X. International Congress of Molecular Medicine

23-27 September 2024
Çeşme-İzmir

CONGRESS FULLTEXT BOOK

Welcome Message

Dear Colleagues,

On behalf of the Organizing Committee I am delighted to invite you to the 10th International Congress of Molecular Medicine that will be held in Çeşme-Izmir, Turkiye on 23rd - 27th of September 2024 under the auspices of the Turkish Society of Molecular Medicine.

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

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

On the other hand, this year, in which we celebrate the 25th anniversary of the Turkish Society of Molecular Medicine, is of particular importance for our congress.

We will be looking forward to meet you in September 2024 for this congress we are organizing for the 10th time this year, with leading scientists in their field and researchers who will make their valuable contributions.

Prof. Dr. Umit Zeybek

Chair of the Turkish Society of Molecular Medicine

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Baran Yusuf

Congress Honorary President, Rector İzmir Institute of Technology

İsbir Turgay

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Çeşme-İzmir

INVITED SPEEKER PROGRAM

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24 September 2024 - Tuesday	
10:30 – 11:30	**PROTOCOL** - **Ümit Zeybek** - Congress President, Head of Turkish Molecular Medicine Association - **Kenan Türkdoğan** - Rector, Alanya Alaaddin Keykubat University - **Yusuf Baran** - Rector, İzmir Institute of Technology, Congress Honorary President **WELCOME NOTE** - **Yusuf Baran** Molecular Mechanisms of Drug Resistance
11:30 - 12:15	**PLENARY LECTURE** **Moderators: Bülent Özpolat** - **Mustafa Djamgöz** Cancer Neuroscience - A New Discipline in Oncology
13:00 – 15:00	**Panel 1: RNA Biology in Health and Diseases** **Moderators: Alper Tunga Özdemir** - **Alper Tunga Özdemir** Chimeric Antigen Receptor (CAR) Approaches in Autoimmune Diseases - **Bilge Özdemir** Mesenchymal Stem Cell Experiences in the Clinic - **Mustafa Diken** mRNA-based Cancer Vaccines - **Elif Diken** Single-Cell Insights into Immunotherapy Efficacy and Mechanisms
15:15 – 16:00	**KEYNOTE LECTURE** **Moderators: Akif Turna** - **Suresh C. Jhanwar** Cancer Genomics in Translational Research and Precision Medicine: A Brief Overview of Current Status and Future Prospects
16:15 – 18:15	**Panel 2: Health Informatics (Bioinformatics) & Artificial Intelligence in Health** **Moderators: Mükerrem Betül Yerer Aycan, Tunç Akkoç** - **Akif Turna** AI in Thoracic Surgery - **Mehmet Baysan** Systematic Analysis of Genetic Variant Identification and Pathogenicity Prediction - **Uzay Görmüş DeGrido** The Fundamentals and Sustainability of Future Science in the Medical and Research Laboratories - **Ceyda Açılan Ayhan** Epidrug Screening Identifies Type I PRMT Inhibitors as Modulators of Lysosomal Exocytosis and Drug Sensitivity in Cancers

25 September 2024 - Wednesday	
09:30 – 10:15	**KEYNOTE LECTURE** **Moderators: Gül Güner Akdoğan** - **Luciano Saso** Pharmacological Modulation of NRF2
10.30 – 12:30	**Panel 3: Advances in Natural Product Biochemistry: in Terms of Basic and Clinical Perspective and Nanomedicine** **Moderators: İlhan Yaylım, Ammad Ahmad Farooqi** - **Ammad Ahmad Farooqi** Targeting of Oncogenic Pathways by Natural Products: Underlying Mechanisms and Proof-of-Concept Animal Model Studies - **Suna Timur** In-Vitro Diagnostics and Nanomedicine - **Mükerrem Betül Yerer Aycan** Nano solutions for Big Problems: Targeted Drug Delivery Systems - **Burcu Okutucu** Biopolmer Based Nanoparticles for Metabolic Diseases
13:00 – 15:30	**Panel 4: Molecular Cancer Medicine; Past & Present, and Future Prospects and Medical Solutions** **Moderators: Hilal Koçdor, Zekiye S. Altun** - **Figen Zihnioğlu** Multifunctional Bioactive Peptides for Cancer Diagnosis and Therapy - **Safiye Aktaş** Fusion Analyzes and Clinical Significance in Next-Generation Sequencing in Tumor Tissue - **Güliz Armağan** A Novel Approach to Drug Resistance in Cancer: Ferroptosis - **Yasemin Soysal** Wound Age Determination in the Perspective of Forensic Biology - **Özkan Doğanay** New Insight into Lung Function Using Novel Contrast MR: from Anatomical to Molecular Imaging
15:45 – 17:45	**Panel 5: Cell Signalling and Metabolism: New Trends in Basic and Clinical Approaches** **Moderators: Nuray Ulusu, Özlem Timirci Kahraman** - **Aylin Sepici Dinçel** Osteporosis Treatment via Wnt Signalling Pathway - **Mutay Aslan** Plasma Sphingolipidomic Profile in Insulin Resistance and Diabetic Dyslipidemia - **Tunç Akkoç** Cancer İmmunotherapy and CarT Cell - **Nihal Karakaş** Stem Cell Treatment Approaches for Covid19 and Possible Viral Pandemics

26 September 2024 - Thursday	
10:00 – 10:45	**KEYNOTE LECTURE** **Moderators: Engin Ulukaya** - **Bülent Özpolat** Development of Novel Targeted Therapies for Solid Cancers
11:00 – 12:30	**Panel 6: Nutrition and Molecular Medicine** **Moderators: Ali Osman Gürol, Gülsen Meral** - **Eugenia Bezirtzoglou** Functional Dairy Foods and Microbiota Modulation - **İrina Danilova** Beta-cells in Diabetes: New Look at the Old Problem - **Christina Tsigalou** Mediterranean Dietary Patterns and Immunonutrition as Potential Intervention upon Gut Microbiome in IBD. Wishful Thinking or a Vision of the Future?
13:00 – 15:30	**Panel 7: Molecular Aspects in Forensic Sciences** **Moderators: Emel Hülya Yükseloğlu, Mutay Aslan** - **Itır Tarı Cömert** Genetic Signatures: Revealing Insights into Criminal Profiling and Violence - **Ceren Özbaşaran Tan** The Psychological Impact of Genetic Testing - **Meryem Ebedi** Exploring Addiction, Violence, and Genetic Insights in Forensic Science - **Nazlı Hölümen** Celebrating Uniqueness: The Power of Forensic Genetics in Individual Identification - **Ömer Karataş** Unveiling the Genetic Code: Exploring Forensic Science at the DNA Level
15:45 – 17:15	**Panel 8: Anti-Cancer Therapies; Cilinical and Molecular Approaches** **Moderators: Banu İşbilen Başok, Aylin Sepici Dinçel** - **Engin Ulukaya** Critical Considerations for Preclinical Evaluation of Newly Synthesized Anticancer Compounds - **Metin Kurtoğlu** Advanced Therapeutic Medicinal Product: Advantages and Disadvantages - **Nuray Ulusu** Metabolic Plasticity & Dormant Status of Cancer Cells

27 September 2024 - Friday	
09:30 – 10:15	**KEYNOTE LECTURE** **Moderators: Semra Demokan** - **Ahmad R. Bassiouny** Role of Long- noncoding RNA in Cancer and Neurodegenerative Diseases
10:30 – 12:30	**Panel 9: Biomarkers: Recent Hallmarks** **Moderators: Banu İşbilen Başok, Canan Cacına** - **Bünyamin Akgül** Long Non-coding RNAs in Cell Death and Survial - **Duygu Aydemir** Impact of the Metabolic Targets on the Cancer Treathment Regarging with Novel Cancer Death Pathways - **Yunus Akkoç** The Role of Autophagy in Tumor Microenvironment
12:30 - 14:00	**Closing and Award Ceremony**

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ORAL PRESENTATION PROGRAM

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24.09.2024 / 13:30-14:45

Oral Presentation Session I

Session Chairs: Özlem Timirci Kahraman, Cem Horozoğlu

- **Effects of Nanoplastics on Locomotor and Acetylcholinesterase Activities of Developing Zebrafish Embryos**
  Zülal Mızrak, Semanur Işıkoğlu, Merih Beler, Gizem Eğilmezer, Ismail Ünal, Derya Cansız, Ebru Emekli Alturfan

- **Relationship between Radio Frequency Electromagnetic Field Exposure and Developmental Pathways to Obesity in Zebrafish Embryos**
  Derya Cansız

- **Investigation of Morphological and Behavioral Alterations in Hypoxia-induced Zebrafish Embryos**
  Efruz Irem Akkuş Yaylamış, Derya Cansız, Ismail Ünal, Merih Beler, Müjgan Cengiz, Ebru Emekli Alturfan

- **Investigation of the Effect of Nanoplastic Exposure on The Oxidant-Antioxidant Balance in Zebrafish Embryos**
  Merih Beler, Ismail Ünal, Derya Cansız, Gizem Eğilmezer, Ebru Emekli Alturfan

- **Differential Effects of Vitamin K1 and Vitamin K1 on Zebrafish Embryogenesis**
  Semanur Işıkoğlu, Zülal Mızrak, Merih Beler, Gizem Eğilmezer, Efruz İrem Akkuş, İsmail Ünal, Derya Cansız, Ebru Emekli Alturfan

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24.09.2024 / 15:00-16:15

Oral Presentation Session II

Session Chairs: Beyza Özçınar, Semra Koçtürk

- **Hedgehog Pathway is a Regulator of Stemness in Her2-Positive Trastuzumab Resistant Breast Cancer**
  Idris Er, Asiye Busra Boz Er

- **Targeting ITGβ3 to Overcome Trastuzumab Resistance in HER2-Positive Breast Cancer: Insights into TGF-β Signaling and Migration**
  Asiye Busra Boz Er, Idris Er

- **Investigation of The Effects of Doxorubicin on MTR, BCAT1 And PHGDH Genes In Mcf-7 Cell Line**
  Arda Kebapcı, Levent Gülüm, Kezban Uçar Çifçi, Merve Nur Al, Yusuf Tutar

- **Pharmacogenomics and Its Influence on Therapy Response and Toxicity**
  Mahmoud Abudayyak, Gül Özhan

- **Investigation of Gene Fusions and Rearrangements In Thyroid Malignancies In The Turkish Population**
  Burcu Çelikel, Gulcin Yegen, Nihat Aksakal, Semen Onder, S. Umit Zeybek

24.09.2024 / 16:45-18:00

Oral Presentation Session III

Session Chairs: Cem Horozoğlu, Gülper Nacarkahya

- **Exploring lipidomic shifts in acute myeloid leukemia cells: The impact of dual inhibition of Mcl-1 and sphingolipid metabolism**
  Melis Kartal Yandim, Mesut Bilgin

- **Effects of PGR gene expression and H770H (rs1042839) mutation on tumor and clinical characteristics in glioma**
  Özlem Kurnaz Gömleksiz, Merve Nur Aksakal, Adil Meric Altınöz, Mahmut Özden, Melih Bozkurt

- **Evaluation of Nrf2 Gene and Keap 1 Enzyme Levels İn Bladder Cancer**
  Mehmet Aydın Dağdeviren, Mehmet Özaslan, Sibel Bayıl Oğuzkan, Ömer Eronat

- **Investigation of Co-expression of lncRNA SNHG1 and miR-153-3p in Tumor and Tumor Microenvironment of Gastric Cancer Cases**
  Şafak Şener, Cem Horozoglu, Hazal Karadag, Asli Yildiz, Mehmet Tolgahan Hakan, Soykan Arikan, Ilhan Yaylim

- **Possible Correlations between OX40 rs17568 A/G Gene Variant and sOX40 serum levels in Gastric Cancer patients**
  Hajir Moosa Mohammed Al Khafaji, Ali Elselmo, İslim Kaleler, Dilara Sönmez, Mehmet Tolgahan Hakan, Cem Horozoğlu, Özlem Küçükhüseyin, Soykan Arıkan, Filiz Akyüz, İlhan Yaylım

25.09.2024 / 13:00-14:15

Oral Presentation Session IV

Session Chairs: Canan Cacına, Özlem Kurnaz Gömleksiz

- **Exploring the role of epigenetic regulators in Paclitaxel-Resistant NSCLC**
  Arda Işıklar, Buse Cevatemre, Hamzah Syed, Ceyda Açılan Ayhan

- **Ylang ylang oil might induce mitophagy in lung cancer cell lines**
  Baris Ertugrul, Göksu Kasarcı Kavsara, Tugba Buse Şentürk, Timur Hakan Barak, Sinem Bireller, Bedia Cakmakoglu

- **Effects of PPAR-gamma Agonists on in-vitro Diabetic Models of Oestrogen-Positive Breast Cancer**
  Melike Sağ, Hülya Yılmaz Aydoğan, Oğuz Öztürk

- **A First Preliminary Report: Potential Implications of IDO1 Expression on Soluble Tryptophan and Tryptophan Catabolites in Gastric Tumors and Tumor Microenvironment**
  Cem Horozoglu, Mehmet Tolgahan Hakan, Safak Sener, Dilara Sonmez Zor, Fikret Aktas, Ozlem Kucukhuseyin, Soykan Arikan, Filiz Akyuz, Ilhan Yaylim

- **New and Effective Compounds for Alzheimer's Disease and Cancer Therapy: Design, Synthesis and Biological Evaluation Studies**
  Kadircan Ural, Ferah Comert Onder

25.09.2024 / 16:45-18:15

Oral Presentation Session V

Session Chairs: Sacide Pehlivan, Gülbu Isıtmangil

- **Investigation of LEP, LEPR Variants, and LEP Methylation In Knee Osteoarthritis**
  Yasemin Oyacı, Dicle Rotinda Özdaş Sevgin, Mustafa Pehlivan, Demirhan Dıraçoğlu, Fatima Ceren Tuncel, Sacide Pehlivan

- **Determining the association between GDF-15 gene variants and Type 2 Diabetes: A hospital-based case-control study**
  Emine Yagci, Gamze Zengin, Cansu Ozbayer, Melike Bayindir Ureten, Medine Nur Kebapci, Irfan Degirmenci, Hulyam Kurt

- **Evaluation of rs1244378045, rs767450259 and rs750556128 Mutations in Terms of Polymorphism in Diabetic Obese and Non-Diabetic Obese Individuals**
  Saadet Busra Aksoyer Sezgin, Sermin Durak, Faruk Celik, Varol Guler, Aysegul Sarıkaya, Umit Zeybek

- **Comparison of Symptoms with Mutation Results in Patients with FMF Preliminary Diagnosis in the Bolu Region**
  Ali Osman Arslan, Murat Dıramalı, Murat Alışık

- **Impact of *IFNAR1* Gene Variations on Epilepsy: Interaction of Carbamazepine and Levetiracetam in Treatment Outcomes**
  Kübra Çiğdem Pekkoç Uyanık, Zeynep Gizem Todurga Seven, Erhan Raşit Agay

- **SCUBE-1 as a biomarker predictor for the home follow up and hospitalization of SARS-CoV-2 patients**
  Mustafa Kerem Özyavuz, Selçuk Eren Çanakçı, Kenan Ahmet Türkdoğan, Faruk Çelik, Mehmet Mesut Sönmez, İbrahim Yılmaz, Ali Osman Arslan, Abdullah Emre Güner, Şakir Ümit Zeybek

26.09.2024 / 15:00-16:15

Oral Presentation Session VI

Session Chairs: Özlem Timirci Kahraman, Özlem Kurnaz Gömleksiz

- ***In Silico*, Synthesis, *In Vitro* Enzyme-Activity of New and Potential Inhibitor Candidates for Alzheimer's Treatment and Their Antiproliferative Effects**
  Merve Sıkık, Ferah Comert Onder

- **Testing of 1.7A2UCOE, 1.2A2UCOE and O.5UCOE Universal Chromatin Opening Elements (UCOE) Derived from Hnrpa2b1-Cbx3 Reference Gene Loci on Human Stem Cells (iPS Cells)**
  Omer Faruk Anakok, Livanur Yücel, Hassana Adamou Ali

- **Effects of the VDR rs757343 (T>A) polymorphism in MODY patients**
  Şura Edanur Sağlam, Beyzanur Şimşek Çetin, Aref Khalkhali, Bita Rostami, Ilhan Satman, Nurdan Gül, Özlem Kurnaz Gömleksiz, Hülya Yılmaz Akdoğan, Deniz Kanca Demirci

- **Impaired CB1 Receptor Function and Endocannabinoid Disruption Enhance Simvastatin-Induced Skeletal Muscle Toxicity**
  Hilal Kalkan, Elisabetta Panza, Ester Pagano, Giuseppe Ercolano, Claudia Moriello, Fabiana Piscitelli, Mónika Sztretye, Raffaele Capasso, Vincenzo Di Marzo, Fabio Arturo Iannotti

- **Photodynamic Therapeutic effects of Verteporfin on foveal retinal vessels in Age-related Macular Degeneration:: A year result of the retrospective study planned for three years**
  Ayhan Önal

26.09.2024 / 16:30-18:00

Oral Presentation Session VII

Session Chairs: Canan Cacına, Karolin Yanar

- **Determination of the relationship between cholinergic activity and autoimmunity in patients with Hashimoto thyroiditis**
  Gulten Ates, Ismail Cem Sormaz

- **Effects of oxidative stress caused by methotrexate and aging on reproductive hormones**
  Şükriye Çalışkan, Şehkar Oktay

- **Comparison of the impact of oleuropein on adult female *Drosophila melanogaster* fed on sucrose and sucrose+fructose media**
  Karolin Yanar, Sevcan Kutlu, Melike Demir, Pınar Atukeren

- **Development and Clinical Validation of an Oxford Nanopore-Based Multigene Panel for the Diagnosis of Carnitine Cycle Defects**
  Gökçe Akan, Mehmet Cihan Balcı, Gülten Tuncel, Meryem Karaca, Hasan Hüseyin Kazan, Ahmet Caglar Ozketen, Ozge Ozgen, Asuman Gedikbaşı, Gulden Fatma Gokcay, Fatmahan Atalar

- **Comparative Evaluation of Immune Marker Expressions in Human Adipose Tissue MSCs Expressing Chimeric Cytokine Receptors**
  Sude Coşkun, Alper Tunga Özdemir, Rabia Bilge Özgül Özdemir, Mehmet Tolgahan Hakan, Nihat Aksakal, Ali Osman Gürol, İlhan Yaylım, Şakir Ümit Zeybek

- **Investigation of Umami Taste TR1/TR3 Receptor Expression Levels in Rat Peripheral Tissues in the NMDAR Hypofunction Model**
  Duygu Vardağlı, Karolin Yanar, Zeliha Emel Zengin

- **Evaluation of time-dependent molecular changes of thrombocyte concentrates prepared by apheresis method**
  Basma Alhamrouni Almuntasir, Basak Adaklı Aksoy, Ayca Dogan

Functional Dairy Foods and Microbiota Modulation

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Democritus University of Thrace, Faculty of Medicine, Laboratory of Hygiene and Environmental Protection, Alexandroupolis, Greece

Functional foods are foods consumed for specific health purposes, because of their constitution in nutrients or other substances which may confer health benefits action for their host. The history of functional foods can be traced back to the first use of cheese and fermented products, that were well known to the Greeks and Romans who recommended their consumption. The fermentation of dairy foods represents one of the oldest techniques for food preservation.

Introduction of the concept is generally attributed to Nobel Prize recipient Eli Metchnikoff, who in 1907 suggested that the "dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes". Metchnikoff correlated the intake of large quantities of Bulgarian fermented milk with the longevity of the Caucasian people.

Since then, multiple strategies and approaches were created for the development of effective functional foods.

Many functional foods now exist in various countries. Regulatory authorities and legislative frameworks in the different countries diverge their features on functional foods. Japan seems to be the country strongly cognizant and supportive of the public health benefits of functional foods, as more than 200 functional foods are proposed marketed under the FOSHU (Foods for Specialized Health Use) legislation. Accordingly, Food and Drug Administration (FDA) in the USA imposed legislation allowing more than 15 food categories grouping different functional foods.

Through centuries fermented cultures were developed from empiric cultures with beneficial action to artificial cultures with particular beneficial action to artificial probiotic cultures with chosen enteric bacteria and finally, since 2000, genetically selected probiotic therapeutic cultures with reinforced properties stimulated the researcher's interest. One of the

most promising areas for the development of functional foods lies in modification of the activity of the gastrointestinal tract by use of probiotics, prebiotics and synbiotics.

The contribution of biotechnology has been very important (a)by selection of new strains, (b) improvement of specific functional properties, (c) nutritional improvement of food, and finally(d) improvement of sensory and textural qualities of the final product.

However, the capital role of functional foods is focused on the stimulation of the host immune system and preservation of the microbial intestinal balance via the "barrier effect'.

Dairy functional foods showed important health benefits, which includes;(1) increased nutritional value, (2) promotion of intestinal lactose digestion, (3) positive influence on intestinal and urogenital flora and finally, (4) prevention and reduction of intestinal tract infections by inhibiting colonization by enteric pathogens.

Probiotics keep a key role as functional dairy foods, as they are designated to protect the integrity of the intestinal system.

Different ways of action are associated with their presence in the intestinal system, such as, competition for space (Spatial arrangement), competition for nutrients found in limited quantities, maintenance of an acid pH on the epithelium, production of H_2O_2, production of antimicrobial substances, organic acids or bacteriocins, synthesis of nutrients reported as sources for energy for epithelial cells or bacteria, immune system stimulation. Moreover, some additional roles are associated to their functionability, as regulation of the gut motility, decreased incidence and duration of diarrhea, maintenance of mucosal integrity, improvement of immune system and reduction of catabolic products eliminated by kidney and liver.

Last years, studies reported their relation to the prevention of colon cancer as having anti – carcinogenic and anti-mutagenic action and some other studies report their anti - allergic activities. It is evident that the most controversial remains that of anticancer activity. As experimentation is based on the profile of specific biomarkers for colon cancer risk in dietary intervention studies. There is no provided direct experimental data for cancer regression in human, because of consumption of dairy lactic cultures.

Balance diet is capital to the incidence of serious metabolic imbalances, such as obesity, type 2 diabetes, hypertension, food allergies and intolerances, gastrointestinal and inflammatory

disorders. Diet based on meat consumption seems to cause changes to the intestinal microbiota which seems to be associated with cancer disease. It is also known that the occurrence of intestinal cancer shows an increasing incidence in developed countries due to the most important consumption of meat. Moreover, there are components in a plant-based diet other than traditional nutrients that can reduce cancer risk.

Yet, their role in prevention of osteoporosis seems to be real, as they stimulate and support production of calcium. They are also known to have an hypocholestaemic action and as such, they compensate us with the feeling of well – being.

Another type of functional dairy foods embeds prebiotics which are non - digestible bioactive substances acting on a beneficial way on the intestinal microflora of the host. Prebiotics encounters the following properties (Gibson, Roberfroid, 1995), as they are; non - hydrolyzed or absorbable in the intestine, metabolized selectively by the beneficial microflora, promote growth and metabolic capacity of the beneficial microbiota, resistant in acids, bile and pancreatic secretions, promote the well - being of the host. They are belonging in the class of carbohydrates as, dietetic fibers non – digestibles. Results of their action include the increase in bacterial mass and volume in the stools, modification of the pH and production of volatile fatty acids and gas. Finally, a mixture of probiotics and prebiotics gave us the *synbiotics* (from the Greek word to live together), which combine both effects. Finally, a mixture of probiotics and prebiotics gave us the synbiotics (from the Greek word to live together), which combine both effects.

The role of functional foods in the maintenance of a healthy intestinal system is well known. However, we have to clarify the specific action of prebiotics and the probiotic microorganisms in regulating not only the balance of the microbial intestinal flora but participating actively in the metabolism.

The intestinal tract have not only as role the selective nutrient absorption ,but it is also furnished with an important signal transduction and information exchange system within our body. Moreover, the intestine is involved in nutrient recognition and signal transduction, and also acts as a neuroendocrine sensor as well as an immunological recognition and presentation system. Together, these complex information exchange systems form a number of signaling

networks that include cells immobilized in organs as well as cells transported via the circulatory system . Functional foods may exert their beneficial effects through these cell communication networks and by interacting with various receptors found along the gastrointestinal tract. However, many non-nutrients, such as dietary fibers and oligosaccharides, exert their effects via signaling pathways other than those found in the intestine.

The human gastrointestinal tract harbors a large variety of microorganisms. Firstly, the human newborn is devoid of bacteria before birth (Bezirtzoglou, Anaerobe,3(2-30;1997). Bacteria start appearing from the first days of life. Within a few hours the newborns develop its normal bacterial flora, which originates principally from the immediate environment, the hospital staff and the effect feeding. The composition of the newborn bowel flora depends on age, race and basically on the diet of the person (Bezirtzoglou, Stavropoulou,17(6);2011).

The different part of the gastrointestinal ecosystem seems to carry different bacterial populations concerning as well qualitative and quantitative differences. Bacterial numbers are increasing as they move down the alimentary tract, where the bacterial population can reach extremely high numbers as more than 10 million bacteria/ ml of fecal fluid. However, specific factors determining the development of the human lactic acid microbiota are not yet completely elucidated and studies focusing on the different strain's distribution in the various human organs could clarify this crucial problem. Attachment to epithelial cells explains Lactobacillus selective adherence to a particular ecosystem (Lazar, Bezirtzoglou, Biotechnol. Letters,9(3);2004). Non-pathogenic anaerobic bacteria belonging to Lactobacillus and

Bifidobacterium genera could inhibit the adhesion and invasion capacity of some enteropathogenic enterobacterial strains (Lazar, Bezirtzoglou, Biotechnol. Letters, 9(3);2004). Members of genera Lactobacillus are normal residents of the complex ecosystem of the human gastrointestinal tract (Bezirtzoglou et al, Rev.Med. Microbiol,8;1997). As discussed already, most interest is focused on their beneficial effect by improving the human intestinal microflora via different actions and called so then probiotics. Moreover, probiotics produce nutrients, bacteriocins, antimicrobial substances, they are able to eliminate toxins and protect food from putrefaction. The rural people still produce unpasteurized fermented milk and other dairy products with live cultures of Lactobacillus, Bifidobacterium and other probiotics by using traditional methods and technology (Vassos et Bezirtzoglou, Cent.Eur.J.Biol.3(1);2008).

However, it is different to have complete information on the nutritional habits of a population. Children are considered to consume more frequently milk and dairy products than adults. Various bacterial species used in fermentation of dairy products often colonize the children in high numbers, such as *L.paracasei, L.delbuckii lactis, L.lactis lactis, Leuconostoc* (Vassos et Bezirtzoglou, Cent.Eur.J.Biol.3(1);2008). Bifidobacterium is also found in the children microflora (10%), as well as in the old persons microflora (5%) sparsely (Bezirtzoglou et al, Biol.Neonate,58(5);1990). Old persons have particular dietetic habits and a key role in their nutrition reserves traditional foods. It is then attended to be inhabited as well by bacterial species used for traditional food preparation. Clearly, healthy subjects in all ages are colonized by a predominant lactoflora (100%) (Vassos et Bezirtzoglou, Cent.Eur.J.Biol.3(1);2008). However, there are differences in the species numbers and distribution during ageing. Stability in Lactobacillus numbers was also observed by other authors (Mikelsaar et al, In: Lactic acic Bacteria (Eds), NY,1988). Furthermore, prolonged biological isolation of healthy persons, as it is the case of space flight or special trainings, cause alterations in the intestinal microflora (Klaus et al., Trends Biotechnol,24(3);2006).

Gastrointestinal infections, bacterial or viral diarrhoeas disease, preudomembranous colitis and antibiotic-associated diarrhoea have been treated successfully by using some pharmaceutical probiotics as *S.boulardii, L.casei GG, L.acidophilus*, and *E.faecium*. Surprisingly, high levels of intestinal Lactobacillus and/or *Enterococcus* were seen in infants with rotavirus diarrhea, as well as in inhabitants of the Chernobyl area after the atomic explosion. Moreover, stress influence seems to provide the gastrointestinal microbiota with putrefactive bacteria and especially increase *C.perfringens numbers* which is found in antagonism with *Lactobacillus*.(Bezirtzoglou et al, Microb. Ecol.,55(4),2008), (Tsiotsias, Bezirtzoglou, MEHD,16,2004), (Bezirtzoglou et al, Microecol Ther,28;1999), (Kostandi, Bezirtzoglou et al, MEHD,18;2006), (Mullie, Bezirtzoglou et al, MEHD,14,2002).

As already mentioned, Lactobacillus were reported to be extremely sensitive to environmental factors, diet, antibiotics (Bezirtzoglou et al, MEHD,20;2008) and stress.

Dissemination of antibiotic resistances into dairy products and other foods and into consumer following overusing of them, could select some bacterial species. In Greece, multiresistance in hospitalized patients and outpatients is reported (Bezirtzoglou et al, J. Inf.

Parasit. Dis; IX (4),2006). The case of outpatients is somewhat interesting, as these patients had not received systematically antibiotics, the observation of frequent multiresistance was unexpected in this group of patients. The reasons for the resistance patterns observed may be due to the feed ingested. It is of substantial interest to note that in our country for improving the quality of animals, antibiotics are added in their food illegally. Antibiotics could be present at high levels in animals and their products ingested by man. It is then conceivable and understandable, the presence of multiresistance observed in most of our isolates which may not be underestimated. However, it is difficult to know if a given bacterial species consists of an autochthonous microorganism inhabiting the gastrointestinal microbiota or a transient one probably associated with food ingested, environmental factors and personal habits. Adhesion of Lactobacillus to the epithelial cells is directly connected to the mucus integrity, as these species can ferment monosaccharides of mucins (Midtvedt et al, Microecol Ther,14;1984) in order to keep their stable population level. Antibiotics can cause breakdown of mucins (Midtvedt et al, Prog. Clin. Biol. Res.181;1985), (Bezirtzoglou, Midtvedt, Microecol Ther,28;1999). Specifically, *Lactobacillus* were reported to be inhibitory to some other microorganisms and this is the basis of their ability to improve the keeping quality and safety of many food products e.g. against *C.perfringens* (Bezirtzoglou et al, Microb Ecol).The production of bacteriocins by LAB has been reported extensively. Bacteriocins, as bactericidal peptides are active against other microbial species. Fermented dairy products are considered as complete food enabling us with the feeling of fullness by causing increase in the human intestinal bacterial content. Probiotic bacteria introduced by food in the large intestine are participating in the fermentation of alimentary derived indigestible carbohydrates. This type of fermentation results in production of short-chain fatty acids (SCFA), lowering circulatory cholesterol concentrations either by inhibiting hepatic cholesterol synthesis or by redistributing cholesterol from plasma to the liver. Cholesterol, as precursor of bile acids participates actively in the de novo bile acid synthesis. Increasingly bacterial activity in the large intestine seems to enhance bile acid deconjugation. It is known that deconjugated bile acids are non-absorbable at the level of intestinal mucosa and therein eliminated. However, Lactobacillus and Bifidobacterium, are more commonly isolated from yoghurts or other fermented dairy products and play important role to the treatment of gastrointestinal infections by reinforcing the intestinal system against invasion and establishment of pathogens. The integrity of the intestine

seems to be largely depending on the presence of a healthy microflora as, probiotic products are involved in promoting the intestinal defense barrier by normalization of intestinal permeability and altered gut microecology. So then, probiotics dairy products preserve the integrity of the intestinal immunological status by stimulating the inflammatory response particularly through production of intestinal immunoglobulin A. Although traditional thinking has been that the presence of one form of bacteria rather than another in the gut is sufficient to produce such actions, more recent evidence suggests that the secondary products produced by the microflora are responsible for the bioactivity that leads to health benefits. These secondary products include short-chain fatty acids (SCFA) (Gibson, J. Nutr.125;1995) (Gibson, Gastrenterol,108;1995), and short bioactive peptides resulting from cleavage from milk or other proteins in the gut (Ramberg, Nutr. J,9;2010) (Hemmings, Gut,19;1978) (Nakamura, J. Dairy Sc.78;1995) This change in thinking will probably stimulate active research in exploring mechanisms that might explain the health benefits of probiotic and prebiotic materials.

The prevailing dogma is that the intestine is associated to an important extend with CYP metabolism, as it is responsible for the extra hepatic metabolism.

It is clear that high microbial charge following intestinal disturbances, ageing, environment or food associated, leads to the microbial metabolism of a drug before absorption. Moreover, it is obvious that knowledge on the intestinal CYP system is of tremendous interest because of its key role in steroid hormone formation, carcinogen activation, and drug metabolism (Bezirtzoglou, MEHD,23;2012)

Based on the fact that many intestinal bacterial strains possess P450 enzymes, the question is raised that if live probiotics express a P450 activity, which of them could eventually influence the drug metabolism and bioavailability? Despite numerous studies, many aspects of the ecological profile of the human lactoflora remain obscure and speculative.

Whatever the underlying explanations and knowledge, it is obvious that more research must be done to explain the know-how, mechanisms together with the therapeutic advantages for the host by the beneficial maintenance of the human intestinal microbiota.

Comparison of Symptoms with Mutation Results in Patients with FMF Preliminary Diagnosis in the Bolu Region

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Abstract

Aim: Familial Mediterranean Fever (FMF) is an autoinflammatory disease in an autosomal recessive manner. Phenotypic effects may vary according to genetic background. It was aimed to investigate the relationship between genotype and symptoms in order to diagnose this disease, which is common in Bolu region. We aimed to compare R202Q, E148Q, M680I, M694V, M694I, V726A, A744S and F479L mutations with disease symptoms in patients with FMF pre-diagnosis.

Method: The sample group consisted of 250 individuals referred or directly applied to Department of Medical Genetics of İzzet Baysal Education and Research Hospital diagnosed with FMF. Mutations were studied with RT PCR. In our retrospective study, data were obtained by scanning patient results.

Result: In our results, we found that the age of onset and age were not related to gender. The age of onset was found to be significant in the complaint of abdominal pain ($p=0.026$). R202Q was found to be associated with abdominal pain, joint pain and chest pain ($p=0.056$, $p=0.042$, $p=0.02$). M694V was found to be significant in relation to skin rash ($p=0.043$). F479L was found to be associated with abdominal pain ($p=0.01$). E148Q was associated with all symptoms in the study.

Conclusion: Our results were generally consistent with the literature. It was observed that R202Q and E148Q mutations were more frequent in Bolu and were statistically significant compared with the symptoms of the disease. So the identification of mutant genes shed light on

the pathogenesis of the disease and determines which symptoms are more common in which mutations will support diagnosis and treatment.

Keywords: FMF, R202Q,E148Q, M694V, PCR, Abdominal pain,

INTRODUCTION

Familial Mediterranean Fever (FMF) is an autoinflammatory disease characterized by recurrent fever, peritonitis, pleuritis, arthritis, or erysipelas-like skin findings, following an autosomal recessive (AR) inheritance pattern. The identification of mutant genes sheds light on the pathogenesis of the disease and aids in its diagnosis and treatment (Ben-Chetrit, Lewy, 1998). FMF is the first rheumatic disease where the causative gene has been identified, and its encoded protein is reported to enhance anti-inflammatory effects or suppress inflammation. Understanding the inflammatory pathway involved here will also help explain the pathogenesis of many rheumatic diseases (Kastner, 1998).

It was first described in 1908 in a Jewish woman patient with recurrent fever and abdominal pain, and it was named “extraordinary recurrent peritonitis” (Janeway, Mosenthal, 1908). The first study involving 10 patients was reported in 1945 (Siegal, 1945). In 1951, it was reported that the disease was familial, and in 1952, it was noted that amyloidosis could develop in these patients (Momou, Cattan, 1952). In 1958, the term "Familial Mediterranean Fever" was first used by Israeli researcher Heller (Heller et al., 1958). In 1997, the MEFV (Mediterranean Fever) gene, located on the short arm of chromosome 16 (16p13.3) and responsible for the disease, was identified (International FMF Consortium, 1997). It is particularly common among Jews, Arabs, Turks, and Armenians (Onen, 2006). The carrier frequency is reported as 1 in 5 among Turks, Jews, and Arabs, and 1 in 7 among Armenians (Lidar, Livneh, 2007). The prevalence of the disease in Turks has been reported as 1 in 1000. Family history is present in 43% of patients (Turkish FMF Study Group, 2005).

The Pyrin-Marenostrin gene (MEFV gene) consists of 10 exons and encodes the 781-amino-acid Pyrin protein. This protein has been reported to suppress inflammation.

The Marenostrin protein is mainly found in neutrophils, where it inhibits inflammation by suppressing neutrophil activation. Pyrin, also expressed in neutrophils, inhibits C5a, an

inflammatory mediator of the complement system. (Centola, 200 & Kastner, 1998).

The symptoms of Familial Mediterranean Fever (FMF) appear in 65% of patients within the first ten years of life and in 90% of patients within the first 20 years (Tamir et al., 1999). It has been reported that in children with late-onset disease, the number of annual attacks is lower, and the course of the disease is milder (Özdel et al., 2016). FMF is characterized by recurrent fever and pain from inflammation in one or more body areas. Attacks typically last 12-72 hours, are self-limiting, and may recur from once a week to once a year. Between attacks, patients are completely normal. The symptoms observed in patients include fever in 82-93%, abdominal pain in 84-93%, chest pain in 14-31%, arthritis in 33-47%, arthralgia in 49%, myalgia in 40-58%, and erysipelas-like erythema in approximately 20% (Aydın et al., 2017).

Fever: It is usually between 38-40˚C and subsides on its own within 12-72 hours. During some attacks, the patient's fever may be high, while in others, it may remain at normal levels. It is more commonly observed in individuals with the M694V homozygous or compound heterozygous mutations (Ozturk et al., 2012).

Abdominal pain: Abdominal pain is caused by aseptic serositis in the peritoneum. The attacks usually last between 24-48 hours. The severity of abdominal pain can range from mild tenderness to acute abdomen symptoms. The pain can be localized or may radiate to the back or groin areas. Abdominal pain is the most common type of attack in FMF, occurring in 95% of cases (Tunca et al., 2005).

Joint Involvement/Pain: Joint attacks are a significant feature of FMF and are commonly seen, occurring in about 75% of cases. Approximately 5% of patients experience prolonged joint attacks lasting longer than a month. The hips and knee joints are most commonly affected. The disease particularly affects the large joints of the lower extremities (knees, ankles) in the form of recurrent attacks (Sohar et al., 1967 & Sarı et al., 2014).

Chest pain: This is caused by pleural inflammation. The pain is usually unilateral and lasts for 24-72 hours. Patients may experience pain during breathing and a decrease in breath sounds on the affected side. Chest pain from pleural inflammation occurs in 25-50% of Turkish, Jewish, and Arab patients, with pleural attacks as the initial symptom in 5% of cases.Patients often experience severe pain that leads to rapid and shallow breathing (Lidar et al., & Schwabe, Peters, 1974).

Skin manifestations: The most common skin finding is erysipelas-like erythema. These lesions are characterized by well-defined red patches. They most commonly appear on the dorsum of the foot and the anterior surface of the tibia. The erythema usually resolves on its own within 2-3 days. In 3-40% of patients, it is generally localized to the skin area between the knee and the ankle (Sarı et al., 2014 & Lidar et al., 2002).

Approximately 200 gene variations have been reported in the FMF database (Infevers, 2024). Most of these mutations are missense mutations, although nonsense mutations and deletions have also been identified. It is stated that 85% of these mutations are localized to exons 2 and 10. The most common mutations include R202Q, M694V, M680I, V726A, M694I, and E148Q. Additionally, with the increasing use of sequence analysis, rare mutations such as A744S and F479L have also become more frequently identified. According to the 2005 data from the Turkish FMF Study Group, the most common mutations in Turkey are R202Q and M694V (Tunca et al., 2005).

R202Q is located in the second exon of the MEFV gene. Studies have directly associated R202Q homozygosity with the FMF phenotype. It is thought that the presence of R202Q along with other mutations can enhance the clinical presentation of FMF. The R202Q polymorphism tends to lead to more pronounced clinical manifestations, particularly when it occurs alongside M694V (Ozturk et al., 2008).

The M694V mutation is reported to cause a more severe course of the disease in FMF patients (Bayram et al., 2015). Both the M694V mutation and recurrent arthritis attacks are significant predisposing factors for amyloidosis development. A study by Nikolay A et al. found that recurrent arthritis attacks increase the risk of amyloidosis by 2.28 times. (Mukhin et al., 2015). The M694V mutation is more common among Turks and Jews, while it is less frequent in Arabs (Tunca et al., 2005). It is observed in approximately 43.5% of the Turkish population, making the M694V allele the most frequently seen allele among Turks (Ayaz et al., 2021).

The V726A mutation has been associated with a milder disease course and the absence of amyloidosis (Tunca et al., 2005). It has been reported as the fourth most common mutation in several studies conducted within the Turkish population (Kocakap et al., 2014). Studies in

various ethnic groups have indicated that V726A homozygotes and compound heterozygotes are not sensitive to amyloidosis. However, Yalçınkaya and colleagues reported cases of amyloidosis in Turkish patients with the V726A mutation. (Yalçınkaya et al., 2000a). While the V726A mutation is the second most common in Arabs (Touitou, 2001), it ranks third among Turkish patients (Tunca et al., 2005).

The M680I variant is a mutation that is commonly found in both the Turkish population and in Middle Eastern countries and Armenia (Touitou et al., 2004). It has been reported that patients who are homozygous for M694I or M680I, or carry combinations of these mutations, exhibit disease behaviors similar to those with homozygous M694V in terms of severity (Yalçınkaya et al., 2000b). Tüzün and colleagues conducted a mutation-symptom study with 110 patients reporting abdominal pain, chest pain, fever, and arthritis, finding the M680I mutation in 8 of 16 patients (50%) with periodic chest pain (Tüzün et al., 2004). In several studies conducted in Turkey, M680I has been reported as the second most common mutation (Abuhandan et al., 2015).

The E148Q mutation is associated with a milder form of the disease (Aksentijevich et al., 1999). As one of the most common variants after M694V, the pathogenic role of E148Q in FMF remains unclear (Marek-Yagel et al., 2009). In some studies, E148Q is regarded as a functional polymorphism associated with atypical FMF, usually presenting a mild phenotype. This variant has also been linked to other recurrent fever and inflammatory syndromes. (Touitou et al., 2004). It is thought that this variant may play a role in enhancing the pathogenic effects in compound heterozygous patients or in complex alleles (Ben-Chetrit et al., 2000).

Studies have shown that M694I heterozygosity is associated with a phenotype related to FMF, and the E148Q variant also accompanies this condition (Aldea et al., 2004). The presence of either the M694I or E148Q mutation alone does not have a strong genetic impact for the diagnosis of FMF; however, their coexistence provides a strong genetic indication for the diagnosis of the disease (Nakamura et al., 2005).

The A744S mutation, which is frequently reported in Arab populations, is rarely observed in the general population (Medlej-Hashim et al., 2004). Among FMF patients with A744S, a family history is less common compared to patients with M694V and M680I mutations.

Furthermore, it has been reported that the A744S mutation is supportive of the diagnosis for prophylactic treatment in the disease (Soylemezoglu et al., 2015). Another FMF mutation, F479L, is infrequently observed in the general population. Depending on ethnic origin, it can lead to varying symptoms in the clinical presentation of FMF. However, its role in the disease is not fully understood. It is believed that the phenotypic effects caused by mutations may vary according to genetic background due to ethnic origin and environmental factors (Bakkaoglu, 2003). Genetic studies allow for the detection of symptoms in the siblings of affected individuals before they manifest. Therefore, genetic studies are of great importance. This study aims to investigate the relationship between genotypic and phenotypic characteristics and symptoms to facilitate early diagnosis of this disease, which is also common in the Bolu region. Based on this information, we aimed to compare the R202Q, E148Q, M680I, M694V, M694I, V726A, A744S, and F479L mutations with disease symptoms in patients suspected of having FMF.

MATERIAL-METHODS:

The sample group for our study was composed of patients with a preliminary diagnosis of FMF who were referred to or directly applied to the Department of Medical Genetics at Bolu Abant İzzet Baysal University, İzzet Baysal Training and Research Hospital.. The study included 250 patients with FMF genetic analysis and relevant information. The patients were selected from those who visited the Department of Medical Genetics between 2015 and 2020, during which the Medical Genetics outpatient clinic was actively operating, and genetic results were available. In our retrospective study, patient data were obtained by screening the results of patients during the active periods of the Medical Genetics clinic. Approval for the study was obtained from the head of the Department of Medical Genetics and the hospital administration. The genetic analyses for FMF were conducted using Real-Time PCR. Written informed consent forms were obtained from all patients. By reviewing the outpatient follow-up files of the patients, demographic characteristics, genetic information, and FMF test results were recorded, and the study was designed accordingly.

The sample size for chi-square test was calculated using G*Power 3.1.9.7 software. When effect size was taken as 0.3 and power as 0.95, the required minimum sample size was

found to be 220. 250 patients were included in the study. Statistical analyses were performed using JASP 0.19.0 program. Categorical variables were evaluated using Chi-square test.

Shapiro-Wilk test was used to check the conformity of continuous variables to normal distribution and mean, standard deviation or median and interquartile range were used in their presentation. Student's t test or Mann-Whitney U test was used for comparisons according to categorical variables and correlation tests were used to examine the relationships between them. The significance level was taken as $p<0.05$.

RESULTS

When we look at the results of our study, first, there was no statistically significant difference in the relationship between age, age of onset, and the gender of the patients (p=0.339, 0.082). It was observed that the age of onset of the disease and the age of the patient were independent of gender. When examining the relationship between the age of onset and symptoms, it was found to be associated with abdominal pain complaints (p=0.026). It was observed that the age of onset was not directly related to other disease symptoms. In the analysis of the relationship between disease symptoms and gender, no statistically significant differences were found (Table 1).

Table 1: The analysis of disease symptoms by gender.

Gender	Symptom (+)		Symptom (-)		p
	Female	Male	Female	Male	
Abdominal Pain	131	58	40	21	0.587
Joint Pain	114	51	57	28	0.744
Fever	63	25	108	54	0.424
Chest Pain	22	11	149	68	0.818
Skin Redness	11	4	160	75	0.672

In the analysis of the relationship between the presence of mutations and the symptoms observed, it was determined that R202Q is associated with abdominal pain, joint pain, and chest pain symptoms (p=0.043, p=0.018, p=0.014). E148Q was found to be associated with abdominal pain, joint pain, fever, chest pain, and skin redness (p=0.011, p=0.005, p=0.029, p=0.002, p=0.049).

M694V was identified as being related to the skin rash/redness finding (p=0.043). The analyses of all mutations related to individual symptoms are presented in Tables 2, 3, 4, 5, and 6. In the results of the analyses conducted, no statistically significant difference was found between the disease symptoms and the mutations M680I, M694I, V726A, and A744S. Additionally, it was observed that F479L was statistically significant in relation to abdominal pain findings (p<0.001).

Table 2: Analysis of abdominal pain symptoms by mutations.

	Abdominal pain (+)			**Abdominal pain (-)**			**p**
	Heterozygous	Homozygous	Wild	Heterozygous	Homozygous	Wild	
R202Q	53	4	132	8	1	52	**0,043**
E148Q	35	5	149	4	0	57	**0,011**
M680I	14	1	174	5	2	54	0,222
M694V	42	11	136	11	2	48	0,538
M694I	2	0	187	0	0	61	0,420
V726A	27	0	162	7	0	54	0,578
A744S	3	0	186	0	0	61	0,322
F479L	0	0	189	4	0	57	**<.001**

Table 3: Analysis of joint pain symptoms by mutations.

	Joint Pain (+)			**Joint Pain (-)**			**p**
	Heterozygous	Homozygous	Wild	Heterozygous	Homozygous	Wild	
R202Q	46	5	114	15	0	70	**0,018**
E148Q	32	5	128	7	0	78	**0,005**
M680I	13	2	150	6	1	78	0,973
M694V	37	8	120	16	5	64	0,775
M694I	2	0	163	0	0	85	0,308
V726A	20	0	145	14	0	71	0,342
A744S	3	0	162	0	0	85	0,211
F479L	2	0	163	2	0	83	0,496

Table 4: Analysis of fever symptoms by mutations.

	Fever (+)			Fever (-)			p
	Heterozygous	Homozygous	Wild	Heterozygous	Homozygous	Wild	
R202Q	27	3	58	34	2	126	0,098
E148Q	18	4	66	21	1	140	**0,029**
M680I	5	0	83	14	3	145	0,296
M694V	19	8	61	34	5	123	0,116
M694I	1	0	87	1	0	161	0,660
V726A	13	0	75	21	0	141	0,690
A744S	1	0	87	2	0	160	0,946
F479L	1	0	87	3	0	159	0,667

Table 5: Analysis chest pain symptoms by mutations.

	Chest pain (+)			Chest pain (-)			p
	Heterozygous	Homozygous	Wild	Heterozygous	Homozygous	Wild	
R202Q	11	3	19	50	2	165	**0,014**
E148Q	10	3	20	29	2	186	**0,002**
M680I	4	1	28	15	2	200	0,324
M694V	10	2	21	43	11	163	0,358
M694I	0	0	33	2	0	215	0,580
V726A	2	0	31	32	0	185	0,175
A744S	0	0	33	3	0	214	0,497
F479L	1	0	32	3	0	214	0,482

Table 6: Analysis Skin Rash/Redness symptoms by mutations.

	Skin Rash/Redness (+)			Skin Rash/Redness (-)			p
	Heterozygous	Homozygous	Wild	Heterozygous	Homozygous	Wild	
R202Q	6	1	8	55	4	176	0,121
E148Q	6	0	9	33	5	197	**0,049**
M680I	2	1	12	17	2	216	0,087
M694V	7	0	8	46	13	176	**0,043**
M694I	0	0	15	2	0	233	0,720
V726A	0	0	15	34	0	201	0,113
A744S	0	0	15	3	0	232	0,660
F479L	0	0	15	4	0	231	0,610

DISCUSSION

FMF is a genetic disorder characterized by recurrent fever and serositis, which is self-limiting in nature. It is typically observed in childhood, with 70-90% of cases occurring in individuals under the age of twenty (Ureten et al., 2010; Ece et al., 2014). While mutations are detected in 80% of patients, 20% may exhibit clinical symptoms without the presence of a detectable mutation (Bakkaoglu, 2003).

Our results were generally consistent with the literature. In Bolu, it was found that the R202Q and E148Q mutations were more common and statistically significant when compared to the symptoms of the disease. In a study conducted by Booth et al., E148Q was reported to be significant in patients with chronic high fever (Booth et al., 2001). It is thought that the phenotypic effects caused by mutations may vary depending on genetic background, ethnicity, and environmental factors. In our study, we aimed to investigate the relationship between the genotypic and phenotypic characteristics of FMF, which is also frequently seen in the Bolu region, and the associated symptoms to enable early diagnosis. Based on this information, we aimed to compare the R202Q, E148Q, M680I, M694V, M694I, V726A, A744S, and F479L mutations with the symptoms of the disease in patients with a preliminary diagnosis of FMF.

In addition to our analysis results, the presence of the rarely seen A744S and F479L mutations in the Bolu region holds significance for the outcomes of our study. Consistent with the literature, the presence of E148Q and M694V was found to be statistically significant in relation to skin rash and redness. Although the literature indicates that M694V and R202Q are also associated with fever, in our study, we found that only E148Q was statistically significant.

Determining the mutations most often linked to certain symptoms could greatly assist in the diagnosis and treatment process.

Key Messages

Familial Mediterranean Fever (FMF) is an autoinflammatory disease marked by recurrent fever, peritonitis, pleuritis, arthritis, or erysipelas-like skin findings, inherited in an autosomal recessive pattern. Identifying mutant genes aids in understanding the disease's pathogenesis and assists in diagnosis and treatment. Our study aimed to explore the relationship between genotypic and phenotypic characteristics and symptoms to enable early diagnosis of this disease, common in the Bolu region.

We compared symptoms in patients with a preliminary diagnosis of FMF with the R202Q, E148Q, M680I, M694V, M694I, V726A, A744S, and F479L mutations.

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Comparison of the impact of oleuropein on adult female Drosophila melanogaster fed on sucrose and sucrose + fructose based media

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ABSTRACT

Drosophila melanogaster is a model organism used for various field of researches such as aging, nutrition, metabolic syndrome, neurotoxicity. Sucrose is a disaccharide consisting of glucose and fructose. It is commonly used as a standard medium for drosophila. Fructose is a monosaccharide. It's low additive levels may beneficial for reproduction capacity. On the other hand, high levels may lead to metabolic syndrome due to high metabolic fate of fructose. Oleuropein is the main phenolic component of Olea europaea L. It has several health beneficial features and it acts as antioxidant, anti-inflammatory, cardio- and neuro-protective agent depends on its consumption amount and usage duration. We aimed to evaluate whether oleuropein has protective effect or not on adult female *Drosophila melonagaster* fed on sucrose and sucrose + fructose media. We prepare 4 groups (n=6, with 25 fruity fly per media) as adult female Drosophila fed on sucrose media, Sucrose + fructose media, sucrose + oleuropein (5 mg/L for four hours after 4 hours of starvation period) media, sucrose + fructose + oleuropein (5 mg/L for four hours after 4 hours of starvation period) media. We analyzed biomarkers of redox homeostasis via spectrophotometric analysis of superoxide dismutase activity (Cu,Zn SOD) and total thiol (T-SH) content as antioxidant biomarkers and advanced oxidation protein products (AOPP) and spectrofluorometric analysis of dityrosine (DT), kynurenine (KYN) and advanced glycation end products (AGEs) as oxidant biomarkers. Our analysis showed that oleuropein (5mg/L for 4 hours after 4 hours of starvation period) acts as pro-oxidant.

INTRODUCTION

Model organisms such as *Caenorhabtidis elegans, Danio reri* oand *Drosophila melanogaster* have been widely used in scientific research (Sandner et al;2022). *Caenorhabtidis elegans,* a nematode, shows 65% homology with the human genome. With a short life cycle of 4 days, genetic manipulation is simple in *C. elegans.* It is especially prominent in scientific studies related to neurons. However, they suffer from several disadvantages, including the absence of a heart, an immune system, and a male-female sex system (Zhang et al.,2020; Johnson 2008). Researchers use the *Danio rerio*, a vertebrate model system, to study neurodegeneration and various diseases. It is advantageous to use *Danio rerio* as a model due to its many gene homologies with mammals, rapid early embryonic development, lower cost compared to rodents, small space requirements for reproduction and storage, and high reproduction capacity Chia et al., 2022; Cansız et al.,2023). *Danio rerio* is more expensive than *C. elegans, and Drosophila melanogaster* is its main disadvantage. Fruity fly*, Drosophila melanogaster,* has been comprehensively well- defined in terms of its genome, transcriptome, proteome, and metabolome, with ongoing advancements still being accomplished (Severino et al., 2023). The structure of the adult fly is functionally similar to the mammalian heart, lungs, kidneys, intestines, reproductive system and brain. There are over 100000 neurons involved in many complex behaviors including feeding. These properties have made this species stand out by distinguishing it from other species. Drosophila melanogaster provides advantages over usual mammalian models, including as enhanced genetic modification capabilities, a virtually limitless supply of experimental subjects at a low cost, and barely regulatory limitations (Manev & Dzitoyeva 2010).

Sucrose is a non-reducing disaccharide composed of glucose + fructose linked via their anomeric carbons (with α-1,2 glycosidic bond) (Finelli,2019). Fructose known as fruit sugar which is one of the monomers of sucrose It is the main sugar required for sperm and adequate levels of fructose required for optimal reproductive capacity. However, at high levels it leads to metabolic syndrome. Due to this property fructose has been widely used for inducing experimental metabolic syndrome model (Dholariya & Orrick, 2022).

The olive, a member of the Oleaceae family, has been applied in various fields including nutrition, cosmetics, medicine, and pharmacology throughout centuries (Selim et al;2022). Olives and olive derivatives comprise triacylglycerols, fatty acids, diverse pigments, aromatic substances, sterols, phenolic compounds, and resinous compounds (Ograş,2022; Lukic et al. ,2021). Recent clinical, experimental, and epidemiological research have demonstrated that the radical scavenging and antioxidant properties of polyphenols in these products are contingent upon dosage and exposure intensity (Barbaro, et al., 2014).

Oleuropein (OL), a powerful constituent of olives, stabilizes reactive oxygen species by building hydrogen bonds with free radicals. OL is the principal phenolic component of the olive tree, constituted by the combination of hydroxytyrosol, elenoic acid, and glucose molecules, with the molecular formula $C_{25} H_{32} O_{13}$ and molecular weight 540.51 g/mol (Sklenarova et al.,2023). Redox homeostasis defined as balanced between oxidant and antioxidant system. Impairment in redox homeostasis result in increased levels of oxidant and reduced scavenger activities/levels of antioxidant systems. The sum of the effect lead to development of oxidative stress. Elevated oxidative stress brings about macromolecular damage such as proteins, carbohydrates, lipids and nucleic acids. We can evaluate the changes in redox homeostasis via usage of various macromolecular damage biomarkers including biomarkers of protein oxidation, glycation, lipid peroxidation and DNA damage (Kotur-Stevuljević et al.,2023; Yanar et al.,2019; Gutteridge & Halliwell, B 2018).

MATERIAL-METHODS

Wild-type Oregon-R strain *Drosophila melanogasters* (10-15 days old) were used in this study. All experimental groups were subjected to roughly 55% humidity and an ambient temperature of 25°C throughout the experimental period. Fruit flies were stored in glass vials. Each vial contains 25 fruity flies). We prepared 4 groups as;

Group 1 (S)- Fruity fly fed with Sucrose media

Group 2 (S+OL)- Sucrose + oleuprein media (5 mg/L for 4 hours after starvation period)

Group 3 (S+F)- Sucrose + fructose media (0.1M)

Group 4 (S+F+OL)- Sucrose + fructose (0.1M) +Oleuropein media.

After the experimental period fruity flies were collected and homogenates (%10) were prepared for biochemical analysis.

Analysis of oxidant biomarkers:

DT: Analysis of DT was performed based on the spectrofluorometric method according to Sadowska-Bartosz et all (2014).

KYN: KYN was analyzed based on the spectrofluorometric method according to Sadowska-Bartosz et al. (2014).

Spectrophotometric analysis of global protein oxidation biomarker as Advanced oxidation protein products (AOPP): AOPP levels were analyzed by the method of Hanasandet al.(2012).

Spectrofluorometric analysis of glycoxidation biomarker as advanced glycation end product(AGEs): AGEs were analyzed according to Sadowska-Bartosz et al. (2014).

Spectrophotometric analysis of antioxidant biomarkers:

Determination of total thiol: The concentration of thiol fractions was determined by Sedlak and Lindsay's method (1968).

Determination of Cu,Zn-superoxide dismutase (Cu,Zn-SOD) activity: Cu,Zn SOD activity was analyzed by Sun et al method (1988).

Determination of total protein content:

Total protein content was measured by Bicinchoninic acid (BCA) assay described by Smith et al.(1985).

Statistical analysis: Statistical analysis was conducted using GraphPad Prism V10. All data are presented as mean ± standard deviation (SD) and median value. Normality was assessed using the Shapiro-Wilk test, and based on the results, either One-Way ANOVA or Kruskal-Wallis tests were employed for group comparisons. The Tukey test was employed for the post hoc analysis. $P < 0.005$ is deemed significant.

RESULTS

Specific protein oxidation biomarkers: S+F group showed increased levels of DT and KYN when compared to S group ($p<0.01$ and $p<0.05$, respectively). DT levels were significantly higher in S+F+OL when compared to S+OL group ($p<0.05$). Both DT and KYN levels significantly elevated in S+OL group and S+F+OL group when compared to corresponding groups ($p<0.0000$ for each) (Figure 1). These results showed OL act as pro-oxidant at 5mg/L.

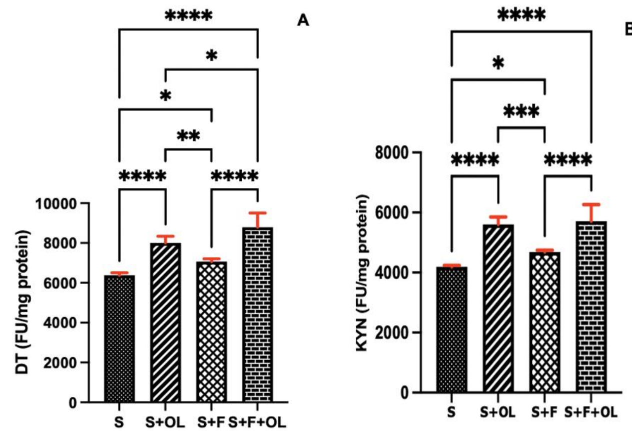

*Figure 1: Specific protein oxidation biomarkers. S: Female Drosophila melanogaster fed on sucrose-based media, S+OL: Female Drosophila melanogaster fed on sucrose + oleuropein-based media, S+F: Female Drosophila melanogaster fed on sucrose+ fructose- based media, S+F+OL: Female Drosophila melanogaster fed on sucrose + fructose + oleuropein-based media.*p<0.05; **p<0.01;***p<0.001;****p<0.000.DT:Dityrosine; KYN: Kynurenine; FU: Fluorescence Unit*

Global protein oxidation biomarker: AOPP levels were higher in S+F group when compared to S and S+OL group.S+F+OL group showed higher AOPP levels when compared to S+F group, S+OL group and S group (p<0.01, p<0.000, p<0.000, respectively) (Figure 2). These results suggests that OL (5mg/L) showed prooxidant effects.

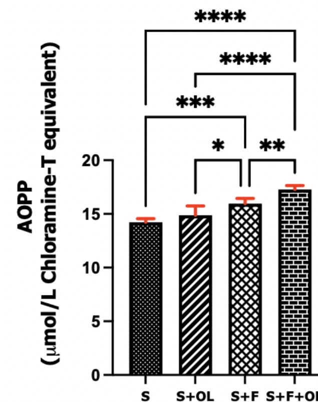

*Figure 2: Global protein oxidation biomarkers .S: Female Drosophila melanogaster fed on sucrose-based media, S+OL: Female Drosophila melanogaster fed on sucrose + oleuropein-based media, S+F: Female Drosophila melanogaster fed on sucrose + fructose- based media, S+F+OL: Female Drosophila melanogaster fed on sucrose + fructose + oleuropein-based media.*p<0.05; **p<0.01;***p<0.001;****p<0.000.AOPP: Advanced oxidation protein products.*

Spectrofluorometric analysis of glycation biomarker: AGEs levels were higher in S+OL group when compared to S and S+F group ($p<0.001$ and $p<0.01$, respectively). S+F+OL group were showed elevated levels of AGEs when compared to S+F and S group ($p< 0.001$ and $p<0.000$, respectively) (Figure 3). Oleuropein (5g/L) exhibited prooxidant effect.

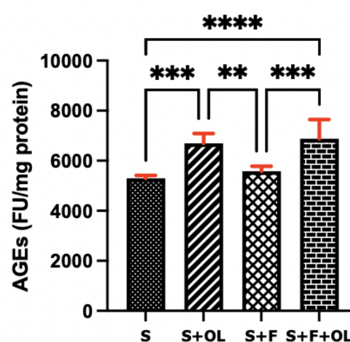

*Figure 3: Advanced glycation end products levels. S: Female Drosophila melanogaster fed on sucrose-based media, S+OL: Female Drosophila melanogaster fed on sucrose + oleuropein-based media, S+F: Female Drosophila melanogaster fed on sucrose + fructose- based media, S+F+OL: Female Drosophila melanogaster fed on sucrose + fructose + oleuropein-based media.*p<0.05; **p<0.01;***p<0.001;****p<0.000.AGEs: Advanced glycation end products level.*

Spectrophotometric analysis of antioxidant parameters: Cu,Zn -SOD activity were significantly lower in S+F group when compared to S group, although in TSH levels only non-significant decrease tendency were seen. S+OL group exhibited significantly higher level of T-SH when compared to S+F+OL group while Cu,Zn SOD activities were not different between two group. In both biomarker S+F+OL group showed lower levels when compared to S+F group ($p< 0.05$(Cu,Zn SOD) and $p<0.000$,(T-SH) respectively), and Cu,Zn-SOD activity significantly diminished in S+OL group when compared to S group (Figure 4). These results indicate that, OL (5 mg/L) act as prooxidant role especially presence of elevated levels of oxidative stress.

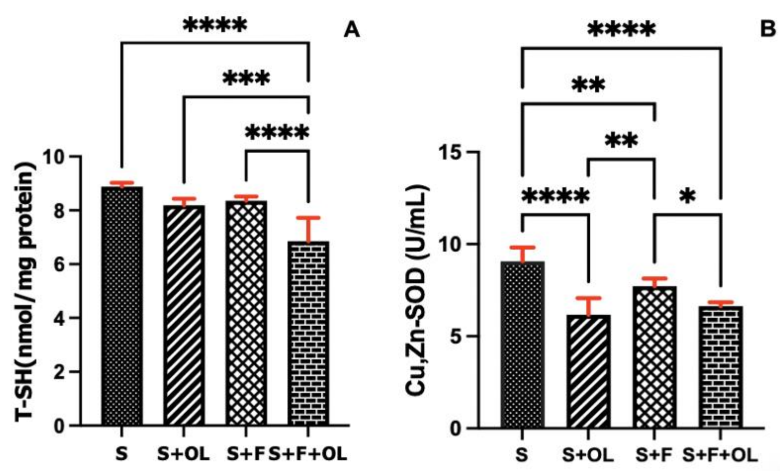

*Figure 4: Antioxidant biomarkers. S: Female Drosophila melanogaster fed on sucrose-based media, S+OL: Female Drosophila melanogaster fed on sucrose + oleuropein-based media, S+F: Female Drosophila melanogaster fed on sucrose + fructose- based media, S+F+OL: Female Drosophila melanogaster fed on sucrose + fructose + oleuropein-based media.*p<0.05; **p<0.01;****p<0.000.T-SH:Total thiol groups; Cu,Zn-SOD: Copper, Zinc superoxide dismutase activity.*

DISCUSSION

Drosophila melanogaster has been extensively used for model organism in various scientific fields including nutrition. Due to its similarities with human being according to the basic biochemical, neurological, physiological properties, the usage of fruity fly provides some advantageous due to easily optimize the experimental processes, higher reproductive capacity, availability to distinct gender, low cost, not requirement for ethic committee allowance (Rocha 2013). However, fruity flies very sensitive to environmental and nutritional changes. The ratio of media content to each other affects all the developmental processes, reproductive capacity and redox homeostasis (Yanar et al,2019). Fruity flies fed on different type of media, however mainly they fed on sucrose-based media. This media may call as standard media. When we compared sucrose concentration of media we see usage of different concentration of sucrose as a standard media (Strilbytska et al,2022;. Rovenko et al.2015; Lushchaket al.,2014). There is no set standard quantity. So, we optimised basal levels of sucrose according to our previous studies (Yanar et al.,2019). During the preparation of media, sucrose hydrolysed to its monomers due to effect of heat. So, we had basal levels of fructose which also increase the reproductive

capacity when we compered to glucose-based media in our previous laboratory findings. Higher levels of fructose used to development of metabolic syndrome in mammals and fruity flies. We add more fructose to our media to evaluate that dose mimic metabolic syndrome or not. Redox homeostasis biomarkers subcategorised as biomarkers of specific and global protein oxidation, glycation, lipid peroxidation, DNA damage and enzymatic and non-enzymatic antioxidant biomarkers. Our results exhibited that fructose led to impairment on redox homeostasis according to each subgroup of biomarkers. We used 10 days -old female fruity flies in our study. The endocrine glands of D. melanogaster at cellular and molecular levels, function in a similar way to those of vertebrate glands (Rocha 2013). Due to possible protective effects of estrogens-homolog, ecdysone, females basal oxidative stress levels were lower than males (we did not show). But they give more response to nutritional changes. We can easily say that females are more sensitive than males although their lower basal oxidative stress levels. Oleuropein (OL), which is the main phenolic phytochemical in olive oil have been used in many beneficial purposes for nutrition, cosmetics, medicine and pharmacology from past to present. Various clinical, experimental and epidemiological studies in recent years have revealed the radical scavenging and antioxidant effects of polyphenols in these products depends on the dosage and exposure time. There are only two studies with oleuropein in fruity flies. One of them used 12 g/L of oleuropein and reported negative effects on development and survival of fruity flies (Güneş & Danacıoğlu, 2018). The other study reported that 0.1 mmol/L of oleuropein (54.5 mg/L) administration resulted in increased lifespan (Atici and Altun, 2021). To avoid any potential toxic effects of OL we prefer to use 5mg/L. The flies were starved for four hours to make sure that oleuropein was consumed. Then we collected our samples. Our results showed advocate results with the Güneş et al study. Further studies are needed to determine the expression and activities of some metabolic enzymes which play important role in carbohydrate metabolism. Acoording to this result relation between redox homeostasis and metabolic alteration can be evaluated. The preliminary results recommend OL should be consumed carefully and further studies to detect the optimum antioxidant dose are needed.

Key messages

1- Drosophila melanogaster has been used as model organism to evaluate relation between redox homeostasis and screening and evaluating the impact of phytochemicals present in the diet.
2- Low levels of fructose beneficial for reproductive capacity while higher doses induce metabolic syndrome
3- OL should be consumed carefully due to its potential prooxidant effect depend on its dosage and duration of administration.

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Impact of *IFNAR1* Gene Variations on Epilepsy: Interaction of Carbamazepine and Levetiracetam in Treatment Outcomes

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ABSTRACT

Epilepsy is a chronic neurological disorder that affects approximately 50 million individuals globally. A key factor in the development of epilepsy is the dysregulation of inflammatory cells and molecules in damaged neuronal tissue. However, the exact mechanisms by which this imbalance in inflammation contributes to epilepsy are not yet fully understood. Therefore, a major focus of epilepsy research is to identify and clarify the interrelated inflammatory pathways involved in both systemic and neurological disorders that could further influence the progression of epilepsy.

Currently available antiepileptic drugs such as carbamazepine and levetiracetam have been found to have anti-inflammatory effects. Our study aimed to examine the relationship between *IFNAR1* gene variations as a risk factor in epilepsy patients using carbamazepine and levetiracetam drugs.

Targeted next-generation sequencing (tNGS) was used to perform molecular genotyping analysis of the *IFNAR1* genes in genomic DNA from 20 epilepsy patients using carbamazepine. and levetiracetam drugs.

According to the MAF value, the relationship between rs2243592:T>G, rs2834196:A>G, rs2856973:T>A, rs2850015:T>C, one of the common variants, and the responses of epilepsy patients to the antiepileptic drugs they use was revealed. A statistically significant association

was found identified between the variants rs2243592: T>G and rs2856973: T>A of the IFNAR1 gene, which is believed to influence sustained drug response (p=0.03 and p=0.05, respectively).

The research findings demonstrate that variations in the *IFNAR1* gene can contribute to modifications in epilepsy progression.

Key Messages: Next-generation sequencing, *IFNAR1*, single nucleotide polymorphism, genetic variation

INTRODUCTION

Epilepsy is a neurodevelopmental disorder that impacts individuals across all age groups. It is estimated that approximately 50 million people worldwide are affected by epilepsy, rendering it one of the most prevalent neurological conditions globally. Epilepsy is a disorder caused by genetic, environmental, and immunological factors. It is known that neural development processes are affected by immune functions (WHO, 2024).

The development of new drugs to control seizures in patients with drug-resistant epilepsy is a crucial area of research. Current treatments are symptomatic; therapies to prevent disease onset or progression are lacking (Vezzani et al., 2015). While recent research has implicated certain signaling pathways in the pathogenesis of epilepsy, the fundamental molecular mechanisms driving disease initiation and progression remain poorly understood (Pitkänen & Lukasiuk, 2011). Recent researches and clinical data from animal models and human brain samples associated with various forms of drug-resistant epilepsy indicate the activation of both innate and adaptive immune responses, as well as the subsequent induction of inflammatory processes (Aronica & Crino, 2011; Vezzani et al., 2011).

Dysregulated activation and resolution of inflammatory cells and molecules within injured neuronal tissue represent a crucial factor in the pathogenesis of epilepsy. However, the precise mechanisms by which this imbalance in inflammation contributes to the development of epilepsy remain unclear. Consequently, one of the primary objectives of epilepsy research is to identify and elucidate the interconnected inflammatory pathways involved in both systemic

and neurological disorders that may further promote the progression of epilepsy (Rana & Musto, 2018).

Genetic variations in several specific genes are known to alter antiepileptic drug (AED) response in different directions. This may be due to genetic variation in several categories of genes, including variation in response to AED therapy, genes affecting drug pharmacokinetics and pharmacodynamics, and genes thought to cause epilepsy. When approaching AED response from a pharmacogenomic perspective, it is very important to investigate the relationships with the numerous genetic variations that affect the pharmacokinetics and pharmacodynamics of these drugs (Balestrini & Sisodiya, 2018). Currently, antiepileptic drugs like carbamazepine and levetiracetam were found to exhibit anti-inflammatory effects (Haghikia et al., 2008; Kambli et al., 2017).

All type I interferons (IFNs) interact with a shared receptor located on the surface of human cells, referred to as the type I IFN receptor. This receptor is composed of two subunits: IFNAR1 and IFNAR2. A cascade of immune responses takes place, mainly involving Janus-activated kinases (JAKs) and tyrosine kinases (Platanias, 2005). The IFNAR1 subunit is expressed by the IFNAR1 gene. *IFNAR1* gene is an important immune response gene that may be immunologically linked to epilepsy. This study aims to evaluate the relationship between *IFNAR1* gene polymorphisms in epilepsy patients with antiepileptic drugs like carbamazepine and levetiracetam with anti-inflammatory effects and to investigate the possible genetic role in disease progression.

MATERIAL-METHODS

The study group consists of 20 patients. The cases were 18–76 years old cases diagnosed with epilepsy. Samples of patients diagnosed with epilepsy were taken from the Republic of Turkey Ministry of Health, Sinan Sipahi Family Health Centre, İstanbul. The protocol was approved by the Ethics Committee of the Haliç University (Approval Number: 2024/78). Firstly, genomic

DNA was isolated from the patient’s peripheral blood. The 12 exons of the *IFNAR1* gene, 3’UTR, and 5’UTR regions of the exons were amplified by PCR method using region-specific primers. The amplified exonic regions were controlled by agarose gel electrophoresis.

Sequence analysis of all exons of the *IFNAR1* gene encoding type I membrane protein that forms one of the two chains of a receptor for interferons alpha and beta was analyzed by next-generation sequencing method on Novaseq 600 next generation sequencing platform (Illumina, San Diego, CA). With the next-generation sequencing method, PCR amplicons were made single-stranded by going through stages such as tagmentation, amplification, and library validation and loaded into Illumina NovaSeq next-generation sequencing device. Raw data analysis was performed. Four single nucleotide polymorphisms (SNPs) of type I IFN receptor 1 (*IFNAR1*) were chosen to analyze their relationships to epilepsy. The statistical analysis four alternative models (genotypes, dominant, recessive and allele) of the study was evaluated using GraphPad Prism version 10.3.1 program (GraphPad Software, La Jolla, CA). Genotype and allele comparisons, as well as their adherence to Hardy–Weinberg Equilibrium (HWE), were assessed using Fisher's exact test. The gene counting method was employed to evaluate allele frequencies. The odds ratio and 95% confidence interval were utilized to assess the risk factors between the groups. A p-value of ≤ 0.05 was regarded as statistically significant.

RESULTS

In our study, all exons of the *IFNAR1* gene were analyzed by next-generation sequencing method. According to the MAF value, the relationship between rs2243592:T>G, rs2834196:A>G, rs2856973:T>A, rs2850015:T>C, one of the common variants, and the responses of epilepsy patients to the antiepileptic drugs they use was revealed. Accordingly, in the rs2243592:T>G dominant model (TG + GG vs.TT), TG + GG carriers (at least 1 G allele carrier) were found to have an increased risk of persistent drug response in epilepsy. However, it was not statistically significant ($p = 0.16$). In the rs2856973:T>A, AA carriers were associated with an elevated drug persistent risk of epilepsy. However, it was not statistically significant ($p = 0.19$). On the other hand, no association was identified rs2834196:A>G and rs2850015:T>C with epilepsy development in the case of the dominant models (Figure 1).

In the rs2856973:T>A recessive model (AA vs. TA+TT), AA carriers were associated with an elevated drug persistent risk of epilepsy. However, it was not statistically significant ($p = 0.19$). Conversely, no association was found with rs2243592:T>G, rs2834196:A>G, and rs2850015:T>C with epilepsy development in the case of the recessive models (Figure 2). In the allele model, rs2243592:T>G variant, the percentage of the T allele was 28.5% and 71.4%,

whereas the G allele was 66.6% and 33.3% in the persistent and rare group with epilepsy, respectively (p=0.03). In the allele model, rs2856973:T>A variant, the percentage of the T allele was 22.2% and 77.7%, whereas the A allele was 54.5% and 45.4% in the persistent and rare group with epilepsy, respectively (p=0.05) (Figure 3).

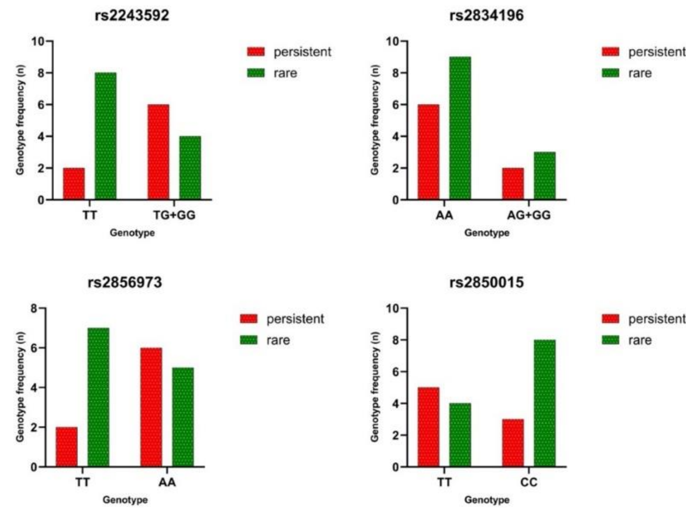

Figure 1. *IFNAR1* Gene Common variants genotype in dominant model among drug persistent and rare groups with Epilepsy

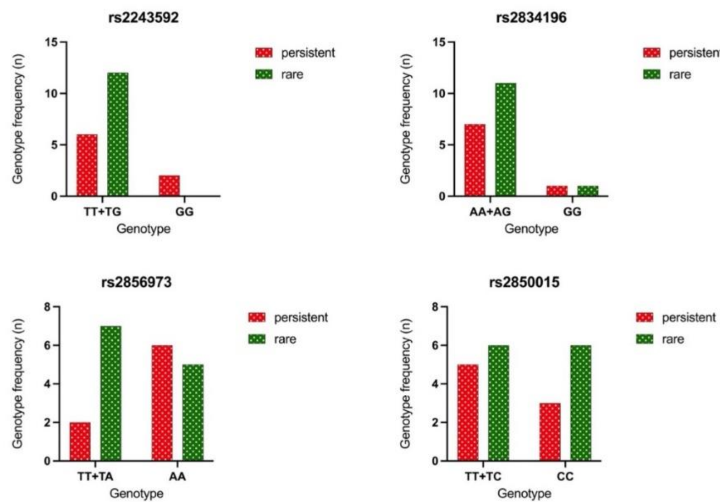

Figure 2. *IFNAR1* Gene Common variants genotype in recessive model among drug persistent and rare groups with Epilepsy

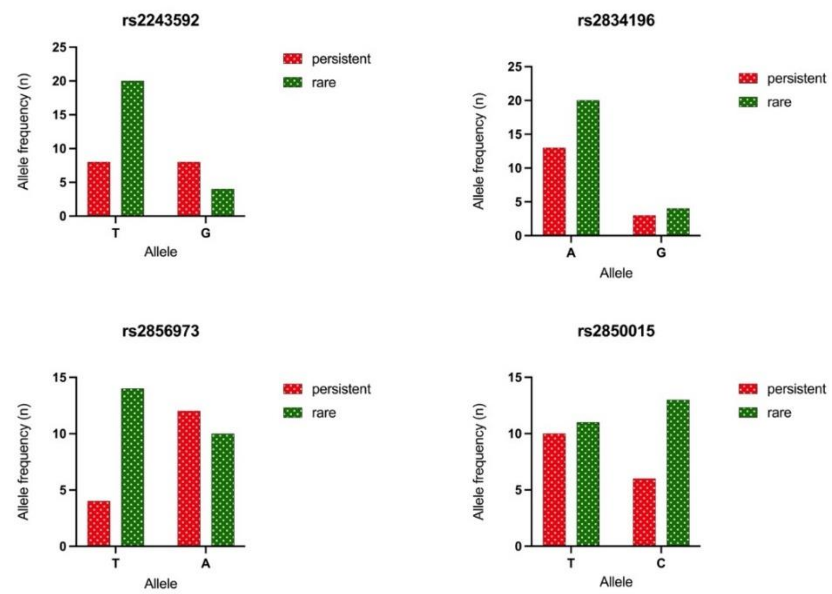

Figure 3. *IFNAR1* Gene Common variants genotype in allele model among drug persistent and rare groups with Epilepsy

DISCUSSION

While various participants have been examined in the recent evidences, the association of these single-nucleotide polymorphisms rs2243592:T>G and rs2856973:T>A with epilepsy patients undergoing treatment with antiepileptic medications such as carbamazepine and levetiracetam has not yet been documented. Therefore, this research is the first rare–persistent study for finding the association of *IFNAR1* gene rs2243592:T>G and rs2856973:T>A in Turkish cases with epilepsy. A statistically significant association was observed for the *IFNAR1* gene rs2243592:T>G and rs2856973:T>A variants, and this association can contribute to persistent drug response. Detecting these polymorphisms in a larger sample size could improve our comprehension of drug responses in epilepsy and facilitate the creation of new treatment protocols.

Acknowledgements

We thank our patients who participated in the study.

Key Messages:

- IFNAR1 gene variations as a risk factor in epilepsy patients using carbamazepine and levetiracetam drugs.

- IFNAR1 gene can contribute to epilepsy progression.
- rs2243592: T>G and rs2856973: T>A polymorphisms of the IFNAR1 gene influence drug response.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The study was conducted by the Declaration of Helsinki on medical research consisting of a human study group. The protocol was approved by the Ethics Committee of the Haliç University (Approval Number: 2024/78).

Data availability

The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

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Determining the Association Between GDF-15 Gene Variants and Type 2 Diabetes: A Hospital-Based Case-Control Study

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ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is one of the most common metabolic disorders worldwide and its development is mainly caused by defective insulin secretion by pancreatic β-cells and the failure of insulin-sensitive tissues to respond to insulin.

Growth differentiation factor-15 (GDF-15) is a member of the transforming growth factor-beta (TGF-β) superfamily that plays multiple roles in various cellular processes. GDF-15 is widely expressed in liver, skeletal muscle, kidney, heart or macrophage, is a direct molecular target of p53 protein and is considered a marker of inflammation and oxidative stress induced by tissue damage, hypoxia and proinflammatory cytokine response. Studies have shown that GDF-15 expression levels can be significantly increased in response to various physiological conditions associated with obesity, insulin resistance, tissue damage, inflammation, diabetes and various malignancies.

Our study aimed to determine the association between GDF-15 rs4808793 and rs1058587 variants and the risk of T2DM. Seventy healthy controls and seventy endometrial T2DM patients participated in this case-control research. Blood samples were used to obtain genomic DNA, and the PCR-RFLP method was used to determine genotypes.

GDF-15 rs4808793 and rs1058587 genotype distributions were found to differ significantly between the T2DM and control groups ($p<0.05$). In addition, while GDF-15 rs1058587 allele frequencies were found to be significantly different between the T2DM and control groups, no significant difference was found in terms of GDF-15 rs4808793 allele frequencies.

As a conclusion, the GDF-15 rs4808793 and rs1058587 variations were determined to be closely associated with T2DM risk.

Keywords: Type II Diabetes, GDF-15, Inflammation, Biomarker

INTRODUCTION

Diabetes mellitus is a chronic, metabolic disease characterized by high blood sugar levels that, over time, damage the blood vessels, heart, eyes, kidneys, and nerves, according to the World Health Organization (WHO). The majority of cases of diabetes mellitus are T2DM, a condition characterized by inadequate insulin secretion by pancreatic islet β-cells, tissue insulin resistance (IR), and an inadequate compensatory insulin secretory response (Galicia-Garcia et al., 2020). 1 in 11 people worldwide are diagnosed with diabetes mellitus, of which approximately 90% have T2DM (Bellary et al., 2021). Patients with T2DM are often characterized by being obese or having a high percentage of body fat, predominantly distributed in the abdominal region. In this condition, adipose tissue promotes IR through various inflammatory mechanisms, including increased free fatty acid (FFA) release and adipokine dysregulation (Galicia-Garcia et al., 2020).

The prevalence of DM and obesity is continuously increasing worldwide. Obesity increases the impact of genetic predisposition and environmental factors on DM. Expansion of adipose tissue and excessive accumulation of certain nutrients and metabolites sabotage metabolic balance through insulin resistance, dysfunctional autophagy, and the microbiome-gut-brain axis. It also worsens immunometabolism dysregulation through low-grade systemic inflammation, leading to rapid loss of functional β cells and gradual elevation of blood sugar (Ruze et al., 2023).

Growth differentiation factor-15 (GDF-15) or macrophage inhibitory cytokine 1 (MIC-1) is a 308 amino acid protein encoded by a 1.2-kilobase transcript of the GDF15 gene located on chromosome 19p13.11 (Xie et al., 2022). GDF-15 has been identified as an adipokine that can regulate appetite and body weight, modulate glucose metabolism, maintain lipid homeostasis, and protect against inflammation and oxidative stress in adipose tissues (Wu et al., 2021). Recent studies have shown a relationship between GDF-15 and carbohydrate metabolism. Experimental studies in mice have shown that GDF-15 improves insulin sensitivity and glucose tolerance. Because of the association between protected hyperglycemia and elevated GDF-15

levels and the beneficial effects of GDF-15 on insulin sensitivity and glucose tolerance, it has been suggested that GDF-15 may have a protective role against the deleterious effects of chronic hyperglycemia associated with diabetes.

Circulating serum GDF-15 levels increase with the development of insulin resistance and the onset of T2DM (Iglesias et al., 2023). Studies have shown that GDF-15 expression levels can increase significantly in response to a variety of physiological conditions associated with obesity, insulin resistance, tissue damage, inflammation, diabetes, and various malignancies (Li et al., 2024).

The link between GDF-15 levels and the risk of type 2 diabetes has not been definitively established due to selection bias and confounding factors such as age, obesity, and pre-existing insulin resistance in the study populations (Wu et al., 2021). Furthermore, although many studies have confirmed that serum GDF-15 levels are associated with the risk of T2DM, there are few studies on the association between GDF-15-associated single nucleotide polymorphisms (SNPs) and the risk of T2DM (Liu et al., 2024).

Our study aimed to determine the relationship between GDF15 rs4808793 and rs1058587 variants and the risk of T2DM.

MATERIAL AND METHODS

Study Population

This case-control study included 70 patients with Type 2 Diabetes Mellitus and 70 healthy controls who applied to the Department of Endocrinology, Eskişehir Osmangazi University, Faculty of Medicine in Eskisehir, Turkey. Informed consent by our protocol, confirmed by the Ethics Committee of Eskisehir Osmangazi University Faculty of Medicine (Approval no: 2024/03), was obtained from all of the patients and control individuals. We actualized the study following the ethical principles of the 1975 declaration of Helsinki. The patient and control group were homogeneous Turkish populations as ethnicity.

DNA Isolation and Quantification

DNA isolation was made using the genomic DNA isolation kit (PureLinkTM, Invitrogen Corporation, Carlsbad, California, USA) and in accordance with the kit protocol. The quantity and purity of DNA samples were evaluated with a NanoDrop (Allsheng, Nano300, Micro-spectrophotometer) spectrophotometer.

Genotyping

The GDF-15 rs4808793 and rs1058587 variants were analyzed by PCR-RFLP. The sequence of primers utilized in the amplification of all of the variants are shown in **Table 1**.

Table 1. Forward and reverse primer pairs designed for PCR.

Gene	SNP	Base change	Forward (5'-3') Primer Sequence	Reverse (5'-3') Primer Sequence
GDF15	rs4808793	-3148 C>G	AGTGAGTCCTTGTGTCTCTTAC	GCAGGCTGGTGTAGAGTC
GDF 15	rs1058587	+2438 C>G	GCAGAATCTTCGTCCGCAC	CCAGCCCAGGTCTTCCAG

The PCR reactions were realized following protocol and all amplification reactions were performed on a thermal Cycler (Thermo Scientific, VeritiPro Thermal Cycler). The PCR mix was equiped in a total reaction volume of 20 µl, consisting of 4 µl master mix (Solis Biodyne, FIREPol Master Mix Ready to Load, 12.5 mM $MgCl_2$), 0,5 µl Reverse and 0,5 µl Forward primer, 14 µl distilled water, and 1 µl genomic DNA. The amplification protocol included initial denaturation at 95°C for the 3 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at the 54-66°C for 30 seconds and an extension at 72°C for a further 60 seconds with a final extension 72°C for 5 minutes, cooling to 4 °C for all variants.

Amplification products were digested with appropriate restriction endonucleases (*Bsr1 for* GDF-15 rs4808793 variants, *AvaII* for GDF-15 rs1058587 variant*)* and analyzed using 3% agarose gel electrophoresis (Invitrogen, UltraPure™ Agarose, Carlsbad CA, 10X TBE Buffer, ABT). The yields were viewed using a (Bio-Rad, ChemiDoc MP Imaging System) and electrophoresis on a 3 percent agarose gel stained with RedSafeTM nucleic acid staining solution (Intron Biotechnology Inc., Seoul, Korea).

Statistical Analysis

The percentage of categorical data was calculated (percent). The examination of the constructed cross tables included Pearson Chi-Square analyses. The analysis was conducted using IBM SPSS Statistics 27.0. p values of less than 0.05 were considered substantial in all of the analyzes.

RESULTS

Genotype Findings

rs4808793 Variant of GDF-15 Gene

When the genotype frequencies of the GDF-15 rs4808793 variant were evaluated, a statistically significant difference was determined between the T2DM and control groups ($p<0.05$). However, no significant difference was determined in terms of allele frequencies ($p>0.05$) (Table 1).

rs1058587 Variant of GDF-15 Gene

When the GDF-15 rs1058587 variant was evaluated in terms of genotype distributions, a statistically significant difference was determined between the T2DM and control groups ($p<0.05$). Similarly, a statistically significant difference was determined between the T2DM and control groups for allele frequencies ($p<0.05$) (Table 1).

Table 2. Genotype and allele frequencies of **GDF-15 rs4808793** and **GDF-15 rs1058587** polymorphisms in the T2DM and Control group.

SNP rs4808793	**Allele**		Statistic p	**Genotype**			Statistic p
	C n (%)	G n (%)		**CC**	**CG**	**GG**	
Control	91 (65)	49 (35)	0.458	23	45	2	**0.006**
T2DM	85 (60.7)	55 (39.3)		27	31	12	
SNP rs1058587	**Allele**		Statistic p	**Genotype**			Statistic p
	C n (%)	G n (%)		**CC**	**CG**	**GG**	
Control	106 (75.7)	34 (24.3)	**0.001**	40	26	4	**0.006**
T2DM	80 (57.1)	60 (42.9)		25	30	15	

DISCUSSION

GDF-15 is widely distributed in cells such as vascular smooth muscle cells, cardiomyocytes, macrophages, fibroblasts, endothelial cells, and fat cells, and in tissues such as the central-peripheral nervous system, vessels, and adipose tissue, and in organs such as the placenta, liver, brain, and heart. GDF-15 has been shown to play important roles in regulating

inflammatory responses, growth, and cell differentiation in these areas. The main sources of GDF-15 release in diabetes are macrophages, white adipose tissue, and liver cells (Berezin, 2016).

Studies have shown that GDF15 may be associated with the development and prognosis of T2DM. Although GDF-15 has been shown to play a role in energy homeostasis and weight loss, has anti-inflammatory properties, and is useful in predicting cardiovascular disease, evidence for the predictive role of elevated GDF-15 in T2DM subjects is still insufficient (Berezin, 2016).

GDF-15 acts as a metabolic regulator, similar to adiponectin and leptin, and has therefore been called cardiokines. Adipokines generally regulate lipid and glucose metabolism, increase insulin sensitivity, regulate food intake and body weight, and protect adipose tissue from chronic inflammation. GDF-15 has been found to reduce food intake, body weight, and adiposity in normal and obesogenic diets and improve glucose tolerance. Serum GDF-15 levels are increased in obese and type 2 diabetic women and correlate with body mass index (BMI), body fat, glucose, and C-reactive protein (Adela & Banerjee, 2015).

GDF-15 has been demonstrated to act as a critical hormone regulating lipid and carbohydrate metabolism (Desmedt et al. 2019). A study conducted by Hong et al showed that GDF-15 level was increased in impaired fasting glucose and type 2 diabetes groups and had a positive correlation with insulin resistance, independent of age and BMI (Hong et al., 2014).

Although studies have confirmed that serum GDF-15 levels are associated with the risk of T2DM, there are few studies on the association between GDF-15-associated single nucleotide polymorphisms (SNPs) and the risk of T2DM.

There are studies in the literature that associate the GDF-15 rs1058587 variant that we evaluated in our study with diseases such as Keshan Disease (He et al., 2018), Systemic Lupus Erythematosus (Xu et al., 2022), Rheumatoid Arthritis (He & He, 2022), Renal Disease (Li et al., 2020). However, there is no study directly associating it with T2DM. In this respect, our study is the first study associating this variant with the risk of T2DM. In addition, in a study conducted in 2022 to analyze the relationship between growth differentiation factor-15 (GDF-15) polymorphisms and catheter infection in patients with diabetic nephropathy (DN), the frequencies of the GG genotype and the G allele in the GDF-15 gene rs1058587 were significantly higher in the DN group with catheter infection compared to the DN group, and the

frequencies of the CC genotype and the C allele were significantly lower compared to the DN group. It was determined that the risk of catheter infection was significantly higher in patients carrying the G genotype in the GDF-15 gene rs1058587 and significantly lower in those carrying the C genotype. As a result of this study, it was determined that the allele in the GDF-15 gene rs1058587 may increase the risk of catheter infection in DN patients (Qing et al., 2022).

In our study, a significant difference was determined between T2DM patients and the control group in terms of both genotype and allele frequencies for the GDF-15 rs1058587 variant. As stated in the above study, the GG genotype and the G allele appear to be risk factors for T2DM.

The second gene variant, GDF-15 rs4808793 variant, whose relationship with T2DM risk we evaluated in our study, has been associated with diseases Thallassemia (Atar et al., 2021), Systemic Lupus Erythematosus (Xu et al., 2022), Coronary Artery Disease (Chen et al. 2021), Rheumatoid Arthritis (He & He, 2022) when literature data are examined.

Diabetes and Hypertension are common diseases that frequently coexist and increase the risk of cardiovascular and renal morbidity and mortality (Ali & Bakris, 2020). On the other hand, high blood pressure exacerbates both micro and macrovascular complications of diabetes mellitus (Rabizadeh et al., 2021). In this sense, although studies directly associating GDF-15 polymorphisms with T2DM risk are limited, studies associating these gene variants with hypertension may also be informative. In the study investigating the relationship between GDF15 gene variants and left ventricular hypertrophy in essential hypertension, three GDF15 variants, -3148C>G (rs4808793), +157A>T (rs1059369) and +2438C>G (rs1058587), were genotyped. As a result of the study, it was determined that only the rs4808793 variant G allele was significantly associated with a lower risk of left ventricular hypertrophy (Wang et al., 2010).

In our study, genotype frequencies for the rs4808793 variant were found to be significantly different between the T2DM and control groups. According to our study results, the GG genotype appears to be a risk factor for T2DM.

Acknowledgements

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Authorship Contributions

Conception and design: E.Y, C.O; Diagnosis and sample collection: N.K, İ.D; Collection and assembly of data: E.Y, C.O, G.Z; Data analysis: E.Y, C.O Data interpretation: E.Y, H.K; Literature search: E.Y, M.B.U; Writing and Presentation: E.Y, M.B.U.

Key Messages

Since inflammation plays an important role in the development and progression of diabetes and diabetes-related cardiovascular complications, inflammatory biomarkers have begun to be used in the diagnostic evaluation of diabetics suspected of having cardiovascular disease. The most commonly used inflammatory biomarker in diabetes is the highly selective C-reactive protein (hs-CRP), which correlates well with cardiovascular complications, poorer metabolic control, and severe hypoglycemia in diabetes, but this biomarker has low sensitivity and specificity. In addition to serial testing of hs-CRP, which is time-consuming, other novel proinflammatory biomarkers such as GDF-15 have the potential to be a useful diagnostic tool for risk stratification in patients with diabetes, including those with T2DM. Scientific data have shown that serum GDF-15 may be a potential marker for identifying people at risk of diabetes and obesity. However, longitudinal studies are needed to identify the early stages of the disease when GDF-15 levels begin to increase in serum. Further research is needed to find out whether GDF-15 plays any role in reducing inflammation or early pathological changes in diabetes and obesity. Studies examining GDF-15 gene variants will also be guiding in this sense and will contribute to the identification of more specific potential biomarkers.

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Testing of 1.7A2UCOE, 1.2A2UCOE and O.5UCOE Universal Chromatin Opening Elements (UCOE) Derived from *Hnrpa2b1-Cbx3* Reference Gene Loci on Human Stem Cells (iPS Cells)

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Abstract

This project aims to provide a universal solution to the problems of low-level binding and inactivation in mammalian cells, such as gene silencing, histone modifications and vector-driven transfection applications, which are frequently encountered in in-vivo and in-vitro gene therapy, genetic modifications and recombinant protein biotechnology studies. The aim of this project is to test the universal chromatin opening potentials of the new generation 1.7A2UCOE and 1.2A2UCOE ubiquitous chromatin opening element models against the methylation and histone modification problems frequently encountered in gene therapy studies on human stem cells and after differentiation into three different tissue types.

Our results showed that the new UCOE designs (1.2kb and 1.7kb UCOE chromatin elements) that we developed, have maintained their expression levels stably on human iPS cells before and after differentiation into three different tissue type cells. In addition, our new 0.5kb design (0.5kb UCOE), produced from another CpG density region of HNRPA2B1 gene, which has not been studied before in the literature, has been observed to display a stable expression level compared to our other designs and even a more stable profile. This result is beyond the target in our project, with the length of 5kb-3kb (without supporting, expression-enhancing cassette regions) and 1.5kb (with supporting, expression-enhancing cassette regions), the new 0.5kb, 1.2kb 1.7kb (without supporting, expression enhancing cassette regions, thus eliminating the potential mutation problem) with a length of 10, 6 and 3 times shorter than the current standard A2UCOE models showed to be more advantageous for gene therapy and recombinant protein production studies.

INTRODUCTION

The aim of this project is to test the resistance of the new generation 1.7A2UCOE-1.2A2UCOE universal chromatin opener elements developed against methylation and histone modification problems frequently encountered in gene therapy studies by differentiating them into different tissue types on human stem cells. The universal chromatin opener element (UCOE) derived from the human HNRPA2B1-CBX3 reference gene locus has been shown to have high reproducibility and stable expression properties even when integrated into extreme heterochromatin regions such as centromeres (Antoniou et al., 2003; Williams et al., 2005). A2UCOE consists of unmethylated CpG chromatin islands encompassing the bidirectional and differentially transcribed HNRPA2B1 and CBX3 promoters. The high reproducibility and stable expression properties of A2UCOE have been proven in many studies both in vitro with lentiviral vectors and more importantly in living organisms, in gene therapy studies with stem cells transplanted into bone marrow. Recently, studies also confirmed its strong resistance to the DNA methylation problem frequently seen in gene transfer (Antoniou et al., 2013; Neville et al., 2017; Zhang et al., 2007).

In my related doctoral project in the field of gene therapy at King's College, the new chromatin opener elements that halve the size of the existing UCOE element, free from additional support enhancers and promoters (thereby eliminating the possible mutation problem), were tested in various cell groups, including mouse stem cell groups (Anakok, 2015; Neville et al., 2017). As a result, it was revealed that the activation capacity of these new UCOE elements we developed became twice as efficient as the previous ones. These new elements will replace the current patent with the advantages mentioned.

The biggest problems encountered in the field of gene therapy are the silencing and inactivation of the transferred gene or chromatin particle as a result of methylation or histone modification and the mutations in the genome of the host cell other than the targeted ones (Sauer et al., 2014; Aubourg, 2016; Zhang et al., 2007). In this sense, the 1.7A2UCOE and 1.2A2UCOE elements we developed have been shown to be resistant to methylation and histone modifications, and to successfully enable the transferred gene to remain active, with pre- and post-differentiation experiments on tetratocarcinoma cells and mouse embryonic stem cells. At the same time, by shortening its length considerably compared to the previous A2UCOE element, extra space has been opened for gene transfer, and the enhancer factors and promoters

in the original A2UCOE structure have been removed, eliminating the risk of uncontrolled mutations (Anakok, 2015; Neville et al., 2017).

The aim of this project is to reveal whether the new UCOE elements can be used in gene therapy studies, primarily by differentiating them into tissue cells on human stem cells, which is a missing part in the literature. When the expected goal is achieved, these new UCOE elements we have developed will replace A2UCOE both in the treatment of genetic diseases in the field of gene therapy and in the industrial production of specific enzymes and proteins with mammalian cells in the in-vitro environment in the fields of biotechnology.

MATERIAL-METHODS

Obtaining 1.2kb and 1.7kb UCOE elements

To obtain 1.2kb and 1.7kb UCOE elements, target chromatin sequences were ordered from the relevant GeneArt company together with control group UCOE vectors, then target chromatin fragments were amplified by PCR method and stored at -200C under sterile conditions. Then, for verification analyses, they were cut with specific restriction enzymes and run in gel electrophoresis to verify length, and the target plasmid in the relevant E. Coli DH5α bacteria was transferred into the vectors with the final stage, plasmid cloning method. Before proceeding to the production stage, plasmid DNAs were cut again with relevant restriction enzymes to verify the placement of UCOE chromatin fragments in the targeted region in the plasmid vector, and the kb sizes of the cut regions were compared with the required values.

Target stem cell group (Induced Pluripotent stem cell) culture, stocking and differentiation into tissue cells.

After the induced human pluripotent stem cell group cells ordered from ATCC company reached us, they were immediately cultured and multiplied according to the protocol specified by the company on the same day and backed up in liquid nitrogen storage.

After the stock backup, our stem cells were multiplied in culture again and verification analyses were performed before proceeding to the next experimental steps. For this purpose, the cells were multiplied in 6 well plates with 1cm2 slides in the wells in a special medium

containing a cell differentiation inhibitor agent recommended by the relevant company and immunofluorescence staining was performed after the colonies reached a significant size. For the staining process, the undifferentiated iPS cell markers SSEA4, Tra-1-60 and Nanog pluripotent antibodies specified in the relevant stem cell protocol were applied with the relevant secondary antibody Alexa Fluor 488 goat anti-mouse and the cells were stained and kept in this solution at +40C overnight, followed by immunofluorescence confirmation analyses under a confocal microscope.

Transfection of the target stem cell group with lentiviral vectors and verification analyses

The induced human stem cells (Human iPS cells) that we produced were then inoculated with our lentiviral vectors carrying our UCOE models that we produced in line with the target in this section.

After 24 and 48 hours, the cultures were first examined under light and fluorescence microscopes to check whether the changing MOI values had a cytotoxic effect. At the end of 48 hours, no significant cell death or morphological change was observed at the MOI-5 value when examined under a light microscope. In order to understand whether the transduction process was successful under a fluorescence microscope, the placement and activation of our target UCOE chromatin groups in the stem cell genomes due to eGFP reporter protein radiation in our cells were checked and recorded.

RESULTS

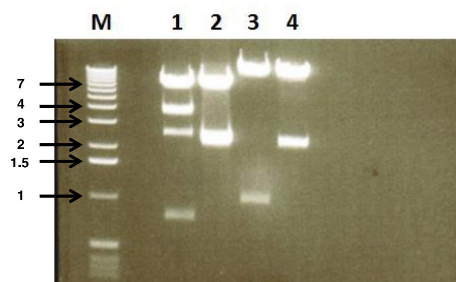


Figure 1. Vector maps of target UCOE lentiviral vector plasmids and identity and integration verification analysis of UCOE chromatin elements after cloning in target vectors.

M: Molecular size marker (kb)

1. GFPVECTOR (UCOE-LESS Negative Control)
2. 1.7kbUCOE
3. 1.3kbUCOE
4. 0.5kbUCOE

After cloning of target vectors and mini-prep plasmid DNA isolation, target lentiviral vector plasmids were cut with the relevant enzymes and run on agarose gel electrophoresis, and the DNA band lengths formed depending on the cut points were compared in order to verify their integration in vector maps.

Culture, stocking and differentiation of target stem cell group (Human Induced Pluripotent Stem Cell) into tissue cells

The culture, stocking and tissue type differentiation of target induced type human stem cell group was applied within the framework of the specified protocols and the culture and stocks of the cells were completed both before differentiation and after tissue type cell differentiation.

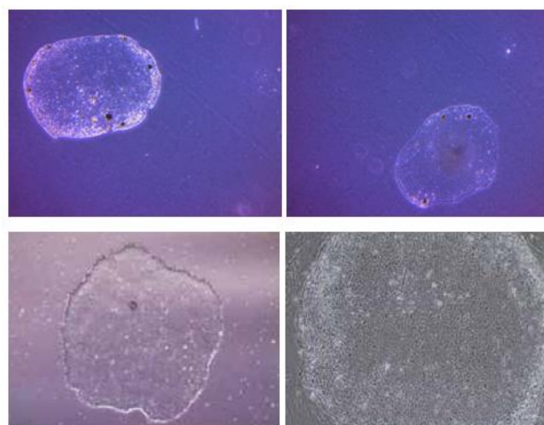


Figure 2. Culture of target iPSc stem cells. 1st and 2nd images are light microscope images of the development of the culture after the first four days. 3rd and 4th images are x40 and x100 magnification images of the clustering formed by the cell groups after the 5th-6th days.

Transfection of the target stem cell group with lentiviral vectors and verification analyses

In this section, the induced human stem cells we produced began to be inoculated with our lentiviral vectors carrying our UCOE models that we produced in line with the target.

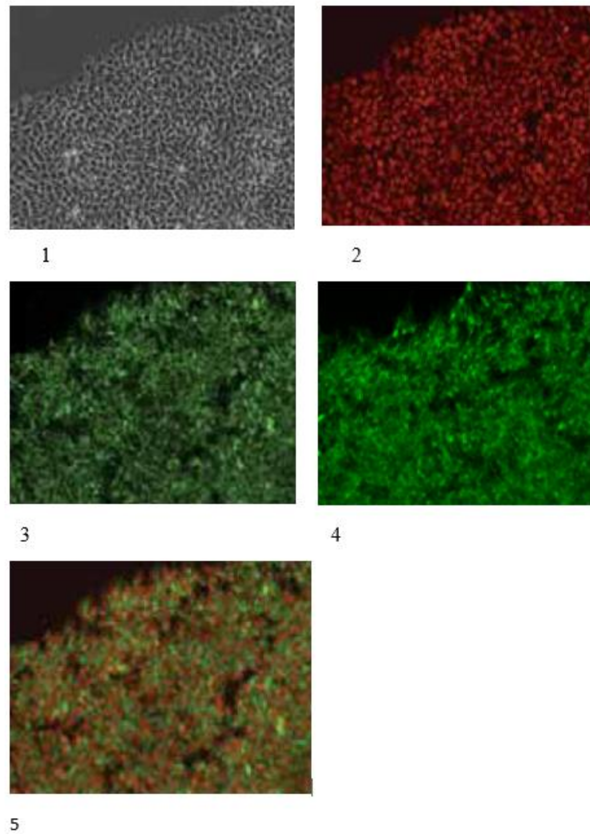


Figure 3. Detection of undifferentiated human iPS cells transfected with lentiviral vectors using immunofluorescence markers.

1, phase contrast microscope image (x10)

2, Nanog antibody (red) confocal staining image

3, Tra-1-60 + SSEA4 (green) confocal staining image

4, Tra-1-60 + eGFP (green and bright green) confocal staining image

5, Nanog + eGFP (red and green) confocal staining image

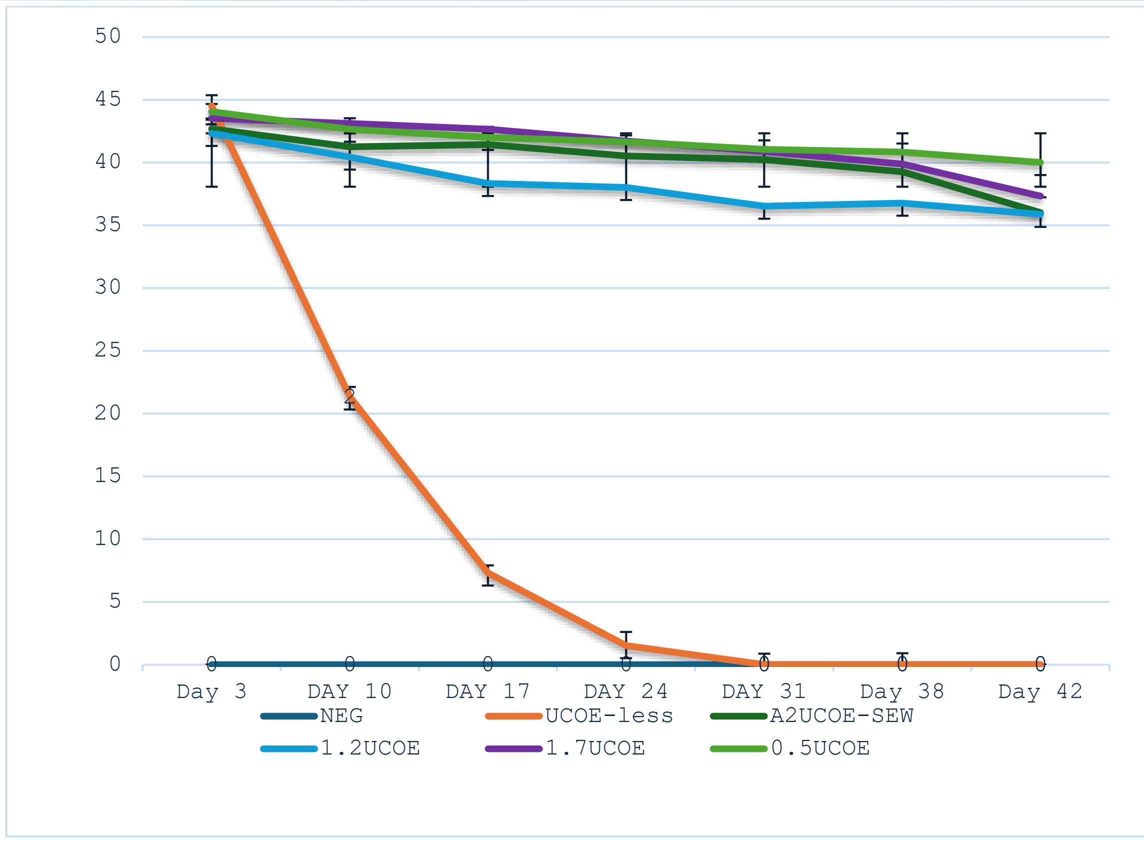


Figure 4. Mean percentage of 'eGFP+' cells measured weekly by flow cytometry during the 0-42 day experiment after transfection of undifferentiated human iPS stem cells with UCOE vectors.

UCOE-less vector carrying no UCOE chromatin is shown in red, A2UCOE-SEW standard UCOE control vector is shown in green, 1.2UCOE 1.2 kb long UCOE model is shown in purple, 1.7UCOE 1.7 kb long UCOE model is shown in blue and 0.5UCOE 0.5 kb long UCOE model is shown in orange (Mean + SEM, n=4; **p<0.01).

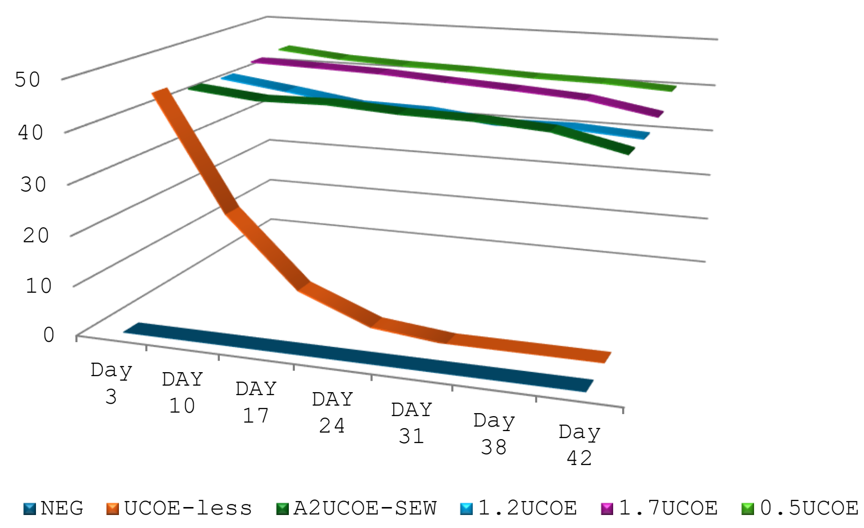


Figure 5. Mean Fluorescence Intensity percentage averages of undifferentiated human iPS stem cells transfected with UCOE vectors

Measured weekly by flow cytometry during the 0-42 day experiment. UCOE-less vector carrying no UCOE chromatin is shown in red, A2UCOE-SEW standard UCOE control vector is shown in green, 1.2UCOE 1.2 kb long UCOE model is shown in purple, 1.7UCOE 1.7 kb long UCOE model is shown in blue and 0.5UCOE 0.5 kb long UCOE model is shown in orange (Mean + SEM, n=4; **p<0.01).

Differentiation of target stem cell groups into 3 different tissue types and keeping them in culture for 2-3 months and performing weekly verification experiments

After successful completion of the experiments of transfecting undifferentiated iPS cells with our target UCOE vectors and observing their activation for 42 days, induced type human stem cells were differentiated into 3 different tissue types (1. Frontal nerve cell type and astrocyte nerve cells, 2. Liver Hepatocyte (Hepatocyte-Like) cell type and 3. Heart muscle cell (Cardiomycet) type cell) and testing whether our UCOE vectors still maintain their activation.

Nervous type cell differentiation

For tissue type differentiation experiments of iPS cells, the frontal nerve cell differentiation protocol was first applied. For this purpose, our stem cells were first re-cultured and inoculated with the same MOI ratios with our target vectors, and after the target GFP-positive ratio was confirmed, the nerve cell differentiation protocol was started to be applied starting from the 5th day.

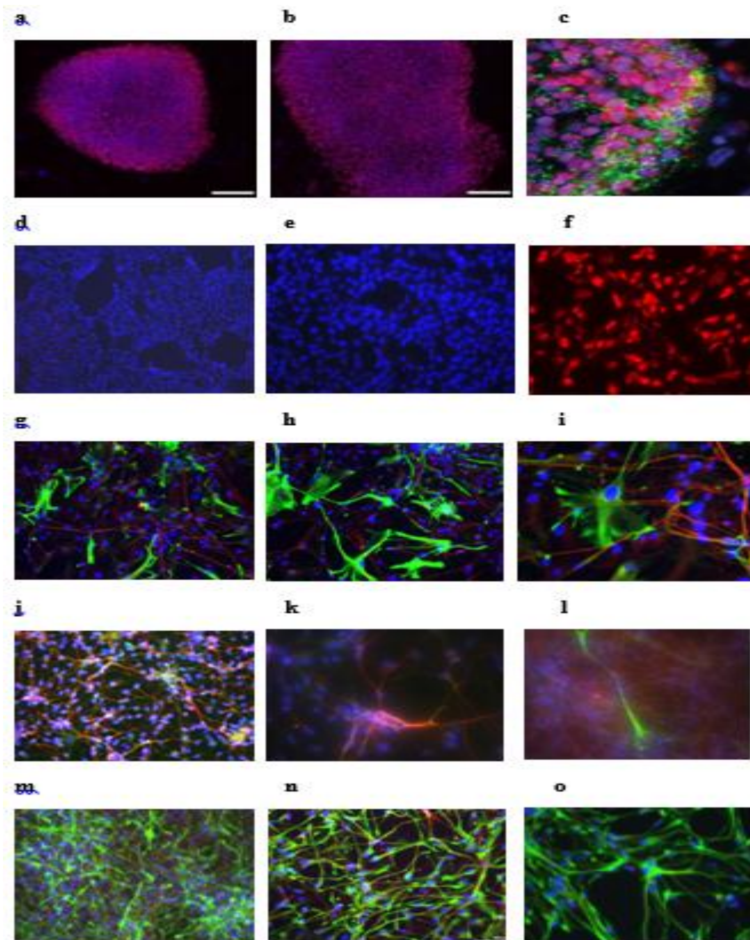


Figure 6. Differentiation of transduced human iPS stem cells into pre-neuronal cell and astrocyte cell types.

a-b: Immunofluorescence confacial image before differentiation (x10), nanog+DAPI (red and blue)

c: Immunofluorescence confacial image before differentiation (x20), nanog+DAPI+eGFP (red, blue and green)

d: DAPI (blue) confacial image after 3rd day pre-neuronal cell differentiation medium and retinoic acid agent application (x10)

e: DAPI (blue) confacal image after 5th day differentiation medium and retinoic acid agent application (x10)

f: Beta-tubulin III (red) confacal image after 5th day differentiation medium and retinoic acid agent application (x20)

Liver Hepatocyte (Hepatocyte–Like) cell type differentiation

After successful testing of nerve cell transformation and target UCOE activation, our iPS cells were also tested for type 2 tissue differentiation hepatocyte type cell transformation experiments.

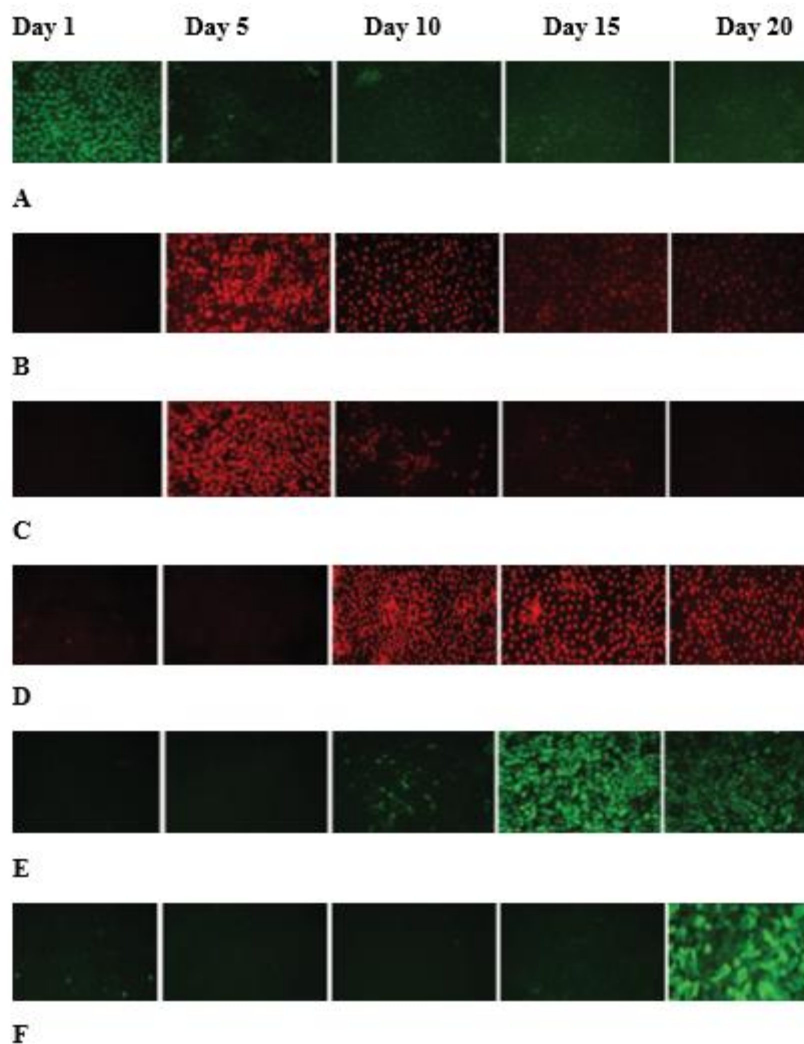


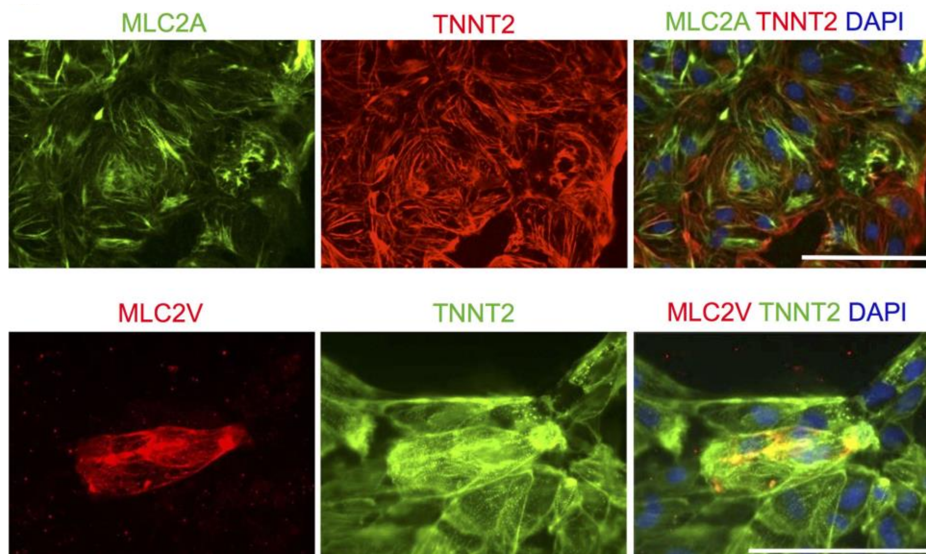
Figure 7. Hepatocyte cell type differentiation of transduced human iPS stem cells.

Confocal images of A-OCT3/4, B-GATA4+FOXA2, C-BMP4, D-FGF2+HNF4, E-AFP, F-Albumin antibody + eGFP expressions before and after hepatocyte type differentiation for 20 days.

Cardiomyocyte Type Differentiation Application

Induced type human stem cells (hiPSCs) were initiated as in the above culture cultures, then dissociated at 1:10 or 1:12 ratios using EDTA and continued to be grown for 4 days.

A



B

Figure 8. Cardiomyocyte type differentiation of transduced human iPS stem cells.

A) Flow cytometry assessment of cardiac troponin T (TNNT2) + eGFP, atrial myosin light chain 2 (MLC2A) + eGFP, and ventricular myosin light chain (MLC2V) + eGFP expressions derived and maintained in cardiomyocytes from day 10 to day 60 of differentiation.

B) Immunofluorescence staining of day 20 cardiomyocytes with the same antibodies used in flow cytometry to demonstrate specificity (Scale bar, 10 μm).

DISCUSSION

Many reports have shown that the HNRPA2B1-CBX3 UCOE fragments tested for expression anti-silencing capacity contain several functional subdomains that are capable of generating an environment for this purpose, both epigenetically and transcriptionally (Neville et al., 2017; Zhang et al., 2010; Dighe et al., 2014; Yu-cheng Kao et al., 2016; Brendel et al., 2012; Talbot et al., 2010). The mechanism by which these regions are completely independent or work together to ensure stable expression is still unknown (Neville et al., 2017). It has been suggested by many similar studies that the 1.5UCOE portion of the 3'UCOE-CORE region, which has both the HNRPA2B1 and CBX3 promoters, provides slightly greater expression stability than fragments that only contain the CBX3 portion (Karagiannis & Eto, 2016; Takahashi et al., 2007). This suggests that the bi-directionally transcribed promoter structure of

the UCOE-CORE model may provide a stronger ability to prevent anti-transcriptional silencing (Ackermann et al., 2014).

In light of all the results obtained, it was observed that the new UCOE designs we developed (1.2kb and 1.7kb UCOE chromatin elements) stably maintained their expression levels in line with the targeted purpose before differentiation and after 2 different types of differentiation in human induced stem cells. In addition, our new 0.5kb long design (0.5kb UCOE) that we produced for trial purposes from another region with CpG (Cytosine-Guanine bases) density on the HNRPA2B1 gene, which has not been studied in the literature, was observed to exhibit the stable expression level observed in our other designs and even a more stable profile (Neville et al., 2017). This result is beyond what was targeted in our project. Compared to the current standard A2UCOE models of 5kb-3kb (without promoter, expression enhancer cassette regions) and 2.2kb-1.5kb (with promoter, expression enhancer cassette regions), our new 0.5kb (without promoter, expression enhancer cassette regions) design, which is 10, 6 and 3 times shorter in length, has become quite advantageous in terms of gene therapy and recombinant protein production studies (Jostock & Knopf, 2012; Wurm, 2004; Betts & Dickson, 2015; Pfaff et al., 2013).

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Key Messages

Inclusion of cis-acting (non-coding DNA which regulate the transcription of neighboring genes) UCOEs in mammalian expression vectors has been shown to improve productivity and facilitate high-level gene expression irrespective of the chromosomal integration site without lengthy gene amplification protocols.

The presence of the UCOE also affords increased recombinant protein expression per vector copy number at reproducible levels, with small variation in expression within and between cell lines transfected with UCOE-containing vectors, compared to cells transfected with non-UCOE-containing vectors.

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A First Preliminary Report: Potential Implications of Ido1 Expression on Soluble Tryptophan and Tryptophan Catabolites in Gastric Tumors and Tumor Microenvironment

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ABSTRACT

AIM: Indoleamine 2,3-Dioxygenase 1 (IDO1)-mediated tryptophan degradation products are associated with the process of carcinogenesis in different cancers. IDO1, whose overexpression at the tumor site causes low survival, metastasis and differentiation, also affects the functions of immune system cells in the tumor microenvironment, leading to suppression of the immune system and thus to the development of tumor-related mechanisms such as metastasis and angiogenesis. In this study, we aim to investigate the relationship between IDO1 and tryptophan/kynurenine pathway in gastric cancer tumor tissue and microenvironment.

METHOD: IDO1 expression was analyzed using quantitative PCR (q-PCR) method in 102 gastric cancer (GC) samples, both tumor tissue and the surrounding microenvironment. Serum levels of tryptophan, kynurenine, and kynurenic acid were measured via High Pressure Liquid Chromatography-Fluorescence Detector (HPLC-FD) in 51 GC patients and 49 healthy controls.

RESULT: IDO1 expression was observed 3.25 times lower compared to the tumor microenvironment ($p=0.05$) in gastric tumors. In GC, tryptophan levels were identified approximately 1.6 times higher at a significant level (AUC: 0.889; cut-off ≤21.57; $p<0.001$).

DISCUSSION: When metabolized through the IDO1 pathway, increased tryptophan accumulation in the gastric tumor and its microenvironment plays a significant role.

INTRODUCTION

Tryptophan is an essential aromatic amino acid which carries an indole ring. It transforms some special products and takes part in protein synthesis in the tissue (Ball, 2009). Tryptophan/kynurenine pathway converts the amino acid tryptophan into several biologically active metabolites. This pathway terminates with three products which are Kynurenic acid, 3-hydroxykynurenine, quinolinic acid and nicotinamide adenine dinucleotide (Macchiarulo, 2009). The first and rate-limiting step in tryptophan/kynurenine pathway involves the transformation of tryptophan into N-formyl kynurenine. This reaction is carried out by three enzymes: tryptophan 2,3-dioxygenase (TDO), indoleamine 2,3-dioxygenase (IDO) and indoleamine 2,3-dioxygenase-2 (IDO-2) (Thackray, 2008). Studies showed that some products which involves in this pathway can play important roles in cancer development. Some catabolites in this pathway can both have antiproliferative effect and proliferative effect on some cancer types (Capece, 2010).

Different amino acids which are involved in the tumor and tumor microenvironment (TME) take role in tumorigenic and anti-tumorigenic activity. The amino acids glutamine, histidine, and tryptophan are lower in gastric cancer plasma samples (GC) compared to those with gastric ulcers (GU) (Jing, 2018). But L-tryptophan can only be detected in GC patients (Ilie-Mihai, 2020). In addition to these differences, according to two studies, tryptophan levels in the gastric juice of GC cases have been found 9 times higher compared to control group and 11.8 times for gastric juice and serum in GC respectively (Deng, 2011; Choi, 2016). But also, it can be seen that kynurenic acid, anthranilic acid and kynurenine/tryptophan ratio increased in serum (Choi, 2016). These results suggest that differences in metabolite levels are frequently reported in gastrointestinal tract cancer (Laviano, 2003).

Kynurenic acid showed an antiproliferative effect in colon cancer, while it was found to have the opposite effect in gastric cancer (Xiang, 2019). Such as kynurenic acid, kynurenine has been shown to have a proliferative effect and its levels are increased in cells resistant to antiproliferative drugs such as 5-Fluorouracil and cisplatin (Wu, 2022). The studies also showed the relationship between kynurenine and Regulatory T (Treg) cells. Li et. al. found that kynurenine can transfer to extracellular matrix and regulates the immune cells (Li, 2019).

Patil et. al. found that IDO1 levels are related with decreased metastasis and recurrence rate (Patil, 2018). In gastric tumors, IDO1 positivity or high mRNA expression causes more severe depth of invasion, increased lymph metastasis and lower survival rates (Zhang, 2013; Liu, 2016). IDO1 is also related with LGALS9A which is responsible for immune control (Mansorunov, 2022). Another study showed significantly high levels of IDO1 positivity in Tumor-infiltrating lymphocytes (TILs) with gastrointestinal cancer (Blakely, 2018). In addition to these results, metanalysis results suggest high IDO1 expression causes low survival, increased differentiation, distant organ metastasis and advanced clinical stage (Herrstedt, 2019).

While Lu et. al. found that IDO1 expression was only detected in half of the cancer patients, %89,8 IDO1 was found in tumor infiltrating lymphocytes with gastric cancer (Patil, 2018; Blakely, 2018). Studies showed that IDO1 expression is associated with decreased CD4+/CD8+ T cell ratio and $CD8^{+}$ T cell dysfunction (Li, 2014; Li, 2020). In IDO1 knock-down mice models, decreased metaplasia and autoantibody levels were found and high IDO1 levels related to angiogenesis, migration and invasion in the H. Pylori-induced animal model study (El-Zaatari, 2018). In gastric tumors, immune cells continuously express IDO1 and different IDO1 expressions affect the immune profile and suppression status in the tumor microenvironment, histological type and tumor localization (Perrot-Applanat, 2022; Yang, 2021). On the other hand, Nishi et. al. suggested that high IDO1 expression resulted with low survival rate in gastric cancer (Nishi, 2018). In addition, this enzyme is responsible for low survival rate, more severe depth of invasion and increased lymph metastasis.

Many studies investigating the effect of tryptophan metabolism on cancer have reported different results. Histopathologic evaluation of the effects of IDO1 inhibitors on gastric tumors is of great importance. Our study aims to evaluate the effects of IDO1 expression and tryptophan metabolites on gastric cancer.

MATERIAL-METHODS

Sample Collection

A total of 100 people, fifty-one diagnosed with cancer and forty-nine controls, participated in the study and two different tissue samples were taken from patients at Çam and Sakura Hospital. Tumor and tumor microenvironment tissue samples were taken from gastric cancer patients. From control group, peripheral blood samples were taken.

Gene Expression

Firstly, tissues were homogenized with trizol via homogenizer. Then RNA extraction was performed using chloroform (Trizol:1/Chloroform:200). RNA was precipitated and purified with absolute isopropyl alcohol and 75% ethanol respectively. After that cDNA synthesized from 1 µg of total RNA using the High-Capacity cDNA Reverse Transcription Kit (LifeTech, Applied Biosystem, USA). TaqMan Gene Expression Assay (LifeTech, Applied Biosystem, USA) was used for real-time quantitative PCR following the manufacturer's instructions. Primers specific for the IDO1 gene were designed. The qPCR conditions were 95 °C for 10 min and then 35 cycles of 95 °C for 15 s, 60 °C for 1 min.

qPCR was performed and Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) was used as the endogenous control. Relative gene expression of the IDO1 gene was calculated using the $2^{-\Delta\Delta CT}$ method.

HPLC-FD Analysis

To examine the levels of tryptophan, kynurenine and kynurenic acid in plasma samples, high pressure liquid chromatography-fluorescence detector (HPLC-FD) was used. It was done according to the method which was developed by Xiang et. al. (Xiang, 2010).

Statistical Analysis

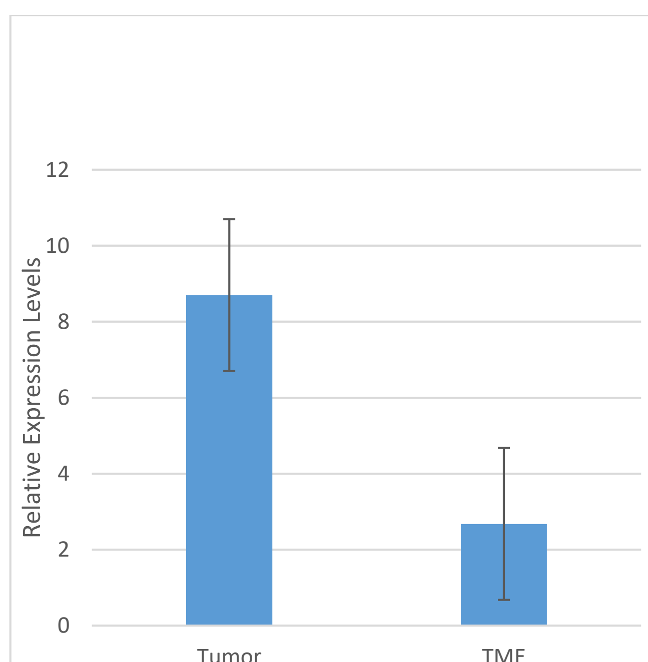
Statistical analyses were conducted using IBM SPSS version 22 and GraphPad Prism version 8, with significance determined at $p<0.05$ for all tests. As the IDO1 expression levels in both tumor and tumor microenvironment did not follow a normal distribution, the Mann-Whitney U test was applied for evaluating them in relation to clinicopathological parameters, while the Student's t-test was used for comparisons of clinicopathological data. Tryptophan and its metabolites were assessed using an independent t-test based on IDO1 regulation categories and patient-control differences. Receiver operating characteristic (ROC) curve analysis was performed for IDO1 and metabolite levels, and their associations were examined using Spearman's correlation analysis.

RESULTS

IDO1 Expression Analysis

According to qPCR and statistical analysis, IDO1 levels were found to be approximately 3.25±0,34-fold times higher in gastric tumors compared to TME (Table 1). According to ROC analyses, IDO1 TME expression could not be discriminated against gastric tumors.

Figure 1. Comparison of Expression Levels of IDO1 in Tumor and Tumor Microenvironment



Tryptophan Levels

In gastric cancer patients, serum tryptophan levels were found 1,6 times higher than control group. In ROC curve analysis, serum tryptophan in GC discriminates against healthy control. These analyzes are shown in table 1-2.

Kynurenine Levels

Serum kynurenine levels were found slightly lower in gastric cancer compared to control samples but not statistically significant (Table 1-2).

Kynurenic Acid Levels

Serum kynurenic acid levels were found higher (approx. 2 times) in gastric cancer samples compared to control group but not statistically significant.

Table 1. Tryptophan, Kynurenine, Kynurinic acid levels, fold change of IDO1 and distribution according to histopathology.

		n (%)	**IDO1 [a] µmol/L ±SE**	P value	**TRP[b] µmol/L ±SE**	P value	**KYN[c] µmol/L ±SE**	P value	**KA[d] µmol/L ±SE**	P value
Group	GC Patients	51 (%51)	3,25±0,34[f]	**0,05**	16,76±1,07	**<0.0001**	1,39±0,03	0,15	0,029±0,017	0,48
	Control	49 (%49)	**-**[e]	-[e]	26,80±0,74		1,42±0,01		0,013±0,002	

Abbreviations: n: Number of patients; SE: Standard Error; a: This expression value is the calculated fold change of tumor tissue compared to tumor-free tissue, taking GAPDH as reference. b indicates TRP-Tryptophan, c indicates KYN-Kynurenin, d indicates KA-kynurenic acid. e: Since IDO1 expression was examined in tumor and tumor microenvironment (TME), the fold change cannot be calculated since the healthy control group does not have a tumor. f: Fold change of IDO1 expression in tumor tissue of GC patients compared to TME

Table 2. Distribution of metabolites according to the regulation status of IDO1 expression in tumor tissue relative to TME

	IDO1	**n (%)**	**Mean±SE**	**p value**
Tryptophan µmol/L	Downregulation	21 (%41.2)	17,597±1,478	**0,025**
	Upregulation	30 (%53,8)	11,964±1,604	
Kynurenine µmol/L	Downregulation	21 (%41.2)	1,292±,058	0,133
	Upregulation	30 (%53,8)	1,426±,051	
Kynurenic Acid µmol/L	Downregulation	21 (%41.2)	0,012±0,004	0,669
	Upregulation	30 (%53,8)	0,011±0,002	

Abbreviations: n: Number of patients; SE: Standard Error

DISCUSSION

In gastric cancer, amino acids and amino acid metabolism play important roles in tumor development within tumor tissue as well as the tumor microenvironment. They can affect tumor development tumorigenic and anti-tumorigenic. Studies on tryptophan levels, one of these

amino acids, have yielded different results (Deng, 2011; Choi, 2016). These conflicting results could stem from the accumulation of tryptophan in tissues, which is only minimally reflected in its soluble levels (Laviano, 2003). When looking at the studies on kynurenine and kynurenic acid levels, it can be seen that the increased levels of these two amino acids so they can be used in clinical approaches (Engin, 2016).

Tryptophan metabolites kynurenine and kynurenic acid show different effects on colorectal and gastric cancer (Walczak, 2011; Xiang, 2019). These different results suggest that kynurenic acid effect is related to anatomical localization and histological type of the tumor (Chen, 2022). Also, tryptophan and the catabolites can inhibit the proliferation of T cells in gastric tumors (Zhang, 2011). On the other hand, apoptosis promoting effects observed. This showed that the tryptophan pathway can be both tumorigenic and anti-tumorigenic (Wu, 2022).

In this study overexpression of IDO1 was found in tumor tissue compared to tumor microenvironment, in contrast tryptophan levels were lower in GC. IDO1 expression shows heterogenicity according to localization and histological type. Recent studies show that overexpression of IDO1 is related to poor prognosis, increased pathological stage and increased depth of lymph node invasion (Li, 2014). In serum, when we found the IDO1 expression in 58.8% of the cases in tumor tissue, another study showed that the overexpression 47.7% in GC.

In this study we didn’t know the distribution of TIL, did not measure the enantiomers of tryptophan separately and didn’t evaluate the epigenetic factors that will affect IDO1 expression. In future studies we aim to reduce these limitations. Eliminating these limitations will enable better elucidation of IDO1-mediated tryptophan metabolism in gastric cancer.

ACKNOWLEDGEMENTS

The authors declare no acknowledgments.

KEY MESSAGES

- ✓ IDO1-mediated tryptophan metabolism products are associated with cancer.
- ✓ IDO1 expression is more dominant in the tumor microenvironment than in the tumor.
- ✓ Tryptophan levels are highly discriminatory between gastric cancer and healthy controls.

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Investıgatıon of Co-Expressıon of Lncrna Snhg1 and Mır-153-3p in Tumor and Tumor Mıcroenvıronment of Gastrıc Cancer Cases

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ABSTRACT

AIM: miR-153 has tumor suppressor effects in many solid organ cancers including gastric cancer (GC). Similarly, LncRNA SNHG1 is overexpressed in many solid organ tumors compared to tumor-free surrounding tissues. Our study aimed to decipher the role of mir-153-3p/ lncRNA SNHG1 axis in the pathophysiological direction in gastric cancer.

METHOD: cDNA synthesis was performed from total RNA in tumor and tumor-free surrounding tissue of 30 GC patients, and transcript levels of SNHG1 and mIR-153-3p were determined by RT-PCR. The transcript levels, clinicopathologic parameters of the patients, and demographic information of the patients were statistically analyzed. Comparison of the levels between tumor and TME, the relationship between fold changes and clinicopathological parameters were evaluated. Spearman correlation test was used to evaluate the co-expression of lncRNA SNHG1 and miR-153.

RESULT: As a result, relative expression of lncRNA SNHG1 levels was determined as 38.9 (fold change) in tumor tissue and 18.4 (fold change) in tumor-free surrounding tissue compared to GAPDH. A 2.12-fold significant increase was detected in tumor tissue compared to surrounding tissue (p=0.02). Relative expression of miR-153-3p levels was determined as 318.07 (fold change) in the surrounding tissue without tumor and 701.55 (fold change) in tumor tissue. The miR-153-3p level was 2.22 times higher in tumor tissue than in the non-tumorigenic surrounding tissue.

DISCUSSION: The correlation of the SNHG1/mir-153-3p axis in gastric cancer and their higher expression in tumor tissue compared to TMC suggest that this axis has a strong oncogenic effect.

INTRODUCTION

Gastric cancer is one of the most common cancers in the world and is highly aggressive with an average survival of less than one year. Gastric cancer is more common in men than in women. The main risk factors for gastric cancer are family history, obesity, diet, smoking and alcohol consumption, Helicobacter pylori and Epstein-Barr virus (EBV) positivity (Machlowska, 2020). Gastric cancer has been characterized in three subtypes. These are named intestinal, diffuse, mixed subtypes. Diffuse subtype involves weakly cohesive cells, diffusely infiltrating the gastric wall without gland formation. Cells with prominent cytoplasmic mucin and a nucleus pushed aside due to the high mucin content have been termed the stony ring type and have been reported to have a poor prognosis in the literature (Chia, 2016). Despite all these histopathologic classifications, studies suggest that the planning of the treatment of the disease is inadequate (Dicken, 2005).

In gastric cancer, some mutations, gene polymorphisms and epigenetic changes can affect various cell signaling pathways and molecular mechanisms that regulate cell proliferation, metastasis and invasion. Recent studies have shown that epigenetic changes, which can also vary with the effect of environmental factors, have been reported as another factor affecting heterogeneity (Goyal, 2019).

Gastric cancer is a heterogeneous disease, and no clear mutation pattern has been identified in gastric tumors. Therefore, classification types differ even within themselves in terms of the response of the disease to treatment. The molecular mechanisms that drive the genesis and development of the disease are critical for the generation of new prognostic and therapeutic targets (Zhang, 2017). In gastric cancer, some mutations, gene polymorphisms and epigenetic changes can affect various cell signaling pathways and molecular mechanisms that regulate cell proliferation, metastasis and invasion. Recent studies have shown that epigenetic changes, which can also vary with the effect of environmental factors, have been reported as another factor affecting heterogeneity. Epigenetic modifications include methylation, acetylation, miRNA, lncRNAs. While these alterations do not cause any change in DNA sequence, they can alter gene expression and can be inherited somatically (Goyal, 2019). Non-protein-coding RNAs, molecules responsible for epigenetic modifications, can be categorized into two primary groups based on their length: short non-coding RNAs and long non-coding RNAs (lncRNAs). Studies have shown that lncRNAs can increase/decrease transcription and

cause a series of conformational changes by interacting with endogenous (ceRNA) RNAs and mi-RNA/m-RNAs (Fang, 2016).

MicroRNAs (miRNAs) are short RNA molecules of 19 to 25 nucleotides. MicroRNAs regulate gene silencing by targeting the 3' untranslated region of mRNA. A single miRNA can target hundreds of mRNAs (Lu, 2018). miRNA can be detected both in different biological fluids and in tissues (Weber, 2010). This makes them candidate biomarkers for the diagnosis of various diseases such as cancer (Sauter, 2011). miRNAs are transcribed in the nucleus by their own promoters or by sharing the promoters of their host genes into a long transcript called pre-miRNA, for which RNA polymerase II is responsible for transcription (Lee, 2002).

Pre-miRNAs are exported from the nucleus via Exportin and Ran-GTP or CRM1, cleaved by DICER and exported to the cytoplasm, transiently forming double-stranded miRNA duplexes of ~21-23 nucleotides (Kurzynska-Kokorniak, 2015). In the cytoplasm, miRNAs can associate with some proteins in a ribonucleoprotein complex called RISC. Mammalian miRNAs are known to regulate approximately 30% of all protein-coding genes (Filipowicz, 2008).

miR-153, a member of the miRNA family, was found to be associated with inhibition of cell proliferation and increased apoptosis (Zhao, 2017). Similarly, in a study by Chen et al. investigating the relationship between miR-153 and response to Sorafenib, Etoposide and Paclitaxel in cell culture models, flow cytometry results showed that high miR-153 expression increased the protein levels of the antiapoptotic proteins Survivin and BCL-2 (Chen, 2015). In another study, miR-153 expression levels were found lower in breast cancer tissue samples and cell lines compared to the normal human breast cell line MCF-10A. Low miR-153 expression was associated with advanced clinical staging and metastasis in breast cancer patients, while overexpression of miR-153 was found to reduce proliferation, migration, invasion and EMT in breast cancer SK-BR-3 and BT-549 cells (Zuo, 2019). When the expression levels of 30 gastric cancer patients in tumor and TME tissue were examined, it was found that lncRNA OIP5-AS1 and ZBTB2 had high expression levels and miR-153-3p had low expression levels. This suggests that OIP5-AS1 deficiency may suppress cell proliferation, migration, invasion and promote apoptosis in gastric cancer cells (Zhi, 2020). These data provide important evidence that the miRNA/lncRNA axis may be co-regulated in gastric cancer.

lncRNAs are non-protein-coding RNA clusters longer than 200 nucleotides that are amplified by RNA Polymerase II. lncRNAs can be subdivided according to their genomic localization and function: Intronic lncRNAs originate from introns of protein-coding genes; intergenic lncRNAs (lincRNA) originate from the region between two protein-coding genes; enhancer lncRNAs (elncRNA) originate from promoter enhancer regions; duplex lncRNAs are localized near a coding transcript of the opposite strand; antisense lncRNAs (aslncRNA) originate from antisense strands of DNA. Functionally, they are classified as signaling, sponge, guide and scaffolding lncRNAs. lncRNAs contain interaction domains for DNA, mRNA, miRNA and proteins and together with these molecules can coordinate various physiological processes that control the flow of genetic information such as chromatin modification, transcription, mRNA stability, post-translational modifications (Skierucha, 2016). lncRNAs are found in the nucleus, nucleolus, cytoplasm and even mitochondria (Rackham, 2011; Cabili, 2015) and their localization is a good indicator of function (Long, 2017). Dysregulation of lncRNAs and their dysfunction can affect a variety of pathologies, including cancer and infectious diseases. lncRNAs can be oncogenes, or they can bind directly to RNA and act as tumor suppressor genes.

Dysregulation of lncRNAs is associated with pathways such as LKB1/AMPK, HIFα, p53, NF-κB and Notch (Zhang, 2013; Liu, 2020; Yang, 2014). Given the role of cell signaling pathways in cancer formation, progression and metastasis, lncRNAs involved in these pathways may influence tumorigenesis in many ways.

Aberrant expression, mutations and SNPs of lncRNAs are associated with tumorigenesis and metastasis (Wang, 2018). SNHG1 is one of the lncRNAs and has clinical importance in many cancers. It is involved in pathways such as EMT, Wnt-B-catenin signaling pathway in important processes such as cell proliferation, invasion and metastasis. There is evidence that lncRNA SNHG1 may be an important marker in cancers (Yang, 2017). SNHG1 is synthesized at much higher rates in many cancer cells than in healthy tissues. Inhibition of SNHG1 decreased cell proliferation. Therefore, SNHG1 may be a potential therapeutic target for cancer. In previous studies, small nucleolar RNA host gene 1 (SNHG1) has been associated with cancers such as colorectal cancer (CRC) (Xu, 2018), liver (Li, 2019), breast (Zheng, 2019). However, the association of SNHG1 expression with gastric cancer remains unclear.

MATERIAL - METHODS

Sample Collection

Samples collected from 30 patients diagnosed with gastric cancer by the General Surgery Clinic of Istanbul Training and Research Hospital. Samples of tumor tissues and tumor microenvironment of the 30 patients identified as a result of pathological examinations were stored in liquid nitrogen.

RNA Isolation

Total RNA extraction is performed using Trizol reagent. Samples are homogenized and RNA is separated from the homogenate by phase separation with chloroform treatment. Total RNA is dissolved in RNAse/DNAse-free water. The extracted total RNA is quantified spectrophotometrically, and RNA quality is checked by 1% agarose gel electrophoresis.

cDNA Synthesis

The detected RNA ratios equalized, and the process carried out through the cDNA synthesis kit. DNA amounts measured spectrophotometrically again after the processes to be carried out in the cDNA synthesis kit, taking into account the mi-RNA and lncRNA specific synthesis feature.

Real time PCR

lncRNA and miRNA specific TaqMan probe assays are performed by following the manufacturer's conditions. GAPDH for lncRNA and RnU6b for mi-RNA are used as reference genes. PCR reactions for reference and target transcripts of 60 samples from 30 patients are set up simultaneously in duplicate for tumor tissue and TME. The ct values obtained are determined as fold change by the $2^{-\Delta\Delta\Delta Ct}$ method for tumor and tumor-free surrounding tissue.

Statistical Analysis

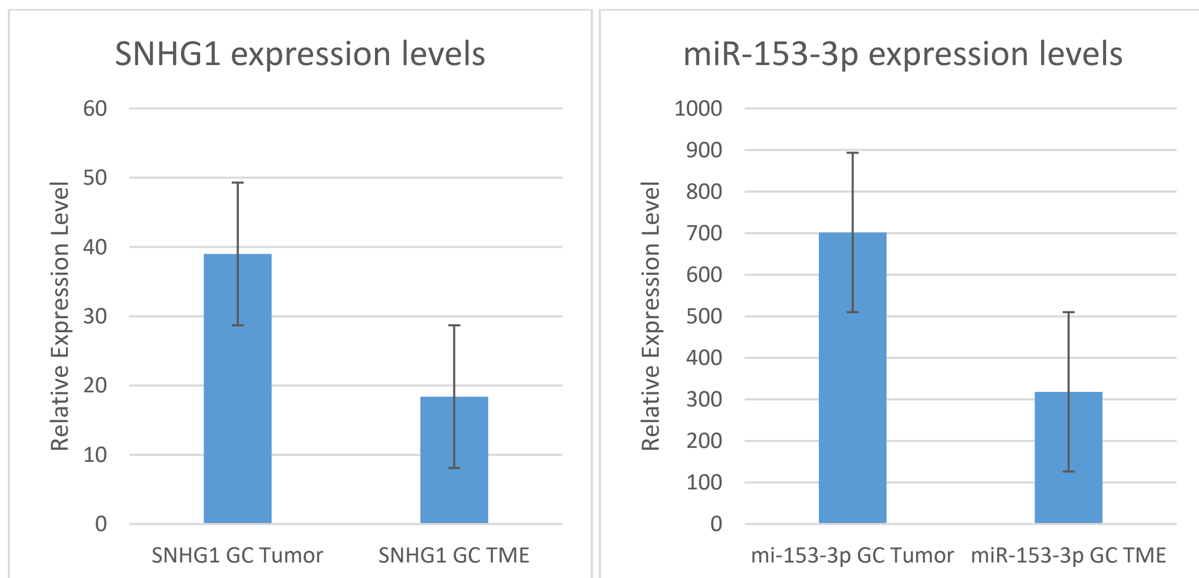
Our results are statistically analyzed using SPSS version 22.

Comparison of levels between tumor and TME and the relationship between fold changes are evaluated by t-test and mann whitney U tests. Spearman correlation test is used to evaluate the co-expression of lncRNA SNHG1 and mir153.

RESULTS

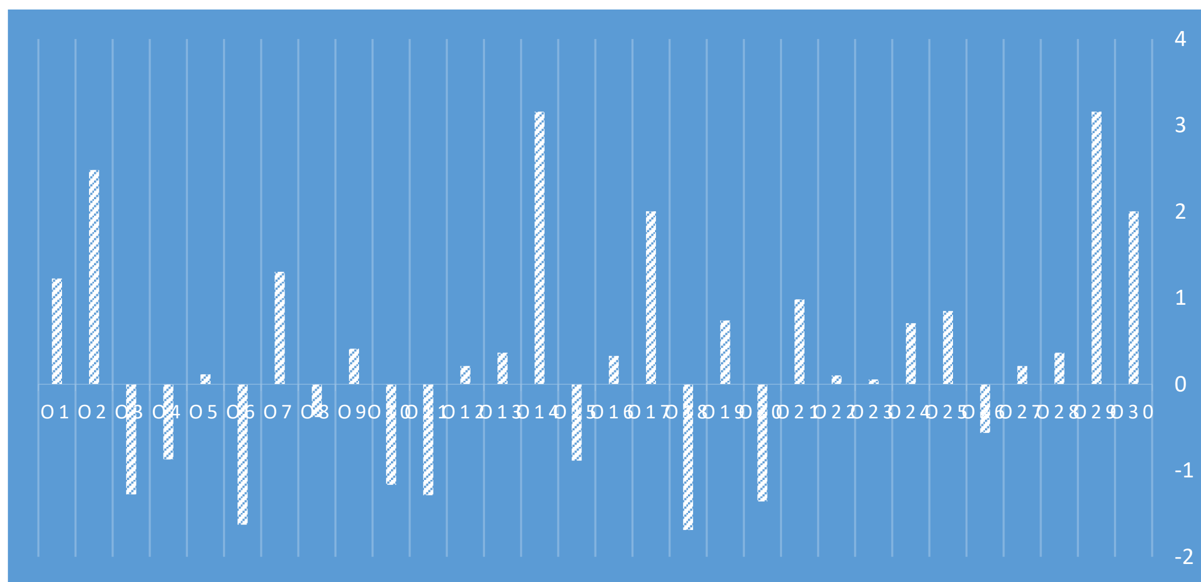
As a result of our study, relative expression of lncRNA SNHG1 levels was determined as 38.9 (fold change) in tumor tissue and 18.4 (fold change) in TME tissue with reference to GAPDH. A 2.12-fold significant increase was detected in tumor tissue compared to surrounding tissue (p=0.02). Also miR-153-3p levels were found approximately 2.2 times higher in tumor tissues compared to TME (Figure 1).

Figure 1. SNHG1 and miR-153-3p expression levels between Tumor and Tumor microenvironment (TME)



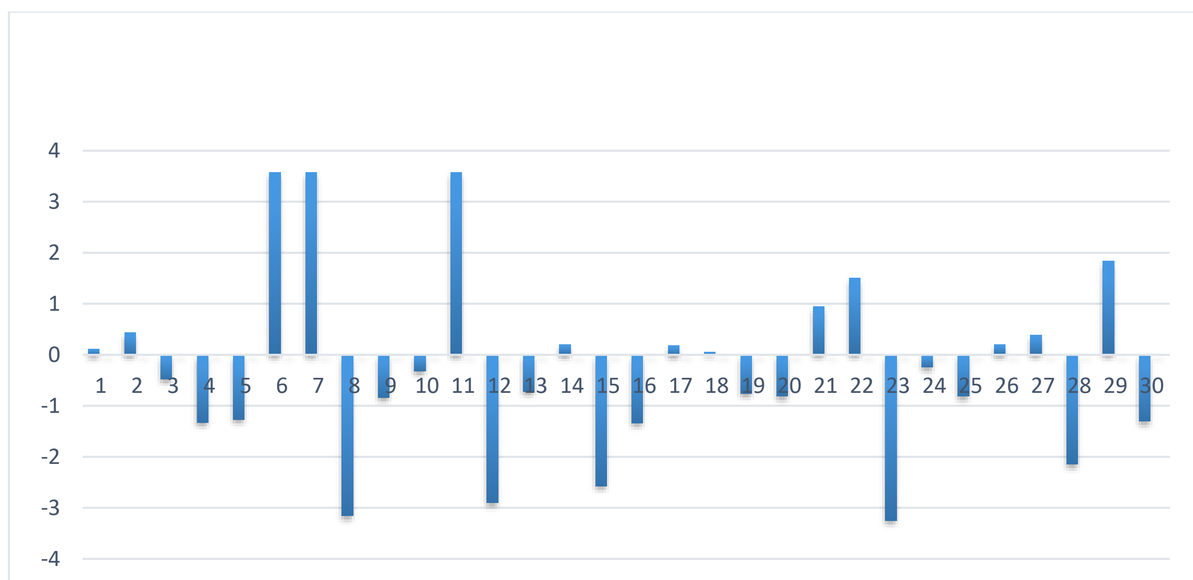
In a comparison between tumor tissue and TME tissue, lncRNA SNHG1 was found to be up-regulated in 20 out of 30 cases, while down-regulation was observed in 10 out of 30 cases (Table 1).

Table 1. Comparison of lncRNA SNHG1 expression levels in tumor tissue compared to TME tissue.



In the comparison of miR-153-3p levels between tumor tissue and TME, up-regulation was identified in 13 of the 30 cases, while down-regulation was observed in 17 cases.

Table 2. Comparison of miR-153-3p expression levels in tumor tissue compared to TME Tissue.



DISCUSSION

In our experiment, both SNHG1 and miR-153 levels found higher levels in GC tumor samples. Zhu and Zou analyzed the expression levels of SNHGs in gastric cancer patients and the data showed that SNHG1/10/11 expression levels were dramatically elevated in gastric cancer tissue samples compared to adjacent normal tissues. In the light of the data obtained, it was shown that cell viability and invasion were suppressed when SNHG1 was silenced with siRNA by selecting the SGC-7901 cell cancer line in which SNHG1 was highly expressed (Zhu, 2020).

In another study, when the expression of DCLK1, Notch1 and SNHG1 in gastric cancer cell lines (N87, SGC7901 and MKN-28) and normal gastric mucosal cells (GES-1) were examined. It was observed that SNHG1 expression was significantly increased in gastric cancer cell lines compared to normal gastric mucosal cells (Liu, 2020). To determine the function of lncRNA-SNHG1 in gastric cancer, RT-PCR was performed to examine lncRNA-SNHG1 levels in 50 pairs of gastric tumor and its adjacent tissues. The results showed that lncRNA-SNHG1 was upregulated in gastric cancer (Hu, 2017).

In another study, the expression levels of SNHG1 in tissues with and without gastric tumors were measured and high SNHG1 expression levels were significantly associated with tumor size, lymph node metastasis and late clinical stage. In addition, high SNHG1 expression was associated with poorer overall survival time. In the cell culture part of the study, SNHG1 downregulation was found to induce cell apoptosis. SNHG1 expression was found to decrease in miR-140-treated cells, while its expression increased in anti-miR-140-treated cells (Guo, 2019). Since SNHG1 levels were also found to be higher in tumor tissues in our study, it can be said that all these studies support our study.

In contrast to these results, the results of a study conducted by Wang et al. suggest that SNHG1 suppresses cell migration and invasion. SNHG1, which was found to be downregulated in gastric tumors, showed a different expression pattern in cell lines. AGS cells had high SNHG1 levels, while SGC-7901, MGC-803 and BGC-823 cells had low SNHG1 levels. SNHG1 knock-out AGS cells showed increased proliferation, migration and metastasis, while western blot results confirmed that SOCS2 protein was increased in SNHG1 overexpressing BGC-823 cells and decreased in SNHG1 AGS cells compared to control cells. Comparison of

the data in gastric tumor tissues revealed that SOCS2 expression was downregulated in tumor tissue compared to TME and positive correlation was found with SNHG1 in gastric tumor tissue (Wang, 2018).

In vitro studies showed that high miR-153 expression decreased cell migration and invasion in MKN-45 cells, while low miR-153 expression promoted SGC-7901 cell migration and invasion. MiR-153 also showed an inverse correlation with SNAI1 expression, which causes EMT in gastric cancer (Zhang, 2015; Wang, 2015). Zhang et al. showed that miR-153 expression levels were clearly lower in gastric tumor tissues than in TME. Similarly, in the cell culture study, data suggesting that its high expression increases invasion were noted.

ACKNOWLEDGEMENTS

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KEY MESSAGES

- ✓ SNHG1 and mir-153-3p show coexpression in gastric cancer.
- ✓ SNHG1 and mir-153-3p are upregulated in gastric tumors according to TME.
- ✓ SNHG1/mir-153-3p axis may be an oncogenic factor for gastric cancer.

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Effects of the *Vdr* Rs757343 (G>A) Polymorphısm in Mody Patıents

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ABSTRACT

Objectives: Vitamin D (VD) levels are associated with insulin resistance and contributes pathogenesis of diabetes. VD functions with its receptor (VDR) and VDR mutations play a role in development of diabetes. We aimed to investigate the effects of *VDR*-rs757343 SNP on metabolic and clinical features in patients with clinically-diagnosed maturity-onset-diabetes-of-the-young (MODY).

Materials and Methods: *VDR*-rs757343 polymorphism is analyzed by PCR-RFLP in 63 patients with MODY and 61 healthy-control subjects. Statistical analyses were performed by SPSS v.24.0.

Results: In the MODY patient group weight (p=0.021), BMI (p<0.001), WC (p<0.001), HC (p<0.001), FBG (p<0.001), HbA1c (p<0.001), TG (p=0.007), GGT (p=0.001), hs-CRP (p<0.001) and fT4 (p=0.039) values were found higher and statistically significant, while HDL-C (p=0,043) value was found lower compared to the control group.

It was observed that TG levels were higher (p=0.008) and TSH levels were lower (p=0.042) in the patients with GG genotype compared with A-allele. BMI (p=0.051) and HC (p=0.050) levels were higher in the control group with GG genotype compared with A-allele.

Higher TG levels (p=0.045) and lower TSH levels (p=0.042) were observed with ANOVA analysis in the patients with GG genotype compared to GA genotype and higher SBP (p=0.030) and lower TSH (p=0.057) levels in those carrying GG genotype compared to the control group with GA genotype.

Conclusions: Our findings show that the *VDR*-rs757343 (G>A) major GG genotype was effective on parameters related to lipid and thyroid metabolism. Therefore, further studies with larger sample sizes are required for stronger findings.

Keywords: MODY, *VDR*, rs757343, SNP, TG, TSH

INTRODUCTION

Maturity-onset diabetes of the young (MODY) is characterized by onset before 25 years of age, sustained pancreatic β-cell function, autosomal dominant inheritance and absence of β-cell autoimmunity (Urakami, 2019). MODY develops through beta cell dysfunction as a result of heterozygous mutations in different genes, including genes encoding transcription factors, enzymes and channel proteins (Shields et al., 2010). To date, 14 different genes related to MODY have been identified and numbered from MODY1 to MODY14, including *HNF4A, GCK, HNF1A, PDX1, TCF2, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11,* and *APPL1* (Tosur & Philipson, 2022).

Vitamin D (VD) is a fat-soluble vitamin. The most important source of VD is sunlight, but it can also be obtained from food. It plays a role in various biological functions, including calcium homeostasis, cell proliferation and differentiation in many target tissues. VD is considered a hormone that exerts its effects through the vitamin D receptor (VDR), a member of the intracellular steroid/thyroid hormone receptor family. It suppresses insulin secretion from pancreatic β cells and promotes glucose tolerance (Al-Hazmi, 2019).

MATERIAL-METHODS

Characteristics of the study groups

This study is included 63 patients with prediagnosed of MODY and 61 healthy individuals from the outpatient clinics of the Department of Internal Medicine and the Department of Pediatrics at Istanbul University. Ethical approval for our study was obtained from the Haliç University Non-Invasive Clinical Research Ethics Committee, dated April 25, 2023, with approval number 123. Information about gender, age, medical history, medications used, BMI, and surgical operations were provided with informed consent form during routine controls.

***VDR* rs757343 Genotying**

DNA isolation was performed using the Epicentre (MCD85201) Genomic DNA Isolation Kit from blood samples. The *VDR*-rs757343 SNP was genotyped with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The forward and reverse primers for *VDR*-rs757343 were 5'-CTTTGGAGCCTGAGAGATGG-'3 and 5'-CTCCAGTCCAGGAAAGCATC-3', respectively. The DNA was amplified with 2X PCR

MasterMix (abm BlasTaq^{TM} G895) under the following conditions; pre-denaturation 95℃ for 5 min, 30 cycles of 95℃ for 30 s (denaturation), 59℃ for 30 s (annealing), 72℃ for 30 s (extension), and a final extension step at 72℃ for 7 min. PCR products were digested with Tru1I (MseI) (Thermo Fisher Scientific ER0981) at 65℃ for 2 hours. Product sizes were 235 bp for the GG genotype, 162 bp and 73 bp for AA genotype and 235 bp, 162 bp and 73 bp for GA genotype.

Statistical Analyses

The clinical and biochemical parameters of the study groups were statistically evaluated using SPSS 24.0 software. *p* values <0.05 were considered statistically significant. Student's t-test was used for comparison between the study groups and ANOVA was used for genotype comparisons involving more than two variables. Odds ratios (OR) and 95% confidence intervals (95% CI) were provided to determine inter-group risk factors. The allele frequency calculations utilized the allele counting method.

RESULTS

In the MODY patient group, weight (p=0.021), BMI (p<0.001), WC (p<0.001), HC (p<0.001), FBG (p<0.001), HbA1c (p<0.001), TG (p=0.007), GGT (p=0.001), hs-CRP (p<0.001) and fT4 (p=0.039) values were found higher and statistically significant, while HDL-C (p=0,043) levels was found lower compared to the healthy control group. Among the participants, 78 were women (62.9%) and 46 were men (37.1%).

Table 1: Characteristics of the study groups.

	Patient	Control	*p* Value
Patient age (year)	40.24 ± 1.55	36.00 ± 1.85	0.082
Height (cm)	1.64 ± 0.01	1.65 ± 0.02	0.488
Weight (kg)	78.40 ± 2.00	70.43 ± 2.76	**0.021**
BMI (kg/m^2)	29.29 ± 0.77	25.17 ± 0.63	**<0.001**
Waist circumference (cm)	97.88 ± 1.97	79.89 ± 3.62	**<0.001**
Hip circumference (cm)	111.45 ± 1.82	99.78 ± 1.66	**<0.001**
SBP (mmHg)	118.34 ± 1.94	116.39 ± 1.37	0.417
DBP (mmHg)	75.18 ± 1.44	76.86 ± 1.03	0.347
Glucose (mg/dL)	162.73 ± 8.96	92.17 ± 1.71	**<0.001**
HbA1C (%)	7.82 ± 0.27	5.61 ± 0.05	**<0.001**
Insulin (µU/mL)	12.93 ± 2.30	13.46 ± 6.58	**0.942**
C peptide (ng/mL)	2.33 ± 0.17	-	**-**
Total Cholesterol (mg/dL)	193.14 ± 6.79	196.98 ± 5.73	0.667
Triglyceride (mg/dL)	179.93 ± 18.26	122.90 ± 9.50	**0.007**
HDL-C (mg/dL)	45.84 ± 2.22	51.71 ± 1.81	**0.043**
LDL-C (mg/dL)	117.98 ± 5.15	117.13 ± 4.75	0.904
VLDL-C(mg/dL)	27.64 ± 1.68	25.04 ± 1.92	0.311
ALT (U/L)	24.55 ± 1.93	20.57 ± 1.82	0.139
AST (U/L)	19.43 ± 1.11	17.95 ± 0.64	0.261
GGT (U/L)	29.51 ± 4.94	10.75 ± 2.06	**0.001**
hs-CRP (mg/L)	5.84 ± 0.94	0.68 ± 0.37	**<0.001**
TSH (mIU/L)	2.14 ± 0.44	12.64 ± 1.41	0.149
Free T4 (ng/dL)	15.87 ± 0.44	12.64 ± 1.41	**0.039**
Uric Acid (mg/dL)	4.25 ± 0.31	4.46 ± 0.31	0.635
Urea (mg/dL)	23.67 ± 1.72	25.65 ± 1.13	0.340
Creatinine (mg/dL)	0.72 ± 0.03	2.81 ± 2.05	0.314
Hemoglobin (g/dL)	12.94 ± 0.24	12.94 ± 0.30	0.997
Hematocrit (%)	38.95 ± 0.69	37.61 ± 1.78	0.399

The degree of significance between groups was examined with student's t test. The values in the table are given as X+SEM. n: number of samples.* Bold p values indicate statistical significance (p<0.05). BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP:Diastolic Blood Pressure; FBG, fasting blood glucose; HDL-C: High-Density Lipoprotein Cholesterol LDL-C: Low-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol; ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma Glutamyl Transferase, hs-CRP: High-Sensitive C-Reactive Protein; TSH: Thyroid Stimulating Hormone; FT4: Free Thyroxine

There was no statistically significant difference in the patient and control groups in terms of genotype and allele distribution of *VDR*-rs757343 polymorphism and it was observed that it is not consistent with HWE. (p<0.05) (Table 2).

Table 2: Distribution of Tru1I-rs757343 SNP genotypes in the study groups.

VDR	GENOTYPE	Control (n=61)	Patient (n=63)
Rs757343 (G>A)	GG	46 (52.9 %)	41 (47.1 %)
Genotype n (%)	AA	1 (100 %)	0 (0 %)
	GA	14 (38.9%)	22 (61.1 %)
Alleles	G	106 (86.9 %)	82 (78.8 %)
	A	16 (13.1 %)	22 (21.2 %)
HWE		0.0	0.0

The degree of significance between groups was examined with the chi-square test. n: number of samples. HWE: Hardy-Weinberg equation

Since the AA genotype was not seen in the patient group, the G allele could not be compared with the AA genotype. However, when the GG genotype was compared with the A allele, it was observed that TG levels were higher (p = 0.008) and TSH levels were lower (p = 0.042) in those carrying the GG genotype in the patient group (Table 3).

Since only 1 AA genotype was seen in the control group, the G allele could not be compared with the AA genotype. However, when comparing the GG genotype with the A allele, it was observed that BMI (p=0.051) and hip circumference (p=0.050) were higher in the GG genotype carriers in those carrying the A allele in the control group (Table 3). This suggests that the GG genotype may be associated with weight status.

Table 3: The effect of the GG genotype of the rs757343 SNP on biochemical and clinical parameters in the study groups.

	Patient			Control		
rs757343 = 2 | rs757343 = 3 (FILTER)	**A allele (GA + AA)**	**GG**	**P value**	**A allele (GA + AA)**	**GG**	**P value**
Patient age (year)	42.32 ± 2.66	39.12 ± 1.91	0.330	35.90 ± 3.51	36.03 ± 2.20	0.976
Height (cm)	1.64 ± 0.02	1.64 ± 0.01	0.788	1.64 ± 0.05	1.65 ± 0.02	0.827
Weigh (kg)	83.29 ± 2.66	75.84 ± 2.64	0.076	64.10 ± 5.10	72.54 ± 3.23	0.188
BMI (kg/m^2)	30.86 0.99	28.47 ± 1.04	0.144	23.06 ± 1.08	25.88 ± 0.73	**0.051**
Waist circumference (cm)	102.55 ± 2.95	95.65 ± 2.44	0.102	75.67 ± 5.70	82.00 ± 4.72	0.446
Hip circumference (cm)	115.27 ± 3.26	109.55 ± 2.12	0.139	95.33 ± 1.45	102.00 ± 1.81	**0.050**
SBP (mmHg)	121.07 ± 3.02	117.07 ± 2.48	0.344	111.36 ± 3.10	118.13 ± 1.41	0.066
DBP (mmHg)	77.14 ± 1.79	74.27 ± 1.94	0.283	73.64 ± 2.34	77.97 ± 1.09	0.115
Glucose (mg/dL)	160.18 ± 12.54	164.13 ± 12.16	0.835	90.86 ± 3.22	92.66 ± 2.04	0.645
HbA1C (%)	7.54 ± 0.36	7.99 ± 0.37	0.420	5.61 ± 0.09	5.61 ± 0.07	0.981
Insulin (µU/mL)	9.10 ± 0.46	14.08 ± 2.91	0.124	-	13.46 ± 6.58	-
C peptide (ng/mL)	2.17 ± 0.18	2.41 ± 0.24	0.500	-	-	-
Total Cholesterol (mg/dL)	179.10 ± 11.19	201.39 ± 8.35	0.114	206.25 ± 12.10	194.20 ± 6.53	0.381
Triglyceride (mg/dL)	128.40 ± 9.54	205.70 ± 26.14	**0.008**	118.08 ± 16.67	124.39 ± 11.41	0.781
HDL-C (mg/dL)	44.41 ± 3.49	46.62 ± 2.89	0.639	57.33 ± 3.95	50.07 ± 1.98	0.118
LDL-C (mg/dL)	115.76 ± 10.14	119.15 ± 5.86	0.758	120.58 ± 9.92	116.15 ± 5.46	0.701
VLDL-C (mg/dL)	25.71 ± 1.95	28.74± 2.39	0.330	23.62 ± 3.55	25.46 ± 2.27	0.692
ALT (U/L)	26.75 ± 3.77	23.39 ± 2.20	0.414	19.50 ± 3.20	20.89 ± 2.19	0.752
AST (U/L)	20.80 ± 2.24	18,61 ± 1.17	0.344	17.42 ± 1.06	18.15 ± 0.78	0.579
GGT (U/L)	21.63 ± 4.56	32.52 ± 6.54	0.333	10.50 ± 0.50	11.00 ± 5.00	-
hs-CRP (mg/L)	6.98 ± 2.26	5.27 ± 0.83	0.490	1.17 ± 0.85	0.32 ± 0.15	0.424
TSH (mIU/L)	2.64 ± 0.33	1.90 ± 0.18	**0.042**	7.40 ± 4.54	2.69 ± 0.43	-
Free T4 (ng/dL)	16.65 ± 0.77	15.48 ± 0.53	0.209	9.51 ± 3.59	13.47 ± 1.50	0.262
Uric Acid (mg/dL)	3.66 ± 0.46	4.49 ± 0.39	0.227	4.60 ± 0.50	4.37 ± 0.47	0.765
Urea (mg/dL)	23.75 ± 2.67	23.63 ± 2.27	0.973	25.91 ± 2.78	25.57 ± 1.23	0.913
Creatinine (mg/dL)	0.71 ± 0.05	0.72 ± 0.04	0.812	0.71 ± 0.04	3.47 ± 2.71	0.314
Hemoglobin (g/dL)	13.16 ± 0.46	12.83 ± 0.27	0.510	12.94 ± 0.81	12.93 ± 0.29	0.994
Hematocrit (%)	39.56 ± 1.37	38.62 ± 0.78	0.517	39.12 ± 2.33	37.02 ± 2.32	0.612

The degree of significance between groups was examined with student's t test. The values in the table are given as X+SEM. n: number of samples.* Bold p values indicate statistical significance (p<0,05).

BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP:Diastolic Blood Pressure; FBG, fasting blood glucose; HDL-C: High-Density Lipoprotein Cholesterol LDL-C: Low-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol; ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma Glutamyl Transferase, hs-CRP: High-Sensitive C-Reactive Protein; TSH: Thyroid Stimulating Hormone; FT4: Free Thyroxine

AA genotype of VDR-rs757343 polymorphism was not observed in the MODY patient group. When the effect of the GG and GA genotypes of the rs757343 SNP on biochemical and clinical parameters was examined with ANOVA analysis in this group, it was observed that TG levels were higher (p=0.045) and TSH levels were lower (p=0.042) in the patients carrying the GG genotype compared to the GA genotype (Table 4). It is speculated that GG genotype can be effective on parameters related to lipid and thyroid metabolism. When the effect of the GG and GA genotypes of the rs757343 SNP on biochemical and clinical parameters was examined with ANOVA analysis in the control group, it was observed that SBP (p=0.030) were higher in the GG genotype while TSH (p=0.057) levels were lower compared to the GA genotype carriers (Table 4).

Table 4: Effect of *VDR*-rs757343 SNP genotypes on biochemical and clinical parameters in the study groups.

	Patient			Control		
Rs757343	**GG**	**GA**	**P value**	**GG**	**GA**	**P value**
Patient age (year)	39.12 ± 1.91	42.32 ± 2.66	0.330	36.03 ± 2.20	34.89 ± 3.61	0.609
Age at onset of DM (year)	27.82 ± 1.24	27.77 ± 1.49	0.982	-	-	-
Height (cm)	1.63 ± 0.02	1.64 ± 0.02	0.788	1.65 ± 0.02	1.63 ± 0.05	0.338
Weight (kg)	75.84 ± 2.64	83.29 ± 2.66	0.076	72.54 ± 3.23	62.96 ± 5.34	0.315
BMI (kg/m²)	28.47 ± 1.04	30.85 ± 0.99	0.144	25.88 ± 0.73	23.07 ± 1.16	0.150
Waist circumference (cm)	95.65 ± 2.45	102.55 ± 2.95	0.102	82.00 ± 4.72	70.00 ± 1.00	0.359
Hip circumference (cm)	109.55 ± 2.12	115.27 ± 3.26	0.139	102.00 ± 1.81	94.00 ± 1.00	0.126
SBP (mmHg)	117.07 ± 2.48	121.07 ± 3.02	0.344	118.13 ± 1.41	111.36 ± 3.10	**0.030**
DBP (mmHg)	74.27 ± 1.94	77.14 ± 1.79	0.359	77.97 ± 1.09	73.64 ± 2.34	0.067
Glucose (mg/dL)	164.13 ± 12.16	160.18 ± 12.54	0.835	92.66 ± 2.04	92.54 ± 2.97	0.166
HbA1C (%)	7.99 ± 0.37	7.54 ± 0.36	0.420	5.61 ± 0.07	5.61 ± 0.09	0.983
Insulin (µU/ml)	14.08 ± 2.91	9.10 ± 0.46	0.383	-	-	-
C peptide (ng/mL)	2.41 ± 0.24	2.17 ± 0.18	0.500	-	-	-
Total Cholesterol (mg/dL)	201.39 ± 8.35	179.10 ± 11.19	0.114	194.20 ± 6.53	208.27 ± 13.07	0.586
Triglyceride (mg/dL)	205.70 ± 26.13	128.40 ± 9.54	**0.045**	124.39 ± 11.41	122.58 ± 17.45	0.706
HDL-C (mg/dL)	46.62 ± 2.89	44.41 ± 3.49	0.639	50.07 ± 1.98	58.18 ± 4.22	0.186
LDL-C (mg/dL)	119.15 ± 5.87	115.76 ± 10.14	0.758	116.15 ± 5.46	119.91 ± 10.85	0.908
VLDL-C (mg/dL)	28.74 ± 2.39	25.71 ± 1.95	0.390	25.46 ± 2.27	25.04 ± 3.57	0.476
ALT (U/L)	23.39 ± 2.20	26.75 ± 3.77	0.414	20.89 ± 2.19	20.45 ± 3.34	0.680
AST (U/L)	18.61 ± 1.17	20.80 ± 2.24	0.344	18.15 ± 0.78	17.42 ± 1.06	0.631
GGT (mg/dL)	32.52 ± 6.54	21.63 ± 4.56	0.333	11.00 ± 5.00	-	0.993
hs-CRP (mg/L)	5.27 ± 0.83	6.98 ± 2.29	0.394	0.32 ± 0.15	1.17 ± 0.85	0.299
TSH (mIU/L)	1.90 ± 0.19	2.64 ± 0.33	**0.042**	2.69 ± 0.43	9.05 ± 5.98	**0.057**
Free T4 (ng/dL)	15.48 ± 0.53	16.65 ± 0.77	0.209	13.47 ± 1.50	6.72 ± 3.21	0.150
Uric Acid (mg/dL)	4.49 ± 0.39	3.66 ± 0.46	0.227	4.37 ± 0.47	4.60 ± 0.50	0.765
Urea (mg/dL)	23.63 ± 2.27	23.75 ± 2.67	0.973	25.57 ± 1.23	25.91 ± 2.78	0.900
Creatinine (mg/dL)	0.72 ± 0.04	0.71 ± 0.05	0.812	3.47 ± 2.71	0.71 ± 0.04	0.570
Hemoglobin (g/dL)	12.83 ± 0.27	13.16 ± 0.46	0.510	12.93 ± 0.29	12.55 ± 0.92	0.389
Hematocrit (%)	38.62 ± 0.77	39.58 ± 1.37	0.517	37.02 ± 2.32	38.03 ± 2.65	0.729

3-variable genotype and biochemical/metabolic comparisons were analysed with ANOVA test. The values in the table are given as X+SEM. n: number of samples.* Bold p values indicate statistical significance (p<0,05).BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP:Diastolic Blood Pressure; FBG, fasting blood glucose; HDL-C: High-Density Lipoprotein Cholesterol LDL-C: Low-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol; ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma Glutamyl Transferase, hs-CRP: High-Sensitive C-Reactive Protein; TSH: Thyroid Stimulating Hormone; FT4: Free Thyroxine

DISCUSSION

According to the genotype distributions in the study groups, when GG allele and A allele carriers compared between the groups, GG genotype was found related with TG, TSH, BMI and hip circumference values and this suggests that the GG genotype may be associated with weight status (Table 4). Similarly, when GG and GA genotypes (no AA genotype in the patient groups) compared between the groups, GG genotypes was determined associated with TG, TSH and SBP and it indicates that GG genotype can be effective on parameters related to lipid and thyroid metabolism (Table 4).

In a Poland study, it has been reported that vitamin D supplementation affects LDL and TG levels in diabetics (Szymczak-Pajor et al., 2020). Additionally, studies conducted in Iran and Iraq have also found a relationship between vitamin D and TG levels (Al-Kashwan et al., 2021). In our previous research on the MODY patient group regarding the relationship between *VDR*-rs757343 polymorphism, we found that TSH and TG levels were significantly different in the patient group compared to the controls and these findings show that the *VDR*-rs757343 (G>A) mutant GG genotype was effective on parameters related to lipid and thyroid metabolism.

In this context, our study suggests that vitamin D-related pathways may be functional in MODY type diabetes.

Key Messages

The Vitamin D Receptor (*VDR*) rs757343 SNP has been investigated for the first time in Turkish patients with Maturity-onset diabetes of the young (MODY) and thyroid and lipid metabolism related parameters were found significantly despite the small size of our study groups. Further studies with larger sample sizes are required for stronger findings regarding the association of MODY and Vitamin D receptor polymorphisms.

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Evaluation of rs1244378045, rs767450259 and rs750556128 Mutations in Terms of Polymorphism in Diabetic Obese and Non-Diabetic Obese Individuals

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Abstract

Obesity is among the important healthcare issues that is becoming more prevalent around the world caused by adipose tissue and is linked to increased diabetes and insulin resistance. The Tumor Necrosis Factor (TNF)-Alpha overexpression in adipose tissue plays important roles in mediating obesity and insulin resistance. Tumor-Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL), TNF family member, is an effect on the development of obesity and diabetes. The rs1244378045, rs767450259 and rs750556128 polymorphisms of TRAIL, were evaluated with the Real-Time Polymerase Chain Reaction Method in the study. It was found that the T-Allele of the rs767450259 had protective roles against diabetic obesity. It was shown that carrying the A-Allele in the genotype distribution of TRAIL rs750556128 polymorphism might increase the risk of obesity in diabetic patients by 1.3-fold. Our study is the first to investigate these polymorphisms in diabetic obese and non-diabetic obese groups and will make significant contributions to the literature.

Keywords: Obesity, diabetes, polymorphism, TRAIL

INTRODUCTION

Obesity is a global health issue characterized by an increase in adipose tissue In addition leads to various diseases such as diabetes and kidney diseases. (Pantalone et al. 2017). Adipocyte hypertrophy causes adipose tissue dysfunction with diabetes (Odegaard and Chawla 2013). Diabetes is a metabolic disease that is characterized by raised blood glucose levels (Mayer-Davis et al. 2018).

Tumor necrosis factor (TNF)-alpha plays an important role in obesity and insulin resistance (Nisoli et al. 2000), and a member of this family, "TNF-related apoptosis-inducing ligand (TRAIL (TNSF10)"), is a Type II transmembrane protein. TRAIL has a protective role against diabetes mellitus (Harith et al. 2013; Koliaki and Katsilambros 2022; Aksoyer Sezgin et al. 2024). Studies modeling diabetes have indicated that TRAIL blockage and deficiency increase the incidence of diabetes, affect glucose tolerance and lead to beta dysfunction (Kang et al. 2010; Harith et al. 2013).

Yu et al. showed us the rs1131580 polymorphism of the TRAIL gene, explaining that the CC genotype may be associated with diabetes and may be a candidate for prognostic evaluation (Yu et al. 2013). In addition, studies examining TRAIL gene polymorphisms associated with diabetes and obesity are quite limited. In our literature review, no studies were conducted in any disease group related to rs1244378045, rs767450259 and rs750556128 polymorphisms of the TRAIL gene. The evaluation of polymorphisms in these gene regions is discussed for the first time in this study.

MATERIAL AND METHODS

We included 3 groups in this study. These; 80 diabetic obese, 80 non-diabetic obese and 80 healthy controls. The study groups were taken from Istanbul University Faculty of Medicine Hospital, Department of Endocrinology and Metabolism. The experimental research was carried out in the Department of Medical Biology and Genetics, Faculty of Medicine, Istanbul Yeni Yüzyıl University. People diagnosed with any chronic disease other than diabetes and obesity and receiving radiotherapy or chemaotherapy treatment were not included in the study. In this study, ethical approval was obtained from the Ethics Committee of Istanbul Yeni Yüzyıl University Science, Social Sciences and Non-Interventional Health Sciences Research (2023/06-1077, 06.06.2023).

Nucleic acid isolations were performed by taking 2x5 ml of peripheral blood from the individual in each group. For this, Robotic Device (RINATM M14) (Bioeksen, Turkey) was used (Kayaaslan et al. 2020). Serum samples were used for, urea, glucose, creatinine,, ALT, AST, LDH, HDL, total cholesterol, triglyceride, LDL and HbA1c analysis (Beckman Coulter Inc., Brea, CA, USA). The primers we designed to amplify the region where polymorphism is observed in gene regions are as follows, and the RT-qPCR protocol is given in Table 1.

rs1244378045

F1/F2	GTTACCTGAGAGGTTCTCTTAATCAT / GTTACCTGAGAGGTTCTCTTAATCAA
R	TCAGCTAGATCAGCGATAGG

rs767450259

F1/F2	TAGAATTGGAATCCTCAGACGTTCT/ TAGAATTGGAATCCTCAGACGTTCA
R	TCAGCTTGATGACCCATAGG

rs750556128

F1/F2	CTTCAACAGTAGAAATCCTTTCCTCA / CTTCAACAGTAGAAATCCTTTCCTCG
R	ATTAATCCGAGCTCGCACCC

Table 1. RT-qPCR Protocol Used for Gene Regions

Reagent	Volume
2xqPCR Mix	5 µL
Forward Primer	0,025 µL
Reverse Primer	0,025 µL
Sybr Green	0,06 µL
H_2O	2,89 µL
DNA	2 µL
Total	10 µL

Step	Temperature	Time	Process
Pre-denaturation	95 °C	5:00	
	95 °C	00:10	
PCR			39 cycles
	65 °C	00:30	
	72 °C	00:20	
Melt Curve	65 °C - 95 °C, by 0.5 °C gradual increase		Melting curve read every 5 seconds

We used SPSS (Statistical Package for Social Sciences for Windows) 29.0 software for all statistical analyses. The 2x2 Chi-Square test was used to compare the differences and significance of gene and allele distributions in the study groups.

RESULTS

The genotype distribution of the TRAIL rs767450259 polymorphism was statistically significant in diabetic obese individuals (p=0.045). The T allele of the rs767450259 polymorphism was found to be significantly higher in the control group when we compared to the diabetic obese group (p=0.016). In the rs750556128 polymorphism, the A allele was found to be significantly higher in the diabetic obese group when we compared to the control (p=0.01) (Table 2).

The allele and genotype distributions of the rs767450259, rs750556128, and rs1244378045 polymorphisms of the TRAIL gene between non-diabetic obese/control groups and between , the diabetic obese/non-diabetic obese groups no statistically significant differences were found (p>0.05)

Table 2. Comparison of genotype and allele distributions of rs767450259, rs750556128, and rs1244378045 polymorphisms in the TRAIL gene in diabetic obese subjects

rs	Genotype and Allel	Control	Diabetic Obese	P	CI 95%
rs767450259	TT	23 (44.2%)	22 (38%)	**0,045**	
	TA	20 (38.5%)	14 (24.%)		
	AA	9 (17.3%)	22 (38%)		
	T Allel	66 (63.5%)	58 (50%)	**0,016**	1.332 (0.140-0.837)
	A Allel	38 (36.5%)	58 (50%)	0,502	0.606-2.781
rs750556128	AA	22 (42.3%)	35 (60.3%)	0,131	
	AG	16 (30.8%)	10 (17.2%)		
	GG	14 (26.9%)	13 (22.4%)		
	A Allel	60 (57.7%)	80 (69%)	**0,01**	1.37 (0.131-0.776)
	G Allel	44 (42.3%)	36 (31%)	0,391	0.64-1.19
rs1244378045	TT	28 (53.8%)	32 (55.2%)	0,506	
	TA	15 (28.8%)	12 (20.7%)		
	AA	9 (17.3%)	14 (24.1%)		
	T Allel	71 (68.3%)	76 (65.5%)	0,379	0.258-1.679
	A Allel	33 (31.7%)	40 (34.5%)	0,889	0.447-2.010

The rs variants listed in the diabetic obese group were examined in terms of biochemical parameters (Table 3).

The analysis of rs12443788045 in diabetic obese group;

- LDH, total cholesterol, HDL and LDL levels in individuals with AA genotype were found to be higher and significant compared to those with T allele (p=0.001, p=0.052, p=0.023 and p=0.038, respectively).
- It was determined that ALT and AST levels were higher in those with the T allele compared to those with AA genotype (p=0.047 and p=0.045, respectively).
- - LDH levels were found to be higher in diabetic obese patients with the A allele compared to those with TT genotype (p=0.02).
- It was found that diabetic obese people with TT genotype had higher ALT and AST levels compared to those carrying the A allele (p=0.004 and p=0.003, respectively).

The analysis of rs767450259 in diabetic obese group;

- Individuals with AA genotype had higher urea and creatinine levels compared to those carrying the T allele (p=0.016; p=0.009).
- Creatine levels of diabetic obese individuals with TT genotype were found to be higher than those with A allele (p=0.0262).
- In diabetic obese individuals, LDL levels of those with the A allele were found to be higher than those with TT genotype (p=0.055).

The analysis of rs750556128 in diabetic obese group;

- Those with the GG genotype were found to have higher urea and creatinine levels compared to those carrying the A allele (p=0.016; p=0.006, respectively).
- LDL levels were found to be higher in diabetic obese patients with GG genotype compared to those with the A allele (p=0.049).
- Triglyceride, total cholesterol and LDL levels were found to be higher and significant in individuals with G allele compared to those with AA genotype (p=0.05, p=0.045 and 0.037, respectively).

Table 3. Comparison of Genotype and Allele Distributions of rs1244378045, rs767450259, rs750556128 Polymorphisms in the TRAIL Gene in the Diabetic Obese Group in Terms of Biochemical Parameters

rs	Genotype and Allele		Age	Glucose	Urea	Creatinine	LDH	ALT	AST	T_Chol	Trig	HDL	LDL	Hba1c	BMI
rs1244378045	**AA genotype**	**Mean ±SE**	57,32 ±1,88	186,05±16,63	33,98 ±3,91	0,76±0,04	211,33±10,86	20,52 ±2,21	17,65 ±1,14	215,73±10,03	200,20±32,32	47,61 ±1,97	131,96±8,08	8,51±0,42	36,04 ±0,99
	T allele	**Mean ±SE**	54,16 ±1,30	188,36±9,82	35,97 ±2,22	0,90±0,05	179,53±4,53	27,27 ±2,30	21,49 ±1,32	197,98±5,57	193,26±16,13	42,66 ±1,32	117,25±4,02	8,58±0,26	34,84 ±0,49
	P value		0,096	0,451	0,326	0,060	**0,001**	**0,047**	**0,045**	**0,052**	0,416	**0,023**	**0,038**	0,447	0,117
	TT genotype	**Mean ±SE**	54,73 ±1,40	196,63±11,91	35,95 ±2,74	0,90±0,06	178,62±5,48	30,04 ±2,97	23,02 ±1,70	204,46±6,95	203,60±20,56	43,24 ±1,49	120,48±4,91	8,63±0,32	34,75 ±0,56
	A allele	**Mean ±SE**	55,33 ±1,67	178,36±11,83	34,91 ±2,72	0,82±0,04	197,96±7,58	20,55 ±1,68	17,61 ±0,87	202,05±7,21	186,12±20,91	44,90 ±1,69	122,37±5,70	8,48±0,31	35,61 ±0,70
	P value		0,391	0,140	0,395	0,138	**0,020**	**0,004**	**0,003**	0,405	0,277	0,230	0,401	0,368	0,169
rs767450259	**TT genotype**	**Mean ±SE**	55,48 ±1,44	186,87±13,71	38,33 ±3,42	0,98±0,09	178,89±5,09	27,63 ±2,09	21,47 ±1,06	196,04±8,20	200,34±20,63	43,80 ±1,91	113,87±5,99	8,59±0,38	35,13 ±0,66
	A allele	**Mean ±SE**	54,74 ±1,52	188,27±10,75	33,66 ±2,27	0,79±0,03	192,63±6,82	24,01 ±2,62	19,78 ±1,51	207,65±6,23	191,87±20,16	44,19 ±1,38	126,10±4,68	8,54±0,27	35,19 ±0,60
	P value		0,369	0,468	0,120	**0,025**	0,057	0,165	0,212	0,130	0,390	0,433	0,055	0,452	0,474
rs750556128	**GG genotype**	**Mean ±SE**	53,43 ±1,78	191,79±16,75	30,92 ±1,58	0,76±0,03	188,44±8,48	23,41 ±2,67	19,19 ±1,09	210,96±8,27	196,72±32,77	44,28 ±1,75	129,85±5,92	8,64±0,39	35,52 ±0,83
	A allele	**Mean ±SE**	55,89 ±1,35	185,54±9,39	37,93 ±2,80	0,92±0,06	186,90±5,73	26,49 ±2,37	21,10 ±1,45	199,08±6,20	194,40±14,53	43,91 ±1,45	116,92±4,68	8,51±0,27	34,98 ±0,53
	P value		0,140	0,363	**0,016**	**0,006**	0,438	0,208	0,186	0,128	0,470	0,439	**0,049**	0,394	0,286
	AA genotype	**Mean ±SE**	54,80 ±1,54	181,50±11,89	38,34 ±3,34	0,94±0,08	185,38±6,67	26,25 ±2,27	19,76 ±0,98	191,52±7,93	168,51±14,19	44,13 ±2,01	113,17±5,45	8,29±0,36	34,65 ±0,60
	G allele	**Mean ±SE**	55,16 ±1,47	191,46±11,46	33,75 ±2,33	0,82±0,04	188,53±6,33	24,91 ±2,55	20,80 ±1,51	210,00±6,22	211,52±21,67	43,98 ±1,33	126,27±4,89	8,72±0,28	35,49 ±0,62
	P value		0,436	0,285	0,126	0,071	0,379	0,360	0,312	**0,037**	**0,050**	0,475	**0,045**	0,176	0,183

Table 4. Comparison of Genotype and Allele Distributions of rs1244378045, rs767450259, rs750556128 Polymorphisms in the TRAIL Gene in the Non-Diabetic Obese Group in Terms of Biochemical Parameters

rs	Genotype and allele	Age		Glucose	Urea	Creatinine	LDH	ALT	AST	T_Chol	Trig	HDL	LDL	Hba1c	BMI
rs750556128	GG genotype	Mean ±SE	43,50 ±3,54	95,38± 4,65	21,70 ±3,18	0,59± 0,06	197,40± 34,03	18,71 ±2,22	17,54 ±0,66	179,67± 7,49	107,33± 25,37	55,14 ±10,4 8	111,57± 11,77	5,36± 0,14	36,31 ±2,20
	A allele	Mean ±SE	42,41 ±2,50	93,60± 2,51	25,57 ±1,95	0,68± 0,03	182,00± 7,33	30,84 ±4,83	24,32 ±2,53	203,41± 9,55	133,50± 28,84	49,36 ±2,06	123,84± 6,91	5,36± 0,07	38,24 ±1,35
	P value	0,424		0,379	0,197	0,097	0,34	0,122	0,103	**0,031**	0,347	0,188	0,219	0,483	0,267
	AA genotype	Mean ±SE	45,37 ±3,25	89,88± 2,13	27,64 ±2,56	0,67± 0,04	183,78± 9,39	32,13 ±7,74	27,79 ±4,52	213,47± 14,44	159,77± 56,10	49,84 ±2,48	129,56± 10,15	5,34± 0,10	37,40 ±1,98
	G allele	Mean ±SE	40,58 ±2,82	96,58± 3,29	23,09 ±2,21	0,66± 0,04	187,08± 15,22	26,27 ±4,35	19,79 ±1,47	188,75± 8,82	107,26± 12,09	50,85 ±3,96	115,77± 7,29	5,37± 0,07	38,26 ±1,45
	P value	0,137		0,069	0,099	0,451	0,433	0,24	**0,029**	0,067	0,147	0,423	0,132	0,426	0,361
rs1244378045	AA genotype	Mean ±SE	46,29 ±3,73	102,29±9, 21	21,33 ±2,40	0,60± 0,03	169,75± 12,52	21,56 ±3,68	22,61 ±2,75	181,67± 22,91	254,17± 138,22	42,98 ±5,92	107,17± 13,18	5,46± 0,15	39,96 ±3,17
	T allele	Mean ±SE	41,92 ±2,44	92,31± 1,91	25,30 ±1,88	0,68± 0,03	189,41± 11,15	29,95 ±4,71	23,11 ±2,46	203,00± 8,64	104,13± 8,36	51,82 ±2,71	124,28± 6,69	5,34± 0,06	37,52 ±1,27
	P value	0,233		0,163	0,241	**0,041**	0,212	0,223	0,465	0,165	0,164	0,1	0,153	0,222	0,228
	TT genotype	Mean ±SE	40,04 ±3,02	91,72± 2,52	25,66 ±2,31	0,70± 0,04	179,09± 9,57	30,92 ±6,17	23,62 ±3,46	203,29± 9,53	93,71±7, 98	50,27 ±2,13	125,83± 6,47	5,27± 0,07	38,13 ±1,75
	A allele	Mean ±SE	46,11 ±2,81	97,00± 3,89	23,65 ±2,54	0,62± 0,04	192,90± 16,96	25,34 ±4,34	22,24 ±1,70	193,43± 14,76	178,73± 55,91	50,66 ±5,54	115,07± 11,71	5,48± 0,10	37,57 ±1,46
	P value	0,082		0,121	0,287	0,069	0,238	0,249	0,374	0,28	**0,042**	0,474	0,195	**0,042**	0,408
rs767450259	AA genotype	Mean ±SE	43,50 ±3,54	95,38±4,6 5	21,70 ±3,18	0,59± 0,06	197,40± 34,03	18,71 ±2,22	17,54 ±0,66	179,67± 7,49	107,33± 25,37	55,14 ±10,4 8	111,57± 11,77	5,36± 0,14	36,31 ±2,20
	T allele	Mean ±SE	42,41 ±2,50	93,60±2,5 1	25,57 ±1,95	0,68± 0,03	182,00± 7,33	30,84 ±4,83	24,32 ±2,53	203,41± 9,55	133,50± 28,84	49,36 ±2,06	123,84± 6,91	5,36± 0,07	38,24 ±1,35
	P value	0,424		0,379	0,197	0,097	0,34	0,122	0,103	**0,031**	0,347	0,188	0,219	0,483	0,267
	TT genotype	Mean ±SE	45,37 ±3,25	89,88±2,1 3	27,64 ±2,56	0,67± 0,04	183,78± 9,39	32,13 ±7,74	27,79 ±4,52	213,47± 14,44	159,77± 56,10	49,84 ±2,48	129,56± 10,15	5,34± 0,10	37,40 ±1,98
	A allele	Mean ±SE	40,58 ±2,82	96,58±3,2 9	23,09 ±2,21	0,66± 0,04	187,08± 15,22	26,27 ±4,35	19,79 ±1,47	188,75± 8,82	107,26± 12,09	50,85 ±3,96	115,77± 7,29	5,37±0,0 7	38,26 ±1,45
	P value	0,137		0,069	0,099	0,451	0,433	0,24	**0,029**	0,067	0,147	0,423	0,132	0,426	0,361

The rs variants listed in the non-diabetic obese group were examined in terms of biochemical parameters (Table 4).

The analysis of rs1244378045 in non-diabetic obese group;

- It was found that the creatinine levels of individuals carrying the T allele were higher and more significant than individuals with AA genotype (p=0.041).
- - Triglyceride and HbA1c levels were found to be higher and k significant in those carrying the A allele compared to those with TT genotype (p=0.042; p=0.042, respectively).

The analysis of rs767450259 in non-diabetic obese group;

- Total cholesterol levels were found to be higher in patients with T allele compared to those with AA genotype (p=0.031).
- It was determined that the AST level of individuals with TT genotype was higher compared to those carrying the A allele (p=0.029).

The analysis of rs750556128 in the non-diabetic obese group;

- It was determined that the total cholesterol levels of individuals with the A allele were higher than those with GG genotype (p=0.031).
- AST levels of those with AA genotype were found to be higher and significant compared to those with G allele (p=0.029).

DISCUSSION

It has been reported that the CC genotype in TRAIL (rs1131580) is associated with type II diabetes in non-alcoholic fatty liver disease (Yu et al. 2013). In a study, Osteoprotegerin rs2073617 T950C polymorphism was examined in pregnant and healthy pregnant women with gestational diabetes before diet treatment and it was reported that there was a significant increase in the distribution of CT genotype between groups. (M et al. 2021).

In addition to these studies, some studies on TRAIL variants, which were studied for the first time in different disease groups, are as follows.

It has been found that the TNSF10 rs9859259 variant is associated with Crohn's disease (Schwarz et al. 2011), the TRAIL rs13074711 variant is associated with breast cancer in black women (Han et al. 2023), and the TNSF10 rs6785617 variant is associated with malignant tumors seen in low-grade ovarian cancer (Charbonneau and al. 2014).

The presence of mutations in rs1244378045, rs767450259, rs750556128 was investigated for the first time in this study. As a result, it was determined that the genotype distribution of TRAIL rs767450259 polymorphism reached a significant level in diabetic obese patients (p=0.045) and the T allele was higher in the control group compared to the diabetic obese group. These data show us that the T allele may have a protective effect against diabetic obesity. In the TRAIL rs750556128 variant, the A allele was found to be higher in the diabetic obese group than in the control group. This proves that carrying the A allele increases the risk of diabetes by 1.3 times in obese individuals. The T allele (rs12443788045) has been associated with high levels of AST and ALT in diabetic obese groups. These data suggest that the T allele

may adversely affect liver function. The A allele (rs12443788045 and rs 767450259) have significant effects on the lipid profile and renal function in both groups. AA genotype and A allele (rs750556128) are associated with lipid profile and kidney function in diabetic obese group. All these data reflect the complex effects of genetic variations on biochemical parameters and show that each polymorphism affects the parameters in different ways. Our study will make important contributions to the literature as it is the first study to evaluate the rs1244378045, rs767450259, rs750556128 polymorphisms of the TRAIL gene.

KEY MESSAGE

1. TRAIL rs750556128 variant the A allele was found to be higher in the diabetic obese group and this proves that carrying the A allele increases the risk of diabetes by 1.3 times in obese individuals.
2. The A allele (rs12443788045 and rs 767450259) have significant effects on the lipid profile and renal function in diabetic obese and non-diabetic obese groups.
3. Our study will make important contributions to the literature as it is the first study to evaluate the rs1244378045, rs767450259, rs750556128 polymorphisms of the TRAIL gene.

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Comparative Evaluation of Immune Marker Expressions in Human Adipose Tissue MSCs Expressing Chimeric Cytokine Receptors

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ABSTRACT:

Mesenchymal stem cells (MSCs) are early-stage cells of mesoderm development and are non-hematopoietic stem cells. MSCs can be easily isolated from bone marrow, umbilical cord, dental pulp and adipose tissue. Due to their strong immunomodulatory abilities, MSCs used in the clinic inhibit pro-inflammatory activity and trigger anti-inflammatory events. Therefore, they are promising in the regulation of immune effects for many diseases, especially autoimmune diseases. With in vitro and in vivo studies, it has been observed that MSCs suppress some immune cells. These are CD4 T cells, cytotoxic and natural killer cells, phagocytes and antigen-presenting cells and B cells. Studies have also shown that MSCs can activate regulatory T and B cells. MSCs exert their immunomodulatory effects against immune cells either through microenvironmental interactions such as cytokines, chemokines and signaling molecules also they secrete or through direct cell contact. While microenvironmental effect is provided by molecules such as HGF, TGFβ, IDO, PGE2. İn addition PD-L1, ICAM-1, CD200, HLA-G molecules expressed on cell surfaces provide immunomodulation through cell contact. Although MSCs are promising for various diseases due to their immunomodulation abilities, the short duration of their effects is a problem. Priming MSCs with various cytokines (such as IFNγ) or environmental conditions increases their immunomodulation ability. In order to make this effect permanent, it is aimed to ensure the continuity of the priming effect realized with IFNγ through the designed chimeric cytokine receptor. For this purpose, immune marker expressions such as IDO, TGF-β, HGF should be evaluated comparatively.

INTRODUCTION:

Mesenchymal stem cells are non-hematopoietic stem cells that are capable of multilineage differentiation and are early-stage cells of mesoderm development (Herger et al., 2024; Wang et al., 2022). Mesenchymal stem cells, which can be easily obtained from many sources, are mostly isolated from sources such as bone marrow, umbilical cord, dental pulp and adipose tissue. MSCs are fibroblast-like cells that adhere to plastic and have self-renewal potential and the ability to differentiate into adipocytes, osteocytes and chondrocytes under in vitro conditions. In addition, while expressing surface markers such as CD44, CD73, CD90, and CD105, MSCs should be negative for surface markers such as hematopoietic markers CD14, CD34, CD45, CD11b, CD79α, and HLA-DR (Huang, Wu & Tam, 2022; Rawat, Gupta & Mohanty, 2019; Merimi et al., 2021). Due to their strong immunomodulatory abilities, the MSCs used in the clinic and they can inhibit pro-inflammatory activity while triggering anti-inflammatory events. Therefore, they are promising cells in the regulation of immune effects for many diseases, especially autoimmune diseases. MSCs provide their immunomodulation effects through cell-cell contact or soluble factors such as cytokines or signaling molecules that they secrete. In vivo and in vitro studies have demonstrated that through these interactions, MSCs affect various immune cells such as T cells, B cells, NK cells, Treg cells and macrophages (Huang, Wu & Tam, 2022; Rawat, Gupta & Mohanty, 2019; Song, Scholtemeijer & Shah, 2020). While MSCs suppress the activities of CD4 T cells such as Th1, Th2, Th17, cytotoxic cells such as CD8 and natural killer cells (NK), phagocytes and antigen-presenting cells such as monocytes/macrophages and dendritic cells, and B cells also they activate regulatory T and B cells (Treg and Breg). Unlike other immune cells, Treg and Breg cells are immunosuppressive cells. Mesenchymal stem cells are therefore involved in supporting these cells and increasing their activity. IL-10 producing Breg cells support the transformation of T cells into Tregs (Weiss & Dahlke, 2019) . On the other hand, they suppress the proliferation and activation of B and T cells by affecting various signaling pathways through the molecules they secrete. MSCs also affect the differentiation and maturation of dendritic cells, which are antigen-presenting cells, inhibiting their activity and affecting their antigen presentation (Jiang & Xu, 2020) . TGF-β (Transforming Growth Factor-beta), HGF (Hepatocyte Growth Factor) and IDO (Indoleamine 2,3-Dioxygenase) are important molecules expressed by MSCs and are involved in the immunomodulation abilities of MSCs. TGF-β produced by MSCs is one of the

most important cytokines contributing to immunomodulation and suppresses MHC-II (major histocompatibility complex II) expression on DCs and T cells. It inhibits the proliferation and activity of T cells and NK cells and promotes Treg and Breg formation. It is involved in the conversion of T cells into Treg cells (Jiang & Xu, 2020; Cagliani et al., 2017). HGF is a cytokine that is involved in many cellular events and is expressed by MSCs. Like TGF-β, it inhibits the activity of T cells and NK cells (Cagliani et al., 2017). IDO is an enzyme involved in tryptophan metabolism and inhibits the activity of NK cells, T cells and dendritic cells by converting tryptophan to kynurenine. It also inhibits TH17 differentiation and promotes Treg formation (Rawat, Gupta & Mohanty, 2019; Jiang & Xu, 2020; Noronha et al., 2019; Harrell, Djonov & Volarevic, 2021). Although the immunomodulation abilities of mesenchymal stem cells are promising, their activity is short-lived. To overcome this problem, pre-conditioning (or priming) is applied. Priming is the stage of preparing MSCs by exposing them to various cytokines and using environmental conditions such as hypoxia. 3D culture is also a new strategy that can be used to improve the immunomodulatory activities of MSCs (Herger et al., 2024; Noronha et al., 2019; Harman, Marx & Van de Walle, 2021). Interferon-gamma (IFN-γ), Tumor Necrosis Factor-alpha (TNFα) and Interleukin-17 (IL-17) are cytokines frequently used for priming. With IFN-γ applying, IDO and Programmed Death-Ligand 1 (PD-L1) levels increase also immunomodulatory molecules for example HGF, Prostaglandin E2 (PGE2) and TGF-β are secreted. These molecules cause an effect on immune cells and trigger immunosuppression. Studies show that NK and T cells are suppressed more effectively in cells priming with IFN-γ (Noronha et al., 2019). However, priming does not cause a sustained effect. Therefore, new strategies need to be developed to make the effect permanent. The chimeric cytokine receptor designed for this purpose aims to ensure the continuity of the priming effect realized with IFNγ.

MATERIAL-METHODS

Mesenchymal stem cells were isolated by the mechanical mincing method from abdominal adipose tissues of healthy male and female volunteers without chronic and immune-related diseases. The isolated cells were cultured under optimal conditions. When they reached 80% confluence, they were suspended and sub-passaged using Trypsin-EDTA and the cells were plated in T75 culture flasks. For the characterization of MSCs obtained by primary culture, they were differentiated into mesenchyme-derived cell types using StemPro™ Adipogenesis and

Osteogenesis Differentiation Kits. For this purpose, cells were cultured in 96 well-flat bottom plates and cultured according to the kit protocols. At the end of the culture, the cells were fixed with formaldehyde and the presence of lipid vesicles with Oil Red O and calcium deposits with Alizarin Red-S were demonstrated. In addition, venous blood samples were collected from healthy volunteers in tubes containing anticoagulant and PBMCs were isolated by Ficol density gradient method and activated with RPMI-1640 medium containing 2% (v/v) PHA-M at 5% CO2 and 37°C for 72 hours. The activation-induced supernatant containing a large amount of cytokines, growth factors and bioactive molecules was collected and used in subsequent experiments as a “proinflammatory medium (PM)”. For priming, MSCs were cultured in a T75 culture flask until 90% confluence and the old medium was removed and cultured for 24 hours with medium containing 100 ng/ml recombinant human IFN-γ. MKHn (cells cultured with normal medium) and MKHifn (cells primed with IFN-γ) were seeded at 3x105 cells/well in 6 well culture dishes and cultured at varying PM concentrations (NC, 10%, 20%, 30%, 40%, 50%) and varying incubation times (30 min, 1, 4, 12 and 24h). At the end of incubation, total RNA was first isolated from the cells (Total RNA Purification Kit - Column Kit Jena Bioscience). After RNA extraction, cDNA synthesis was performed using NEB ProtoScript® II First Strand cDNA Synthesis Kit (Germany; E6560S). After cDNA extraction, NEB Luna® Universal qPCR Master Mix (M3003) kit was used for Real-Time PCR. Then HGF, IDO and TGF-β expression levels were analyzed.

RESULTS:

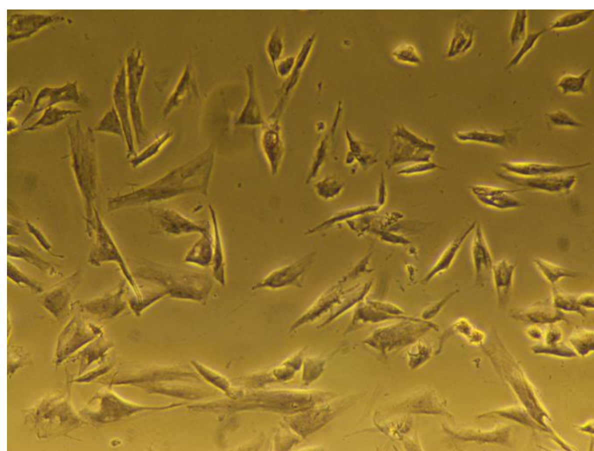


Figure 1: After Alizarin Red-S staining of negative control well of osteogenesis differentiation assay of YD-MSCs.

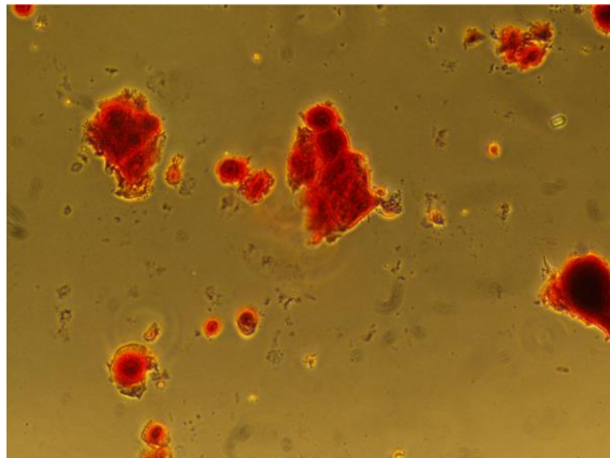


Figure 2: After Alizarin Red-S staining of positive control well of osteogenesis differentiation assay of YD-MSCs.

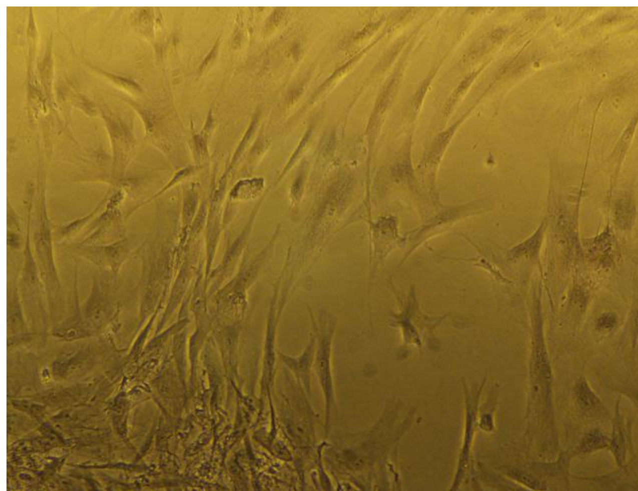


Figure 3: After Oil Red O staining of negative control well of adipogenesis differentiation assay of YD-MSCs.

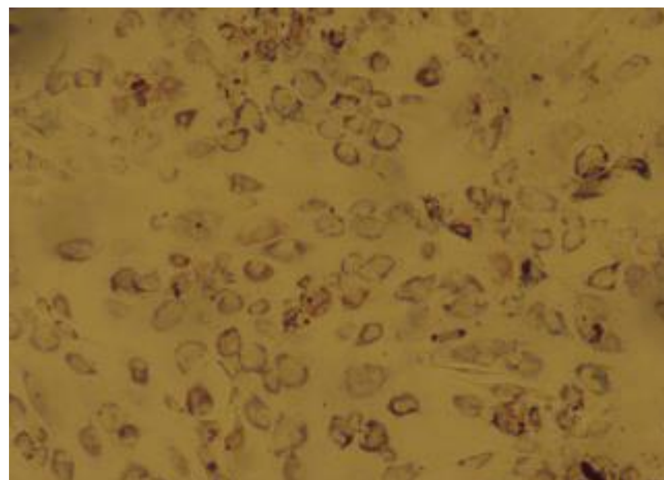
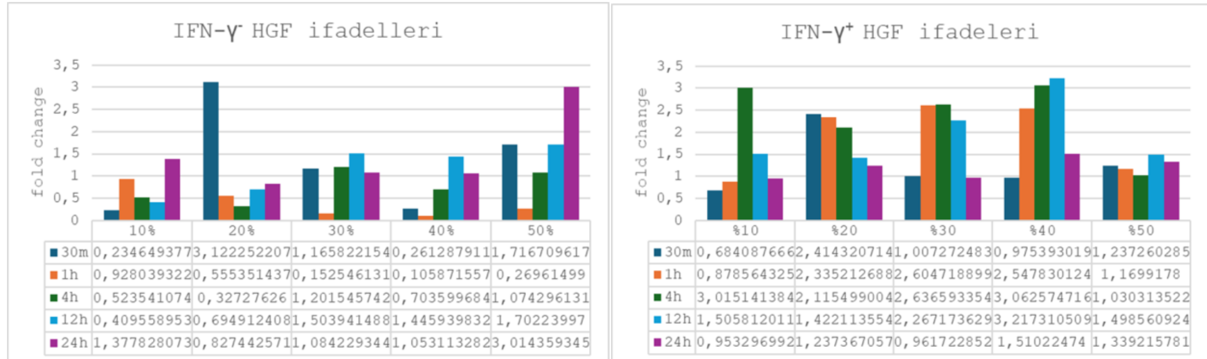


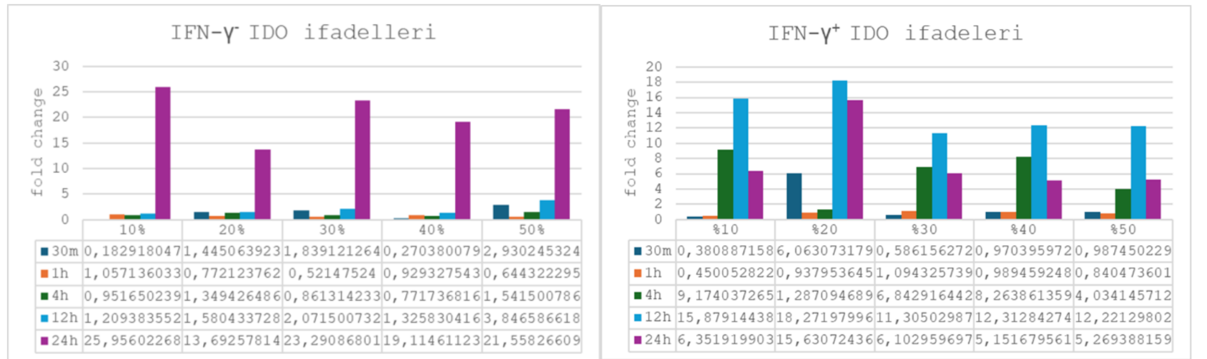
Figure 4: After Oil Red O staining of positive control well of adipogenesis differentiation assay of YD-MSCs.



	10%	20%	30%	40%	50%
30m	0,234649377	3,122252207	1,165822154	0,261287911	1,716709617
1h	0,928039322	0,555351437	0,152546131	0,105871557	0,26961499
4h	0,523541074	0,32727626	1,201545742	0,703599684	1,074296131
12h	0,409558953	0,694912408	1,503941488	1,445939832	1,70223997
24h	1,377828073	0,827442571	1,084229344	1,053113282	3,014359345

	%10	%20	%30	%40	%50
30m	0,684087666	2,414320714	1,007272483	0,975393019	1,237260285
1h	0,878564325	2,335212688	2,604718899	2,547830124	1,1699178
4h	3,015141384	2,115499004	2,636593354	3,062574716	1,030313522
12h	1,505812011	1,422113554	2,267173629	3,217310509	1,498560924
24h	0,953296992	1,237367057	0,961722852	1,51022474	1,339215781

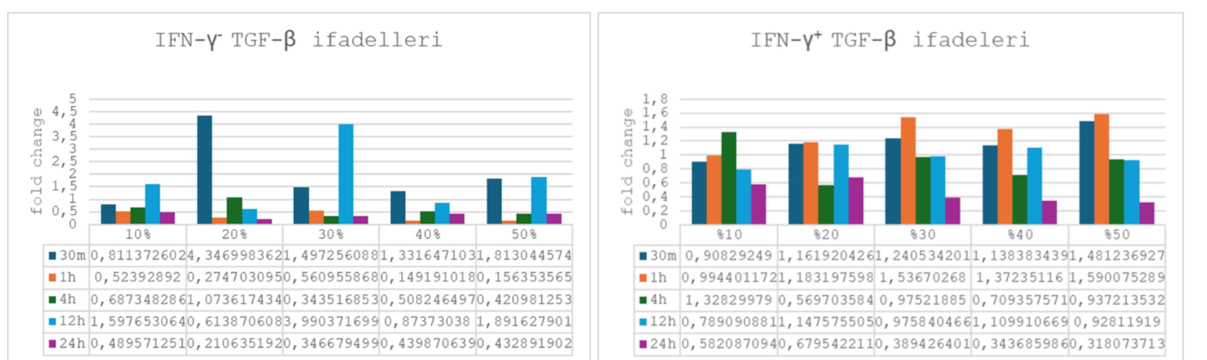
Figure 5: Graphs of HGF expression in MSCs not pre-conditioned and pre-conditioned with IFN-γ.



	10%	20%	30%	40%	50%
30m	0,182918047	1,445063923	1,839121264	0,270380079	2,930245324
1h	1,057136033	0,772123762	0,52147524	0,929327543	0,644322295
4h	0,951650239	1,349426486	0,861314233	0,771736816	1,541500786
12h	1,209383552	1,580433728	2,071500732	1,325830416	3,846586618
24h	25,95602268	13,69257814	23,29086801	19,11461123	21,55826609

	%10	%20	%30	%40	%50
30m	0,380887158	6,063073179	0,586156272	0,970395972	0,987450229
1h	0,450052822	0,937953645	1,094325739	0,989459248	0,840473601
4h	9,174037265	1,287094689	6,842916442	8,263861359	4,034145712
12h	15,87914438	18,27197996	11,30502987	12,31284274	12,22129802
24h	6,351919903	15,63072436	6,102959697	5,151679561	5,269388159

Figure 6: Graphs of IDO expression in MSCs without and with pre-conditioning with IFN-γ.



	10%	20%	30%	40%	50%
30m	0,811372602	4,346998362	1,497256088	1,331647103	1,813044574
1h	0,52392892	0,274703095	0,560955868	0,149191018	0,156353565
4h	0,687348286	1,073617434	0,343516853	0,508246497	0,420981253
12h	1,597653064	0,613870608	3,990371699	0,87373038	1,891627901
24h	0,489571251	0,210635192	0,346679499	0,439870639	0,432891902

	%10	%20	%30	%40	%50
30m	0,90829249	1,161920426	1,240534201	1,138383439	1,481236927
1h	0,994401172	1,183197598	1,53670268	1,37235116	1,590075289
4h	1,32829979	0,569703584	0,97521885	0,709357571	0,937213532
12h	0,789090881	1,147575505	0,975840466	1,109910669	0,92811919
24h	0,582087094	0,679542211	0,389426401	0,343685986	0,318073713

Figure 7: TGF-β expression graphs in MSCs without and with pre-conditioning with IFN-γ.

DISCUSSION

MSCs are cells with certain characteristics and these characteristics are used in their identification. MSCs are plastic-adherent cells, similar to fibroblasts, express surface markers such as CD44, CD73, CD90 and CD105, but do not express surface markers such as CD14, CD34, CD45, CD11b, CD79α, HLA-DR, which are hematopoietic markers. In addition to self-renewal, they can also differentiate into different cell types such as adipocytes, osteocytes and chondrocytes. MSCs can be isolated from various sources (Huang, Wu & Tam, 2022; Rawat,

Gupta & Mohanty, 2019; Merimi et al., 2021). As in the present study, it is easily possible to isolate MSCs from adipose tissue. However, it is necessary to confirm the MSCs obtained by primary culture. For this reason, firstly, the MSCs should be characterized and only then the cells should be used in various experiments. In our study, after isolating MSCs from adipose tissue, we examined them under a microscope and observed fibroblast-like cells that adhered to plastic. In the next step, we used differentiation kits for differentiation into adipocytes and osteocytes and examined their differentiation for 14 days. After 14 days, we evaluated the differentiation by staining. In the process of adipogenesis, MSCs are first transformed into pre-adipocytes by various cytokines and agents and then fat droplets begin to form. In this process, intracellular lipid storage mechanisms are activated and mature adipocytes are formed as a result of differentiation. Oil Red O is a red dye that binds to lipids accumulated in adipocytes and enabling detection under the microscope (Kraus et al., 2016; Hassanlou, Meshgini & Alizadeh, 2019). In the process of osteogenesis, similar to adipogenesis, various stimuli and initiate the process (Zhang et al., 2012). MSCs first transform into pre-osteoblasts and begin to secrete calcium and phosphate as maturation continues. The combination of calcium and phosphate with the collagen matrix supports hard bone tissue. Alizarin Red S stain also detects the released calcium deposits (Donzelli et al., 2007). Through the kits we used and the staining we performed at the end of the experiment, we detected the color formations under the microscope and accepted that these cells were MSCs (Figure 2,4). We plan to continue characterization studies using flow cytometry in the next stages.

Mesenchymal stem cells are clinically important stem cells capable of immunomodulation. Their ability to suppress the immune system by targeting immune cells through cell-cell contact or microenvironmental interactions makes them important for autoimmune diseases (Huang, Wu & Tam, 2022; Rawat, Gupta & Mohanty, 2019; Song, Scholtemeijer & Shah, 2020). MSCs realize these abilities thanks to molecules such as TGF-β, IDO and HGF that they secrete. The ability of mesenchymal stem cells are short-lived and there is a need to prolong this effect. Priming is a useful procedure to increase this ability. The application of various cytokines or chemical agents, the use of environmental conditions such as hypoxia or the application of 3D culture conditions are priming methods that have been performed in recent years (Herger et al., 2024; Noronha et al., 2019; Harman, Marx & Van de Walle, 2021). In order to examine the effect of this procedure, PBMCs were isolated by

collecting EDTA blood from healthy volunteers. PBMCs are important cells of the immune system and can be activated by PHA-PHM. As a result of activation, PBMCs secrete various proinflammatory cytokines and create an inflammatory environment. We activated PBMCs and collected the supernatants, thus obtaining a proinflammatory medium, which we administered to the MSCs at varying ratios and incubation times to examine their responses under inflammatory conditions. Then, we performed the same experiment by priming the MSCs with IFN-γ, which is the most widely studied cytokine. Since TGF-β, IDO and HGF are cytokines secreted by MSCs and involved in immunomodulation, we evaluated the expression levels of these molecules at the end of the experiment. We aimed to examine the responses of unprimed and primed MSCs to different inflammatory signals and the molecules such as IDO, TGF-β, HGF secreted in this process and the effect of priming effect on the immunomodulatory abilities of MSCs. As a result of our experiments, when we compared the HGF, IDO and TGF-β expressions of MSCs without IFN-γ with their expressions after IFN-γ stimulation, we observed that HGF, IDO and TGF-β expressions increased more rapidly and under lower inflammation conditions (Figure 5,6,7). These data suggested that pre-conditioning with IFN-γ may be more sensitive in the response of MSCs to inflammation. In the next phase of our study, we will evaluate the responses of MSCs that expressing chimeric IFN-γ receptors and try to obtain data on whether continuous IFN-γ stimulation is superior to current approaches.

ACKNOWLEDGEMENTS

The authors declare no acknowledgments.

Key Messages:

- Mesenchymal stem cells are capable of immunomodulation and therefore hold promise for autoimmune diseases.
- Their immunomodulation ability is mediated by secreted cytokines (such as HGF, IDO and TGF-β) or cell-cell contact.
- Priming with IFN-γ can be used to increase the immunomodulatory ability of MSCs.

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