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Clinical Research Article

Prevalence of Endocrine Manifestations and GIST in 108 Systematically Screened Patients With Neurofibromatosis Type 1

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Abstract

Context: In patients with neurofibromatosis type 1 (NF1), guidelines suggest screening for pheochromocytoma by metanephrine measurement and abdominal imaging, which may lead to the discovery of gastroenteropancreatic neuroendocrine tumors (GEP-NETs) and their differential diagnosis, gastrointestinal stromal tumors (GISTs). Other endocrine manifestations such as follicular thyroid carcinoma and primary hyperparathyroidism have also been reported in a few cases.

Objective: This study aimed to describe prevalence and clinical presentation of these manifestations through systematic screening in a large cohort of patients.

Methods: In this monocentric retrospective study, 108 patients with NF1 were included and screened for endocrine manifestations and GISTs. Clinical, laboratory, molecular profile, pathology, and morphologic (abdominal computed tomography scan and/or magnetic resonance imaging) and functional imaging were collected.

Results: Twenty-four patients (22.2% of the cohort, 16 female, mean age 42.6 years) presented with pheochromocytomas that were unilateral in 65.5%, benign in 89.7%, and with a ganglioneural component in 20.7%. Three female patients (2.8% of the cohort, aged 42-63 years) presented with well-differentiated GEP-NETs, and 4 (3.7%) with GISTs. One patient had primary hyperparathyroidism, 1 patient had medullary microcarcinoma, and 16 patients had goiter, multinodular in 10 cases. There was no correlation between pheochromocytoma and other NF1 tumoral manifestations, nor correlations between pheochromocytoma and *NF1* genotype, despite a familial clustering in one-third of patients.

Conclusion: The pheochromocytoma prevalence in this NF1 cohort was higher (>20%) than previously described, confirming the interest of systematic screening, especially in young women. The prevalence of GEP-NETs and GISTs was about 3%, respectively. No phenotype–genotype correlation was observed.

Key Words: type 1 neurofibromatosis, pheochromocytoma, gastroenteropancreatic neuroendocrine tumor

Abbreviations: CT, computed tomography; GEP-NET, gastroenteropancreatic neuroendocrine tumor; GIST, gastrointestinal stromal tumor; MPNST, peripheral nerve sheath tumor; MN, metanephrine; MRI, magnetic resonance imaging; NET, neuroendocrine tumor; NF1, neurofibromatosis type 1; NMN, normetanephrine; PET, positron emission tomography; PHEO, pheochromocytoma; TSH, thyrotropin; US, ultrasound.

Neurofibromatosis type 1 (NF1) is the most common phakomatosis, with a prevalence of 1/4000 [1]. This autosomal dominant disease is caused by mutations in the *NF1* gene located on the long arm of chromosome 17 (17q11.2) [2]. This gene encodes for the protein neurofibromin, a GTPase-activating protein leading to the inactivation of

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RAS that dysregulates both the mitogen-activated protein kinase and phosphatidylinositol-3-kinase–Akt–mammalian target of rapamycin signaling pathways [3, 4]. The most prevalent manifestations of NF1 include skin neurofibromas, café-au-lait spots, iris hamartomas, and optic nerve glioma [5]. Additionally, NF1 predisposes individuals to cardiovascular diseases, orthopedic manifestations, neurologic features, and neoplasia, including malignant peripheral nerve sheath tumors (MPNSTs) or central nervous system tumors [6-8].

Pheochromocytoma (PHEO) is the most frequent endocrine manifestation of NF1 [9, 10], with a reported prevalence between 2.9% and 14.6% [11-13]. The only available prospective study found 7.7% of PHEOs in 156 patients screened systematically [14]. The American College of Medical Genetics (ACMG) recommends screening all patients over 30 years old, during pregnancy, and/or presenting with paroxysmal hypertension and/or hypertension associated with specific manifestations of PHEOs [15]. The French National Authority for Health guidelines recommend systematically screening all patients with symptoms, before surgery, pregnancy, or childbirth, and all patients over 35 years of age every 5 years in the entire NF1 population [16].

Sixty-three cases of neuroendocrine tumors (NETs) related to NF1 have been reported in the literature in the last 20 years [9]. Most of these NETs were gastroenteropancreatic (GEP) including duodenal somatostatinomas. The prevalence of these tumors is, however, unknown. Additionally, gastrointestinal stromal tumors, usually benign and predominantly located in the ileum/jejunum, have been described in patients with NF1 [17, 18].

Other endocrine diseases have been reported in patients with NF1, including an increased relative risk of thyroid cancer [19] and cases of primary hyperparathyroidism and pituitary adenoma [9]. It is unclear if these associations with NF1 are coincidental.

A better description of the prevalence and the clinical presentation of endocrine manifestations and GIST would help to refine the follow-up of patients with NF1. The aim of this study is to evaluate their prevalence in a cohort of patients with NF1 who had been systematically referred for endocrine screening at a reference center for rare diseases. Furthermore, the association between the endocrine manifestations and genotype of patients was studied.

Patients and Methods

Study Design

This retrospective, observational, single-center, cohort study was conducted in accordance with the methodology MR-003 of the French data protection authority. All patients with NF1 over 18 years of age who had been referred to the multidisciplinary competence center for NF1 at a large university hospital between January 2000 and July 2022 were included in the study.

Clinical, Laboratory, and Imaging Assessments

The diagnosis of NF1 was established using National Institute of Health criteria established in 1988 [5] and/or genetic analysis. In our institution, patients with NF1 were systematically offered a multidisciplinary assessment (cutaneous, ophthalmologic, neurologic, and endocrinologic). The standardized endocrine evaluation included a physical examination, and routine and hormonal laboratory testing for PHEOs, thyroid disorders, GEP-NETs, hyperparathyroidism, and pituitary gland dysfunction, as well as abdominal imaging. The following information were retrospectively extracted from patients' electronic medical files:

1. *Physical*: sex, age, body mass index, family history, café-au-lait spots, freckling, neurofibromas, iris hamartomas, history of neoplasia and/or neurologic disease,
2. *Laboratory testing*: plasma and/or urine methoxylated derivatives were measured by liquid chromatography coupled with tandem mass spectrometry and analyzed according the reference interval specific for age [20]. Chromogranin A, thyroid hormones (thyrotropin [TSH], free thyroxine, free triiodothyronine, calcitonin), and pituitary axis hormones (cortisol and adrenocorticotropin at 0800 hours and 2400 hours, insulin-like growth factor1, prolactin, follicle-stimulating hormone, luteinizing hormone, testosterone, and sex hormone–binding globulin [for men]) were measured using an immunochemiluminometric assay. Estradiol (for women) and digestive hormones (pancreatic polypeptide, glucagon, somatostatin, vasoactive intestinal peptide, gastrin) were measured using a radioimmunoassay.
3. *Imaging*: Helical computed tomography (CT) scan (MX Twin Flash, Marconi Medical Systems, Cleveland, OH, USA) before and after iodine contrast product injection and/or 1.5 Tesla magnetic resonance imaging (MRI) (Philips Intera, Best, The Netherlands) was performed, as well as thyroid ultrasound (US) with linear 9 to 13 MHz probes (Toshiba Aplio XG SSA790A, Tokyo, Japan). In case of abnormal hormonal or morphologic results, functional imaging (^{123}I-MIBG scintigraphy, ^{18}F-DOPA positron emission tomography [PET]-CT, octreoscan, ^{68}Ga-SSA PET-CT, and/or ^{18}FDG PET-CT) was performed.

Genetic Analysis

Genetic testing for the *NF1* gene was conducted in patients who provided written informed consent according to French law. Experiments were performed at the next-generation sequencing facility of Cochin Hospital (Public Assistance–Paris Hospitals), as previously described [21]. Briefly, all exons and flanking intronic regions of the *NF1* and *SPRED1* genes were amplified with a custom-made panel (IAD35072, Thermo Fischer Scientific) and sequenced on NextSeq 500 (Illumina). Every variant was validated through Sanger sequencing or multiplex ligation-dependent probe amplification, and quantitative polymerase chain reaction for gene dosage.

Definitions of Endocrine Manifestations and Tumors

The malignancy of PHEO was defined by the presence of lymph node or distant metastasis [22]. The aggressiveness of GEP-NETs was evaluated by the World Health Organization NET classification of 2019 [23]. Goiters were defined as a thyroid volume of 18 mL for women and 20 mL for men.

To investigate the phenotype–genotype correlation, a disease severity score was arbitrarily defined by counting the number of NF1 features. A score of 1 was assigned for each NF1 feature as a whole, including the presence of malignant or benign tumors, such as subcutaneous, internal, and/or plexiform neurofibromas. PHEOs were excluded from this score to analyze the tumor phenotype of patients according to the presence or absence of this condition.

Statistical Analysis

The statistical analysis was conducted using R software (version 4.0.5) and GraphPad Prism software (version 8.0.1.244). The normality of variable distributions was verified using a Shapiro–Wilk test. Quantitative variables were expressed as means (SD) when the distribution was normal, or as medians (interquartile range) when it was not. Categorial variables were expressed as number (percentage). To compare percentages, Fisher's exact test was used, and the t-test or Wilcoxon test was used to compare quantitative variables based on the normality of variables distribution. The level of statistical significance was set at $P < .05$.

Results

General Characteristics of the Cohort

A total of 113 patients with NF1 were referred to the endocrinology department over the 22.5-year period. Five patients refused evaluation and were excluded, resulting in a final sample of 108 patients from 91 index cases (Fig. 1). The mean age of the cohort was 37.5 ± 14.2 years and 63 (58.3%) of the patients were female (Table 1). One-third of patients had no family history of NF1.

General Manifestations of NF1

The majority (85%) of the patients had multiple café-au-lait spots and/or cutaneous neurofibromas, and 74.6% had freckling (Table 1). Only 10% of the cohort had no cutaneous manifestation. Iris hamartomas were present in 73% of patients. Of the 71 patients who underwent a whole-body MRI, 22.6% had plexiform neurofibromas, with half being single and half being multiple.

Five patients (4.8%) had a history of optic nerve glioma treated by surgery and radiotherapy. Schwannomas were observed in 5.5% of cases, as well as central nervous system tumors, including 3.7% of pilocytic astrocytomas. MPNSTs were observed in 2.7% and meningiomas in 2.9% of patients. Other neurologic features associated with NF1 were observed, including 8.3% of patients with epilepsy and 5.6% with migraine. Other tumoral manifestations such as breast, lung, and skin carcinomas, sarcomas, and blood malignancies were observed in 2% to 3% of the cohort.

Pheochromocytomas

Four patients had a previous history of surgically treated PHEO (unilateral for 3 patients and bilateral for 1). Abdominal imaging and plasma and/or urine methoxylated derivative measurements were performed for the entire cohort except in the patient already operated on for bilateral PHEO. Adrenal nodules were identified in 24 patients. Four of these 24 patients had imaging characteristics suggestive of an adrenal adenoma with spontaneous density <10 HU and normal methoxylated derivatives or other adrenocortical hormone levels. Consequently, they did not have functional imaging and were considered as incidental adenomas. The remaining 20 patients had hypervascular adrenal nodules, with elevated levels of methoxylated derivatives in 18 of them. Functional imaging (^{123}I-MIBG scintigraphy and/or ^{18}F-DOPA PET-CT) was positive in 20 patients who subsequently underwent surgery with pathologic diagnosis of PHEO.

Finally, 24 patients (22.2%; P1-P24) of the cohort were diagnosed with PHEO (Table 1). The mean age at diagnosis was 42.6 ± 16.5 years, and the proportion of females was significantly higher in patients diagnosed with PHEOs than in those without (66.7% vs 55.9%, $P < .001$). Of the 24 patients with PHEOs, 16 (66.7%) had elevated blood pressure and/or were being treated with antihypertensive drugs, compared with 20 patients (23.8%) of the group without PHEOs ($P < .001$). In the PHEO group, symptoms suggesting PHEO were described for 17 patients. When considering the association of both hypertension and clinical symptoms, only 2 patients with PHEO were totally asymptomatic.

In the PHEO group of 24 patients, plasma and/or urine MN or NMN level above 4, between 2 and 4, between 1- and 2-fold or below the upper limit of the normal was observed in respectively 14, 6, 2, and 2 patients. In the non-PHEO group of 84 patients, an increase in the metanephrine (MN) or normetanephrine (NMN) level above 2, between 1- and 2-fold ,or below the upper limit of the normal was observed in, respectively, 0, 14, and 70 patients. The mild increase in MN or NMN in some patients of the non-PHEO group was assigned to the stress of hospital admission or to drug interferences in some cases, since no abnormality was identified on abdominal imaging.

A total of 29 PHEOs were reported in 24 patients (Table 2). The median size of PHEOs was 32 mm (range from 5 to 158 mm). Nineteen patients had a single PHEO, 2 patients had 2 foci in their right adrenal gland, and 3 patients had bilateral PHEOs; all synchronous. Three PHEOs (10.3%) were malignant with node and/or distance metastasis. Seven PHEOs (24.1% of the 29 PHEO) had a ganglionic component, including 1 case of malignant PHEO. All cases of PHEOs were treated via adrenalectomy, most commonly via laparoscopy (87.5%).

Gastroenteropancreatic Neuroendocrine Tumors

Before the screening, 1 female patient (P25) was surgically treated at the age of 49 years for a jejunal NET discovered during the assessment of abdominal pain (Table 3). Abdominal imaging, performed in 108 patients, resulted in the detection of single or multiple GEP lesions in 9 patients including P25. These patients underwent ^{68}Ga-SSA PET-CT or octreoscan imaging, which was positive in 3 patients, including P25 who was aged 75 years at the time and refused any further investigations. The 2 other patients underwent echo-endoscopic biopsies, which led to the confirmation of a NET. The 2 patients diagnosed with NET (P2 and P26) were female, aged 42 and 63 years, and were surgically treated. P2 had a 20-mm somatostatinoma of the ampulla of Vater associated with venous and lymphatic tumor emboli. P26 had a well-differentiated, grade G2 (Ki67 10%), 50-mm duodenal NET, with lymph node invasion. She had metachronous liver metastasis treated firstly with somatostatin analog, then a Mitogen-activated Extracellular signal-regulated Kinase (MEK) inhibitor (Trametinib) before her death after 5 years of follow-up.

Enteropancreatic hormones were normal in 83 out of 87 measured patients, including the 2 patients diagnosed with a GEP-NET. Two patients had moderate increase of vasoactive intestinal peptide levels (38 and 72 pmol/L [normal range <30]) and 1 had elevated glucagon (464 pg/mL [normal range <250]) without abdominal abnormality on morphologic and functional imaging.

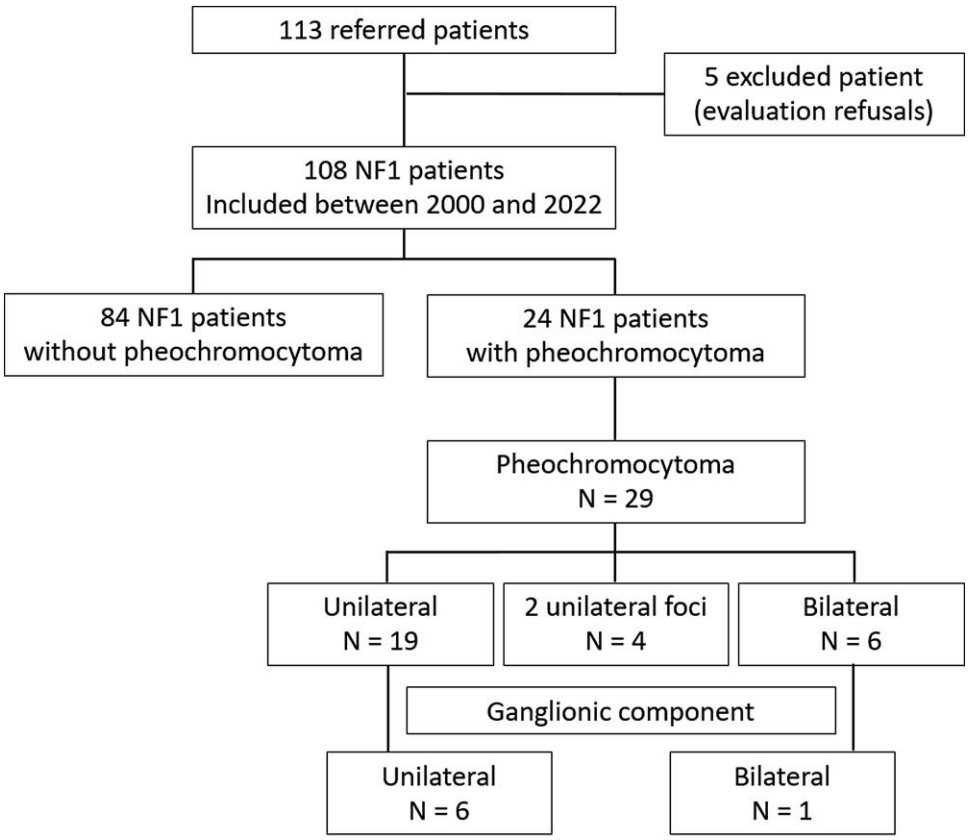

Figure 1. Flow chart of the study. NF1, neurofibromatosis type 1.

Table 1. Characteristics of the cohort

Characteristics of patients with NF1	Total cohort n = 108		Patients with pheochromocytoma n = 24		Patients without pheochromocytoma n = 84		
General							
Age (years)	108	37.5 ± 14.2	24	42.6 ± 16.5	84	36.1 ± 13.3	**$P < .05$**
Sex (F)	108	63 (58.3%)	24	16 (66.7%)	84	47 (55.9%)	**$P < .01$**
BMI (kg/m²)	107	24.5 ± 5.1	22	23.4 ± 4.5	84	24.8 ± 5.2	$P = .477$
NF1 familial history	105		23		82		
None		34 (33%)		5 (21.7%)		29 (35.4%)	$P = .193$
≥ 1 affected relative		71 (67%)		18 (78.3%)		53 (64.6%)	$P = .269$
Skin and ophthalmologic							
Café-au-lait spots	103	91 (88.3%)	20	18 (90%)	83	73 (88%)	$P = .492$
Cutaneous NF	104	96 (92.3%)	22	20 (90.1%)	82	76 (92.7%)	$P = .406$
Subcutaneous NF	100	58 (53.7%)	21	13 (61.9%)	79	45 (57%)	$P = .355$
Plexiform NF	77	18 (23.4%)	11	0 (0%)	66	18 (27.3%)	$P = .637$
Freckling	59	44 (74.6%)	13	9 (69.2%)	46	35 (76.1%)	$P = .600$
Iris hamartomas	73	46 (63%)	16	10 (62.5%)	57	36 (63.2%)	
Optic nerve glioma	104	5 (4.8%)	23	0 (0%)	81	5 (6.2%)	
High blood pressure	107	37 (34.6%)	24	16 (66.7%)	83	21 (25.3%)	**$P < .01$**

Data are presented as mean ± SD, n, or n (%). Comparisons were performed between patients with and without pheochromocytoma. Results significantly different were highlighted in bold.
Abbreviations: BMI, body mass index; NF, neurofibroma; NF1, neurofibromatosis type 1.

Table 2. Detailed data of patients with NF1 diagnosed with pheochromocytoma

Patient	Year of diag	Age at diag	Sex	HBP	Symptoms	Laboratory tests	Morphologic imaging	Functional imaging	Size (mm)	Histologic examination
1	2000	26	M	No	No	Urinary MN 1.8N, MNM 1.6N	Right (MRI)	Right (MIBG)	28	Benign pheo.
2	2003	42	F	No	Headache	Increased plasma methox. Derivates Urinary MN 5.2N, NMN 4.5N	Left (CT)	Left (MIBG)	5	Benign pheo.
3	2004	21	F	Yes	No	NA	Left (MRI)	NA	90	Malignant pheo. with lung metastasis
4	2008	27	M	No	Headache	Plasma MN 2.2N, NMN 1.6N Normal urinary methox. derivates	2 right foci (CT and MRI)	Right (FDOPA and FDG-PET)	15 and 17	2 foci of benign pheo. (Ki 67: 2%)
5	2009	20	F	Yes	Dyspnea, chest pain	Plasma MN 1.8N, NMN 3N Urinary MN 5N, NMN 3N	Right (CT)	Right (MIBG)	34	Benign pheo.
6	2009	43	F	Yes	Palpitation, flush	Plasma MN 20N, NMN 3N	Bilateral (CT and MRI)	Bilateral (MIBG)	R: 70; L: 40	Benign pheo.
7	2010	45	F	Yes	No	Plasma MN 2.4N, NMN 1.6N Urinary MN 1.6N, NMN 1.2N	2 right foci (CT and MRI)	Right (MIBG and FDG- PET)	15 and 10	Benign pheo.
8	2010	49	F	No	Palpitations dyspnea	Plasma MN 5N, NMN 1.3N Urinary MN 5.5N, NMN normal	Bilateral (CT)	Bilateral (MIBG)	L: 28; R: 25	Benign bilateral pheo. (Ki 67: 2%)
9	2011	66	F	Yes	Headache	Plasma MN 5N, NMN 2.7N	Left (CT and MRI)	Left (MIBG)	35	Benign pheo.
10	2011	55	F	Yes	Headache, anxiety, palpitations	Plasma MN 12.8N, NMN 3.8N Urinary MN 13N, NMN 1.5N	Left (CT and MRI)	Left (MIBG)	45	Benign pheo. (Ki 67: 4%)
11	2011	28	F	No	No	Normal plasma methox. derivates	Right (CT and MRI)	Right (FDOPA and FDG-PET)	25	Benign pheo. (Ki 67: 10%)
12	2011	56	M	Yes	Sweating	Plasma MN 4N, NMN 28N Urinary MN 6.8N, NMN 28N	Right (CT)	Right (MIBG and FDG-PET)	105	Encapsulated malignant pheo. with mesenteric, node and spine metastasis (Ki 67: 30%)
13	2012	25	F	Yes	Palpitations	Plasma MN 70N, NMN 21N Urinary MN 96N, NMN 278N	Left (CT)	Left (MIBG)	158	Malignant pheo. with node metastasis (Ki 67: 8%)
14	2012	22	F	No	Dyspnea	Plasma MN 1.9N, NMN normal Urinary MN 2.5N, NMN normal	Left (CT)	Left (MIBG and FDOPA-PET)	30	Benign pheo.
15	2012	32	F	Yes	Palpitations, sweating	Plasma MN 11N, NMN 13N Urinary MN 9N, NMN 5.8N	Bilateral (CT)	Bilateral (MIBG)	R: 40; L: 50	Benign pheo
16	2015	56	M	Yes	No	Increased plasma and urinary methox. derivates	Left (CT)	Left (MIBG)	40	Benign pheo
17	2015	68	M	Yes	Palpitations, weight lost	Plasma MN 3N, NMN 10N Urinary MN 2N, NMN 4.4N	Right (CT)	Right (FDG-PET)	55	Benign pheo

(*continued*)

Table 2. Continued

Patient	Year of diag	Age at diag	Sex	HBP	Symptoms	Laboratory tests	Morphologic imaging	Functional imaging	Size (mm)	Histologic examination
18	2017	34	M	Yes	No	Plasma MN 4.5N, NMN 4.4N Urinary MN 3.6N, NMN 1.4N	Right (CT and MRI)	Right (MIBG)	40	Benign pheo.
19	2018	75	F	Yes	No	Plasma MN 10N	Right (CT and MRI)	Right (MIBG)	32	Benign pheo. (Ki 67: 1%)
20	2018	59	M	Yes	No	Plasma MN 2.5N, NMN 4.4N Urinary MN 3.5N, NMN 3N	Right (CT)	Right (MIBG and FDG-PET)	17	Benign pheo. (Ki 67: 2%)
21	2018	45	M	Yes	Sweating and palpitations	Normal plasma and urinary methox. derivates	Left (CT and MRI)	Left (MIBG)	15	Benign pheo.
22	2019	61	F	No	Palpitations	Plasma MN 1.8N, NMN 1.2N; Urinary MN 1.5N, NMN 2N	Left (CT and MRI)	Left (MIBG)	10	Benign pheo. (Ki 67: 1%)
23	2021	31	F	Yes	Palpitations Headache	Plasma MN 200N, NMN 274N; Urinary MN 120N, NMN 87N	Right (CT and MRI)	Right (FDOPA-PET)	145	Benign pheo. (Ki 67: 1%)
24	2022	36	F	No	Palpitations, chest pain	Plasma MN 25.5N, NMN 6N; Urinary MN 20N, NMN 3N	Right (CT and MRI)	Right (FDG-PET)	85	Benign pheo. (Ki 67: 5%)

CGA, chromogranin A; CT, computerized tomography; diag., diagnosis; FDG, ^{18}fluorodeoxyglucose; FDOPA, ^{18}fluoro-dihydroxyphenylalanine; HBP, high blood pressure; Methox, methoxylated; MIBG, ^{123}I-metaiodobenzylguanidine; MN, metanephrine; MRI, magnetic resonance imaging; NA, not available; NMN, normetanephrine; PET, positron emission tomography; pheo, pheochromocytoma; PPI, proton pump inhibitor; F, female; M, male.

Table 3. Detailed data of patients with NF1 diagnosed with GEP-NET

Patient	Age	Year of surgery	Sex	Location	Size (mm)	Histologic examinations	Laboratory tests	Recurrence	Associated pathologies
25	49	1992	F	Small bowel	NA	NA	NA	2 suspicious nodules of small bowel	Mammary carcinoma, thyroidectomy for unknown reason
2	42	2003	F	Ampulla of Vater	20	Somatostatinoma, venous and lymphatic emboli	Normal pancreatic and gut hormone levels	No	Pheochromocytoma, uterine benign leiomyoma
26	63	2015	F	Duodenum	50	Well-differentiated NET, grade G2, lymphatic emboli Ki 67: 10%	Normal pancreatic and gut hormone levels	No	Mammary carcinoma

Abbreviations: GEP, gastroenteropancreatic; NA, not available; NET, neuroendocrine tumor; NF1, neurofibromatosis type 1.

Table 4. Detailed data of patients with NF1 diagnosed with a GIST

Patient	Age	Year of surgery	Sex	Location	Size [mm]	Histologic examinations	recurrence risk (class. of Joensuu and Miettinen)	Recurrence	Associated pathologies
7	52	2015	F	Ileum	NA	GIST, Ki 67: 2%	NA	No	Pheochromocytoma
27	42	2016	F	2 jejunal, 1 ileal	2 525 and 4	GIST well-differentiated, <5 mitoses/field	Low risk	No	Absence epilepsy
20	59	2017	H	Jejunal	2 to 5	Multiple GISTs, well-differentiated, only few mitotic figures	Low risk	No	Pheochromocytoma, small bowel schwannoma
28	59	2018	F	Gastric	70	Spined cells, 10 mitoses/field	High risk	No	Hashimoto disease

Abbreviations: GIST, gastrointestinal stromal tumors; NA, not available; NF1, neurofibromatosis type 1.

Finally, 2.8% (3/108) of patients of this cohort had a GEP-NET detected on abdominal imaging.

Gastrointestinal Stromal Tumors

One male patient (P20) had a history of multiple GISTs of the jejunum, diagnosed at age 59 years in the context of enteric ischemia. These GISTs were well-differentiated, with low mitotic count (Table 4). Six patients from whom GEP lesions were detected had negative ^{68}Ga-SSA PET-CT or octreoscan imaging. These 6 patients had a ^{18}FDG PET-CT that did not show hypermetabolism of the lesions, leading to the suspicion of GIST. Two female patients (P20 and P27), aged 42 and 59 years, underwent echo-endoscopic biopsies that confirmed the GIST. They were surgically treated. During the investigation, P7 was surgically treated in emergency for ileal necrosis, which confirmed GIST. No further exploration was performed for the remaining 3 patients.

Finally, a total of 3.7% (4/108) of the cohort had a histologically confirmed GIST. The patients were aged 41-59 years at diagnosis. Three patients had well-differentiated GIST of the small intestine, including 2 patients with multiple lesions. One patient had a single GIST in the stomach with atypical spindle cells and 10 mitoses per field. The risk of recurrence according to the classifications of Joensuu [24] and Miettinen [25] was low for P20 and P27, high for P28, and was not available for P7. Two patients (P7 and P20) also had PHEO.

Thyroid Axis

Seventy-nine patients of the cohort had a neck US. Twenty-three patients (41.7%) had nodules detected by US: 1 patient had 2 suspicious nodules (marked hypoechogenicity and irregular contours), 2 patients had moderate hypoechogenic nodules, and 20 patients had isoechogenic or hyperechogenic nodules. Two patients had a fine needle aspiration indication, which finally showed benign nodules (Bethesda II). Three patients (2.8%) underwent total thyroidectomies: 1 for toxic multinodular goiter, 1 for multinodular goiter with cervical discomfort, and 1 for 2 suspicious macronodules. Pathologic analysis revealed an incidental medullary microcarcinoma for the latter, while the 2 macronodules were benign. Sixteen patients (20.3%) had goiter detected by US: 6 homogeneous goiters and 10 multinodular goiters. TSH levels were normal in most patients (101/108), including 9 patients with levothyroxine supplementation at the first evaluation. Two patients had a TSH level in the low range and 4 patients had a slightly increased level that remained below 10 µUI/L

Primary Hyperparathyroidism

Plasma calcium and phosphorus levels were measured in 107 patients, including urine calcium parathyroid hormone and 25-$(OH)_2$-vitamin D in 78 patients. Only 1 female patient was diagnosed with primary hyperparathyroidism. US of the neck coupled with ^{123}I-MIBI scintigraphy and ^{18}F-choline PET-CT

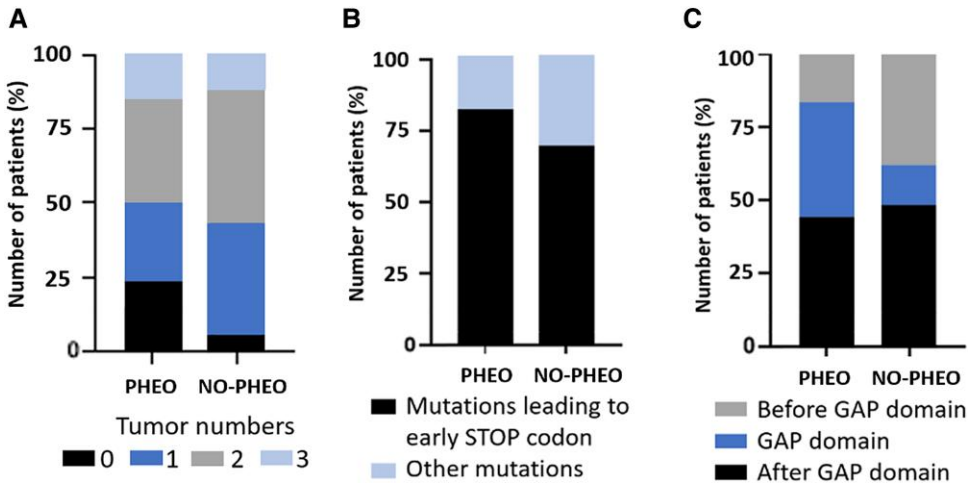

Figure 2. (A) Patients subgroups according to the presence of PHEO and the score of tumoral severity (1 point in presence of subcutaneous neurofibromas, 1 point in presence of internal and/or plexiform neurofibromas, 1 point for each type of benign tumor and 1 point for each type of malignant tumor). (B, C) Patient subgroups according to the presence of PHEO and respectively the *NF1* gene mutation type and the location of the mutation.

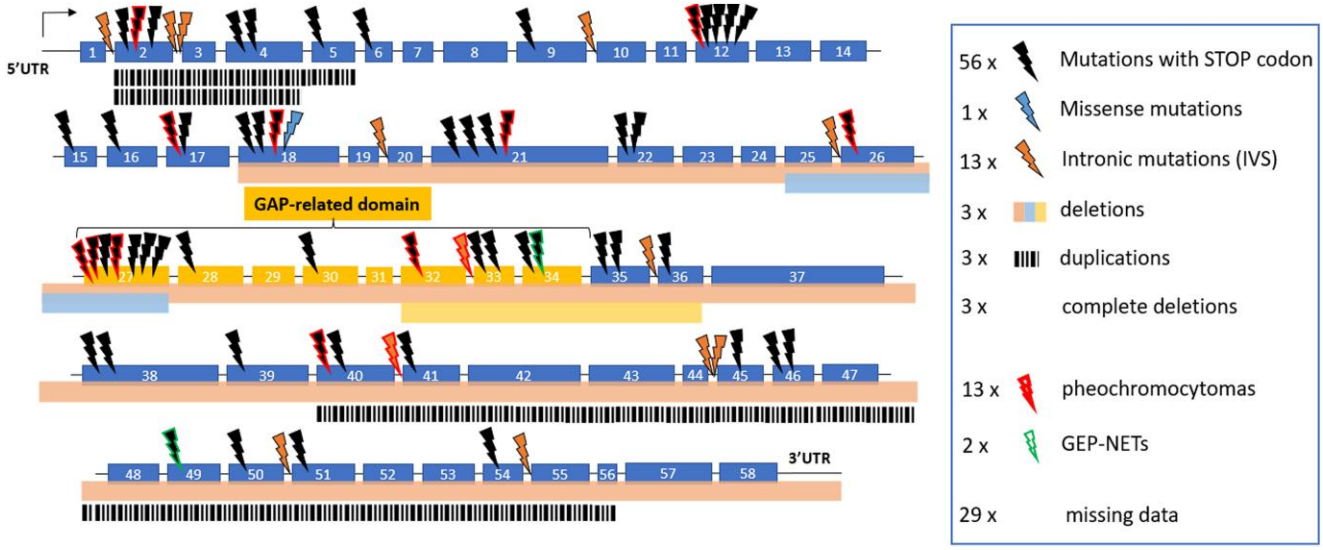

Figure 3. Schematic representation of the *NF1* gene with location and type of mutations of the 79/108 genotyped patients of the cohort.

found 2 parathyroid nodular lesions (1 upper left and 1 lower left). A variation of uncertain significance of the multiple endocrine neoplasia type 1 gene (class 3 according to the recommendations of the ACMG) was identified in this patient.

Pituitary Axis

One hundred and one patients had anterior pituitary hormone work-up. Additionally, 14 patients underwent pituitary MRI due to abnormal hormone levels. However, no pituitary adenoma was observed. One patient had thyrotropin, corticotropin, and gonadotropin deficiencies secondary to the management of an optic nerve glioma that had been treated since childhood.

Phenotype and Genotype Correlation

Because there is a skin phenotype at risk for MPNSTs [26], we investigated the association between the presence of a specific skin phenotype and the occurrence of PHEO, but found no association between the presence of a PHEO and the number of skin or ophthalmologic manifestations of NF1 (Table 1). The severity of the diseases assessed by the number of tumoral manifestations was not significantly different between patients with and without PHEO (Fig. 2A).

Genotyping was available for 79 patients, with 13/24 in the PHEO group and 66/84 in the non-PHEO group (Fig. 3). Small insertions or deletions (indel) represented 30.4% of the mutation, splice alterations accounting for 18.9% (with frameshift (n = 9) or in-frame (n = 6) consequences), nonsense mutation for 36.7%, missense mutations for 1.3%, deletion or duplication larger than 1 exon for 7.6% of patients, and complete deletion of the gene for 3.8% of patients. We also identified 1 deep intronic mutation with frameshift consequence. In total, over 95% of the mutations were truncated variants that resulted in a loss of function of the neurofibromin protein. We found no significant correlation between the location or type of mutations and the presence of a PHEO (Fig. 2B and 2C). Interestingly, 3 NF1 families had at

least 2 members with NF1-related PHEO, corresponding to about one-third of PHEOs.

Discussion

This study is the first real-life study to provide an overview of the spectrum of endocrine manifestation in patients with NF1 after systematic screening performed in a reference center. Prevalence of PHEO was 22.2% of the cohort while no patient was diagnosed with thyroid follicular carcinoma and only 1 patient with primary hyperparathyroidism. In addition, abdominal imaging led to diagnosis of GEP-NETs in 2.8% and GISTs in 3.7% of the patients.

The prevalence of PHEOs in our cohort was higher than previously reported in the literature (2.9-14.6%) [9, 11, 12, 27], while the prevalence of GIST was lower [28, 29]. Only 1 study has assessed prospectively the prevalence of PHEO in a series of 156 patients, with a prevalence of 7.7%. Interestingly, 58.3% of the patients were female in our cohort, which is slightly higher than in the prospective study [14]. Female predisposition to PHEO development has been reported independently of genetic background [30]. In addition, estrogen receptors have been described in neurofibromas, explaining their higher development during pubertal period and pregnancy in female patients [31]. Therefore, a particular role of estrogen on the development of PHEO in patients with NF1 cannot be excluded. Taken together, the high female proportion in our cohort could partially explain the high PHEO prevalence. In addition, our cohort (mean age 37.5 years) is slightly younger than in Képénékian's prospective cohort [14] (mean age 42.6) with a younger mean age of PHEO detection (42.6 years in our cohort vs 53-55 years in their cohort) [14]. Interestingly, the usual age of diagnosis of PHEO in NF1 is estimated to be between 40 and 45 years old [9], which is consistent with our results. Indeed, Képénékian et al excluded from their analysis patients with NF1 with a history of PHEO [14], on the one hand, but, on the other hand, also 78 out of the 234 patients initially included because both abdominal imaging and urinary methoxylated derivates were lacking. Moreover, of their 156 patients with full screening, 20 patients had only abdominal US for the imaging screening of adrenals [14]. Taken together, we can suppose that the prevalence of PHEO in the prospective study by Képénékian et al is underestimated by the lack of consideration of previous symptomatic PHEOs and lack of detection of nonsecreting PHEOs.

The description of the PHEOs in our cohort led to interesting findings. If 79.2% of the patients had unilateral a PHEO consistently with a previous study [9], 2 patients (8.3% of the patients with PHEO) had 2 foci of PHEO in the same adrenal gland. This has been reported to our knowledge in only 1 patient with NF1 [12]. The median size of the PHEO in our cohort was 32 mm, in the study by Képénékian et al 17.5 mm, while in a recent review of the literature it was estimated at 58 mm [9]. This suggests that systematic screening leads to earlier detection and management of PHEO. Additionally, 24.1% of PHEO cases had a ganglioneural component, which has been reported in only few cases in the literature [12, 32]. In a systematic review reporting 90 cases of PHEO with a ganglioneural component, 19% were found to have NF1 [33]. Ganglioneuromas, like PHEOs, are tumors originating from crest cells [34], and NF1 may participate in the development of adrenal ganglioneuroma. However, the mitogen-activated protein kinase and phosphatidylinositol-3-kinase pathways do not appear to be involved in the pathogenesis of ganglioneuromas [34]. Finally, in our cohort, only 10.3% of PHEOs were malignant as previously described [35].

The prevalence of GIST was at least 3.7% in our cohort as compared with 7% in a Swedish registry study [28] and 6% in a Japanese prospective cohort with systematic abdominal CT scan [29]. Interestingly, in the study by Képénékian et al, incidental GIST were diagnosed in 1.7% patients of the cohort [14]. Diagnosis of NF1-related GIST is often made after 50 years [17]. The lower prevalence observed in our cohort compared with the Swedish registry study [28] and the Japanese study [29] could be explained by the younger age of our cohort (mean age 37.5 years); however, the lower prevalence observed in the previous French prospective study [14] suggests that our cohort may have a more severe phenotype, which could also explain the higher prevalence of PHEO. Interestingly, while NF1-associated GISTs are usually indolent with a low mitotic rate and a good prognosis [16, 29], histopathologic report showed a high mitotic index rate suggestive of high risk of recurrence in 1 patient of the present cohort. Of note is the multiplicity of GISTs in some patients, raising differential diagnosis with ileal NET.

Our study is the first to estimate the prevalence of GEP-NETs discovered on abdominal imaging in a large cohort of patients with NF1. The prevalence observed in our cohort (2.8%) was notably lower than the reported prevalence of 12% in von Hippel–Lindau syndrome and over 30% in patients with multiple endocrine neoplasia type 1 who have reached 40 years of age [36, 37]. Consistently with our findings, NF1-related GEP-NETs are usually located in the ampulla of Vater and then the duodenum, and are well differentiated [9].

Our results do not support the hypothesis of an increased risk of thyroid carcinomas in patients with NF1. Additionally, the prevalence of goiter and nodules in our cohort was similar to that observed in the general population [38]. Interestingly, 1 patient in our cohort was diagnosed with thyroid medullary carcinoma and another with primary hyperparathyroidism. The association of these endocrine diseases and NF1 has been described in a few case reports [9, 39, 40], but the pathophysiologic link has not been proven. Unfortunately, we could not analyze the tumor of the patients to look for a second hit in the *NF1* gene as observed for tumor suppressor genes and reported in tumor-related NF1 [41].

In addition to the phenotype of this cohort, NF1 genotyping data were available for most patients, which is not frequent in a disease easy to recognize clinically. The prevalence of missense mutations seems to be lower in our cohort (1.3%) than in a large French cohort (7%) [42], with consistent results for the prevalence of other types of genetic alterations [42]. Also, most of the mutations identified in our cohort were truncated variants that generated a loss of function of neurofibromin, potentially leading to a more severe phenotype. However, we did not find any genotype–phenotype correlation in our cohort. This might be related to a lack of potency related to the relatively small size of our series. Nevertheless, to the best of our knowledge, no genotype–phenotype correlation has been shown in NF1, including in a recent large French database study of 439 patients with NF1 [42]. Note, however, that endocrine manifestations, especially PHEO, the most prevalent type, were not included in the manifestations analyzed in this French database study

[42]. Here, we did not observe endocrine manifestations in the 9 patients with large or complete gene deletions. We did not observe correlation between the type or the location of the *NF1* mutations and the presence of PHEO. Further studies are needed in larger cohorts to confirm the absence of correlation between PHEO and patients' genotype.

Due to the retrospective design of the study, we cannot exclude selection bias leading to an overestimation of the prevalence of PHEO in our cohort. In addition, as a large referral center, we cannot exclude that patients of our cohort may have shown more serious forms than in other centers. Another limitation is the absence of systematic ^{68}Ga-SSA PET-CT, echo-endoscopy, and gastroduodenal fibroscopy, which might underestimate the prevalence of GEP-NETs and GISTs. Nevertheless, besides these possible biases, our study might also be a real-life picture, especially of the prevalence of PHEO in NF1 in a specific area (north of France). This prevalence may be higher than in other regions for some geographical, environmental, social (large families), or genetic (modifier genes) factors, modulating the expression of NF1 disease.

Conclusion

In patients with NF1, systematic screening of PHEO in real life disclosed high prevalence (>20%) of this tumor diagnosed at a younger age and at a smaller size. Systematic abdominal imaging also led to a significant detection rate of GEP-NETs and GISTs. Early detection of these tumors and subsequent management would limit the occurrence of complications in these patients and confirm the pertinence of the new guidelines. Radiologists should be informed that GET-NETs and GISTs are some of the NF1 manifestations that can be detected on abdominal imaging. Other rare endocrine manifestations, such as thyroid carcinoma and primary hyperparathyroidism, may be sporadic and do not require systematic investigation unless suggestive signs are present. We did not observe genotype–phenotype correlations for endocrine manifestations, despite a trend toward familial clustering of PHEOs.

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Disclosures

The authors declare that they have no competing interest concerning the topic of this manuscript.

Data Availability

The authors can provide original data on demand.

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