Pancreatic Neuroendocrine Neoplasm Associated with a Familial *MAX* Deletion

Authors

Sandrine Petignot[1], Adrian F. Daly[1], Emilie Castermans[2], Esther Korpershoek[3], Irène Scagnol[4], Pablo Beckers[2], Vinciane Dideberg[2], Vincent Rohmer[1], Vincent Bours[2], Albert Beckers[1]

Affiliations

1 Department of Endocrinology, Liège Université, Liège, Belgium
2 Department of Human Genetics, Liège Université, Liège, Belgium
3 Department of Pathology, Erasmus University Medical Center, Rotterdam, The Netherlands
4 Department of Pathology, Liège Université, Liège, Belgium

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Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence

Prof. Albert Beckers MD, PhD
Chief, Department of Endocrinology Centre Hospitalier Universitaire de Liège, Liège Université
4000 Liège
Belgium
Tel.: +32 4 3667083, Fax: +32 4 3667261
albert.beckers@chuliege.be

ABSTRACT

Most pancreatic neuroendocrine neoplasms (pNEN) occur sporadically but they can also occur as part of multiple endocrine neoplasia type 1 (MEN1). *MAX* was originally described as an inherited pheochromocytoma-paraganglioma risk gene, but also has recently been implicated in pituitary tumorigenesis. Here we describe the first case of a pNEN associated with an inherited *MAX* gene deletion in a family with endocrine tumors. The patient was a male carrier of an intragenic exon 3 deletion inherited from his father who had recurrent pheochromocytomas and a macroprolactinoma. The patient underwent screening and hormonal studies but no pheochromocytoma-paraganglioma, pituitary or renal tumors were identified. However, abdominal magnetic resonance imaging (MRI) identified a 1 cm lesion in body of the pancreas. The lesion was hyperintense on T2-weighted signal, and there was hyperfixation of the tumor on 68Ga-DOTANOC PET-CT images. No biochemical evidence of pancreatic hormone excess was identified. Following a guided biopsy, a pathological diagnosis of a low grade pNEN was made and immunohistochemistry showed loss of MAX nuclear staining. Genetic analysis of the tumor tissue indicated copy number neutral loss of heterozygosity consistent with uniparental disomy. This is the first reported case of a *MAX* deletion associated pNEN and strengthens the argument that *MAX* may represent an inheritable multiple endocrine neoplasia risk gene. Further analysis of germline and somatic *MAX* mutations/deletions in large cohorts of unexplained NEN cases could help clarify the potential role of MAX in NEN etiology.

Introduction

Pancreatic neuroendocrine neoplasms (pNEN) have an incidence of 0.48 cases per 100 000, and the frequency is rising [1]. While they are usually sporadic, pNENs can occur in the setting of multiple endocrine neoplasia type 1 (MEN1) and hence they are the subject of active surveillance in that setting [2]. Other genetic syndromes that are rarely associated with pNENs include von Hippel–Lindau disease, neurofibromatosis type 1 (NF1), MEN4, Lynch and Cowden syndrome [3–9].

In 2011, Comino Mendez et al. identified *MAX* as a risk gene for the development of hereditary pheochromocytoma [10]. Germline mutations in *MAX* lead to the development of sporadic and familial pheochromocytoma-paragangliomas and *MAX* acts as a tumor suppressor gene in the MYC/MAX/MXD1 pathway [11]. While germline *MAX* genetic changes account for a small proportion of all known genetic forms of pheochromocytoma-paragangliomas, they appear to have an aggressive phenotype. Burnichon et al. reported that pheochromocytoma-paragangliomas patients with

MAX mutations had an earlier age at onset as compared with non-mutated cases and *MAX* associated tumors are much more frequently bilateral or have multiple tumors occurring within the same gland [11]. Until recently the tumoral phenotypes associated with germline *MAX* mutations and rearrangements were limited to pheochromocytoma, paraganglioma and kidney neoplasms [12, 13]. In primary tumors and cell cultures derived from small cell lung cancer, a neuroendocrine tumor, somatic *MAX* mutations and deletions with concurrent loss of heterozygosity (LOH) were found to occur in 6 % of cases [14]. Furthermore, two patients with gastrointestinal intestinal stromal tumors (GIST) that were negative for *KIT*/*PDGFRA*/*BRAF*/*SDHx* abnormalities (quadruple wild-type) were reported as having somatic truncating mutations in *MAX* [15].

An association between *MAX* and the development of pituitary adenomas (acromegaly or prolactinoma) has been described recently [16, 17]. We described three cases of intragenic germline deletions in *MAX* that were not identified on Sanger sequencing but were established with multiplex ligation-dependent probe amplification (MLPA). Those cases had aggressive features with early onset, recurrence, bilateral pheochromocytomas or metastatic disease, in keeping with established *MAX* related characteristics [11, 17]. In one kindred, the deletion was inherited by the patient's son from his father [17]. Subsequent screening of this 31-year old male, who had no medical history, was undertaken to identify tumors in known sites related to *MAX* mutations. Unexpectedly, abdominal imaging studies revealed a pancreatic mass, which was further investigated and characterized.

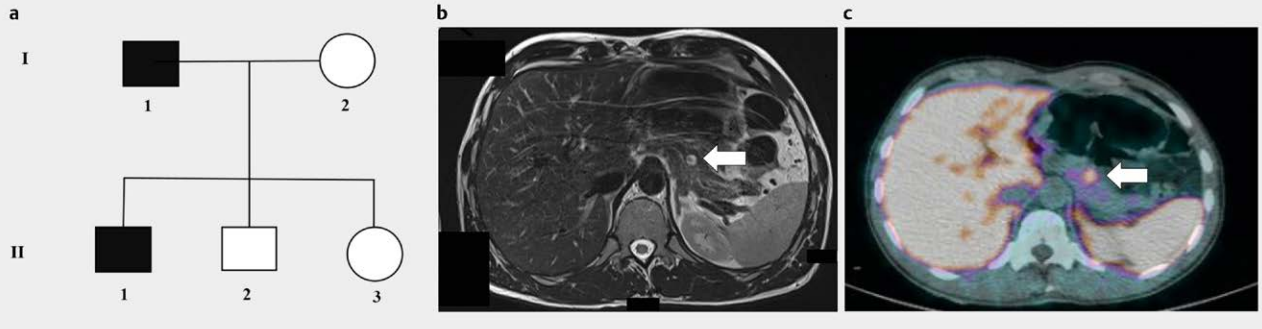
Statement of Ethics

The patient provided informed consent and the study was approved by the Ethics Committee of the CHU de Liège.

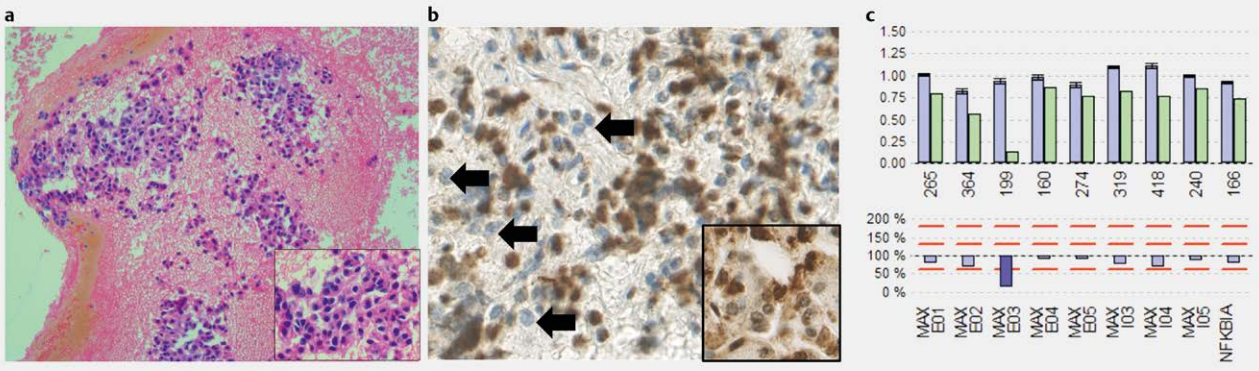
Methods and Results

As we reported previously, the patient's father had a history of recurrent pheochromocytoma and a prolactinoma in the setting of a germline intragenic exon 3 deletion in *MAX* [17]. The pheochromocytoma tissue had been shown to have LOH at the *MAX* locus that differed between the initial tumor and the recurrence (18 years later), indicating separate somatic "second hit" events affecting the wild-type *MAX* allele [17]. Family genetic studies including MLPA had identified the son as a carrier of the identical germline exon 3 *MAX* deletion as his father (▶ **Fig. 1a**). Screening studies were performed and included biochemical and hormonal analyses of adrenal and pituitary function, hematological, renal and liver function tests. All were normal. Abdomino-thoracic and pituitary magnetic resonance imaging (MRI) were performed and no evidence of pheochromochromocytoma/paraganglioma, pituitary adenoma, or kidney tumors was identified. On the abdominal MRI a 1 cm lesion in body of the pancreas was identified, which was hyperintense on T2 weighted signal (▶ **Fig. 1b**). An ^{18}F-fluorodeoxyglucose-positron emission tomography-CT (^{18}FDG-PET-CT) scan showed no enhanced uptake. There was hyperfixation of the tumor on ^{68}Ga-DOTANOC PET-CT images, indicating strong SST_2 expression (▶ **Fig. 1c**). Neither biochemical evidence nor signs/symptoms of pancreatic hormone excess were identified. The patient provided informed consent and the study was approved by the Ethics Committee of the CHU de Liège.

To further investigate the lesion, a percutaneous ultrasound-guided fine-needle aspiration (FNA) biopsy was performed. Hematoxylin and eosin staining showed aggregations of cells with eccentric nuclei, salt and pepper chromatin pattern and a granular, eosinophilic cytoplasm (▶ **Fig. 2a**). The tissue was positive for anti-CD56, Chromogranin A and Synaptophysin and no mitoses were seen. A pathological diagnosis of a low grade pancreatic neuroendocrine tumor was made (G1 grade; Ki-67: 1–2 %, mitotic index: 0). Immunohistochemistry of the FNA material for MAX was performed as previously described [12]; this showed neuroendocrine cells that exhibited loss of MAX nuclear staining in the setting of other normally-stained cells (▶ **Fig. 2b**). Genetic analyses were also performed on the pNEN FNA tissue DNA; MLPA showed LOH and an apparent homozygous deletion of the exon 3 of MAX gene (▶ **Fig. 2c**). The MLPA results and the paternal inheritance pattern strongly point copy neutral LOH involving the MAX locus due to paternal uniparental disomy (UPD) at chromosome 14q as has been



▶ **Fig. 1** Panel **a** shows the genealogical tree of the family. The father (**I1**) had a pheochromocytoma at 32 years of age that recurred at the age of 50 and a prolactinoma that was diagnosed at the age of 49 years. His son (**II1**) had a pancreatic neuroendocrine tumor discovered during screening at the age of 32. Both **I1** and **II1** were diagnosed with an intragenic deletion of exon 3 in *MAX*. Other family members were tested and had a wild-type *MAX* sequence and MLPA. Panel **b** shows the location of the pNEN (arrow) as a hyperintense lesion in the body of the pancreas on a T2-weighted MRI. Panel **c** shows intense uptake in the tumor (arrow) on ^{68}Ga-DOTANOC PET-CT.



▶ **Fig. 2** Panel **a** shows an image of the hematoxylin and eosin stain of the tissue obtained following fine needle biopsy of the pancreatic lesion. The biopsy material shows groups of abnormal cells with generally eccentric nuclei and an eosinophilic cytoplasm. Inset is a high magnification image of a section of the tumor cells. Arrowed on Panel **b** are groups of tumoral cells that were negative for MAX staining (blue nuclei and negative cytoplasm) interspersed with groups of normal cells. Inset is an image of a positive control from a normal pancreas section demonstrating strong positive (brown) nuclear and moderately positive cytoplasmic staining. Panel **c** illustrates the MLPA finding on pNEN tumor DNA showing an apparent homozygous deletion in exon 3 in the *MAX* gene. The upper histograms show each probe coverage in the kit controls (blue) and in the patient's sample (green).

demonstrated in familial cases of MAX-related pheochromocytoma and renal oncocytoma [11, 12].

The patient remains under close clinical follow-up and is currently asymptomatic. On abdominal MRI at six months post-diagnosis the tumor remains stable and in light of the low grade, size <2 cm, low Ki-67 score, non-functional status and patient wishes, the patient is being managed with active surveillance [18].

Discussion

This is, to the best of our knowledge, the first case of a gastroenteropancreatic NEN associated with an inherited germline *MAX* mutation or deletion. Originally *MAX* mutations were described in association with pheochromocytoma, and subsequent research has further defined the clinical phenotype which can be bilateral and aggressive [10, 11, 19]. Since then *MAX* has been implicated in a growing number of sporadic and familial cancers, many which have a neuroendocrine origin. Emerging evidence suggests that inactivating *MAX* genetic abnormalities appears to lead to tumor risk at multiple endocrine and non-endocrine tissues, including pheochromocytoma, paraganglioma, renal tumors, pituitary adenomas, and GIST and SCLC [10–17, 19, 20]. Clustering of tumors within the same patient and/or kindred with *MAX* mutations includes pheochromocytoma-paraganglioma, pituitary adenoma, and renal tumors [10–12, 16, 17].

The past decade has seen a large volume of fundamental research on the genetics and genomics of NEN in general and pNEN in particular. The study of inherited or familial disorders provided early and important insights into pNEN pathogenesis, including sporadic disease [21]. For example, comprehensive analyses have identified mutations in genes such *MEN1*, *VHL*, *TSC1*, *TSC2*, and *PTEN*, which cause individual syndromic diseases, as also playing an integral role in the development of sporadic pNET [21–23]. In addition, mutations in the *ATRX* and *DAXX* genes that are involved in telomere length regulation via histone 3.3 deposition are frequently found in pNEN [2]. Subsequent work has expanded the list of recurrent genetic alterations, chromosomal loss/gain patterns and epigenetic profiles and certain pathway groupings are now evident, including, *MEN1*-related alterations, telomeric changes (*ATRX*/*DAXX*), abnormal cell-cycle regulation (e. g., *CDKN1B*), PI3K-mTORpathway disorders, and disordered chromatin remodeling or DNA and base repair dysregulation [21]. While these large-scale studies have not identified *MAX* mutations/deletions as a major contributor to sporadic pNEN pathogenesis, it remains to be seen if *MAX* intragenic copy number variations represent a contributory factor in a subgroup of cases. Taking the findings of the current study into account, it seems reasonable to suggest that surveillance of previously identified *MAX* carriers could be expanded to include a wider range of potential target tumors. As sporadic pheochromocytoma-paraganglioma cases without known family history can have unsuspected germline mutations in *MAX*, similar tumor risk related to *MAX* might be present in sporadic cases of NEN, pituitary adenoma, among others [24]. Genetic analyses of large NEN and other tumor banks should assess for intragenic deletions and complex rearrangements of *MAX*, which can be missed by some sequencing driven approaches [17].

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Conflict of Interest

The authors declare that they have no conflict of interest.

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