



T.C.

YEDİTEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF PEDIATRIC DENTISTRY

**INVESTIGATION OF THE RELATIONSHIP
BETWEEN ENAMEL DEGENERATIVE DISEASES
AND ENAM GENE POLYMORPHISMS**

DOCTOR OF PHILOSOPHY THESIS

DENTIST

BURCU AKSOY ÖZGÜVEN

İSTANBUL-2023



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Ph.D. THESIS

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DECLARATION

I declare that this thesis is my own work from its planning to the conclusion, and I have cited pieces of information from previous research and studies while retaining the property rights of these sources, which I list in the list of references. I further declare that this thesis has not been submitted for any previous degree. The work was done under the guidance of Professor Dr. Didem ÖZDEMİR ÖZENEN.

Burcu AKSOY ÖZGÜVEN



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LIST OF SYMBOLS AND ABBREVIATIONS

A: Adenine nucleotide

AI: Amelogenesis Imperfecta

AIHHT: Amelogenesis Imperfecta Hypoplastic-Hypomaturation with Taurodontism

AIT: Amelogenesis Imperfecta with Taurodontism

AMBN: Ameloblastin gene

AMEL: Amelogenin gene

AMELX: Amelogenin gene encoded by X chromosome

AMELY: Amelogenin gene encoded by Y chromosome

ARAI: Autosomal recessive Amelogenesis Imperfecta

C: Cytosine

DEJ: Dental Enamel Junction

DNA: Deoxyribo Nucleic Acid

DNTPs: Deoxynucleotide Triphosphates

EDD: Enamel Degenerative Disease

EDTA: Ethylene Diamine Tetraacetic Acid

EMSP1: Enamel Matrix Serine Proteinase 1 gene (also known as *KLK4* gene)

ENAM: Enamalin gene

FAM83H: Family with sequence similarity 83 member H gene

FPM: First Permanent Molars

G: Guanine

KLK: Kallikrein gene

MMP20: Enamelysin (Matrix metalloproteinase20) gene

MIH: Molar Incisor Hypomineralization

n: number

PCR: Polymerase Chain Reaction

RT-PCR: Real-Time Polymerase Chain Reaction

SNP: Single Nucleotid Polymorphism

T: Thymine

TDO: Tricho-dento-osseous

XAI: X-Linked Amelogenesis Imperfecta

°C: Degrees Celcius

ABSTRACT

Aksoy Özgüven, B. (2023). Investigation of the Relationship Between Enamel Degenerative Diseases And Enam Gene Polymorphisms. Yeditepe University, Institute of Health Science, Department of Pediatric Dentistry, Ph.D. Thesis, Istanbul.

The Human Genome Project and advances in molecular genetics have made substantial progress in the identification of genes implicated in the pathogenesis of human diseases. In addition, these projects include dental diseases that affect enamel and dentin formation as well as tooth anomalies. These complex mechanisms are regulated by genes and influenced by epigenetic and environmental factors. Abnormalities in the developmental pathways may result in decreased tissue production and/or poor mineralization quality. Developmental enamel defects may be inherited as a result of mutations in the genes that code for enamel proteins or as a characteristic of more general familial conditions. The genes that encode the enamel matrix proteins are *Enamelin (ENAM)* and *Ameloblastin (AMBN)* (4q11-q21), *Tuftelin (TUFT)* (1q21-31), *Matrix Metalloproteinase20 (MMP20)* (11q22), and *Kallikrein4 (KLK4)* (19q13.3-q13.4). The *Enamelin* gene (*ENAM*) encodes a matrix protein that is essential for tooth enamel formation. Amelogenesis imperfecta (AI) refers to a group of inherited conditions in which the developmental defects are restricted to the teeth and enamel. This thesis study aims to investigate the possible association between single nucleotide polymorphism (SNP) rs12640848 in *ENAM* gene and susceptibility to Enamel Degenerative Disease (EDD) in the group of Turkish population. The two groups were investigated enamel degenerative disease patients (n=20) and the control groups (n=20), using real time PCR and statistical analysis of data was performed by the SPSS program. This study is focused on the genotypic and allelic distribution of this SNP (rs12640848). The results revealed no significant relationship between the genotype distributions for the *ENAM* gene (rs12640848) polymorphism between the patient and control groups (p=0.052). However, the distributions of allelic A carrier and noncarrier for the *ENAM* gene (rs12640848) between the patient and control groups, there was significantly higher frequency of A carriers in patient group (p=0.025). In conclusion, the identification of genes associated with AI, along with the ongoing documentation of mutations responsible for different forms of both AI and enamel generative diseases, will enhance our understanding of the molecular basis underlying enamel anomalies.

Keywords: *ENAM* gene, Amelogenesis Imperfecta, Single nucleotide polymorphism

ÖZET

Aksoy Özgüven, B. (2023). Mine Dejeneratif Hastalıkları ile Enam Gen Polimorfizmleri Arasındaki İlişkinin Araştırılması. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü Çocuk Diş Hekimliği Anabilim Dalı, Doktora tez, İstanbul.

İnsan Genomu Projesi ve moleküler genetikteki gelişmeler, insan hastalıklarının patogeneğinde yer alan genlerin tanımlanmasında önemli ilerlemeler kaydetmiştir. Ayrıca bu projeler arasında diş anomalilerinin yanı sıra mine ve dentin oluşumunu etkileyen diş hastalıkları da yer almaktadır. Bu karmaşık mekanizmalar genler tarafından düzenlenir ve epigenetik ve çevresel faktörlerden etkilenir. Gelişim yollarındaki anormallikler, doku üretiminin azalmasına ve/veya düşük mineralizasyon kalitesine neden olabilir. Gelişimsel mine kusurları, mine proteinlerini kodlayan genlerdeki mutasyonların bir sonucu olarak veya daha genel ailevi durumların bir özelliği olarak kalıtsal olabilir. *Enamelin (ENAM)*, *Ameloblastin (AMBN)* (*4q11-q21*), *Tuftelin (TUFT)* (*1q21-31*), *Matrix Metalloproteinase20 (MMP20)* (*11q22*), ve *Kallikrein4 (KLK4)* (*19q13.3-q13.4*) isimli genler, diş minesi matriks proteinlerini kodlamakla sorumludur. *Enamelin (ENAM)* geni, diş minesi oluşumu için gerekli olan bir matris proteinini kodlamaktadır. Amelogenesis İmperfekta (AI), gelişimsel kusurların dişler ve mine ile sınırlı olduğu bir grup kalıtsal durumu ifade etmektedir. Bu tez çalışmasında, bir grup Türk popülasyonunda *ENAM* genindeki tek nükleotid polimorfizmi rs12640848 ile Mine Dejeneratif Hastalığı arasındaki olası ilişkiyi araştırmayı amaçlandı. Mine dejeneratif hastalığı olan hastalar (n=20) ve kontrol grupları (n=20) olmak üzere iki grup real time PCR kullanılarak incelendi ve verilerin istatistiksel analizi SPSS programı ile yapıldı. Bu çalışmada, rs12640848 tek nükleotid polimorfizminin genotipik ve alelik dağılımına odaklanıldı. Çalışmanın bulgularına göre hasta ve kontrol grupları arasında *ENAM* gene (rs12640848) polimorfizmi için genotip dağılımları arasında anlamlı bir ilişki saptanmadı (p= 0,052). Bununla birlikte, hasta ve kontrol grupları arasında *ENAM* geni (rs12640848) için alelik A taşıyıcısı ve taşıyıcı olmayan dağılımları, hasta grubunda anlamlı derecede daha yüksek A taşıyıcısı sıklığı olduğu saptandı (p=0,025). Sonuç olarak, AI ile ilişkili genlerin tanımlanması hem AI hem de mine dejeneratif hastalıklarının farklı formlarından sorumlu mutasyonların devam eden genetik incelemeleriyle birlikte, mine anomalilerinin altında yatan moleküler dayanak hakkındaki anlayışımızı geliştireceği ileri sürülebilmektedir.

Anahtar kelimeler: *ENAM* geni, Amelogenesis İmperfekta, Tek nükleotid polimorfizmi

1.INTRODUCTION AND PURPOSE

Developmental anomalies of dental hard tissues, various gene defects have been associated with environmental and systemic factors encountered in the uterus and postpartum. Many of them were considered idiopathic. Their etiology has not yet been completely clarified. Understanding the mechanisms of tooth development is necessary for learning the pathogenesis of developmental dental anomalies and designing new methods for their diagnosis and treatment (1).

Enamel is an unusual tissue that has developed over millions of years to aid in the survival of numerous species. In humans, enamel changed to create the tooth crown's external protective layer (2). A number of studies to date have examined the effect of genes on the structure and function of enamel (3). Over 115 genetic variants known to affect amelogenesis are associated with enamel phenotypes characterized by a reduction in enamel quantity or mineralization (2).

Environmental, genetic, and epigenetic factors can affect the creation of enamel and play an essential role in resistance or risk for developmental disorders and dental caries, a complex disease. Recognizing why and how enamel is affected, as well as the clinically observed enamel phenotypes, helps with diagnostics, prognostication, and the choice of treatment methods that are suitable to the specific tissue defects (e.g., inadequate amount, reduced mineral, decreased insulation, and hypersensitivity) (4).

The aim of this study was to identify the role of the *ENAM* gene polymorphism in patients with Amelogenesis Imperfecta which is an Enamel Degenerative Disease.

The hypothesis of this study (H_1) was that there is a relationship between *ENAM* gene polymorphism and Enamel Degenerative Diseases. The null hypothesis of this study (H_0) was that there is no significant relationship between *ENAM* gene polymorphisms and Enamel Degenerative Diseases

2.GENERAL INFORMATION

2.1. Development of Dental Hard Tissues

2.1.1. Odontogenesis

There are two different types of cells within the composition of teeth: oral ectodermal cells, which originate from the enamel organ and ectomesenchymal cells, which originate from the dental papilla. The enamel organ produces enamel, whereas the dental papilla produces dentin. Interactions between these epithelial and mesenchymal cells are critical for initiation and development of the teeth. Despite the fact that tooth creation is a constant process, it is distinguished through sequence of plainly recognizable phases referred to as the bud, cap and bell stages. Every single stage is determined by the form of the enamel organ generated from ectodermal tissue, which is a component of the growing tooth (5).

Tooth formation occurs near the end of the fifth week human pregnancy. Teeth formation is initiated by a localized thickening inside the main epithelial bands. Due to the fact that the underlying ectomesenchyme is more dynamic than the epithelial cells, the ectomesenchymal cells rapidly develop epithelial outgrowths. While those cells form, newly emerging structure follows the morphological phases of tooth formation, described as: bud, cap and bell. The epithelium's formation and development determine the future state of the tooth crown (6).

At the onset of the bud stage, the ectodermal cells are encompassed by a surge of mesenchymal cells, resulting in a compact and circular formation. As the epithelial bud expands in dimension, its surface becomes concave, marking the initiation of the cap stage (6). Following the process of differentiation, the ectodermal structure transforms into the enamel organ and maintains its connection to the lamina. As the ectomesenchyme is encompassed by mineralized tissue, it evolves into the dental papilla, which subsequently develops into the tooth pulp. The dental follicle is the connective tissue that surrounds these two structures (5). The tooth germ is a result of a collective process of the enamel organ, dental papilla, and dental follicle (6, 7). The enamel organ is a component of the ectoderm that is responsible for the formation of tooth enamel. In the cap and bell stages, signals from the enamel organ dictate the size and shape of the dental crown (7, 8). The dental papilla is important for the production of

pulp and dentin and is derived from the mesenchyme. The periodontal membrane, cementum, and alveolar bone are all formed by the dental follicle, which is a mesenchymal cell condensation that surrounds these tissues (8).

During the bell stage of tooth development, the papilla and enamel organ undergo morphodifferentiation and histodifferentiation (7). At this stage, the inner enamel epithelial cells are determined by the tooth's shape. The outer enamel epithelial cells cover the enamel organ, while the inner enamel epithelial cells turn into ameloblasts that create the enamel of the tooth crown. Stellate reticulum cells, which are star-shaped and have cytoplasmic processes, are located between these two layers of cells. Odontoblasts develop at the dental papilla's periphery during the bell stage. In various aspects, these cells differ from neural crest and ectomesenchymal cells. Dentin is formed as the odontoblasts develop. The developed ameloblasts deposit an enamel matrix after several increments of dentin have grown. Amelogenesis is always preceded by dentinogenesis (5).

2.1.2. Dentinogenesis

Dentinogenesis is a well-ordered process in which ectomesenchymally-derived cells termed odontoblasts gradually mineralize the organic predentine matrix. The predentine (10–40 µm thick) is an unmineralized area that contains type I collagen and divides the mineralisation border from the odontoblast cell bodies (9). Within 24 hours predentin becomes calcified and called dentin (5).

2.1.3. Amelogenesis

Enamel development begins with cells generated from the tooth organ's internal enamel epithelium following the initiation of dentinogenesis. Amelogenesis is divided into three specific functional stages: secretory, transition, and maturation stages (10).

2.1.3.1. The Stage of Secretion

When the enamel matrix is built down, ameloblasts secrete large quantities of enamel matrix proteins, which serve as a scaffold and allow hydroxyapatite crystals to grow relatively quickly. Dentin crystals interdigitate with the earliest hydroxyapatite crystals that develop the development of a small cytoplasmic extension on the secretory end of ameloblasts, the Tomes'

processes, is an essential event for the creation and structure of enamel (11). The initial enamel created is composed of 65% water, 20% organic matter (protein), and 15% inorganic materials (hydroxy). This phase of secretion is repeated until all enamel tissue has been secreted. Meanwhile, the matrix is composed of two distinct types of proteins. The former is a hydrophobic proline-rich amelogenin, while the latter is an acidic enamelin. The resulting matrix is composed of long, thin layers of hydroxyapatite crystals. The molecular weight of the enamel proteins reduces as the matrix is secreted. This occurs as a result of extracellular proteolysis, which causes the crystals to develop. As a result, the enamel matrix is 30% calcified and has a soft structure (12).

2.1.3.2. The Stage Of Transition

After getting the optimum enamel thickness, at a point determined by the genetic program, secretory ameloblasts enter the maturation phase with a short transitional phase that significantly reduces the amount of enamel proteins secreted (1, 13). The transitional phase lasts only 19 hours during the ameloblasts' 30-day life cycle. Apoptosis occurs in more than 50% of ameloblasts during the transitional and maturation stages (1). It is believed that secretory ameloblasts with a diameter of around 4 μm are destroyed during the transitional phase to make way for mature ameloblasts with a diameter of approximately 7 μm , larger bases, and wavy and flat ends. At the conclusion of the transitional phase, a basement membrane is created and ameloblasts are joined to it. The newly synthesized basement membrane contributes to the management of enamel maturation's internal and external transport (13).

2.1.3.3. The Stage Of Maturation

The thickness and length of these mineral bands rapidly expand throughout the maturation stage due to the elimination of enamel proteins by the existing proteinases. As a result, a mineralized texture with a minimum of 95% inorganic structure is created. The remaining 1-2 percent is made up of leftover proteins, while the remainder is made up of water (14). Enamel becomes more calcified and porous. Mineral ions such as calcium and phosphate are used to restore the degraded enamel matrix proteins. The absence of organic matrix and consistent mineral development distinguish enamel tissue from other hard tissues (12). At the growth center, the maturation phase begins when the enamel reaches its full thickness at the cusp apex. This process follows the matrix's direction of development. It begins at the enamel-

dentin border and works its way out to the outer surface. This occurs more rapidly than matrix secretion (0.04-0.05 mm per day). At this stage, the protein material is removed selectively. Enamelins with a high molecular weight persist. The enamelins remain securely bonded to the apatite crystal surfaces. The majority of the water has evaporated. The enamel is severely calcified but remains extremely porous (14). The pH value of the fluid around the enamel crystals oscillates between values less than 6 and 7.2 during the development process. Crystals having a larger carbonate content, which are more acid soluble, are eliminated selectively during the cycle's lower pH section. As a result, an evolutionary process occurs throughout the maturation phase, in which minerals with a low acid stability are gradually replaced by more acid-resistant apatites. This process occurs in the mouth as well, and with time, the enamel becomes more resistant to caries (15).

2.1.4. Enamel Matrix Protein

Beginning before dentin biomineralization and continuing until the end of its secretory phase, ameloblasts periodically secrete enamel protein (15). Ameloblasts utilize different kinds of molecule such as amelogenin, ameloblastin and enamelin as well as matrix enzymes such as kallikrein-4 and matrix metalloproteinase-20 for enamel matrix recreation, formation and maturation. Three primary structural proteins comprises amelogenin (80-90 percent of total enamel protein), ameloblastin (5-10 percent), and enamelin (1-5 percent). The assemblies formed by these proteins at the mineralization front seem to be accountable for incremental lengthening of existing crystallites (10).

2.1.4.1. Amelogenin (AMEL)

Amelogenin is secreted just prior to dentin calcification by ameloblasts (1). Around 90% of enamel proteins are composed of the amelogenin protein. By protein sequencing and cDNA cloning, the amelogenin protein was identified for the first time. It comprises around 30% proline, 13% glutamine, 10% leucine, and 8% histidine (16). It is primarily composed of hydrophobic molecules (1).

The amelogenin protein is critical for the correct formation of enamel. It is predominantly secreted by the amelogenin gene on the X-chromosome. Amelogenin secretion from the amelogenin gene on the Y-chromosome has been found at a rate of approximately

10%. (17). Amelogenesis imperfecta (AI) is a developmental enamel condition caused by a mutation in the amelogenin gene on the X-chromosome (10). *Gibson et al. (18)* examined mice lacking the Amel gene; they observed the development of hypoplastic, opaque, and fragile enamel and concluded that the Amelogenin protein operates an essential role in the regulation of enamel thickness and crystal structure.

2.1.4.2. Ameloblastin (AMBN)

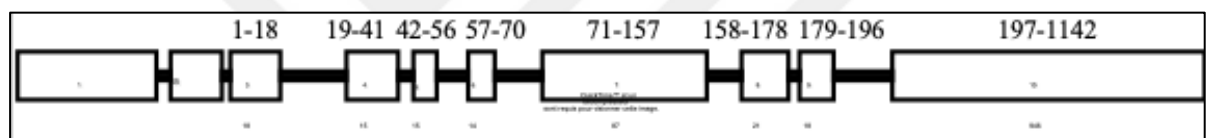
Japanese researchers identified Ameloblastin (AMBN) through the isolation and analysis of protein compounds found in the immature enamel of porcine teeth, uncovering the unique amino acid profiles within (19). Ameloblastin, encoded by the *AMBN* gene on chromosome 4q13.3, contributes to approximately 5 percent of the total protein released during enamel development and is the second most abundant protein in enamel tissue (20). AMBN is a protein specific to teeth whose activities are unknown. AMBN is an enamel matrix protein that is secreted by cells originating from the inner enamel epithelium. AMBN are produced by the root epithelial sheath, presecretory, secretory, and mature ameloblasts, as well as for brief periods in preodontoblasts (21). Ameloblastin is considered to play a role in crystal growth because it is found near to the Tomes appendages, which is where ameloblasts secrete and crystal growth occurs (22). There are several theories regarding the role of this substance. Some propose that it serves as a building block for the prismatic structure of growing enamel crystals, while others suggest that it plays a role in ameloblast adhesion. Additionally, it may function as a growth factor or signaling molecule (23).

2.1.4.3. Enamelin (ENAM)

Scientists in Japan first found enamel in while analyzing porcine enamel extracts (24). Enamelin (ENAM) is the largest and least abundant enamel protein (1-5 percent). It is a glycosylated, phosphorylated protein that is predominantly expressed in ameloblasts (25). Enamelin, a key component in the formation of tooth enamel, has a significant contribution from glycosylations, which make up roughly one-third of its apparent molecular weight. Its presence is limited to the mineralization front, suggesting a direct involvement in crystal elongation. Enamelin is initially cleaved at its C-terminus, but the majority of cleavage products do not accumulate in the enamel matrix. The few that do are found in the rod and interrod enamel from the DEJ to the enamel surface, but are sparse in the sheath space. The

Enamelin and *Ameloblastin* genes, both implicated in the development of autosomal types of Amelogenesis imperfecta, are situated on chromosome 4q in a region previously associated with the disorder (15).

Enamelin is required for the normal production of tooth enamel but is not required for any other process. Autosomal dominant AI with localized hypoplastic (thin) enamel is caused by mutations in a single copy of the *Enamelin* gene (*ENAM*, 4q13). In its simplest form, it manifests as well-defined enamel pits on the tooth surface (26). Horizontal bands of thin enamel are seen in some cases, most notably in the cervical portion of the crown. When both alleles are deficient, hardly no enamel forms. The structure of the human *ENAM* is seen in Figure 2.1 (24). *ENAM* mutations are classified using a defined nomenclature. (26). This technique begins counting nucleotides and amino acids at the initiation of translation (not from the N-terminus of the secreted protein) (24).



Ex/Int	Nucleotides	Amino Acids	Ex/Int	Nucleotides	Amino Acids
Exon 1	221: 2-222	Non-coding	Exon 7	261: 5566-5826	87: Met ⁷¹ -Gln ¹⁵⁷
Intron 1	320: 223-542		Intron 7	1263: 5827-7089	
(Exon 2)	61: 543-603	Non-coding	Exon 8	63: 7090-7152	21: Ala ¹⁵⁸ -Gln ¹⁷⁸
Intron 2			Intron 8	135: 10555-10689	
Exon 3	114: 699-812	18: Met ¹ -Leu ¹⁸	Exon 9	54: 9048-9101	18: Arg ¹⁷⁹ -Gly ¹⁹⁶
Intron 3	3603: 813-2927		Intron 9	4171: 9102-13272	
Exon 4	69: 2928-2996	15: Val ¹⁹ -Gln ⁴¹	Exon 10	4804: 13273-18076	946: Asn ¹⁹⁷ -Ala ¹¹⁴²
Intron 4	110: 2997-3106				
Exon 5	45: 3107-3151	15: Met ⁴² -Glu ⁵⁶			
Intron 5	757: 3152-3908				

Figure 2.1: *ENAM* gene structure (ref seqs: cDNA, NM_031889.2; and gene, NG_013024.1)

2.1.4.4. Enamelysin (MMP20)

Enamelysin (MMP20) is a matrix metalloproteinase that is produced from a gene on chromosome 11 and is associated to other matrix metalloproteinases (27). The enamelysin cDNA sequences allow for the derivation of the proteinase's primary structure, which consists of a signal peptide, a propeptide domain, a catalytic domain, a linker, and a hemopexin-like

domain. Enamel proteinases are found in significant levels. MMP20 was originally called Enamelysin due to the fact that it was expressed only in the enamel organ of developing teeth. MMP20 was later revealed to be expressed at extremely low levels in odontoblast (28) and the large intestine approximately 5000-fold lower than in the secretory enamel organ. MMP20 was expressed in developing ameloblasts and secreted into the enamel (29). MMP20 was the first proteinase to be definitively identified in 1998 as being expressed by enamel organ ameloblasts and released into the developing enamel matrix (21).

2.1.4.5. Kallikrein (KLK)

Kallikrein 4 (KLK4) is a protein encoded by a gene located on chromosome 19 (30). KLK4 was initially isolated from developing pig enamel in 1984 (31). In the course of events, the cDNA of human KLK4 was procured from the growth of teeth. KLK4 is originally produced by odontoblasts during the progression of tooth formation. The expression of KLK4 in the enamel organ commences suddenly in the conversion phase ameloblasts and endures throughout the maturation phase (32). During the initial stages of maturation, *KLK4* degrades enamel proteins, expediting their detachment from the enamel matrix and facilitating the hardening process. Hypothetically, if KLK4, formerly Enamel matrix serine proteinase 1 (EMSP1), were to malfunction, the enamel matrix would not be effectively reabsorbed and the crystallites would not fully thicken, leading to a hypomaturational manifestation of Amelogenesis Imperfecta (33).

2.2 Developmental Defect of Dental Enamel Formation

It results from diseases that occur during the enamel's development; it can be caused by a variety of reasons, including structural abnormalities, genetic defects, and environmental factors (34). Anomalies in the enamel can be symptomatic of both environmental and systemic disorders. To diagnose an enamel deficiency, a thorough history of the patient's clinical history, possible exposure to environmental causes, and family history is required. Problems in the genes producing enamel proteins frequently result in enamel malformations without affecting other areas of the body, however enamel defects are related with a variety of hereditary disorders (35). Clinically, developmental enamel defects frequently result in esthetic degeneration, increased tooth sensitivity, increased susceptibility to caries, increased wear, and erosion (34).

2.2.1. Hereditary Enamel Defects

Hereditary enamel defect; it is characterized as defects in the organic matrix and mineralization of the hard tissue during odontogenesis. Enamel deformities are frequently caused by mutations in the genes encoding enamel proteins (35).

2.2.1.1 Amelogenesis Imperfecta

Amelogenesis imperfecta (AI) is a large variety of hereditary illnesses defined by abnormalities in the development of tooth enamel in the absence of any systemic or generalized disease (36). The process of diagnosis involves excluding any external or environmental factors, identifying a probable pattern of inheritance, observing the physical characteristics of the individual, and considering the timing of tooth development in order to rule out any developmental abnormalities (37). It is a hereditary abnormality of enamel formation that affects the structure, quantity, and content of enamel in primary and permanent teeth. It is often inherited in an autosomal dominant or autosomal recessive manner; X-linked crossovers are uncommon (38).

The frequency of AI as reported in the literature varies significantly. According to studies, values range from 43:10,000 in Turkey to 14:10,000 in Sweden, 10:10,000 in Argentina, and 1.25:10,000 in Israel (10).

The enamel discoveries in AI are diverse, encompassing hindered enamel growth (known as hypoplastic AI) and shortcomings in mineral and protein content (known as hypomineralized and hypomature AI). In cases of the latter, the enamel demonstrates a range of deterioration, appearing opaque white, yellowish, or brown. Once the enamel cap is removed, the dentin may be exposed to a varying degree (35). The afflicted teeth may be discolored, sensitive, or susceptible to decay prior to or following eruption (37).

AI is a genetic disorder that is caused by a single gene mutation or by a microdeletion or chromosomal defect (37). Mutations are caused by five genes or changes in their expression; *AMEL*, *ENAM*, *MMP20*, *KLK4* and *FAM83H*. In contrast to other AI-related genes, *FAM83H* does not encode an extracellular matrix protein. Its location within the cell, as well as its function, are unknown (10).

Clinical Description

Clinical appearance of AI show a wide range of clinical diversity, which is determined by the pattern of inheritance, the mutation involved, matrix protein expression, and metabolic alterations caused by the mutations (10).

- *ENAM*- Varying degrees of hypoplasia range from local pitting to widespread enamel thinning. Numerous hypoplastic phenotypes are possible based on the mutation and its effect on the protein.
- *AMELX*- Defects in maturation and mineralization. Teeth that are clearly abnormal, having disordered, hypoplastic enamel. Phenotypes are variable and range from hypoplasia to hypomaturational/hypomineralisation.
- *KLK4 and MMP20*- Defects in the enamel's ultimate crystallisation or maturation.
- *FAM38H*- Autosomal dominant amelogenesis imperfecta with hypocalcified amelogenesis. Enamel thickness is normal, however the mineral content is lowered (10).

Although AI is considered a non-syndromic defect, several additional dental defects such as pulp calcification, taurodontism, delayed eruption, and occasionally gingival overgrowth may be related with the enamel defect (39). The maxillary and mandibular bones may develop abnormally, typically resulting in open-bite malocclusion and, less frequently, prognathism (40).

Classification

There are several categories for Amelogenesis Imperfecta, but the most generally used is the one established by Witkop and Sauk in 1976, which defines both the genetic and clinical characteristics of Amelogenesis imperfecta (41). Table 2.1 shows that Witkop's classification in 1988, amelogenesis imperfecta may be classified into 4 major categories and 15 subcategories based on clinical characteristics and inheritance (42).

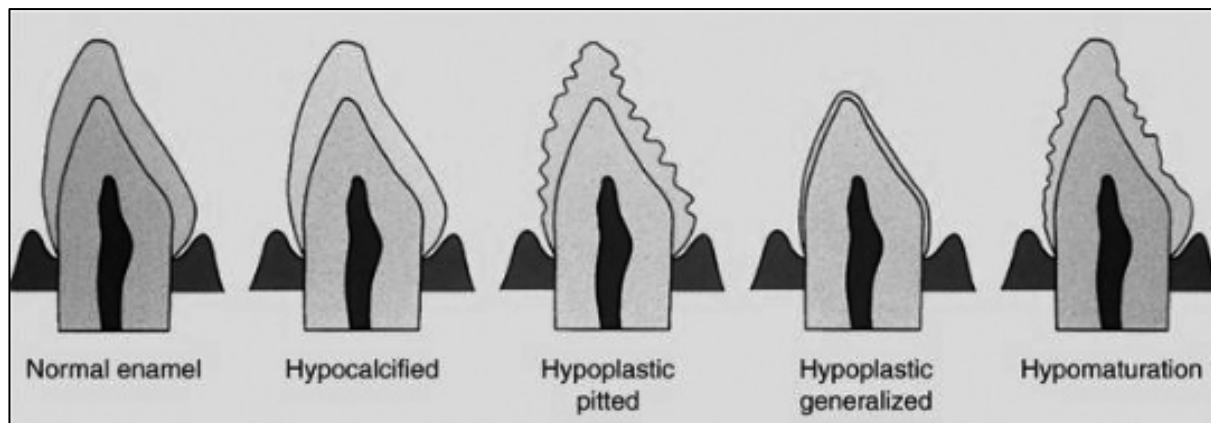


Figure 2.2. Amelogenesis Imperfecta. Diagram of enamel defects of basic types (43)

Figure 2.2. demonstrates the basic types of enamel defects seen in AI. The hypocalcified type has a normal thickness, a smooth surface, and a lower degree of hardness. The hypoplastic pitted kind has an average thickness, a pitted surface, and an average hardness. In hypoplastic generalized type, the thickness is decreased, the surface is smooth, and the hardness is normal. In hypomaturational, the thickness is normal, the surface is chipped, the hardness is reduced, and the coloring is opaque white (43).

Table 2.1. Classification of Amelogenesis imperfecta proposed by Witkop (42)

Type I- Hypoplastic	
IA	Hypoplastic, pitted autosomal dominant
IB	Hypoplastic, local autosomal dominant
IC	Hypoplastic, local autosomal recessive
ID	Hypoplastic, smooth autosomal dominant
IE	Hypoplastic, smooth X-linked dominant
IF	Hypoplastic, rough autosomal dominant
IG	Enamel agenesis, autosomal recessive
Type II- Hypomaturation	
IIA	Hypomaturation, pigmented autosomal recessive
IIB	Hypomaturation, X-linked recessive
IIC	Hypomaturation, snow-capped teeth, X-linked
IID	Hypomaturation, snow-capped teeth, autosomal dominant
Type III- Hypocalcified	
IIIA	Autosomal dominant
IIIB	Autosomal recessive
Type IV- Hypomaturation-hypoplastic with taurodontism	
IVA	Hypomaturation-hypoplastic with taurodontism, autosomal dominant
IVB	Hypoplastic-hypomaturation with taurodontism, autosomal dominant

2.2.1.1.1 Autosomal Dominant Inheritance

One or more members of each generation in a family are affected by autosomal dominant AI (ADAI). Affected individuals in the same family may present with consistent or variable clinical symptoms resulting in subtle or marked changes (37). The ENAM gene, responsible for coding Enamelin, is often impacted and can result in thin enamel and tooth gaps. Certain families may display irregular or depressed enamel due to ENAM mutations (35, 37). In cases of insufficient enamel matrix, the enamel will be firm, transparent, and resistant to wear. However, enamel that is easily lost, reduced in size, and sensitive is considered to have a "cheesy" consistency (37).

2.2.1.1.2. Autosomal Recessive Inheritance

If there is known consanguinity in a family with an affected individual, autosomal recessive AI (ARAI) should be taken into consideration. Intermarriage within the family may be more common in certain ethnic and cultural groups, which may result in encountering AI more often (44).

Enamel phenotypes, which may be dose-dependent, could be caused by ENAM mutations. Generalized hypoplastic AI is inherited as a recessive trait, while localized enamel pitting is inherited as a dominant trait (45).

Autosomal recessive hypomaturational Amelogenesis imperfecta has recently been associated with the *KLK4* gene, the first mutation identified in the *Kallikrein* gene family, which maps to chromosome 19q13.4 (46).

A *matrix metalloproteinase 20* gene (*MMP-20*) mutation in the region 11q22.3–q23 has been linked to autosomal recessive pigmented hypomaturational Amelogenesis imperfecta. (47).

2.2.1.1.3. X linked Inheritance

X-linked amelogenesis imperfecta (XAI) (Amelogenesis imperfecta 1, hypoplastic type, AIH1) shows the classic X-linked inheritance pattern (48). X-linked AI accounts for less than 5% of all AI situations (49). Amelogenin is encoded by the Xp22.1e p22.3 (*AMELX*) and Yp11.2 (*AMELY*) genes. *AMELX* has been found to have fifteen mutations. There has been no record of a harmful mutation in *AMELY*, and no report of AI being inherited through the Y chromosome (50). Female heterozygotes have a 50% chance of passing on the mutant gene to their children of either sex. Males and females are affected in markedly different ways by the disease. Males exhibit the characteristic completely (48).

XAI may have teeth with a thin coating of enamel that is normal in color and translucency, or they may have enamel that is normal in thickness but is poorly mineralized, resulting in loss of translucency and/or a yellow-brown discoloration, with or without opacities. (48). Teeth appear different from yellow to brown, with or without opacities. The teeth are varied in size, ranging from normal to microdontia, depending on the thickness of the enamel.

A smooth or rough surface is acceptable. In female carriers, a unique striated pattern of AI is evident, showcasing distinct columns of both normal and pathological ameloblast activity, which are the result of the actions of either regular or faulty ameloblast clones (48, 51).

2.2.1.1.4. Syndromes Associated with Amelogenesis Imperfecta

AI is a hereditary disorder that can occur alone or in association with other symptoms in syndromes. It is either caused by a single gene mutation or by a microdeletion or chromosomal abnormality (48).

In clinical practice, about 50% of individuals with AI of either X-linked or autosomal heredity have a skeletal anterior open bite. While such a relationship may be considered a condition, it does not exist in any categorization. The meaning of this frequent relationship remains unknown (52).

The inclusion of AI with taurodontism (AIT) in various phenotypic classifications of AI is justified by the inherent variability of the condition. Despite exceeding the precise definition of AI, its ectodermal nature warrants its inclusion in any classification of the disorder. In addition to 'curly hair' and skeletal abnormalities including bone sclerosis, AIT and the Tricho-Dento-Osseous (TDO) syndrome have significant similarities. While the hair alterations may reflect a shared ectodermal deficiency in TDO, the bone abnormalities are more difficult to explain through a similar route because they imply a mesodermal problem. TDO is caused by a *DLX3* gene mutation (53). There is a discrepancy in genetic makeup between AIT and TDO, as one study suggests, (54) while another asserts that TDO and Amelogenesis imperfecta hypoplastic-hypomaturation with taurodontism (AIHHT) are linked through the *DLX3* gene.(55).

2.2.2 Molar Incisor Hypomineralization

Tooth hypoplasia is a developmental disorder that affects the quality and quantity of tooth structure. Molar Incisor Hypomineralization (MIH) is a kind of tooth hypoplasia which is defined as hypomineralization of one to four permanent first molars that is commonly linked with affected incisors and is caused by a systemic etiology (56).

The following diagnostic criteria and clinical appearance of the defects have been agreed upon:

- Permanent first molars and incisors. From one to all four permanent first molars (FPM), the enamel is hypomineralized. Simultaneous inclusion of the permanent incisors is possible. MIH must effect at least one FPM. Additionally, the anomalies might be found in the second primary molars, incisors, and canine tips. Where more molars and incisors are affected, the abnormality is more severe.
- Demarcated opacities. The affected teeth have well-defined opacities in the occlusal and buccal crown regions. Color and size of the faults vary. The color might range from white, creamy or yellow to brownish. The defect may be minor or may take up a significant portion of the crown. It is advised that problems smaller than one millimeter in diameter are not reported.
- Enamel disintegration. The degree to which the hypomineralised opaque regions are porous varies. When severely affected enamel is exposed to masticatory stresses, it quickly decays, exposing unprotected dentine and encouraging caries formation.
- Atypical restorations. FPM and incisors with restorations that display similar extensions to MIH should be considered affected.
- Tooth sensitivity. The damaged teeth may be described as sensitive on a frequent basis, ranging from a slight reaction to external stimuli to spontaneous hypersensitivity; these teeth are frequently difficult to anaesthetize.
- Extracted teeth. MIH can be defined for extracted teeth only if there are notes in the records or defined opacities on the other FPM. Otherwise, MIH cannot be diagnosed (57).

In clinical terms, hypomineralized enamel may be fragile and porous, resembling discolored chalk or old Dutch cheese. Enamel defects can range in color from white to yellow to brownish, but they always show a distinct distinction between damaged and healthy enamel. The porous, brittle enamel is readily chipped away during mastication. Occasionally, the loss of enamel (post-eruptive enamel disintegration) occurs so quickly following eruption that it seems as though the enamel was never created. Post-eruptive enamel breakdown can present with symptoms similar to hypoplasia, but the distinction lies in the smooth margins of

normal enamel in hypoplasia versus the uneven borders in cases of post-eruptive enamel collapse (58).

MIH is frequently mistaken for fluorosis or amelogenesis imperfecta. It is differentiated from fluorosis by the fact that its opacities are defined, as compared to the diffuse opacities characteristic of fluorosis, and by the enamel structure (fluorosis is caries resistant and MIH is caries prone). Fluorosis is mostly caused by the excessive fluoride exposure throughout a period of time. The question of whether to diagnose a patient with Amelogenesis imperfecta (AI) or Molar incisor hypomineralization (MIH) may be one of definition. It is important to note that only the most severe cases of MIH result in molar teeth that are equally affected and resemble those of AI. In general, MIH defects tend to be more asymmetrical in both molars and incisors. AI may also cause taurodont molars on radiographs and often runs in families (58, 59).

2.3. Mutation and Polymorphism

2.3.1. Mutation, Definition and Types

Mutation is defined as the spontaneous modification of the nucleotide sequences or DNA organization (60). Mutations are permanent changes in DNA nucleotide sequences that affect the phenotype. The protein or enzyme generated by DNA is reduced by structural damage in the DNA chain caused by X-rays, radiation, UV rays, certain medicines, chemicals, and rapid temperature changes. Thus, a living thing's structure may be altered by proteins, and its metabolism can be altered by enzymes (61). Theoretically, it might be useful to distinguish between mutations at three different levels:

- Mutations that affect the number of chromosomes in a cell without disrupting the chromosomes themselves (chromosome mutations)
- Mutations that alter specific sections of a chromosome, resulting in changes to the copy number of subchromosomal regions or the structural arrangement of one or more chromosomes (regional or subchromosomal mutations)
- Mutations that involve modifications to the DNA sequence, such as substitutions, deletions, or insertions, ranging from a single nucleotide to a limit of approximately 100 kb (gene or DNA mutations) (60).

2.3.2. Polymorphism and Definition

Two individuals' DNA sequences are 99.9% similar. Individual genetic diversity results from minor changes in DNA sequences. Genetic polymorphism is the presence of at least two alleles for a specific gene locus in a population. It affects the anatomical and physiological aspects of individuals, their vulnerability to illness, and their genetically determined phenotypic variety. If nucleotide changes in DNA have an effect on the phenotype, this is referred to as a mutation, and it is the cause of the formation of inherited illness. Changes in nucleotides that have no effect on the phenotype are known as polymorphism and contribute to genetic diversity in the population. Polymorphism can also be defined as DNA alterations involving a single nucleotide that do not result in illness (60, 62).

2.3.2.1. Single Nucleotide Polymorphism (SNP)

Single nucleotide polymorphisms (SNPs) are the simplest and most prevalent polymorphisms. Typically, a locus defined by an SNP has just two alleles, which correspond to the two distinct bases occupying that place on the chromosome. SNPs are prevalent, but their distribution across the genome is unequal; the majority of SNPs are located in introns, which are noncoding regions of the genome. Over 100,000 exonic SNPs have been identified to far for the set of protein-coding genes (62).

The rsID number is a one-of-a-kind designation ("rs" followed by a number) that researchers and databases use to identify a specific SNP. It is the naming system for most SNPs and stands for Reference SNP cluster ID (62).

2.3.3. Method Used to Identify Genetic Polymorphisms and Mutations

2.3.3.1. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is an enzymatic process that amplifies in vitro a particular region of genomic DNA from any organism. The aim of the PCR technique is to recreate this DNA area specifically and selectively by repeatedly synthesizing the target DNA sequence between two DNA primers unique to the region. Consequently, the DNA concentration is raised to a molecularly detectable level (60).

Each PCR consists of the following four components:

1. Template DNA: The DNA sequence that will be replicated. As with genomic DNA, template DNA is frequently a complex composite of numerous sequences. Alternatively, any DNA molecule containing the target sequence may be utilized. Simultaneously, the initial copy of DNA created from RNA using reverse transcriptase can be used for PCR (63).
2. Oligonucleotide primers: Typically, each PCR requires a pair of oligonucleotide primers. These are chemically synthesized short DNA molecules with a single strand. At both edges of the sequence to be amplified, complementary primary sequences corresponding to the DNA strand are placed as complementary base pairs (63).
3. DNA polymerase enzyme: In PCR, numerous DNA polymerase enzymes can be utilized. All of them are thermostable and can withstand temperatures as high as 100°C when necessary. Taq DNA polymerase is the most widely-used enzyme. DNA polymerase's function in PCR is to copy DNA molecules. The enzyme binds to the single-stranded DNA and synthesizes the complementary double-stranded DNA. DNA polymerase requires a brief region of double-stranded DNA in order to initiate synthesis of the new sequence. In PCR, this is accomplished by attaching oligonucleotide primers to both ends of the sequence to be amplified, thereby generating brief double-chain regions. Thus, the primers direct the DNA polymerase to replicate only the DNA sequence of interest (63).
4. Deoxynucleotide triphosphates (dNTPs): Each reaction requires four dNTPs (dATP, dGTP, dTTP, dCTP) to be used by DNA polymerase in the synthesis of newly synthesized DNA (63).

For mutation analysis, PCR enables fast amplification of particular sequences and cloning of specific genes from DNA samples. PCR is used in fields such as DNA sequence analysis, diagnosis of hereditary diseases, genetic typing of forensic materials (maternity and paternity determination), agriculture (determination of seed purity), and systematic and evolutionary research (diagnosis of various living species in nature, determination of polymorphism between species) (60).

2.3.3.2 Real-Time Polymerase Chain Reaction (Real-Time PCR)

Real-Time Polymerase Chain Reaction (RT-PCR) is a method that enables DNA amplification to identify its products in a single tube. RT-PCR enables to monitor the quantity of DNA product in each cycle of the PCR reaction. This implies that the entire procedure is completed very rapidly and in a few hours (63).

The most typical applications for RT-PCR are converting the copy number of DNA received from biological samples to numerical values and quantitatively calculating the quantity of mRNA. In addition to its use for these purposes, it also has areas of use in studies such as identifying single point mutations, identifying pathogens, identifying DNA damage, methylation detection, SNP analysis, and detecting chromosomal disorders (64).

Many methods can be used to accurately monitor the number of new copies of the target DNA molecule obtained during the PCR reaction. All of these methods require a 'thermal cycler' modified with a spectrophotometer based on the measurement of fluorescence generated during PCR. The quantity of DNA synthesis in the PCR reaction is measured at each cycle using the probe labeled with a fluorescent label, which is capable of emitting fluorescent light when bound to the target sequence. Specialized chemical processes are also required to design these instruments (63). Since RT-PCR is conducted in a closed system, this method can be safely investigated with a large number of samples with very little risk of contamination (64).

3. MATERIALS AND METHODS

3.1. Sample Selection and Definition

In this study 40 blood and serum samples of patients were divided into two group; the patient group (n = 20), who was diagnosed as EDD and healthy control group (n = 20). Blood and serum samples of the patient and control group were collected from the patients who referred to Yeditepe University, Faculty of Dentistry. In this study inclusion criteria include: patient with EDD, patient with EDD in a pediatric patient and/or family, and patient and family willingness to provide a blood sample. Exclusion criteria include a lack of EDD, the presence of a systemic disease or a distinct condition, and the patient's and family's refusal to provide blood samples. Informed consent form was obtained from all patients. The control group was included the healthy individuals applied to the same clinic for examination. The ethical approval was obtained prior to the study from Yeditepe University Clinical Research Ethical Committee (Ethics Committee Decision No: 644).

3.2. Collection of Peripheral Blood Samples

Samples of peripheral venous blood were collected from the forearms of all research participants using a needle (Quick-Fit Multi-Sample 21G 0.8x40 mn) and 4.5 ml vacuum tubes (Greiner Bio-one Vacuette) containing ethylene diamine tetraacetic acid (EDTA) solution. Blood samples were maintained in a deep freezer at -20°C until DNA extraction methods were performed.

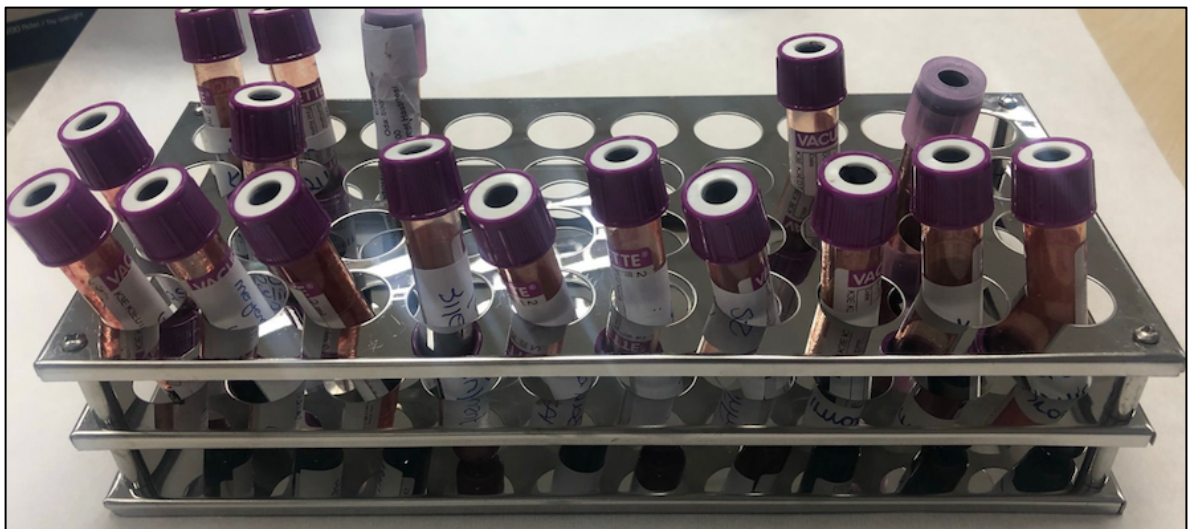


Figure 3.1: Blood samples for patients and control groups

3.3. Materials and Devices Used in Experiment

3.3.1. Materials

The DNA Isolation system mixture, which has a pH 8.8, contains 10.5mM Tris-Cl, 10.5mM NaCl, 10.5nM EDTA, 8M Guanidiniumhydrochloride and 1.12mg/ml Proteinase K (iPrep Purelink, Invitrogen, Thermo Fisher Scientific Inc).

3.3.2. Devices

DNA isolation kit (iPrep Purelink) and DNA isolation robot (iPrep Purelink, Invitrogen), NanoDrop 2000 Spectrophotometer (Thermo Scientific), TaqMan Genotyping Assay and TaqMan SNP Genotyping Master Mix, Real time PCR (Fast Real Time 7500 , Applied Biosystems), Mini Centrifuge (Wealtec), Plate Centrifuge (Hettich), Microplate washing device (WHYM200, Powen Medical co.) , Ultra-pure H₂O (Pure lab option Q, Elga), Vortex (V.I. plus Biosan), +4°C Refrigerator (Haier), -20°C Refrigerator (Haier), pipette Kit (Thermo Fisher Scientific Inc.) devices were used in this study.

3.4.Methods

3.4.1. DNA Isolation from Blood

EDTA tubes were used to collect 5 ml blood samples from the control and patient groups in the study. The collected samples were stored at a temperature of +4°C. The iPrep DNA isolation kit and iPrep DNA isolation robot were used to separate DNA from blood. The iPrep DNA isolation kit and iPrep DNA isolation robot operate on the following principles:

- At any given moment, 13 blood samples could be processed.
- The kit required 350 µl of blood (for each sample)
- The kit included all of the necessary solutions for isolating DNA from blood cells. When these solutions were required, they were used automatically.
- From each of the 13 samples, the robot could isolate 150 µl DNA.
- The isolated DNA samples were kept at +4°C until they were needed (65).



Figure 3.2: The iPrep DNA isolation kit

3.4.2. DNA Purity Identification

A spectrophotometer was used to determine the purity and concentration of DNA isolated using the iPrep DNA Isolation kit and robot. For this, we applied the NanoDrop device. The wavelengths 260 nm and 280 nm were used to determine the quality of DNA. The DNA purity value was calculated by dividing the values measured at 260 nm wavelengths by the values measured at 280 nm wavelengths. When this number was 1.8, it indicated that the isolated DNA was pure, however when it was below 1.8, it indicated protein content, and when it was over 1.8, it indicated the presence of RNA (66).

For DNA concentration identification the below calculation was used;

$$\text{DNA concentration: } OD_{260} \times 50 \mu\text{l/ml} \times \text{dilution rate (100)}$$

The value of DNA at the 260 nm was accepted to be 1 Optic Density (OD).

Spectrophotometer (NanoDrop 2000, Thermo Scientific Inc) device;

1. The computer that Nanodrop was connected to, was turned on and the spectrophotometer application was started.
2. By selecting the DNA analysis section from the table on the screen, a command was given to measure the DNA in the sample.

3. A routine wavelength verification test was performed. After the process was finished, it was ensured that there was no foreign or contaminant material in the Nanodrop sensor.
4. The handle of the Nanodrop was opened and the sensors were cleaned. At the end of the procedure, the arm was gently closed.
5. Before measuring the density of DNA samples, a blank is taken. For this process, the Nanodrop arm was lifted and 1.5 μl of blank solution is placed on the sensor with a micropipette.
6. After the blank solution was released, the arm was lifted down and the blank button in the computer application was selected.
7. At the end of the process, the Nanodrop sensors were cleaned again and 1.5 μl of sample was added to the sensor and the arm was lifted down.
8. DNA concentration was measured by selecting the measuring section in the upper left corner of the computer application.
9. At the end of all processes, before closing the program, a blank was taken and the sensors were thoroughly cleaned.



Figure 3.3: NanoDrop 2000 Spectrophotometer (Thermo Scientific)

3.4.3 Genotyping by Real Time PCR

Using a 7500 Fast Real-Time PCR (Applied Biosystems) instrument, polymerase chain reaction (RT-PCR) was utilized for genotyping. As primers and probes, ‘TaqMan single nucleotide polymorphism (SNP) Genotyping Assays’ were utilized. Molecular beacons were used in Real-Time PCR to identify SNPs. When these molecules were hybridized with a supplemental target, they produced fluorescent light. Because of the linking DNA sequences, molecular beacons opened to supplement target regions when additional target areas were present. The wavelengths of the molecular beacons utilized in Real-Time PCR for allele-specific detection, which included wildtype and mutant alleles, were different.

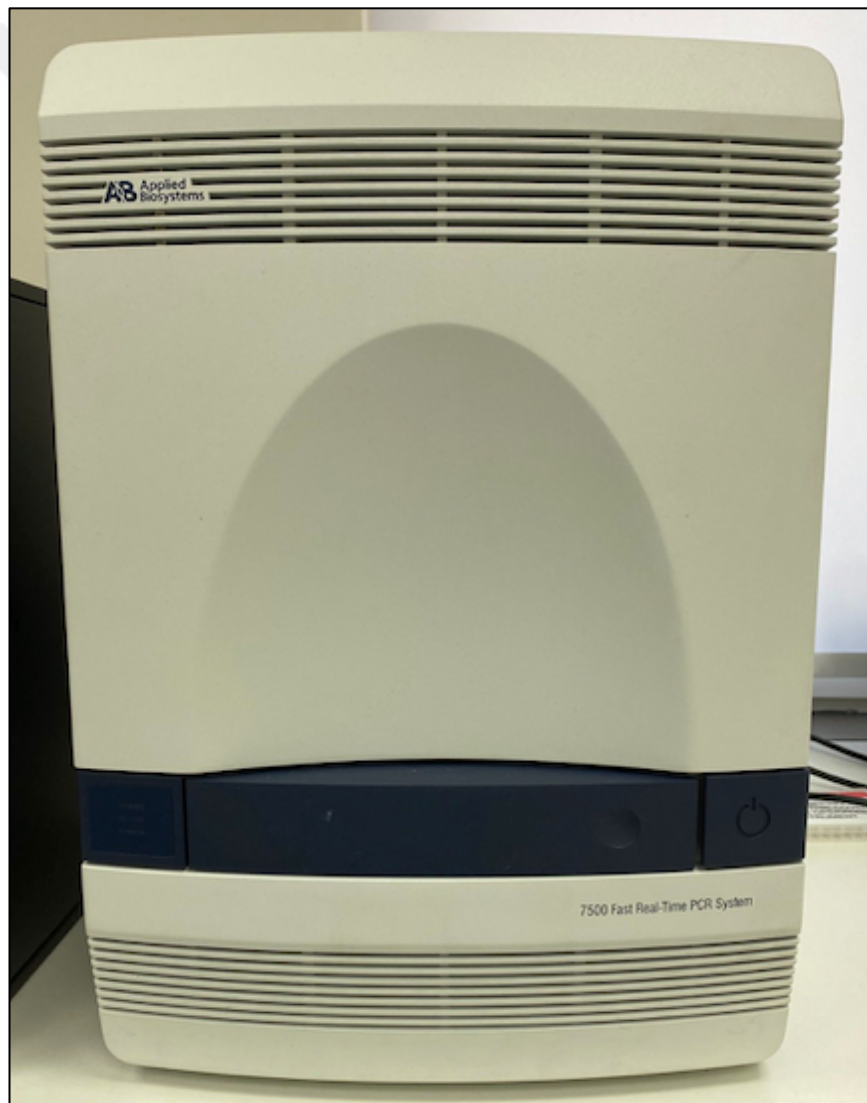


Figure 3.4: Real time PCR (Fast Real Time 7500, Applied Biosystems)

3.4.4 Real Time Protocol

Table 3.1 contained the Real-Time PCR reagents as well as the reaction mix. The protocol determined the total volume of each sample.

Table 3.1. The reaction mixture for the Real-Time PCR

The Agent	Quantity of Agent
Master Mix	5
TaqMan Genotyping Assay	0.25
DNase, RNase Free water	3.75
Template DNA	1

For Real-Time PCR, the conditions were set by waiting 10 minutes at 95°C, denaturing for 15 seconds at 92°C for each cycle, and connecting/elongation for 1 minute at 60°C for each cycle. Denaturation and connecting/elongation were accomplished for 40 cycles, as shown in Table 3.2.



Figure 3.5: TaqMan Genotyping Assay

Table 3.2. The Real-Time PCR Conditions

		40 Cycles	
	Hold	Denaturation	Annealing/Extension
Temperature	95°C	92°C	60°C
Duration	10 minutes	15 seconds	1 minutes

3.4.5. Statistical Analysis

In this study, numerical results were obtained using the Student's t-test. When PCR experiments were performed and allelic discrimination data was received, all data were imported into SPSS 26.0. In addition, the other clinical parameters had been collected and entered into the SPSS document. The analyzes of the data obtained from the genotyping study using Chi-Square and Fisher's Exact Tests were made with SPSS 26.0 program. The genotype distribution and allele frequencies in the groups were statistically analyzed using Chi-square and Fisher's Exact Tests. The significance value of the results was accepted as $p < 0.05$. The values obtained from the Chi-Square (χ^2) analysis are shown as percentages and numbers.

4. RESULTS

4.1 Statistical Evaluation of Real-Time PCR Results

The software of the 7500 Fast-Real Time PCR system automatically examined allelic discriminations. The dyes in the probes were responsible for taking the measurements and interpreting the fluorescence radiation. Some samples, meanwhile, were indistinguishable. Two distinct dyes that correspond to the FAM color blue and the VIC color red were used in this study. For analyzing FAM and VIC dyes, ROX provides as a reference color. Radiance curve analysis and interpretation were used to evaluate allelic discrimination.



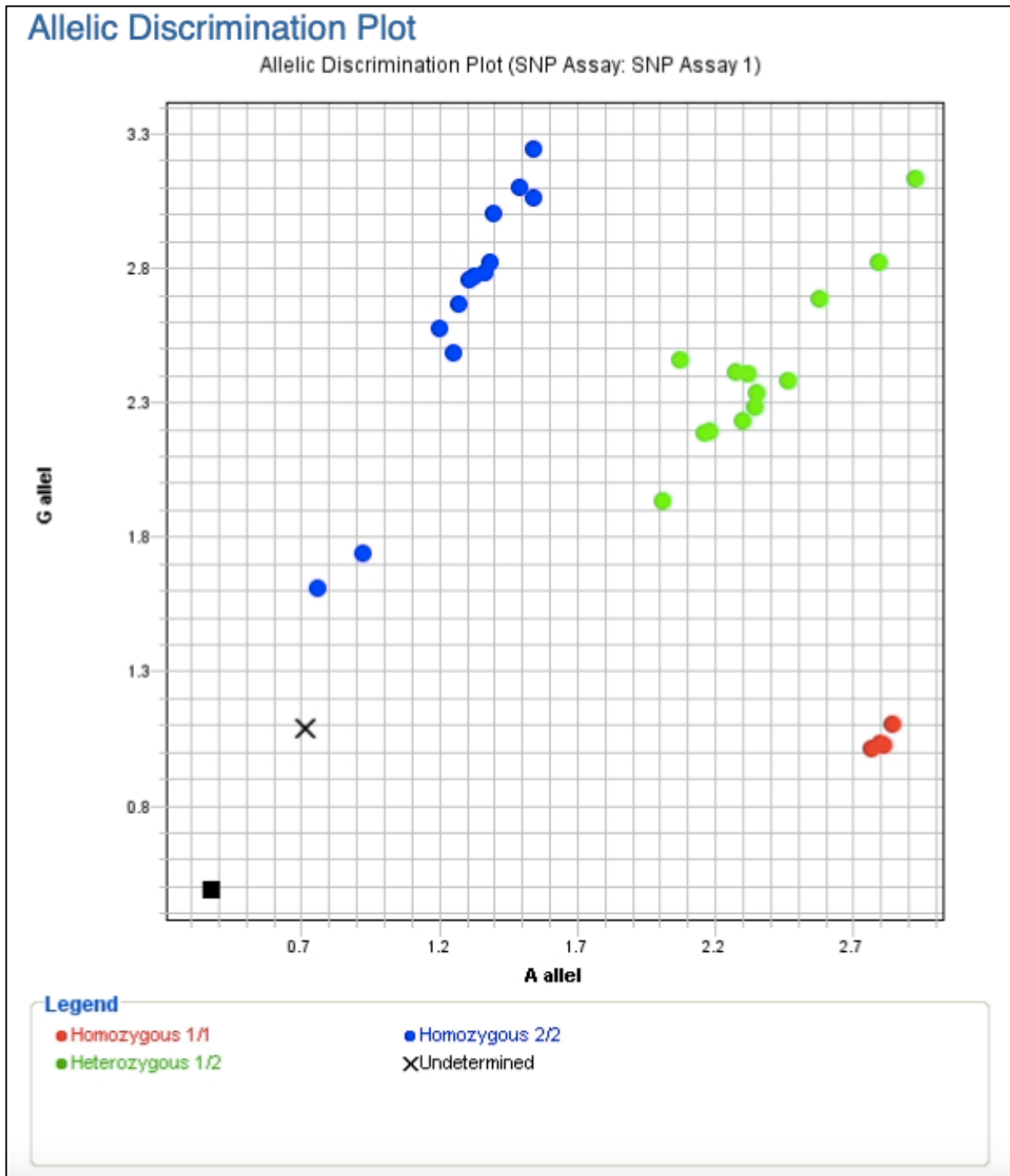


Figure 4.1: Allelic discrimination analysis of *ENAM* genotype

(Note that: AA: Homozygote Wild Type AG: Heterozygote Type GG: Homozygote Mutant Type)

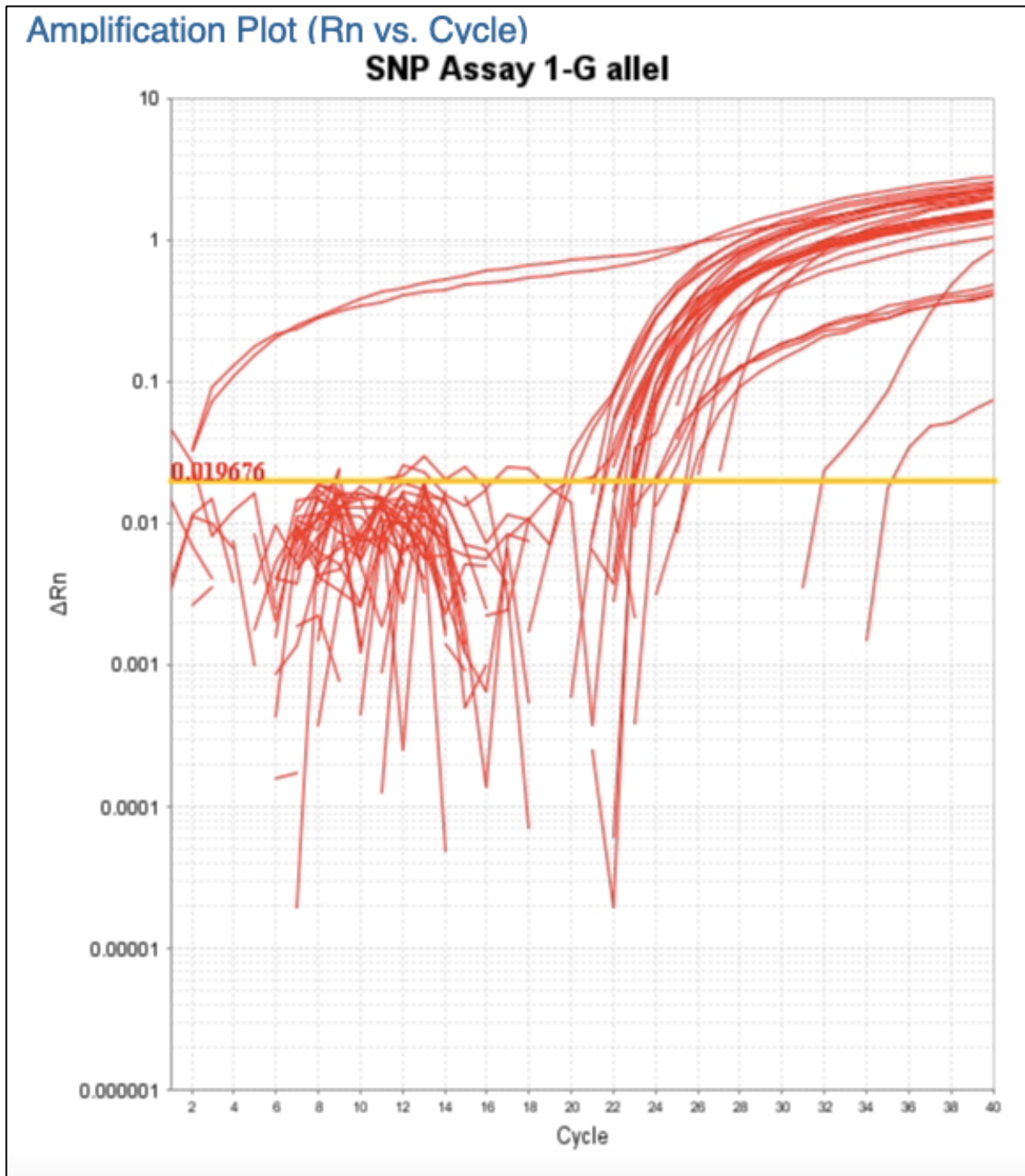


Figure 4.2: Amplification plot display of Allele G.
(Note that threshold value (0.019676) is shown as a **yellow** line)

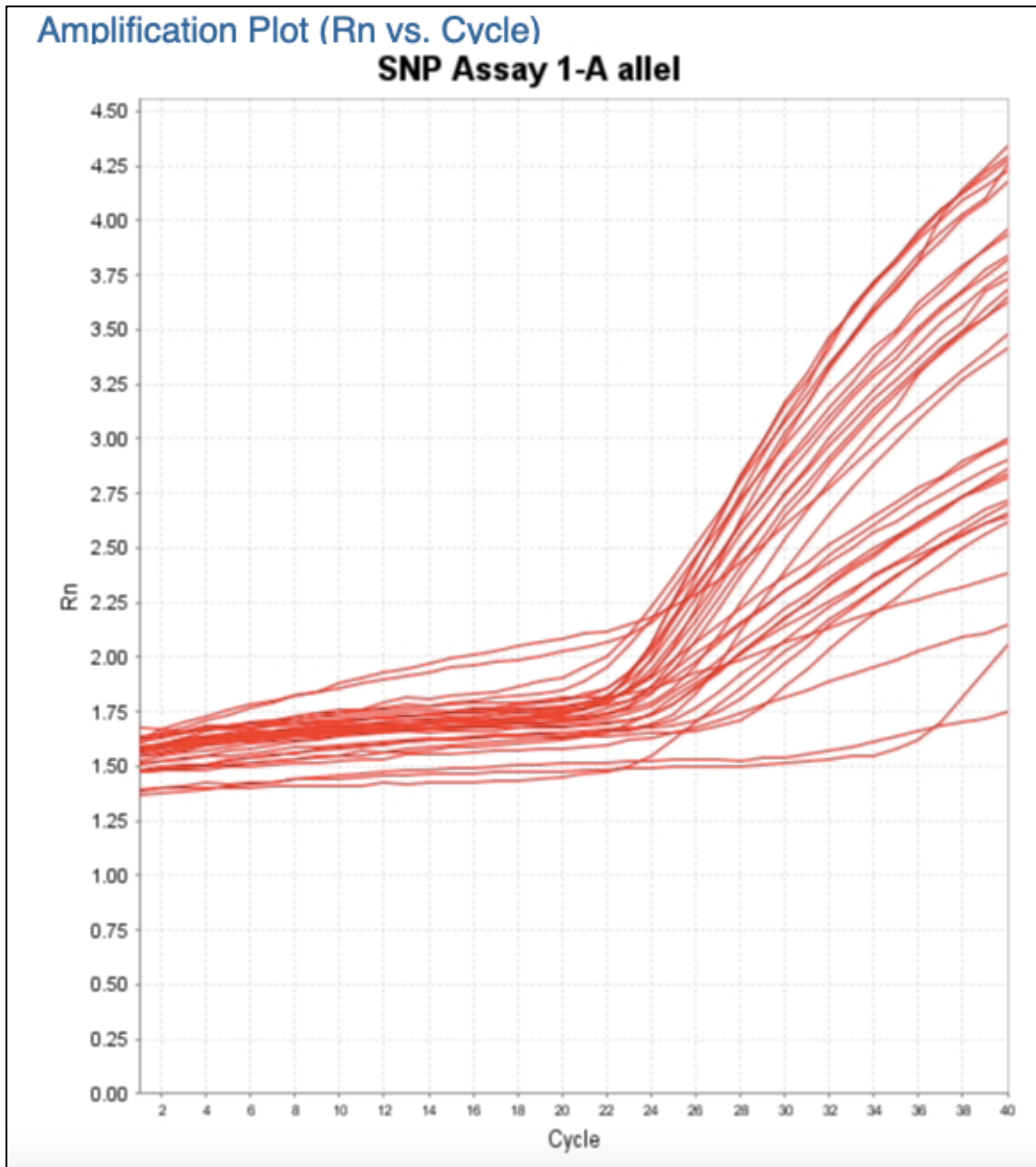


Figure 4.3: Amplification plot display of Allele A

4.2. Analysis of Genotype and Allele Related to the Patient and Control Groups

In this study, the *ENAM* gene polymorphism (rs12640848) in a particular area of *ENAM* was examined in patients with EDD and the control group of healthy individuals. According to this study, there is no significant variations in the genotyping distribution of patients and

healthy individuals ($p=0.052$) (Table 4.1). Chi square (χ^2) and Fisher's Exact test were used in the statistical analysis.

Table 4.1. The genotype distributions for the *ENAM* gene (rs12640848) polymorphism between the patient and control groups

ENAM Genotypes	Control group n=20	Patient group n=20	p-value
AA Genotype	10% n=2	10% n=2	^b0.052 $\chi^2=5.900$
AG Genotype	15% n=3	50% n=10	
GG Genotype	75% n=15	40% n=8	

^bChi-Square Test used for comparison of patients and control group; * $p<0,05$; n:number of samples

The percentages of homozygous mutant type (AA), heterozygous type (AG) and homozygous wild type (GG) in the control group were found to be correspondingly 10%, 15%, and 75% according to the experiment's results. On the other hand, the percentages of homozygous mutant type (AA), heterozygous type (AG) and homozygous wild type (GG) in the EDD patient group, respectively, were determined to be 10%, 50%, and 40% (Table 4.1.).

Table 4.2. The distributions of genotypes AA carrier and noncarrier for the *ENAM* gene (rs12640848) between the patient and control groups

Genotype AA	Control group (n=20)	Patient group n=20	p-value
AA- noncarrier	90% n=18	90% n=18	^b 1.00
AA- carrier	10% n=2	10% n=2	

^bChi-Square Test used for comparison of patients and control group; * $p < 0,05$; n:number of samples

Comparing the control and patient groups for the AA genotype was given in Table 4.2 AA carrier group was equal in both the patient group and the control group at the same time. There was no significant difference between the control and patient group ($p=1$).

Table 4.3. The distributions of genotypes AG carrier and noncarrier for the *ENAM* gene (rs12640848) between the patient and control groups

Genotype AG	Control group (n=20)	Patient group n=20	p-value	Odd Ratio (OR)	95% Confidence Interval
AG- noncarrier	85% n=17	50% n=10	^b 0.041	5.667	1.254-25.606
AG- carrier	15% n=3	50% n=10			

^bChi-Square Test used for comparison of patients and control group; * $p < 0,05$; n:number of samples

As it was shown in Table 4.3. evaluation of the AG noncarrier and AG carrier groups, there was a significant differences between group regarding AG heterozygote genotype ($p=0.041$). The carrying heterozygote type AG genotype increase the risk of EDD in 4 times (OR=5.667, %95 CI 1.254-25.606).

Table 4.4. The distributions of genotypes GG carrier and noncarrier for the *ENAM* gene (rs12640848) between the patient and control groups

Genotype GG	Control group (n=20)	Patient group n=20	p-value	Odd Ratio (OR)	95% Confidence Interval
GG- noncarrier	25% n=5	60% n=12	<i>^b0.025</i>	<i>0.222</i>	<i>0.58-0.858</i>
GG- carrier	75% n=15	40% n=8			

^bPearson Chi-Square Test; * $p<0,05$; n:number of samples

There was a significant differences between non carrier and carrier groups ($p=0.025$). To EDD, mutant type (GG) homozygote genotype can lead to 4.5 times effect (OR=0.222) than compared with other genotypes (Table 4.4.). The risk of EDD reduced in GG genotype 4.5 times. Therefore carrying GG homozygote genotype could have protective effect.

Table 4.5. The distributions of allelic A carrier and noncarrier for the *ENAM* Gene (rs12640848) between the patient and control groups

Alleles distribution	Control group n=20	Patient group n=20	p-value	Odd Ratio (OR)	95% Confidence Interval
A-noncarrier	75% n=15	40% n=8	<i>^b0.025</i>	<i>4.500</i>	<i>1.166-17.373</i>
A-carrier	25% n=5	60% n=12			

^bChi-Square Test used for comparison of patients and control group; * $p < 0,05$; n:number of samples

There was significantly higher frequency of A carriers in patient group ($p=0.025$). According to statistical analysis, having A allele has 4.5 times ($OR=4.500$) more risk for EDD.

Table 4.6. The distributions of allelic G carrier and noncarrier for the *ENAM* gene (rs12640848) between the patient and control groups

Alleles distribution	Control group (n=20)	Patient group n=20	p-value	Odd Ratio (OR)	95% Confidence Interval
G-noncarrier	10 % n=2	10 % n=2	<i>^b1.00</i>	<i>1.00</i>	<i>0.127-7.893</i>
G-carrier	90 % n=18	90 % n=18			

^bPearson Chi-Square Test; * $p < 0,05$; n:number of samples

Having G allele does not show any advantage in patient with EDD (Table 4.6.).

Overall, carrying the heterozygote genotype (AG) increases the risk of EDD by 5.667 times ($p=0.041$). G allele carrying does not demonstrate any benefit in patient. In addition, having the A allele increases risk by 4.5 times compared to not having it. Due to the absence of the A allele, the homozygote GG genotype (OR=0.222) might have a protective effect.



5. DISCUSSION

The results of this thesis revealed the rejection of the null hypothesis (H0) and the confirmation of the alternative hypothesis (H1), demonstrating a significant link between *ENAM* gene polymorphisms and Enamel Degenerative Diseases.

The science of genetics is advancing on a daily basis, and the etiology of diseases is explored in greater detail with the aid of new technological advancements, thereby advancing our understanding of their causes. After the completion of the Human Genome Project, it was revealed which genes encode which proteins, and thus, the genetic background of a protein-related disorder and disease began to be explored (67).

Dental hard tissue may be impacted by mineralization deficiencies (68). Diseases characterized by a prevalent alteration of the dentition are typically genetic, i.e. inherited. Specific diseases such as amelogenesis imperfecta (AI), dentinogenesis imperfecta (DI), and dentin dysplasia (DD) are hereditary alterations of dental hard tissues (69). In addition, dental changes may be accompanied by the symptoms of syndromes such as osteogenesis imperfecta or Tricho-odonto-onycho-dermal syndrome (70). Patients with Amelogenesis Imperfecta were included in the present study.

Amelogenesis imperfecta (AI) is a hereditary disease which affects both primary and permanent dentition due to the genetic origin of the formation of dental hard tissues (37). AI affects both primary and permanent dentition, so no age range was selected for this study.

Amelogenesis imperfecta affects the enamel of all teeth in affected individuals, regardless of chronology, and sometimes in conjunction with other general conditions. Enamel can be hypoplastic, hypomineralized, or both, and affected teeth can be discolored, sensitive, or susceptible to disintegration after eruption (posteruptive destruction) or prior to eruption (idiopathic resorption) (37). *Lee et al.* (71) selected four Turkish families with hypomaturation AI and used whole exome sequencing to analyze *KLK4* gene mutations. And also, *Yu et al.* (72) examined whole-exome sequencing to identify the genetic etiology of one Chinese family with hypoplastic amelogenesis imperfecta (AI) and to investigate the relationship between genotype and phenotype. The identified mutation was confirmed by Sanger sequencing.

In this present study, the enamel structures of Amelogenesis imperfecta patients were not clinically classified as hypoplastic or hypomineralized.

There is no certain therapy protocol available for Amelogenesis imperfecta patients. Increased sensitivity to heat and cold, decreased aesthetic appearance and discoloration of mandibular and maxillary anterior teeth, and masticatory difficulties are the most common complaints of patients with AI (68). **Parekh et al.** (73) investigated the effect of AI variations on the quality of life of 61 patients with AI. For approximately 90% of patients, tooth discoloration was their primary concern. Reduction of sensitivity was the most important reason for 74% of patients getting dental treatment, whereas improvement of tooth size was essential for 60% of patients. Early diagnosis of AI can lead to a better consequences of treatment strategies (68). Therefore genetic tests support early diagnosis of AI.

This study was designed as a genetic base to enlight enamel degenerative diseases in the Turkish population.

In genetic base studies, polymorphisms in specific genes have been evaluated. Polymorphism in genes may be the cause of individual genetic variation resulting from changes in DNA sequences (74). Some genes such as *Amelogenin (AMEL)*, *Ameloblastin (AMBN)*, *Enamelin (ENAM)*, *Enamelysin (MMP20)* and *Kallikrein 4 (KLK4)* play role to produce enamel (75). Mutations in these genes disrupt molecular processes, leading to a range of enamel abnormalities, including a deficiency in quantity (hypoplasia), a change in substance (e.g., hypomineralization), and a change in form (76). **Zhang et al.** (77) examined the hypothesis that ENAM mutations produce autosomal dominant or recessive amelogenesis imperfecta (AI). Sanger sequencing was utilized to confirm the sequence changes and their association with the clinical phenotype in their study, and they discovered that mutations in ENAM induced AI. Also, **Ozdemir et al.** (47) investigated the mutations in the genes encoding the two enamel proteases, *Matrix Metalloproteinase-20 (MMP20)* and *Kallikrein-4 (KLK4)* that have been identified in a single family with autosomal-recessive hypomaturation AI.

In this thesis, since enamel abnormalities were examined, the *ENAM* gene was highlighted.

A mutation in *ENAM* gene encoding the enamel-specific protein enamel has been identified as the cause of autosomal dominant Amelogenesis Imperfecta (78). **Kim et al.** (79)

investigated the potential genotype-phenotype relationships with AI patients and the role of enamelin in enamel development. They detected two *ENAM* mutations in families with hypoplastic autosomal-dominant AI and studied the enamel morphologies that resulted. Additionally, **Hart et al.** (80) demonstrated that mutations in exon 10 of *ENAM* are the cause for Amelogenesis Imperfecta.

Therefore, the aim of this study was to identify the role of *ENAM* gene polymorphism in the patients with Amelogenesis Imperfecta.

SNPs are the simplest and most common polymorphisms. SNP locus has two alleles that match the two nucleotides on the chromosome. **Gerreth et al.** (81) aimed to demonstrate the association between dental caries and single nucleotide polymorphisms (SNPs) in the *ENAM* gene. The study was conducted on 96 children from Poznan. They found to have a substantial association between the rs12640848 marker of the *ENAM* gene and caries susceptibility in their primary teeth. **Divakar et al.** (82) evaluated the association of *ENAM* gene SNP (rs12640848) polymorphism and susceptibility of dental caries development risk in South Indian population. **Linhartova et al.** (83) investigated the relationship between 187 children aged 2–6 years with primary dentition caries and *ENAM* polymorphism (rs12640848) in Czech children. Also, **AlMarshad et al.** (84) evaluated the association between single nucleotide polymorphisms (SNPs) in *ENAM* (rs12640848) gene and early childhood caries (ECC) in 360 Saudi preschool children.

This study did not investigate the association between single nucleotide polymorphisms (SNPs) in the *ENAM* (rs12640848) gene and dental caries. No data were collected for this research. This patient-control study is the first to examine the association between the *ENAM* gene SNP polymorphism (rs12640848) and AI in the Turkish population.

Deoxyribonucleic acid (DNA), which contains the components of the fundamental building blocks that contain the genetic codes of all living beings and distinguishes species from one other, is required in molecular epidemiology investigations. **Isbir et al.** (85) investigated the relationship between the lipid profiles in their serums and the apolipoprotein E (apo E) gene polymorphism by taking blood into tubes containing EDTA from patients (35) with Alzheimer's disease and healthy (29) individuals. **Yilmaz et al.** (86) examined the potential significance of Kallikrein 10 (*KLK10*) genotype and allele frequencies in prostate cancer

predisposition using EDTA-containing blood samples. For this reason, blood samples were taken from patients to obtain DNA samples in this study.

Genotyping is performed using the 7500 Fast-Real Time PCR (Applied Biosystems) instrument with Real-Time Polymerase Chain Reactions (RT-PCR). "TaqMan single nucleotide polymorphism (SNP) Genotyping Assays" are used as site-specific primers and probes. Molecular markers are used to identify SNPs in Real-Time PCR. *Yılmaz et al.* (87) genotyped the *CASP9* (rs 1052576) polymorphism in blood samples from prostate cancer patients (n=69) and controls (n=76) by using real-time polymerase chain reaction using the 7500 Fast-Real Time PCR instrument (Applied Biosystems).

Similar to this study, the 7500 Fast-Real Time PCR (Applied Biosystems) was used to determine genotypes in this study.

Rajpar et al. (78) analyzed a family with an autosomal-dominant, smooth hypoplastic form of AI and demonstrated that a splicing mutation at the splice donor site of intron 8 of the gene encoding the enamel-specific protein enamelin is responsible for the observed phenotype. At the splice donor site of intron 8, nt841+1 (G→A) direct sequencing revealed a heterozygous mutation. This mutation was not found in 200 control patients.

Similarly, this study identified a mutation in the intron 8 (G→A) region of the *Enam* gene (SNP rs12640848).

Limitations of this study

- All of the participants in the study were selected from the Turkish population. This resulted in the study being conducted only on Caucasians and not the whole population.
- In this study, patients with Amelogenesis imperfecta were included. Patients with other enamel degenerative diseases who did not visit our clinic could not be included.
- The limited number of patients and controls is one of our study's most significant limitations.
- The fact that the medical history and age of the patients are not exactly known due to the collection of blood samples beforehand.

6. CONCLUSION

This thesis study investigated the possible relationship between SNP rs12640848 in the *ENAM* gene and Enamel Degenerative Disease in a group of Turkish population;

1. The rs12640848 genotypes distribution has no significant difference between the patient and the control groups ($p>0.05$).
2. AG noncarrier and AG carrier groups, there was a statistically significant difference between group regarding AG heterozygote genotype ($p<0.05$).
3. There were distributions of allelic A carrier and noncarrier for the *ENAM* gene (rs12640848) between the patient and control groups, with a significantly higher frequency of carrier A in the patient group ($p<0.05$).
4. According to statistical analysis, having the A allele has a 4.5 times ($OR=4.500$) higher risk for EDD.
5. Due to the absence of the A allele, the homozygote GG genotype ($OR=0.222$) might have a protective effect.

AI requires an early diagnosis for the purpose of minimizing its negative effects. Referral to a pediatric dentist is essential for dental rehabilitation of the teeth degeneration particularly in addressing teeth attrition, decay, and loss of vertical dimension. Consistent dental examinations are essential for maintaining healthy gingiva and teeth as well as detecting any signs of disease in its earliest stages.

In conclusion, the identification of AI associated genes as well as the ongoing documentation of these mutations responsible for various forms of either AI or enamel generative diseases will contribute to a deeper comprehension of the molecular foundation of enamel anomalies.

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8.APPENDECIES

APPENDIX-1: ETHICAL APPROVAL FORM



Sayı : 37068603-6100-15-1247
Konu: Klinik Araştırmalar
Etik kurul Başvurusu hk.

30/06/2016

İlgili Makama (Didem Özdemir Özenen)

Yeditepe Üniversitesi Diş Hekimliği Fakültesi Pedodonti Anabilim Dalı Doç. Dr. Didem Özdemir Özenen'in sorumlu olduğu "**Diş minesini Dejenaratif Hastalıkları ile ENAM Gen Polimorfizmlerinin İlişkisinin Araştırılması**" isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası (1240 kayıt Numaralı KAEK Başvuru Dosyası), Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından 29.06.2016 tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki ismi belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (**KAEK Karar No: 644**).

Prof. Dr. Turgay ÇELİK
Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurulu Başkanı

APPENDIX-2: ROW DATA

ENAM_genotype * patient_vs_control

Crosstab					
			patient vs control		Total
			control	AI	
ENAM_genotype	AA	Count	2	2	4
		% within ENAM_genotype	50.0%	50.0%	100.0%
		% within patient vs control	10.0%	10.0%	10.0%
		% of Total	5.0%	5.0%	10.0%
	GA	Count	3	10	13
		% within ENAM_genotype	23.1%	76.9%	100.0%
		% within patient vs control	15.0%	50.0%	32.5%
		% of Total	7.5%	25.0%	32.5%
	GG	Count	15	8	23
		% within ENAM_genotype	65.2%	34.8%	100.0%
		% within patient vs control	75.0%	40.0%	57.5%
		% of Total	37.5%	20.0%	57.5%
Total		Count	20	20	40
		% within ENAM_genotype	50.0%	50.0%	100.0%
		% within patient vs control	100.0%	100.0%	100.0%
		% of Total	50.0%	50.0%	100.0%

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	5.900 ^a	2	.052
Likelihood Ratio	6.141	2	.046
Linear-by-Linear Association	2.658	1	.103
McNemar-Bowker Test	.	.	. ^b
N of Valid Cases	40		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 2.00.

b. Computed only for a PxP table, where P must be greater than 1.

Symmetric Measures					
		Value	Asymptotic Standard Error ^a	Approximate T ^b	Approximate Significance
Measure of Agreement	Kappa	.000	.025	.000	1.000
N of Valid Cases		40			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Risk Estimate	
	Value
Odds Ratio for ENAM_genotype (AA / GA)	a

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

ENAM_genotypeAA * patient_vs_control

Crosstab					
			patient vs control		Total
			control	AI	
ENAM_genotype AA	yok	Count	18	18	36
		% within ENAM_genotypeAA	50.0%	50.0%	100.0%
		% within patient vs control	90.0%	90.0%	90.0%
		% of Total	45.0%	45.0%	90.0%
	var	Count	2	2	4
		% within ENAM_genotypeAA	50.0%	50.0%	100.0%
		% within patient vs control	10.0%	10.0%	10.0%
		% of Total	5.0%	5.0%	10.0%
Total		Count	20	20	40
		% within ENAM_genotypeAA	50.0%	50.0%	100.0%
		% within patient vs control	100.0%	100.0%	100.0%
		% of Total	50.0%	50.0%	100.0%

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2- sided)	Exact Sig. (1- sided)
Pearson Chi-Square	.000 ^a	1	1.000		
Continuity Correction ^b	.000	1	1.000		
Likelihood Ratio	.000	1	1.000		
Fisher's Exact Test				1.000	.698
Linear-by-Linear Association	.000	1	1.000		
McNemar Test				.000 ^c	
N of Valid Cases	40				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 2.00.

b. Computed only for a 2x2 table

c. Binomial distribution used.

Symmetric Measures					
		Value	Asymptotic Standard Error ^a	Approximate T ^b	Approximate Significance
Measure of Agreement	Kappa	.000	.095	.000	1.000
N of Valid Cases		40			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for ENAM_genotypeAA (yok / var)	1.000	.127	7.893
For cohort patient_vs_control = control	1.000	.356	2.809
For cohort patient_vs_control = AI	1.000	.356	2.809
N of Valid Cases	40		

ENAM_genotypeGA * patient_vs_control

Crosstab					
			patient vs control		Total
			control	AI	
ENAM_genotype GA	yok	Count	17	10	27
		% within ENAM_genotypeGA	63.0%	37.0%	100.0%
		% within patient vs control	85.0%	50.0%	67.5%
		% of Total	42.5%	25.0%	67.5%
	var	Count	3	10	13
		% within ENAM_genotypeGA	23.1%	76.9%	100.0%
		% within patient vs control	15.0%	50.0%	32.5%
		% of Total	7.5%	25.0%	32.5%
Total		Count	20	20	40
		% within ENAM_genotypeGA	50.0%	50.0%	100.0%
		% within patient vs control	100.0%	100.0%	100.0%
		% of Total	50.0%	50.0%	100.0%

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.584 ^a	1	.018		
Continuity Correction ^b	4.103	1	.043		
Likelihood Ratio	5.812	1	.016		
Fisher's Exact Test				.041	.020
Linear-by-Linear Association	5.444	1	.020		
McNemar Test				.092 ^c	
N of Valid Cases	40				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.50.

b. Computed only for a 2x2 table

c. Binomial distribution used.

Symmetric Measures					
		Value	Asymptotic Standard Error ^a	Approximate T ^b	Approximate Significance
Measure of Agreement	Kappa	.350	.139	2.363	.018
N of Valid Cases		40			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for ENAM_genotypeGA (yok / var)	5.667	1.254	25.606
For cohort patient_vs_control = control	2.728	.970	7.671
For cohort patient_vs_control = AI	.481	.271	.856
N of Valid Cases	40		

ENAM_genotypeGG * patient_vs_control

Crosstab					
			patient vs control		Total
			control	AI	
ENAM_genotype GG	yok	Count	5	12	17
		% within ENAM_genotypeGG	29.4%	70.6%	100.0%
		% within patient_vs_control	25.0%	60.0%	42.5%
		% of Total	12.5%	30.0%	42.5%
	var	Count	15	8	23
		% within ENAM_genotypeGG	65.2%	34.8%	100.0%
		% within patient_vs_control	75.0%	40.0%	57.5%
		% of Total	37.5%	20.0%	57.5%
Total		Count	20	20	40
		% within ENAM_genotypeGG	50.0%	50.0%	100.0%
		% within patient_vs_control	100.0%	100.0%	100.0%
		% of Total	50.0%	50.0%	100.0%

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2- sided)	Exact Sig. (1- sided)
Pearson Chi-Square	5.013 ^a	1	.025		
Continuity Correction ^b	3.683	1	.055		
Likelihood Ratio	5.134	1	.023		
Fisher's Exact Test				.054	.027
Linear-by-Linear Association	4.887	1	.027		
McNemar Test				.701 ^c	
N of Valid Cases	40				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 8.50.

b. Computed only for a 2x2 table

c. Binomial distribution used.

Symmetric Measures					
		Value	Asymptotic Standard Error ^a	Approximate T ^b	Approximate Significance
Measure of Agreement	Kappa	-.350	.146	-2.239	.025
N of Valid Cases		40			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for ENAM_genotypeGG (yok / var)	.222	.058	.858
For cohort patient_vs_control = control	.451	.204	.998
For cohort patient_vs_control = AI	2.029	1.072	3.842
N of Valid Cases	40		

ENAM_alleleA * patient_vs_control

Crosstab					
			patient_vs_control		Total
			control	AI	
ENAM_alleleA	yok	Count	15	8	23
		% within ENAM_alleleA	65.2%	34.8%	100.0%
		% within patient_vs_control	75.0%	40.0%	57.5%
		% of Total	37.5%	20.0%	57.5%
	var	Count	5	12	17
		% within ENAM_alleleA	29.4%	70.6%	100.0%
		% within patient_vs_control	25.0%	60.0%	42.5%
		% of Total	12.5%	30.0%	42.5%
Total		Count	20	20	40
		% within ENAM_alleleA	50.0%	50.0%	100.0%
		% within patient_vs_control	100.0%	100.0%	100.0%
		% of Total	50.0%	50.0%	100.0%

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2- sided)	Exact Sig. (1- sided)
Pearson Chi-Square	5.013 ^a	1	.025		
Continuity Correction ^b	3.683	1	.055		
Likelihood Ratio	5.134	1	.023		
Fisher's Exact Test				.054	.027
Linear-by-Linear Association	4.887	1	.027		
McNemar Test				.581 ^c	
N of Valid Cases	40				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 8.50.

b. Computed only for a 2x2 table

c. Binomial distribution used.

Symmetric Measures					
		Value	Asymptotic Standard Error ^a	Approximate T ^b	Approximate Significance
Measure of Agreement	Kappa	.350	.146	2.239	.025
N of Valid Cases		40			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for ENAM_alleleA (yok / var)	4.500	1.166	17.373
For cohort patient_vs_control = control	2.217	1.002	4.908
For cohort patient_vs_control = AI	.493	.260	.933
N of Valid Cases	40		

ENAM_alleleG * patient_vs_control

Crosstab					
			patient vs control		Total
			control	AI	
ENAM_alleleG	yok	Count	2	2	4
		% within ENAM_alleleG	50.0%	50.0%	100.0%
		% within patient_vs_control	10.0%	10.0%	10.0%
		% of Total	5.0%	5.0%	10.0%
	var	Count	18	18	36
		% within ENAM_alleleG	50.0%	50.0%	100.0%
		% within patient_vs_control	90.0%	90.0%	90.0%
		% of Total	45.0%	45.0%	90.0%
Total		Count	20	20	40
		% within ENAM_alleleG	50.0%	50.0%	100.0%
		% within patient_vs_control	100.0%	100.0%	100.0%
		% of Total	50.0%	50.0%	100.0%

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2- sided)	Exact Sig. (1- sided)
Pearson Chi-Square	.000 ^a	1	1.000		
Continuity Correction ^b	.000	1	1.000		
Likelihood Ratio	.000	1	1.000		
Fisher's Exact Test				1.000	.698
Linear-by-Linear Association	.000	1	1.000		
McNemar Test				.000 ^c	
N of Valid Cases	40				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 2.00.

b. Computed only for a 2x2 table

c. Binomial distribution used.

Symmetric Measures					
		Value	Asymptotic Standard Error ^a	Approximate T ^b	Approximate Significance
Measure of Agreement	Kappa	.000	.095	.000	1.000
N of Valid Cases		40			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for ENAM_alleleG (yok / var)	1.000	.127	7.893
For cohort patient_vs_control = control	1.000	.356	2.809
For cohort patient_vs_control = AI	1.000	.356	2.809
N of Valid Cases	40		

9. CURRICULUM VITAE

Personal Information

Name	BURCU	Surname	AKSOY ÖZGÜVEN
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Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Master	Dentistry	Yeditepe University Faculty of Dentistry	2018
University	Dentistry	Yeditepe University Faculty of Dentistry	2018
High school	-	F.M.V Nişantaşı Işık High School	2012

Languages	Grades
English	75 (YÖKDİL)

Program	Level
Microsoft Office	Excellent