VIII. INTERNATIONAL CONGRESS OF MOLECULAR MEDICINE

09-12 NOVEMBER 2021

Molecular Aspects of Human Wellness

CONGRESS ABSTRACT BOOK

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Dear Colleagues,

On behalf of the Organizing Committee I am delighted to invite you to the 8th International Congress of Molecular Medicine that will be held in Istanbul, Turkey on 9th – 12th of November 2021 under the auspices of the Turkish Society of Molecular Medicine and Istanbul Yeni Yüzyil University, Faculty of Health Sciences.

Congress program will consist of outstanding lectures, including keynote talks, plenary sessions, oral & poster presentations & exhibition.

The congress biennial of molecular medicine is an important forum for researchers and clinicians from Turkey and all around the world to focus on the latest developments in molecular medicine.

Trends, technologies and clinical applications in areas including, “Tumor Biology”, “Anti-Cancer Agents”, “Sportive Performance and Genetic”, “Nutrition and Epigenetic Aspects”, “Metabolic Syndrome”, “Prospective Methods in Molecular Medicine“, Molecular Aspects in Diabetes” , “Molecular Metabolic Syndrome” shall be discussed during the congress.

Istanbul Yeni Yüzyil University aims to bring the scientists needed by the society into the country and to become a higher education institution and centre of excellence connected to Atatürk's principles and reforms, aiming to educate knowledgeable, contemporary, equipped and experienced young people who are useful to the society.

Taking into account the developments in the world, this university considers itself responsible for the upbringing of the workforce trained at associate, undergraduate, graduate and doctoral level to exceed the level of civilization academically and technologically and to contribute to researches and studies. To this end, Istanbul Yeni Yüzyil University provides universal education in different disciplines in cooperation with stakeholders such as government institutions, private sector and civil society institutions and organizations with its expert and experienced academic staff.

We are looking forwards to meet you in Istanbul on November 2021 for this outstanding congress and we hope you will enjoy scientific sessions, as well as Turkish hospitality and all the beauty of the Istanbul.

Prof. Dr. Ümit Zeybek

Chair of the Turkish Society of Molecular Medicine,
Istanbul University, Department of Molecular Medicine,
Aziz Sancar Institute of Experimental Medicine,
Istanbul, Turkey

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Main Theme

- Molecular Aspects of Human Wellness

Main Scientific Topics

- Tumor Biology
- Anti-Cancer Agents
- Sportive Performance and Genetic
- Nutrition and Epigenetic Aspects
- Metabolic Syndrome
- Prospective Methods in Molecular Medicine
- Molecular Aspects in Diabetes
- Molecular Metabolic Syndrome

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ATHLETIC PERFORMANCE AND OUR GENETIC STRUCTURE

E. Hülya YÜKSELOĞLU
İ.U.C. Institute of Forensic Sciences and Legal Medicine

In recent years, genetic studies have accelerated and have started to apply into new fields within every area at an unprecedented pace. Many unknowns related with genes and DNA matrixes that constitute these have started to be solved. This has become a race in a science world and prominent notions in robotic approaches like artificial intelligence have tried to make similar into protein array that found in DNA structures.

At the present time, the studies which conducted with athletes are gaining significance gradually. Many disciplines are working together in order to increase the sportive performance and upper-level competition achievement of athletes. For maintenance of our country's successes on sports field and for carrying them to the upper level, the studies conducted by athletes should be multidisciplinary. Until now, the common consideration about the feature development of elite athlete and high performance of athletes, they have depended on special trainings and nutrition programs. Now, environmental factors like them are not seen adequate for characterizing the state of human physical performance. Nowadays, complex physical performance phenotype is considered as another determinant factor for genetic tendency determinants. Genetic tendency is not the most important factor but it has huge importance on characterization of an individual as an elite.

In human genetic map, it is known that many genes related with physical performance take part. The small polymorphic differences with the contribution of environmental factors, cause discrepancy among individuals by affecting the physiological functions. Single nucleotide polymorphisms (SNP), indicate the < %1 of the polymorphisms within general population. This may cause the gaining distinctive supremacy of an athlete from another athlete. In literature, during the determination of genetic tendencies, researches are conducted on the genes which are more important in the assessment of an athlete's performance and that affect characteristics like maximum oxygen volume (VO_2 max), endurance performance features like heart pumping strength, fat-free mass which is an indicator of skeleton muscle mass.

According to the polymorphisms of these genes, durability of an athlete or tendency of an athlete into which strength sports, can be foreseen. Myostatin, erythropoietin, growing hormone, nitric oxide synthase, vascular endothelial growth factor, angiotensin transforming enzyme, angiotensinogen, monocarboxylate carrier 1, insulin like growth factor-1, peroxisome proliferator active receptor, and alpha-actinine-3 genes can be sorted as an example for athletic performance related genes. To illustrate, ACE DD and ACTN3 XX genotypes were found at high frequency in strength athletes as well as it can be foreseen that individuals who have this polymorphism can be more successful in strength sports. In people who have PPARA C allele, existence of fast tighten muscle fibrils at a high rate also may indicate individuals who have this polymorphism may prone to strength sports.

There are different molecular technology alternatives for screening of these genes. The most of these alternatives are based on microarray, new generation sequencing (NGS), polymerase chain reaction and classic Sanger high cost DNA sequence analysis, but quantitative PCR (qPCR) which not based on DNA sequence analysis is also be used. In the achievements

of professional atheletes and successful athletes, there are coexistence of more than one factors. When they are examined one by one, the most effective variables are thought as performance of an athlete, athlete's tendency to preferred sport, sportive and physiological capacity, and physical health.

There is a belief about sportive performance that located in the most important place during talent choosing, it is constituted by acquired environmental factors and innate genetic heritage as well as it is aimed that enhancing the athlete's potential which athlete should have it hereditarily, to the highest level by training in order to be successful in a sport branch. In other words, interactions of the building blocks of biological gift equipments like race, gender, genetic substructure, intelligence, locomotor, neuromuscular, anatomic structure, psychological state and physical well-being, will be the determinant of future performance. When studies are examined, athlete phenomenon is assessed as a whole of psychological features with environmental and biological factors, as well as all of these characteristics should be emphasized in researches. As well as the controversial ''athletes not born equal'' quote of Sir Roger Bannister who is the first person run one mile under four minutes, ethnic origin of people can be seen advantageous sometimes when compared with others. For example, it can be seen that West African runners, East Africans, and Asians are successful on short distances, marathon, and swimming respectively. In this genomic era, explaining the genes that play role in tendency of people for specific sports, and genetic studies in this direction will be enlightening. In conclusion, studies in this field is very important not just for tendency to sport or improvement of personal training programs, they are also important for preventing from unwanted cases like sudden athlete death or keeping them at minimum rate. Being aware of these factors which affect individual success in different sport disciplines also within our country, will provide huge benefit as well as it will provide making healthier decisions for future. Because the factors which effective on formation of athletic performance and improvement of it, like muscle type, cardiovascular capacity, oxygenation of tissues, training resistance and psychology of an athlete, are known as controlled by our genes, some genes related with sports have been studied frequently while very little study on many genes is available. In this scope, more researches on the genes which studied lesser are required. Besides, assessment of the storage and sharing of individuals' genetic data by relevant persons should be beneficial.

Today, relationship of 120 gene with athletic performance has been showed. It has been discovered that some of them show differences in endurance athletes and strength athletes. It has been showed that endurance athletes carry the one type of genes like ACE, ACTN, PPARA at high frequency while strength athletes carry the different type of it. Being hereditary at high rate of sportive performance is highly important evidence. To illustrate, height that is critic for many sport branches, show inheritance at 80% rate. Studies show that body type which is another important characteristic in sport, is also hereditable. Additionally, it is expressed that aerobic endurance is inheritable at 50% rate as well as muscle strength and power are also inheritable at 30-83% rate. Nonetheless, in some conduct studies, genetic differences between endurance athletes with strength athletes have been determined.

Muscle fibril type rate showing that individuals have tendency for which sport, is also seen related with genetic differences of an individual. ACE, ACTN3, PPARA, PARGC1A and Gly482 are the most examined genes for their relations with endurance

performance. The relationship of endurance performance with total 77 genes including these genes has been examined until today. ACE gene is one of the most studied genes and is showed its relation with endurance sports. ACE gene encodes angiotensin-1 transforming enzyme. This enzyme is found in 17th chromosome of renin-angiotensin system that responsible from the control of blood pressure by regulating the body fluid level, and this gene sequence may show difference between individuals. ACE I (Insertion) allele is formed by addition of excessive 287 base pair in a region of ACE gene, as well as ACE D (Deletion) allele is formed by the lack of this part. There is a relationship between ACE I/I genotype with endurance performance and in the conducted researches; it has been showed that ACE I/I genotype is found at high frequency in English professional ≥ 5000 m runners, Spanish professional bicycler and long distance runners, Italian Olympic endurance athletes, long distance swimmers and high successful marathon runners from different nations. PPARA (Peroxisome proliferator activated receptor α) is a gene that is researched frequently for its relation with endurance features of an athlete. With the replacement of guanine nucleotide by cytosine (G/C, rs4253778) in the sequence of PPARA gene which located within 22nd chromosome; DNA sequences that show difference among individuals form in this gene and they are called as polymorphism.

In a detailed study in 2016, genotypes of 760 endurance athlete and 1792 sedentary individual were examined. Result of research has shown that in endurance athlete individuals who have G allele in their both two chromosomes (homozygote G) is at higher frequency when compared with sedentary individuals. Creatine kinase (CKM) which is an essential enzyme for energy support in muscles, is coded from CKM gene found in 19th chromosome. It was found that an adenine/guanine polymorphism in CKM gene have a relationship with sport performance. In Russian endurance athletes, it was showed that CKM AA genotype was at higher frequency in comparison with sedentary. The studies that examine CKM gene-sport performance relationship is quite a few, as well as novel studies are required for clarifying this relationship. In addition, there are polymorphisms among individuals on ADRB3 gene which play a thermoregulatory role in skeleton muscle.

Conducted studies show that muscle strength is affected by heredity at 30-80% rate. In researches, it is determined that maximum oxygen intake, cardiac mass and structure are inherited at 40-70% rate as well as capacity is inherited at 30-90% rate. Relationship of 43 genes with power/strength has been shown. There are researches on a vast number of genes related with power/strength performance. D allele of ACE gene cause high ACE activity. Circulatory ACE activity is significantly related with isometric and isokinetic quadriceps muscle strength. Studies determine that D allele is relevant with high muscle strength, high muscle volume and high fast-twitch muscle fibril rate. D allele was found at high frequency in Russian and European short distance swimmers. Similarly, D allele was found at high frequency in goalkeepers which is an extreme strength and power required position in football. Conducted studies showed that D allele is resulted in high hand strength within teenager girls as well as increment in standing long jump within middle school kids. As similar, it was seen that D allele frequency was higher in Lithuanian and Iranian strength/power athletes when compared with sedentary. There is a common genetic diversity for ACTN3 gene. When arginine (R) amino acid in 577th place of ACTN3 gene sequence replace with a stop

codon (X), ACTN3 protein cannot be encoded and this prevents the production of functional α-actinin-3 protein. In the first conducted research, it was shown that ACTN3 XX genotype is lower in Australian strength athletes in comparison with sedentary as well as XX genotype was not observed in Olympic athletes. There are studies that examine the relationship between strength athletes with C allele of PPARA gene which mentioned in endurance related genes. It was shown that individuals who have PPARA C allele, they have higher left ventricular mass as a response to exercise when compared with the ones who have G allele. It was seen that PPARA C allele is found at high frequency in Russian strength athletes and these athletes have fast-twitch muscle fibril at a high rate. PPARA C allele was found related with high hand strength.

Gene or cell doping has defined as ‘‘Non-therapeutic use of cells, genetic materials, and the genes which have capacity to increase athletic performance’’ by World Anti-Doping Agency (WADA). In 2003, International Olympic Committee and WADA included gene doping into forbidden applications list. Principal of gene therapy bases on giving a therapeutic gene that can compensate abnormal gene or non-existent gene, into a cell. Treatment of serious diseases is not the only use of gene therapy, as well as it can be used for reconstitution of athlete’s bodies in order to gain better performance. Gene therapy can be appropriate for some diseases but the ones want to get benefits from these advances in sportive field should be aware of that science proceed rapidly about revealing the gene doping. Developments in genetic field can be used by athletes for being better in play areas. For this reason, being better, faster, stronger than others and charm of doing this by genes, may be attractive rather than being oppose for it.

Genetic factors are directly related with many components which known that it is important within performance in sportive performance, visual performance, hearing, balance, muscle structure and emotional states, like endurance, strength, power, speed, muscle fibril composition.Talent selection is an important phase for subjecting young athletes to appropriate and personalized training program. This selection is mostly performed according to physical and physiological characteristics of an individual as well as his/hers sport performance. In this scope, genetic and psychological tests are shown as alternative methods that give information about having tendency of individuals to which sport type during talent selection phase by researchers.

Today, there are some genetic tests done by frequently worked genes like ACTN2, ACE. According to the polymorphisms of these genes, tendency of an athlete into which one of endurance or strength sports can be foreseen. To illustrate, ACE DD and ACTN3 XX genotypes are found at high frequency in strength athletes and it can be foreseen that the individuals who have this polymorphism may be more successful in strength sports. In an individuals who have PPARA C allele, fast tighten muscle fibrils are found at high frequency, so it may be estimated that individuals who have this polymorphism more prone to strength sports. In conclusion; not just for tendency to sport or improvement of personal training programs, studies in this area is also very important for preventing from unwanted cases like sudden athlete death or keeping them in a minimum rate. Knowing these factors that affect individual success in different sport disciplines also within our country, will provide huge as well as it will provide making healthier decisions for future. Because the factors which effective on formation of athletic performance and improvement of it, like

muscle type, cardiovascular capacity, oxygenation of tissues, training resistance and psychology of an athlete are known as controlled by our genes, some genes related with sports were studied frequently while very little study on many genes is available. In this scope, more researches on the genes that studied lesser are required. Besides, assessment of the storage and sharing of individuals' genetic data by relevant persons should be beneficial.

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INVESTIGATION OF HEPATIC AND INTESTINAL RESPONSES TO ROTENONE AND ACETIC ACID IN ZEBRAFISH

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INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease known in the world. In the pathology of this disease, loss of dopaminergic neurons is observed. With the increase in the elderly population in the world, the number of people suffering from PD is expected to increase.

Parkinson's disease has two classes of symptoms as motor and non-motor. Clinically, symptoms such as tremor, slowness in movements, body stiffness, and balance problems are accepted as motor symptoms, while symptoms such as sleep disturbance, gastrointestinal symptoms (constipation), olfactory disorder, depression and pain are also known as non-motor symptoms.

Although pain was mentioned in the original definition of PD by James Parkinson, it is one of the most frequently neglected and most common non-motor symptoms with no really effective treatment. Depending on the epidemiological study considered, it has a prevalence between 30% and 83% of patients [1-4]. Clinical data have shown that both pain thresholds and pain tolerance are significantly lower in Parkinson's patients compared to the control population, and these patients may suffer from a variety of different pains [4,5].

The dopaminergic system in zebrafish has become increasingly popular in recent years, is well characterized both in embryonic and adult periods. For this reason, zebrafish and its embryo are accepted as suitable models for neuronal research. One of the most commonly used chemicals to create a PD model is rotenone. Rotenone is a common chemical that belongs to the rotenoid family and is also used as a pesticide. The lipophilic nature of rotenone allows it to easily cross the blood-brain barrier. It inhibits proteosome activity in the cell and causes the formation of reactive oxygen species. It has been shown that motor functions, olfactory functions, and dopamine levels decrease in adult zebrafish exposed to rotenone [6].

Pain is defined by the International Association for the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with or described in terms of actual or potential tissue damage”[7]. The main function of pain is to be a warning signal that protects the organism from harm or minimizes injury. The second main function of pain occurs after injury. Internal mechanisms exacerbate pain intensity, location and duration. Pain radiates from the damaged area to the undamaged areas and continues during healing [8,9].

Although pain is a non-motor symptom in PD, it sometimes occurs before motor symptoms and the diagnosis of the disease difficult. Since pain is a very important symptom in PD, clarifying its mechanism is important for the early diagnosis of this disease. The aim of our study is to examine the redox system in the hepatic and intestinal tissues of zebrafish that were treated with acetic acid, rotenone and acetic acid with rotenone

METHODS

Zebra Fish Breeding

Wild-type male/female zebrafish (AB/AB strain) were housed in ZebTEC aquarium rack system (Tecniplast, Italy). Zebrafish were kept in disease-free conditions at 27 ± 1 °C under a light/dark cycle of 14/10 h and they were fed twice a day. In the study, 4 groups were formed as control group, acetic acid group, rotenone group, , rotenon and acetic acid groups, and 15 fish were placed in each group.

Rotenone and Acetic acid Exposure

Fish in the rotenone group (R) were exposed to 5 µg/L rotenone (Sigma, USA) which was dissolved in 0.1% dimethyl sulfoxide (DMSO) (Sigma, USA). For rotenone exposures, 5µg/ml was added to 7 liter aquariums, and the aquarium water was changed every 3 days for 30 days. Zebrafish in acetic acid group (AA) and in the rotenone + acetic acid group (ROT + AA) were exposed to 5 % acetic acid solution with intraperitonal injection (5µl). At the end of 4 weeks, fish were anesthetized and euthanized by decapitation followed by rapid removal of the liver and intestinal tissues for biochemical analysis.

Biochemical analyses

For the biochemical analyses liver and intestines were taken from anesthetized fish and they were homogenized in physiological saline to prepare 10% (w/v) homogenates. Protein levels were determined using the Lowry method and biochemical parameters were evaluated per protein [10].

Lipid Peroxidation Level (LPO)

The level of malondialdehyde (MDA) in homogenates prepared with liver tissues was determined using thiobarbituric acid as a reactive substrate. An extraction coefficient of 1.56 x 105 M^{-1} cm^{-1} was used and LPO was expressed as nmol MDA / mg protein as MDA equivalent [11].

Nitric Oxide Level (NO)

Nitric oxide (NO) was determined by the Miranda method, which is based on the reduction of nitrate to nitrite with vanadium (III) chloride [12]. In an acidic environment, nitrite and sulfonylamide react with N-(1-Naphthyl) ethylenediamine dihydrochloride and a complex diazonium compound is formed. The color complex was measured at 540 nm with a spectrophotometer and the results were expressed as nmol NO/mg protein.

Superoxide Dismutase Activity (SOD)

A method based on the ability of SOD to potentiate the action of riboflavin-sensitive o-dianicidin was used to determine SOD activities in liver homogenates, and the absorbance of the product was measured at 460 nm by spectrophotometer [13]. Final absorbance was calculated by

measuring absorbances at 0 and 8 minutes of illumination. Results were expressed as U/mg protein.

Glutathione S Transferase Activity (GST)

The activity of GST was determined based on the spectrophotometric evaluation of the absorbance at 340 nm of the product formed by the conjugation of GSH and 1 - chloro - 2,4 - dinitro - benzene [14].

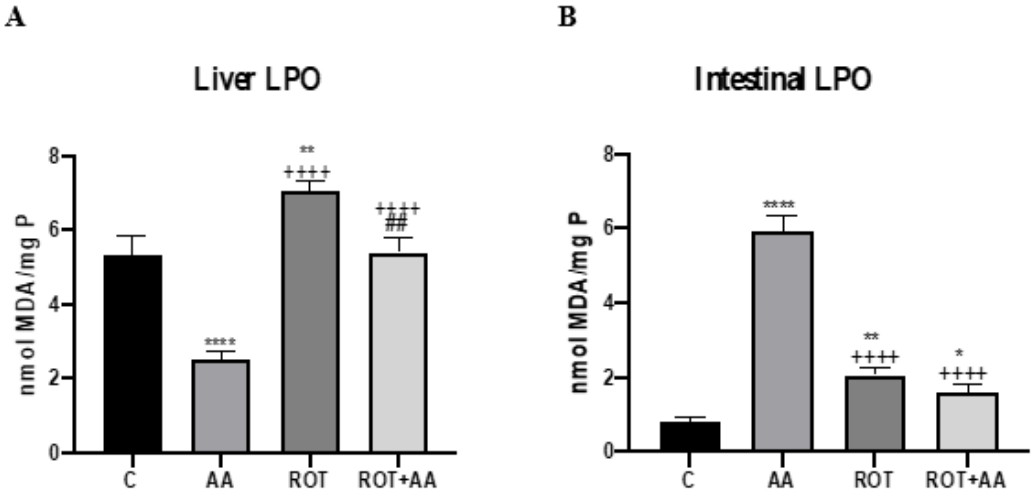

Figure 1: Liver lipid peroxidation (LPO) levels (nmol MDA/mg P) of the groups (A). Intestinal lipid peroxidation (LPO) levels (nmol MDA/mg P) of the groups (B) Data presented are mean ± SD. Significant difference is indicated by an asterisk. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ Significantly different from the C group; ++++$p < 0.0001$ Significantly different from the AA group; ## $p < 0.01$. Significantly different from the ROT group. C Control Group, AA Acetic Acid Group, ROT: Rotenone Group; ROT +AA Rotenone + Acetic Acid Group

RESULTS AND DISCUSSION

MDA levels in liver decreased in AA groups compared to the control group.On the other hand, MDA levels increased in the ROT group compared to the control group. Compared to the AA group, MDA levels increased in the ROT and ROT+AA groups. Compared to the ROT group, MDA levels decreased in the ROT+AA group. The decreased liver MDA levels in acetic acid injected groups compared to the ROT group suggests that acetic acid reduces lipid peroxidation. Compared to the control group, lipid peroxidation was also observed to be decreased in the AA group (Figure 1A).

When compared with the Control group intestinal MDA levels increased significantly in all groups. Compared to the AA group, the MDA levels in the ROT and ROT+AA groups were significantly decreased (Figure 1B).

Unlike the liver tissue, the increase in the intestinal MDA levels of the acetic acid treated group suggests that acetic acid does not have the same effect on lipid peroxidation in the intestinal tissue. An increase was observed in the groups exposed to only rotenone and rotenone and acetic acid compared to the control. According to these results, it is seen that intestinal tissue responds differently to the intraperitoneal exposure of acetic acid than liver tissue.

Hepatic NO levels were significantly increased in the ROT and ROT+AA groups when compared to the control group.

When compared to the ROT+AA group, NO levels were significantly reduced in the ROT group (Figure 2A).

Intestinal NO levels increased significantly in all groups when compared to the control group. It was significantly decreased in the AA and ROT+AA groups compared to the ROT group (Figure 2B).

with the ROT group, SOD activity was significantly increased in the AA and ROT+AA groups (Figure 3A).

When compared to the control group, intestinal SOD activity increased in the AA injection groups, while it decreased significantly in the ROT group (Figure 3B).The superoxide radical that triggers hyperalgesia is neutralized by superoxide dismutase (SOD) enzyme [16]. In PD, changes can be observed in the levels of NO, GST, and SOD,

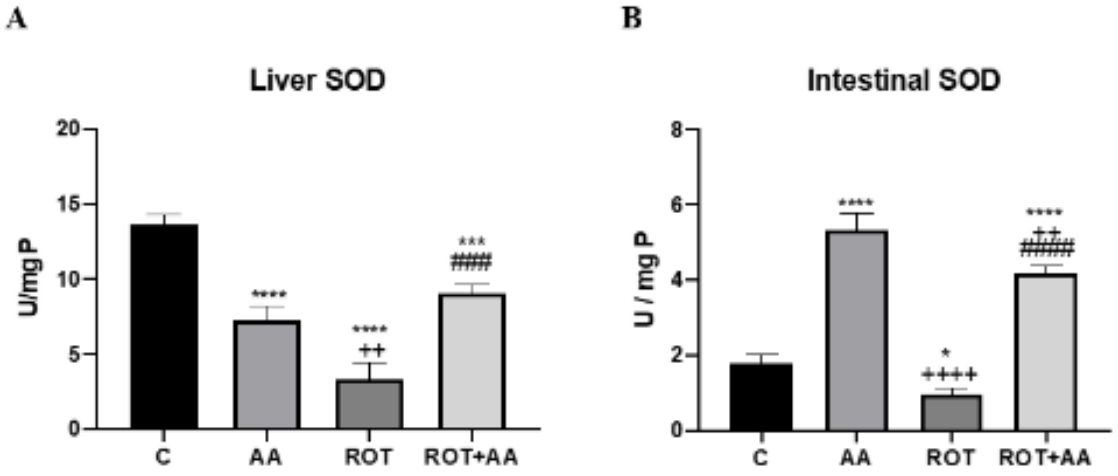

Figure 3: Liver Superoxide Dismutase (SOD) levels of the groups (A). Intestinal Superoxide Dismutase (SOD) levels of the groups (B). Data presented are mean ± SD. Significant difference is indicated by an asterisk. **** $p < 0.0001$, *** $p < 0.001$. Significantly different from the C group; *$p<0.1$, ***$p < 0.001$ ****$p < 0.0001$ Significantly different from the AA group; $^{++}$ $p < 0.01$. $^{++++}p < 0.0001$. Significantly different from the ROT group $^{###}p<0.001$, $^{####}p<0.0001$. C Control Group, AA Acetic Acid Group, ROT: Rotenone Group; ROT +AA Rotenone + Acetic Acid Group

NO is synthesized from L-arginine and is involved in the modulation of pain [15]. In our study, NO levels increased in the liver tissues in the ROT and ROT-AA groups compared to the control. On the other hand, in intestinal tissues, NO level, known as a pain modulator, increased in all groups compared to the control, and NO levels in tissues differed according to the groups.

SOD activities in liver decreased in all groups when compared to the control group. Compared

which act as modulators in the antioxidant system [17]. In our study, SOD activity increased in the groups exposed to acetic acid in intestinal tissues compared to the control. In addition, SOD activity was decreased in all groups in liver tissues compared to the control. SOD activity differed between groups according to tissues. GST activity in the hepatic tissues of zebrafish increased significantly in all groups when compared to the control group. Compared to the ROT group, GST

activity was significantly reduced in the acetic acid injection groups (Figure 4A).

GST levels, which are included in the antioxidant system, change in cases of increased oxidative stress such as inflammation, neurodegenerative diseases and pain formation [18]. In the results of GST activity in intestinal tissues of zebrafish, GST activity increased in all groups compared to the control. Compared to the ROT group, GST activity decreased in the acetic acid injection groups (Figure 4B). In both intestinal and liver tissues, GST levels were increased in all groups compared to control.

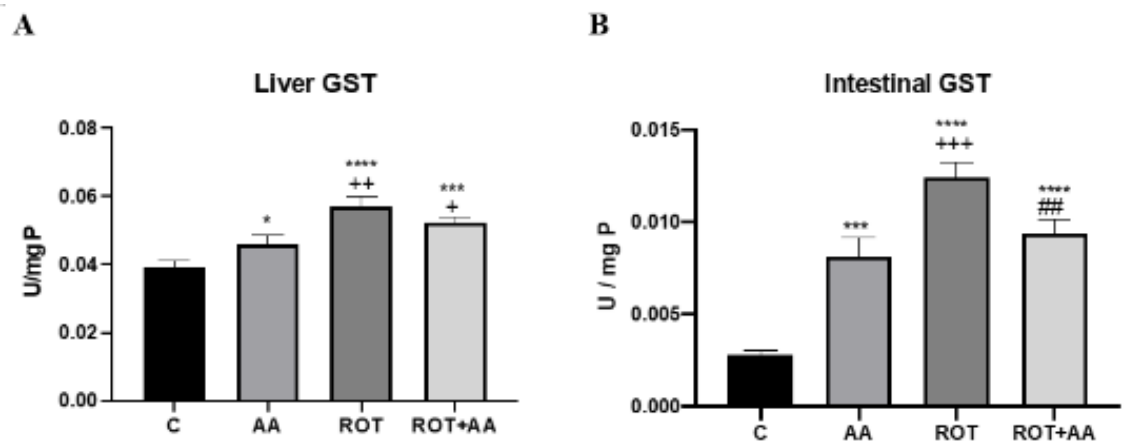

Figure 4: Liver Glutation *S*-Transferase (GST) levels of the groups (A). Intestinal Glutation *S*-Transferase (GST) levels of the groups (B). Data presented are mean ± SD. Significant difference is indicated by an asterisk. **** $p < 0.0001$, *** $p < 0.001$. Significantly different from the C group; *$p<0.01$, ***$p < 0.001$, ****$p < 0.0001$ Significantly different from the AA group; $^{+}p < 0.1$, $^{++}p < 0.01$, $^{+++}p < 0.001$. Significantly different from the ROT group $^{\#\#}p<0.01$. C. Control Group, AA: Acetic Acid Group, ROT: Rotenone Group; ROT +AA Rotenone + Acetic Acid Group

CONCLUSION

In our study, acetic acid induced-pain was generated for the first time in PD model in zebrafish.

Our results showed that liver and intestinal tissues respond differently in terms of oxidant-antioxidant status when acetic acid is administered in case of rotenone toxicity. Further studies are needed to examine iluminate the mechanism of pain in PD model.

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LABORATORY AND IN SILICO ANALYSIS OF THE PATHOGENIC VARIANT OF INTERLEUKIN-17 (rs763780) IN PATIENTS DIAGNOSED WITH COVID-19

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INTRODUCTION

Since the first case was announced, Corona virus disease 2019 (COVID-19) has been among the major infectious events of the century, causing close to 250 million confirmed cases and close to 5 million deaths worldwide (1). Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2) virus emerges as the causative agent and specifically affects patients aged 65 years and older with comorbid burdens (2,3). In particular, the patient population with hypertension, chronic respiratory and heart diseases, diabetes mellitus, renal failure and malignancy is defined as the most severely affected group (4,5).

Cytokine storm is a hyperactive immune response characterized by the release of interferons, interleukins, tumor-necrosis factors, chemokines, and several other mediators (6). It has been reported that a cytokine storm is associated with the deterioration of many infectious diseases, including severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). In the course of COVID-19 disease, macrophages, dendritic cells and Th1, Th2 and Th17 lymphocytes become overactive. In addition, overproduction of IL-1β, IL-6, IL-10, IL-12, IL-15, IL-17, IL-18, IL-23, IL-27, and IFN-α cytokines causes to the cytokine storm associated with disease severity. (7-9).

Interleukin-17 (IL-17) is produced by T helper 17 (Th17) cells and is involved in numerous immune and inflammatory processes. Th17 cells play a predominant role in mucosal cell integrity and may infiltrate the central nervous system through its cytokine production's direct effects. Six members of the IL-17 family were identified (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F). A IL-17F gene is located on chromosome 6p12.3 (10).

IL-17 levels were found to be increased in intensive-care COVID-19 patients compared to controls (11). When the literature is reviewed, there is no study examining the association of IL-17F (7488 A/G = rs763780) gene polymorphism with COVID-19. To our knowledge, this is the first study that has examined the relationship between IL-17F (rs763780) gene polymorphism and the pathogenesis of COVID-19. Therefore, this study aims to investigate the relationship between the IL-17F gene polymorphism in patients with COVID-19 by comparing genotype distributions of IL-17F gene between patients and healthy controls considering clinical parameters.

In the in silico analysis part of our study, Gencard, HaploReg4, rVarBase and SNP & GO programs

were used to predict the pathogenetic effect of IL-17F. Analyzed on the basis of HaploReg4; It has been reported that it is a functional gene variant that causes missense or amino acid difference, causes motif changes in Lmo2-complex, Mtf1 proteins, and its frequency is 0.05, 0.07, 0.14, 0.06 in Africa, America, Asia and Europe, respectively (12). In rSNP and rVarBase systems; It has been reported that it has a role as an RNA-binding protein and is associated with the disease by its weak transcription (13,14).

METHODS

Patient Selection

In this study, 200 patients with a positive PCR test result and 78 patients with a negative PCR test result with a diagnosis of COVID-19, who were admitted to the COVID-19 center of a university hospital between April and June 2020, and 100 healthy individuals without any known comorbidity to create a control group were included. Healthy controls were comprised of individuals who were negative for Sars-Cov-2 antibody (Sars-Cov-2 IgM, IgG) and two PCR results taken 48 hours apart were negative.

All of the PCR-negative COVID-19 patients had typical COVID-19 pulmonary involvement on their initial CT. It is possible to define this patient group as patients with a diagnosis of COVID-19 who showed false negativity in the first examinations before hospitalization.

DNA isolation and genotyping

Blood samples were taken into EDTA tubes, and DNAs were isolated from the collected blood samples by using the Plus Blood Genomic DNA Purification Kit according to manufacturer recommendations (Gene Mark). The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to determine the genotypes of *IL-17F* (rs763780) single nucleotide polymorphisms. PCR was performed using a forward (F: 5'-GTG TAG GAA CTT GGG CTG CAT CAA T-3') and reverse (5'-AGC TGG GAA AAA CAA AC-3') primers (15). For *IL-17F* (rs763780), a 470 bp fragment was amplified, which was then digested with NlaIII restriction enzyme (New England Biolabs) overnight at 37^{o}C.

Statistical Analyses:

Statistical analysis was performed using IBM SPSS version 21.0 (IBM Corp. released 2012; Armonk, NY, USA). Quantitative data (clinical parameters of COVID-19 patients, and *IL-17F* genotype distributions) represented as descriptive statistics included the mean, standard deviation, frequency, and percentage. The Pearson chi-square or Fisher's exact test analyzed comparisons of *IL-17F* genotype distribution of patients. The suitability of continuous variables to normal distribution was evaluated by using the Shapiro-Wilk test. Since the variables did not have a normal distribution, intergroup comparisons of continuous variables were performed by Mann Whitney U testing. Statistical significance was accepted as $p < 0.05$ for the results of all analyses.

RESULTS

The total distribution of the GA and AA genotypes of IL-17F/rs763780 was 14% and 86% in the COVID-19 PCR (+) patient group, 20.5% and 79.5% in the COVID-19 PCR (-) patient group, and 13% and 87% in the healthy controls. When the genotype and allele distributions of IL-17F/rs763780 polymorphism were compared in COVID-19 PCR (+) patients and control groups, it was found that there was no statistically significant difference in neither genotype nor allele distribution (P>0.05) (Table 1).

Table 1: Comparison of frequencies of IL-17F/rs763780 gene variants between PCR (+) COVID-19 patients and healthy controls

	Genotype	PCR (+) COVID-19	Healthy Control	OR Exp (B)	95% CI	p*
		n=200 (%)	n=100 (%)			
IL 17 7488 A/G	AA	172 (86)	87 (87)			
	AG	28 (14)	13 (13)	0.907*	0.446-1.842*	0.786*

*:OR (95%CI) was adjusted by age and sex, &Fisher's Exact Test.

When the genotype and allele distributions of IL-17F/rs763780 polymorphism were compared in COVID-19 PCR (-) patients and control groups, it was found that there was no statistically significant difference in neither genotype nor allele distribution (P>0.05) (Table 2).

Table 2: Comparison of frequencies of IL-17F/rs763780 gene variants between PCR (-) COVID-19 patients and healthy controls

	Genotype	PCR(-) COVID-19	Healthy Control	OR Exp (B)	95% CI	p*
		n=78 (%)	n=100 (%)			
IL 17 7488 A/G	AA	62 (79.5)	87 (87)			
	AG	16 (20.5)	13 (13)	0.558*	0.247-1.256*	0.159*

*:OR (95%CI) was adjusted by age and sex, &Fisher's Exact Test.

When PCR (+) patients were divided into two separate clinical subgroups as severe and mild, no significant differences were found in or group in terms of the distribution of IL-17F/rs763780 genotypes (P>0.05) (Table 3).

Table 3: Distribution of IL-17F/rs763780 genotypes between clinical subgroups: severe or mild infection

	Genotype	Severe	Mild	OR Exp (B)	95% CI	p*
		n=94 (%)	n=106 (%)			
IL 17 7488 A/G	AA	82 (87.2)	90 (84.9)			
	AG	12 (12.8)	16 (15.1)	0.904*	0.353-2.313*	0.833*

*:OR (95%CI) was adjusted by age and sex, &Fisher's Exact Test.

When patients were divided into two separate clinical subgroups as exitus/alive according to their last condition during their 28-day follow-up, no significant differences were found in or group in terms of the distribution of IL-17F/rs763780 genotypes (P>0.05) (Table 4).

Table 4: Distribution of IL-17F/rs763780 genotypes between clinical subgroups: exitus or alive during the 28 day follow-up

	Genotype	28 gün ex	alive	OR Exp (B)	95% CI	p*
		n=9 (%)	n=191 (%)			
IL 17 7488 A/G	AA	9 (100)	163 (85.1)			
	AG	0 (0)	28 (14.6)	0.853&	0.805-0.905&	0.365&

*:OR (95%CI) was adjusted by age and sex, &Fisher's Exact Test.

When patients were divided into two separate clinical subgroups as those with intensive care unit follow-up or not during their hospitalization,

no significant differences were found in or group in terms of the distribution of IL-17F/rs763780 genotypes (P>0.05) (Table 5).

Table 5: Distribution of IL-17F/rs763780 genotypes between clinical subgroups: need for intensive care or being inpatient

	Genotype	Needfor intensive care	inpatient	OR Exp (B)	95% CI	p*
		n=16 (%)	n=184 (%)			
IL 17 7488 A/G	AA	15 (93.3)	157 (84.3)			
	AG	1 (6.7)	27 (15.7)	0.369*	0.044-3.118*	0.360*

*:OR (95%CI) was adjusted by age and sex, &Fisher's Exact Test.

DISCUSSION

In clinical studies, proinflammatory cytokine storms that progress to multi-organ dysfunction and death have been observed in COVID-19 patients. In COVID-19 patients at risk of cytokine storm, tocilizumab, a monoclonal antibody that blocks the effect of IL-6, has been used and has been observed to be effective (16,17).

IL-17 is a cytokine that may be associated with IL-6, especially in cases of viral infection. In a study, it was found that the increase in IL-6 level promotes the generation of Th17 cells and IL-6 and IL-17 synergistically promote viral persistence by protecting virüs infected cells from apoptosis (18). Researchers observed that increased IL-17 in COVID-19 patients in intensive care compared to non-ICU patients (11).

Holster et al. reported that IL-17F rs763780 polymorphism is associated with asthma in children and may increase the risk of asthma after bronchiolitis (19). Rolandelli et al. They defined the IL17F rs763780 polymorphism as a biomarker of tuberculosis susceptibility and advanced disease severity in the Argentine population and reported that they thought that IL17F might be a critical cytokine in tuberculosis immunity (20).

Our study is the first to investigate the relationship of the IL-17F/rs763780 pathogenic variant with COVID-19 in the Turkish population. These results indicate that the IL-17F/rs763780 variant is not associated with COVID-19 susceptibility in the Turkish population. Further studies, both at the whole gene level and in larger groups and different ethnicities, are needed to determine the impact of this variant on the risk of developing COVID-19.

KEY MESSAGES

- Cytokine storm is associated with the severity of COVID-19.
- IL-17 is involved in numerous immune and inflammatory processes.
- IL-17F rs763780 polymorphism is not associated with COVID-19 in Turkish population.

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EXAMINATION OF CXCL12 EXPRESSION REGULATION AND ASSOCIATION WITH MULTIPLE MYELOMA AND OTHER DISEASES

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INTRODUCTION

Approximately 50 chemokines have been identified in humans (1). Chemokines are protein molecules with a molecular weight of 8-12 kD and multiple domains. Chemokine genes are located at specific loci. CC chemokine genes 17q11.2-12 and C-X-C chemokine genes are also located at 4q13 locus. Stromal cell-derived factor 1 is a chemokine protein encoded by the CXCL12 gene on chromosome 10 in humans (2). It is ubiquitously expressed in many tissues and cell types. CXCL12 has a ligand relationship with the CXCR4 receptor. In adulthood, CXCL12 plays an important role in angiogenesis by recruiting endothelial progenitor cells (EPCs) from the bone marrow via a CXCR4-dependent mechanism (3).

CXCL12 is expressed in many tissues, including the brain, thymus, heart, lung, liver, kidney, spleen, and bone marrow. CXCL12 is a potent chemotactic for lymphocytes (4). During embryogenesis, it directs the migration of hematopoietic cells from the fetal liver to the bone marrow and the formation of large blood vessels. It has also been shown that CXCL12 signaling regulates CD20 expression on B cells. CXCL12 is also chemotactic for mesenchymal stem cells and is expressed in the domain of inflammatory bone destruction, where it mediates its suppressive effects on osteoclastogenesis (6).

This protein belongs to the intercrine alpha family. SDF-1 is produced in two forms, SDF-1α/CXCL12a and SDF-1β/CXCL12b, by alternative splicing of the same gene. Chemokines are characterized by the presence of four conserved cysteines that form two disulfide bonds. The CXCL12 proteins belong to the group of CXC chemokines whose first cysteine pair is separated by an intervening amino acid. In addition, the first 8 sites of the CXCL12 N-terminus serve as a receptor binding site, but only Lys-1 and Pro-2 are directly involved in activation of the receptor. Meanwhile, the RFFESH motif (residues 12-17) in the loop region functions as a docking site for CXCL12 receptor binding (7).

It plays a role in functional states such as embryogenesis, angiogenesis, development of the immune system, development of infection, tissue homeostasis, tumor growth, metastasis. All chemokine receptors are membrane-bound molecules and contain 7-transmembrane domains in their structures and form pairs with G-proteins. Chemokine receptors are “G protein-coupled proteins” and are expressed on leukocytes. Chemokines initiate intracellular signaling by binding to specific G-protein-coupled cell surface receptors on target cells and induce cell migration and activation. Up to 20 chemokine receptors have been identified to date (8).

Substances such as PDGF, VEGF-A released from platelets after the stimulus that initiates the inflammatory process in the tissue outside the vessel (bacteria, surgery, ag-ab complex, etc.), chemokines such as CXCL1, CXCL5, CXCL7, CXCL4 in the CXC type, act against the invading microorganism in the first stage. They are effective in creating barriers (9). Under the inhibitory effect of IL-1 and TNF-, the production of CXCL12, which is synthesized from fibroblasts and keratinocytes, gradually decreases until the 6th day. Then, from the 14th day onwards, a large number of lymphocytes accumulate in the region under the effects of CXCL9 and CXCL10. A population of non-hematopoietic cells expresses markedly high levels of CXCL12 as well as SCF, FOXC1 and EBF3 in human adult bone marrow (10).

CXC chemokine ligand (CXCL) has bone marrow-specific mesenchymal stem cells called reticular (CAR) cells that interact strongly with leptin. Receptor-expressing (Lepr+) cells are the main component of HSC niches in murine bone marrow (11). CAR cells have been shown to be specialized mesenchymal stem cells characterized by several distinctive features, including much higher expression of the LEPR and HSC niche factors. Required for the maintenance of HSCs, CXCL12, stem cell factor (SCF) and transcription factors are expressed relative to other cell types such as forked box C1 (FOXC1) and early B-cell factor 3 (EBF3) (12).

MM is a plasma cell malignancy characterized by infiltration and growth of malignant plasma cells in the bone marrow (BM). MM is the most common type of cancer in plasma cells. The relationship between CXCR4 and CXCL12 is crucial for targeting MM cells to the protective BM niche (13). On the other hand, CXCL12 associated with HIV, WHIM Syndrome, immunodeficiency, cardiovascular disease, cancer types (14).

MATERIAL AND METHOD

We use The BioGrid Database/Pathway Commons for gene-protein and pathway interaction, GSEA/MSigDB for gene set enrichment analysis, TargetScan/miRDB for miRNAs targeting CXCL12, Blood eQTL Browser / BIOS / mQTLdb for targeting CXCL12, GRASP and GWAS for disease associations of CXCL12 and miRNA region SNPs.

RESULTS

The BioGrid Database identified 31 genes interaction with CXCL12, including 8 additional CXCL genes in the vicinity of CXCL12. We selected the miRNAs targeting CXCL12, and determined their other target genes using Target Scan. The results nearly significant as the co-expressed gene set suggesting that miRNA’s play a major role in the regulation of CXCL12 expression. The most significant co-expression pattern was noted in the bone marrow followed by other organs (brain, kidney, heart, lung and skin). The gene set enrichment analysis of the co-expressed gene set on the GSEA/MSigDB tool suggested enrichment of genes involved allergic

disease, arthritis, autoimmune disease of musculoskeletal system, bone inflammation disease (FDR<5E-06). Examination of disease associations of SNPs from each miRNA gene region in GWAS databases yielded results for B-lymphoblastic leukemia/lymphoma (P<7E-40). SNPs acting as eQTL/meQTL in blood for CXCL12 showed GWAS associations with; respiratory system disease, intestinal disease, combined immunodeficiency, multiple sclerosis, hepatit (P<8E-06).

CONCLUSION

We found strong evidence for miRNA-mediated CXCL12 expression, the variants near miRNA's showed stronger genetic associations with lymphoblastic leukemia/lymphoma, respiratory system disease, intestinal disease, combined immunodeficiency, multiple sclerosis, hepatit. miRNAs showed stronger genetic associations with inflammatory, immune disorders and MM. We conclude that the role played by CXCL12 is stronger in autoimmunity, inflammation and possibly in MM. This study shows the feasibility of preliminary dry laboratory projects before launching wet laboratory experiments.

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EFFECTS OF TESTOSTERONE ON HEPATIC REDOX SYSTEM AND LOCOMOTOR ACTIVITY

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INTRODUCTION

Hormone replacement therapy (HRT) can be applied for any conditions like infertility, hypogonadism, post-menopausal period to make up for the lack of hormones in the body or complete them. It is also used for the transition process with transgender people to facilitate body adaptation. In this situation that is started at the request of transgender people, the aim of hormone replacement therapy is to change secondary sex characteristics. Testosterone is a steroid hormone that stimulates secondary sex characteristics and affects bone density, libido, muscle mass, body composition and fat metabolism. The assigned gender of trans men at birth is female (afab) but the gender they identify is a man. Exogenous testosterone use is observed in trans men from a certain period of their transition process and they use these preparations for many years. The preparations that they use provide masculinization while suppressing female secondary sex characteristics. Deeping of the voice, increase in body hair, enlargement of the clitoris, change in fat composition are observed with the effects of exogenous testosterone (Goldstein et al, 2019; Unger C, 2016).

Sustanon is an anabolic androgenic steroid medication (Sustanon 250mg/ml) and it is a useful and popular medication in hormone replacement therapy. It is an oil-based preparation and applied with intramuscular injection on the body. In HRT with transgender men, the injection is repeated every 14- 21 days (Aljeboori and Majhool, 2017; Allouh and Aldirawi, 2012; Unger C, 2016**)**.

In this study, it was aimed to determine the short-term effects of Sustanon treatment in zebrafish which is the most commonly used preparation in hormone replacement therapy for trans men.

METHODS

Wild-type AB/AB strain zebrafish were housed in an aquarium rack system (ZebTEC, Tecniplast, Italy) at 27-28 ± 1 °C under a 14/10 h light/dark cycle. Animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) (Louhimies, 2002). Fish were fed with 20 mg of commercial fish food (Tetramine) per day. Tetramine content is minimum 11% crude oil, 51% crude protein, 2.3% calcium, 1.5% phosphorus, maximum 15% ash, 3% crude fiber and 6.5% moisture. The granule size is 0.36–0.65 mm and its energy is 3.39 kcal / g.

Zebrafish (Totally 14) were divided into two groups as the Control (Vehicle) (C), and Sustanon group (S). The Control group consisted of 6 fishes and the Sustanon group consisted of 8 fishes. The testosterone dose was determined according to

literature as 880 ng/5 microliters for 1 g animal (Jianzhen et al., 2019). Testosterone solution (Sustanon 250 mg/ml) was diluated with sunflower oil for the determined dose. The solution was applied by using Hamilton injector and testosterone was injected intramuscularly on the back of the animals, from the top of the tail fin. 24h after injection, a behavior test was performed and the locomotor activities were recorded by the camera. Then the animals were anesthetized in iced water and sacrificed to remove the hepatopancreas (liver and pancreas) tissues and they were homogenized in physiological saline to prepare 10% (w/v) homogenates. After centrifuging briefly the supernatant was separated and used for the biochemical parameters. The recorded videos for locomotor activity were analyzed using automated computer programs (Kinovea and ToxTrac).

Biochemical Analyses

For the biochemical analyses supernatant was used. Total protein levels were measured according to the method of Lowry and the results were expressed per protein (Lowry et al., 1951). The method of Yagi was used to measure malondialdehyde (MDA) levels as thiobarbituric acid reactive substances as the end product of lipid peroxidation (Yagi, 1981). GST catalyzes the conjugation of GSH and GST activity was determined using a spectrophotometer at 340 nm (Habig and Jacoby 1974). Acetylcholinesterase analysis was determined by using Ellman method, it is used for determination of cholinesterase activity and also for monitoring of the ACH hydrolysis by acetylcholinesterase (AChE) of tissue extracts (Ellman et al., 1960). Nitric oxide (NO) was determined by the method of Miranda which is based on reducing nitrate to nitrite by vanadium (III) chloride. The colored complex was measured at 540 nm by a spectrophotometer and results were expressed as nmol NO/mg protein (Miranda et al., 2001).

Data Analysis

The required power and sample sizes used in this experiment were defined using statistical power analysis to detect minor effects. Statistical analysis was performed by using GraphPad Prism 9.0 (GraphPad Software, San Diego, USA). The normality of the distribution was verified using the Shapiro-Wilk test. To compare the groups One-Way ANOVA test was used which was followed by Tukey's multiple comparison tests. The data obtained were given as the mean ± standard deviation. P value less than 0.05 was regarded as significant.

RESULTS

Average speed and swimming distance are indicators of locomotor activity in zebrafish. In our study when compared with the Control group, both average speed and swimming distance increased significantly in the Sustanon Group ($p < 0.05$) (Figure 1A and 1B). The primary function of acetylcholinesterase is to catalyze and promote the breakdown of the neurotransmitter acetylcholine.

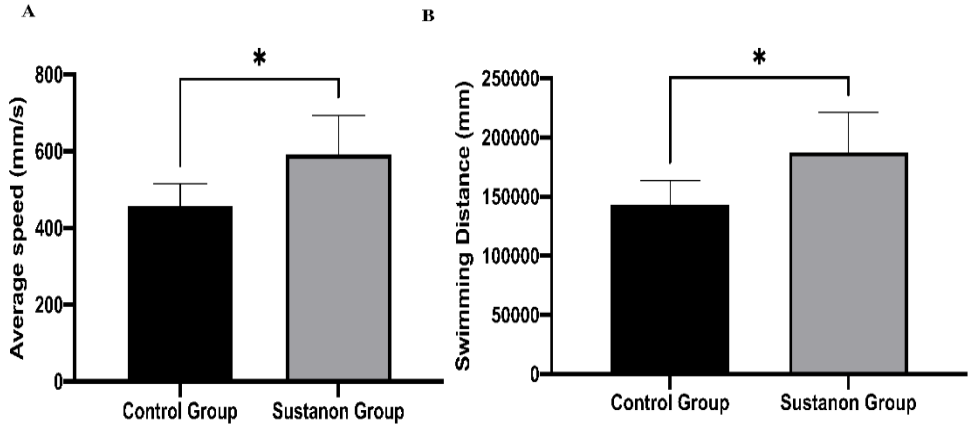

Figure 1: Locomotor activities of the Control and Sustanon groups. A: Average speed of the groups B: Swimming distances of the groups. * $p<0.05$ significantly different from the Control group.

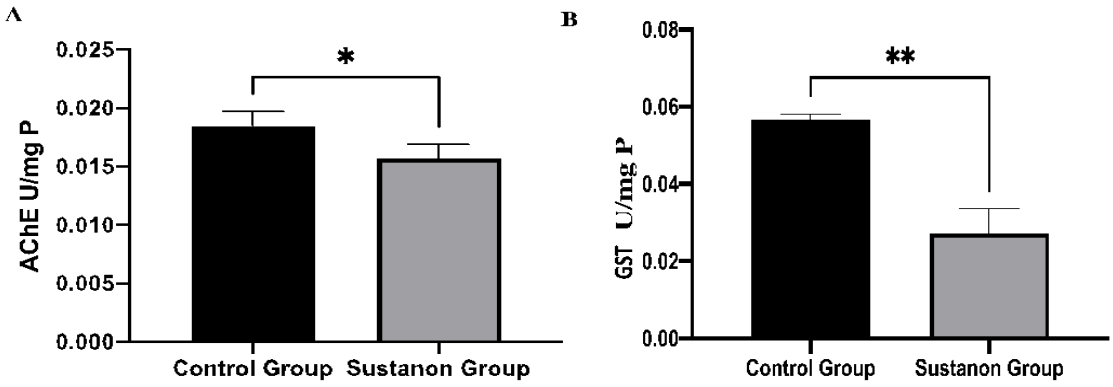

Figure 2: Acetylcholinesterase (AChE) and Glutathione S-transferase (GST) activities of the Control and Sustanon groups. * $p<0.05$; ** $p<0.05$ significantly different from the Control group.

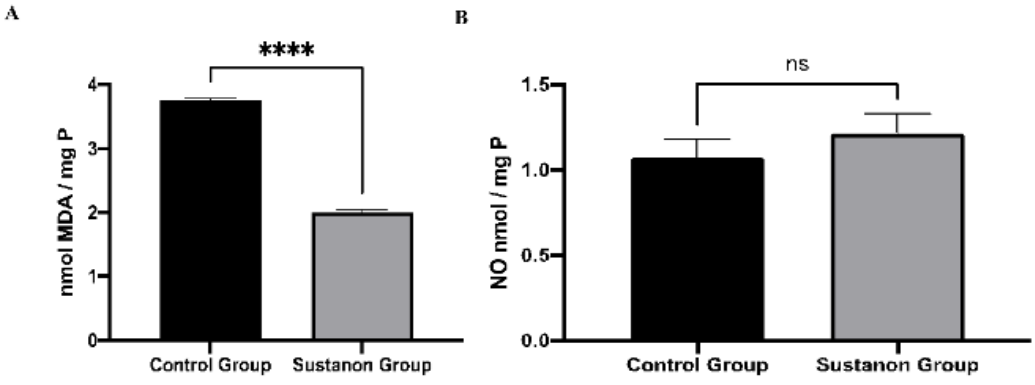

Figure 2: Malondialdehyde (MDA) levels as index of lipid peroxidation (A) and Nitric oxide (NO) (B) levels of the Control and Sustanon groups. **** $p<0.0001$ significantly different from the Control group.

In our study Sustanon treatment significantly decreased AChE activity when compared with the Control group (Figure 2A) ($p < 0.05$). GST activity decreased significantly in the Sustanon group when compared with the control group ($p < 0.01$) (Figure 2B). LPO is an index oxidative stress and Sustanon treatment significantly decreased LPO levels when compared with the Control group ($p < 0.0001$) (Figure 3A). On the other hand, the slight increase observed in the NO levels of the Sustanon treated group was not statistically significant ($p > 0.05$) (Figure 3B).

DISCUSSION

There are conflicting reports on the effects of testosterone on lipid metabolism. In our study Sustanon treatment decreased the levels of LPO and the activities of GST and AChE in liver. Low testosterone levels in men have been related with the development of metabolic syndrome. testosterone has been reported to stimulate primarily extrahepatic and not hepatic lipid oxidation in humans (Birzniece et al., 2009). GSH-related enzymes such as GST have been reported be subject to modulation by androgenic and estrogenic influences (Igarashi et al., 1984). GST enzymes are a family of detoxification isoenzymes present in different tissues including the testis and that conjugate many toxic substrates to glutathione. Among these substrates are carcinogens, mutagens and products of oxidative processes. In the present report we show that GSTalpha is expressed in somatic testicular Leydig cells and Sertoli cells. In our study GST activity decreased significantly in the Sustanon group when compared with the control group. Sertoli cells exert a protective role and are under endocrine control to ward against toxic agents. In our study, decreased hepatic GST activity may be related with decreased LPO levels. Testosterone was shown to be a negative modulator of butyrylcholinesterase but not of AChE, in male mouse sera (Durrant et al., 2012). However in our study AChE activity in liver decreased in T treated zebrafish. The concentration and period of testosterone administration may influence the results and may be the reason of different results obtained from different study models. Testosterone has been shown to stimulate physical activity behavior in male mice by acting on central DA pathways. Accordingly in our study locomotor activities increased testosterone treated zebrafish. Although AChE activity has been positively correlated with locomotor activity, in our study in contrast to decreased AChE activities, testosterone treatment increased average speed and swimming distance. This difference might be due to the determination of hepatic AChE activity instead of blood or brain AChE activity as hepatic AChE may be involved in other intercellular and intracellular regulatory mechanisms.

CONCLUSION

Studies on hepatic lipid metabolism and oxidation of testosterone have conflicting results. In the preliminary studies we conducted on testosterone, we determined that testosterone did not increase or even decreased hepatic lipid peroxidation at the

dose we applied, and accordingly, GST activities were also decreased. We plan to continue our studies by examining the effects of testosterone treatment in different stress situations and at different concentrations.

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COMPARISON OF BETWEEN CETP AND ANKK1 GENE VARIATIANTS BY USING QPCR AND DNA SEQUENCING IN OBESE PATIENTS

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INTRODUCTION

Obesity can be defined as a disorder in the system that regulates body weight. As obesity increases, the risk of obesity-related hypertension, diabetes, cardiovascular diseases, stroke, infertility and cancer. Anatomical distribution of body fat has a significant impact on associated health conditions. It has been seen in studies that genetics has a decisive importance in obesity. (1). Genetic predisposition is effective in obesity at a rate of 20% to 80%. (2). Many genes and mutations have been identified that can express the predisposition to obesity, the development of obesity, and the link of obesity with metabolic processes. (3). Cholesteryl ester transfer protein (CETP) mediates lipid exchange between lipoproteins. The physiological role of is to replace triglycerides in VLDL or LDL particles with cholesteryl esters in HDL. Also, CETP gene plays a key role in HDL-C metabolism and CETP performs neutral lipid exchange (cholesteryl ester and triglyceride transfer) between lipoproteins from HDL-C to other lipoproteins and subsequently lipid exchanges resulting in cholesterol uptake by hepatocytes, known as reverse cholesterol transport. (4,5)

Ankyrin Repeat- and Kinase Domain-Containing Protein 1(ANKK1); Controls the synthesis of dopamine in the brain. Changes in ANKK1 activity have been investigated in many studies in which it is shown that there is a relationship between ANKK1 variations and neuropsychiatric disorders such as addiction, eating habits/addiction Literature review shows polymorphisms that can express the link of obesity predisposition, obesity development and obesity with metabolic processes. Also, Dopamine receptors are reduced in obese individuals compared to weak individuals, This suggests that dopaminergic signaling contributes to the development of obesity. A mutation in ANKK1 can cause disruption in the dopamine pathway and lead to the formation of obesity. (6-8)

We aimed, to investigate CETP and ANKK1 gene variations, which are considered risky from obesity susceptibility genes, by using DNA sequencing and qPCR analysis methods.

METHODS

The study consists of 200 patients who came to Istanbul Faculty of Medicine, Department of Internal Medicine and Department of General Surgery with the complaint of obesity. Blood and buccal mucosa swab samples were taken from the participants. The patients were determined

according to the body mass index (BMI) of the patients. Non-obese, overweight, obese, and morbidly obese. DNA isolation was performed by a silica DNA column-based method. In order to test the success of the DNA isolation method, DNA was isolated from 100 different blood samples and the quality of the obtained DNA was measured by spectrophotometric methods. After DNA isolation, variatiants in related genes was investigated by using DNA sequencing and qPCR analysis methods. The design of oligos targeting SNPs was performed with the Primer 3 and Primer Blast bioinformatics tools. Also, our study targets the SNPs that are associated with BMI and genetic risk scores. Polymorphisms associated with the genes for which we have performed PCR and DNA sequence analysis and SNP analyzes, both within the framework of projects and routine applications, have been taken into account. The results were evaluated in line with statistical analysis.

Table 1: Interpretation of SNP analysis results

Gene	Risk	Low risk	Normal	Clinical risk
ANKK1	TT	TC	CC	Decrease in the number of dopamine binding sites in the brain and cause excessive appetite
CETP	AA	AG	GG	Predisposition to weight gain associated with abdominal obesity and HDL-cholesterol levels.

RESULT

As a result of the analysis of the correlation between the mutation profiles and BMI values of the participants, a statistically significant relationship was found between the BMI and the mutation profile ($p<0.05$). Results in qPCR and DNA sequence analyzes were found to be compatible. CETP and ANKK1 gene variants were found to give corelative results in obese and morbidly obese individuals in both analysis methods. Interpretation of SNP analysis results in CETP and ANKK1 genes are given in Table 1. Comparison of polymorphism q PCR and DNA sequence analysis results in ANKK1 and CETP

Table 2: Comparison of polymorphism q PCR and DNA sequence analysis results in ANKK1 and CETP genes in terms of risk ratios (Some Part)

Test results for ANKK1 (R= Risk, LR=Low Risk, N= Normal)				
Patients	**Results**	**DNA Sequence Analysis**	**qPCR**	
			R	**LR**
A1	LR	TC	31	28
A2	N	CC	23	23
A3	LR	TC	26	19
A4	R	TT	18	22
A5	LR	TC	33	25
A6	N	CC	23	23
A7	R	TT	28	33
A8	R	TT	25	33
A9	LR	TC	25	22
A10	R	TT	23	28
A11	R	TT	22	26
A12	LR	TC	29	25
A13	R	TT	25	30
A14	N	CC	23	23
A15	R	TT	26	32
A16	R	TT	18	22
A17	LR	TC	32	28
A18	LR	TC	29	24
A19	LR	TC	33	28
A20	LR	TC	27	23
A21	LR	TC	27	20
A22	LR	TC	32	28

Table 3: Body Mass Index (BMI) values of the participants and mutation profiles determined by Major Component Analysis (PCA) (Some Part)

Participant	PCA	BMI	Participant	PCA	BMI
A1	-0.16	24.8	C1	-0.086	33.3
A2	-0.216	20.9	C2	-0.067	33.3
A3	-0.276	23.5	C3	-0.075	31.5
A4	-0.216	19.3	C4	-0.086	31.3
A5	-0.344	22.8	C5	-0.034	34.9
A6	-0.232	20.6	C6	-0.081	30.6
A7	-0.264	21.2	C7	-0.086	34.6
A8	-0.216	18.7	C8	-0.034	34.9
A9	-0.344	22.5	C9	-0.082	32.4
A10	-0.32	24.6	C10	-0.047	33.3
B1	-0.07	25.2	D1	-0.083	40.8
B2	-0.038	26.7	D2	-0.083	36.0
B3	-0.07	27.6	D3	-0.084	44.1
B4	-0.072	26.8	D4	-0.076	39.5
B5	-0.07	27.6	D5	-0.068	39.0
B6	-0.067	29.0	D6	-0.083	35.6
B7	-0.062	25.2	D7	-0.084	45.3
B8	-0.075	26.8	D8	-0.073	38.3
B9	-0.073	29.2	D9	-0.066	35.3
B10	-0.073	29.2	D10	-0.048	35.2

genes in terms of risk ratios (table 2). Body Mass Index (BMI) values of the participants and mutation profiles determined by Major Component Analysis (PCA) are given in Table 3. Comparison of results obtained from blood and cheek epithelium Table 4.

CONCLUSIONS

Although obesity is a visual problem, the main problem is diabetes, heart attack, fatty liver, high blood pressure, paralysis, sleep apnea, infertility and many types of cancer. (9). According to WHO data for 2014, It is reported that throughout the world, being overweight in adults over the age of 18 has reached 39%, and that 13% of them are obese, and it is increasing globally (10). According to the study of Satman et al., It has been reported that one out of every three people in Turkey has reached the obesity limit (11). In recent studies, it has been seen that genetics has a decisive importance in obesity. Children given to foster parents, when their body weights were examined, were found to be compatible with the biological family (1).

BMI is affected by many SNPs with minor effects (12). The results obtained in our study were also compatible with the literature. As a result of the analysis

Table 4. Comparison of results obtained from blood and cheek epithelium

SNP		DNA Extraction from Blood		DNA Extraction from Cheek Epithelium	
		QPCR		QPCR	
		Allele Type		Allele type	
		Risk	Normal	Risk	Normal
		Cq	Cq	Cq	Cq
rs1800497	Sample No:1-6	26.28	26.31	33.09	33.12
rs5882		29.97	36.06	36.78	Neg
rs1800497		32.82	27.27	39.63	34.05
rs5882		32.77	27.32	39.58	34.1
rs1800497		26.32	32.63	33.13	39.41
rs5882		26.45	26.25	33.26	33.06
rs1800497		29.8	35.89	36.61	Neg
rs5882		26.49	32.58	33.3	39.36
rs1800497		36.08	30.69	Neg	37.47
rs5882		28.74	28.54	35.55	35.35
rs1800497		28.57	28.6	35.38	35.41
rs5882		36.25	30.74	Neg	37.52

of the correlation between the mutation profiles and BMI values of the participants, a statistically significant relationship was found ($p<0.05$). The aim of this study was to determine the polymorphisms of blood and buccal mucosa swab samples with different analysis methods (qPCR and DNA sequence) that can provide comprehensive identification of the genetic obesity risk in individuals. In addition, it is aimed to establish a panel with other similar obesity susceptibility genes. CETP and ANKK gene variants, which carry potential risk of obesity, were determined by two analysis methods to be important in Obesity. As a result, variants in the CETP and ANKK1 genes are associated with the development of obesity and metabolic processes of obesity. Changes in ANKK1 activity may provide an alternative explanation for neuropsychiatric disorders (2). it has been reported in many studies that polymorphisms in the CETP gene affect plasma lipid levels (13). Screening for mutations in genetic obesity risk in individuals It will be of great benefit in the preliminary diagnosis of the disease and in the follow-up of the metabolic process. It is aimed to create a general panel with CETP and ANKK1 and other similar obesity predisposition genes. In this sense, we believe that the study will shed light on other studies.

Key Messages:

Obesity can be defined as a disorder in the system that regulates body weight. Obesity can be defined as a disorder in the system that regulates body weight. Genetic predisposition is effective in obesity at a rate of 20% to 80%. Therefore, We investigated CETP and ANKK1 gene variations, which are considered risky from obesity susceptibility genes, by using DNA sequencing and qPCR analysis methods.

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INTERRACTION OF APOA2/APOA5 GENE VARIATIANTS BY USING QPCR AND DNA SEQUENCING IN OBESE PATIENTS

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INTRODUCTION

Obesity can be defined as a disorder in the system of regulating body weight, characterized by excessive accumulation of body fat. As obesity increases, the risk of obesity-related hypertension, diabetes, cardiovascular diseases, stroke, infertility and cancer. Anatomical distribution of body fat has a significant impact on associated health conditions. It has been seen in studies that genetics has a decisive importance in obesity. (1). Genetic predisposition is effective in obesity at a rate of 20% to 80%. (2). Many genes and mutations have been identified that can express the predisposition to obesity, the development of obesity, and the link of obesity with metabolic processes. One of these genes is Apoliprotein A2 (APOA2). The protein synthesized by the APOA2 gene is the second most abundant protein of the high-density lipoprotein particles. showed that in both mice and humans, the APOA2 gene is linked to a gene that controls plasma levels of apoA-II and that the APOA2 gene or its product influences, by an unknown mechanism, plasma levels of free fatty acids (FFA) (3). Triglyceride and HDL-C levels were significant predictors of apoA-II levels (4). Another obesity-related gene is Apoliprotein A5 (APOA5). It is known that APOA5 has a role in determining plasma triglyceride levels independent of age (5). Polymorphisms in APOA5 is strongly associated with plasma triglyceride concentrations.(6). The APOA5 gene polymorphism is associated with Hypertriglyceridemia, Hyperlipoproteinemia, Type V and Hypertriglyceridemia (7-9).

We aimed to evaluate APOA2 and APOA5 gene variations, which are considered risky from obesity susceptibility genes, by using DNA sequencing and qPCR analysis methods.

METHODS

The study consists of 200 patients who came to Istanbul Faculty of Medicine, Department of Internal Medicine and Department of General Surgery with the complaint of obesity. Blood and buccal mucosa swab samples were taken from the participants. The patients were determined according to the body mass index (BMI) of the patients. Non-obese, overweight, obese, and morbidly obese. DNA isolation was performed by a silica DNA column-based method. In order to test the success of the DNA isolation method, DNA was isolated from 100 different blood samples and the quality of the obtained DNA was

measured by spectrophotometric methods. After DNA isolation, variatiants in related genes was investigated by using DNA sequencing and qPCR analysis methods. The design of oligos targeting SNPs was performed with the Primer 3 and Primer Blast bioinformatics tools. Also, our study targets the SNPs that are associated with BMI and genetic risk scores. Polymorphisms associated with the genes for which we have performed PCR and DNA sequence analysis and SNP analyzes, both within the framework of projects and routine applications, have been taken into account. The results were evaluated in line with statistical analysis.

RESULT

As a result of the analysis of the correlation between the mutation profiles and BMI values of the participants, a statistically significant relationship was found between the BMI and the mutation profile ($p<0.05$). Results in qPCR and DNA sequence analyzes were found to be compatible. APOA2 and APOA5 gene variants were found to give corelative results in obese and morbidly obese individuals in both analysis methods. Interpretation of SNP analysis results in APOA2 and APOA5 genes are given in Table 1. Body Mass Index (BMI) values of the participants and mutation profiles determined by Major Component Analysis (PCA) are given in Table 2. Comparison of

Table 1: Interpretation of SNP analysis results

Gene	Risk	Low risk	Normal	Clinical Risk
APOA2	CC	TC	TT	Predisposition to fat accumulation around the waist and abdomen. Predisposition to obesity development with increase in body mass index in connection with saturated fatty acid
APOA5	GG	GA	AA	Predisposition to triglyceride-dominated fat accumulation, increased body mass index, and obesity. Susceptibility to high levels of total cholesterol, LDL-cholesterol, systolic and diastolic blood pressure and triglyceride levels, and low HDL-cholesterol levels during the fasting period.

Table 2: Body Mass Index (BMI) values of the participants and mutation profiles determined by Major Component Analysis (PCA) (Some Part)

Participant	PCA	BMI	Participant	PCA	BMI
A1	-0.16	24.8	C1	-0.086	33.3
A2	-0.216	20.9	C2	-0.067	33.3
A3	-0.276	23.5	C3	-0.075	31.5
A4	-0.216	19.3	C4	-0.086	31.3
A5	-0.344	22.8	C5	-0.034	34.9
A6	-0.232	20.6	C6	-0.081	30.6
A7	-0.264	21.2	C7	-0.086	34.6
A8	-0.216	18.7	C8	-0.034	34.9
A9	-0.344	22.5	C9	-0.082	32.4
A10	-0.32	24.6	C10	-0.047	33.3
B1	-0.07	25.2	D1	-0.083	40.8
B2	-0.038	26.7	D2	-0.083	36.0
B3	-0.07	27.6	D3	-0.084	44.1
B4	-0.072	26.8	D4	-0.076	39.5
B5	-0.07	27.6	D5	-0.068	39.0
B6	-0.067	29.0	D6	-0.083	35.6
B7	-0.062	25.2	D7	-0.084	45.3
B8	-0.075	26.8	D8	-0.073	38.3
B9	-0.073	29.2	D9	-0.066	35.3
B10	-0.073	29.2	D10	-0.048	35.2

results obtained from blood and cheek epithelium (Table 3).

Table 3. Comparison of results obtained from blood and cheek epithelium

SNP		DNA Extraction from Blood		DNA Extraction from Cheek Epithelium	
		QPCR		QPCR	
		Allele Type		Allele type	
		Risk	Normal	Risk	Normal
		Cq	Cq	Cq	Cq
rs5082	Sample No:1-6	29.76	34.54	36.57	41.32
rs662799		29.86	29.66	36.67	36.47
rs5082		31.25	25.49	38.06	32.27
rs662799		32.58	25.98	39.39	32.76
rs5082		26.24	26.27	33.05	33.08
rs662799		27.57	27.37	34.38	34.18
rs5082		26.28	31.06	33.09	37.84
rs662799		31.09	37.4	37.9	Neg
rs5082		28.53	28.56	35.34	35.37
rs662799		37.59	29.4	Neg	36.18
rs5082		34.73	28.91	41.54	35.69
rs662799		27.61	32.39	34.42	39.17

CONCLUSIONS

Although obesity is a visual problem, the main problem is diabetes, heart attack, fatty liver, high blood pressure, paralysis, sleep apnea, infertility and many types of cancer (10). According to WHO data for 2014, It is reported that throughout the world, being overweight in adults over the age of 18 has reached 39%, and that 13% of them are obese, and it is increasing globally (11). In recent studies, it has been seen that genetics has a decisive importance in obesity. Children given to foster parents, when their body weights were examined, were found to be compatible with the biological family (1). BMI is affected by many SNPs with minor effects (12). The results obtained in our study were also compatible with the literature. As a result of the analysis of the correlation between the mutation profiles and BMI values of the participants, a statistically significant relationship was found ($p<0.05$). The aim of this study was to determine the polymorphisms of blood and buccal mucosa swab samples with different analysis methods (qPCR and DNA sequence) that can provide comprehensive identification of the genetic obesity risk in individuals. In addition, it is aimed to establish a panel with other similar obesity susceptibility genes APOA2 and APOA5 gene variants, which carry potential risk of obesity, were determined by two analysis methods to be important in Obesity. As a result, variants in the APOA2 and APOA5 genes are associated with the development of obesity and metabolic processes of obesity. Showed that in both mice and humans, the APOA2 gene is linked to a gene that controls plasma levels of apoA-II and that the APOA2 gene or its product influences, by an unknown mechanism, plasma levels of free fatty acids (FFA) (3). Triglyceride and HDL-C levels were significant predictors of apoA-II levels (4).

In a molecular study of an American Caucasian familial hypercholesterolemia with 1,135 members, it was reported that a mutation in the LDLR gene of the SNP in the promoter of the APOA2 gene causes hypercholesterolemia and affects low-density lipoprotein (LDL) levels (13). APOA5 haplotypes are found in approximately 16% of Caucasians and are associated with increased plasma triglyceride concentrations (14). In studies to examine the effect of APOA5 variants

on plasma lipids, among 2,808 healthy middle-aged male subjects homozygous for the S19W and -1131T-C SNPs in APOA5 had 52% and 40%higher triglycerides, respectively, compared to common allele homozygotes (15). In a Chinese study, a variant in the APOA5 coding region was reported to cause hypertriglyceridemia (8). The APOA5 gene polymorphism is associated with Hypertriglyceridemia, Hyperlipoproteinemia, Type 5 and Hypertriglyceridemia (8, 9, 14). Based on this information, we thought that APOA2 and APOA5 gene variants with potential obesity risk are important in Obesity, and we studied these genes with two analysis methods. As a result, variants in the APOA2 and APOA5 genes are associated with the development of obesity and metabolic processes of obesity. It is aimed to create a general panel with CETP and ANKK1 and other similar obesity predisposition genes. In this sense, we believe that the study will shed light on other studies.

Key Messages:

Obesity can be defined as a disorder in the system of regulating body weight, characterized by excessive accumulation of body fat. Genetic predisposition is effective in obesity at a rate of 20% to 80%. Therefore, We investigated APOA2 and APOA5 gene variations, which are considered risky from obesity susceptibility genes, by using DNA sequencing and qPCR analysis methods.

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INVESTIGATION OF THE EFFECT OF TRAIL IN OBESE PATIENTS

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INTRODUCTION

Obesity has become most common healthcare problems worldwide in recent years. Europe Cardiovascular Disease Statistics (ATLAS) explained that obesity is most common type of inflammatuar decease in males (22,9%), and in females (35,8%) in Turkey [1]. The common etiological factors includes nutritional disorders, inactive life, environmental and genetic factors [2]. In addition, obesity is associated with changes in the amount of secretion of adipokines-adiponectin and Tumor Necrosis Factor(TNF)-alpha [3].

Tumor necrosis factor-associated apoptosis-inducing ligand (TRAIL), which is a member of the TNF-ligand family, has important roles in the pathogenesis of obesity and obesity-related diseases [4]. The previous studies suggested that TRAIL plays protective roles against obesity in *in-vitro* systems and in mice-models. However, a direct role for TRAIL is not fully established yet for obesity in human [5,6].
In the literature, the results of studies considering the role of TRAIL in obesity are quite conflicting [5–8], therefore, in the present study, we aimed to determine the expression levels of TRAIL in obese patients and its role in the risk of obesity development.

MATERIALS AND METHODS

Participants

This case-control study includes 160 individuals (80 obese patients and 80 healthy controls). The control group was selected from healthy individuals without any pathological findings and the patient group was consisted of patients diagnosed with obesity followed by Istanbul University Hospital.

All participants in the study provided their written consent prior to the study. This study approved with the Helsinki Declaration. The study protocol was confirmed by both the Research Fund of Istanbul University (Project No: TDK-202036578).

Expression Level of TRAIL

TRAIL expression level was obtained with RT-PCR method. Firstly, the izolation of the nucleic acid was carried out by robotic device (RINA™ M14). Secondly, it was performed with cDNA isolation by commercial kit. Lastly, the RT-PCR protocol was applied.

Statistical Analysis

All the statistical analyses were carried out using the Mann– Whitney U test. Estimation of relative risk was determined by calculating confidence intervals and odds ratio (OR). $p< 0.05$ was accepted as statistically significant.

RESULTS

Accordingly, the control and patient groups have no difference of age and sex (*p>0.05*). In the present study, the means of expression levels of TRAIL in control patients versus obese group were 54.57 ± 288.39 → 0.26 ± 0.56 ng/ml (*p<0.001*), respectively. However, according to age and gender when we examine expression levels of TRAIL, no significant diference was obtained *(p> 0.05).*

CONCLUSIONS

Obesity has become most common public healthcare problems worldwide in recent years. Among various inflammatory disease obesity has significant priority with high mortality and morbidity rates [1]. In addition to common risk factors, the relationship of TRAIL with obesity has begun to be investigated more in the last decade. Moreover, in literatüre it was shown that TRAIL has important roles in the pathogenesis of obesity and obesity-related diseases [4].

Bernardi et al. (2012) showed that weekly injections of TRAIL in wild-type mice placed on a high-fat diet resulted in smaller fat mass compared to controls. [6]. Similarly, in our study, the expression level of TRAIL was significantly higher in the control group than the patient group which suggested ta protective role for TRAIL in obesity. Moreover, the distribution of TRAIL expression was found increase in control group compared to obese patients and fold change was estimated at approximately 54.57 fold (2-ΔΔCt method) ($p<0.001$).

As it was well known that life style activations including intake of high-energy foods, body mass index (BMI), genetic predisposition, endocrine disorders, inflammatory, psychiatric diseases and use medicine for them and insufficient physical exercise were important factors that affect obesity [9]. Accordingly, in our study, at least in order to eliminate the effect of drug use in obesity, individuals who did not have any pathological disease and did not use drugs due to a different metabolic or inflammatory disease other than obesity were included in the study while forming the patient and control group.

In order to reach a definite conclusion, further studies with wider metabolic groups, increasing the number of cases and including larger clinical data and biochemical parameters are needed.

Acknowledgements

The study protocol was confirmed by both the Research Fund of Istanbul University (Project No: TDK-202036578).

Key Messages

- There is relationship between expression level of TRAIL and obesity.
- TRAIL has a protective role against obesity.
- TRAIL has a protective role of adipose tissue dysfunction.

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EVALUATION OF MUTATION PROFILES OF PLIN AND POMC GENES IN DIFFERENT BMI GROUPS

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INTRODUCTION

Obesity is generally rooted in a complex interplay between genetic and environmental factors such as culture, socioeconomical status, and lifestyle, leading to an alarming health concern in the 21st century (1-3). Over the past two decades, the worldwide prevalence of overweight and obesity has risen dramatically, mainly driven by socioeconomical and lifestyle changes manifested by lower energy expenditure (physical activity) and increased usage of energy-rich food sources, especially refined carbohydrates (1, 4). Uncorrected obesity unfavorably impacts all aspects of physiological functions, lowers quality of life, and increases the risk of illness and health-care burden worldwide (5).

Obesity is an anthropometric trait resulting from a complex interplay of genetic and environmental factors. The gene-environment interactions are generally responsible for changes in gene expression and epigenetic modifications leading to excess body fat and obesity (6). obesity does not usually follow the Mendelian rule of inheritance and genetic factors only explain a small proportion of the development of obesity (7). The obesity trait may be triggered by both single genes (monogenic) and multiple genes (common or multifactorial obesity). In addition to genetic factors, epigenetic contributions (i.e., modifications pre- or posttranslation) also play an essential role in obesogenesis. Loss-of-function mutations in genes including LEP, LEPR, MC4R, PCSK1, ADCY3, hypothalamic proopiomelanocortin (POMC), and SIM1 have all been associated with monogenic obesity (8).

POMC (Proopiomelanocortin)

POMC is a protein precursor to many other hormones and neu- ropeptides . It is released by hypothalamic neurons in re- sponse to insulin and leptin . The protein is cleaved into sev- eral smaller peptides vital to normal function such as adrenocor- ticotropic hormone (ACTH), α-MSH, and β-endorphin, among others .

PLIN (Perilipin)

It encodes a protein localized on the surfaces of lipid droplets in adipocytes, steroid-producing cells, and atherosclerotic plaque formations. It plays a key role in the cellular regulation and mobilization of triglyceride accumulation (9-10).

MATERIALS AND METHODS

In the study we used peripheral blood from 200 patients who applied with the complaint of obesity.

Samples were grouped as 50 patients Non-obese (BMI<25)-Overweight (25≤BMI<30)-Obese (30≤BMI<35)-Morbid obese (35≤BMI). we analyzed PLIN and POMC genes by using DNA sequencing and qPCR methods. The isolation of the nucleic acid was carried out by robotic device (RINA™ M14). It was performed with cDNA isolation by commercial kit. Lastly, the RT-PCR protocol was applied.

Statistical Analysis

All the statistical analyses were carried out using the Mann– Whitney U test. Estimation of relative risk was determined by calculating confidence intervals and odds ratio (OR). $p< 0.05$ was accepted as statistically significant.

RESULTS

As a result of, the mutation profiles and BMI values of the participants, a statistically significant relationship was found between the BMI and the mutation profile (r=0.628, p=0.00; statistical significance p<0.05). The results obtained by qPCR and DNA sequence analysis showed 100% agreement.

PLIN and POMC gene variants were found to give corelative results in obese and morbidly obese individuals in both analysis methods.

CONCLUSIONS

Obesity, defined by an excess of body fat impacting on health, is a complex disease resulting from the interaction between many genetic/epigenetic factors and environmental triggers. Investigating genetic drivers in the development of obesity is an important area of focus, as genetics move to the forefront of medicine and personalized treatment evolves.

Recent human studies suggest POMC DNA methylation is influenced by maternal nutrition in early pregnancy and associated with childhood and adult obesity.

PLIN and POMC genes are associated with the development of obesity and metabolic proceses of obesity. As technology evolves to allow for detailed characterization of genetic mutations, large, well-controlled studies of genetic mutations from multi-ethnic populations, and their subsequent outcomes on surgical and non-surgical weight loss, must be undertaken. Further re- search must be done in order to fully understand the implica- tions and prognostic predictors, as well as appropriate man- agement of these diseases.

Acknowledgements

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Key Messages

- There is relationship between qPCR and DNA sequence analysis
- There is important for the detection of polymorphisms that can provide a comprehensive definition of obesity risk

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INVESTIGATION OF THE EFFECT OF PACLITAXEL ON MIR-221 EXPRESSION IN BREAST CANCER STEM CELLS

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INTRODUCTION

Breast cancer is the most common malignancy and the leading cause of death among women in the world. Despite the development of early diagnosis methods and advances in treatment in recent years, it is still an important cause of mortality and morbidity (Adhami et al. 2018). It is known that many cancer types, including breast cancer, are driven by a population of cells displaying stem cell characteristics. These cells, called cancer stem cells (CSCs) or tumor initiating cells, are known to not only promote tumor initiation and growth, but also mediate tumor metastasis and therapeutic resistance (Yu et al. 2012). On the other hand, Breast cancer stem cells (BCSCs), consist of a small subpopulation of cells within the tumor mass that exhibit stem cell-like properties and are responsible for tumor development, recurrence, and metastasis in breast cancer (Liu et al. 2012). The complexity of control of gene expression in BCSCs is mediated by numerous signaling pathways, often triggered by extracellular signals, mutations, and epigenetic control. Recent studies show that abnormal activation of these pathways causes tumor development, increased tumor aggressiveness and treatment resistance in breast cancer (Takebe et al. 2011). Therefore, there is still a need for research and clinical use of new molecular biomarkers in the treatment of breast cancer. MicroRNAs (miRNAs) are non-coding RNAs consisting of 18-24 nucleotides. By inhibiting translation or regulating the stability of target mRNAs; they control cell proliferation, differentiation, invasion and apoptosis. Recent evidence shows that miRNA dysregulation in breast cancer is associated with CSCs and related signaling pathways (Zhang et al. 2014). In addition, chemotherapy; It is the most common and effective method used in the treatment of breast cancer. However, it is common for patients to fail to respond to treatment during chemotherapy or for cancer to recur after treatment. Although more than one anticancer drug is generally applied to achieve success in breast cancer treatment, drug resistance acquired later or existing before treatment greatly hinders success in breast cancer chemotherapy (Magee et al. 2012). Paclitaxel (taxol) is an important chemotherapeutic agent used in the treatment of solid tumors including breast, ovarian, lung, head and neck cancers. Paclitaxel, one of the taxoid group drugs from plant alkaloids, binds to the β-tubulin subgroup of microtubules during mitosis, prevents the conversion of microtubules into

tubulins and the destruction of spindle fibers formed during mitosis, and stops cell division. Although there is frequent resistance to various chemotherapeutic agents, including paclitaxel, in breast cancer metastasis and recurrence, the mechanism of resistance of breast cancer cells to paclitaxel is not fully understood (Zhou et al. 2010). Although miRNAs have proven to have important roles in drug resistance and tumorigenesis, the development of paclitaxel resistance and the interaction between miRNAs is still a topic of research. On the other hand, after determining that miRNAs act as regulators of gene expression, these RNA molecules were evaluated as both potential therapeutic targets and candidate diagnostic and prognostic indicators.

MATERIAL AND METHODS

In the current study, following paclitaxel treatment, differences in expression levels of let-7a, miR-155, miR-10b, miR-221, miR-222, miR-335, miR-145, miR-200c, miR-21 and miR-125b, which are important in breast cancer development, were investigated in parental MCF-7 and MCF-7s cells. In this direction, the obtaining of breast cancer cancer stem cells and the characterization of these cells were provided. Then, the cytotoxic activity of paclitaxel was determined and its effect on the spheroid structure was observed. However, parental MCF-7 and MCF-7s cells were treated with 15.93 μM dose of paclitaxel for 24 and 48 hours, and RNA isolation and cDNA synthesis were performed from these cells. Subsequently, changes in the expression levels of let-7a, miR-155, miR-10b, miR-221, miR-222, miR-335, miR-145, miR-200c, miR-21 and miR-125b were statistically evaluated in Sabiosciences PCR-Data Analysis (RT^2 profiler PCR array data analysis version 3.5) web-based program (StepOnePlus™, Real-Time PCR, Applied Biosystems™).

RESULTS AND DISCUSSION

Results revealed that, miR-221 was 28.37-fold upregulated after 24 h of paclitaxel treatment in MCF-7s cells *($p=0.0007$).* This finding, supported by further functional analyzes, may reveal that miR-221 can play an important role in breast cancer and cancer stem cell biology and can be used as an effective treatment option.

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PSYCHOLOGY, PHYSIOLOGY AND SPORTS WITH TODAY'S PERSPECTIVE: MULTIDISCIPLINARY STUDY

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Sports has come to its current form by passing some stages such as being a way of life in the change from the first societies to modern societies, capturing the aesthetic and physical beauties, being a source of entertainment for the elites, preparing for wars and being a means of cold war. Within this change, sports started to gain scientific direction from the 20th century onwards due to changing social conditions. As the field matures, interests in sport and exercise psychology research and practices spread across different cultures and geographic locations. Similar growth in interests could have been observed in Turkey. With the increase in the importance given to sports and athletes and the successes in the Olympics, it can be predicted that these studies will increase even more. Sport is a lifestyle for professional athletes. It is a battle of power and success within its own world. Achievements and failures are a life that provides a balance and confronts the athlete with the facts.

It was not until the 1980s that the term ‘exercise psychology’ was widely used in the international literature. Exercise and sport psychology began in the 1890s with a series of sport-related psychological experiments. Dr. Coleman Griffith, a professor at the University of Illinois, created the first sport psychology course, text, and laboratory in the 1920s. The field has continued to grow, particularly in the last two decades. Issues related to training, licensure, ethics, image, and job opportunities continue to be problematic, but the future of the profession is bright.

Sports has come to its current form by passing some stages such as being a way of life in the change from the first societies to modern societies, capturing the aesthetic and physical beauties, being a source of entertainment for the elites, preparing for wars and being a means of cold war. Within this change, sports started to gain scientific direction from the 20th century onwards due to changing social conditions.

Sport is a lifestyle for professional athletes. It is a battle of power and success within its own world. Achievements and failures are a life that provides a balance and confronts the athlete with the facts.

It is defined as “representing a convergence of exercise science and psychology . . . [and] is concerned with (1) the application of psychological principles to the promotion and maintenance of leisure physical activity (exercise), and (2) the psychological and emotional consequences of leisure physical activity”. Rejeski and Brawley (1988) and Matarazzo's (1980) definition of health psychology to exercise: “the application of educational, scientific, and professional contributions of psychology to the promotion, explanation, maintenance, and enhancement of behaviors related to physical work capacity”. Today, the phrase ‘physical work capacity’ might

better be changed to ‘physical activity’ or even ‘physical activity for health’.

The role of exercise psychology, however, is less clearly demarcated. The research concerning physical activity and public health has adopted a behavioral medicine approach, of which psychology is just one part. The important thing is that we are trying to create a conceptual distance between psychologies relevant for the understanding of competitive behaviors (sport psychology) from that of physical activity for health (exercise psychology). Exercise psychology at its simplest can be defined as the study of a) the psychological correlates or determinants of physical activity, b) psychological factors and strategies influencing physical activity behavior change, and c) the psychological outcomes of involvement or non-involvement in physical activity.

There are multiple factors in the success of professional athletes and successful athletes. When these are examined one by one, the performance of the athlete, his abilities to the sport and his sporting capacities are considered as the most effective variables. However, it would be more accurate to distinguish them as internal and external factors. Internal factors determine the limits of the person while external factors are effective in reaching these limits. While the innate characteristics of the athlete change at a very low level due to external factors, their physiology becomes more decisive on the way to adulthood.

Physical activity behavior change requires knowledge of physical health outcomes, behavioral measurement issues, environmental influences, and social/cultural context. As such, the field of exercise psychology may be only one part of what is now known as ‘behavioral medicine’.

It is well-documented that regularly engaging in exercise and physical activity brings physical and mental benefits for individuals. In terms of physical benefits, individuals who are more active: (a) have lower rates of all-cause mortality, coronary heart disease, high blood pressure, stroke, type 2 diabetes, metabolic syndrome, and colon and breast cancer; (b) have a lower risk of hip or vertebral fracture; (c) have a higher level of cardiorespiratory and muscular fitness; (d) are more likely to maintain weight, have healthier body mass and composition, and (e) have delayed disease onset (WHO, 2010). As for mental health, regularly participating in physical activity and exercise slow down cognitive decline, reduce symptoms of depression, schizophrenia, and dementia; and improves mood (WHO, 2019). Because physical activity and exercise provide many benefits, many scholars have devoted themselves to exercise science research areas such as exercise physiology, biomechanics, motor learning and control, and our main focus – exercise psychology.

In recent years, with the development of genetic science as well as environmental factors affecting athletes' performance, genetic factors have become one of the main research topics. The combination of psychological factors and genetic factors and supporting each other will constitute an important

model for athlete health, performance development and success.

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