

# Chromosomal Aberrations in Minor Salivary Gland Pleomorphic Adenoma

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**Purpose:** Cytogenetic analysis of a pleomorphic adenoma (PA) arising in the major salivary glands, in particular the parotid, is well documented, with chromosome 8 being the most commonly involved aberration, mainly in t(3;8). However, cytogenetic studies of PA in the minor salivary glands (MSGs) are rare and, to the authors' knowledge, only 3 reports have been published. The authors investigated the cytogenetic abnormalities of a series of 6 PAs arising from MSGs and compared these with published findings from the parotid gland to determine whether the karyotype was the same in the 2 sites.

**Materials and Methods:** Six fresh samples of MSG PA were examined by classic cytogenetic analysis. The tissue was minced and cultured in RPMI-1640 medium. The cells were fixed after 2 to 8 days of culture and analyzed according to standard procedures. More than 25 metaphases were analyzed on G-banded slides, and the karyotype was described according to International System for Human Cytogenetic Nomenclature guidelines.

**Results:** The spectrum of chromosomal aberrations found in the MSG PAs was similar to those reported in the major salivary glands in all 6 cases.

**Conclusions:** Cytogenetically, there would seem to be no clear differences in PAs arising from the major salivary glands versus the MSGs. It is unknown whether the underlying tumorigenesis and chromosomal aberrations of PAs from major salivary glands and MSGs are similar, although the proportion of malignant tumors arising from the MSGs is much larger compared with the parotid. Further studies are needed in this area.

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Pleomorphic adenoma (PA) is a benign tumor originating from the major salivary glands and minor salivary glands (MSGs). It is the most common type of salivary gland tumor, accounting for more than 60% of all salivary gland neoplasms and approximately 80% of all benign tumors of the major salivary glands and MSGs. Although most common in the parotid gland,

PAs may occur in any of the salivary glands. Intraorally, PAs may be found in the soft or hard palate, upper lip, buccal mucosa, or tongue.<sup>1-3</sup>

Characteristically, PA presents as a very slowly growing and painless tumor. The reported age range for PA is the fourth to sixth decades and is rarely seen in children. Although it can grow to a huge size in the parotid gland, it seldom reaches a size larger than 1 to 2 cm in diameter in the MSGs.<sup>4-6</sup>

The histologic features of the tumor are pleomorphic and include sheets of epithelial cells, some of which have formed ducts, myxomatous tissue, and cartilage. Metaplastic squamous epithelial cells are relatively common. Carcinoma ex-PA (CA-ex-PA) is a malignant transformation of a pre-existing PA and has been reported in the major salivary glands and MSGs.<sup>7,8</sup> Surgical excision is the treatment of choice.

Cytogenetic studies of PAs have discerned abnormal karyotypes in up to 70% of cases, with nonrandom involvement of 8q12, the locus of PA pleomorphic adenoma gene 1 (*PLAG1*).<sup>9,10</sup> Highly specific chromosome translocations, namely t(3;8)(p21;q12), and other abnormalities, such as 12q13-15 rearrangements and trisomy 8, have also been reported.<sup>11-16</sup>

Most cytogenetic studies on PAs are from tumors arising in the major salivary glands, mainly the pa-

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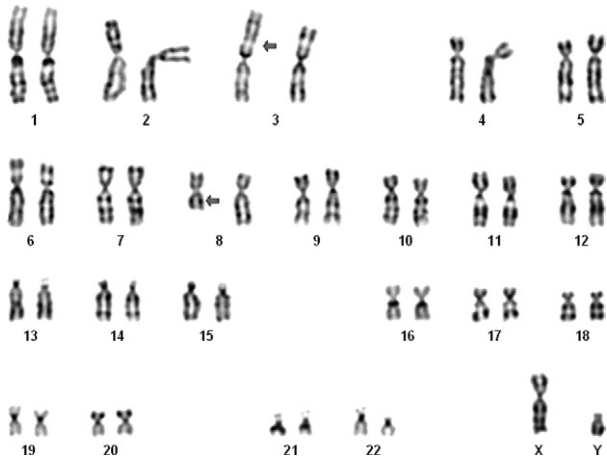
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**FIGURE 1.** Case 1 karyotype: 46,XY,t(3;8)(p21;q12).

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rotid. To the authors' knowledge, only 3 cases of cytogenetics of PAs arising in the MSGs have been reported. In 1 case of PA in the junction between the soft and hard palate, its karyotype was 45,X,-Y[4]/46,XY[16]<sup>14</sup>; in the 2 other cases in the palate, the karyotypes were 46,XX,t(3;8)(p21;q12)[6] and 46,XX,t(5;8)(p13;q12-13)[8].<sup>13</sup>

Several significant differences exist in PAs arising in the major salivary glands versus the MSGs. Apart from differences in size,<sup>6</sup> the proteomics of their secretions are very different.<sup>17</sup> Also, the percentage of malignant tumors arising from the MSGs is much larger compared with the parotid and submandibular glands.<sup>8,18,19</sup> The purpose of the present study was to investigate the cytogenetic abnormalities of a series of PAs affecting the MSGs and compare these with published findings of PAs arising in the major salivary glands to see if the 2 entities have the same karyotype. The authors hypothesized that the tumorigenesis and chromosomal aberrations of PAs from the major salivary glands and MSGs would differ. The specific aims of the study were to karyotype PAs of the MSGs and characterize their chromosomal aberrations.

## Materials and Methods

### PATIENTS

Consecutive patients seen at the Oral and Maxillofacial Surgery Clinic, Soroka University Medical Center, Be'er Sheva, Israel were included in the study. PA was diagnosed by characteristic pathologic features. This study was approved by the Soroka Medical Center ethics committee.

### CYTOGENETICS

A fresh sample of each lesion was also examined by classic cytogenetic analysis, as previously de-

scribed.<sup>20-23</sup> The tissue was minced and cultured in RPMI-1640 medium, supplemented with antibiotics, glutamine, and 10% fetal calf serum, and incubated at 37°C in 5% CO<sub>2</sub> humidity. The cells were fixed after 2 to 8 days of culture and analyzed according to standard procedures. More than 25 metaphases were analyzed on G-banded slides, and the karyotype was described according to the International System for Human Cytogenetic Nomenclature guidelines.<sup>24</sup>

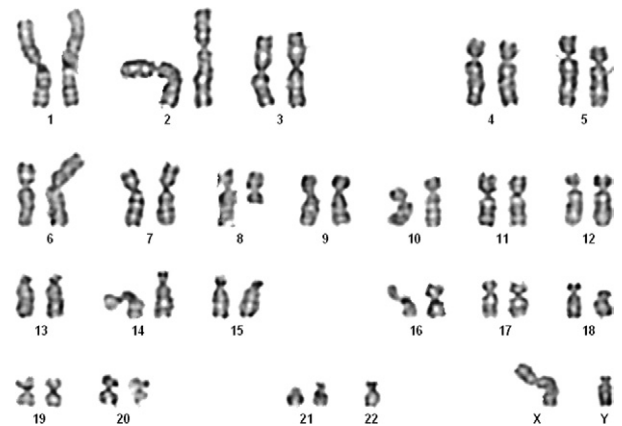
## Results

There were 5 male patients and 1 female patient, with a mean age of 48.3 years (range, 31 to 81 yrs). The affected sites included 2 in the upper lip, 2 in the hard palate, 1 in the soft palate, and 1 in the buccal mucosa. Histopathology showed the characteristic architecture of PA in 5 cases and CA-ex-PA in 1 case (Figs 1 to 3). The cytogenetic results for all 6 patients are summarized in Table 1, showing a spectrum of chromosomal aberrations, which were similar to PAs arising in the major salivary glands.

## Discussion

The present study concerned a small group of patients and any gender predilection cannot be determined. It is unclear whether the pathogenesis of MSG PA differs from tumors arising in the major salivary glands. Because the percentage of malignant tumors arising from the MSGs is much larger compared with that from the parotid gland, this has clinical relevance. Cytogenetic studies of PAs from major salivary glands and MSGs may be helpful in this regard.

The cytogenetics of PA of the major salivary glands is well documented.<sup>11,14,25-28</sup> About 70% of parotid



**FIGURE 2.** Case 3 karyotype: 45,XY,t(6;8)(p21;q12),t(14;22)(q11;q11). Note that t(14;22)(q11;q11) is a constitutional chromosomal change.

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**FIGURE 3.** Case 6 karyotype of carcinoma ex pleomorphic adenoma: 48,Y,-X,-4,-5,,+der7t(7;?) [q32?],+der8t(8;?) [q22?],-10,-11,der11t(11?) ,der12t(12;?) [p12.2?],+13,+der13t(13;?) [(q14.2?)],-14,-15,-18,+21,+22,+m1,+m2,+m3,+m4,+m5,+m6,+m7.

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gland PAs show an abnormal karyotype in 1 of the following 3 patterns: 1) rearrangement of 8q12 (39% of cases); 2) rearrangement of 12q13-15 (8% of cases); and 3) sporadic, clonal changes not involving 8q21 or 12q13-15 (23% of cases).<sup>29</sup> The 2 most commonly involved chromosomes are 8 and 12. Most abnormalities are translocations of chromosome 8, mainly with chromosome 3. Other chromosomes, such as 5, 9, 14, and 21, are less frequently involved. There are several reports describing changes just in chromosome 8.<sup>25</sup>

Cytogenetic findings on PAs of the MSG are very rare.<sup>13,14</sup> The karyotypes of the 3 reported cases were 45,X,-Y[4]/46,XY[16], 46,XX,t(3;8)(p21;q12)[6], and 46,XX,t(5;8)(p13;q12-13)[8]. A missing Y chromosome is common in many tumors and is not specific to PAs. There are reports on a missing Y chromosome in parotid gland PA.<sup>14</sup> Translocations t(3;8) and t(5;8) are among the most common karyotypes seen in parotid PA, so there is nothing unique in the previously reported findings in MSG PAs.

Of the 6 presented cases with MSG PA, 1 case was t(3;8), similar to the most common finding of PAs in the major glands. The other cases with der8t(6;8), t(8;10), and t(11;12) have been reported in the major glands.<sup>28</sup> One of the present cases with t(4;12)(q21; q22) has not been previously described. However, because chromosome 12 is involved in the pathogenesis of PA and is reported to be translocated with several chromosomes, such as Y, 1, 2, 3, 5, 8, 9, and 12,<sup>28</sup> it is not that surprising to find a translocation with chromosome 4.

CA-ex-PA is a carcinoma arising from a primary or recurrent benign PA. Molecular studies have shown that the development of CA-ex-PA follows a multistep model of carcinogenesis, with the progressive loss of heterozygosity at chromosomal arms 8q, 12q, and 17p. There are specific candidate genes in these regions that are associated with particular stages in the progression of CA-ex-PA. Also, many genes that regulate tumor suppression, cell cycle control, growth factors, and cell-cell adhesion play a role in the development and progression of CA-ex-PA.<sup>7</sup>

The case of CA-ex-PA of the buccal mucosa had complex and multiple chromosomal aberrations and clonal evolution, a finding similar to those reported in major glands.<sup>7,18,30</sup> Taking the 3 previously reported MSG PAs and the 6 evaluated in the present study together, no specific karyotypic characteristics can be found. All the different aberrations that have been found in MSGs have been reported in tumors arising in the major salivary glands, mainly the parotid. Thus, based on this small group of tumors, it can be hypothesized that, cytogenetically, there are no clear differ-

ences between the two groups.

**Table 1. PATIENT DETAILS, HISTOPATHOLOGY, AND CYTOGENETICS OF MINOR SALIVARY GLAND TUMORS**

Patient Number	Age/Gender	Site	Histopathology	Karyotype
1	31/M	Upper lip	PA	46,XY,t(3;8)(p21;q12)
2	36/M	Upper lip	PA	46,XY,der8(p?)
3	54/M	Hard palate	PA	45,XY,t(6;8)(p21;q12),t(14;22)(q11;q11)
4	51/F	Hard palate	PA	46,XX[22]/46,XX,t(4;12)(q21;q22)[4]
5	37/M	Soft palate	PA	45,XY,t(8;10)(q13;q23),t(11;12)(p15.3;q23),-18,-19,-21,-22+,m1,+m2,+m3[cp9]/idemX2[cp11]
6	81/M	Buccal mucosa	CA-ex-PA	34-55,Y,-X,-4,-5,-7+,der7t(7;?) (q32?),-8,-8+,der8t(8;?) (q22?),-9,+9,-10,-11-11,+der12t(12;?) (p12.2?)+,13+,der13t(13;?) (q14.2?),-14,-15,-17,-19+,21+,22+,m1,+m2,+m3,+m4,+m5,+m6,+m7,+m8,+m9[cp32]/idemX2[cp30]

Abbreviations: CA-ex-PA, carcinoma ex pleomorphic adenoma; F, female; M, male; PA, pleomorphic adenoma.

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ences in PAs arising from the major salivary glands versus the MSGs.

MSG secretions are different from those of major salivary glands, suggesting a wide range of host-protective proteins that play a pivotal role in oral tissue homeostasis, influencing infectious diseases and anti-cancer protection in rats and humans.<sup>17,31</sup>

Mark et al<sup>14</sup> reported that 66% of benign and 25% of malignant salivary gland tumors had abnormal karyotypes. It does not make sense that benign tumors have greater genomic instability than malignant ones, and further work is needed in this area to understand the pathogenesis more fully.

In conclusion, PAs arising in the MSGs have similar karyotypes to those found in the major glands, suggesting that cytogenetically, at least, these tumors are the same entity. The number of cases in the present study is small and may not be adequate to detect significant differences between MSGs and the major glands.

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