Odontogenic Myxoma Harbors Widespread Loss of Heterozygosity and Not Trisomies

To the Editor:

Odontogenic myxoma, also termed myxoma of the jaw, is a rare and locally aggressive tumor type that occurs in the maxilla and mandible. Recently, Kleijn et al[1] discovered that odontogenic myxoma shows a distinctive DNA methylation profile and recurrent copy number alterations, based on genome-wide DNA methylation microarray data in a series of 12 cases. This novel finding marks a significant advance for the field, as it is the initial description of recurrent genetic alterations in odontogenic myxoma, a tumor type for which genetic drivers have remained elusive. The tumors that underwent DNA methylation testing occurred in patients aged between 17 and 49 years, and it was concluded that "copy number profiling showed recurrent whole-chromosome gains (trisomies) of chromosomes 5, 8, and 20 in all cases, and of chromosomes 10, 12, and 17 in all except 1 case." Here, we offer an alternative interpretation of the copy number findings, supported by previously published results,[2] as well as new results discussed below.

Previously, Odintsov et al[2] studied a series of 46 myxomas of the craniofacial bones. Sixteen of them were classified in the study as "infantile sinonasal myxoma," a distinctive craniofacial myxoma type that involves the anterior maxillary bone, and generally occurs in children at most 3 years of age, and harbors WNT signaling pathway alterations in *CTNNB1* and *APC*, without identified recurrent copy number changes.[2] The remaining 30 myxomas occurred in a more heterogeneous patient cohort, aged 7 to 84 years, with both mandibular and maxillary tumors diagnosed as (fibro) myxoma of the jaw and odontogenic myxoma. Seven of these 30 myxomas were sequenced using a hybrid-capture next-generation DNA sequencing assay (OncoPanel v3.1) that covers 447 genes, and ~2000 common single nucleotide polymorphism (SNP) sites,[3] and the sequenced tumors lacked recurrent genomic alterations, possibly due to the heterogeneity of analyzed cases and the small sample size.

Re-examination of these prior sequencing results showed 1 myxoma (case #3 in the cohort of mandibular tumors) to harbor similar relative copy number changes as reported by Kleijn et al.[1,2] This 15-year-old male patient was diagnosed with a destructive mandibular lesion that was surgically resected. Microscopic examination demonstrated a markedly hypocellular, myxoid spindle cell neoplasm with scattered stromal capillary channels (Fig. 1A). The neoplasm was composed of bland, bipolar, and stellate fibroblasts, with scattered stromal deposits of ropey collagen (Fig. 1B). DNA sequencing was negative for pathogenic or likely pathogenic small nucleotide variants, small insertions or deletions, and large structural variants. The normalized copy number plot showed above-baseline $\log_2$ copy ratios of chromosomes 4, 5, 8, 10, 12, 13, 16, 17, 19, 20, and 21 (Fig. 1C). Similar relative copy number changes were reported in several myxomas by Kleijn et al.[1] Both the DNA methylation array testing of Kleijn and colleagues and the DNA sequencing of Odintsov and colleagues yielded relative copy number data, such that either the chromosomes demonstrating relative loss or those demonstrating relative gain could be at the diploid baseline. While the relative copy number gains could reflect trisomies as proposed by the Kleijn and colleagues, an alternative explanation would be relative copy number losses of the inverse set of chromosomes 1, 2, 3, 6, 7, 9, 11, 14, 15, 18, and 22.

Allele frequency data can readily discriminate between gain and loss patterns, because trisomies and whole-chromosome losses are expected to alter the distribution of allele frequencies at heterozygous loci, such that there is a correlation between the copy number changes and loss of heterozygosity. The MethylationEpic v1.0 and v2.0 microarrays used by Kleijn et al[4] were not designed to interrogate allele frequency, because only a small proportion of the probes overlapped with regions of single nucleotide polymorphisms, and because the probes associated with known single-nucleotide polymorphisms were excluded from analysis. For the myxoma discussed above that was sequenced by Odintsov and colleagues, examination of the variant allele frequency plot showed loss of heterozygosity on chromosomes 1, 2, 3, 6, 7, 9, 11, 14, 15, 18, and 22, consistent with their haploidization (Fig. 1D). Accordingly, chromosomes 4, 5, 8, 10, 12, 13, 16, 17, 19, 20, and 21 demonstrated retained heterozygosity, consistent with an even number of these chromosomes and inconsistent with trisomies.

SNP array testing is a comparative technique that quantifies allele frequencies, thereby enabling the estimation of absolute chromosome numbers. To verify loss of heterozygosity orthogonally, and to estimate the number of chromosomes in the tumor, we performed SNP array testing using methods described previously in detail.[5] In brief, DNA was extracted from formalin-fixed paraffin-embedded tissue and analyzed using OncoScan Array (Thermo Fisher, Waltham, MA). SNP array testing demonstrated loss of heterozygosity of chromosomes 1, 2, 3, 6, 7, 9, 11, 14, 15, 16p (subclonal), 18, 19 (subclonal), and 22, confirming the DNA sequencing findings (Fig. 2A–B). The detected allele frequencies were consistent with multiple whole chromosome losses, with an estimated near haploid modal number of 34 chromosomes and with no detected whole chromosomal gains (including no trisomies).

Corroborating this finding, one of the myxomas previously reported in Kleijn et al[1] (case 10 in that study) was reanalyzed herein using a commercially available SNP array panel (Life and Brain, Bonn, Germany). It was found to harbor loss of heterozygosity of chromosomes 1, 2, 3, 6, 7, 9, 11, 13, 14, 15, 18, and 22 (Fig. 2C–D), instead of trisomies in the inverse set of chromo-

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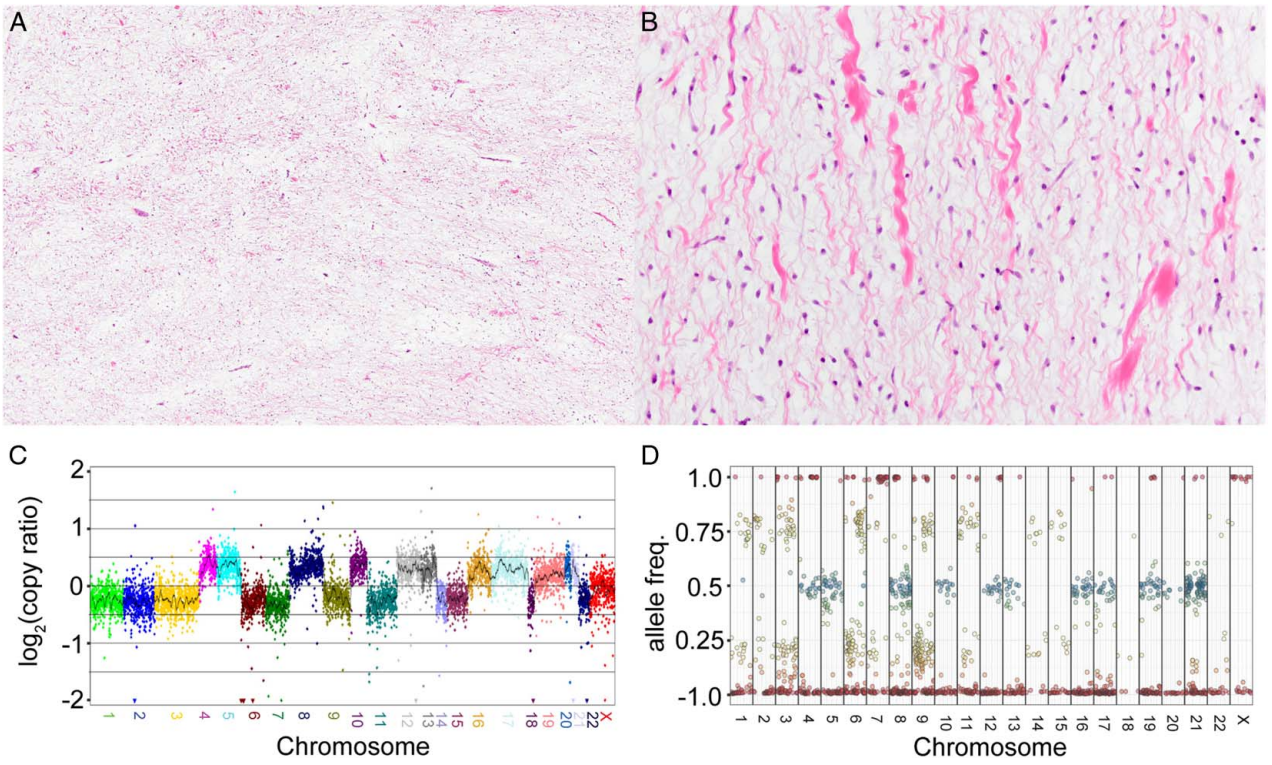

FIGURE 1. Mandibular myxoma with hypodiploid state and multiple whole-chromosomal losses. A, At low power, this mandibular myxoma is a markedly hypocellular, myxoid spindle cell neoplasm with scattered stromal capillary channels. B, The tumor shows bland, fibroblastic cytomorphology, with bipolar and stellate cells. There are scattered stromal deposits of ropey collagen, a common finding among odontogenic myxomas. C, Log_2 (copy number ratio) from DNA sequencing data demonstrates relative loss of chromosomes 1, 2, 3, 6, etc., compared with chromosomes 4, 5, 8, etc. These data are relative, such that they could be explained by relative copy number loss of chromosomes 1, 2, 3, etc., or relative gains, including trisomies, of chromosomes 4, 5, 8, etc. D, Variant allele frequency data. Allele frequency estimates around 0.5 are consistent with an even mixture of alleles (ie, retained heterozygosity), while estimates skewed above and below the 0.5 line are heavily enriched for specific SNPs, consistent with loss of heterozygosity.

somes. The only whole-chromosome difference between the 2 tumors that underwent SNP array testing was loss of heterozygosity of chromosome 13.

Extensive haploidization is thought to be potentially detrimental to cell survival, and many tumor types undergo subsequent whole-genome endoreduplication.[6] Whole genome endoreduplication results in restoration of the number of lost chromosomes with excessive doubling of chromosomes that were not lost previously; it cannot, however, revert the allelic imbalance. While in these 2 reported cases, there was no specific indication of endoreduplication in the results of the SNP array, it is nonetheless possible that endoreduplication could occur in a subset of odontogenic myxomas with genomic near-haploidization. A cytogenetic study of more cases would be needed to make this determination.

Taking together the novel, recurrent findings reported by Kleijn and colleagues and the results reported herein, odontogenic myxoma joins the growing list of neoplasms characterized by recurrent extensive haploidization: inflammatory rhabdomyoblastic tumor (previously referred to as "inflammatory leiomyosarcoma"),[7,8] *SETDB1*-mutated pleural mesothelioma,[9] giant cell glioblastoma,[10] oncocytic carcinomas of the thyroid, parathyroid, and adrenal glands,[11] and acute lymphoblastic leukemia,[12] to name a few. It is important to distinguish extensive haploidization from multiple trisomies to elucidate which chromosomes house genes that might drive tumor biology. The drivers of tumorigenesis are expected to be among the genes harboring loss of heterozygosity.

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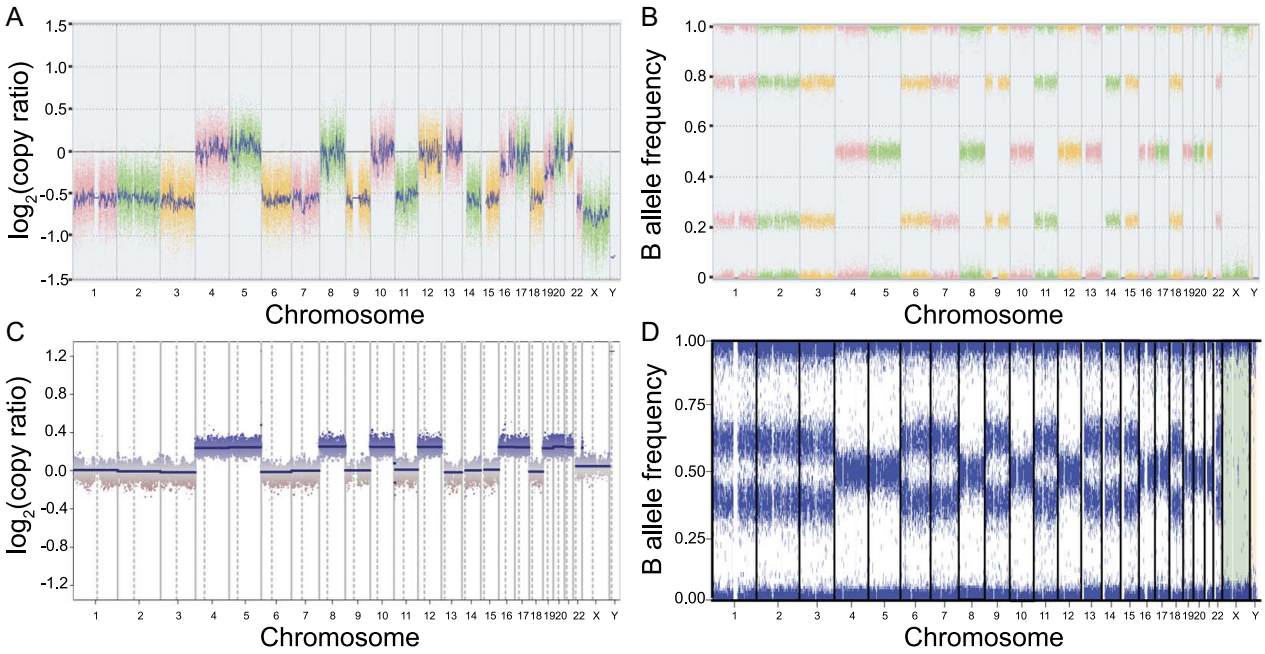

FIGURE 2. Single nucleotide polymorphism (SNP) array testing results of odontogenic myxomas from 2 independent study cohorts. A and B, SNP array data for the mandibular myxoma in a 15-year-old male patient previously described by Odintsov et al.[2] A. Log_2(copy number ratio) plot corroborates the findings of the DNA sequencing panel (Fig. 1C–D). B, B-allele frequency plot of SNP array data for the myxoma in A, consistent with loss of heterozygosity of chromosomes 1, 2, 3, 6, 7, 9, 11, 14, 15, 16p (subclonal), 18, 19 (subclonal), and 22. C and D, SNP array data for the maxillary myxoma in a 25-year-old male previously described by Kleijn et al.[1] C. Log_2(copy number ratio) from SNP array testing recapitulates the relative copy number pattern described previously by Kleijn et al. D, B-allele frequency plot of SNP array data for the myxoma in C, consistent with loss of heterozygosity of chromosomes 1, 2, 3, 6, 7, 9, 11, 13, 14, 15, 18, and 22. These 2 tumors show almost entirely overlapping findings by SNP array testing, and both are consistent with widespread loss of heterozygosity.

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