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Short Report

Familial small-intestine carcinoids: Chromosomal alterations and germline inositol polyphosphate multikinase sequencing

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ABSTRACT

Background: Familial small-intestine neuroendocrine tumors (SI-NETs) are an exceptional inherited entity. Underlying predisposing mechanisms are unelucidated, but inositol polyphosphate multikinase (*IPMK*) gene alterations might promote their tumorigenesis.
Methods: A retrospective-prospective nationwide cohort was constituted, by including patients with proven SI-NETs and at least one relative with the same disease. We performed constitutional and somatic *IPMK* sequencing, and somatic DNA comparative genomic hybridization (CGH).
Results: We included 17 patients from 8 families, who were characterized by high prevalence (57%) of multiple SI-NETs, and high frequency of distant metastases (82%) and carcinoid syndrome (65%). No *IPMK* mutation was found in constitutional or tumor DNA. CGH array revealed recurrent chromosome-18 deletions but no alteration in the *IPMK* region.
Conclusion: We report here the first European series of patients with familial SI-NETs. Predisposing mechanisms may not involve the *IPMK*-encoding sequence or chromosomal region and might not differ from those of sporadic SI-NETs.

1. Introduction

Well-differentiated small-intestine neuroendocrine tumors (SI-NETs) are usually sporadic [1,2]. Their occurrence in at least 2 blood relatives defines familial SI-NETs, which is a rare, previously unrecognized inherited entity. Familial SI-NETs were initially described in rare case reports and large-scale epidemiologic studies, with an estimated prevalence of 2.6–3.7% among all SI-NETs [3–5]. The relative risk of developing SI-NETs may be 4- to 13-fold higher among relatives of SI-NET patients in the general population vs. 194-fold for affected families [4,5]. Due to its extreme rarity, familial SI-NETs were poorly characterized clinically and pathologically until the recent report of the US National Institute of Health (NIH) series [6,7] and the description of a germline deletion in the inositol polyphosphate multikinase (*IPMK*) gene promoting SI-NET tumorigenesis [6].

We aimed to confirm this entity's existence by reporting on a relatively large nationwide cohort of patients with familial SI-NETs, who underwent *IPMK* sequencing and comparative genomic hybridization (CGH) of their primary tumor DNA to move toward the genetic characterization of this exceptional condition.

2. Material and methods

Since February 1st 2012, we have collected all known familial SI-NET cases in France, from the *Groupe d'Étude des Tumeurs Endocrines* (GTE) and the *Fédération Francophone de Cancérologie Digestive* (FFCD) networks. This retrospective-prospective observational cohort included patients who were >18 years old, had histologically proven SI-NETs, and at least 1 blood (1st- and/or 2nd-degree) relative with the same disease. All clinical information (demographic, cancer history, tumor extension) was collected

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retrospectively, from medical records, from the initial diagnosis, and prospectively until 31 December 2013. SI histologic specimens from patients who underwent surgical resection were collected and analyzed centrally in an expert center for macroscopic and microscopic characteristics, including differentiation, depth invasion (WHO 2010), and vascular, perineural and lymph-node invasion. Immunohistochemical studies included synaptophysin, chromogranin A and MIB-1 (monoclonal M724001, Dako™) labeling. Tumor grade (WHO 2010) was defined according to the Ki67 index. Data collection and analyses were approved by the GTE institutional review board.

After obtaining written informed consent, we collected venous blood samples from index cases, as well as frozen histologic specimens of SI primary tumors, for nucleic acid extraction. High-molecular-weight DNA was prepared by standard proteinase K digestion followed by phenol–chloroform extraction from whole-blood leukocytes or frozen tumor samples. The quality of the nucleic acids was determined by electrophoresis through agarose gels and ethidium bromide staining. Comprehensive screening of *MEN1* and *VHL* gene mutations in all patients included DNA sequencing of coding exons and their intervening sequence boundaries for small genetic changes and multiplex ligation-dependent probe-amplification analysis to detect copy-number variations (full-gene deletion or one/several exon deletion(s)). Moreover, 244 K whole-genome array–CGH was performed to exclude any large genomic rearrangements in those genes. Then, genomic DNA was amplified with primers specific to the 6 *IPMK*-coding exons and their intervening sequence boundaries. Polymerase chain reaction (PCR) used the *Taq*Man PCR Core Reagent Kit (Applied Biosystems™). Mutations were screened with bidirectional DNA sequencing of purified PCR products using the ABI BigDye® terminator sequencing kit (Applied Biosystems™) on an ABI Prism® 3130 automatic DNA sequencer (Applied Biosystems™). Sequences were aligned with Seqscape® analysis software (Applied Biosystems™). Primer oligonucleotide sequences and PCR conditions are available on demand.

Genome-wide 400 K array–CGH was performed to identify potential genetic rearrangements in tumor DNAs. To detect tumor-specific aberrations, each tumor DNA was individually hybridized on whole human-genome 400 K microarrays (Agilent Technologies™), using the patient's matched genomic leukocyte DNA as the reference. Arrays were scanned with a DNA microarray scanner (G2565BA, Agilent Technologies™). The results were visualized and analyzed with Feature Extraction® and CytoGenomics® (Agilent Technologies™) softwares.

3. Results

Between January 1, 2012 and December 31, 2013, 17 patients from 8 families were included in our cohort, with a median follow-up of 52 months (range 1–438) from initial diagnosis. Five (29%) patients had a history of a second cancer, which were different from SI-NET and consisted in breast cancer, colonic cancer, gastric cancer, tricholeucocytic leukemia and, most interestingly, pulmonary carcinoid, respectively. First-degree relatives with other malignancies were identified in 7/8 kindreds (Table 1). SI surgery was performed on 15/17 patients, including 14 for whom a histologic specimen was available. All SI-NETs were well differentiated, were mostly located at the terminal ileum, and were multiple in 57% of patients. The majority of SI-NETs displayed features of aggressiveness, including invasion beyond subserosa (T4 stage), vascular and/or perineural invasion, and lymph-node metastases.

Pedigrees were suggestive of autosomal-dominant inheritance with incomplete disease penetrance (Fig. 1). Among the 11 patients who were alive at the time of the study, 9 underwent blood sampling for genetic analysis and frozen histologic specimens of SI primary tumors were available for 5 patients, representing a total of 10 patients from 7 families. No *MEN1* or *VHL* gene abnormality, including large genomic rearrangements, was found in constitutional DNA. Furthermore, no *IPMK* coding-sequence mutation was found in germline or tumor-DNA samples from all 10 patients.

Table 1
Descriptive information for 17 patients from 8 families with familial SI-NETs.

	N = 17
Male sex, *n* (%)	9 (53)
Age at diagnosis, years, *mean* ± *SD* (*range*)	58 ± 14 (30–80)
Personal cancer history, *n* (%)	5 (29)
Familial cancer history in 1st-degree relatives, *n* (%)	15 (88)
Distant metastases, *n* (%)	14 (82)
Liver	13 (76)
Bone	5 (29)
Peritoneum	3 (18)
Lung	2 (12)
Breast	1 (6)
Pancreas	1 (6)
Carcinoid syndrome, *n* (%)	11 (65)
Somatostatin-receptor scintigraphy uptake, *n* (%)[a]	12 (100)
Largest primary tumor location, *n* (%)	
Ileum (without its terminal portion)	3 (18)
Terminal ileum	9 (53)
Ileocecal valve	5 (29)
Tumor multiplicity, *n* (%)[b]	8 (57)
No. of tumors, *mean* ± *SD* (*range*)[b]	8.7 ± 20.9 (1–80)
No. of tumors (without the case with 80 tumors), *mean* ± *SD* (*range*)[b]	3.2 ± 3.9 (1–14)
Largest tumor diameter (mm), *mean* ± *SD* (*range*)[b]	22.5 ± 10.7 (10–45)
Depth invasion, *n* (%)[b]	
T2	2 (14)
T3	3 (21)
T4	9 (64)
Vascular extension, *n* (%)[b]	11 (79)
Perineural invasion, *n* (%)[b]	10 (71)
Lymph-node invasion, *n* (%)[b]	13 (93)
Well-differentiated, *n* (%)	14 (100)
Tumor grade, *n* (%)[b]	
Grade 1	11 (79)
Grade 2	3 (21)

[a] 12 patients underwent somatostatin-receptor scintigraphy.
[b] No histologic sample was available for 3 patients.

Using 400 K high-resolution oligonucleotide array-CGH, genome-wide molecular characterization of the 5 frozen SI-NETs found chromosome-18 deletions in 4/5 tumors (Fig. 2). It was the only somatic alteration in 3/5 tumors (Fig. 1); 1/5 tumors also had a partial 16q deletion; in the remaining tumor, without chromosome-18 deletion, chromosome-11p and -11q were partially deleted. The suppressor genes *MEN1* (11q13), *TSC2* (16p13.3) and *CDH1* (16q11) were not located in the deleted regions. No other chromosome alterations were found in the 5 analyzed tumors, especially, no evidence of deletions in the regions carrying *IPMK* (10q21), *TP53*, *ATRX*, *DAXX*, *CDKN1B* or *RB1* loci.

4. Discussion

We report here the first European series of patients with familial SI-NETs, and confirm the existence of this previously unrecognized inherited entity. Overall, the clinical and histologic characteristics of patients with familial SI-NETs are apparently similar to those of patients with sporadic SI-NETs [6,8]. The frequent personal and familial histories of other cancers suggested a familial predisposition for malignancies [4]. The frequency of multiple SI-NETs (57%) was higher than usually reported for sporadic SI-NET

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