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Loss of heterozygosity and absence of MAX immunostaining in a prolactinoma associated with multiple endocrine neoplasia type 5 (MEN5)

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Abstract

Background Multiple endocrine neoplasia type 5 (MEN5) is an emerging syndrome caused by germline pathogenic variants involving the *MYC Associated Factor X (MAX)* gene. Affected individuals typically have pheochromocytomas, often bilateral, at a relatively early age. In *MAX* pheochromocytoma cohorts, pituitary adenomas are rarely reported. The role of *MAX* as a tumor suppressor gene in the pituitary gland has not been directly proven to date.

Methods The propositus came from a pheochromocytoma kindred with a germline pathogenic *MAX* variant c.97 C>T (p.R33*). In his late thirties he developed asynchronous bilateral pheochromocytomas and underwent bilateral adrenalectomy. At age 46, he developed hyperprolactinemia (45.1 µg/L; 3x ULN) and increased IGF-1 (460 ng/mL; 1.9x ULN). Total testosterone was low (1.5 ng/mL) as was LH (1.2 IU/L). Pituitary MRI showed a microadenoma (6 mm), which was resected and his prolactin, IGF-1, and testosterone levels normalized. A Pituitary adenoma was confirmed on pathology, which showed positivity for prolactin only and a Ki67 of 2%.

Results *MAX* immunohistochemical staining was lost in the pituitary adenoma cells. Tumoral DNA analysis (120X read depth) showed that at the *MAX* locus the pathogenic variant c.97 C>T constituted > 90% of the sequencing reads supporting tumoral loss of heterozygosity (LOH).

Conclusions Loss of MAX staining and the identification of tumor LOH at the *MAX* locus confirms pituitary adenomas as a component tumor in the emerging MEN5 syndrome due to germline pathogenic *MAX* variants.

Keywords Pituitary adenoma · MEN5 · Pheochromocytoma · Genetics · MAX

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Introduction

Multiple endocrine neoplasia type 5 (MEN5) is an emerging endocrine cancer syndrome caused by germline pathogenic variants in the *MYC Associated Factor X* (*MAX*) gene. This gene on chromosome 14q23.3, encodes MAX, a member of the basic helix-loop-helix leucine-zipper family of transcription factors. MAX was identified in the early 1990's as a partner and regulator of the MYC proto-oncogene [1, 2]. Given its important role in regulating MYC and other partners, disruption of MAX expression has been implicated in various neoplasms, including the PC12 pheochromocytoma cell line [3–7].

The most prominent *MAX*-associated cancers in humans are pheochromocytomas-paragangliomas [8, 9]. *MAX*-related pheochromocytomas occur at a young age, are often bilateral or multiple, recurrent and are malignant in 19% of cases [10, 11]. Increased clinical focus on *MAX* led to the identification of rarely associated tumors including pituitary adenomas, neuroblastomas and pancreatic neuroendocrine tumors [12–16]. This expanded tumor phenotype led to the classification of *MAX* as a multiple endocrine neoplasia predisposition gene [17].

MEN5-associated pituitary adenomas are usually somatotropinomas mixed GH-prolactin positive tumors, mammosomatotropinomas or prolactinomas, which can occur at a young age [12, 18]. Until recently the implication of *MAX* as a tumor suppressor gene in the human pituitary gland had not been directly proven due to lack of somatic genetic studies of resected pituitary tissue in these usually historical cases. Freie et al. recently demonstrated in a mouse model that *Max* inactivation led to tumorigenesis across multiple neuroendocrine tissues, including pituitary adenomas [19]. Here we describe tumoral loss of heterozygosity (LOH) at the *MAX* locus accompanied by loss of MAX immunohistochemical staining in a prolactinoma in a patient from an extensive MEN5 kindred with a germline pathogenic *MAX* variant.

Methods

The subject came from a family with a known history of pheochromocytoma (Fig. 1a). On his paternal side, his grandfather, an aunt, an uncle and a female first cousin had been diagnosed with pheochromocytomas. Interestingly, a son of this female first cousin had been diagnosed with a prolactinoma as a teenager. The subject and his affected family members were positive for a germline pathogenic variant in *MAX* c.97 C>T (p.R33*). Aged 37, he developed hypertension associated with excessive sweating, anxiety and nervousness, and CT imaging identified a 15 mm left adrenal mass. An adrenalectomy identified a pheochromocytoma. Four years later, a similar presentation led to the diagnosis of a right-sided pheochromocytoma, and he underwent an adrenalectomy. He was replaced with hydrocortisone and fludrocortisone, although 60 mg/day hydrocortisone was required to control symptomatic fatigue.

Aged 46, a hormonal profile revealed hyperprolactinemia (45.1 ng/ml; 3x ULN) and an increased IGF-1 (460 ng/mL; 1.9x ULN). Total testosterone level was low (1.5 ng/ml; normal range 2.5–8.4 ng/ml), sex hormone binding globulin was 21.4 nmol/l (normal range: 14.4–70.7 nmol/l), LH was 1.2 IU/L, while FSH (3.2 IU/L) was normal. His blood pressure was 135/80 mmHg and urinary and plasma catecholamines were normal. Other hormonal and biochemical parameters were unremarkable (ACTH was low due to chronic 40 mg/day hydrocortisone). He reported mild erectile dysfunction but no specific signs or symptoms of hyperprolactinemia or acromegaly. Densitometry showed osteopenia of the femoral neck. A chest CT scan was normal. Due to the elevated IGF-1 and prolactin, he underwent an oral glucose tolerance test (OGTT) which showed a nadir GH level of 0.26 µg/L. A pituitary MRI showed a right sided lesion of 6 mm in maximum diameter that was consistent with a microadenoma without evidence of cavernous sinus invasion (Fig. 1b, c).

The subject elected for neurosurgery and underwent a transsphenoidal resection of the pituitary lesion, which confirmed a pituitary adenoma. Postoperative follow-up was uneventful and after three months his prolactin (4.5 ng/ml), IGF-1 (210 ng/ml NR:68–237 ng/ml), total testosterone (3.4 ng/ml), LH (3.2 IU/L) and FSH (3.2 IU/L) were normal. The nadir GH at 120 min was 0.24 ng/mL on repeat OGTT.

Immunohistochemistry

The anti-MAX antibody was ab101271 (Abcam) and was used at a dilution of 1:400 with heat-induced antigen retrieval with citrate for 40 min (H1/40) on a Leica Bond Rxm auto-stainer (all reagents from Leica).

Genetic analysis

DNA was extracted from formalin fixed paraffinized tumor tissue and genomic DNA was fragmented for library preparation. The indexed libraries were sequenced to at least 100X coverage with 2 × 150 chemistry on MGI sequencing platform. The primary and secondary analyses were performed on an Illumina DRAGEN platform, and sequences were aligned to the human reference genome (GRCh37/hg19). Bioinformatic analyses were performed using CNVkit and data were visualized using Integrated Genomics Viewer (version 2.19.6) [19, 20].

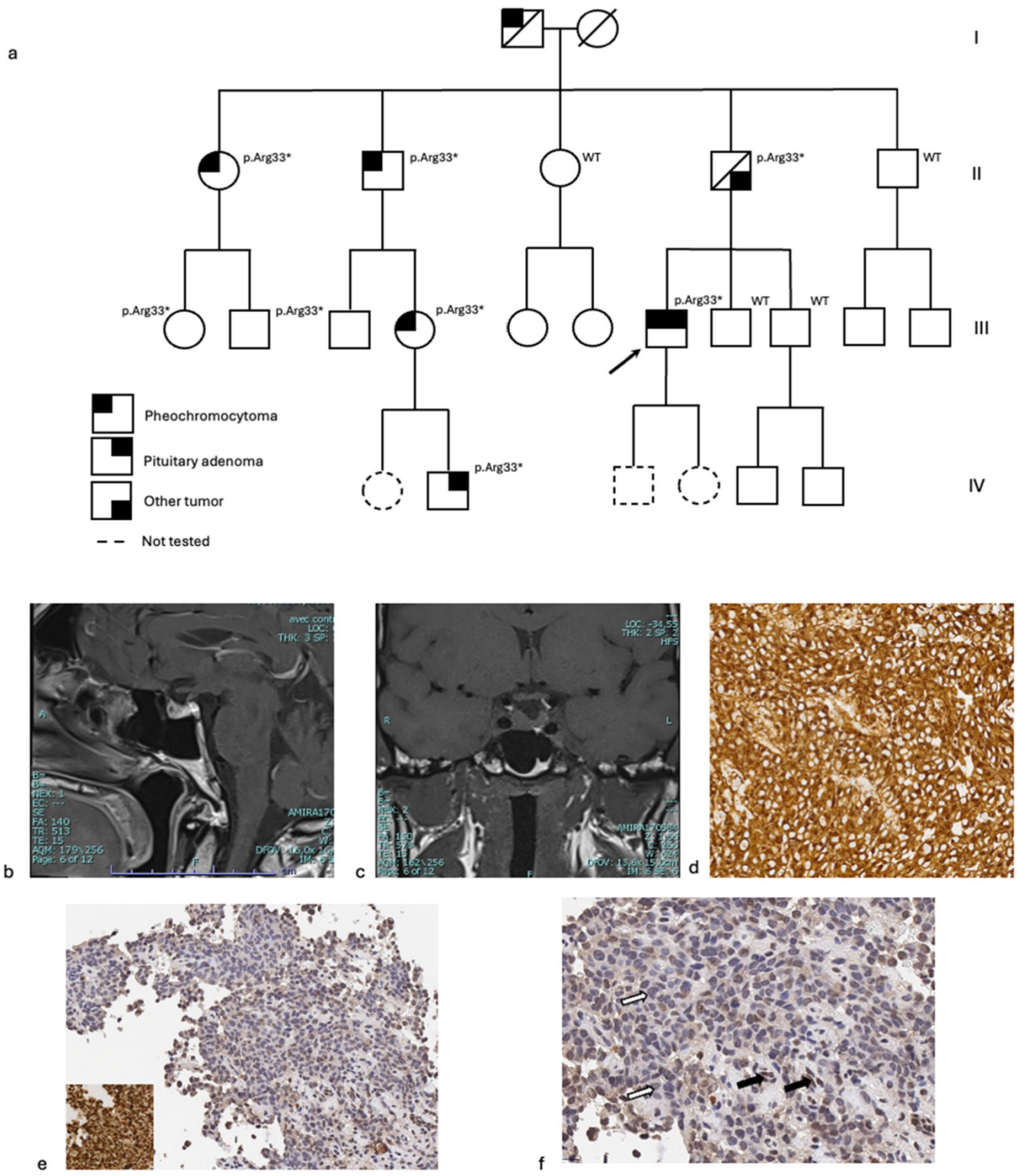

Fig. 1 (**a**) Genealogical tree of the kindred affected with a germline pathogenic c.97 C>T (p.R33*) *MAX* variant. The propositus (III-7) is indicated with an arrow. His father was affected with other cancers including skin cancer and a hematological malignancy. MRI images of the pituitary microadenoma in subject III-7, including preoperative, T1-weighted sagittal (**b**) and coronal (**c**) images. Immunohistochemical studies of resected pituitary adenoma showing (**d**) uniform, intense prolactin staining (40x magnification); (**e**) negative (blue) nuclear staining for MAX in tumoral cells at 20x magnification, while inset image shows intense positive (brown) nuclear staining from a control *MAX* wild-type pituitary adenoma; (**f**) negative (blue; white arrows) nuclear staining for MAX in adenoma cells (40x) contrasts with positive staining of endothelial cells (filled arrows)

Consent

Subjects provided informed consent for genetic and immunohistochemical analyses and the Ethics Committee of the University of Liège provided ethical approval for the genetic studies (Number: B707201420418).

Data Availability

All data produced in the present study are available upon reasonable request to the authors.

Results

Immunohistochemistry

The resected tissue consisted of monomorphic tumor cells with clear or eosinophilic cytoplasm, rounded nuclei with granular salt-and-pepper chromatin; hyperplasia was absent and the Ki67 was 2%. Cytokeratin AE1-AE3 immunostaining showed diffuse cytoplasmic positivity and rare dot-like staining pattern in tumor cells. Tumor cells were intensely positive for prolactin (Fig. 1d), and negative for GH and other pituitary hormones; mixed positivity for GH, ACTH, LH and FSH was only seen in some pituitary fragments. Immunohistochemistry for MAX showed negative nuclear staining in the pituitary tumoral cells (Fig. 1e and f).

Genetic analysis

Sequencing of the somatic DNA extracted from the tumor tissue (read depth 120x) showed that the *MAX* c.97 C>T (p.R33*) pathogenic variant was present. No other variants in the *MAX* gene were noted. There was marked allelic imbalance between the reference and variant alleles with 90.83% of the sequencing reads consisting of the c.97 C>T pathogenic variant. As the allelic imbalance far exceeded the threshold (>25% difference) between reference and variant alleles, this indicates that the wild-type *MAX* allele was lost and loss of heterozygosity (LOH) in the tumoral tissue was present.

Discussion

As a newly defined entity, the clinical characteristics and constituent tumors of MEN5 are still being clarified. After pheochromocytomas, which are seen in >90% of those with deleterious germline *MAX* variants, pituitary adenomas are one of the more frequently observed tumors in MEN5 [11]. Including the current case, 11 patients with *MAX*-related pituitary adenomas have been described in the peer-reviewed literature, accounting for about 8% of cases [11, 18]. Clinically, -*MAX* related pituitary adenomas range from mild disease to aggressive phenotypes like pituitary gigantism [12, 18]. To date, somatotropinomas, mammosomatotropinomas, and prolactinomas have been identified in this setting, which are of PIT1 lineage. In mice with conditional *Max* knockout in neuroendocrine compartments, pituitary tumor phenotypes are more variable [19]. *MAX*-associated pituitary adenomas have only been encountered in the syndromic setting in individuals/kindreds with pheochromocytomas and other tumors and deleterious germline *MAX* variants likely play no major role in the pathogenesis of isolated pituitary adenomas [21].

We add new evidence regarding the role of pathogenic *MAX* variants in pituitary adenomas in MEN5. As a tumor suppressor gene, deleterious germline variants in *MAX* represent a first hit, which must be accompanied by a somatic second hit that alters the wild-type allele. In *MAX*-related pheochromocytomas, tumoral LOH is seen, abrogating normal MAX protein expression and signaling. Here we show a similar mechanism in MEN5-associated pituitary adenomas, with somatic DNA analysis showing >90% of sequencing reads in the resected tissue as bearing the pathogenic c.97 C >T (p.R33*) variant. This led to loss of MAX immunostaining, which has not been reported previously in MEN5-related pituitary adenomas. *MAX* is a paternally imprinted gene and pheochromocytomas are inherited in families along the paternal line, as occurred here [8]. Systematic use of MAX immunohistochemistry and somatic genetic studies play a key role in better defining the full range of other tumors that consitute MEN5 in subjects with germline *MAX* pathogenic variants.

Taken together, the demonstration of somatic LOH in the pituitary adenoma, accompanied by absent tumoral MAX immunostaining provide the final step in confirming the pathophysiological link between germline deleterious *MAX* variants and pituitary tumorigenesis in MEN5.

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Data availability All data produced in the present study are available upon reasonable request to the authors.

Declarations

Competing interests The authors declare no competing interests.

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