man, concentrating on potential affiliations that may clarify causal connections between early misfortune and consequent maltreatment of liquor, nicotine, and illegal medications [41]. A noteworthy emphasis of this paper is on formative/worldly factors, perceiving that medication misuse is a "formative issue" in which there are windows of helplessness when presented to medications of maltreatment are bound to prompt maltreatment and reliance. To this structure, the creators include new proof for the presence of delicate periods in which discrete mind districts are maximally helpless with the impacts of pressure and underscore the considerable slack period that may intercede between the season of presentation and appearance of unfavorable outcomes [39].

On a similar line of research, the paper by gives proof in cocaine addicts that a past filled with youth disregard and poor parent-youngster connection may in part add to a complex neurobiological confusion including the neuroendocrine pivot and dopamine framework dysfunctions, assuming a critical job in addictive and full of feeling issue defenselessness [42]. Without a doubt, these creators present exploratory proof demonstrating the neurobiological changes basic substance misuse weakness and their communication with early affliction. Results demonstrate that a decrease of the dopamine metabolites, higher basal dimensions of pressure hormones, and a fundamentally higher rate of mental manifestations, describes subjects detailing early antagonistic family encounters, contrasted with those portrayed by great parental holding [43].

2.2.2 Genetic Predispositions

Family, twin, and appropriation studies have immovably settled the jobs of the two qualities and condition in mental issue. It stays troublesome, nonetheless, to discover qualities for these scatters, and to describe the specific ecological conditions under which depression rise. The purpose behind this trouble lies in the perplexing idea of mental issue. Numerous disarranges – in the same way as other ordinary physiological conditions (e.g., circulatory strain) and subjective capacities (e.g., knowledge) – presumably result from the consolidated activity of different qualities of little impact together with an assortment of natural variables. Moreover, hereditary and ecological components associate with one another in complex approaches to impact phenotype. At the end of the day, singular qualities and natural variables apply their belongings just through communication with different qualities and other ecological elements [44]. The issue is never again one of nature versus sustain; rather, we should ask: how do qualities and condition collaborate to create a conduct phenotype?

Quality condition cooperation happens when ecological impacts on a characteristic contrast as per an individual's hereditary inclinations, or when an individual's hereditary inclinations are communicated contrastingly in various situations. Connection wonders are significant. By overlooking communications, genuine hereditary and ecological impacts can be darkened, which prompts false negative outcomes and, all the more for the most part, to conflicting discoveries in the writing [47].

The resulting exchange starts with a thought of methodological and estimation issues including quality condition associations, with models concerning mental and neurological conditions. This will be trailed by an agent survey of interactions in mental issue utilizing twin, reception and affiliation structures. At long last, quality condition collaborations will be considered in chose neurodevelopmental issue (autism and schizophrenia) to feature their capability to reveal insight into fundamental etiologic instruments in this class of mental conditions [48].

TABLE 2.2: List of Candidate Gene Associated with Depression

Genes	**Impact**	**Reference**
5-HTT	Plays role in post-traumatic stress disorder	Kuzelova, 2010
BDNF	Plays role in pathophysiology of depression	Chen, 2011
TPH2	Associated with neuropsychiatric disorder spectrum	Zhang, 2016
FKBP5	Risk factor for stress related psychiatric disorder	Binder, 2009
HTR2A	Role in antidepressant action mechanism	McMohan. 2006

2.3 Obesity Induced Depression

Numerous investigations have surveyed the relationship between mental disease and obesity and have assessed different models of obesity treatment and conduct change in patients with dysfunctional behavior. A constructive relationship between mental issue and the improvement of obesity has been exhibited for various mental issues, including state of mind issue, character issue, and schizophrenia [49]. These relationships shift from 1.2–1.5 for significant sadness and bipolar issue to 2.8–3.5 for schizophrenia. So also, obesity has been distinguished as one of the regular comorbid conditions related with bipolar issue.

The relationship among obesity and mental issue are unpredictable and, in all likelihood, include a mix of poor self-care of life, disease related manifestations, lower salary, metabolic reactions of different mental prescriptions, and perhaps the resulting advancement of state of mind issue identified with impacts of the disgrace related with obesity. While the definite causal and transient connections between these 2 factors stay to be completely clarified, the requirement for satisfactory

weight related consideration in the essential consideration setting for patients with mental issue is clear [50].

While clearly not in charge of the full relationship between psychological sickness and obesity, psychotropic prescriptions are a notable contributing component to weight rates among mental patients. Weight addition is a symptom of antipsychotic meds that influences 15% to 72% of patients being treated for schizophrenia, and antipsychotic drug has been related with weight gain in patients with bipolar issue also. Weight increase is known to be most noteworthy with clozapine and olanzapine contrasted and other antipsychotic operators. Be that as it may, the sum total of what antipsychotics have been related with weight gain in treatment-gullible patients; antidepressants and state of mind stabilizers have additionally been related with weight gain [51].

Most of outpatient intercessions went for decreasing obesity among patients with mental disease have been examined in patients with extreme mental issue, most usually schizophrenia and maniacal issue [43-47]. Current rates of screening for metabolic hazard factors by either specialists or essential consideration doctors are low, and numerous specialists have called for more prominent observing by psychological well-being suppliers of weight-related parameters (midsection perimeter, BMI, metabolic parameters, change in BMI) for every mental patient just as screening for metabolic hazard factors in patients taking antipsychotic prescriptions. This methodology ought to likewise be offered by essential consideration doctors who care for such patients. Also, patients ought to be urged to screen and graph their very own weight [52].

As expanding quantities of mental health patients are treated in the network instead of in the inpatient setting, consideration must be paid to the physical soundness of these patients in standard clinical experiences. Different intercessions for prior obesity and mental drug instigated weight gain in mental patients have been contemplated. One meta-investigation concentrating on patients with an assortment of insane issue demonstrated that different nonpharmacologic way of life

mediations (counting diet, physical action, guiding, and data sessions) were commonly ready to decrease weight by a normal of -0.98 BMI focuses contrasted with control patients who got treatment of course.

While this decreease in the BMI does not meet the limit of 5% body weight decrease required to characterize these as "effective" mediations, such an adjustment in body weight can in any case have a critical positive effect on the metabolic disturbances all the more ordinarily found in this patient population.71 Similarly, specialists advocate for the combination of way of life changes into instruction and treatment programs for patients with mental illness[48].

Cognitive behavioral treatment, bunch advising, and persuasive meeting are on the whole conduct ways to deal with the treatment of heftiness that have appeared huge level of accomplishment in accomplishing and keeping up weight reduction, despite the fact that upkeep of weight reduction has reliably demonstrated testing with these procedures [49]. Improvement of long-haul weight support with these treatment modalities has been connected to broadened times of treatment and development, blends of different dietary and physical movement procedures, and expanded patient-supplier contact. A portion of these techniques have been examined among patients with psychological maladjustment, uncovering comparative outcomes.

Pharmacotherapy has likewise appeared in patients with psychological instability; be that as it may, achievement has been restricted by critical drug symptoms and long-haul weight recaptures [50]. Concerning pharmacologic supporters of stoutness in the number of inhabitants in patients with psychological maladjustment, specialists have exhorted that suppliers not be hesitant to consider exchanging mental drugs in patients who have picked up $> 5\%$ of starting body weight on treatment or who have indications of metabolic symptoms, for example, hyperglycemia or hyperlipidemia [51].

Stress physical or mental both can have a role in development of white adipose tissue, and results in obesity. This trigger of obesity could exploit gut brain axis, or through chronic inflammation. Stress also activates HPA i.e. Hypothalamus

pituitary Adrenal Axis where adrenal gland secretes hormones to combat stress especially cortisol. Autonomous nervous system is directly involved in stress combat through fight or flight response of sympathetic nervous system. It is a well-established fact that cortisol produced over a long period of time in response to chronic stress is associated with increase BMI.

On the other hand, mental stress triggers inflammatory pathways through cytokines, resulting systemic inflammation and neuroinflammation. These cytokines produce adipokines resulting in adiposity. Neuro inflammation triggers synaptic plasticity and neurogenesis resulting in depression. As summarized in Figure 2.4, chronic inflammation, HPA axis and synaptic plasticity pathways trigger both onset of obesity and depression, in this way both of these diseases are interlinked and trigger onset of each other and often occur as comorbidity [52].

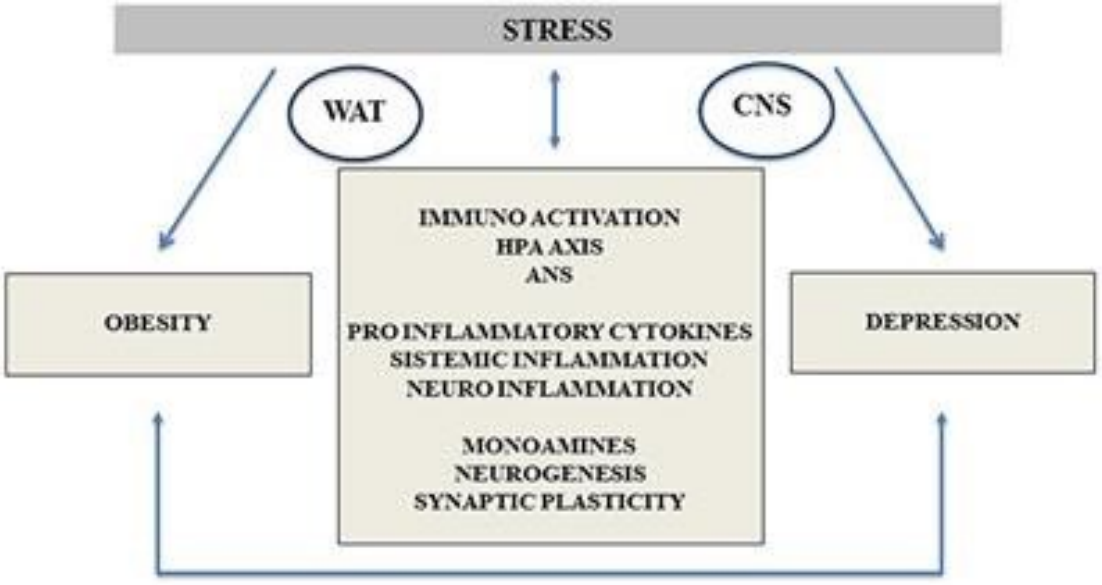

FIGURE 2.4: Association between Obesity and Depression where both act as risk factor and outcome for each other [7]. where CNS means Central Nervous System and WAT stands for White Adipose Tissue, HPA means Hypothalamus pituitary Adrenal Axis, ANS means Autonomous Nervous System

2.4 Brain-Derived Neurotrophic Factor

Due to its role in brain development and appetite control, BDNF is considered one of the most important candidate gene for analysis of obesity, depression and

obesity induced depression.

2.4.1 Gene and Protein Structure

BDNF gene consists of four 5' exons and one 3' exon. 5' exons are linked with promoters that are far from each other while 3' exon encodes mature BDNF protein. BDNF have 50% amino acid identity with NGF, NT-3 and NT-4/5 which consists of a signal peptide following the initiation codon and pro-region consisting of N-linked glycosylation site [49].

2.4.2 Role of Brain-Derived Neurotrophic Factor

Metabolic disorder including hypertension, hyperlipidemia, and focal corpulence alongside diabetes have been distinguished as hazard factors for mental sicknesses including sadness and schizophrenia. An assortment of elements including oxidative pressure, dysregulation of the HPA hub, awkwardness of synapse frameworks, aggravation, and neuro-progression including neurodegeneration and diminished neurogenesis, have been proposed to be engaged with the pathogenesis of obesity related mental infections [51].

Then again, as BDNF adds to the guideline of both synaptic pliancy and vitality digestion including sustaining conduct the neurotrophin has been perceived as a key focus to explain the connection among metabolic and mental disease [52]. The last piece of this audit covers BDNF and its impact on weight and mental illness in connection to abstain from food. Significantly, there are numerous reports and surveys concerning the impact of nourishment, for example, omega-3 unsaturated fats and amino acids, on discouragement. Here, we center more around the valuable impacts of flavonoids and zinc, as they can possibly actuate the BDNF/TrkBsystem [53].

Inverse to energy intake, energy consumption (by exercise, and so on.) is considered to improve mind work. In obesity, diminished articulation dimensions

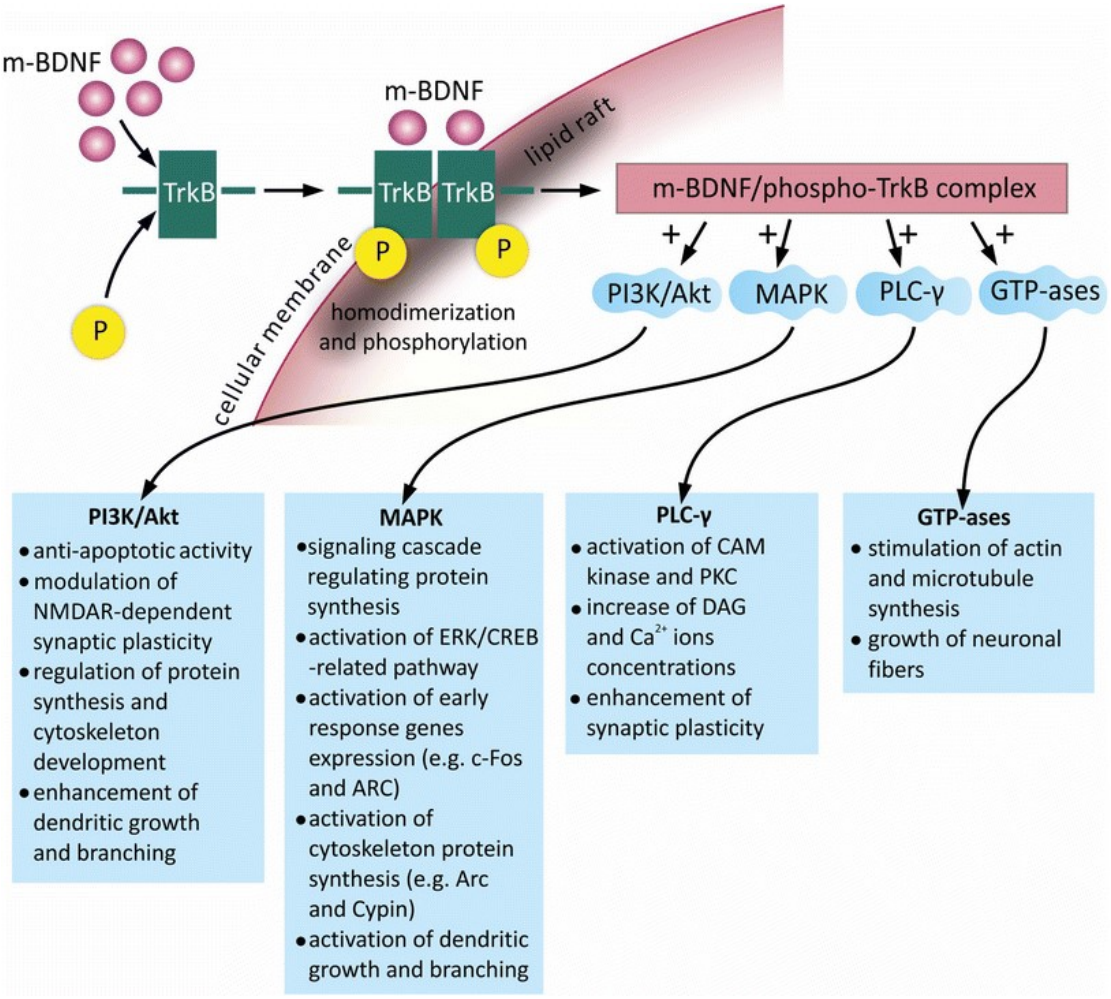

FIGURE 2.5: Mechanism of Action of BDNF and Its Impact on Neuronal Differentiation and Growth.

of neurotrophins (NGF and BDNF) and related receptors (TrkA and TrkB, individually) evoked by a HFD were watched, through weakening of neurotrophin downregulation was accomplished via preparing and additionally expending a typical calorie diet [56]. A conceivable component behind exercise-subordinate BDNF acceptance has been illustrated. Wrann et al. discovered that activity upregulated hippocampal BDNF through animating peroxisome proliferator-enacted receptor gamma coactivator 1-alpha (PGC-1α), which adds to mitochondrial biogenesis and oxidative digestion. Knockdown of fibronectin type III space containing protein 5 (FNDC5), an objective quality of PGC-1α, reduced BDNF articulation with no effect on other development factors (CNTF, GDNF, NGF, and IGF-1), recommending that BDNF assumes a job in vitality digestion by means of the PGC-1α/FNDC5-intervened pathway, (Figure 2.5) [57].

TABLE 2.3: Allele and Genotype Distribution for G196A of BDNF in Healthy Subjects in Different Ethnicity.

Ethnicity	**Cohort**	**Allele Freq**	**Genotype**			**Ref**
		A(Met) %	**G(Val) %**	**A/A**	**G/A**	**G/G**	
Asian	Korea	46.3	53.7	23.4	45.9	30.7	Pivac et al. 2009
	Japan	39	61	15	47	38	Fukumoto et al. 2009
	China	45.4	54.6	21.3	48.1	30.5	Bian et al. 2005
Caucasian	Romania	19.3	80.7	4	30.5	65.5	Vulturar et al. 2016
	Poland	15	85	1	30	69	Nowak et al. 2014
	Croatia	19.5	80.5	3.4	32.4	64.2	Pivac et al. 2009
	Italy	20.6	79.4	4.3	32.6	63.1	Geerini et al. 2009
	USA	18.8	81.2	4	29.6	66.4	Zhang et al. 2006

Table 2.3 continued from previous page

Ethnicity	**Cohort**	**Allele Freq**	**Genotype**				**Ref**
		A(Met) %	**G(Val) %**	**A/A**	**G/A**	**G/G**	
	Finland	13	87	2	24	75	Vepsal ainen et al. 2005
	Spain	19	81	3.7	30.7	6.6	Comb arros et al. 2004

BDNF level, especially a decreased level in brain is indication of antidepressant activity. Low levels of BDNF in untreated patients with depression were found in contrast to the high level of BDNF in treatment group and in control group. Treatment group was the group of patients suffering with depression and were at antidepressant therapy [78]. A similar study indicated an increase in BDNF levels after treatment in patients with Major depressive disorder contrary to common belief [79]. Anti-depressant effect of BDNF administration was found in animal models and support that increase level of BDNF controls depression [80].

Chapter 3

Materials and Methods

3.1 Methodology and Techniques

This chapter includes the methodological steps preferred to achieve aims and objectives of the project.

3.1.1 Study Population and Data Acquisition

List of questions related to biochemical and physical parameters and their standard ranges were finalized in consultation with physician and experts from Tehsil Headquarter Hospital Kahuta. Sampling camps were organized; blood samples along with necessary information were collected. In order to determine the prevalence of obesity induced depression, the inclusion criteria were to consider any individual who is at least 18 years of age, whereas individuals with any physical injury or major infections involving long term treatments were excluded. Pregnant women and physically disabled individuals were not considered as well. In order to calculate the prevalence and correlation at a regional level, following formula was used to calculate the overall sample size [63].

$$Sample\ Size = \frac{Z^2 * p(1-p)/e^2}{Z^2 * p(1-p)/e^2 N}$$

N is the population size, p is sample population, e is margin error and Z is the z-score the number of standard deviations. An estimated sample size of 382 subjects was calculated using the above formula with 5% margin error, 95% level of confidence and 1.96 z-score. During sampling process, 400 samples were collected.

3.1.2 Determination of Obesity

Body mass index (BMI), a standard measure to determine the level of obesity, was calculated using formula Kg/m^2 in Microsoft excel registered version. Subjects were classified into six categories such as underweight, normal, overweight, obese class 1, obese class 2, and obese class 3 as per WHO recommendation for Asian population. In order to calculate BMI, weight and height of subjects were calculated as by given methods.

An analog weight measuring device was used to measure weights of subjects. Before measurement weight machine was calibrated and 0 error was removed by adjusting pointer to exactly zero. Weight measuring machine was placed on a hard surface. Subjects were asked to remove extra clothing like heavy jackets, shawls, and shoes. They were asked to stand straight on both feet so that equal force was applied on the machine. Readings were taken twice and mean was calculated for each subject. Measured weight was recorded on each questionnaire in Kilograms.

10-meter rod was purchased and fixed straight with a wall from floor subjects were asked to remove shoes and high heels in case of the female patient. They were asked to stand straight with face direction not too lower or high. A steel ruler was used to press hairs of subjects and note down the exact height in inches. Height was recorded on a questionnaire of each subject and converted into meters.

Waist circumference was measured with an anthropometric tape applied horizontally at a level laterally midway between the iliac crest and the lowest lateral portion of the rib cage and interiorly the umbilicus [64].

3.1.3 Collection of Blood Samples

Each subject was asked to sit relaxed; a suitable site for vein puncture to collect blood, by tiding the tourniquet 3 to 4 inches above was selected for insertion of the syringe on the subject arm or back side of Hand. After putting gloves vein was palpated. The vein was selected, cleaned in a circular motion, after the area was cleaned; it was touched or palpated again. Subjects were asked to make a fist and avoid pumping the fist. Patient's arm was firmly gripped using the thumb to draw the skin stretched and anchor the vein. The needle was inserted into the lumen of the vein. There should be an angle of 15-30 degree with the arm surface; Syringe was filled for 5CC blood. Tourniquet was removed first than needle from the patient's arm was removed using a swift and backward motion. Alcohol swab was placed immediately on the puncture site and the patient was asked to apply adequate pressure to avoid the formation of a hematoma. After holding pressure for 1-2 minutes, 5ml blood from each subject was collected in 5 CC Syringe which was stored at 4℃ in EDTA vacutainers.

3.1.4 DNA Extraction

DNA was extracted through the organic method. Extracted DNA was analyzed for quality on agrose gels. DNA was extracted through the following given procedure:

3.1.4.1 RBC Lysis

RBC lysis was done by adding 50 μl of 1x Triton-X and 900 μl TKM 1 to 300 μl of blood in an autoclaved 1.5 ml Eppendorf. This mixture was incubated at 37 ℃ for 5 minutes to lyse the RBCs. Cells were centrifuged at 8000 rpm for 3 minutes and the supernatant was discarded. We repeated this step 2-3 times with decreasing amount of 1x Triton-X till RBC lysis was completed and a white pallet of WBCs was obtained.

3.1.4.2 Cell Lysis

300μl of TKM 2 and 40 μl of 10% SDS was added to the pellet obtained through RBCs lysis. After thorough mixing, the mixture was incubated at 37°C for 5 minutes. After incubation 100 μl of 6M NaCl was added and the mixture was vortexed to precipitate the proteins.Cells were centrifuged at 8000 rpm for 5 minutes.

3.1.4.3 DNA Precipitation

The supernatant obtained in the last step was transferred into a new Eppendorf tube containing 300 μl of isopropanol.DNA was precipitated by inverting the eppendrof slowly.Further, the Eppendorf were centrifuged at 8000 rpm for 10 minutes to pellet down the DNA. The supernatant was discarded, and 100μl of 70% ethanol was added to the Eppendorf and mixed slowly to remove any excess salts. Finally, the tubes were centrifuged at 8000 rpm for 5 minutes to pellet down the DNA. The supernatant was discarded and DNA was air dried. After thorough drying, 50 μl of TE buffer was added to dissolve the DNA.DNA will be stored at 40℃ for years without disintegration.

Obtained DNA was run on 6% agarose gel electrophoresis to detect the quality of extracted DNA. Then gel was observed under UV illuminator to visually detect the DNA [66].

3.1.5 Determination of Depression

Depression can be determined through interview by using DSM-IV (Diagnostic and Statistical Manual for Mental Disorders-Version IV) criteria [65].

Depressed mood and/or loss of interest or pleasure in life activities for at least 2 weeks and at least five of the following symptoms that cause clinically significant impairment in social, work, or other important areas of functioning almost every day.

TABLE 3.1: Depression Screening

No.	Questions
1	In past weeks, how often you felt down or depressed?
2	Do you have any thoughts of suicide?
3	How is your sleep? Are you taking sufficient sleep?
4	Do you prefer to stay at home rather than doing new things and going out?
5	Have you ever bothered by little interest in doing things?

3.2 Association of Obesity Induced Depression with G196A Gene Variant of BDNF

Genotyping and genetic analysis was performed using Polymerase chain Reaction followed by Restriction Fragment Length Polymorphism (PCR-RFLP). The fragment of DNA around genetic variation was amplified using primer set 5'TGTATTCCTCCAGCAGAAAGAGAA 3' and 5'AAAGAAGCAAACATCCGAGG AC 3'.

For amplification reaction mixture as summarized in Table 3.2 was used. Thermal cycler conditions for the PCR reaction were denaturation at 95 ℃ for 10 minutes, followed by 35 cycles of annealing at 60C and extension at 72 ℃the product was analysed at 1.5% agarose gel. The amplified fragment of 243 bp was then digested with restriction enzyme AflIII (restriction site ACRYGT) which cleaves A variant into two fragments of 168bp and 75 bp. The G variant remains undigested. After getting PCR product enzyme digestion was done according to optimized procedure by taking 10µl PCR product, 18µl nuclease free water was added with the quantity of 2µl tango buffer and 1.5µl AflIII enzyme (Figure 3.1). Product was mixed gently and incubated for 3 hours. The reaction mixture was then analysed on 2% agarose gel.

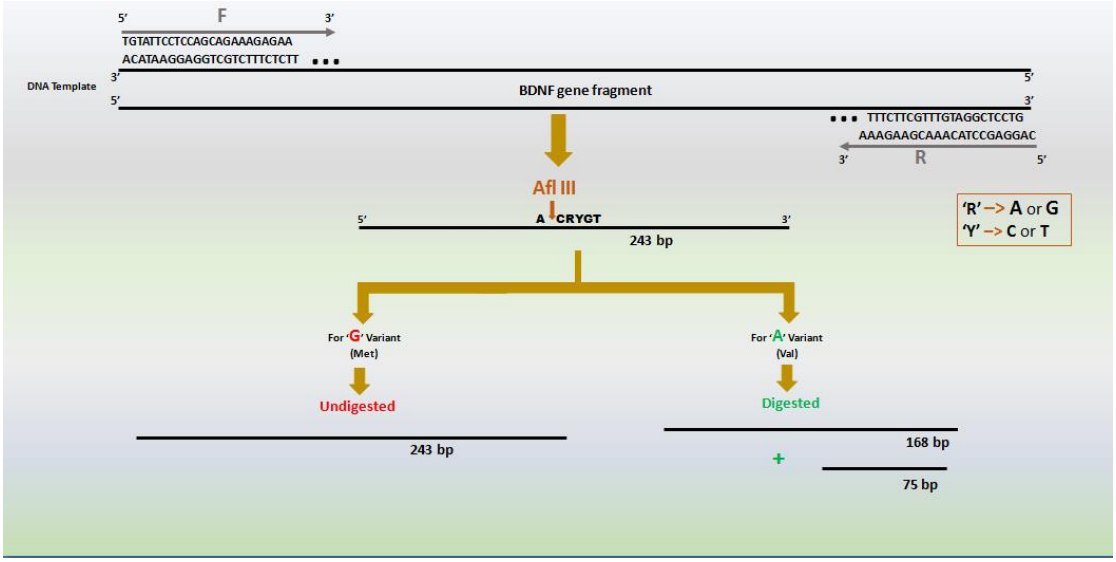

FIGURE 3.1: PCR-RFLP Assay Designed for Genotyping of G196A Gene Variant.

TABLE 3.2: PCR Master Mix Ingredients.

Material	**Quantity**
10X reaction buffer	1µl
dNTPs	1µl
Taq polymerase	0.6µl
$MgCl_2$	1µl
Forward primer	0.8µl
Reverse primer	0.8µl
DNA	1.5µl
H_2O	4.6µl

3.3 Statistical Analysis

The statistical analysis was done by using SPSS. Prevalence in the form of a percentage, Pie charts, and bar charts are plotted using MS Excel. Chi-square test was used to determine the significant association of a variable with each other.

Chi-square test of independence/ chi-square test of association determines if there is an association between two variables i.e. whether the variables are independent or related. This test utilizes a contingency table (two-way table, where one variable appears as row and other as a column), where each variable must have at least two categories. The key result of the chi-square test in SPSS is Pearson Chi-Square and association is determined based on P-value. A p-value greater than 0.05 is considered insignificant. The correlation was calculated to determine the association of with depression and genotype. Odds ratios and relative risk were calculated to determine the exposure of odds outcome.

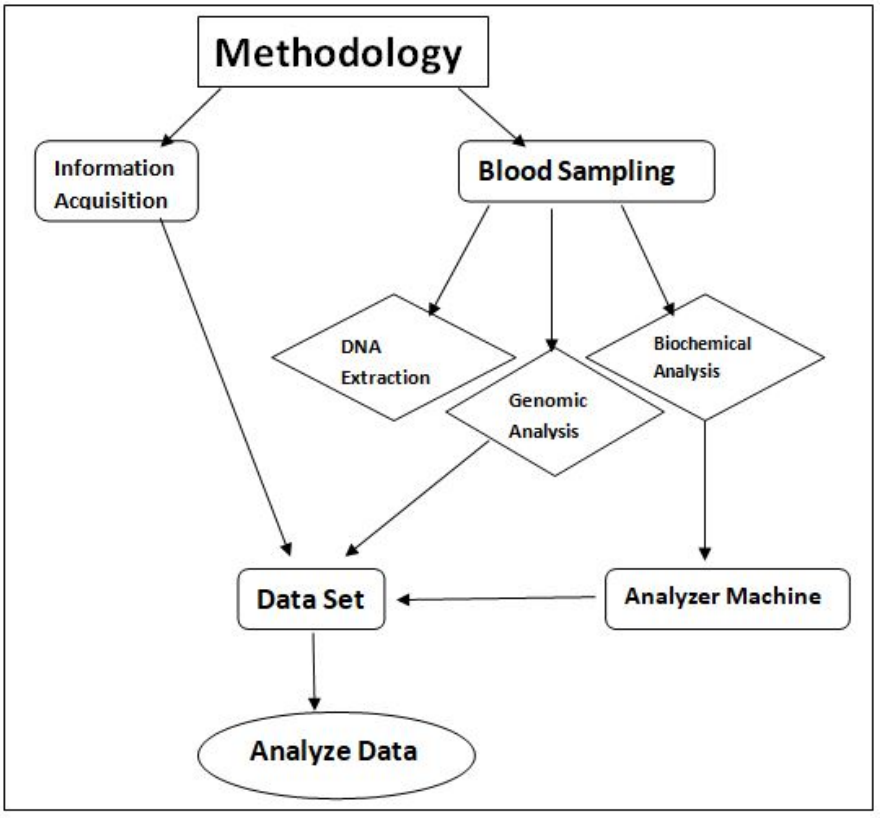

FIGURE 3.2: Methodological Steps Followed to Determine Association of G196A Variant of BDNF with Obesity Induced Depression.

Chapter 4

Results and Discussion

Brain derived neurotrophic factor (BDNF) is an important influencer on serotonergic pathways, dopaminergic pathways and non-adrenergic pathways. In this regard, it is common in many research findings that level of BDNF is lower in people with depressive disorders. Researches have confirmed that BDNF has a major role in molecular mechanism involve in onset of depression. It is also evident that if BDNF is given to animals it produces anti-depressant effects. [66]. These evidences reinforce the concept that BDNF is associated with depression.

On the other hand, BDNF is also considered to play a significantly important role in regulation of BMI i.e. it plays a regulatory role in onset of obesity. Gene variant rs6265 i.e. substitution of G196A and translated as VAL66Met, is well reported to be associated with weight gain. Although the results from various population are contradictory regarding a particular association of A or G allele as risk variant [67].

This population-based study is designed to determine the association of BDNF gene variant rs6265 with obesity and weight gain. As obesity is considered as mother all diseases, and depression associated with obesity is talk of the town, the study also focuses on obesity induced depression and significance of rs6265 in its onset.

4.1 General Characteristics of Sampled Population

In order to avoid any biasness, samples were chosen randomly. Sampling technique used was simple random sampling, that ensures that each individual in the population is chosen completely by chance and each individual in the population has equal probability to be chosen. To collect samples, various sampling camps were organized in the selected population i.e. Kahuta region of Rawalpindi district of Punjab province. It was calculated that 382 samples would be a significant number to ensure proper representation of the selected population.

The study design was approved by Ethical review committee of Department of Bioinformatics and Biosciences, Capital University of Science and Technology and Data acquisition form was designed in coordination with physicians from Kahuta district hospital. The data acquisition form was designed with three specific section, first general information including age, gender, second section was to ensure obesity i.e. BMI calculation and lipid profile, third section was about questions related to determine depression. As there is no specific molecular or biochemical test available to determine depression, therefore DSM IV criteria was used.

Out of collected 400 collected samples, 357 subjects were examined, rest were excluded because of either in sufficient data or in ability to get genotyping results. Out of total 357 samples, 181 were male (50.7%) and 176 were female (49.3%) (Fig 4.1) which indicated that male and female samples were almost same. Mean age of sampled population was 41.63 years that ranges from 18 years to 88 years. The mean age is above 40 years because the inclusion criteria was based on the fact that any individual below the age of 18 will not be considered secondly obesity and associated metabolic diseases including diabetes and cardiovascular diseases have late onset that motivated people of this age group to participate in the sampling.

Mean weight of overall studied individuals was 68.71 kg height was 150.13 M and BMI was 30.69 kg/m, which lies in obesity range. Although the sampling was

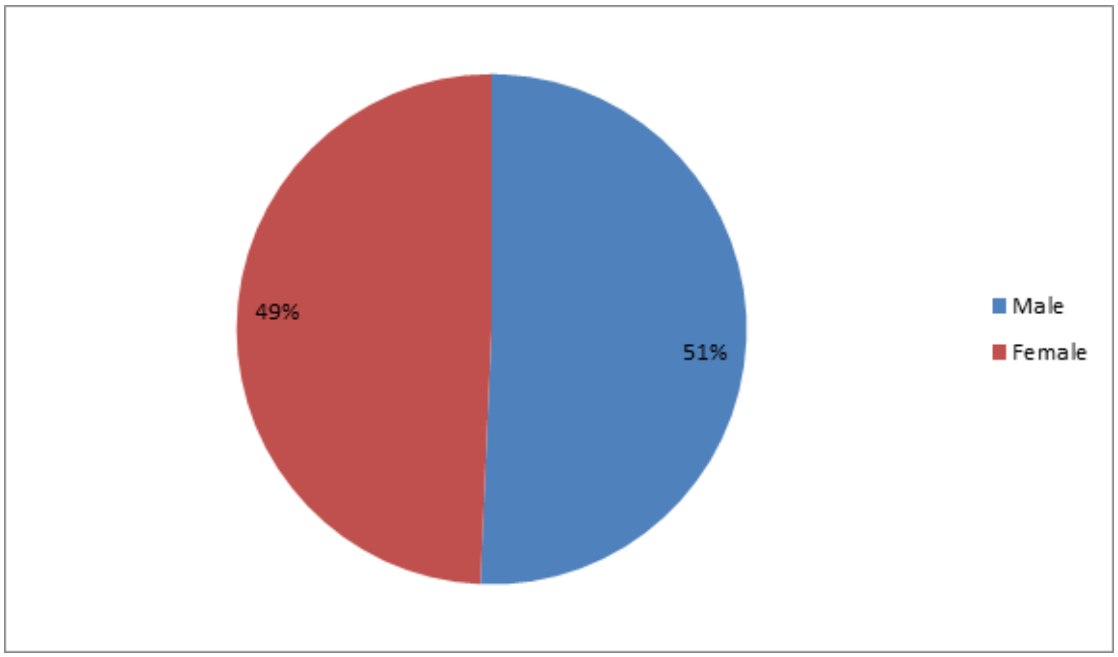

FIGURE 4.1: Ratio of male and female individuals in sampled population, where out of 357 sampled individuals 181 individuals were male and 176 individuals are female.

planned to follow simple random sampling but the sampling method i.e. organization of sampling camps could have triggered the response only in people who have symptoms of metabolic syndrome or obesity. Table 4.1 includes mean and standard deviation values for gender, age, weight, height and BMI. Mean value here signifies arithmetic mean i.e. the average or the central value, Standard deviation is the statistical measure to show dispersion or deviation in the data.

TABLE 4.1: General Characteristics of Population Under Study

	No. of samples	Mean	Std. Deviation
Age	357	41.63	17.404
Weight (kg)	357	68.71	16.259
Height (m)	357	150.13	10.802
BMI (kg/m^2)	357	30.48	6.980

4.2 Prevalence of Obesity and Obesity Induced Depression

Obesity is a complex and multi-factorial disease; it is accumulation of fats on different body parts that can lead to many other diseases. Obesity can be classified on the basis of Body Mass Index (BMI) which is a standard measurement to determine degree of obesity [58]. According to WHO, there are different classes of obesity where the ranges of classes varies between Asian and European population. Obesity is divided into three classes, class I is the first stage where Obese individuals show increase in BMI from normal range, Class II is the next stage and last and Class III is the level of BMI from where problems and associations of other diseases start (Table 4.2). BMI is useful for population-based study due to its acceptance at large scale in defining specific categories of body mass [59].

Table 4.2: Classification of Obesity as Per Criteria of WHO for Asian Population

BMI	Condition
>18-24.9 Kg/m^2	Normal
>25-29.9 kg/m^2	Overweight
>30kg/m^2	Obese
>30-34.9 kg/m^2	Obese Class I
>35-39.9kg/m^2	Obese Class II
>40kg/m^2 or >35kg/m^2	Obese Class III

BMI for all the samples i.e. 357 subjects were calculated and classified as WHO criteria for Asian population. WHO provides two different classifications, one for European population and other for Asian population. It was observed that mean BMI (Table 4.1) is 30.48 which is on higher side but when classified as WHO criteria (as depicted in figure 4.1), 40% of the subjects were with normal weight range while 28% were overweight. While depicting overall population 26% were found obese out which 15% lies in the category of obese class 1, 18% in obese class

2 while 3% were in obese class 3.6% of the population was underweight (Figure 4.2.).

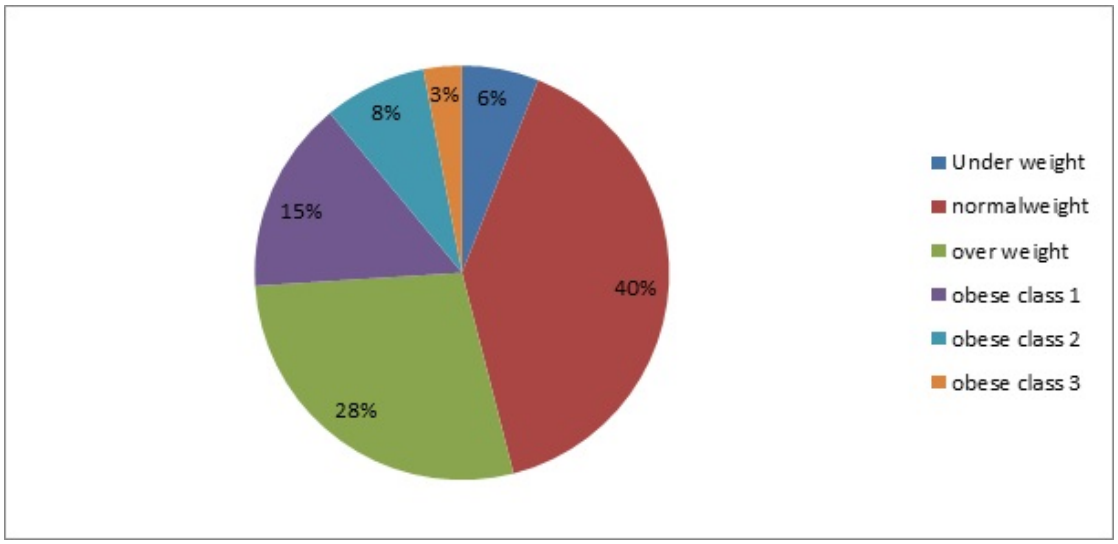

FIGURE 4.2: Classification of Obesity as per Criteria of WHO for Asian population. Classification is Based on BMI Values.

Obesity is considered global issue as indicated by WHO in 2016 survey [68] that 24% of world population is either overweight or obese. This global trend has been translated in Pakistan, in 2006 it was reported that about one fourth of Pakistani population both adults and children are in category of overweight or obese [69]. The study results were further endorsed by various other studies conducted in Pakistan [70]. It is often indicated that disease burden of obesity is quite high as it is associated with various metabolic and physiological disease. In Pakistan prevalence of obesity is quite high making it an epidemic [71]. Prevalence is alarming in children [72] and even more of concern in women within reproductive age.

Depression is one the common condition that remains undiagnosed and untreated. As compared to past it is diagnosed due to increase awareness and concern in general population. Depression may be episodic because of any major life change [60]. So far there is no specific biochemical or physiological diagnostic test available. Depression can be diagnosed through DSM-IV criteria which requires questions to be asked in order to determine eight parameters (Table 4.3).

TABLE 4.3: DSM-IV Criteria to Diagnose Depression by Cross Questioning.

	DSM-IV Criteria
A.	Emotional or behaviour symptoms in response to stressors.
B.	Depressed mood or irritability.
C.	Markedly diminished interest or pleasure in most or all activities.
D.	Significant weight loss (or poor appetite) or weight gain, or failure to gain appropriate weight.
E.	Insomnia or hypersomnia.
F.	Feelings of worthlessness or excessive or inappropriate guilt.
G.	Diminished ability to think or concentrate, or indecisiveness.
H.	Recurrent thoughts of death (not just fear of dying), or suicidal ideation, plan, or attempt.

Based on the DSM IV criteria, questions were designed and samples population was categorized into depression or no depression categories. It was also observed among the subjects that was about 39.8%. depression was not further categorized into severe or mild or any co-morbidity such as anxiety etc [Figure 4.3].

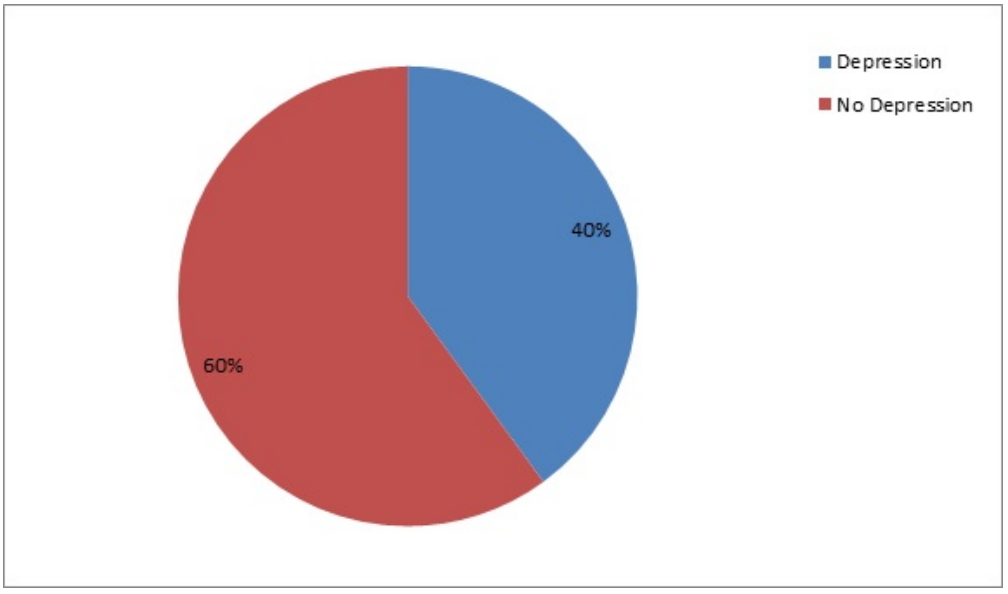

FIGURE 4.3: Prevalence of Depression in Overall Sampled Population. Depression was Determines Based on DSM IV Diagnostic Criteria

Depression is considered among four major disease which affect the world population and it is considered that about 350 million people of various ages and ethnicity are suffering with depression [73]. It is a common psychological state characterized with the presence of irregular sleep patterns, irregular food and appetite patterns, inability to concentrate, lack of decision making and even increase in temptation towards suicide. It is estimated that by 2030, depression would the most significant contributor towards disease burden [74].

In Pakistan, although most cases of depression even sever depression remain un reported, situation is worsened by consultations with spiritual healers and Peers. It is estimated that risk of development of depression in general population is up to 25% with chances to increase to 33% in people suffering with chronic disease and social stigmas. It is also calculated that the risk is 10-25% in females and 5-12% in males [75]. The 37.8% general prevalence of depression was reported in Karachi earlier in 2017 [76].

As it is discussed earlier that prevalence of depression is significantly high in individuals with chronic diseases and social stigma. Obesity unfortunately fulfils both. Metabolic disorders or hormonal changes which regulate increase in BMI and body weight sometime feel uncontrollable and person suffering with obesity also experiences social stigmas increasing the vulnerability for depression to quite a high level. Increase in day to day stresses and trigger in Hypothalamus Pituitary Adrenal Axis (HPA) results in increase in level of cortisol in blood. This has a profound impact on desire to eat and hence weight gain. This type of weight gain in response to stress is equally prevalent in both males and females [77].

In order to determine the prevalence of obesity induced depression particularly, depression onset was determined among obese and non-obese individuals. Both obesity and depression have a two-way relationship. It is very difficult to establish that what causes what, obesity could be the reason of depression and anxiety while depression can also lead to irregular food intake resulting in obesity.

Table 4.4: Association of Obesity with Depression in Sampled Population

		Depression	No Depression
Obese	Count	70	111
	%	38.67	61.32
Non Obese	Count	71	101
	%	41.27	58.72

It was found that out of 181, 70 obese individuals have shown symptoms of depression while 111 subjects have not shown any symptom [Table 4.4]. Total samples chi square value estimated through Pearson's chi square is 0.617 which is more than 0.05 that shows no significant association of obesity with depression in sampled population. A similar study conducted in 2016 in Karachi and employed school children. In order to determine psychological health state DSM IV criteria was used. It was found that a significant symptom of depressionis evident in children who lie in obese or over weight category [63].

The reasons of the contradiction could be the age, where kids and teenage adults are far more concerned about their appearances. It is well reported that incidence of depression is far more in obese girls of teenage than boys at same age [64]. Another study conducted in 2016 targeting university students between 18 -25 years and association between obesity and stress among them [65] concluded that girls suffer more with the stress and criticism than boys of same age.

Depression could be outcome of various stresses, appearance and body weight could be one of many stresses. The samples collected in this study belonged to lower income and mid income categories, where various other stresses could have a significant impact on the onset of depression. Lack of job opportunities, lack of health care facilities, law and order situation are among the major contributing factors in onset of depression. As no molecular analysis was done to ensure that depression is outcome of obesity, and no exclusion was made in analysis to ensure that depression is only outcome of obesity, the contradictions in results of this study from already reported researches is observed.

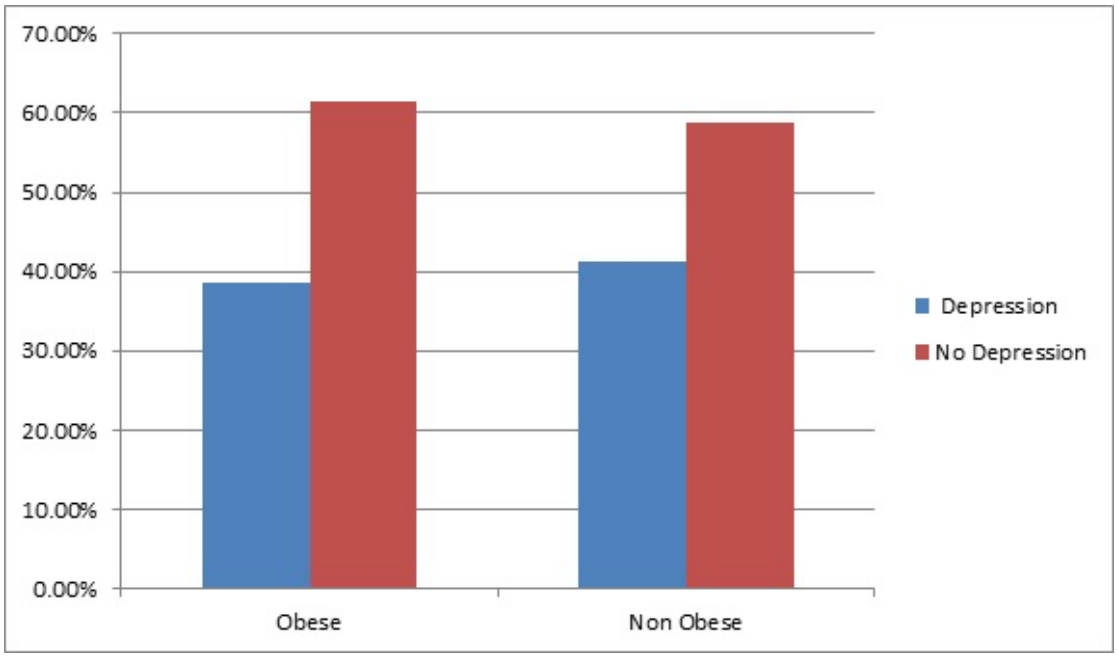

FIGURE 4.4: Prevalence of Depression Among Obese and Non obese Individuals Where x Axis Shows Disease State (Obese or Non obese) and y Axis Show Number of Individuals.

4.2.1 Genotyping of G196A Gene Variant Of BDNF

Most of the complex human diseases or traits are outcome of interactions between environment and genetic susceptibility. In order to identify genetic susceptibility variants, genome wide association studies play a significant role. Genome wide association study and HapMap project has highlighted various gene variants which are associated with obesity, diabetes type II, stroke and even cancer. Data related to genotype frequencies of these variants and genotype-phenotype variations among populations provide significant association and genetic susceptibility information related to that population [81].

An exponential increase in prevalence of complex diseases and their disease burden along with their impact on life expectancy has raised concerns to highlight probable risk factors associated with these complex disorders. Population genetics believes that with increase in population, the accumulation of rare genetic variants occur in population sets. Population specific gene variants are important in determining genetic susceptibility among that population. Obesity a global epidemic has also become a major threat to general health in Pakistan as well. Change in

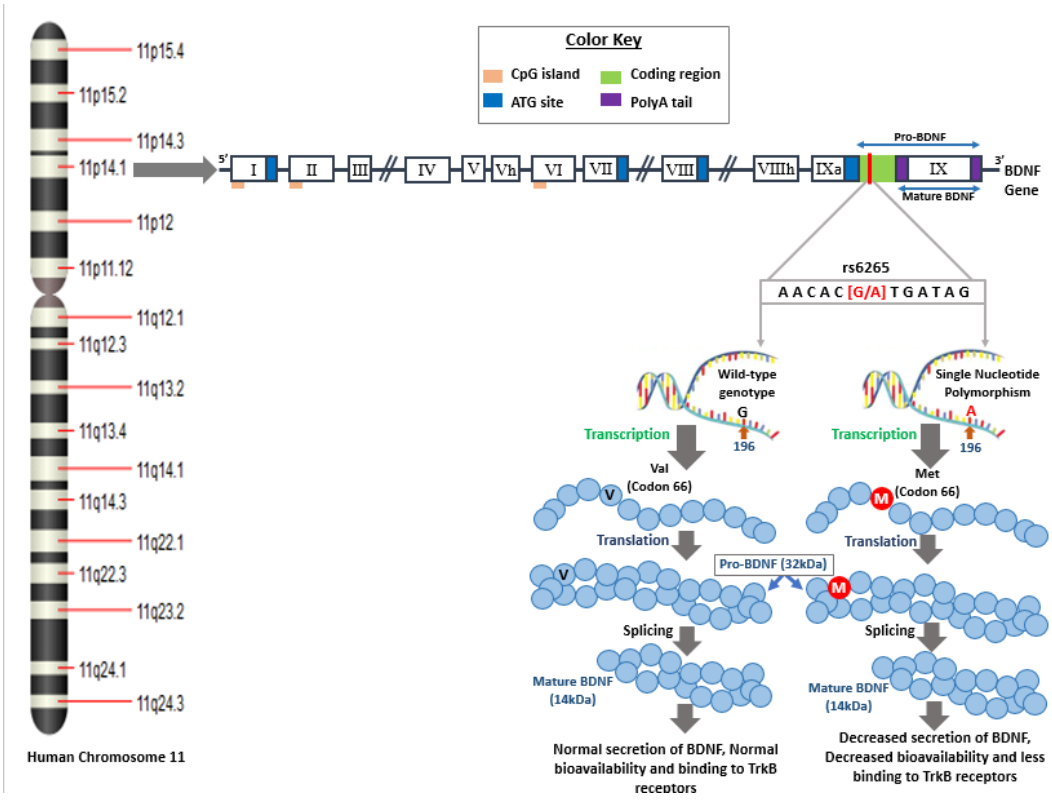

FIGURE 4.5: The Location of G196A on BDNF Gene and Resultant Changes in Expression of BDNF Protein.

lifestyles, food habits, exercise opportunities have obviously contributed in obesity prevalence but roles of genes cannot be undermined. Various studies have been conducted in Pakistan to determine the risk alleles in major contributing genes [82].

BDNF is one of the most important candidate gene to be studied in Pakistani population in relation to obesity and depression. Both of these diseases are characterized as complex phenotypes where interaction between environment and gene play a significant role. Secondly association or interaction between obesity and depression is well established where BDNF is reported to be involved in onset of both disorders. In this study, general prevalence of rs6265 gene variant of BDNF is studied among sampled population. This gene variant is amongst well reported gene variants and only few reports have been generated from Pakistani population regarding its prevalence.

The study design utilizes polymerase chain reaction followed by Restriction fragment length polymorphisms analysis (PCR RFLP assay) for polymorphism detection. PCR RFLP is one of the most commonly used technique for assessment of single nucleotide polymorphism. Restriction enzyme *Afl III* cuts at A variant leaving G variant undigested. The difference in G and A allele was evident from the band length on agarose gel (figure 4.5). Genotyping results were recorded and later analyzed statistically.

4.3 Allele Frequency in Overall Population

Allele frequency means that how common an allele is within population usually expressed in fractions or percentages. The significance of the frequency distribution of alleles is determined by Hardy Weinberg Equation that shows how constant the allele will remain from one generation to next generation. Deviations from Hardy Weinberg Equilibrium principle make allele as a significant genetic marker.

In case of rs6265, two alleles are present i.e. allele A and allele G. Presence of allele G ensures production of normal level of BDNF and is usually considered wildtype allele while allele A produces a variant resulting in decrease in secretion of BDNF. Allele A is usually less frequent but allelic distribution frequency of A allele varies among populations and it varies between 0-72%. The low frequency of A allele is supported by various other studies including one conducted in Mexican children and adolescents in 2018 [83].

In this study, allelic frequency was calculated and A allele was found more frequent as shown in Figure 4.6. Allele frequencies were calculated by dividing the number of times a particular allele was observed in sampled population by the total number of copies of alleles. Out of total 712 alleles 383 A alleles making 0.53 or 53%. G allele on the other hand was 329 out of 712 making 0.46 or 46% (Figure 4.6).

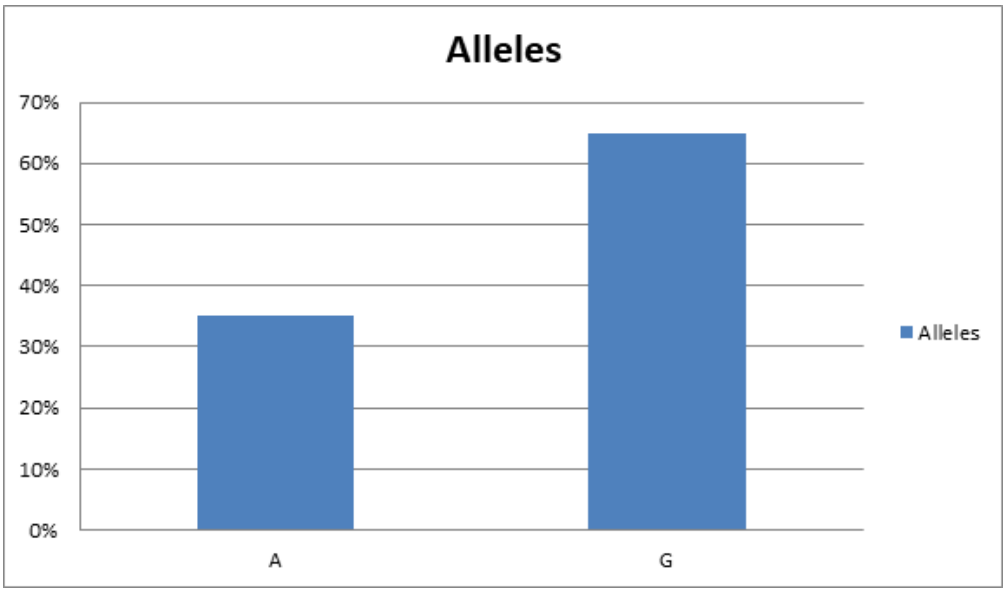

FIGURE 4.6: Allele Frequency in overall population where x axis show allele and y axis indicates frequency in percentage. The frequency was determined using PCR RFLP assay.

4.3.1 Association of G196Agene Variant of BDNF with Obesity

Obesity is a complex disorder where genetic susceptibility along with disbalance in energy intake and energy expenditure in addition to lack of exercises regulate weight gain. Among regulators of weight gain, BDNF possess an important place. BDNF is expressed in various tissues but its concentration in brain is quite high. BDNF plays critical role in development and function of brain especially it controls anorexigenic function i.e. control on appetite. Amongst various gene variants of BDNF rs6265 is studied most as it contributes towards cell specific secretion, localization and intracellular processing of BDNF. Its association and distribution among various complex diseases especially obesity is well studies [84].

G196A also referred as rs6265 or val66Met polymorphism is the most studied gene variant of BDNF in obesity. The genotype distribution was analyzed among obese and non-obese samples. Total 356 samples were analyzed and it was found that individuals who were non obese and possess AA homozygous allele constituted 12% of total population and 25% of non-obese population. Similarly, individuals with genotype GG homozygous and non-obese phenotype constituted 11% of total

Population and 23% of non-obese population. Heterozygous individuals i.e. with genotype AG and phenotype of non-obese constituted 24% of total Population and 50 of non-obese population.

In case of obese individuals 33% of obese individuals were homozygous AA i.e. 17% of total Population, 20% were homozygous GG i.e. 10% of total Population, 24% of total population were obese and possess heterozygous AG genotype and represent 46% of obese samples. Results are summarized in Table 4.5 and presented graphically in Figure 4.7 showing association G196A with Obesity.

TABLE 4.5: Genotypic Distribution of Rs6265 Among Obese and Non-Obese Individuals of Sampled Population.

BDNF				
		AA	GG	AG
Non Obese	Count	44	41	87
	%	25.58	23.83	50.58
Obese	Count	61	37	86
	%	33.15	20.10	47.63

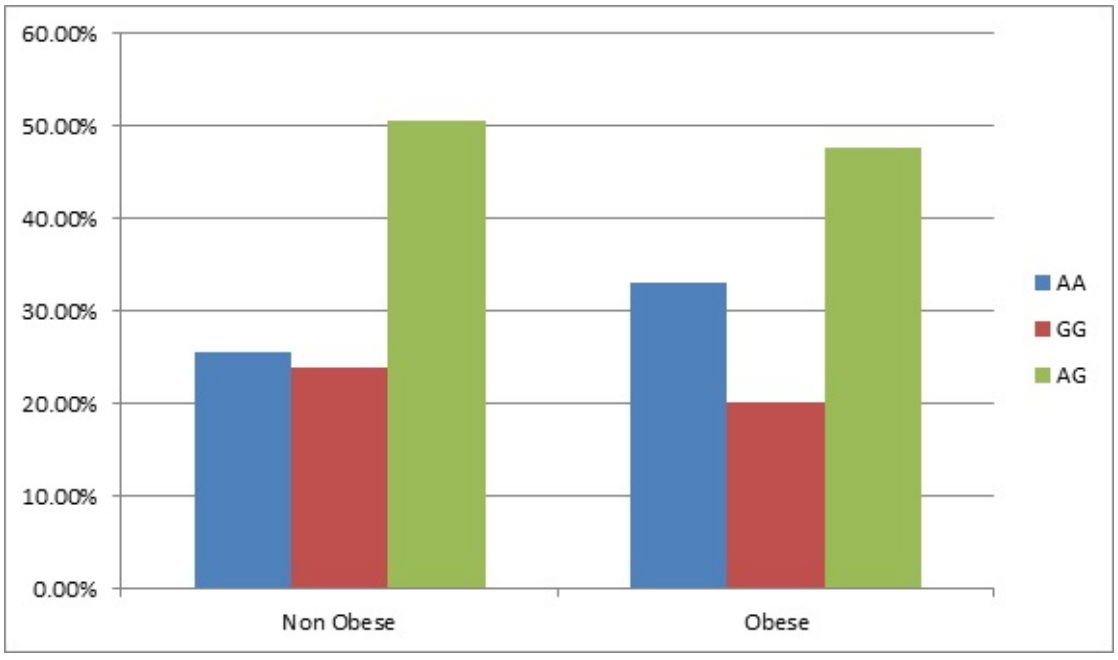

FIGURE 4.7: Association of G196A With Obesity Where X Axis Indicate Obese or Non-Obese Conditions While Y Axis Indicates Number of Individuals.

Chi square analysis was performed to determine P value which was found to be 0.278 which is quite higher than 0.05 indicating no significant association between genotype and obesity. Results are graphically indicated in Figure 4.5. most of the samples collected were found to be heterozygous at this locus. AA genotype was found to be slightly higher in obese individuals i.e. 33% in comparison to 25% in non-obese individuals. This variation in genotype is not enough to conclude that which genotype could be considered more prevalent in obese individuals.

4.3.2 Association of Obesity with Allele Frequency

As in genotyping a major number of sampled populations was heterozygous therefore allele frequency was considered to get a better picture and find association between A allele and obesity. Total 712 allele i.e. total 356 individuals who are diploid were analyzed. Out of total 383 A alleles 175 were found in obese individuals either in homozygous or heterozygous condition making 45%, and rest 55% i.e. 208 out of 712 were found in obese individuals. Similarly, 169 out of 329 G alleles were found in non-obese individuals i.e. 51% and 160 out 329 G alleles i.e 49% were found in obese individuals as summarized in Table 4.6. and graphically represented in Figure 4.8.

Table 4.6: Association of Gene Variant with Obesity

Allele				
		A	G	Total
Non Obese	Count	175	169	344
	%	50.8	49.1	
Obese	Count	208	160	368
	%	56.5	43.4	

It is usually expected that A allele would be more prevalent in obese individuals as this allele is related with decrease secretion of BDNF and lack in appetite control resulting in weight gain. In this study 55% of A alleles were present in obese

individuals. As discussed earlier the frequency of alleles varies between population, similarly the estimation of risk allele is also contradictory in various population. A allele is mostly reported as a risk allele [85]. However, in contradiction the frequency of G allele and even consideration of G allele as risk allele is also reported and no association between obesity with any of allele is also reported [86].

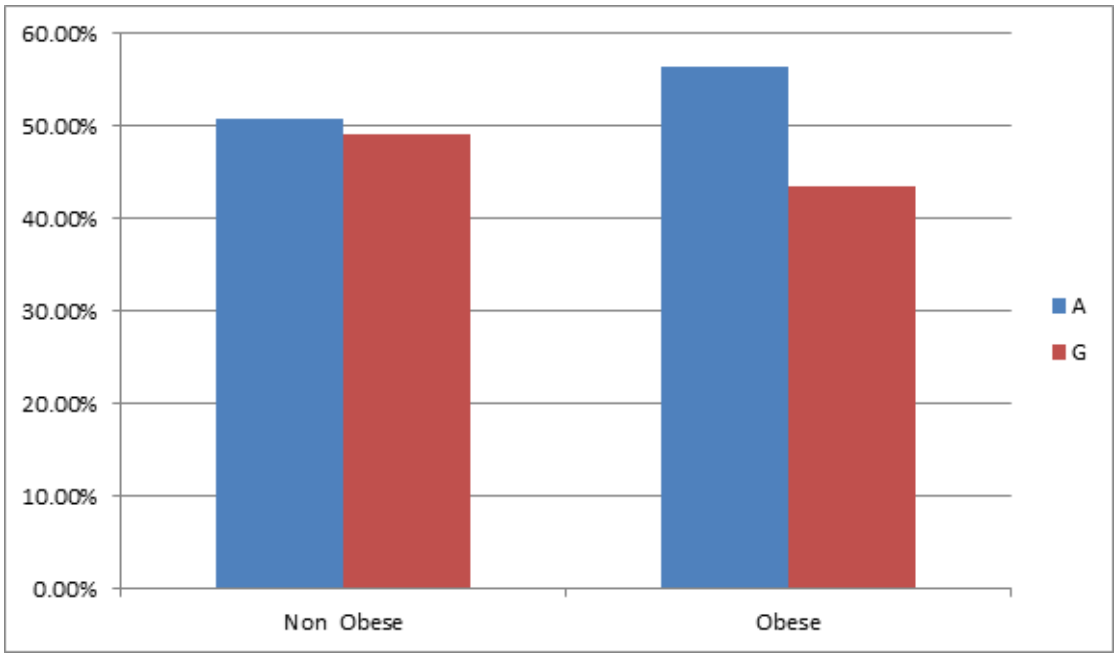

FIGURE 4.8: Association of Obesity and Gene Variant G196A where x Axis Shows Disease State (Obese or Non-obese) and y Axis Show Number of Individuals.

It is reported that people with gene variant A are more likely to be obese as compared to people with G variant [87], but difference is very small. It is not yet determined that how these two different alleles of rs6265 are contributing in obesity and there is no molecular explanation in determination of risk alleles. Reason for these contradictions could be that obesity is a complex phenotype executed by gene environment relationship, gene-gene interactions, allele distribution among various populations and analysis and processing of data with various statistical tools [88].

4.3.3 Association of G196A with Depression

No specific genes associated with depression are reported but it is quite evident that 40-50% susceptibility to have depression is due to genes [89]. Although the role of BDNF in onset of depression is consistent, yet BDNF is considered as an important candidate gene for depression. The concept is that acute and chronic stresses including inflammations, trigger cytokine productions which in turn decrease the BDNF level in blood. Decrease in BDNF, a major neurotrophin, results in atrophy of hippocampus (part of brain involved in regulation of emotions) [90].

Evidence is quite high that level of BDNF decrease as a result of environmental stresses [91], increase in BDNF level with infusions of antidepressants in rats [92] and in humans [93]. There are many reports which indicate that BDNF has no significant role in onset of depression [91]. On the other hand, reports are also there indicating that increase in blood levels of BDNF are associated with depression [94].

As various altered forms of BDNF results in variable phenotypes therefore, analysis of various gene variants of BDNF within population could be of significant information. In this regards G196A gene variant of BDNF is well studied with respect to depression. Similar to BDNF in general variation in association results are observed among different populations. Out of two alleles G allele produces Valine and results in normal secretion of BDNF, even this allele is reported as risk allele in Caucasian American population [93].

Allele A is correspond to methionine, and results in decreased secretion of BDNF and usually considered as risk allele for depression in various populations including Caucasians [94], elderly Chinese population [94] Japanese subjects [95] and many more. In contradictions to all these associations, no associations between depression and G196A has also been reported among various populations including Chinese [96], Korean [97] and European populations [98].

In this study, a cross tabulation was performed between depression and polymorphism to determine the presence of any association between depression and

G196A. All the sampled individuals were ensured to be healthy and were not suffering with any disability or infection which required long term medical treatment. Absence of any neurological diseases, psychiatric disorder was confirmed as required by DSM-IV criteria. Pregnancy and hormonal supplements intake were also excluded.

Out of 354 total studied samples 141 individuals fulfilled DSM IV criteria and were classified to have depression phenotype, while 213 were control or individuals with no depression symptoms. 40% individuals of all sampled individuals were with depression symptoms while 60% lies in control group. Homozygous AA genotype was found in 59 individuals with no depression symptoms making 16% of whole sampled population and 27% amongst individuals with no depression symptom. Homozygous GG genotype was found in 41 individuals with no depression symptoms making 11% of whole sampled population and 19% amongst individuals with no depression symptom. 113 individuals i.. e 31% of total sampled population and 53% of individuals with no depression symptoms were heterozygous at this locus and posse's AG genotype.

In case of individuals with depression 45 individuals were homozygous AA i.e. 12.7% of total sampled population and 31% of the population with depression. Homozygous GG was found in 36 individuals making 10% of total population and 25.5% of samples with depression symptoms. Among cases with depression 42% i.e. 60 individuals making 16% of total population were heterozygous AG, as summarized in table 4.7 and graphically represented in Figure 4.9.

Table 4.7: Number of Depressive and Non-depressive Individual Associated with G196A

BDNF				
		AA	GG	AG
No Depression	Count	59	41	113
	%	27.69	19.24	53.05
Depression	Count	45	36	60
	%	31.91	25.53	42.55

Total samples chi square value estimated through Pearson's chi square is 0.138 which is more than 0.05 that shows no significant association of G196A with depression in sampled population. It was observed that in both cases of depression and in controls i.e. individuals with no depression symptoms, heterozygous were more frequent comprising 42% in depression phenotype and 53% in non-depression phenotype. Although from literature review it was expected that GG would be more prevalent in samples with depression phenotype [62] but this study indicated that there is no significant difference among GG and AG frequency statistically but difference in their prevalence was observed. GG phenotype constituted 25.5% in depression while only 19% in non-depression phenotype. Similarly, AA genotype was also prevalent among depression phenotype i.e. 31% in comparison to control group where AA comprise 27%.

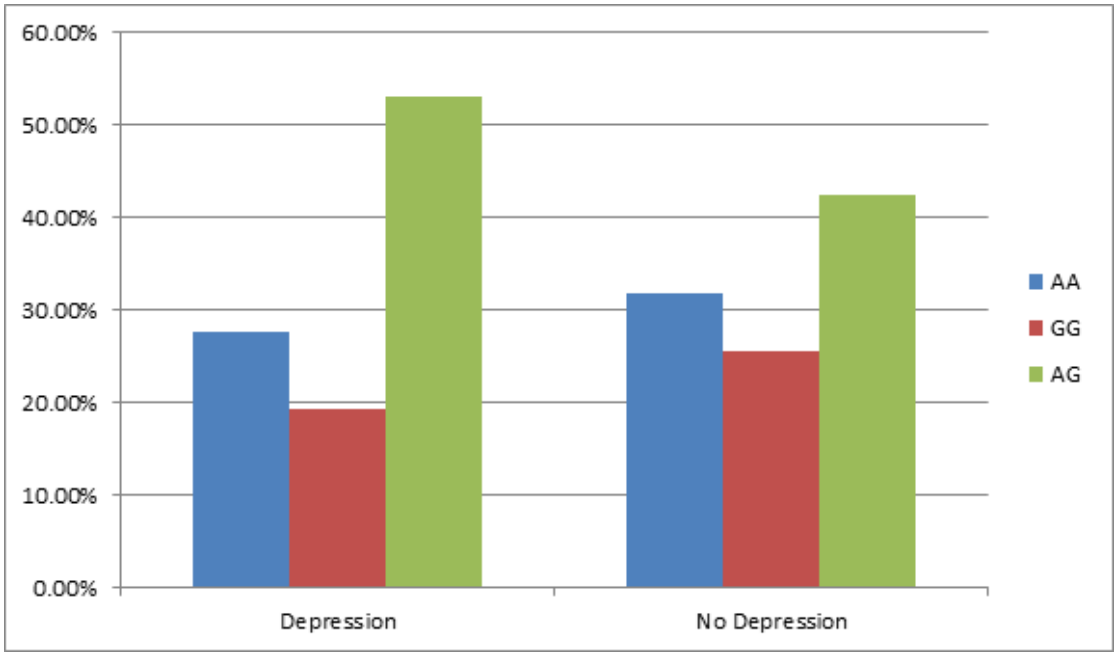

FIGURE 4.9: Association of G196A with Depression Where X Axis Shows Disease State (Depression or No Depression) and Y Axis Shows Number of Individuals.

4.3.4 Association of G196A Gene Variant of BDNF with Depression

As no significant conclusion could have been drawn on genotype and phenotype correlation, allele frequencies of each allele and its association with depression was calculated. Total alleles which were studied in this case were 708 out of which 381 were A and 327 were G comprising 53% A and 47% G. In overall population A allele was found more prevalent. Distribution of these allele within phenotypes was analyzed and it was found that 231 alleles were found in individuals with no phenotypic symptoms of depression while 150 A alleles were found in individuals with Depression phenotype.

TABLE 4.8: Association of Depression with Gene

Allele				
		A	G	Total
Non Depression	Count	231	195	426
	%	54.22	45.77	100
Depression	Count	150	132	282
	%	53.1	46.8	100

Represented in Table 4.8 that the allelic frequencies of A among individuals with depression is 0.40 i.e. 40%, while in individuals without depression symptoms is 060 i.e. 60%. The frequency of G allele was 0.59 i.e. 59% in individuals without depression and 0.41 i.e. 40% in depression phenotype. The results indicate that A allele is more frequent in depression phenotype and G allele act as protective allele as shown in figure 4.10. The results were consistent with already done studies [99]. The significant value is 0.00 which is less than 0.05 that shows a significant association of depression with variant A.

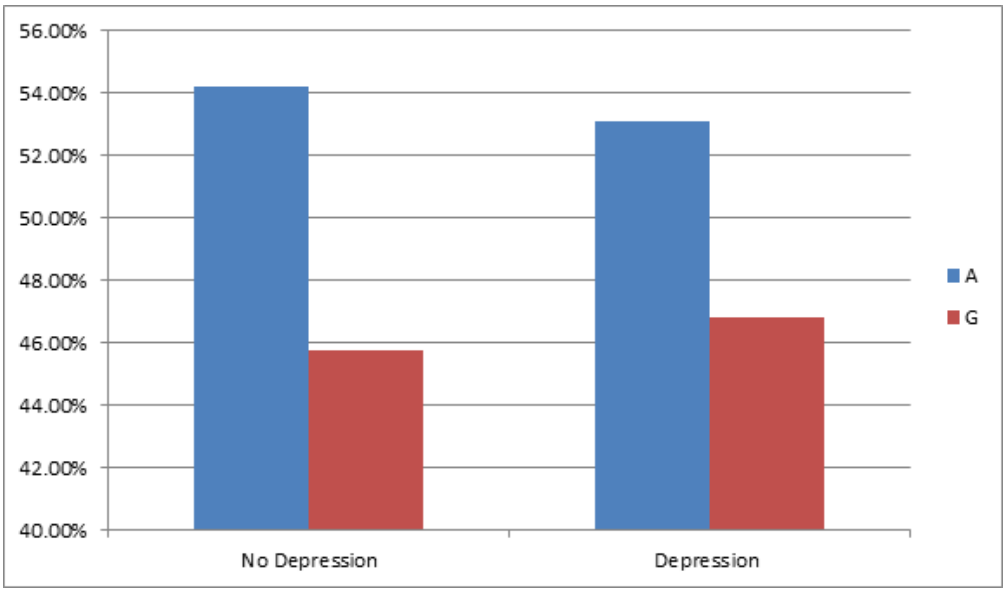

FIGURE 4.10: Association of G196A with Depression Where X Axis Shows Disease State (Depression or No Depression) and Y Axis Shows Number of Individuals.

4.3.5 Association of G196A with Obesity Induced Depression

BDNF plays a significant role in nervous system development and proper functioning. It exclusively gives brain anorexigenic capacity i.e. regulation of appetite [9]. It is also evident that nuclear modification in BDNF can result in dysregulation of appetite resulting in weight changes and dietary issues. Various gene variants of BDNF are reported to influence metabolism and appetite such as G196A. This variant can have a significant influence on intracellular property, movement, distribution and secretion of BDNF. It has also been reported to be associated in mental health issues including seizures, OCD (Obsessive Compulsive Disorder) and bipolar disorder [11].

In this study it was observed that out of 357 individuals only 19% i.e. 70 individuals showed signs of both obesity and depression. Out of these 70 individuals 24 were genotypically homozygous with allele A. 14 were homozygous G and 32 individuals were heterozygous AG. Results are summarized in Table 4.9 and graphically represented in Figure 4.11.

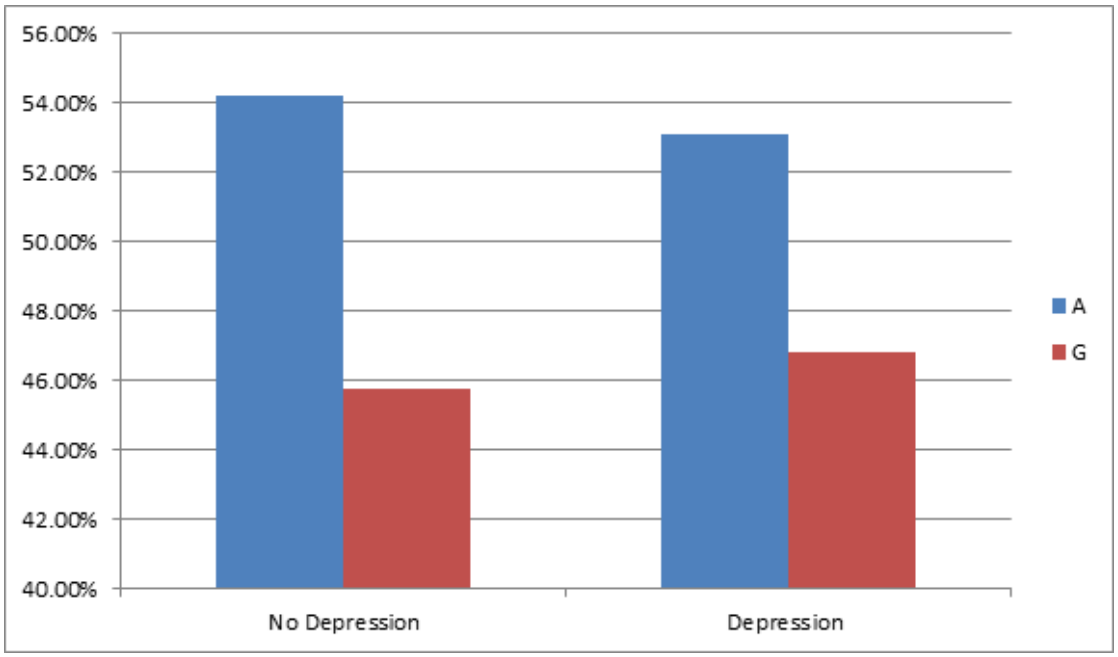

FIGURE 4.11: Association of Obesity Induced Depression with Allels

TABLE 4.9: Association of G196A with Obesity Associated Depression

Allele					
			AA	GG	AG
Obesity+ Depression	Yes	Count	24	14	32
		%	34.28	20	45.71
	No	Count	36	22	53
		%	32.43	19.81	47.74

Chapter 5

Conclusions and Recommendations

Obesity is a multifactorial phenotype, occur due to a complex interplay between genetics and environment. In Genetics, obesity again is complex phenotype which could be monogenic, multigenic, non-syndromic or syndromic. Among many genes causing susceptibility BDNF is one responsible for monogenic obesity, its direct role is unclear but it is assumed that it causes abruption in regulation of appetite. In support of association between obesity and BDNF a particular association between gene variant G196A has also determined with obesity and it is found that the association remains in consistent among various populations. Although Met variant is associated with abnormal packaging of BDNF, but it is still un clear that how the gene variant interacts with environmental stimuli to cause obesity.

Depression is among the complex psychiatric diseases which are controlled by both genetic and environmental factors. There is a long list of environmental factors which can contribute to onset of depression, similarly the list of candidate genes is also long (As summarized in Literature review section). Among this long list BDNF is found strongly associated with depression phenotype. A genetic variation in BDNF, i.e. 196G/A which causes Valine to Methionine substitution at codon 66 is present in pro BDNF. This variation results in reduced activity of BDNF

and hinders its roles in neuroplasticity. This gene variant, a polymorphism is well documented to be associated with onset of depression and its Met allele is found more frequent in individuals suffering with depression.

This investigation was performed on 357 samples. Out of 400, 357 samples were considered for further analysis as samples with insufficient or in complete information were discarded. Sampling was performed in Kahuta region of Rawalpindi and special sampling camps were organized where anthropometric and physiochemical measurements were collected. Biochemical tests were performed to confirm disease. PCR RFLP assay was performed for genotyping The total included number of the individuals in the study is 357. Out of which 176 (49.2%) comprises males and 181 (50.7%) comprises females. The total limit of the subjects was under 80 years. The mean age of the subjects was 41.63 years. The total BMI mean value of the sub jects was 26.3. Thus, keeping in mind the results of calculated BMI value, it is said that the overall scenario of Kahuta region presents overweightness. Therefore, the people of Kahuta are not Obese. It is also observed that systolic diastolic blood pressure is under control. But the Blood Random Sugar level was observed as above the normal level. This can be due to the high-level consumption of soft drinks, juices, tea and sweets and there can be low level of physical activities, such as sport, habit of not walking etc. It was observed that the respondents were divided in 3 age groups, teen age, young adults and adults. This observation was not based on workload. If observing under the classification criteria that WHO BMI prescribed the percentage of underweight subjects were 6%. The normal individuals weight rate was 40%. The percentage of overweight individuals was 28%. The obese class 1 was created and 15% individuals were comprised in this class because their BMI level was above average. The other category was created and named as obese class II, their percentage was 8%. The third category was named as obese class iii and the percentage of the category was 3%.

It is found that G196A (rs6265) gene variant of BDNF plays role in causing obesity and depression. BDNF is an important constituent of brain and has a major role in causing obesity. It is found as major participant in regulation of food intake

by raising the anorexigenic effects. BDNF G196A (rs6265) is involved in obesity development. However, gene variant G and A are associated with Obesity and depression, other relevant factors are also involved in their cause.

It should be noted that the indication of obese subjects was based on 40. Those who crossed 30, they were categorized as obese. In this regard, in Kahuta the 26% of the individuals were noted as obese. In this way, the risk factor in obese is greater and it is 0.897. Likewise, male ratio is 0.943 and female ratio is 95% odds ratio of inflammation mediated obesity is 1.058 and risk estimated is 0.132 time greater with 95% confidence interval, the correlation of obesity and inflammation is 0.05 P-value level. Reluctantly, there was no any important correlation or bondage found among obesity, depression and inflammation. Out of 357 subjects the depression spread in Kahuta region is 39.4%. in this regard, the most common Depression observer is Hypertension in the region. Likewise, 8% is the occurrence of systolic and distolic hypertension. Odd ratio of males and female for depression is 0.332 and risk of depression in males is 1.803 time greater and in female 0.599 time greater . The strong association of depression is with BMI . The total number of obese subjects was 181 and out of the total number the depressed individuals were 70 and the non depressive individuals were numbered 111. In this way, the research conducted suggests that important correlation between obesity and depression was at 0.05 p-value level. Likewise, depression and Obesity are also related with each other at the 0.05 p-value . Phi and Crammer's test suggesting week association between obesity and depression. In this regard, SAH Bokhari (2015) also reported significant association of obesity with depression in South-Asia population from 2001 - 2012. The experiments conducted in Kahuta region with strong correlation with spss test indicate that there is week bondage between obesity and anxiety but there is negative relation with depression. Alvaro Commacho (2013) also reported involvement of various kinds of inflammatory mediator molecules in causing symptoms of anxiety and depression It is mandatory to spread the message of health consciousness from Inflammatory disorders, for example, a diet that affects people and limiting physical practices. There can be various tools to achieve the afore-mentioned Assessment of Intrinsic Smal l Signal

Parameters 51 target such as Social as well as press media, the local headmen, people related to religion, people linked to health and educational communities. These are the basic points to make people able to spread the health issues related to inflammatory disorders. In this way, research scholars can also perform their duty to highlight the main causes of the above-mentioned disorders.

There is a need to aware people about health risks by taking unhealthy food. We also need to guide them about importance of physical activities to maintain healthy life styles. Healthy lifestyle ultimately leads to relax mind which can help to maintain mental health along with physical health. Education and health departments can play a major role in spreading this awareness among people of different sectors.

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Appendix A

PIDNo:

QUESTIONNAIRE FOR RESEARCH PROJECT

Project Title: Association of G196A (rs6265) Gene Variant of BDNF With Obesity Associated Depression

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: _____

BIODATA: (This information provided by Patient will be confidential)

First Name: _____ Mid Name: _____

Last Name: _____ DOB _____ Gender:_____

Age _____ Contact No: (Office) _____

Home: _____ Email:_____

Permanent Address:

Address: _____

1. <u>ANTHROPOMETRIC MEASUREMENT</u>

Weight (kg)	
Height (m)	
BMI (kg/m2)	
Blood Sugar mmol/L	
Total cholesterol (TC)	
Triglycerides (TG) (mmol/l)	
HDL-C (mmol/l)	
LDL-C (mmol/l)	
CRP	
Myoglobin	
CK-MB	

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP C Reactive Protein; CK-MB, Creatine Kinase-MB.

2. OBESITY, CVD AND DIABETES COMPLAINTS

High blood pressure	Yes	No	Diabetes	Yes	No
CVD	Yes	No	Eating disorder	Yes	No

3. FAMILY HISTORY

Obese Persons in family

Father	Sister	Uncle	Mother's Sister
Mother	Brother	Aunty	Mother's Brother

4. PHYSICAL ACTIVITY

Morning walk	Evening walk	Work at home	Outing

5. DIETARY HISTORY

Breakfast	Lunch	Dinner

6. SOCIAL AND PERSONAL HISTORY

Do you have children? No / Yes - How many?	
Education:	
Marital status:	Single / Married /Separated / Divorced
Job:	Part time /Full time

7. MEDICAL/CLINICAL HISTORY

Medication to control obesity	Yes / No
Diet plan to control obesity	Yes / No
Any surgery if yes when or for what	Yes / No
Medicins using for any other diesease	Yes / No
Smoking or counsuption of anyother tobaco product	Yes / No

8. SAMPLES

Blood Sample: _____

Thank you for completing the questionnaire please return it to _____

Department of Health and Life Scinece, 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 Signature Date

Appendix B

FIGURE 1: Madical Camp for Sample Collection

FIGURE 2: Madical Camp for Sample Collection