Original Article

Supernumerary marker chromosome 15 in a male with azoospermia and open bite deformity

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Abstract

Supernumerary marker chromosome 15 (sSMC[15]) is the most frequent marker chromosome, and it is generally regarded as unimportant if it does not contain the Prader–Willi/Angelman syndrome critical region (PWACR). The clinical importance of the larger markers in association with the critical region is mentioned in almost all reports related to marker chromosome 15, and smaller markers are solely associated with minor dysmorphic features, azoospermia and recurrent miscarriages. However, these small sSMC(15)s without the PWACR may also determine a specific phenotype. A dysmorphic examination of an azoospermic patient in a genetics clinic was performed and was followed by a peripheral blood lymphocyte chromosomal analysis according to standard cytogenetic methods. Nucleolar region (NOR) banding, C-banding, fluorescence in situ hybridization and a molecular investigation of Y-microdeletions were also performed. The clinical evaluation identified dysmorphic features accompanied with azoospermia and severe ‘Angle Class II, Division 1 Open Bite Deformity’. The molecular cytogenetic study revealed the small sSMC(15). In addition, a Y-microdeletion analysis showed that the azoospermia was not the result of a deletion. Although the presented case might represent a coincidental example of supernumerary marker 15 and mandibular anomaly association, the condition may also define a specific phenotype that may be more than azoospermia. This condition may be characterized by infertility, malar hypoplasia, mandibular anomaly, keloid formation and minor dysmorphic features.

Keywords: auriculocondylar syndrome, azoospermia, infertility, isodicentric 15q, open bite deformity, small supernumerary marker chromosome 15

1 Introduction

Marker chromosomes are frequently supernumerary and occur at a frequency of about 0.3–0.5/1 000 in humans [1–5]. The most common supernumerary marker chromosome (sSMC) in humans is sSMC(15), and it accounts for as much as 50%–60% of all sSMCs observed [3, 5, 6]. In addition, sSMC(15)s occur predominantly as small pseudodicentric chromosomes that are termed psu dic(15; 15) or inv dup(15) [7, 8].

Carriers of sSMC(15) without euchromatin were found to be normal, but this kind of sSMC(15) has been detected with a high incidence among infertile males [5, 7, 9, 10]. It is currently thought that spermatogenic impairment due to the presence of sSMCs is the cause of oligospermia [11]. In familial cases, females usually
present with normal fertility, but maternal transmission of sSMC(15) may lead to infertile male offspring [9].

The presence of the Prader–Willi/Angelman syndrome critical region (PWACR) on sSMC(15) is the main determinant of the sSMC(15) phenotype. PWACRs containing sSMC(15), which are large sSMCs, cause highly variable clinical phenotypes that are dependent on the location of breakpoints, the copy number of the PWACR region and the ratio of mosaicism; the clinical picture frequently includes hypotonia, motor and speech delay, seizures, moderate to severe learning disability, autism and Prader–Willi/Angelman syndromes [1, 4, 7, 8, 12–18]. It is generally considered that patients with small sSMC(15)s, which do not contain PWACRs, have no clinical symptoms [8]. Small sSMC(15)s may arise during spermatogenesis [1, 19]. Individuals with sSMC(15) also seem to be at increased risk for uniparental disomy of the two normal copies of chromosome 15, which results in the Prader–Willi or Angelman syndromes [9].

In this report, we present an azoospermic man with small sSMC(15) and having distinguishing dysmorphic features. We have attempted to define the possible phenotype of this relatively common marker genotype.

2 Materials and methods

2.1 Subject

One azoospermic male with dysmorphic features and his healthy parents were studied.

2.1.1 Case history

An 18-year-old Turkish male was referred for genetic evaluation because of azoospermia. He was born at term to a 38-year-old, gravida 7, para 7 woman after an uncomplicated delivery. The postnatal history was unremarkable except for bilateral cryptorchidism, and the developmental milestones during childhood were reached appropriately. The parents were not consanguineous, and the family history of the patient was not important except for the sudden death of two brothers in the neonatal period. In his medical history, our patient had orchiopexy and orthodontic operations at 16 years of age.

At his physical examination, he was 176 cm tall (50–75th percentile), 64 kg (25–50th percentile) and his head circumference was recorded as 56 cm (10–25th percentile). He had articulation problems, a triangular face, synophris, a high arched palate, hypoplastic teeth, attached ear lobes, a right-sided simian crease and a keloid scar on the left palm (Figure 1A, B).

The orthodontic history of the patient began with the chief complaint of the supernumerary tooth beyond the incisors and speech problems related with his dentition. A long face and convex soft tissue profile were also noted (Figure 2A–E). The clinical oral and dental examination revealed that he was in the permanent dentition stage, with the exception of the upper left central deciduous incisor. There was a supernumerary tooth beyond the right central incisor. The anterior occlusion showed a severe open bite, and there were generalized and severe hypoplasias on the upper and lower incisors and canines. In addition, polydiastemas were observed in both arches because of the small teeth, according to the dental arches.

2.2 Methods

2.2.1 Radiological investigation

After the identification of azoospermia, an evaluation of the patient’s urogenital system by ultrasonography was performed.

2.2.2 Pathological examination

A testicular biopsy was performed. Radiological examination of the oral region and a cephalometric analysis were also performed.

2.2.3 Cytogenetic study

Karyotyping of peripheral blood lymphocytes from the patient and his parents was performed through Giemsa–Trypsin (GTG) banding. C-banding and nucleolar region (NOR) banding were also used for the investigation of constitutive heterochromatin and satellite DNA, respectively.

2.2.4 Molecular cytogenetic studies

Fluorescence in situ hybridization was used for the investigation of the origin of the marker chromosome, and the technique was performed using the commercial Prader–Willi/Angelman (SNRPN) Region Probe with cep 15 as a control probe (Cytocell, Cambridge, USA).

2.2.5 Molecular study

A Y-microdeletion study was performed using a commercial kit (Promega, Madison, WI, USA) for azoospermia factor region (AZFa, AZFb, AZFc)-specific primers.
3 Results

3.1 Radiological investigation

Ultrasonography of the urogenital system revealed normal anatomical structures.

An examination of the oral region showed that two supernumerary teeth existed between the right and left incisors. The left supernumerary tooth was blocking
the eruption path of the left central incisor, which was impacted (Figure 2F–H). Cephalometric analysis revealed a severe dolichofacial class II skeletal pattern with an anterior open bite owing to the micrognathic and retrusive mandible relative to the anterior cranial base and maxilla (Figure 2F–H).

3.2 Pathological examination

Testes dimensions for the right and left testicles were 30 × 26 × 10 mm and 15 × 12 × 8 mm, respectively. The following testicular biopsy showed Sertoli-cell-only syndrome.

3.3 Cytogenetic studies

Karyotyping of the patient revealed 47, XY, + mar by GTG banding. C-banding and NOR banding identified the bisatellited isodicentric nature of the marker chromosome. Karyotyping of the father and the mother of the patient determined the parental origin of the supernumerary marker; the mother was also established to be the carrier for sSMC(15). The mother had no dysmorphic features.

3.4 Molecular cytogenetic study

In the FISH study, all of the interphase and metaphase cells had three signals specific to the centromere of chromosome 15 owing to sSMC(15) (Figure 3). Commercial probes specific for SNRPN 15qter regions (Cytocell, USA) showed the absence of signals on the sSMC.

3.5 Molecular study

The molecular study for microdeletions of azoospermia factors on the Y chromosome exhibited an intact constitution.

4 Discussion

Although the sSMC(15) is the most common marker chromosome in humans, a clear description of the phenotype of patients is restricted. The difficulty is mainly a result of the variability of breakpoints; thus,
the content of the markers, as a general approach the sSMC(15)s, is classified as large (containing PWACR) or small (without PWACR). The classification may be done easily through commercial PWACR-specific FISH probes, but more detailed molecular classifications are also offered [4, 15].

The patients with small sSMC(15) are expected to be normal except for recurrent miscarriages in some females and an increased risk of oligo- or azoospermia in males [7, 11]. All small de novo sSMC(15)s studied to date have been maternally derived [1, 7, 14]. The low level of disomy 15 in the sperm of carrier males may reflect selection against the sSMC during meiosis/spermatogenesis and could account for these observations [7, 19]. Our patient had azoospermia, but he also had a history of bilateral cryptorchidism, and the result of the testicular biopsy was consistent with Sertoli-cell-only syndrome. In addition, the patient had a severe Angle Class II Open Bite Deformity. Zou et al. [20] reported a 16-month-old girl with ring sSMC(15), microstomia, overbite and micrognathia. Cockwell et al. [18] reported a 19-year-old male with inv dup(15), malar hypoplasia, an anterior crossbite, micrognathia and a high arched palate. In contrast to our case, the maxillomandibular anomalies of these patients may be regarded as mild. However, the patient reported by Cockwell et al. did have some common clinical features with our patient (Table 1), and a comparison of these two cases may give useful clues about the full sSMC(15) phenotype in males. In addition to distinct clinical features, the karyotype of the patient presented by Cockwell et al. was fascinating because of the dynamic mosaicism; the patient had seven types of sSMC(15)s, and they mentioned that the ‘derivative’ sSMCs were all smaller than the proposed progenitor inv dup(15). Furthermore, all of the sSMCs contained chromosome 15 sequences, and some contained the PWACR [18].

In addition to previously reported cases, auriculo-condylar syndrome, which is characterized by ear malformations (question mark ears) and hypoplastic mandible, is worth discussing, because it has some

| Table 1. A comparison of the clinical findings between the presented case and the patient reported by Cockwell et al. [18]. |
|--------------------------------------------------|
| sSMC(15) with dynamic mosaicism, some of the markers contain PWACR | sSMC(15) without PWACR |
| Delayed mental motor development | – |
| Seizures | – |
| Allergic rhinitis | – |
| Brachycephaly | – |
| Wide forehead | – |
| Long face | + |
| Scarce eyebrows | – |
| Prominent nose | + |
| Low-set cupped ears | – |
| Malar hypoplasia | + |
| Short philtrum | – |
| Thick lower lip | + |
| Micrognathia, anterior crossbite | Retromicrognathia, severe angle class II open biteb |
| High arched palate | + |
| Short neck | – |
| Keloid | + |
| Lipoma | – |
| Kyphoscoliosis | – |
| Hyperextensible elbows | – |
| Slender hands | – |
| Inadequate virilization | Azoospermia, Sertoli-cell-only syndrome |

aAbnormal positioning of upper and lower incisors at sagittal plane. 
bAbnormal positioning of upper and lower incisors at vertical plane. 
"Includes only the findings of physical examination; the results of the laboratory investigations and radiological examinations are not mentioned. PWACR, Prader–Willi/Angelman syndrome critical region."
clinical features in common with our case: micrognathia, abnormal palate, speech articulation difficulties, crowded teeth, malocclusion, open/cross bite, respiratory difficulties due to orofacial malformations, mandibular condyle hypoplasia, an asymmetric mandible, short mandibular rami, a small mandibular coronoid process and temporomandibular joint abnormalities [21]. Although the dysmorphic features other than the mandibular anomalies were not seen in our patient, it is worth investigating a possible relationship between auriculo-condylar syndrome, in which the etiology is unidentified, and sSMC(15).

In this report, we have further defined the phenotype of males with sSMC(15) and reported the most severe maxillomandibular anomaly in this group of patients. However, the condition may also represent a coincidental association, and the + inv dup(15) might be unrelated to the mandibular anomaly in the patient. Future reports are needed for clarification of marker chromosome 15 and its phenotype.

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