49, XXXXY: The Role of *MSX*1 Gene

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ABSTRACT

49, XXXXY syndrome is the most severe and rare form of Klinefelter syndrome. The number of cases reporting oral and dental findings of this syndrome is very little. The aims of our case report are to present oral and dental findings of the syndrome about which we have limited knowledge, to support other case reports, and to evaluate the relationship between this syndrome in which hypodontia is seen and *MSX1* gene that is associated with hypodontia. The clinical and radiographical examination of a 11-year-old patient who was taken to the practitioner for his retarded development at the age of 1 and diagnosed as 49, XXXXY syndrome by chromosome analysis and had characteristics of this syndrome were done. Congenital missing of permanent teeth, hypertaurodont primary and permanent teeth, delayed tooth development and mandibular prognatizm were observed in the examination. Whether there are any mutations in the *MSX1* gene was researched, and the association with this syndrome was evaluated.

Keywords: XXXY syndrome, hypodontia, klinefelter syndrome, *MSX1*, taurodontism

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INTRODUCTION

Klinefelter syndrome is defined as male hypogonadism arising from two or more X chromosomes and one or more Y chromosomes (1). It is the most common chromosomal anomaly and it's most severely and rarely recorded constitution is 49, XXXXY (2). In this type of the syndrome of which prevalence is approximately 1 in 85000 newborn males, there are very few number of cases available where oral and dental findings have been reported (3, 4).

Symptoms of the syndrome have been reported to include mental retardation, cardiac anomalies, hypogonadism, small penis, abnormal scrotum, cryptorchism, limited elbow flexion/extension, radioulnar synarthrosis, clinodactyly, coxa valga, genua valga, gap between first and second toes, flatfeet, age-related decline in bone, hardened cranial sutures, defect in capitate bone, thoracic kyphosis, and scoliosis. On the other hand, craniofacial features of the syndrome include ocular hypertelorism, upslanting palpebral fissures, epicanthic folds, diplopia, a broad flat nose, malformed ears, and mandibular prognathism (5). Among the oral and dental findings reported, there are bifid uvula or cleft palate (4, 6), congenitally missing permanent teeth (7, 8), taurodontism (8-10), spade-shaped cutting teeth,[9] and dentine defects (6).

In recent years, numbers of dental studies making use of genetics have been increased and both patients and physicians have been provided with more information regarding these kinds of diseases. However, although numerous authors have reported distinctive features of this syndrome, genetic studies related to the syndrome are limited. Differences in the clinical manifestations of the syndrome with regard to age are associated with changes in hormonal levels. Redundancy of X chromosomes leads to androgen deficiency. Loss of feedback inhibition of pituitary gland depending on this deficiency results in elevated levels of gonadotropin (11). Taurodontism, also included among dental findings, is associated with the

redundancy of X chromosomes (12, 13). The most studied and emphasized gene responsible for missing teeth, which is also counted among the dental findings of this syndrome, is *MSX1*. *MSX1* is a transcription factor closely associated with genetic network regulating dental development. Mutations revealed in *MSX1* which is synthesized during the development and migration of neural crest cause missing teeth.

In our case report, it is aimed to present oral and dental findings accompanying this syndrome, which we have a limited knowledge about. Several cases with Klinefelter syndrome accompanied by missing teeth are reported, but no relationships of those with MSX1 are presented. Role of *MSX1* gene, which is associated with missing teeth in this syndrome, will be evaluated as well.

CASE REPORT

Anamnesis of 11-year-old patient named K.K., who was brought in to our clinic due to toothache and widespread dental caries, revealed that the patient had applied to a physician because of the complaint of growth retardation at 1 year of age and been diagnosed with 49, XXXXY syndrome via chromosomal analysis. Parents suggesting that they are not relatives although dwelling in the same village reported that no similar findings were found in their other 2 children older than K.K. and in their relatives. Mother stated that she received an iron-containing medicine during her pregnancy, that she delivered a baby normally by induced labor two days after her due date, and that the baby weighted 2500 gr. It was learned that the patient with mental retardation by 75-80% started to speak and walk at 5 years of age. Ocular hypertelorism, upslanting palpebral fissures, epicanthal folds, diplopia, a broad flat nose (Fig 1A), limited elbow flexion/extension, kyphosis (Fig 1B), small penis, abnormal scrotum, and palmar and plantar hyperkeratotic areas were recorded.

In the oral examination, prognathic mandibula, poor oral hygiene and widespread dental caries (Fig 2) were detected.

In the radiographic examination, it was observed that he lacked teeth 18, 15, 14, 12, 22, 25, 28, 38, 35, 34, 44, 45, 48, and had hyper and mesotaurodont deciduous and permanent teeth (Fig 3). Using Haavikko method, dental age of the patient was determined to be 1.64 years less than the chronological age. The patient was within the pp2 balance period in terms of skeletal development according to hand-wrist x-rays. When his hand-wrist radiography was assessed, he was found to be 7 years of age.

Having informed the patient and the parents about the procedure of blood sample drawing, the treatment planned to be performed, and possible problems, their written consents were obtained.

Primarily, a prevention program was created due to him being fed frequently by a soft diet, his incapability to maintain oral hygiene, the low socio-economical level of the family, and problems experienced in cooperation. The patient was trained by parents on teeth brushing and received dietary recommendations. In the patient with whom no complete cooperation could be established, composite restoration of permanent maxillary left central incisor, compomer restoration of primary maxillary and mandibular left canines, and metal-reinforced glass ionomer-cement restoration of permanent first molars, permanent maxillary left first premolar, primary mandibular first molars, primary mandibular right second molar and primary mandibular left canine were performed. Primary maxillary second molars and primary mandibular left second molar have deep caries and excessive loss of substance, and primary mandibular lateral incisors present within the mouth were scheduled to be extracted (Figure 4). Following the necessary treatments and extraction procedures, a follow-up program was created for the patient receiving fluorine in order to maintain oral hygiene, check tooth restorations, and monitor the period of growth and development.

Written informed consent was obtained from the patient for take and use of the blood and DNA samples for diagnostic purpose. Genomic DNA was extracted from whole blood samples for molecular analyses by using DNA isolation kit (Gentra). Amplification of polymerase chain reaction (PCR) was performed using overlapping primer sequences (http://genetics.uiowa.edu/publications/peterj/PeterMSX190902.html), which were designed as to include two coding exons of *MSX1* gene and exon-intron binding sites. These sequences consisted of all coding sequences of exon1 and exon2, 468 nucleotides beginning from 5' end of the start codon (whole 5' UTR), and 1073 nucleotides after stop codon (whole 3' UTR).

Volume of 25 µl was used for PCR reaction, and 50 ng DNA, 0.2 mM dNTP, 2.5mM $MgCl_2$, and, 10 pmol and 1U Taq DNA polymerase enzymes for each primer sequence were applied. PCR conditions were realized as 40 seconds at 95°C, 35 seconds at 58°C, 40 seconds and 35 cycles at 72°C, and the final elongation stage was performed at 72°C for 5 seconds. PCR products obtained were exposed to direct DNA sequence analysis by ABI 3100 DNA sequencer (Applied Biosystems). As the result of these analyses: it was determined that 2nd exon of the patient was subjected to complete deletion. Deletion occurred in a sequence including a total of 696 bç (del 4,864,207 – 4,864,902) (Figure 5). It covered a part of intronic region between exons, which consisted of 221 bp and was close to 2nd exon; the whole coding sequence of 2nd exon with 443 bp and the following sequence of 42 bp belonging to 3' UTR.

RESULT AND DISCUSSION

Among the oral and dental findings of the syndrome, no bifid uvula, cleft palate, spade-shaped cutting teeth and dentine defects were recorded in our patient. Prognathic mandible,

missing teeth and findings of taurodontism were included among the symptoms which might be observed related to this syndrome. Depending on the mutations of *MSX1* gene, the most frequently recorded missing teeth are the permanent second premolars and permanent third molars (14). More rarely, losses of permanent maxillary first premolars, permanent mandibular first molars, permanent maxillary lateral incisors and permanent mandibular central incisors might be seen. Deciduous teeth development was normal in general (15). Consistent with these findings, teeth 18, 15, 14, 12, 22, 25, 28, 38, 35, 34, 44, 45, 48 were determined to be missing in our patient. Deciduous and permanent molar teeth of the patient exhibit hyper and mesotaurodontism. Deeply localized pulpal floor in taurodontic teeth and the complex structure of root-canal system complicate the use of canal tolls, the determination of canal localization and the filling of canals; thus, an indication for teeth extraction is usually established. Missing teeth reveal aesthetic and psychological problems by affecting teeth development and mandibular bone, and cause a negative effect on the growth-development period of individuals.

MSX homebox genes are expressed in epithelial or mesenchymal cells during morphogenesis. *MSX1* and *MSX2* genes in vertebrates are expressed in embryonic tissues, such as mandibular and dental tissues, which show epithelial and mesenchymal interaction during morphogenesis. *MSX1* gene localized between 4,861,406 - 4,865,663 nucleotides on 4th chromosome codes a protein including 297 amino acids.

As the result of the mutation occurred, 156 amino acids coded only by 1st exon were found as normal, but the remaining 141 amino acids could not be coded. While amino acids of protein included between 166-225 constitute DNA binding site, this region is completely disappeared due to mutation; thus, there are no DNA binding sites in the protein.

MSX1 protein is responsible for the repression of gene transcription by interacting with TATA-box binding protein (TBP). A new "premature *MSX1* protein" reveals due to a

structural change in the gene of which function is considered to affect especially the craniofacial development and odontogenesis.

We believe that this new protein could not perform its function due to the completely disappeared DNA binding site and generates the primary reason for missing teeth recorded in the patient. Moreover, we think that other clinical findings observed in the patient may also have arisen from the premature protein because of the fact that *MSX1* is a transcription factor effective on the early development of neural crest.

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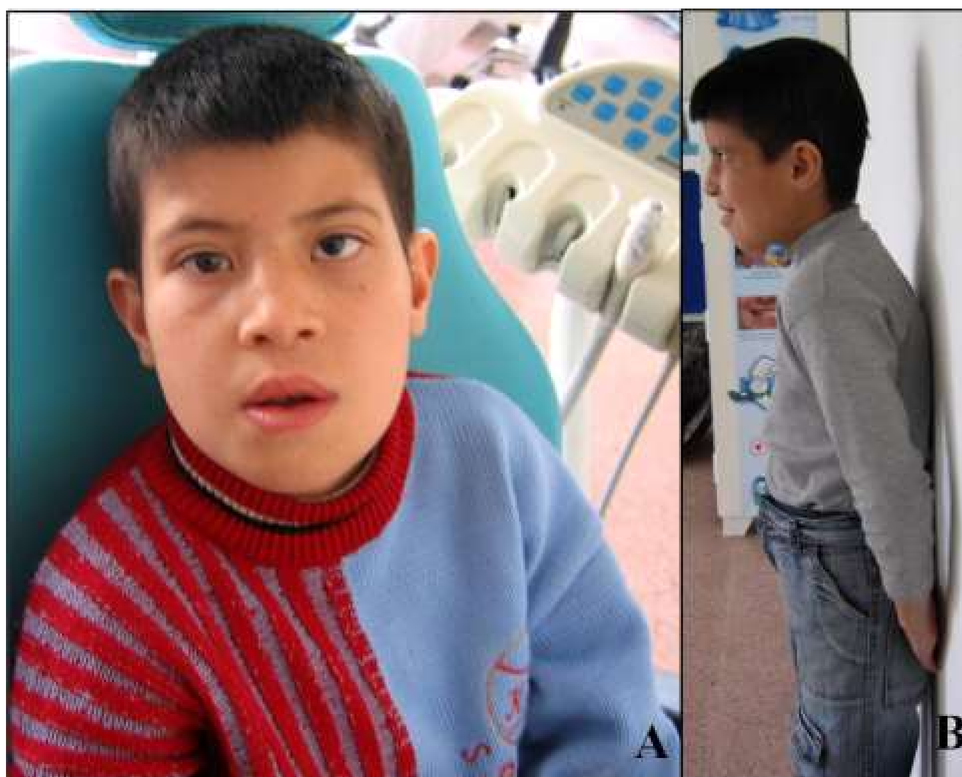

Fig 1: Patient with Klinefelter's syndrome has an ocular hypertelorism, upslanting palpebral fissures, epicanthal folds, a broad flat nose (A) and kyphosis (B).

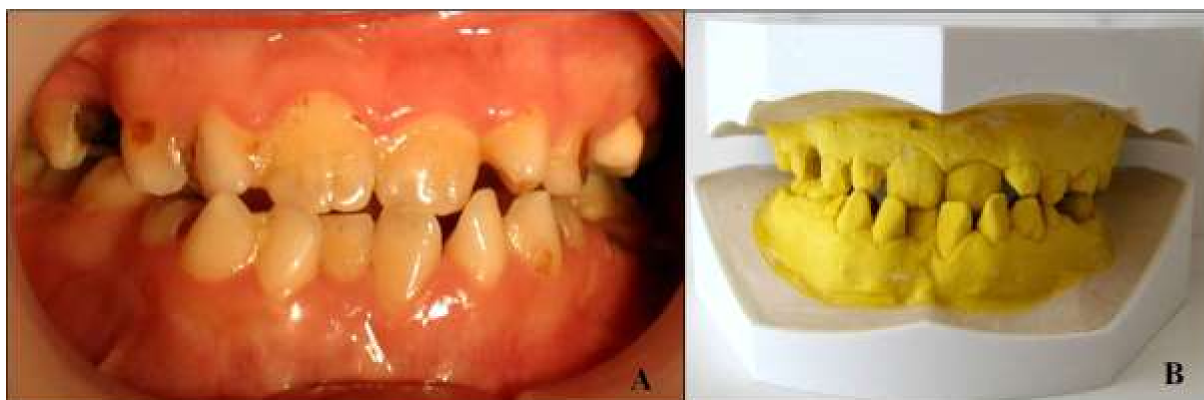

Fig 2: Intraoral view of the patient before dental treatment (A) and the appearance of the stone model (B).

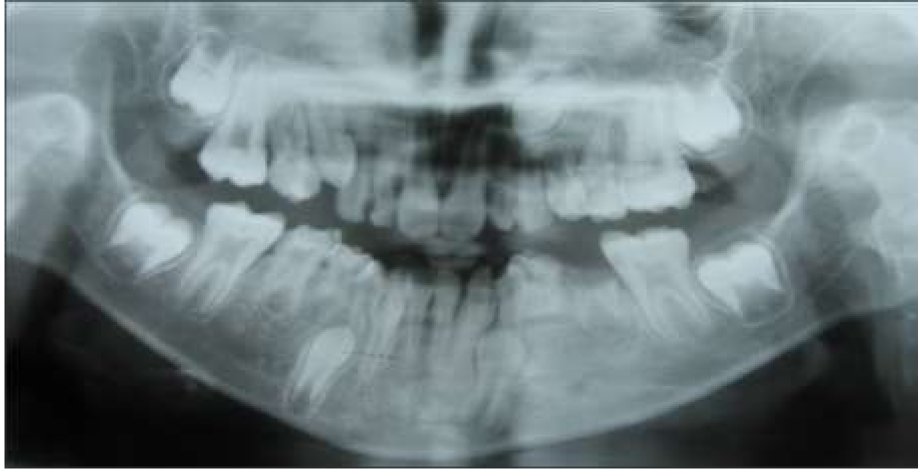

Fig 3: The radiographic appearance of the patient before dental treatment.

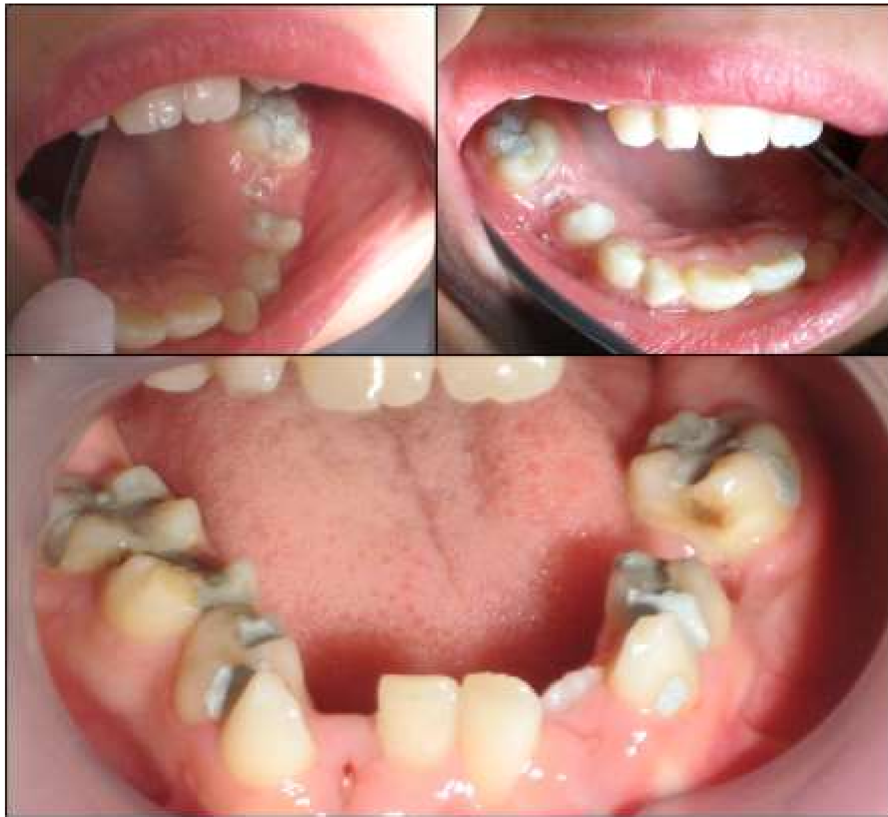

Fig 4: Intraoral view of the patient after dental treatment

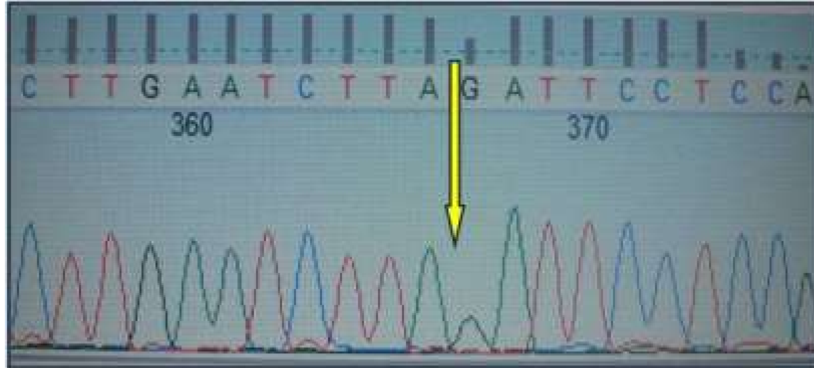

Fig 5: DNA sequence chromatogram in forward direction showing the 696bp deletion (del4,864,207 - 4,864,902) of *MSX1* gene that encompass the whole of the 2nd exson. Arrow mark indicates the range of deletions occurred. While the series at the left side of the arrow are belonging to the intronic region, the series at the right side are belonging to the 3' UTR region.