



ASSESSMENT STUDY OF LEPTIN LEVELS AND LEPTIN RECEPTOR GENE IN THE PATIENTS OF CARDIAC DISEASE IN TIKRIT

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Abstract

Background : Cardiovascular diseases refer to occur a disorders in cardiac and blood vessel. There are three main types of cardiac disease depending on the degree of effect and the member affecting it. The heart can suffer from several diseases such as coronary syndromes, angina, arrhythmia, Cardiomyopathy, heart failure, Inflammatory heart disease and ischemic heart disease. The brain may suffer from brain diseases such as hemorrhagic stroke, ischemic stroke, deep vein thrombosis, hypertensive heart disease and pulmonary embolism (WOH, 2016). The recent data also showed that the average of global death is due increase in percentage of CVDs, with percentage of 41% from 1990 to 2013 (16) (15), It has become the leading cause of death of 175 million people annually, and other factors may increase the risk of heart disease such as smoking, physical inactivity, Drinking alcohol and eat unhealthy diets (WHO, 2016). Heart disease and stroke can be prevented by early detection for persons who their age range between 40 and 79 who suffer from High blood pressure or Diabetes mellitus and their timely treatment (WOH, 2002). Obesity is a major cause of heart disease as well as oxidative stress and bacterial infections (17). The leptin is a peptide hormone with molecular weight of (16 Kda) consisting of 176 amino acid and expressed in many tissues such as lymphatic tissue, placenta and ovaries. There is increasing interest in the potential role of leptin in the cardiac system and blood vessel. In the current study, leptin levels were compared in patients who suffer atherosclerosis and patients who suffer Angina. **Aim of Study**: This study aims to compare the effect of leptin, leptin receptor gene and lipids levels in infected persons with angina, atherosclerosis, and comparing them with healthy persons. **Materials and methods**: The study was conducted in Tikrit Teaching Hospital (intensive care unit (ICU) where the levels of leptin and lipids were determined for 60 patients. The samples were divided into three groups (patients Infected with atherosclerosis, patients Infected with angina, control group or healthy group) A part of the blood samples were also taken and stored in a blood preservative solution to isolate the DNA from it. **Results** : The results showed significant differences at significant levels $P < 0.01$ and $P < 0.05$ in leptin levels, leptin gene and lipids levels in infected persons with angina, atherosclerosis, and comparing them with control group. **Conclusions**: The results showed a relationship between increase leptin concentrations with increase age and increase leptin concentrations with gander. This shows us how important leptin and its role in metabolizing the body.

Introduction

Leptin is produced by white adipose tissue. It regulates all levels of energy, the rate of energy consumption and many hormones. Leptin provides the functional link between the immune system and energy balance, it is the first discovered lipids Kinetics, it was discovered in 1994 by Zhang *et al.*, Matsui *et al.*, (2012), it is a peptide hormone with a molecular weight of (16 Kda) consisting of 176 amino acid and existing in many tissues, has the ability to affect specific genetic factors caused by obesity or diabetes type II or insulin resistance.

Leptin is derived from the Greek word (LIPTOS), which means thin, a term that refers to the main functional role for hormone. Leptin has two main functions, increasing food intake and increasing the rate of energy loss by increasing acid oxidation (Hall *et al.*, 2012; Hall *et al.*, 2011; Hou *et al.*, 2010). Leptin is also called adipose tissue hormone Sloan *et al.*, (2011) and the effect of leptin is shown by receptors in the hypothalamus (Hou *et al.*, 2010). Gene leptin is a gene that encodes a specific protein secreted by lipid cells into the blood vessels and plays an important role in the secretion of energy and the internal balance of the body. Leptin is also linked to the

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brain's leptin receptor, which acts as a blood vessels trajectory of the gene that inhibits nutrition and stimulates energy exchange. This gene is involved in immune regulation, inflammatory response, Blood formation, internal balance, reproduction, bone-building and wound healing (Farooqi and Rahilly 2014), Mutations in this gene and its regulatory regions cause severe obesity and Morbid obesity with Hypogonadism in patients. A mutation in this gene was associated with diabetes (type II) (Witham *et al.*, 2012). Leptin receptors are membrane proteins that belong to the Cytokines receptor family and have several forms distributed in different tissues. Leptin and its receptor are known to play an important role in glucose metabolism. For example, leptin activates its effects on insulin and reduces diabetes by improving the sensitivity of peripheral insulin and absorb glucose. For the leptin receptor six different forms (from ObRa to ObRf) (Nyman *et al.*, 2013). All of these forms have a range extracellular, as for in the inside of the cells, they differ for each form. ObRb has a long arm and participate in the transmission of signals while ObRa has the ability to transmit some signals either short form function is linked to the link and transfer of leptin in the plasma and either through the blood-brain barrier or through renal leptin clearance. In addition, there are other pivotal pathways that have a significant variation in leptin effects on pancreatic b-cell (Mahmod *et al.*, 2013).

Subjects and Methods

This study was conducted in Tikrit teaching Hospital. The 47 patients were diagnosed with heart disease (Atherosclerosis, Angina) and 13 control group (healthy persons). The samples were divided into three groups:

- 1- The control group, which included 13 persons (5 males and 8 females).
- 2- The group of patients infected with Arteriosclerosis, which included 34 patients (16 males and 18 females).
- 3- The group of patients infected with angina, which included 13 patients (4 males and 9 females).

Leptin concentrations were identified using ELISA technology and using kit tests supplied by SunLong Biotech Co with serial number: SL1052Hu.

The molecular study

The molecular study was conducted through the following steps:

- 1- Isolate the DNA from the blood, its purity and concentration were determined using the Nano Drop.
- 2- Use PCR to determine the polymorphism of leptin gene by using the Restriction Enzymes.

DNA extraction:

The method of Bartlett and White, (2003) was used to extract the DNA from blood samples stored in the ACDS solution. Genotyping was performed according to (Gotoda *et al.*, 1997) using the following primer sequences: rs1137101: forward, -50AAA CTC AAC GAC ACT CTC CTT30-, reverse, -50TGA ACT GAC ATT AGA GGT GAC30-, The conditions for PCR were 94 °C for 3 min, and then 94°C for 30 s, 54°C for 30 s and 72°C for 30 s for 40 cycles, and extra extension at 72°C for 10 min. The PCR results were 101bp for rs1137101. Polymorphism was determined by the digestion of PCR product for 17 h using MspI enzymes (Biolabs-New England Inc.) for rs1137101 (Q223R), Gel electrophoresis with 3% agarose was performed to analyze the digestion. The digested product for rs1137101, the Q allele was 101 bp and the R allele was 70 and 30bp.

Statistical analysis

Data was analyzed using statistical product selective solution (SPSS) program, which analyzed by paired t-test. $P \leq 0.05$ was taken as statistically significant. Correlation coefficient was calculated between serum leptin and heart disease.

RESULTS AND DISCUSSION

Fig. 1 shows the concentrations of leptin in patients infected with heart disease compared with the control group, where significant differences were observed at a significant level of $P < 0.05$. Fig. 2 shows the effect of leptin levels on male and female patients, where the results showed that there were significant differences at a significant level of $P < 0.05$. The results also show the rise of leptin concentrations in women infected with angina compared to males infected with angina at a significant level $p < 0.05$, While the results showed a significant increase in concentrations of leptin in males infected with atherosclerosis compared to women infected

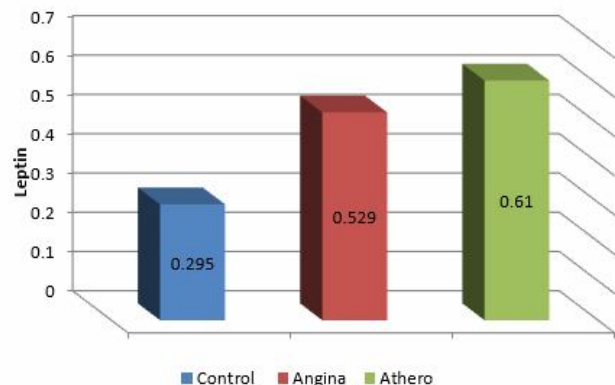


Fig. 1: The different in leptin levels

with atherosclerosis. As for the age groups have showed significant differences at a significant level of $p < 0.05$ in

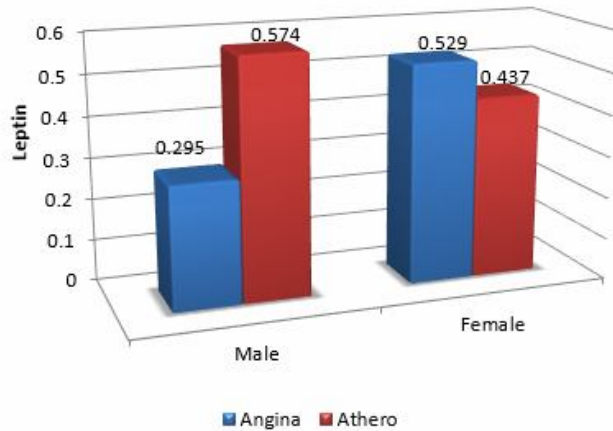


Fig. 2: The different LP between M&F.

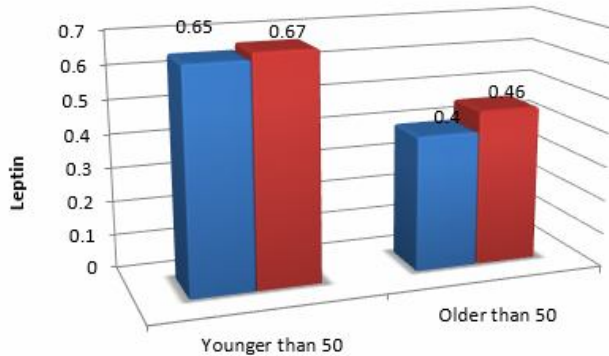


Fig. 3: The different LP in aging

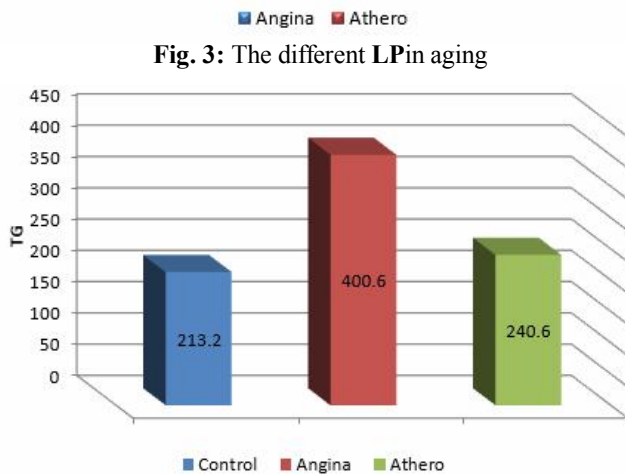


Fig. 4: The different in TG levels

the 50 age group and over compared to other age groups. Genetic results in tables (1, 2, 3) showed significant differences between the Allele frequency and the three genotypes (AA, AG, gg).

Leptin is considered a hormone produced by the Adipose tissue and plays an important role in the regulation of body weight (Nyman *et al.*, 2013). However, many studies have indicated that leptin has an important

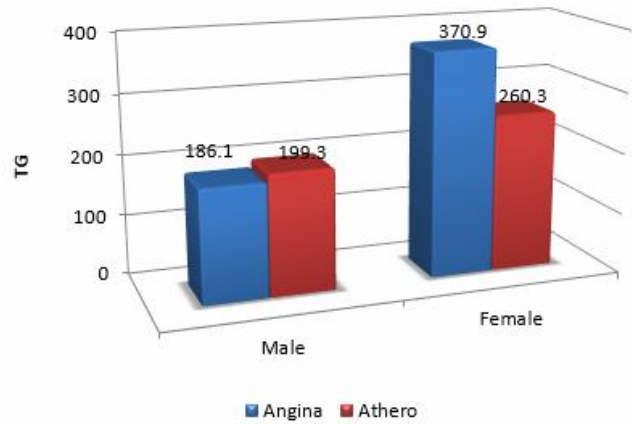


Fig. 5: Show the different TG between M&F

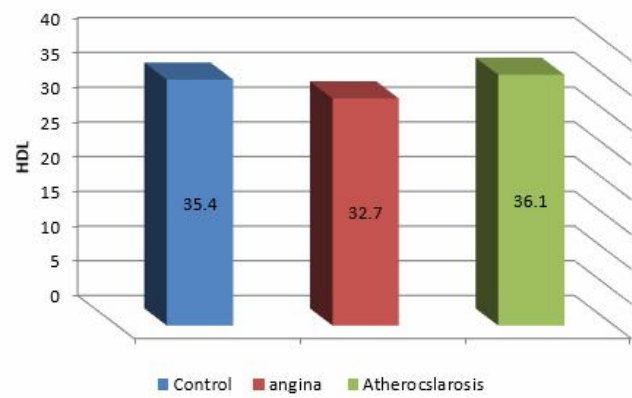


Fig. 6: The different in HDL levels

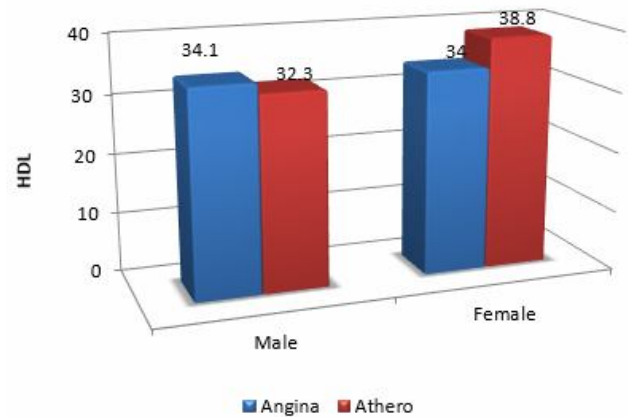


Fig. 7: The different HDL in M&F

role in the cardiovascular system through regulating metabolism and heart functions (Witham *et al.*, 2012). It explained the role of leptin in the regulation of heart function, and the rise of leptin concentrations in persons infected with heart disease have also been an important indicator in the identification of some heart disorders. Several studies have been conducted on leptin, but some of them were used to determine the effect of leptin on the body. In the current study, comparisons were conducted in the leptin concentrations between male and

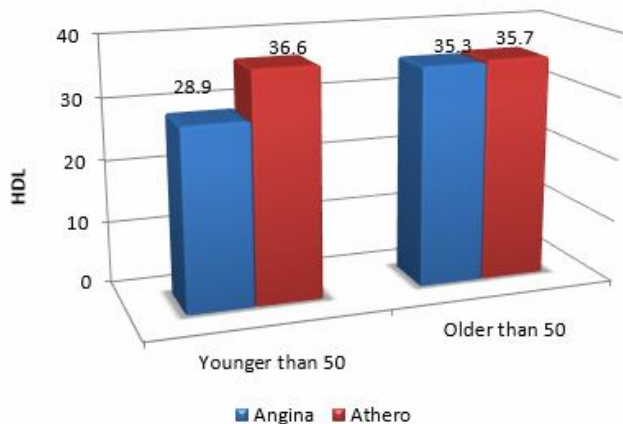


Fig. 8: The different HDL in aging

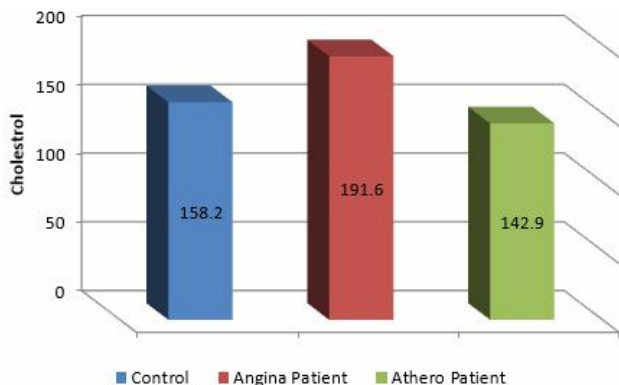


Fig. 9: The different CH

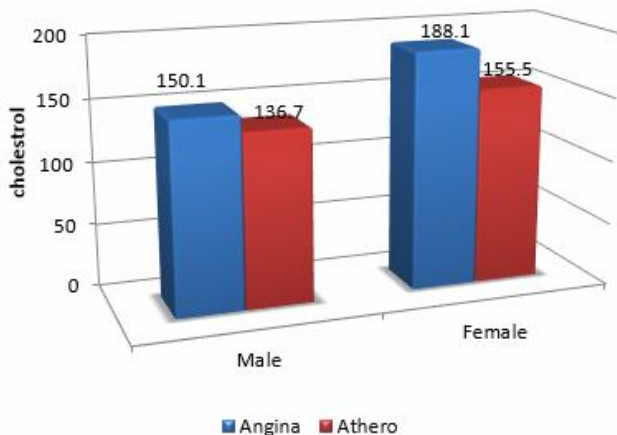


Fig. 10: The different CH in M&F

female who infected with heart disease (angina, atherosclerosis), and noting which is more influential on males and females. The results showed higher concentrations of leptin in women compared to males. This is due to increase the lipid mass in women compared muscle mass while males have a greater muscle mass than lipid mass and that is why gander is the most important factor affecting the concentrations of leptin in plasma. In addition, high concentrations of fat were found in women compared to males. This is due to the effects

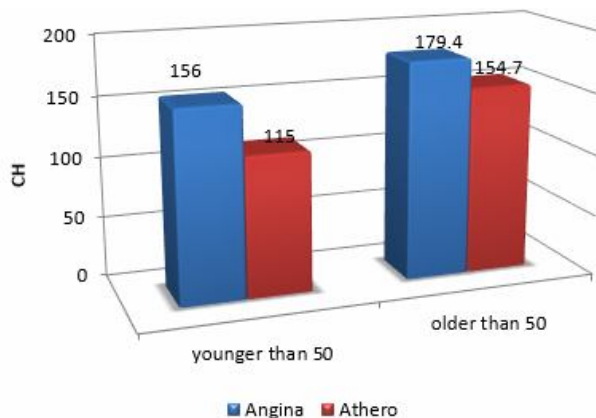


Fig. 11: The different in CH in aging

Table 1: The different between AA & Ag

Feature	gg (M ± SD)	Ag (M ± SD)
LEPTIN	0.60±0.23	0.45±0.23
Cholesterol	192±46	171±61
HDL	37±11	31±9
TRI	375±238	324±208

Table 2: The different between AA & gg

Feature	gg (M ± SD)	Ag (M ± SD)
LEPTIN	0.60±0.23	0.26±0.13
Cholesterol	192±46	158±36
HDL	37±11	33±7
TRI	375±238	210±83

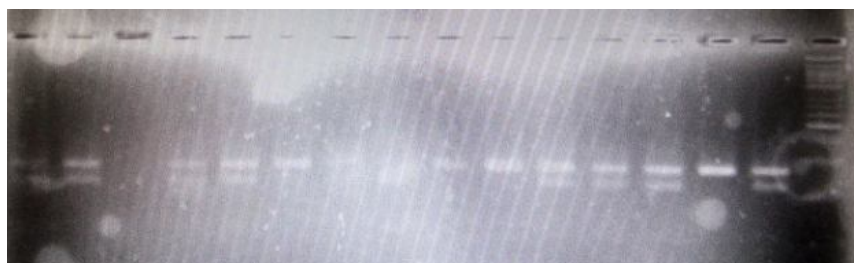
Table 3-1: The different between Ag & gg

Feature	gg (M ± SD)	Ag (M ± SD)
LEPTIN	0.60±0.23	0.45±0.55
Cholesterol	192±46	171±61
HDL	37±11	31±9
TRI	375±238	324±208

of phylogenetic sex hormones, which leads to an increase in body fat (Mahmod *et al.*, 2013), especially cholesterol and triglycerides. These factors are associated with increasing leptin concentrations in women compared with males. The results showed increase in the leptin concentration in males infected with atherosclerotic compared to women infected with atherosclerosis. While the results showed increase in the leptin concentrations in women infected with angina compared to males infected with angina. This variability in leptin concentrations in males, Determination of low levels of leptin in males may be due to a condition called leptin resistance in the body that results in a decrease in leptin concentrations. Since leptin passes through the Blood-brain barrier to reach the hypothalamus, when a defect occurs, the leptin resistance is increased. Most people with obesity suffer from high levels of leptin in the blood



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(Yusuf *et al.*, 2014). Genetic results indicate that there is a mutation in the leptin gene that causes heterogeneity in leptin concentrations. This results agree with Roth *et al.*, (2015); Yusuf *et al.*, (2014) indicated that there is a defect in the structure of leptin and its secretion due to obtaining a mutation in the genetic factor causing obesity. Genetic results indicated that the leptin gene mutation caused a change in the molecular structure of the leptin, thus inhibiting the gene expression process, it cannot express itself in the correct manner and the results of the current study agree with the results of (Sloan *et al.*,) noted that the gene expression process was discouraged when a change occurred in the form of the leptin molecule (1-9). The results showed there were statistically significant differences in the concentration of cholesterol between the persons group infected with angina and the persons group infected with atherosclerosis compared to the control group and the rise cholesterol in women infected with atherosclerosis and women who suffer from angina compared with males. The increase in cholesterol concentration may be due to increased cholesterol absorption by the intestine due to cholesterol activity. This is due to the pattern of nutrition, which is a cause of high blood lipids, and high cholesterol in women may be the result of insulin resistance, which increases the activity of lipase enzyme fat cells Thus increasing the amount of cholesterol. In addition, persons infected with heart disease are more likely to accumulate fat in the blood vessels causing them atherosclerosis as well as persons suffering from angina are more likely to have high cholesterol. Labbé SM, (2014) noted that persons infected with heart disease have high levels of cholesterol. The

causes of high cholesterol in the body may be many reasons or a condition associated with Coronary artery disease such as diabetes, where studies have confirmed a relationship between persons infected with diabetes (type II) and atherosclerosis, which increases the development of atherosclerosis, or as a result of liver diseases, leading to the inability to take advantage of cholesterol to convert it to HDL or LDL (Nyman *et al.*, 2013). As for the concentration of triglycerides, the results indicated that there were statistically significant differences at the $P < 0.05$ level between the three groups, where the concentration of triglycerides increased in patients. This increase may be due to diabetes.

Previous studies have confirmed that triglyceride concentrations are associated with increased Diabetes (Type II). In addition, diabetic patients and heart patients have higher levels of triglycerides than healthy persons. Other studies have indicated a positive relationship between high levels of triglyceride and insulin resistance, and increased triglycerides in the body lead to low HDL in the blood. The results showed a significant difference in the concentration of triglycerides between females and males. High concentrations of triglycerides due to the defect in thymus may cause more excess fat in the blood to create alternatives to energy sources, and high concentrations in women are due to the effect of sex hormones, especially estrogen, where low levels of levels concentration of estrogen increase the concentration of triglyceride and vice versa when increase the levels concentration of estrogen lower triglycerides by increasing the rate of metabolism. The genetic results have been used in the PCR-RFLP technique to detect the genetic diversity of leptin, which is characterized by its ability to detect any heterogeneity of the DNA bar, which is studied, even if the variability at the level of a single nitrogenous base, This is done by distinguishing segments of different sizes on the same site from the DNA tape. This method enables us to distinguish the location of the mutation in the studied DNA. The PCR product was also cut to the leptin gene using the Restriction Enzymes (MSP11). The optimum concentration of the band was 10 units of the enzyme/sample. After that, the electrophoretic was then conducted for PCR reaction after cutting for patient and healthy samples on Agarose with concentration of 2%. The results of the transfer of the PCR product by the MSPII showed

three types of genotypes: the AA model represented by band with the molecular weight of (101bp), the Heterozygous mutant genotype AG represented by bands (101-70-30) bp) and homozygous mutant GG represented by two bands (70-70 bp). The substitution mutation for the nitrogenous base A was transformed it to the nitrogenous base G at the cutting location rs1137101 for the leptin gene, Where the enzyme works on cutting the bands resulting from the multiplication with same size 101bp to two pieces the first 70bp and the second 30bp. A single band of 101bp will appear when PCR is transferred because there is no sequence required for the Restriction enzyme (MSP11) to cut it. In the case of a person having a genotype Ag, one of the alleles will not be cut because it does not have the cutting location. The second allele will be cut into two pieces and it will appear to us three bands When PCR is transferred first 101bp represents the natural allele and the remaining two are 70bp and 30 bp representing the mutation allele, In case of possession of the genotype gg will show us the first two bands 70bp and the second 30bp. The results indicate to a mutation in the leptin gene and mutations can also cause leptinobine and early obesity. Also caused: low growth hormone, thyroid gland, thyroid gland hormone, hypothyroidism (TSH), hyperinsulinemia with insulin resistance, fat deficiency, high degrees of Hepatolenticular Degeneration, absence of secondary sexual growth, Hypothyroidism and arrhythmia (Jin *et al.*, 2013). The results confirm that persons who have the genotype gg have high fat accumulation as well as statistically significant differences in levels of leptin and fat. This confirms the presence of the mutation. In addition, we can detect early readiness to develop heart disease and metabolic diseases. A defect in the biological structure and thus a defect in the function of leptin.

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