CASE REPORT

49, XXXXY: The Role of MSX1 Gene

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ABSTRACT

49, XXXXY syndrome is the most severe and rare form of Klinefelter syndrome. The number of cases of oral and dental findings with 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 the MSX1 *gene that is associated with hypodontia. A clinical and radiographic examination was performed on an 11-year-old patient who had been taken to a practitioner at age 1 for delayed development, diagnosed with 49, XXXXY syndrome by chromosome analysis, and exhibited characteristic features of the syndrome. Congenital missing permanent teeth, hypertaurodont primary and permanent teeth, delayed tooth development and mandibular prognathism were observed in the examination. Whether there were any mutations in the* MSX1 *gene was researched, and the association with this syndrome was evaluated.*

Keywords: Hypodontia, Klinefelter syndrome, *MSX1*, taurodontism, XXXY syndrome.

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 its most severe and rarely recorded constitution is 49, XXXXY (2). In this type of syndrome, of which prevalence is approximately 1 in 85,000 newborn males, there are very few 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, flat feet, age-related decline in bone, hardened cranial sutures, defect in capitate bone, thoracic kyphosis, and scoliosis. 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, the number of dental studies which have made use of genetics have increased and both patients and physicians have been provided with more information regarding these 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 the 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 emphasised 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 the genetic network regulating dental development. Mutations

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revealed in *MSX1*, which is synthesised during the development and migration of neural crest, cause missing teeth.

Our case report is aimed to present oral and dental findings on this syndrome, for which we have limited knowledge. Several cases of Klinefelter syndrome accompanied by missing teeth are reported, but no relationships of those with *MSX1* have been presented. The role of the *MSX1* gene, which is associated with missing teeth in this syndrome, will be evaluated as well.

CASE REPORT

Anamnesis of an 11-year-old patient named K.K., who was brought into our clinic due to toothache and widespread dental caries, revealed that the patient had seen a physician because of the complaint of growth retardation at one year of age and had been diagnosed with 49, XXXXY syndrome *via* chromosomal analysis. The parents, who stated that they are not relatives although they lived in the same village, reported that no similar findings were observed in their other two children, who were older than K.K., or among their relatives. The mother stated that she received an iron-containing medicine during her pregnancy, that she delivered the baby normally by induced labour two days after her due date, and that the baby weighed 2500 g. It was learned that the patient, with mental retardation of 75%–80%, started to speak and walk at five years of age.

In the general examination, ocular hypertelorism, upslanting palpebral fissures, epicanthal folds, diplopia, a broad, flat nose (Fig. 1A), limited elbow flexion/extension, kyphosis (Fig. 1B), a 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 the Haavikko method, the 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 seven years of age.

After informing the patient and the parents about the procedure of blood sample drawing, the planned

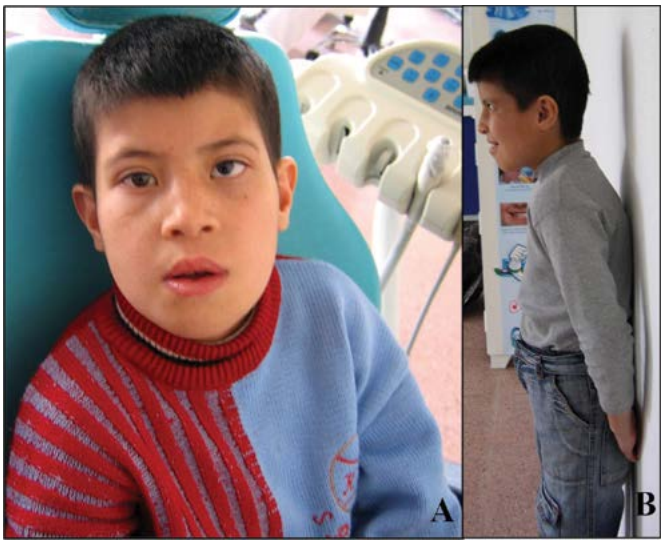

Fig. 1: Patient with Klinefelter's syndrome has ocular hypertelorism, upslanting palpebral fissures, epicanthal folds, a broad flat nose (A) and kyphosis (B).

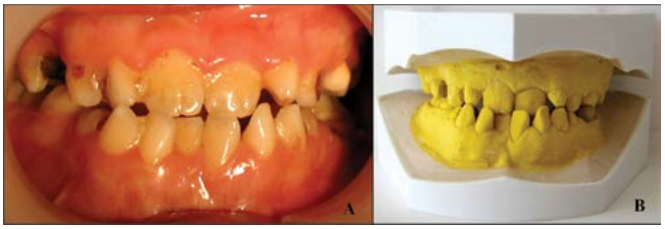

Fig. 2: Intraoral view of the patient before dental treatment (A) and the appearance of the stone model (B).

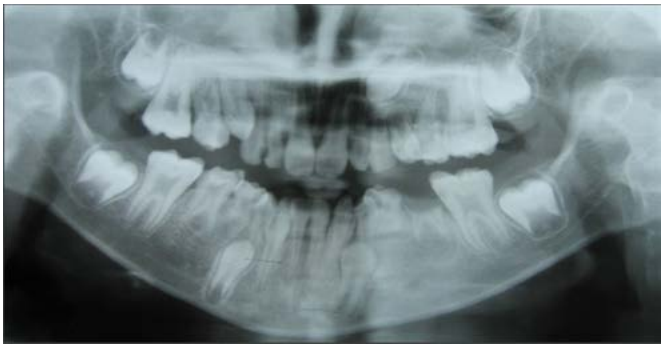

Fig. 3: The radiographic appearance of the patient before dental treatment.

treatment to be performed, and possible problems, their written consent was obtained.

A prevention programme was created because the patient had to be fed frequently with a soft diet, was incapable of maintaining oral hygiene, came from a family with low socio-economic status, and experienced problems with cooperation. The patient was trained by his parents in teeth brushing and received dietary recommendations. No complete cooperation could be established with the patient. However, 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 (Fig. 4). Following the necessary treatments and extraction procedures, a follow-up programme was created for the patient to receive fluorine to maintain oral hygiene, to check tooth restorations, and to monitor the period of growth and development.

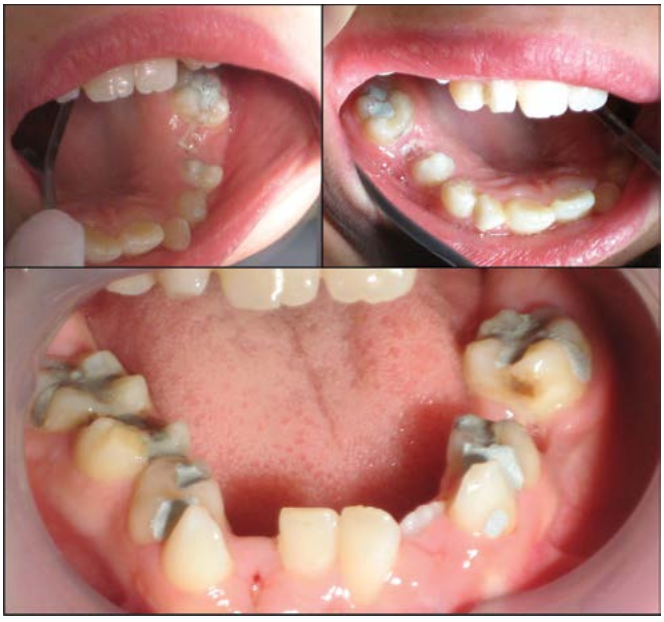

Fig. 4: Intraoral view of the patient after dental treatment.

Written, informed consent was obtained from the patient to take and use 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, Qiagen, Valencia, CA, USA). Amplification of polymerase chain reaction (PCR) was performed using overlapping primer sequences (http://genetics.uiowa.edu/publications/peterj/PeterMSX190902.html), which were designed to include two coding exons of the *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 the stop codon (whole 3′ UTR).

The volume of 25 µl was used for PCR reaction, and 50-ng DNA, 0.2-mM dNTP, 2.5-mM $MgCl_2$, and 10-pmol and 1U Taq DNA polymerase enzymes for each primer sequence were applied. Polymerase chain reaction conditions were carried out 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. Polymerase chain reaction products obtained were exposed to direct DNA sequence analysis by ABI 3100 DNA sequencer (Applied Biosystems, Thermo Fisher, Foster City, CA, USA). As a result of these analyses, it was determined that the second exon of the patient was subjected to complete deletion. The deletion occurred in a sequence including a total of 696 bp [del 4 864 207–4 864 902] (Fig. 5). It covered a part of the intronic region between exons, which consisted of 221 bp and was close to the second exon; the whole coding sequence of the second exon with 443 bp, and the following sequence of 42 bp belonging to 3′ UTR.

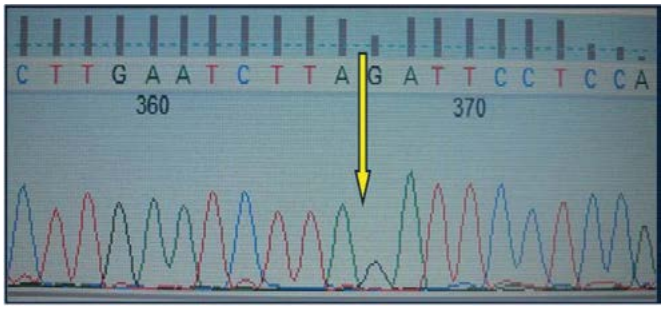

Fig. 5: DNA sequence chromatogram in forward direction showing the 696 bp deletion (de l4 864 207 – 4 864 902) of *MSX1* gene that encompasses the whole of the second exon. The arrow mark indicates the range of deletions that occurred. While the series at the left side of the arrow are belonging to the intronic region, and the series at the right side are belonging to the 3' UTR region.

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 the *MSX1* gene, the most frequently recorded missing teeth are the permanent second premolars and the 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 exhibited hyper- and mesotaurodontism. Deeply localised pulpal floor in taurodontic teeth and the complex structure of the root canal system complicate the use of canal tolls, the determination of canal localisation 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 homeobox 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. The *MSX1* gene, localised between 4 861 406–4 865 663 nucleotides on the fourth chromosome, codes a protein including 297 amino acids.

As a result of the mutation which occurred, 156 amino acids, coded only by the first exon, were found as normal, but the remaining 141 amino acids could not be coded. While amino acids of protein, included between 166 and 225, constitute a DNA binding site, this region 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. A new 'premature *MSX1* protein' revealed a structural change in the gene which is considered to especially affect the craniofacial development and odontogenesis.

We believe that this new protein could not perform its function due to the complete disappearance of DNA of the binding site, and it 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 in the early development of neural crest.

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