Pancreatic Neuroendocrine Neoplasm Associated with a Familial MAX Deletion

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 pheochromocromocytoma/paranganglioma, 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 18F-fluorodeoxyglucose-positron emission tomography-CT (18FDG-PET-CT) scan showed no enhanced uptake. There was hyperfixation of the tumor on 68Ga-DOTANOC PET-CT images, indicating strong SST2 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](https://example.com/fig1.png)  **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 68Ga-DOTANOC PET-CT.

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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 as 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-mTOR pathway 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.
References


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