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EVALUATION OF VITAMIN D RECEPTOR (VDR) GENE POLYMORPHISMS (*Fok*I, *Taq*I AND *Apa*I) IN A FAMILY WITH DENTINOGENESIS IMPERFECTA

Dentinogenesis imperfecta Type II (DGI-II) is a condition inherited as an autosomal dominant trait and characterized by abnormal dentine structure affecting both the primary and secondary dentitions. The genetic etiology of the disease still remains unclear, suggesting a genetically heterogeneous background. The aim of this study is to manifest briefly DGI-II and to investigate the association between BsmI, TaqI and FokI polymorphisms of Vitamin D receptor (VDR) gene and dentinogenesis imperfecta type II in a Turkish family by PCR-RFLP methodology. The affected mother and her two affected daughters were bb for BsmI polymorphism, whereas her unaffected son and her husband were Bb for the same polymorphism. One of the affected children was tt, the rest of the family were Tt for TaqI polymorphism, and all of the enrolled subjects were FF for FokI polymorphism. As a conclusion, BsmI polymorphism bb seems to be associated with (DGI-II) , but should be examined in larger numbers in order to be considered as a risk factor.

Key words: *dentinogenesis imperfecta, vitamin D receptor gene, polymorphism.*

Introduction. Dentin matrix is secreted by odontoblasts in the development of dental tooth germs following a series of interactions between the oral ectoderm and underlying neural-crest-derived ectomesenchyme. The dentin matrix mineralizes following its secretion. Subsequently, odontoblasts migrate toward the pulp; leaving odontoblast processes within the matrix [1]. Resulting dentin has unique structural and mechanical properties. The mineral content and microhardness of dentin are between those of enamel and bone, allowing it to support enamel and to endure masticatory forces. Odontoblast processes organize the structure of the mineralized matrix into peritubular and intertubular regions and project cellular capabilities, such as the reception of sensory information and responses against noxious stimuli through the entire thickness of dentin and to the dentin and to the dentino-enamel junction [2].

While hereditary conditions affecting dentin have long been observed, the 'hereditary opalescent dentin' was adopted to describe conditions of diseases consisting of only dental phenotypes [3]. Likewise, the term 'dentinogenesis imperfecta' was introduced to describe the common dental phenotypes of osteogenesis imperfecta and hereditary opalescent dentin [4].

According to the classification proposed by Shields et al. [5], dentinogenesis imperfecta (DGI) was originally classified into three subgroups—types I, II and III (DGI-I-III) — based on various patterns of dentin defects. Dentinogenesis imperfecta type I (DGI-I) (MIM 125490) was further classified into a syndromic form with osteogenesis imperfecta (OI), which was further classified into collagenous and noncollagenous forms. The classification was based on compartmentalizing the phenotypic variation into groups. Recently, Kim and Simmer [6] proposed revising the Shields classification of DGI. They pointed out that dentin dysplasia type II (DD-II), DGI-II (MIM 125490), and DGI-III (MIM 125500) represented allelic diseases with dentin sialophosphoprotein (DSPP) mutations that highlighted differences in clinical severity. Dentinogenesis imperfecta type II is an autosomal-dominant dentin disorder referred to as opalescent dentin with almost complete penetrance. The estimated prevalence of DGI-II is between 1:6.000 and 1:8.000 in the USA [7, 8]. Both deciduous and permanent dentitions are affected in DGI-II and exhibit short roots, bulbous-shaped crowns caused by cervical constrictions, and the root canals and pulp chambers are usually obliterated [9]. Histological analysis shows dysplastic appearing dentin with irregular dentin tubules and areas completely lacking dentin tubules.

Recent studies have shown that defects in the dentin sialophosphoprotein (DSPP) gene cause DGI-II [9—13], DGI-III [14], and DD-II [15]. To date, despite the existence of other candidate genes for hereditary dentin defects [16], no disease-

causing mutations outside of the DSPP gene have been identified [17].

Tooth formation and dentin development requires genetic and environmental factors in which both of the factors play crucial roles. One of the environmental factors that effect dentin mineralization is Vitamin D [18] which maintains calcium and phosphorus level. Vitamin D is a fat-soluble vitamin and regulates variety of biological processes like bone and dentin development via its' ligand dependent receptor, vitamin D receptor (VDR) [19]. VDR gene (OMIM 601769) encodes nuclear receptor and variations on the receptor, which are caused by polymorphisms on the VDR gene, can alter the response of the cells against Vitamin D stimulation [20]. The aim of this case report is to investigate the association between polymorphisms of the VDR gene polymorphism and its possible association with dentinogenesis imperfecta type II in a Turkish family.

Material and method. *Case Description*. A family with two (4 and 3 years olds, case I and II, respectively) girls and one six years old boy, their mother and father were referred to the Department of Oral Diagnosis and Radiology, Faculty of Dentistry, Marmara University with complaints of sensitivity of their teeth. All family members were healthy with no significant medical history. During oral examination, teeth of affected girls and mother were observed to have opalescence appearance on transillumination (Fig. 1–3). The panoramic radiographs confirmed the anomalies of the teeth in question (Fig. 4, 5). Therefore, the patients were referred to the Department of Pedodontics of the same university.

During the clinical examination, teeth of case I were observed to have attrition and have serious enamel loss (Fig. 1) except second primary molars's occlusal surface. Panoramic radiograph of case I revealed that all of the decidious teeth were affected and have bulbous and obliterated pulp chambers (Fig. 4). Her younger sister, case II, exhibited yellowish discoloration (Fig. 2). No anomaly was detected on the teeth of the 6-years-old brother but it was observed that most of the teeth had decays (Fig. 6). The youngest sibling was a baby (6-month-old) and her teeth had not erupted yet.

The teeth of the mother were highly abraded. Her panoramic radiograph revealed that all of the teeth showed bulbous and obliterated pulp chambers,

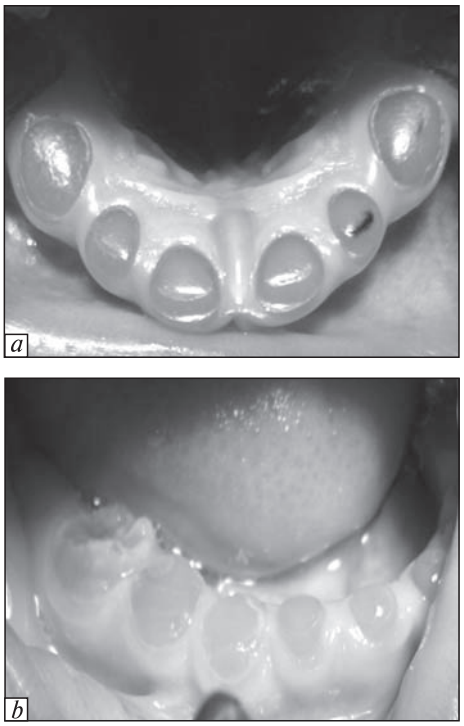

Fig. 1. Intraoral view of case I, revealing loss of enamel: *a* – the maxillary; *b* – mandibular arch

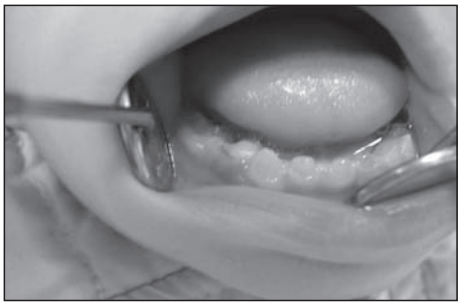

Fig. 2. Intraoral view of the mandibular arch of case II

and that root canals were dramatically reduced. In addition, she had complaints of severe dental sensitivity (Fig. 3, 5). All members of the family denied other symptoms such as bone fragility or hearing loss. According to the characteristic clinical findings, DGI-II was diagnosed in this family.

VDR Genotyping. Informed consent was provided from all participants before taking part in this study according to the Helsinki Declaration. For the genetic analysis DNA was extracted from buccal cells by using Quick Extract DNA Extraction Solution (Epicentre, USA). The *Bsm*I,

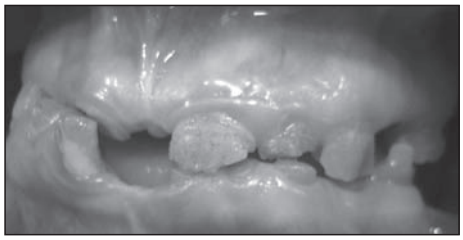

Fig. 3. Intraoral view of the dentitions, revealing serious loss of enamel and vertical dimensions of mother

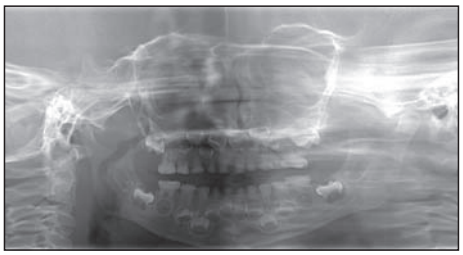

Fig. 4. Panoramic radiograph of case I: teeth with pulp obliteration, bulbous crowns and short, malformed roots

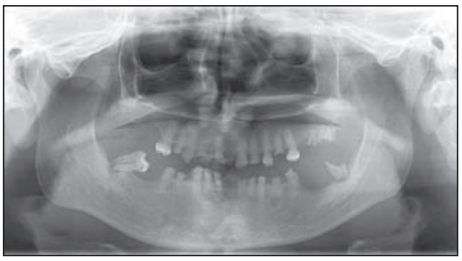

Fig. 5. Radiographic evaluation of the affected mother

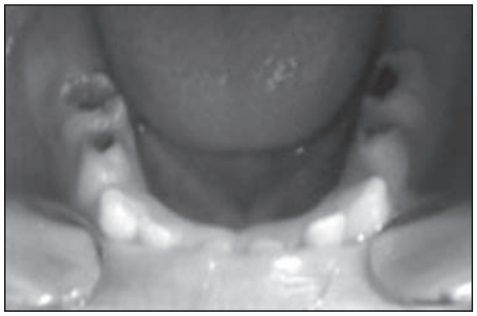

Fig. 6. Clinical evaluation of the unaffected 6-years of brother

*Taq*I and *Fok*I polymorphisms of the VDR gene was determined by PCR-RFLP method as previously described [21]. Amplicons lacking the restriction enzyme sites were donated as B, T and F alleles, in contrast presence of the cutting sites were donated as b, t and f alleles for *Bsm*I, *Taq*I and *Fok*I polymorphisms, respectively.

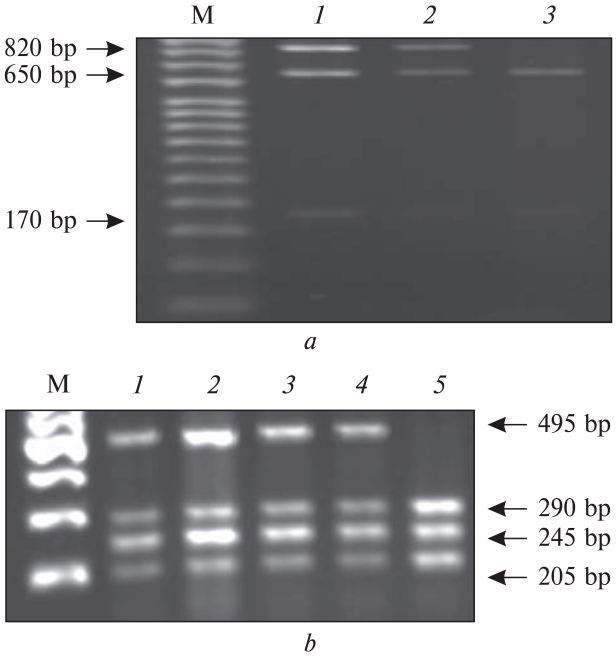

Fig. 7. Digestion profiles of VDR gene; *a* – by *Bsm*I enzyme, M: 100 bp molecular marker; *1*, *2*: Heterozygous genotype; *3*: Mutant genotype; *b* – by *Taq*I enzyme, M: 100 bp molecular marker; *1–4*: Heterozygous genotype; *5*: Mutant genotype

For *Bsm*I polymorphism, affected mother and two children were bb whereas unaffected father and child were Bb. For *Taq*I, one of the affected children was tt whereas the rest were Tt (Fig. 7). All the family members were FF for *Fok*I polymorphism.

Discussion. Developmental defects of the human dentition are not common and can adversely affect the physical and psychological health of children. In the present study, family with DGI-II was examined in the view of medical history, clinical evaluation, radiographic assessment and VDR polymorphisms.

In the family history, it was find out that younger sister, her mother, their relatives including aunts and uncle had the same appearances in their teeth but her older brother and her father, also paternal family were not affected.

The enamel hypermineralization is independent of extracellular hypocalcemia, so Zhang et al. [18] theorized that vitamin D acts on local enamel

mineralization. The direct action of vitamin D on the target cells usually functions through genomic and nongenomic pathways. Early enamel mineralization may be secondary to the absence of hormone-dependent VDR actions in ameloblasts. In osteoblasts lacking VDR, Sooy et al. [22] reported positive regulation of osteogenic potential.

The present study including the Turkish family with DGI-II lets us consider the *Bsm*I B allele as a risk factor. Molecular mechanism of how *Bsm*I polymorphism affects VDR expression is still unclear but the B allele was reported to have a linkage with short PolyA formatting allele [23]. *Bsm*I polymorphism is located in intron 8, near 3'-UTR. Because the 3' UTR may modulate mRNA stability and degradation, this polymorphism was reported to alter the level of mRNA [21]. Thus, in our family, presence of B allele seems to play a protective role for DGI-II and bb genotype can be a risk factor for the formation of DGI-II.

*Taq*I polymorphism is located in exon 9 near to 3'-UTR, and is believed to regulate PolyA formation [23]. The molecular function of this polymorphism is still unclear. In our family only one of the affected children was tt, all the other family members were Tt. We assume that this polymorphism may cause DGI-II only with the other accumulated mutation(s), not alone.

*Fok*I polymorphism is located in exon 2 and has a metabolic activity by having an important role in transcriptional activation of VDR gene [21]. All of the members were FF for the *Fok*I polymorphism, indicating that this polymorphism doesn't have any affect in formation of DGI-II, in the examined family.

Preventing the abrasion of the erupted tooth and establishing an appropriate vertical dimension are the outcomes of the proper treatment of this condition. This treatment will enable clinicians to preserve the function, aesthetics and normal growth of the teeth. It was reported that individuals with DI in primary dentition might not have DI in the permanent dentition [24]. Severe DI can be treated at two stages in deciduous teeth. If diagnosed early, stage I is around 18–20 months and stage II is 28–30 months. This will be curbed at its onset in deciduous dentition and pave the way for a healthier permanent dentition [25].

Developmental defects of enamel and dentin are frequently observed in the pediatric dental clinic. In our cases, because of the ages of the children, considered stainless steel crown restorations for preventing tooth loss could not be applied. As soon as the cooperation is gained, topical fluoride applications can be made in every 6 months to reduce sensibility.

Conclusion. This is the first report including DGI-II and VDR *FokI*, *Taq*I and *Apa*I polymorphisms in a Turkish family. There may be several SNPs effecting dentin and enamel development, polymorphisms of VDR are only one of them. Although the exact mechanism of the VDR polymorphisms is still unknown, bb genotype of *Bsm*I polymorphism may be a causative and diagnostic tool for DGI-II, alone or with other gene alterations. The other polymorphisms of VDR gene didn't have any important affect in the etiology of DGI-II in the examined Turkish family.

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ИЗУЧЕНИЕ ПОЛИМОРФИЗМА ГЕНА РЕЦЕПТОРОВ ВИТАМИНА D (VDR) FokI, TaqI И ApaI В СЕМЬЕ С НЕПОЛНЫМ ДЕНТИНОГЕНЕЗОМ

Неполный дентиногенез II типа (DGI-II) – состояние, которое наследуется как аутосомный доминантный признак и характеризуется как аномальная структура дентина, которая затрагивает первичный и вторичный рост зубов. Генетическая этиология заболевания все еще остается неизученной, но предполагается, что имеет генетически гетерогенную основу. Целью работы было с помощью PCR-RFLP оценить DGI-II и изучить связь между полиморфизмом *BsmI*, *TaqI* и *FokI* гена VDR и неполным дентиногенезом II типа в одной из турецких семей. Затронутые болезнью мать и две дочери были рецессивными гомозиготами bb в отношении полиморфизма *BsmI*, в то время как здоровый сын и муж были гетерозиготами Bb. По *TaqI* полиморфизму одна из больных дочерей была tt, а остальные члены семьи – Tt. Все перечисленные члены семьи были FF по полиморфизму *FokI*. Очевидно, полиморфизм bb *BsmI* связан с DGI-II, однако необходимо исследование большей выборки, чтобы считать его фактором риска.

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