Somatic IDH1 mutation in a pituitary adenoma of a patient with Maffucci syndrome

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Maffucci syndrome is a rare disease characterized by multiple enchondromas and soft-tissue hemangiomas. Additionally, neuroendocrine tumors including pituitary adenomas have been described in these patients. The underlying genetic etiology lies in somatic mosaicism of mutations in isocitrate dehydrogenase 1 (IDH1) or isocitrate dehydrogenase 2 (IDH2). This report describes a patient with Maffucci syndrome who presented with intracranial tumors of the skull base and suprasellar region. The patient underwent resection of both intracranial tumors, revealing histopathological diagnoses of chondrosarcoma and pituitary adenoma. DNA sequencing of the tumors was performed to identify common IDH1/2 mutations. Clinical, radiological, and biochemical assessments were performed. Genotypic studies used standard Sanger sequencing in conjunction with a target-specific peptide nucleic acid to detect IDH1 mutations in tumor tissues. DNA sequencing demonstrated identical IDH1 mutations (c: 394C > T) in both tumors.

To the authors’ knowledge, this report provides the first genetic evidence for the inclusion of pituitary adenomas among tumors characterizing Maffucci syndrome. In patients who are newly diagnosed with Maffucci syndrome, it is appropriate to monitor for development of pituitary pathology and neuroendocrine dysfunction.

http://thejns.org/doi/abs/10.3171/2015.4.JNS15191

KEY WORDS pituitary adenoma; isocitrate dehydrogenase; somatic mosaicism; Maffucci syndrome; oncology

Maffucci syndrome and Ollier disease (OMIM 166000, ICD-10 Q78.4) are noninheritable conditions that are characterized by multiple enchondroma formation. Unlike Ollier disease, patients with Maffucci syndrome also form soft-tissue hemangiomas. Patients typically present during the 1st decade of life with asymmetrical skeletal deformities and limb-length discrepancies, and may require surgery. Up to 40% of patients undergo malignant transformation of enchondromas into chondrosarcomas. Recently, it was shown that individuals with Maffucci syndrome and Ollier disease harbor somatic mosaicism of mutations in isocitrate dehydrogenase 1 (IDH1) or isocitrate dehydrogenase 2 (IDH2).10

Maffucci syndrome was originally characterized as enchondromatosis with hemangioma. However, additional tumors have been reported in these patients, including lymphangiomas, pancreatic adenocarcinomas, biliary adenocarcinomas, osteosarcomas, and mesenchymal ovarian tumors.2,13,17,20 Additionally, intracranial tumors including astrocytomas, olfactory neuroblastosomas, malignant choromas, spindle cell hemangioendotheliomas, and pituitary adenomas have been described.3,22,26 However, the only genetic evidence to demonstrate causality in these associations has been limited to an IDH1-mutated ovarian fibroma and an IDH2-mutated anaplastic astrocytoma in Ollier disease and Maffucci syndrome, respectively.13,22

We describe a patient with Maffucci syndrome who presented with 2 intracranial tumors: a jugular foramen chondrosarcoma and a pituitary adenoma. Both tumors exhibited identical IDH1 mutations and represent the first genetic evidence of pituitary adenoma formation in Maffucci syndrome. Therefore, pituitary adenomas should be included among tumors in Maffucci syndrome that arise from somatic IDH1/2 mutations.
Methods

Study Oversight

This study was approved by the institutional review board of Beijing Tiantan Hospital of Capital Medical University (Beijing, China). The index patient provided written informed consent.

Immunohistochemistry

Routine H & E staining was performed on formalin-fixed tissue specimens to confirm histopathological diagnosis. Immunohistochemical staining was performed using the Bond automatic stainer and Bond ready-to-use antisynaptophysin antibody (both from Leica). Images were obtained at 200x magnification using a Nikon Eclipse Ci microscope with a Nikon DS-Fi2 camera.

DNA Extraction

DNA was extracted from formalin-fixed, paraffin-embedded tumor tissues using the QIAamp DNA FFPE Tissue Kit (Qiagen).

Polymerase Chain Reaction Conditions

Standard polymerase chain reaction (PCR) experiments contained 200–500 ng genomic DNA, 25 μl Taq 2x Master Mix (New England BioLabs), and 0.25 μl each of forward and reverse primers (100 μM) in a final volume of 50 μl. The PCR conditions were as follows: denaturation at 94°C for 15 minutes; followed by 40 cycles of 94°C for 30 seconds (denaturation), 55°C for 30 seconds (primer annealing), and 68°C for 60 seconds (extension), with a final extension step at 68°C for 5 minutes.

Peptide Nucleic Acid Design and PCR Conditions

The peptide nucleic acid (PNA) designed to detect wild-type IDH1 was produced by PNA Bio. The sequence of the PNA was CATCATAAGGTGTACATGCTT-Lys-Lys. The 2 terminal lysine residues were added for improved solubility. The PCR experiments contained 200–500 ng genomic DNA, 25 μl Taq 2x Master Mix (New England BioLabs), 0.25 μl each of forward and reverse primers (100 μM), and 0.5 μl PNA (100 nM) in a final volume of 50 μl. The PCR conditions were as follows: denaturation at 94°C for 15 minutes; followed by 40 cycles of 94°C for 30 seconds (denaturation), 68°C for 60 seconds (PNA hybridization), 55°C for 30 seconds (primer annealing), and 72°C for 60 seconds (extension), with a final extension step at 72°C for 7 minutes.

Detection of R132 Mutation With Nested PCR and PNA Application

PCR amplification to introduce a site-directed mutation was initially performed, using 100 nM PNA as described above (forward primer ACCAACGACCAATGCCCA, reverse primer GTGGTATGGGCTGCTATT). PCR products were purified using QIAquick PCR columns (Qiagen). Subsequently, PCR amplification was repeated with the PNA, using nested primers (forward primer TGGGAAAATCAACCAATGGC, reverse primer TT GCCGATGGGTAGAC). After purification, PCR products were analyzed by gel electrophoresis and subjected to Sanger sequencing for IDH1 mutations, using the nested forward primer. The GenBank (National Center for Biotechnology Information) accession number for IDH1 is NM_005896.2.

Case Report

History and Examination

A 28-year-old man presented with a 3-year history of voice hoarseness and dysphagia, and a 6-month history of left-sided blurred vision. His medical history was significant for development of multiple palpable nodular masses of the left hand beginning at 7 years of age (Fig. 1A), later accompanied by formation of subcutaneous blue, soft, nontender masses, diagnosed as hemangiomas on physical examination (Fig. 1A, inset). Radiographs of the left hand showed skeletal phalangeal malformation with calcific nodules and multiple enchondromas (Fig. 1B). Chest CT demonstrated enchondromas of the bilateral ribs and scapulae (Fig. 1C). Technetium-99m methylene diphosphonate whole-body bone scintigraphy revealed foci of intense tracer uptake, involving the right humerus, right ulna, right femur, right fibula, left humerus, left radius, left hand, left femur, left fibula, and left tibia (Fig. 1D). Based on these findings, the patient was diagnosed with Maffucci syndrome.

Neurological examination revealed left-sided deficits in hearing and gag reflex, bilateral temporal hemianopia, and decreased visual acuity (left eye 20/30; right eye 20/50). Brain MRI demonstrated 2 heterogeneously enhancing lesions, 1 in the left jugular foramen (Fig. 2A and B) and the other in the suprasellar region (Fig. 2C and D). The latter was suggestive of a pituitary macroadenoma. Serum prolactin (PRL), insulin-like growth factor-I, growth hormone (GH), cortisol, adrenocorticotropic hormone (ACTH), thyroid hormone, thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were normal. A diagnosis of Maffucci syndrome associated with a nonfunctional pituitary adenoma was proposed.

Operation and Postoperative Course

A subtotal resection of the contrast-enhancing left jugular foramen lesion through a retrosigmoid approach was performed, resulting in significant improvement of the patient’s dysphagia and auditory acuity. Histopathological review of the surgical specimen diagnosed a chondrosarcoma. Ten months after the first operation, the patient underwent gross-total resection of the suprasellar lesion via a subfrontal approach, revealing a diagnosis of pituitary adenoma. His postoperative recovery was uneventful other than transient polyuria. At the 2-year follow-up, the patient’s voice hoarseness and visual acuity were significantly improved.

Pathological Examination and DNA Sequencing

Pathological examination of the jugular foramen specimen revealed a Grade II chondrosarcoma, characterized by moderate cellularity with occasional mitotic figures, cellular atypia, and increased mucoid-myxoid degeneration of chondroid matrix (Fig. 2E). Histopathological review of the pituitary adenoma demonstrated a highly cellular...
tumor with prominent monomorphism and loss of the normal reticulin meshwork (Fig. 2F). Immunohistochemical staining for synaptophysin confirmed this tumor to be of neuroendocrine origin (Fig. 2F, inset). Additional immunohistochemical investigation was negative for GH, PRL, LH, FSH, TSH, and ACTH (not shown).

Sanger sequencing of DNA from the pituitary adenoma revealed a c.394C > T mutation, a substitution of arginine at codon 132 with cysteine (R132C) (Fig. 3 center). To confirm these results, we performed repeat Sanger sequencing on significantly limited, remaining tissue, using nested PCR technique and a custom-designed PNA against the wild-type IDH1 sequence (Fig. 3 lower). Use of PNA has been demonstrated to be an effective PCR

**FIG. 1.** Clinical presentation of index patient. A photograph of the index patient’s left hand is shown (A). Numerous palpable nodules were evident, which had been present since childhood. Multiple hemangiomas spread throughout his body were found, including his right buttock (inset). A radiograph of the left hand showed calcific nodules and lytic lesions of the phalanges, characteristic of enchondromas (B). 3D anterior (upper panel) and posterior (lower panel) reconstruction of a chest CT showed additional osseous lytic lesions (C). Whole-body bone scintigraphy revealed multiple areas of increased radiotracer uptake, suggestive of enchondromatosis (D). Figure is available in color online only.

**FIG. 2.** Radiographic and histological tumor characteristics. Axial (A) and sagittal (B) sections of a T1-weighted MRI study obtained after contrast administration demonstrated a heterogeneously enhancing lesion encasing the left jugular foramen of the skull base. Axial (C) and sagittal (D) T1-weighted MRI studies obtained after contrast administration showed a strongly enhancing suprasellar mass. A representative image of the H & E–stained sample (original magnification ×200), excised from the jugular foramen, exhibited increased cellular atypia amid a degenerative muco-myxoid chondroid matrix, suggestive of a Grade II chondrosarcoma (E). An H & E–stained photomicrograph (original magnification ×200) of tissue from the suprasellar mass showed monomorphic cellularity and disorganized reticulin meshwork (F). There was prominent positive staining for the neuroendocrine marker, synaptophysin, confirming neuroendocrine origin and diagnostic of pituitary adenoma (inset). Figure is available in color online only.
clamp of wild-type sequences in samples where mutated cells are sparse.\textsuperscript{6} Similarly, chondrosarcoma tissue was very limited but using the same techniques, a c.394C > T (R132C) mutation was detected (Fig. 3 upper).

**Discussion**

Although pituitary adenomas in patients with Maffucci syndrome have been previously described,\textsuperscript{27} none established causality of pituitary adenoma in Maffucci syndrome through analysis of IDH1/2 mutations. Our report of a pituitary adenoma sharing an identical IDH1 mutation with a chondrosarcoma in a patient with Maffucci syndrome supports the inclusion of pituitary adenomas among tumors characterizing Maffucci syndrome. Further investigations may reveal IDH1/2 mutations in other tumor types reported to arise in patients with Maffucci syndrome.

Excluding the current study, 11 cases of pituitary adenoma in patients with Maffucci syndrome have been described and are shown in Table 1.\textsuperscript{5,8,10,11,14,15,19,20,23,27,28} Among these, the majority (6/11) presented solely with visual field deficits, as did our patient. Further, none exhibited symptoms or evidence from immunohistochemical staining and/ or laboratory testing of hormonal abnormalities, indicative of a predominance of nonfunctional pituitary adenomas in this patient population. All patients except for 2 underwent resection, 1 of whom was diagnosed postmortem.\textsuperscript{5} The other patient was treated with radiation only and was without recurrence at the 3-year follow-up.\textsuperscript{19} There were 2 more cases of tumor recurrence within 3 years after surgical removal of the pituitary adenoma.\textsuperscript{10,28} Further, similar to the patient described by Miki et al.,\textsuperscript{20} there was little evidence of sellar enlargement upon review of imaging from our patient, which, although atypical for a pituitary adenoma, does not exclude its diagnosis.\textsuperscript{25} Taken together, the clinical course of our patient is consistent with these previous studies, i.e., he presented solely with visual field deficits secondary to a nonfunctional pituitary adenoma and responded well to surgical treatment alone.

To our knowledge, the current case represents the first case of an IDH1-mutated pituitary adenoma. Balss et al. analyzed 23 samples of sporadic nonfunctional pituitary adenomas and failed to detect IDH1 mutations, using direct DNA-sequencing techniques.\textsuperscript{4} Similarly, Ikota et al. performed immunohistochemical staining for IDH1 mutations in 42 pituitary adenoma samples, of which 2 exhibited 10%–30% positive immunoreactivity.\textsuperscript{9} However, positive staining in these samples was confined to the cytoplasm. Because of this, the authors stated these find-
the onco-metabolite, D-2-hydroxyglutarate, promoting de-
metabolic pathways via mutant enzymatic production of
lignancies.21 As this report suggests,
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Maffucci syndrome may affect cells beyond mesenchymal
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ings should be considered negative, given the absence of concomitant nuclear staining, which is required for immu-
histochemical diagnosis of IDH1 mutations.
Maffucci syndrome was originally theorized as a con-
dition of mesodermal dysplasia.18 However, our study and
Moriya et al.22 question this theory because pituitary ad-
nomas and astrocytomas arise from neuroectodermal tis-
sues. Our finding of the same IDH1 mutation in the chon-
drosarcoma (mesodermal origin) and pituitary adenoma (neuroectodermal origin) suggests IDH1/2 mutations are
early postzygotic events in Maffucci syndrome, occurring
prior to gastrulation.1,22 It is unclear which cell types are
affected and harbor tumorigenic potential in Maffucci
syndrome. Other endocrine tumors have been described in
Maffucci syndrome, including thyroid adenoma, para-
thyroid adenoma, pheochromocytoma, and paragangli-
a. Further, endocrine tumor syndromes have been
attributed to mutations in cellular metabolism genes, in-
cluding succinate dehydrogenase (SDH), hypoxia-induc-
ible factor 2-alpha (HIF2A), multiple endocrine neoplasia
1 (MEN1), rearranged during transfection (RET), and Von
Hippel-Lindau (VHL). IDH1/2 mutations similarly alter met-
abolic pathways via mutant enzymatic production of the
onco-metabolite, D-2-hydroxyglutarate, promoting de-
velopment of low-grade gliomas and hematopoietic ma-
lignancies.21 As this report suggests, IDH1/2 mutations in
Maffucci syndrome may affect cells beyond mesenchymal
lineage, including neuroectodermal cells that may form
tumors. IDH1/2 sequencing of more neuroendocrine tu-
mors from patients with Maffucci syndrome will be re-
quired to answer this question.
In summary, we provide sufficient genetic evidence for
the inclusion of pituitary adenomas among tumors that
arise from IDH1 mutation mosaicism in Maffucci syn-
drome. Because IDH1/2 mutations in Maffucci syndrome
predispose cells to, rather than incite, tumorigenesis, it is
yet to be seen whether IDH1 and IDH2 represent cancer-
susceptibility genes, similar to SDH, MEN1, and RET.

Acknowledgments

We thank Dr. Guilin Li (Beijing Neurosurgical Institute,
Capital Medical University, Beijing, China) and Dr. Abhik Ray-
Chaudhury (National Institute of Neurological Disorders and
Stroke, NIH, Bethesda, MD) for their assistance in histopatho-
logical analysis. We also thank Drs. Liwei Zhang, Zhen Wu, and
Sumin Geng (Beijing TianTan Hospital, Capital Medical Univer-
sity, Beijing, China) for their editing of the case presentation. This
work was supported by the Intramural Research Program of the
National Institute of Neurological Disorders and Stroke at the NIH
as well as the National Natural Science Foundation of China (No.
81341059) and Beijing Nova program (No. 2012033).

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Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Zhuang, Hao, Zhang. Acquisition of data: Zhuang, Hao, Hong, Feng, Chittiboina, Zhang. Analysis and interpretation of data: all authors. Drafting the article: all authors. Critically revising the article: all authors. Reviewed submitted version of manuscript: Zhuang, Hao, Hong, Feng, Zhang. Approved the final version of the manuscript on behalf of all authors: Zhuang. Administrative/technical/material support: Hao, Feng. Study supervision: Zhuang, Zhang.

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