



## *Annexes*

## ANNEXE 1

Duong TA, **Sbidian E**, Valeyrie-Allanore L, Vialette C, Ferkal S, Hadj-Rabia S, Glorion C, Lyonnet S, Zerah M, Kemlin I, Rodriguez D, Bastuji-Garin S/Wolkenstein P. **Mortality associated with Neurofibromatosis 1: a cohort study of 1895 patients in 1980-2006 in France.** *Orphanet Journal of rare diseases*, 2011, 4:6-18

RESEARCH

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# Mortality Associated with Neurofibromatosis 1: A Cohort Study of 1895 Patients in 1980-2006 in France

Tu Anh Duong<sup>1,2</sup>, Emilie Sbidian<sup>1,3,5</sup>, Laurence Valeyrie-Allanore<sup>1,4,5</sup>, Cédric Valette<sup>6</sup>, Salah Ferkaï<sup>3,4,7</sup>, Smail Hadj-Rabia<sup>8</sup>, Christophe Glorion<sup>9</sup>, Stanislas Lyonnet<sup>10</sup>, Michel Zerah<sup>11</sup>, Isabelle Kemlin<sup>12</sup>, Diana Rodriguez<sup>12,13</sup>, Sylvie Bastuji-Garin<sup>3,4,5†</sup> and Pierre Wolkenstein<sup>1,4,5†</sup>

## Abstract

**Background:** Neurofibromatosis 1 (NF1), a common autosomal dominant disorder, was shown in one study to be associated with a 15-year decrease in life expectancy. However, data on mortality in NF1 are limited. Our aim was to evaluate mortality in a large retrospective cohort of NF1 patients seen in France between 1980 and 2006.

**Methods:** Consecutive NF1 patients referred to the National French Referral Center for Neurofibromatoses were included. The standardized mortality ratio (SMR) with its 95% confidence interval (CI) was calculated as the ratio of observed over expected numbers of deaths. We studied factors associated with death and causes of death.

**Results:** Between 1980 and 2006, 1895 NF1 patients were seen. Median follow-up was 6.8 years (range, 0.4-20.6). Vital status was available for 1226 (65%) patients, of whom 1159 (94.5%) survived and 67 (5.5%) died. Overall mortality was significantly increased in the NF1 cohort (SMR 2.02; CI, 1.6-2.6;  $P < 10^{-4}$ ). The excess mortality occurred among patients aged 10 to 20 years (SMR 5.2; CI, 2.6-9.3;  $P < 10^{-4}$ ) and 20 to 40 years (SMR 4.1; 2.8-5.8;  $P < 10^{-4}$ ). Significant excess mortality was found in both males and females. In the 10-20 year age group, females had a significant increase in mortality compared to males (SMR 12.6; CI, 5.7-23.9; and SMR 1.8; CI, 0.2-6.4; respectively). The cause of death was available for 58 (86.6%) patients; malignant nerve sheath tumor was the main cause of death (60%).

**Conclusions:** We found significantly increased SMRs indicating excess mortality in NF1 patients compared to the general population. The definitive diagnosis of NF1 in all patients is a strength of our study, and the high rate of death related to malignant transformation is consistent with previous work. The retrospective design and hospital-based recruitment are limitations of our study. Mortality was significantly increased in NF1 patients aged 10 to 40 years and tended to be higher in females than in males.

## Background

Neurofibromatosis 1 (NF1; MIM#162200) is an inherited autosomal dominant disorder with an incidence of 1 in 2500-3000 births [1]. NF1 is fully penetrant by 8 years of age. According to the National Institutes of Health (NIH), the diagnosis of NF1 requires at least two of the following seven criteria: six or more café-au-lait spots measuring at least 5 mm in prepupertal patients and 15

mm in postpubertal patients, multiple axillary freckles, two or more neurofibromas (NFs) of any type or one plexiform neurofibroma, Lisch hamartomas, optic pathway glioma, bone dysplasia, and at least one affected first-degree relative [2]. The phenotype of NF1 varies substantially across patients.

The NF1 gene on chromosome 17q11.2 encodes the tumor suppressor protein neurofibromin. Loss of this protein is associated with an increased risk of developing tumors [3]. In a 12-year follow-up study of 70 adult NF1 patients in Sweden, life expectancy was decreased by 15 years compared to the general population, and

\* Correspondence: pierre.wolkenstein@hmn.aphp.fr

† Contributed equally

<sup>1</sup>AP-HP, Hôpital Henri-Mondor, Service de Dermatologie, F-94010 Créteil, France

Full list of author information is available at the end of the article



malignancy was the main cause of death [4]. A 1986 study in a Danish cohort of 212 patients diagnosed 42 years earlier showed excess mortality with an increase in malignancies in the males but not in the females, compared to the general population [5]. Several clinical features such as internal or subcutaneous NFs have been shown to predict mortality in NF1 patients [6,7]. In a cohort of 448 NF1 patients in the UK, the overall risk of cancer was increased 2.7-fold compared to the general population, and malignant peripheral nerve sheath tumor (MPNST) was the leading cause of death [8]. A proportional mortality study based on death certificate data in the US from 1983 to 1997 demonstrated that NF1 patients were 34 times more likely to have a malignant connective or soft tissue neoplasm listed on their death certificate than individuals without NF1 persons (proportional mortality rate, 34.3; 95% confidence interval [95%CI], 30.8-38) [9]. This study also showed an about 15-year decrease in life expectancy in NF1 patients [9].

Nevertheless, data on mortality in NF1 are limited. Our aim was to evaluate mortality in a large cohort of patients with NF1 seen in France between 1980 and 2006. We computed standardized mortality ratios (SMRs). We evaluated risk factors for death and causes of death.

## Patients and Methods

### Definitions and study cohort

The study cohort was composed of consecutive patients meeting NIH criteria for NF1 [2] who were referred to the hospital departments of the Paris conurbation that constitute the National French Referral Center for Neurofibromatosis (dermatology departments at the Henri Mondor and Necker-Enfants Malades hospitals and neuropsychiatric department at the Trousseau hospital). We identified NF1 patients referred to these departments between January 1, 1980, and December 31, 2006, in the NF1 Network Database maintained by the National French Hospital Database (PMSI). Data from all sources were linked and compared to eliminate duplications and resolve discrepancies.

The study was approved by the Ile-de-France IX ethics committee, Paris, France. The study complied with Helsinki guidelines.

### Data collection

For each patient, age at the beginning of the study period, sex, and clinical features were abstracted from the medical charts and/or database. Detailed information was available on the cardinal dermatological features of NF1 (café-au-lait spots, freckles, Lisch nodules, and cutaneous and plexiform neurofibromas). We also recorded the following features as present or absent:

orthopedic complications (scoliosis and pseudoarthrosis), neurological abnormalities (hydrocephalus and high T2 signal intensity lesions on magnetic resonance imaging of the brain), and renal artery stenosis. We recorded all available data on tumors known to occur frequently in NF1 patients (central nervous systems tumors, MPNSTs, and pheochromocytomas).

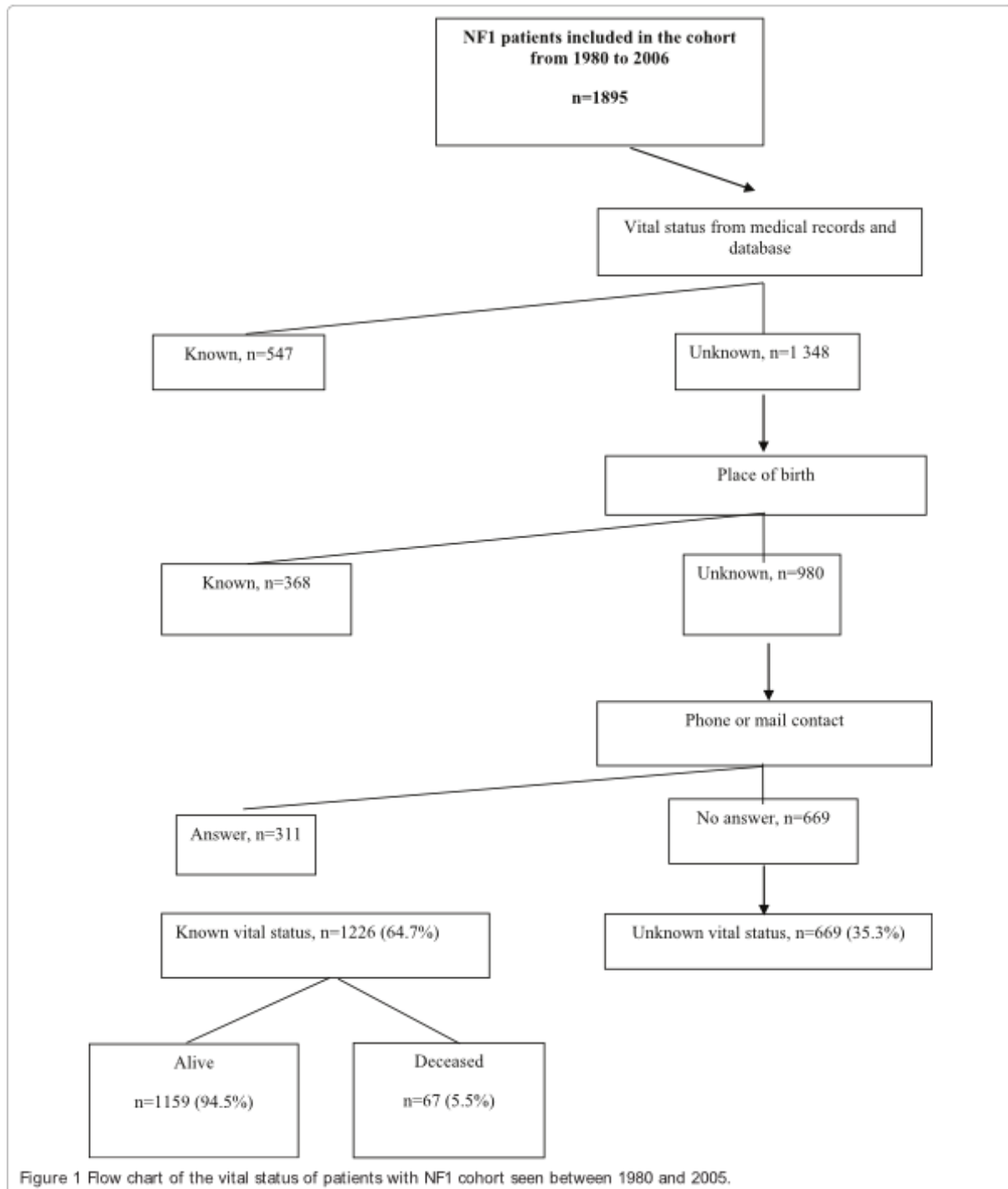
### Mortality assessment

Our goal was to determine vital status as of December 31, 2006. We first consulted the medical records and network database. Then, we searched the National French Mortality Database (INSERM CépIDC) for information on patients whose place of birth was known; we checked this information by calling the town hall of the place of birth. Finally, we sought to contact patients by phone and mail. Patients whose vital status was still unknown after these three steps were classified as having an unknown vital status (unknownVS group) and other patients as having a known vital status (knownVS group) (Figure 1).

### Statistical analysis

Data were double entered and analyzed using STATA software version 11 (Stata Corporation, College Station, TX). Quantitative variables are reported as median (range) and qualitative variables as number (%).

We compared the unknownVS and knownVS groups using univariate analysis (chi-square test, or Fisher exact test where appropriate, and nonparametric Mann-Whitney test). Because age influences the prevalence and number of NF1 features as café-au-lait spots and neurofibromas, we computed age-adjusted odds ratios (aORs) with their 95% confidence intervals (95%CI) separately for each variable. In the knownVS group, we computed the standardized mortality ratio (SMR) as the ratio of observed over expected deaths. SMR values greater than 1 with a CI that does not include 1 indicate excess mortality. The expected number of deaths was obtained by applying the mortality rates in France from 1980 to 2006 (by 5-year age groups and 5-year calendar periods, separately in females and males) to the appropriate person-years in the cohort. We considered the following age groups: younger than 10 years, 10-20 years, 20-40 years, 40-60 years, and 60 years or older. The 95%CI of the SMR was computed assuming a Poisson distribution [10]. The chi-square test of dispersion of the Poisson distribution was calculated. Sensitivity analyses were performed. First, to avoid overestimation of mortality, we computed the SMR for the overall cohort, assuming that patients in the unknownVS group were alive on December 31, 2006. Second, to minimize survivorship bias (bias related to underestimation of mortality in childhood due to patient attrition), we computed the



SMR for patients who were born after January 1, 1980, the beginning of our study period.

## Results

### Study cohort

Between January 1, 1980, and December 31, 2006, 1895 NF1 patients were seen at the National French Referral Center for Neurofibromatosis. Their median follow-up was 6.8 years [0.4-20.6]. At inclusion, median age was 17.7 [0-76] years, and 549 (44.8%) patients were younger than 18 years (Table 1). At the end of the study period, median age was 25.9 years [2-82].

Vital status was known for 1226 (65%) of the 1895 patients (Figure 1). At last follow-up, patients in the unknownVS group had a median age of 25.4 years [0.2-68] (Table 1). Compared to the unknownVS group, the knownVS group had significantly higher prevalence of freckles (aOR, 1.7; 95%CI, 1.3-2.2) and MPNSTs (aOR, 2.7; 95%CI, 1.6-4.7) (Table 1). The prevalence of optic pathway gliomas was nonsignificantly higher in the knownVS group (OR, 1.3; 95% CI, 0.9-1.7; P = 0.11).

Of the 1226 patients in the knownVS group, 1 159 (94.5%) survived and 67 (5.5%) died.

### Causes of death

The median age at death was 31.7 years [0-79.9], and 46.3% of patients who died were males. We did not reported any death from the same family. Compared to

survivors, nonsurvivors had non significantly fewer café-au-lait spots (aOR, 0.6; 95%CI, 0.3-1.1) and significantly more neurofibromas (aOR, 3.1; 95%CI, 1.2-8.4), plexiform neurofibromas (aOR, 1.8; 95%CI, 1.1-3.0), MPNSTs (aOR, 31.2; 95%CI, 17.4-55.9) and pheochromocytomas (aOR, 4.3; 95%CI, 1.1-16.3; Table 2).

The cause of death was known for 58 (86.6%) patients and was related to NF1 in 56 (96.6%) of them. These fatal complications of NF1 included MPNSTs (n = 33, 60%), central nervous system tumors (n = 8, 14%), spinal cord compression by neurofibromas (n = 2, 3%), organ compression by neurofibromas (n = 5, 9%), and pheochromocytomas (n = 2, 3%).

### Standardized mortality ratios

Compared to the general population, overall mortality was significantly increased in the NF1 cohort (SMR, 2.02; 95%CI, 1.6-2.6; P < 10<sup>-4</sup>). The excess mortality was found in two age groups, 10-20 years (SMR, 5.2; 95%CI, 2.6-9.3; P < 10<sup>-4</sup>) and 20-40 years (SMR, 4.1; 95%CI, 2.8-5.8; P < 10<sup>-4</sup>; Table 3). Significant excess mortality was observed in both males (SMR, 1.8; 95% CI, 1.2-2.5; P = 0.01) and females (SMR, 2.9; 95%CI: 2.0-4.0; P < 10<sup>-4</sup>; Table 4). In the 10-20 year age group, mortality is higher in females (SMR, 12.6; 95%CI, 5.7-23.9) than in males (SMR, 1.8; 95%CI: 0.2-6.4).

Assuming that patients in the unknownVS group were alive at last follow-up did not substantially change the

Table 1 Characteristics of the NF1 patients according to whether their vital status was known or unknown

Clinical features*	KnownVS group n = 1226	UnknownVS group	OR (95%CI) ‡	P value
	No (%)†	No (%)†		
Age, years (n = 1893)	25.9 [2-82]□	25.4 [0.2-68]□	-	0.19
Male gender (n = 1 894)	579 (47)	304 (46)	0.9 (0.8-1.1)	0.57
Familial case (n = 1382)	515 (57)	278 (57)	1.0 (0.8-1.3)	0.95
At least 6 café-au-lait spots (n = 1889)	1 046 (86)	584 (87)	0.9 (0.7-1.2)	0.56
Freckles (n = 1 871)	1 043 (86)	509 (78)	1.7 (1.3-2.2)	<10 <sup>-4</sup>
Neurofibromas (n = 1879)	923 (76)	495 (75)	1.0 (0.8-1.3)	0.87
Plexiform neurofibromas (n = 1865)	361 (30)	175 (27)	1.2 (0.9-1.4)	0.20
Lisch nodules (n = 1463)	382 (40)	204 (40)	1.1 (0.8-1.2)	0.87
Renal artery stenosis (n = 1361)	5 (1)	3 (1)	0.7 (0.2-3.1)	0.70
Scoliosis (n = 1373)	231 (25)	104 (24)	1.1 (0.8-1.4)	0.72
Nonunion (n = 1835)	45 (4)	20 (3)	1.2 (0.7-2.0)	0.57
Hydrocephalus (n = 1827)	32 (3)	23 (4)	0.7 (0.4-1.2)	0.22
OBNI (n = 801)	210 (40)	105 (38)	1.0 (0.7-1.4)	0.96
Optic pathway glioma (n = 1812)	161 (14)	69 (11)	1.3 (0.9-1.7)	0.11
MPNSTs (n = 1842)	80 (7)	16 (3)	2.7 (1.6-4.7)	<10 <sup>-4</sup>
Pheochromocytoma (n = 1836)	13 (1)	7 (1)	1.3 (0.5-3.7)	0.61

Abbreviations: VS, vital status; OR, odds ratio; 95%CI, 95% confidence interval; OBNI, high T2-signal intensity lesions on magnetic resonance imaging of the brain; MPNST, malignant peripheral nerve sheath tumor.

\* Values in parentheses are the numbers of patients for whom data were available.

□ Age at the end of the study period in patients whose vital status was known and age at last follow-up in those whose vital status was unknown status

† Percentages may not total 100, because of missing data.

‡ The ORs were adjusted for age.

Data are numbers (%) or median [range].

**Table 2** Characteristics of the survivors and nonsurvivors among the 1226 NF1 patients whose vital status was known

Clinical features*	Survivors	Nonsurvivors	OR (95%CI)‡	P value*
	n = 1159 No (%)†	n = 67 No (%)†		
Age, years, median [range]	25.5 [2-74]	31.7 [0-80]	-	0.008
Male gender	548 (47)	31 (46)	1.0 (0.6-1.7)	0.98
Familial case (n = 897)	498 (58)	17 (53)	0.7 (0.3-1.4)	0.62
At least 6 café-au-lait spots (1220)	998 (86)	48 (74)	0.6 (0.3-1.1)	0.07
Freckles (n = 1217)	985 (86)	58 (88)	1.1 (0.5-2.4)	0.74
Neurofibromas (n = 1215)	863 (75)	60 (90)	3.1 (1.2-8.4)	0.02
Plexiform neurofibromas (n = 1209)	333 (29)	28 (42)	1.8 (1.1-3.0)	0.02
Lisch nodules (n = 954)	366 (40)	16 (40)	0.9 (0.5-1.9)	0.89
Renal artery stenosis (n = 601)	5 (1)	0 (0)	-	0.60
Scoliosis (n = 934)	222 (25)	9 (18)	0.6 (0.3-1.3)	0.23
Nonunion (n = 1 194)	43 (4)	2 (3)	0.7 (0.2-3.2)	0.70
Hydrocephalus (n = 1 190)	31 (3)	1 (2)	0.6 (0.1-4.4)	0.60
OENI (n = 526)	202 (40)	8 (30)	0.6 (0.3-1.5)	0.30
Optic pathway glioma (n = 1 172)	151 (13)	11 (17)	1.2 (0.6-2.6)	0.58
MPNST (n = 1198)	44 (4)	36 (55)	31.2 (17.4-55.9)	<10 <sup>-4</sup>
Pheochromocytoma (n = 1193)	10 (1)	3 (5)	4.3 (1.1-16.3)	0.03

Abbreviations: OR, odds ratio; 95%CI, 95% confidence interval; OENI, high T2-signal intensity lesions on magnetic resonance imaging of the brain; MPNST, malignant peripheral nerve sheath tumor.

\*Values in parentheses are the numbers of patients for whom data were available.

†Percentages may not total 100, because of missing data.

‡The ORs were adjusted for age.

Data are numbers (%) or median [range].

excess mortality in patients aged 10 to 20 years (SMR, 3.3; 95%CI, 1.6-5.8;  $P = 0.01$ ) or 20 to 40 years (SMR, 2.4; 95%CI, 1.6-3.4;  $P = 0.001$ ). When we confined the analysis to the 633 patients born after January 1, 1980, we found excess mortality in patients aged 10 to 30 years (SMR, 3.9; 95%CI, 2.3-6.3;  $P < 10^{-4}$ ). The excess mortality in this subgroup seemed higher than in the overall cohort.

### Discussion

Significant excess mortality was found in our overall cohort and in the subgroups aged 10 to 20 and 20 to 40 years. We did not find excess mortality in the subgroups aged more than 40. It could be related to lack of power due to a very low number of persons over 60. Excess mortality was significant in both females and males; in the 10-20 year age group, excess mortality was significantly higher in the females than in the males. The vast

majority of deaths were due to malignancies and other complications of NF1.

We retrospectively identified consecutive NF1 patients referred to the French National Referral Center for Neurofibromatoses. An important strength of our study is that all patients had a definitive diagnosis of NF1. Furthermore, the overall prevalence of NF1 manifestations in our cohort was consistent with previous reports, [11] indicating that our cohort was representative of the NF1 population. We used multiple sources of information to determine vital status. Nevertheless, vital status was known for only about two-thirds of the patients, which may have resulted in information bias. However, to avoid overestimating mortality, we conducted an analysis assuming that all patients whose vital status was unknown were alive at last follow-up. We again found significant excess mortality among NF1 patients aged 10 to 40 years compared to the general population.

**Table 3** Number of deaths (n) and SMRs among the NF1 patients by age group

Age, years	Person- Years	Observed Deaths	Expected Deaths	SMR	95%CI
<10	11 905.5	4	42	0.95	0.3-2.4
10 to <20	8875	11	21	5.2	2.6-9.3
20 to <40	10 395	31	7.6	4.1	2.8-5.8
40 to <60	3657.5	11	11.6	0.9	0.5-1.7
≥60	480	10	7.7	1.3	0.6-2.4

SMR, standardized mortality ratio (observed deaths/expected deaths); 95%CI, 95% confidence interval.

**Table 4** Number of deaths (n) and SMRs among the NF1 patients, by age categories (years), by sex

Age, years	Person-Years	Male				Female				
		Observed Deaths	Expected Deaths	SMR	95%CI	Person-Years	Observed Deaths	Expected Deaths	SMR	95%CI
<10	5569.5	1	1.8	0.6	0.01-3.1	6336	3	1.8	1.7	0.3-4.8
10 to <20	4000	2	1.1	1.8	0.2-6.4	4875	9	0.7	12.6	5.7-23.9
20 to <40	4262.5	19	4.4	4.3	2.6-6.7	6132.5	12	2.6	4.7	2.4-8.2
40 to <60	1272.5	5	5.5	0.9	0.3-2.1	2385	6	4.8	1.3	0.5-2.7
≥60	162.5	4	4.8	0.8	0.2-2.1	317.5	6	2.7	2.3	0.8-4.9

Another limitation of our study is the retrospective design, which may have resulted in survivorship bias with underestimation of mortality due to higher values for both patient attrition and mortality in the older patients. However, when we conducted an analysis confined to patients born after the beginning of our study period, we still found significant excess mortality compared to the general population. Finally, our hospital-based recruitment may have led to a bias toward patients with more severe forms of NF1 and, therefore, to overestimation of the SMR. However, our findings are consistent with previous reports [4,5,9].

Among the 58 patients who died and in whom the cause of death was known, 56 died from NF1 complications, chiefly malignancies (MPNSTs in 60% and cerebral tumors in 14%). Our results are consistent with those of a prospective study of the incidence of cancer in NF1 patients in the UK [8]. In this study, the overall risk of cancer was 2.7 times higher in NF1 patients than in the general population, and increases were found in central nervous system tumors and connective tissue tumors, as well as in breast cancer in women younger than 50 years (standardized incidence ratio, 4; 95%CI, 1.1-10.3). The risk of mortality related to breast cancer was not reported. In our study, we did not assess the prevalence of breast cancer, which was not the reported cause of death in any of the patients. Similarly, in a death certificate study done in the US, NF1 patients were 34 times more likely to have a malignant connective or other soft tissue neoplasm listed on their death certificate than patients without NF1 [9]. The proportionate mortality ratios for malignancies were 6.07 for patients who died between 10 and 19 years of age and 4.93 for those who died between 20 and 29 years of age. Proportionate mortality ratios were also increased for central nervous system tumors and vascular disease. The authors concluded that the impact of NF1 on mortality from malignancies and vascular disease was focused on patients younger than 40 years of age [9]. In our cohort, cardiovascular disease was not reported as a cause of death. Our chart review did not assess cardiovascular morbidity and we also did not estimate the cardiovascular risk in NF1 patients.

In our study, MPNSTs were the leading cause of death (n = 33, 60%). NF1 is widely recognized as a risk factor for MPNSTs, and the lifetime risk of MPNSTs in NF1 patients has been estimated at 8%-13% [1,12]. In an earlier study, we also found that MPNST was the main cause of death and that risk factors for MPNST were the absence of cutaneous neurofibromas, presence of at least two subcutaneous neurofibromas, and facial asymmetry [6]. Further studies confirmed that subcutaneous neurofibromas predicted mortality (OR, 3.6; 95%CI, 1.2-11.3) [7] and that internal plexiform neurofibromas were strongly associated with MPNST (OR, 18.06; 95% CI, 4.55-73.4) [13]. We did not have detailed information on these features in our patients and were therefore unable to assess their potential relationship to mortality. Nonsurvivors had fewer café-au-lait spots than did survivors. Furthermore, excess mortality was confined to patients aged 10 to 40 years. These two variables are among the four used in the NF1 score for predicting internal neurofibromas (age ≤30 years, fewer than six café-au-lait spots, no cutaneous neurofibromas, and two or more subcutaneous neurofibromas) [14]. Internal neurofibromas are strongly associated with MPNSTs [14].

In our study, median age at death was 31.7 years [0-77.9], that is, considerably younger than in previous studies. However, our patients were young, with a median age of 25.9 years at the time of the analysis. In a Japanese death certificate study, mean age at death from neurofibromatosis was 43 years, but NF1 and NF-2 were not clearly separated [15]. In a death certificate study done in the US, mean age at death in NF1 patients was 54.4 years, that is, 15.7 years younger than in the general population [9]. In this study, the authors used multiple-cause mortality files compiled from US death certificates and identified 3770 cases of presumed NF1 among the 32 722 122 deaths recorded from 1983 to 1997 [9].

Mean age at death in our study was lower in females than males, in keeping with an earlier study [5]. We also found that excess mortality was significantly greater in females than in males between 10 and 20 years of age. Sex hormones may affect the NF1 phenotype. Immunostaining



studies identified the progesterone receptor in neurofibromas [16]. Furthermore, orthotopic xenografts of an immortal human NF1-derived Schwann cell line into the sciatic nerves of female mice with severe combined immunodeficiency produced MPNSTs, and tumor cell proliferation was decreased in ovariectomized mice and restored by estrogen or progesterone replacement therapy [17]. Additional experiments by the same group supported a role for estrogen and progesterone on the growth of MPNST obtained by xenografting [18]. Finally, *in vitro* and *in vivo* studies on MPNST cell lines or MPNST xenografts showed that tamoxifen inhibited malignant cell growth in an estrogen-receptor-independent manner [1,19].

In conclusion, our evaluation of SMRs indicates significant excess mortality in NF1 patients compared to the general population. Although the retrospective design and hospital-based recruitment are limitations of our study, the predominance of cancer among causes of death is consistent with earlier work. We found that excess mortality was confined to the 10-40 year age group and that females in the 10-20 year age group had significantly greater excess mortality than males. Additional studies following our cohort ageing might accurately assess mortality above 40 years in NF1 patients.

#### Abbreviations

NF1: Neurofibromatosis 1; NF2: Neurofibromatosis 2; NFs: Neurofibromas; MPNST: Malignant peripheral Nerve sheath tumor; CI: Confident Interval; aOR adjusted Odds Ratio; SMR Standardized mortality ratios

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#### Author details

<sup>1</sup>APHP, Hôpital Henri-Mondor, Service de Dermatologie, F-94010 Créteil, France. <sup>2</sup>Université Paris Est, F-94010 Créteil, France. <sup>3</sup>APHP, Hôpital Henri-Mondor, Pôle Recherche Clinique-Santé Publique, F-94010 Créteil, France. <sup>4</sup>APHP, Hôpital Henri-Mondor, Centre de référence des Neurofibromatoses, F-94010 Créteil, F-94010, France. <sup>5</sup>Université Paris Est, LIC EM4393 (Laboratoire d'Investigation Clinique), F-94010 Créteil, France. <sup>6</sup>Unité Recherche Clinique, APHP, Hôpital Henri-Mondor, F-94010 Créteil, France. <sup>7</sup>Université Paris Est, INSERM, Centre d'Investigation Clinique 006, F-94010 Créteil, France. <sup>8</sup>APHP, Hôpital Necker-Enfants Malades, Service de Dermatologie, Centre de référence des Maladies Génétiques à Expression Cutanée F-75743 Paris, Université Paris-Descartes, France. <sup>9</sup>APHP, Hôpital Necker-Enfants Malades, Service d'Orthopédie, F-75743 Paris, France. <sup>10</sup>APHP, Hôpital Necker-Enfants Malades, Service de Génétique, F-75743 Paris, France. <sup>11</sup>APHP, Hôpital Necker-Enfants Malades, Service de Neurochirurgie, Paris F-75743, France. <sup>12</sup>APHP, Hôpital Trousseau, Service de Neuropédiatrie, Paris F-75571, France. <sup>13</sup>Université Pierre et Marie Curie, Paris, France.

#### Authors' contributions

SBG, PW and LA have made substantial contributions to conception and design. SBG, PW, TAD, SF, CV and ES have made substantial contributions to acquisition of data, or analysis and interpretation of data. SBG, PW, TAD and ES have been involved in drafting the manuscript or revising it critically for important intellectual content. All the authors have given final approval of the version submitted for publication.

#### Competing interests

The authors declare that they have no competing interests.

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## ANNEXE 2

**Sbidian E**/Bastuji-Garin S, Valeyrie-Allanore L, Ferkal S, Lefaucheur J-P, Drouet A, Brugière P, Combemale P, Barbarot S, Wolkenstein P. **At-risk phenotype of NF-1 patients: a multicentre case-control study.** *Orphanet Journal of rare diseases*, 2011, 13;6:51.

RESEARCH

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# At-Risk Phenotype of Neurofibromatose-1 Patients: A Multicentre Case-Control Study

Emilie Sbidian<sup>1,2,3†</sup>, Sylvie Bastuji-Garin<sup>1,2,4\*†</sup>, Laurence Valeyrie-Allanore<sup>1,3,4</sup>, Salah Ferka<sup>2,5</sup>, Jean P Lefaucheur<sup>6,7</sup>, Alain Drouet<sup>8,11</sup>, Pierre Brugière<sup>9</sup>, Cédric Valette<sup>10</sup>, Patrick Combemale<sup>11,12</sup>, Sébastien Barbarot<sup>13</sup>, Pierre Wolkenstein<sup>1,3,4</sup> and for NF France Network<sup>1</sup>

## Abstract

**Objectives:** To assess associations between subcutaneous neurofibromas (SC-NFs) and internal neurofibromas in patients with neurofibromatosis type 1 (NF-1) and to determine whether the association between SC-NFs and peripheral neuropathy was ascribable to internal neurofibromas.

**Patients and methods:** Prospective multicentre case-control study. Between 2005 and 2008, 110 NF-1 adults having two or more SC-NFs were individually matched for age, sex and hospital with 110 controls who had no SC-NF. Patients underwent standardized MRI of the spinal cord, nerve roots and sciatic nerves and an electrophysiological study. Analyses used adjusted multinomial logistic regression (ORa) to estimate the risk of the presence of internal neurofibromas or peripheral neuropathies associated with patients presented 2 to 9 SC-NFs, at least 10 SC-NFs as compared to patients without any (referential category).

**Results:** Cases had a mean age of 41 ( $\pm$  13) years; 85 (80%) had two to nine SC-NFs and 21 (19%) at least ten SC-NFs. SC-NFs were more strongly associated with internal neurofibromas in patients with ten or more SC-NFs than in patients with fewer NF-SCs (e.g., sciatic nerve, aOR = 29.1 [8.5 to 100] vs. 4.3 [2.1 to 9.0]). The association with SC-NFs was stronger for diffuse, intradural, and > 3 cm internal neurofibromas than with other internal neurofibromas. Axonal neuropathy with slowed conduction velocities (SCV) was more strongly associated with having at least ten SC-NFs (aOR = 29.9, 5.5 to 162.3) than with having fewer SC-NFs (aOR = 4.4, 0.9 to 22.0). Bivariate analyses showed that the association between axonal neuropathy with SCV and sciatic neurofibromas was mediated by the association between SC-NFs and sciatic neurofibromas.

**Conclusion:** The at-risk phenotype of NF-1 patients (i.e. NF-1 patients with SC-NFs) is ascribable to associations linking SC-NFs to internal neurofibromas at risk for malignant transformation and to axonal neuropathies with slowed conduction velocities. Axonal neuropathies with SCV are particularly common in patients with at least ten SC-NFs.

Registration details: ORPHA86301

## Introduction

Neurofibromatosis type 1 (NF-1 [MIM 162200]) is a common autosomal dominant disorder associated with increased morbidity and mortality[1]. Neurofibromas are the hallmark of NF1. They are benign tumors that arise from connective tissue of nerve sheaths, especially the

endoneurium. We used the classification proposed by Riccardi which defines four categories of neurofibromas [2]: (i) cutaneous neurofibromas, presenting as an exophytic tumor moving with the skin on examination, (ii) subcutaneous neurofibromas that lie deeper in the skin, do not move with it, are firm to palpation and may be tender (iii) internal or deep neurofibromas may involve any nerve anywhere along its length and are not palpated. They are therefore identified later in their course of growth (iv) plexiform neurofibromas may involve multiple fascicles and branches, and extend into

\* Correspondence: sylvie.bastuji-garin@mn.aphp.fr

† Contributed equally

<sup>1</sup>Université Paris Est (UPEC), LIC EA4393 (Laboratoire d'Investigation Clinique), F-94010 Créteil, France

Full list of author information is available at the end of the article



surrounding structures. Clinical investigation identifies thickened hypertrophic skin, hyperpigmentation of tissue and subcutaneous tumor [2,3].

Subcutaneous neurofibromas (SC-NFs) were independently associated with mortality among adults with NF-1 in two independent populations from France [4] and North America [5]. The main causes of death were spinal cord compression by internal neurofibromas and malignant peripheral nerve-sheath tumours (MPNSTs) developed from pre-existing internal neurofibromas [4,5]. A recent study demonstrated that having at least two SC-NFs was independently associated with having internal neurofibromas [6]. However, this association needs to be characterised in detail. Furthermore, a strong association between peripheral neuropathy and SC-NFs has been reported [7]. We hypothesized that this association was related to an association between SC-NFs and internal neurofibromas located along the nerve roots.

The primary objective of this prospective matched case-control study was to characterise the association between SC-NFs and internal neurofibromas in patients routinely investigated with magnetic resonance imaging (MRI), the most sensitive method for detecting internal neurofibromas and assessing their features (e.g., type, distribution, location, and size). The secondary objective was to determine whether peripheral neuropathy was associated with having internal neurofibromas along the nerve roots. Peripheral neuropathy was detected using a routine electrophysiological study.

## Patients And Methods

### Study design

This case-control study was conducted prospectively from February 2005 to December 2008 in three hospital centres in France. For a type 1 error of 0.05, 100 cases and 100 controls provided 90% power for detecting odds ratios (ORs) greater than 3 for factors having a 20% prevalence in the general population of adults with NF-1. We decided to select 110 cases and 110 controls to allow for some patients being excluded secondarily (e.g., because of contraindications to MRI). Controls were matched individually to cases on age ( $\pm 3$  years), sex, and hospital. The study was approved by the Ile-de-France IX (Paris, France) ethics committee, and all cases and controls gave their informed consent.

### Cases

The cases were identified among adults with NF-1 in our NF-network database. They met diagnostic criteria for NF-1 established at the National Institutes of Health Consensus Development Conference ( $n = 1\ 099$ ) [8]. Among patients aged 17 years or older ( $n = 748$ ), we included probands and patients with sporadic disease ( $n$

= 515) who had at least two SC-NFs ( $n = 192$ ), defined as palpable nodules along peripheral nerves under the skin. We did not include patients having less than two SC-NFs ( $n = 259$ ), pregnant women, and patients with contraindications to MRI (e.g., cardiac pacemaker, ferromagnetic or electronically operated stapedial implant, haemostatic clip, or metallic splinters) ( $n = 64$ ).

Among these 192 potential cases, 110 cases were selected at random for study inclusion.

### Controls

For each case, we looked for a control individually matched on age, sex, and centre in the NF-network database. Potential controls were probands or sporadic NF-1 patients who were at least 17 years old and had no SC-NFs and no contraindications to MRI. When we found several controls appropriate for the same case, we selected one at random. Thus, 110 controls were selected.

### Data collection

Data, including demographic information (age, sex and body mass index) and clinical features were collected during routine clinical assessments at the neurofibromatosis clinics by one dermatologist at each centre (SF, PC, SB) (Table 1). The skin lesions were described in detail: number of café-au-lait spots, cutaneous neurofibromas (0, 1, 2-9, 10-99,  $\geq 100$ ), and SC-NFs (0, 2-9, 10-99,  $\geq 100$ ); and count and location of plexiform neurofibromas defined as benign peripheral nerve-sheath tumours involving multiple nerve fascicles or branches

Table 1 Characteristics of adults with at least two subcutaneous neurofibromas (cases) and controls

Clinical features	Cases n = 106	Controls n = 102	p value*
Matching variables			
Female gender	62 (59)	62 (61)	
Age (years)	41 ( $\pm 13$ )	41 ( $\pm 14$ )	
Clinical variables			
Familial case	56 (47)	56 (45)	0.76
No cutaneous neurofibromas	13 (12)	4 (4)	0.03
Plexiform neurofibromas	66 (62)	49 (48)	0.04
Large café-au-lait spots (number)	8.6 ( $\pm 4.8$ )	10.1 ( $\pm 10.1$ )	0.07
No freckles	21 (20)	12 (12)	0.11
Lisch nodules	48 (81)	45 (80)	0.89
Scoliosis	46 (43)	42 (41)	0.75
Headache	38 (36)	41 (40)	0.52
Epilepsy	2 (2)	2 (2)	0.97
Learning disabilities	58 (57)	52 (49)	0.26

\*p value obtained using chi-square test or Fisher exact or non-parametric Kruskal-Wallis test as appropriate

Data are numbers (%) or means  $\pm 1$  standard deviation

of major peripheral nerves. Most of the plexiform neurofibromas were cutaneous or subcutaneous and were identified clinically. Other items were coded as present or absent. Orthopaedic complications (scoliosis), neurological abnormalities (headache, epilepsy, learning disabilities), and endocrinological disorders (hypertension) were also recorded.

#### Identification and characterisation of internal neurofibromas

All cases and controls had a standardized MRI study of the spinal cord, nerve roots, and sciatic nerve. MRI was performed using a field strength of 1.5-T. T1- and T2-weighted nonenhanced sequences (coronal plane) including short-tau inversion-recovery (STIR) sequences were acquired using a slice thickness of 4 mm with no gap. The image matrix was 512/256. Three coronal images (cervico-thoracic spine, thoraco-lumbo-sacral spine, and pelvis/thighs) were acquired to characterize the internal neurofibromas. MRI data were reviewed by a senior radiologist (PB) who was unaware of the clinical features. We recorded the presence of internal neurofibromas, their type (along the spinal cord or sciatic nerve roots), their distribution (focal or diffuse), their size (< 3 cm or  $\geq 3$  cm), and their location (intradural or extradural).

#### Electrophysiological study

All cases and controls underwent a standardized electrophysiological study to look for peripheral neuropathy. The investigation included sensory/motor nerve conduction study for the superficial/deep peroneal nerves and the sural/tibial nerves at both lower limbs and for the median and ulnar nerves at one upper limb. The electrophysiological data were reviewed by a senior physiologist (JPL) who was unaware of the clinical features. Patients were classified as having either no neuropathy (normal nerve conduction study) or an axonal distal, sensory-predominant polyneuropathy (characterized by a bilateral and symmetric reduction of sensory nerve action potential amplitude at the lower limbs with less marked abnormalities regarding motor nerve conduction parameters and upper limb results). Among axonal neuropathies, we differentiated those with and without slowed conduction velocities (SCVs). Neuropathies with and without SCVs shared the same presentation in terms of action potential amplitude reduction, but neuropathies with SCVs additionally showed moderate but diffuse decrease of motor nerve conduction velocities, very homogeneously, without any increased temporal dispersion or conduction block. Therefore, we assumed that neuropathies with SCVs were primarily axonal because they did not present any relevant criteria in favour of a demyelinating process [9]. We also determined the presence of proximal/nerve root involvement ("radiculopathy") according to a selective alteration (absence or

prolonged latency) of the F-waves and H-reflexes at the lower limbs.

#### Statistical analysis

Data were double-keyboarded and analysed using STATA software version 11 (Stata Corporation, College Station, TX, USA). All tests were two-tailed and  $p$  values < 0.05 were considered statistically significant. Quantitative variables are reported as mean  $\pm$  standard deviation (SD) and qualitative variables as number (%).

First, we compared cases and controls using univariate analysis (chi-square test or Fisher exact test as appropriate). Then, logistic regression models were used to estimate ORs adjusted for matching variables (aORs) with their 95% confidence intervals (95% CIs) for each characteristic of internal neurofibromas (spinal cord or sciatic nerve root, intra or extradural, diffuse or focal, size, and size category < 3 cm or  $\geq 3$  cm). Similarly, we estimated aORs with their 95% CIs separately for each type of peripheral neuropathy and radiculopathy. Then, to test our hypothesis that the association between SC-NFs and peripheral neuropathy was related to internal neurofibromas along the nerve roots, bivariate analyses including peripheral neuropathies and internal neurofibromas were performed. Finally, to assess whether the number of SC-NFs was associated with internal neurofibromas and/or peripheral neuropathy, cases were classified into two groups based on whether they had two to nine SC-NFs or at least ten SC-NFs. The risk associated with each of these categories versus the controls (no SC-NFs) was estimated using adjusted multinomial logistic regression (aOR). Control patients were the referential category.

#### Results

Between 2005 and 2008, 110 cases were matched for age, sex, and hospital to 110 NF-1 controls. Four cases and eight controls were excluded secondarily because their MRI scans were not interpretable. Table 1 reports the characteristics of the 106 cases and 102 controls. The cases had a mean age of 41 ( $\pm 13$ ) years; 59% were females, 85 (80%) had two to nine SC-NFs and 21 (19%) at least ten SC-NFs. There were no significant differences between cases and controls for age and sex (matching variables) or clinical features (e.g., dermatological characteristics, orthopaedic complications, and neurological abnormalities) except the cutaneous and plexiform neurofibromas. Significantly greater proportions of cases than controls had no cutaneous neurofibromas ( $p = 0.03$ ) and at least one plexiform neurofibroma ( $p = 0.04$ ). Cases had fewer large café-au-lait spots compared to controls but the difference was not statistically significant ( $p = 0.07$ ).

Table 2 summarises the results of the univariate analysis. The presence of SC-NFs was strongly associated

Table 2 Presence of internal neurofibromas in cases and controls

	Cases, n = 106 (%)	Controls, n = 102 (%)	Odds Ratio* (95%CI)	p value**
Paraspinal neurofibromas	54 (51)	20 (20)	4.3 (2.2 - 8.2)	< 10 <sup>-4</sup>
Distribution				
None	52 (49)	82 (81)	1	
Focal	26 (25)	16 (15)	2.6 (1.2 - 5.3)	
Diffuse	28 (26)	4 (4)	14.7 (3.8 - 57.3)	< 10 <sup>-4</sup>
Location				
None	52 (49)	82 (81)	1	
Extradural	37 (35)	17 (16)	3.2 (1.6 - 6.4)	
Intradural	17 (16)	3 (3)	8.8 (2.3 - 33.9)	< 10 <sup>-4</sup>
Size				
None	52 (49)	82 (81)	1	
< 3 cm	30 (28)	14 (14)	3.4 (1.6 - 7.2)	
≥3 cm	24 (23)	6 (5)	6.3 (2.3 - 17.4)	< 10 <sup>-4</sup>
Sciatic neurofibromas	50 (47)	13(13)	6.1 (2.9 - 13.0)	< 10 <sup>-4</sup>
Distribution				
None	63 (60)	90 (88)	1	
Focal	15 (14)	9 (9)	2.7 (1.1 - 6.8)	
Diffuse	28 (26)	3 (3)	15.4 (4.0 - 59.7)	< 10 <sup>-4</sup>
Size				
None	56 (53)	89 (88)	1	
< 3 cm	33 (31)	9 (9)	5.8 (2.5 - 13.8)	
≥3 cm	17 (16)	4 (4)	6.8 (2.1 - 22.2)	< 10 <sup>-4</sup>

\* Odds ratio with 95% confidence interval (95% CI) by logistic regression adjusted for matching variables (age, sex, hospital)

\*\*p value from logistic regression

with having paraspinal neurofibromas (aOR, 4.3; 95%CI, 2.2 to 8.2) and sciatic neurofibromas (aOR, 6.1; 95%CI, 2.9 to 13). The associations tended to be stronger for diffuse vs. focal paraspinal neurofibromas, intradural vs. extradural neurofibromas, and internal neurofibromas measuring at least 3 cm vs. less than 3 cm. Similarly, for sciatic neurofibromas, diffuse distribution and tumour size of at least 3 cm showed trends toward stronger associations with SC-NFs than focal distribution and size smaller than 3 cm, respectively. Finally, the presence of axonal neuropathy with SCVs was significantly associated with having SC-NFs (aOR, 7.7; 95%CI, 1.6 to 36.6) (Table 3).

Radiculopathies were not significantly associated with the presence of SC-NFs (Table 3).

The joint analysis identified no interactions between variables associated with the presence of SC-NFs. A strong association was found between the presence of sciatic neurofibromas and that of axonal neuropathy with SCVs (aOR = 22.1; 95% CI, 4.2 to 115.3). In the bivariate analysis including axonal neuropathy with SCVs and sciatic neurofibromas, axonal neuropathy with SCVs was no longer associated with SC-NFs (aOR, 3.0; 95%CI, 0.6 to 15.1; p = 0.18). The association between axonal neuropathy with SCVs and SC-NFs persisted only in the subgroup with sciatic neurofibromas (aOR, 2.00; 95%CI, 1.01 to 4.12; vs. aOR, 0.69; 95%CI, 0.24 to 4.12 in the subgroup without sciatic neurofibromas). Thus, the association between SC-NF and axonal neuropathy with SCVs was due to the presence of internal sciatic neurofibromas.

Table 3 Presence of peripheral neuropathies in cases and controls

	Cases n = 106 (%)	Controls n = 102 (%)	Odds Ratio* (95%CI)	p (chi <sup>2</sup> )
Peripheral Neuropathies				
none	85 (80)	94 (92)	1	0.009
axonal	7 (7)	6 (6)	1.3 (0.4 - 4.0)	
axonal with SCVs**	14 (13)	2 (2)	7.7 (1.6 - 36.6)	
Radiculopathies	24 (23)	14 (14)	1.8 (0.9 - 3.8)	0.09

\*Odds ratio with 95% confidence interval (95% CI) by logistic regression adjusted for matching variables (age, sex, hospital)

\*\* slowed conduction velocities

In the multinomial logistic regression analysis, patients with at least ten SC-NFs had a significantly higher risk of paraspinal neurofibromas (aOR, 82; 95%CI, 10.4 to 647.9) compared to patients with two to nine SC-NFs (aOR, 2.7; 95%CI, 1.4 to 5.3) (Figure 1). This dose-effect relationship was observed for distribution, location, and size (Figure 1, Table 4). Similar dose-effect relationships were found for sciatic neurofibromas, particularly regarding distribution, location, size (Figure 1, Table 4). Finally, axonal neuropathies with SCVs were more common in patients with at least ten SC-NFs (aOR, 29.9; 95%CI, 5.5 to 162.3) than in patients with two to nine SC-NFs (aOR, 4.4; 95%CI, 0.9 to 22.0) (Figure 1).

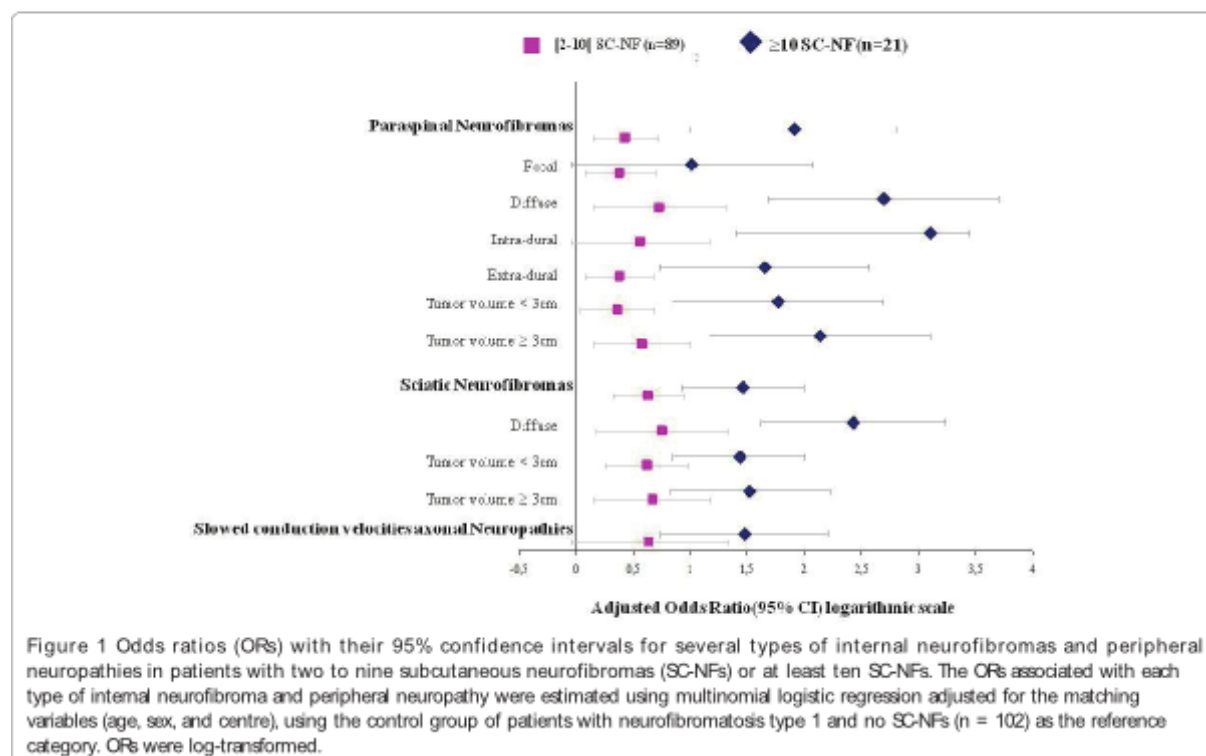
### Discussion

This study shows that sub-cutaneous neurofibromas were significantly associated with having paraspinal and sciatic neurofibromas detected by routine MRI. The strength of the association tended to be higher when the paraspinal and sciatic neurofibromas were diffuse, intra-dural, and at least 3 cm in diameter. Moreover, paraspinal and sciatic neurofibromas were significantly more common in patients having at least ten SC-NFs than in patients having two to nine SC-NFs. A dose-effect relationship was also found for distribution, location, and size of the internal neurofibromas. Finally, our data indicate that the strong association between SC-NFs and

axonal neuropathy with SCVs is due to the association between SC-NFs and internal neurofibromas.

Neurofibromatosis-1 (NF1) is a common disease with an increased propensity for developing both benign and malignant tumors [10]. NF1 has been reported to be associated with a 15-year decrease in life expectancy [1]. One of the aims of the Réseau NF-France was to identify clinical predictive factors for mortality, in order to better adjust the follow up and propose new treatments for eligible patients. We previously demonstrated in two different NF1 populations, from France [4] and North America [5], that the presence of at least 2 subcutaneous neurofibromas was associated with a higher risk of death. The main causes of death were compression of neighboring organs due to internal neurofibromas, and malignant peripheral nerve-sheath tumours developing from preexisting internal neurofibromas.

The main concern in the long-term clinical management of adults with NF-1 is the identification of patients at high risk for MPNSTs developed from pre-existing internal neurofibromas. At present, no effective treatment is available for MPNST untreatable by surgery. New treatments are usually approved based on proof of efficacy in a controlled trial. When conducting controlled trials, an important consideration is patient selection based on severity of illness. Controlled trials in adults with NF-1 should target patients who have a



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**Table 4 Association between subcutaneous neurofibromas (SC-NFs) and several types of internal neurofibromas and peripheral neurofibromas**

	No SC-NFs (n = 102) Controls	2-9 SC-NFs (n = 85) OR** (95%CI)	≥10 SC-NFs (n = 21) OR** (95%CI)
Paraspinal neurofibromas	1	2.7 (1.4 - 5.3)	82 (10.4 - 647.9)
Distribution			
None	-	1	1
Focal	1	2.4 (1.2 - 5.0)	10.3 (0.9 - 120.0)
Diffuse	1	5.4 (1.4 - 20.4)	492.0 (48.4 - 5006.2)
Location			
None	-	1	1
Intradural	1	3.7 (0.9 - 15.0)	1270.0 (25.6 - 2849.8)
Extradural	1	2.4 (1.2 - 4.8)	45.0 (5.4 - 374.2)
Size			
None	-	1	1
< 3 cm	1	2.3 (1.1 - 4.9)	58.6 (6.9 - 494.0)
≥3 cm	1	3.8 (1.4 - 10.4)	136.7 (14.9 - 1253.9)
Sciatic neurofibromas	1	4.3 (2.1 - 9.0)	29.1 (8.5 - 100.0)
Distribution			
None	-	1	1
Focal	1	2.9 (1.2 - 7.0)	-
Diffuse	1	5.7 (1.5 - 21.7)	266.8 (41.6 - 1712.7)
Size			
None	-	1	1
< 3 cm	1	4.2 (1.8 - 9.8)	27.2 (7.2 - 103.2)
≥3 cm	1	4.7 (1.4 - 15.5)	33.4 (6.6 - 167.6)
Neuropathies with SCV***	1	4.4 (0.9 - 22)	29.9 (5.5 - 162.3)

\*Reference category

\*\*Odds ratio with 95% confidence interval (95%CI) by multinomial logistic regression adjusted for matching variables (age, sex, hospital), using the control group of patients with neurofibromatosis type 1 and no SC-NFs (n = 102) as the reference category.

\*\*\* Slowed conduction velocities

high-risk phenotype defined by the presence of SC-NF. Patients with a high risk of internal neurofibromas must be therefore identified as potential new therapies are being developed. For instance, there are at least seven phase II/III trials in which the internal neurofibromas are being treated by targeted therapy such as ranibizumab, niltotinib... (clinicaltrials.gov). In addition, we believe that targeted therapy will be of greater efficacy on non-transformed internal neurofibromas. At present time, an annual careful clinical examination is recommended for all patients with NF1 in order to detect clinical symptoms associated with MPNSTs such as pain, neurological deficit and enlargement of a pre-existing peripheral nerve sheath tumour [11]. Routine MRI screening, which is highly reliable for detecting internal neurofibromas, is not recommended in patients without the alarming clinical signs mentioned above [12]. It is therefore critical to precisely clinically identify the patients with the maximum likelihood of having internal neurofibromas and to closely follow them by it iterating MRI. The first step is to assess recognizable clinical features as potential predictors of morbidity and mortality in order to further improve the clinical management of

NF1. We recently developed a clinical score for predicting the presence of internal neurofibromas in adults with NF1. The NF1Score, computed via a linear combination of four variables (age ≤ 30 years, fewer than six café-au-lait spots, no cutaneous neurofibromas, and two or more SC-NFs), can be used to accurately predict the presence of internal neurofibromas in NF-1 patients and help target patients for now emerging clinical trial [6]. The high point scoring of the NF1Score is the presence of SC-NFs. SC-NFs were highly associated with having internal neurofibromas (OR, 4.7; 95%CI, 2.1-10.5) [6]. The second step, our present work, was to confirm and explain a detailed and unbiased characterisation of the links between internal neurofibromas and SC-NFs. To our knowledge, this was the first controlled study involving a detailed characterization of the relationship between SC-NFs, internal neurofibromas, and peripheral neuropathies in adults with NF-1.

The internal validity of this study depends on many factors, including the unbiased recruitment of cases and controls and the accuracy of the information obtained about the presence of internal neurofibromas. MRI, an extremely reliable method for detecting internal

neurofibromas, was performed routinely in all our cases and controls. Assessment bias was minimized by having the MRI data reviewed by senior radiologist (PB) who was unaware of the clinical data. Assessment bias was minimized by having the electrophysiological data reviewed by senior physiologist (JPL) who was unaware of the MRI data. Clinical information regarding the neuropathy was not available. However, according to our experience the diffuse symmetric peripheral neuropathies related to NF-1 are asymptomatic or pauci-symptomatic with minor sensory manifestations [7].

As with all hospital-based case-control studies, selection bias cannot be excluded. However, the prevalence of the various clinical features in the cases were similar to those in the controls (except for the number of cutaneous neurofibromas) and to those reported previously in NF-1 patients, indicating that our study population was representative of NF-1 patients [13]. Regarding the number of cutaneous neurofibromas, the association linking the absence of cutaneous neurofibromas to the presence of SC-NF is in agreement with a previous study showing higher mortality among NF-1 patients who had no cutaneous neurofibromas [4].

In summary, this study shows a strong association between the presence of internal neurofibromas and the presence of SC-NF. The increased risk in NF-1 patients with subcutaneous neurofibromas (high risk phenotype) can be ascribed in part to the associations between SC-NFs and internal neurofibromas (prone to transformation to MPNSTs) and the presence of a neuropathy with SCVs. The prevalence of neuropathy with SCVs is particularly high in patients with at least ten SC-NFs. To better understand the patho-physiological processes in the high risk phenotype of NF-1 patients, the next step would be to perform genotype-phenotype correlation studies. A more severe clinical phenotype has been reported in NF1 patients carrying genomic microdeletions involving NF1 and surrounding gene compare to patients with mutations restricted to the NF1 genes. A recent study confirmed in a large cohort that NF1 microdeletion patients have a significantly higher incidence of learning disabilities and facial dysmorphism than patients with intragenic NF1 mutation [14]. However no association were found between SC-NFs and MPNSTs and NF1 microdeletion patients.

Further studies are therefore needed to find others genes beside NF1 implied in the internal NF development. One of the major aims of such studies would be to identify key targets for novel drugs' development.

#### List of Abbreviations

aOR adjusted Odds Ratio; CI: Confident Interval; MPNST: Malignant peripheral Nerve sheath tumor; MRI: magnetic resonance imaging; NF-1:

Neurofibromatosis 1; SC-NF(s): Sub-Cutaneous Neurofibroma(s); SCVs: Slow Conduction Velocities; SD: standard deviation

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#### Author details

<sup>1</sup>Université Paris Est (UPEC), LIC EA4393 (Laboratoire d'Investigation Clinique), F-94010 Créteil, France. <sup>2</sup>Assistance Publique-Hôpital Paris (AP-HP), Hôpital Henri-Mondor, Pôle Recherche Clinique-Santé Publique, F-94010 Créteil, France. <sup>3</sup>Assistance Publique-Hôpital Paris (AP-HP), Hôpital Henri-Mondor, Service de Dermatologie, F-94010 Créteil, France. <sup>4</sup>Assistance Publique-Hôpital Paris (AP-HP), Hôpital Henri-Mondor, Centre de référence des Neurofibromatoses, F-94010 Créteil, France. <sup>5</sup>INSERM, Centre d'Investigation Clinique 006, F-94010 Créteil, France. <sup>6</sup>Assistance Publique-Hôpital Paris (AP-HP), Hôpital Henri-Mondor, Service de Physiologie-Explorations fonctionnelles, F-94010 Créteil, France. <sup>7</sup>UPEC, EA 4391 (Excitabilité Nerveuse et Thérapeutique), F-94010 Créteil, France. <sup>8</sup>Service de Neurologie, Hôpital d'Instruction des armées, Lyon, F-69000, France. <sup>9</sup>Assistance Publique-Hôpital Paris (AP-HP), Hôpital Henri-Mondor, Service de Neuroradiologie, Créteil, F-94010, France. <sup>10</sup>Assistance Publique - Hôpitaux de Paris (AP-HP), Hôpital Henri Mondor, Unité de Recherche Clinique. <sup>11</sup>Service de Dermatologie, Centre Léon Bérard, Lyon, F-69000, France. <sup>12</sup>Réseau NF-Rhône-Alpin, Centre Léon Bérard, Lyon, F-69000, France. <sup>13</sup>Service de Dermatologie, CHU Hôtel Dieu, Nantes, F-44200, France.

#### Authors' contributions

PW conceived and designed the study, and is guarantor for the study. SBG contributed to the concept and design, and supervised analysis and interpretation of the data. ES performed the data analyses and wrote the initial draft of the article, to which all the authors contributed. All authors had full access to the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have given final approval for the final version to be published. The NF-France network contributed to the patients' inclusion.

#### Competing interests

ES, SBG, LVA, SF, JPL, AD, FB, PC, SB, PW have non-financial interests that may be relevant to the submitted work.

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## NF-1Score: A Prediction Score for Internal Neurofibromas in Neurofibromatosis-1

Emilie Sbidian<sup>1,2</sup>, Pierre Wolkenstein<sup>1,3,4</sup>, Laurence Valeyrie-Allanore<sup>1,3,4</sup>, Diana Rodriguez<sup>4,5,6,7</sup>, Smail Hadj-Rabia<sup>5,8,9</sup>, Salah Ferkal<sup>2,10</sup>, Jean-Philippe Lacour<sup>11</sup>, Jean-Claude Leonard<sup>12</sup>, Luc Taillandier<sup>13</sup>, Stéphanie Sportich<sup>14</sup>, Philippe Berbis<sup>14</sup> and Sylvie Bastuji-Garin<sup>1,2,4</sup>, Members of the NF France Network

NF-1 is associated with a 15-year decrease in life expectancy. Internal neurofibromas are associated with increased morbidity and mortality through malignant transformation and compression of neighboring organs. Our purpose was to develop and to validate a clinical score for predicting internal neurofibromas in adults. The development sample comprised 208 patients and the validation sample 191 patients. The score was developed using logistic regression. Discrimination and calibration of the model were evaluated. Four variables were independently associated with internal neurofibromas: at least two subcutaneous neurofibromas (odds ratio (OR) 4.7, [2.1-10.5]), age  $\geq$  30 years (OR 3.1, [1.4-6.8]), absence of cutaneous neurofibromas (OR 2.6, [0.9-7.5]), and fewer than six café-au-lait spots (OR 2.0 [0.9-4.6]). The score computed by linear combination of the rounded coefficients of these four variables ranged from 0 to 40 (mean, 12.8  $\pm$  10.8). The probability of internal neurofibromas was computed as  $\exp(\Delta 2.93 \times 0.11 \text{Score}) / \exp(1 + \Delta 2.93 \times 0.11 \text{Score})$ . Probabilities agreed well with the observed frequencies indicating good calibration, and discrimination was adequate (AUC-ROC, 0.75) in both data sets. The presence of internal neurofibromas can be accurately predicted using a simple clinical score. Further work will establish the score threshold that identifies patients at high risk for complications.

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### INTRODUCTION

Neurofibromatosis-1 (NF-1 [MIM 162,200]) is a common autosomal dominant disorder that is associated with both morbidity and mortality (Rasmussen et al., 2001). Internal neurofibromas are among the main causes of adverse outcomes (Tucker et al., 2005). These tumors may cause spinal cord compression, and about 10% of them undergo transformation to malignant peripheral nerve-sheath tumors

(MPNSTs), which are among the main causes of death in adults with NF-1 (Rasmussen et al., 2001). Internal neurofibromas arise from the spinal nerve roots and may extend into surrounding structures to involve multiple fascicles and branches. They are generally asymptomatic (Tongard et al., 1998), being identified only upon imaging studies. Accurate data can be obtained using magnetic resonance imaging (MRI), which shows large diffuse paraspinal tumors with high signal intensity on T2-weighted and T2 fat-saturation images contrasting with normal or, more often, low signal intensity on T1-weighted images (Drouet et al., 2004; Mautner et al., 2006). Clinical indicators of malignancy are persistent or increasing pain, enlargement of the tumor, and neurological deficiencies (Valeyrie-Allanore et al., 2005). MPNSTs carry a poor prognosis. Diagnosis is often delayed, as imaging studies are not performed routinely as part of the follow-up of patients with NF-1. Consequently, metastatic spread is common and survival is limited. The main prognostic factor may be tumor size, which governs the chances of curative surgery (Doom et al., 1995). Therefore, an early diagnosis is a principal objective. As MPNSTs develop from internal neurofibromas, a score predicting the presence of internal neurofibromas might improve the early diagnosis of MPNSTs, as close monitoring could be offered to patients with high-risk score values.

The purpose of this study was to develop a clinical score for predicting the presence of internal neurofibromas in patients with NF-1 and to assess its performance by evaluating discrimination and calibration.

<sup>1</sup>Université Paris 12, LIC EA4393 (Laboratoire d'Investigation Clinique), Créteil, France; <sup>2</sup>Pôle Recherche Clinique-Santé Publique, AP-HP, Hôpital Henri Mondor, Créteil, France; <sup>3</sup>Service de Dermatologie, AP-HP, Hôpital Henri Mondor, Créteil, France; <sup>4</sup>Centre de Référence des Neurofibromatoses, AP-HP, Hôpital Henri Mondor, Créteil, France; <sup>5</sup>Réseau NF-Île-de-France, France; <sup>6</sup>Service de Neuropédiatrie, AP-HP, Hôpital Armand Trousseau, Paris, France; <sup>7</sup>UPMC Université Paris 06, Paris, France; <sup>8</sup>Service de Dermatologie, Centre MAGEC, AP-HP, Hôpital Necker-Enfants Malades, Paris, France; <sup>9</sup>Faculté de Médecine Paris 5, Paris, France; <sup>10</sup>INSERM, Centre d'Investigation Clinique 006, AP-HP, Hôpital Henri-Mondor, Créteil, France; <sup>11</sup>Service de Dermatologie, Hôpital l'Archet 2, Nice, France; <sup>12</sup>Service de Pédiatrie, Institut Calot, Berck Hôpital Central, Berck, France; <sup>13</sup>Service de Neurologie, Nancy, France and <sup>14</sup>Service de Dermatologie, Hôpital Nord, Marseille, France

Correspondence: Sylvie Bastuji-Garin, Service de Santé Publique, Hôpital Henri-Mondor, Créteil Cedex 94010, France. E-mail: [sylvie.bastuji-garin@hmn.aphp.fr](mailto:sylvie.bastuji-garin@hmn.aphp.fr)

Abbreviations: AUC-ROC, area under the receiver-operating characteristic curve; CI, confidence interval; MPNSTs, malignant peripheral nerve-sheath tumors; MRI, magnetic resonance imaging; NF-1, neurofibromatosis type-1; OR, odds ratio; ROC, receiver-operating characteristic

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## RESULTS

### Study populations

The characteristics of the development and validation samples are reported in Table 1. The development sample comprised 208 patients, 122 females (59%), and 86 males with a mean age of 41.10 ( $\pm$  13) years (range, 20–78). Internal neurofibromas were present in 46 (22%) patients. The validation sample was composed of 191 patients, 108 females (57%), and 83 males with a mean age of 40.3 ( $\pm$  13) years (range 17–72), of whom 39 (20%) were classified as having internal neurofibromas. There were 304 patients whose internal neurofibroma status was unknown and who therefore were not included in the validation sample; their characteristics were not significantly different from those of the validation sample (data not shown).

### Model development

In the univariate analyses, five variables were associated, or nearly associated, with internal neurofibromas: age  $\geq$  30 years, at least two subcutaneous neurofibromas, fewer than six café-au-lait spots, absence of cutaneous neurofibromas, and absence of freckles (Table 2). No significant interaction was observed between these parameters. Absence of freckles was strongly associated with the other variables and was not independently associated with internal neurofibromas in the multivariate analysis. The four remaining variables

were independently associated with internal neurofibromas (Table 3). Calibration was excellent (Hosmer-Lemeshow statistic  $\chi^2$ 2.4; df  $\chi^2$ 4; P  $\chi^2$ 0.66) and discrimination was good (area under the receiver-operating characteristic curve (AUC-ROC)  $\chi^2$ 0.75; 95% confidence interval (CI), 0.7–0.8). The b-coefficients derived from the four independent predictors were multiplied by 10 and rounded to the nearest integer (Table 3). Loss-of-fit related to the rounded coefficients was negligible (Hosmer-Lemeshow statistic  $\chi^2$ 4.5; df  $\chi^2$ 7; P  $\chi^2$  0.5). Discrimination was similar (AUC-ROC  $\chi^2$ 0.75; 95% CI, 0.68–0.82). Table 4 shows the sensitivity and the specificity of the different cut-off levels of the score in the development sample. False-positive and false-negative rates can be deducted from sensitivity and specificity (1 – specificity and 1 – sensitivity, respectively). The score was then computed by means of a linear combination of the rounded coefficients: Score  $\chi^2$  10 (age  $\geq$  30 years)  $\chi^2$  10 (absence of cutaneous neurofibromas)  $\chi^2$  15 (X 2 subcutaneous neurofibromas)  $\chi^2$  5 (o 6 café-au-lait spots). Each factor was assigned the value 1 if present or 0 if absent. Therefore, the score could range from 0 to 40. The mean score was 12.8 ( $\pm$  10.8). An equation based on the logistic regression model was developed to convert the score into a probability of having internal neurofibromas in the following manner:  $\exp(\hat{A}2.93 \chi^2 0.11 * \text{Score}) / \exp(1 \chi^2 0.11 * \text{Score})$ .

### Model assessments

**Internal validation.** The shrinkage coefficients obtained by bootstrapping methods were similar to those based on the logistic regression model:  $a^0 \chi^2 a^* 1.05$  and  $b^0 \chi^2 b^* 0.98$ .

**External validation.** The score maintained adequate discrimination and calibration (Hosmer-Lemeshow statistic  $\chi^2$ 12.7; df  $\chi^2$ 9; P  $\chi^2$ 0.2; AUC-ROC  $\chi^2$ 0.73) when it was applied to each member of the validation sample. Table 5 shows the predicted risk of internal neurofibromas for each possible score.

## DISCUSSION

Our study shows that easily recognizable clinical features can be used to predict the risk of internal neurofibromas among adults with NF-1. Four factors were independently associated with internal neurofibromas: at least two subcutaneous neurofibromas, absence of cutaneous neurofibromas, fewer than 6 café-au-lait spots, and age  $\geq$  30 years. The NF-1 Score was computed as follows: 10 (age  $\geq$  30 years)  $\chi^2$  10 (absence of cutaneous neurofibromas)  $\chi^2$  15 (X 2 subcutaneous neurofibromas)  $\chi^2$  5 (o 6 café-au-lait spots). This score had excellent calibration and good discrimination.

Special attention was given to selecting the development sample. Our goal was to select a population of NF-1 patients systematically investigated with MRI, as this method is highly reliable for detecting internal neurofibromas. As part of the case-control study that included the patients in the development sample, the MRI data were reviewed by senior radiologists who were masked to the clinical features, to minimize assessment bias. Furthermore, the prevalence of the various clinical features in the development sample was

**Table 1. Characteristics of the patients with NF-1 included in the development (n=208) and validation (n=191) samples**

Clinical feature	Development sample n=208	Validation sample n=191
Female gender	122 (59)	108 (57)
Age $\geq$ 30 years	48 (23)	46 (24)
Familial case	112 (54)	127 (67)
X 2 Subcutaneous neurofibromas	106 (51)	111 (61)
No cutaneous neurofibromas	19 (9)	30 (16)
Plexiform neurofibromas	114 (55)	95 (50)
o 6 Café-au-lait spots	43 (21)	58 (30)
No freckles	39 (19)	31 (16)
Scoliosis	87 (42)	90 (47)
Pseudarthrosis	6 (3)	6 (3)
Headache	79 (38)	77 (40)
Epilepsy	4 (2)	8 (4)
Hydrocephalus	3 (1)	7 (4)
Learning disabilities	109 (52)	98 (51)
Facial asymmetry	20 (10)	13 (7)
Hypertension	17 (8)	23 (12)
Internal neurofibroma	46 (22)	39 (20)

Abbreviation: NF-1, neurofibromatosis-1.  
The data are the numbers of patients (%).

**Table 2. Univariate analysis in the development sample (n=208) of factors suspected to be associated with internal NFs**

	Internal neurofibroma		Odds ratio (95% CI) <sup>1</sup>	P-value <sup>2</sup>
	No (n=162)	Yes (n=46)		
Female gender	97 (60)	25 (58)	1.3 (0.6-2.4)	0.502
Age ≥ 30 years	30 (18)	18 (39)	2.8 (1.4-5.9)	0.004 <sup>3</sup>
Familial cases	88 (54)	24 (52)	0.9 (0.5-1.8)	0.800
X 2 Subcutaneous neurofibromas	70 (43)	36 (78)	4.7 (2.1-10.6)	o 10 <sup>-4</sup> <sup>3</sup>
No cutaneous NF	9 (6)	10 (22)	4.7 (1.7-12.8)	0.001 <sup>3</sup>
Flexiform NF	90 (56)	24 (52)	0.9 (0.5-1.7)	0.684
o 6 Café-au-lait spots	28 (17)	15 (33)	2.3 (1.1-4.9)	0.024 <sup>3</sup>
No freckles	26 (16)	13 (28)	2.1 (0.9-4.5)	0.061 <sup>3</sup>
Scoliosis	68 (42)	19 (41)	1.0 (0.5-1.9)	0.935
Pseudarthrosis	4 (2)	2 (4)	1.8 (0.3-10.2)	0.502
Headache	59 (36)	20 (43)	1.3 (0.7-2.6)	0.385
Epilepsy	2 (1)	2 (4)	3.6 (0.5-26.9)	0.175
Hydrocephalus	3 (2)	0	0	0.353
Learning disabilities	83 (51)	26 (57)	1.2 (0.6-2.4)	0.527
Facial asymmetry (n=122/78)	12 (10)	5 (6)	0.6 (0.21-1.87)	0.397
Hypertension	13 (8)	4 (9)	1.1 (0.3-3.5)	0.883

Abbreviations: CI, confidence interval; NF, neurofibromatosis.

<sup>1</sup>Odds ratios with their 95% CIs were estimated using logistic regression.

<sup>2</sup>P-value by  $\chi^2$ -test or Fisher's exact test, as appropriate.

<sup>3</sup>Variables yielding P-values less than 0.15 were entered into a multiple logistic regression model.

The data represent the numbers of patients (%).

**Table 3. Factors independently associated with internal NFs in the multivariate analysis in the development sample (n=208)**

	Odds ratio	95% CI <sup>1</sup>	P-value	b-Coefficient <sup>2</sup>	Points
Age ≥ 30 years old	3.1	1.4-6.8	0.006	1.12	10
No cutaneous neurofibromas	2.6	0.9-7.5	0.08	0.95	10
X 2 Subcutaneous neurofibromas	4.7	2.1-10.5	o 10 <sup>A4</sup>	1.55	15
o 6 Café-au-lait spots	2.0	0.9-4.6	0.08	0.71	5

Abbreviations: CI, confidence interval; NF, neurofibromatosis.

<sup>1</sup>Confidence interval.

<sup>2</sup>b-Coefficients of the factors independently associated with internal neurofibromas.

consistent with those previously reported in NF-1 patients (Friedman and Birch, 1997), indicating that the development sample was representative of NF-1 patients. A cohort study design is generally used to assess predictors to minimize information bias (Coste et al., 1996). However, NF-1 is a rare disease and routine MRI screening is not recommended in everyday clinical practice (Pinson et al., 2001). By taking the development sample from a case-control study in which all patients underwent MRI, we improved the reliability of internal neurofibroma diagnosis as compared with a cohort study.

The validation sample was composed of patients included in the Réseau NF-France database. Selection bias seems unlikely, as the prevalences of NF-1 features were consistent with those reported previously in other populations of NF-1 patients (Friedman and Birch, 1997). Furthermore, the

features in non-included patients whose internal neurofibroma status was unknown were similar to those in the validation sample. However, absence of routine MRI or computed tomography in the validation sample may constitute a limitation of our study. MRI or computed tomography was performed only when routine imaging studies (chest radiograph and abdominal sonogram) or clinical symptoms suggested the presence of internal neurofibromas. This may have resulted in verification bias. Furthermore, we had no data on the size or location of internal neurofibromas in the validation sample.

To validate the NF-1Score, we used a rigorous procedure involving both internal validation (shrinkage) and external validation. Shrinkage of the regression coefficients (a and b) to correct for over-optimism in the model may help to make

**Table 4. Sensitivity and specificity values of the different cut-off levels of the NF-1Score obtained in the development sample**

Cut-point	Development sample (n=208) AUC-ROC=0.75	
	Sensitivity (%)	Specificity (%)
X 0	100	0
X 5	91	37
X 10	87	43
X 15	80	53
X 20	50	83
X 25	41	91
X 30	26	98
X 35	11	99
X 40	2	100

Abbreviations: AUC-ROC, area under the receiver-operating characteristic curve; NF-1, neurofibromatosis-1.

**Table 5. Probabilities of the presence of internal NFs according to the NF-1Score level in the validation sample (191 patients)**

Probability of having internal neurofibromas	NF-1Score	Internal neurofibromas (n=39)		No internal neurofibromas (n=152)	
		Observed	Expected	Observed	Expected
0.051	0	3	2.0	36	37.0
0.083	5	2	1.1	11	11.9
0.133	10	3	1.7	10	11.3
0.207	15	6	11.6	50	44.4
0.308	20	8	8.0	18	18.0
0.430	25	7	9.5	15	12.5
0.561	30	3	5.1	6	4.0
0.684	35	2	4.1	4	1.9
0.787	40	5	5.5	2	1.5

Abbreviation: NF-1, neurofibromatosis-1.

models more transferable (Steyerberg et al., 2004). The results of our internal and external validations in an independent sample indicated satisfactory performance, with good calibration and discrimination. Therefore, from a methodological point of view, this score could be considered as a good prediction tool. Although such probability models cannot be used reliably to predict outcome in individual patients, the information provided by such models using easily recognizable clinical features could be useful for clinical decision-making. For instance, a probability of 0.56 (NF-1Score ¼30) means that approximately 56 out of 100 patients with this score would be expected to have internal neurofibromas. One cannot say whether any specific individual patient will be one of the 56 patients who may have internal neurofibromas or one of the 44 such patients who may have not.

Clinicians often have to discuss whether or not an imaging would be useful. That is, which NF-1 patients need MRI to detect asymptomatic MPNTs. Thus, although MRI is not systematically recommended, faced with a patient with a high NF-1Score value, physicians would be alerted and mandate a radiological follow-up. Whether systematic radiological investigations are required to evaluate the potential risk of MPNTs in this new population at risk remains to be investigated. Furthermore, introduction of new treatment is usually preceded by the demonstration of its effectiveness in a controlled trial. An important aspect of the conduct of such trials is the ability to define and control the severity-of-illness of the patients studied.

In choosing the appropriate "cut-off" score that defines high risk, there is a trade-off between a score that confers high sensitivity or high specificity. A high cut-off score that gave high specificity would lose sensitivity, thereby missing many patients who have internal neurofibromas. However, a low score, with high sensitivity, might select too many patients as high risk, which would be of no practical benefit. Therefore, different thresholds should be defined according to the clinical purpose.

Subcutaneous neurofibromas were independently associated with internal neurofibromas in our study, in keeping with earlier data (Tucker et al., 2005). Furthermore, patients with subcutaneous neurofibromas were at higher risk for mortality in two different NF-1 populations, from France (Khosrotehrani et al., 2003) and North America (Khosrotehrani et al., 2005), respectively. None of the other three factors identified in our study have been reported previously to be associated with internal neurofibromas. However, the association linking absence of cutaneous neurofibromas to internal neurofibromas is in agreement with a previous study in which mortality was higher among NF-1 patients who had no cutaneous neurofibromas (Khosrotehrani et al., 2003). The association between the presence of internal neurofibromas and age younger than 30 years is consistent with a report that most MPNSTs may develop from pre-existing internal neurofibromas, with the risk being highest at about 30 years of age (Evans et al., 2002). Conversely the association between presence of internal neurofibromas and fewer than six café-au-lait spots has never been reported even though four individuals with multiple spinal tumors and no café-au-lait spots but with NF-1 mutation have been identified (Kaufmann et al., 2001). The two features in the score, namely, absence of cutaneous neurofibromas and fewer than six café-au-lait spots, are unusual in NF-1 patients. Presence of at least two neurofibromas of any type and at least six café-au-lait spots are the diagnostic criteria for NF-1. However, all the patients in our study had a definitive diagnosis of NF-1. In patients younger than 30 years, the combination of internal neurofibromas, fewer than six café-au-lait spots, no cutaneous neurofibromas, and at least two subcutaneous neurofibromas constitutes a phenotype that is both distinct from classical NF-1 and particularly severe. Recently, examination of the phenotypic correlations between affected relatives in 750 NF-1 patients from 275 multiplex families collected through the NF-France Network provided evidence that genetic modifiers, unlinked to the NF-1



locus, contribute to the variable expressivity of the disease (Sabbagh et al., 2009). However, biological factors that influence the NF-1 risk of morbidity-mortality are not known.

In sum, we developed a simple scoring system (the NF-1Score) that accurately predicted the presence of internal neurofibromas in patients with NF-1. The NF-1Score could be used to identify NF-1 patients who require particularly close monitoring for internal neurofibromas.

## MATERIALS AND METHODS

### Population and study samples

Patients meeting the diagnostic criteria for NF-1 established at the National Institutes of Health Consensus Development Conference (Conference statement, 1988) were included prospectively by the French NF network (Réseau NF-France). We used two samples for our study. The development sample, used to develop the prediction score, comprised 208 adults (17 years or older) included in an ongoing prospective multicenter case-control study (begun in 2005) designed to assess whether subcutaneous neurofibromas were associated with several types of internal neurofibromas. All patients in this sample were carefully investigated by MRI to determine with confidence whether internal neurofibromas were present. The validation sample was composed of the 191 NF-1 patients who were prospectively included in the Réseau NF-France database from June 2003 to June 2008, and who met the following criteria: 17 years or older, probands or sporadic disease, known internal neurofibroma status, and non-inclusion in the above-mentioned case-control study (Figure 1). The study was approved by the institutional review boards of Île-de-France IV (Paris, France) and informed consent was obtained from all patients. The study adhered to the Declaration of Helsinki Principles.

### Data collection

Demographic information (age and sex) and clinical features recorded in the databases were collected during routine clinical assessments at neurofibromatosis clinics (Table 1). Detailed information was available on the dermatological characteristics: number of café-au-lait spots (0, 1, 2, 3, 4, 5, 6, X 7), number of cutaneous

neurofibromas (0, 1, 2-9, 10-99, X 100), number of subcutaneous neurofibromas (0, 1, 2-9, 10-99, X 100), and number and location of plexiform neurofibromas. Among clinical features, we selected those known to be associated with mortality: absence of cutaneous neurofibromas, facial asymmetry, at least two subcutaneous neurofibromas, and male gender (Khosrotehrani et al., 2003, 2005). We recorded the following features as present or absent: orthopedic complications (scoliosis and pseudarthrosis), neurological abnormalities (headache, epilepsy and learning disabilities), hypertension, and renal artery stenosis.

### Classification of patients: identification of internal neurofibromas

All patients in the development sample underwent standardized MRI of the spinal cord and nerve roots using non-contrast-enhanced T1- and T2-weighted sequences (coronal plane) and short-tau inversion-recovery (STIR) sequences. Paraspinal neurofibromas were characterized as diffuse or focal and as  $\leq 3$  or  $> 3$  cm. Patients were classified as having internal neurofibromas if they had at least one diffuse paraspinal neurofibroma or at least one focal paraspinal neurofibroma measuring at least 3 cm. Patients with neither criterion were classified as not having internal neurofibromas (Drouet et al., 2004; Mautner et al., 2006).

In the validation sample, MRI was not performed routinely (Pinson et al., 2001). MRI or computed tomography is usually recommended when there is evidence of internal neurofibromas on other imaging studies (chest radiograph or abdominal sonogram) or when symptoms such as pain or neurological deficits suggest internal neurofibromas. Patients in the validation sample were classified as having internal neurofibromas when the variable "paraspinal neurofibromas" was coded "yes" in the database and as not having internal neurofibromas when this variable was coded "no". No information was available on the size or location of internal neurofibromas in the validation sample.

### Statistical analysis

Data were analyzed using STATA software version 8 (Stata, College Station, TX). All tests were two-tailed and P-values no greater than

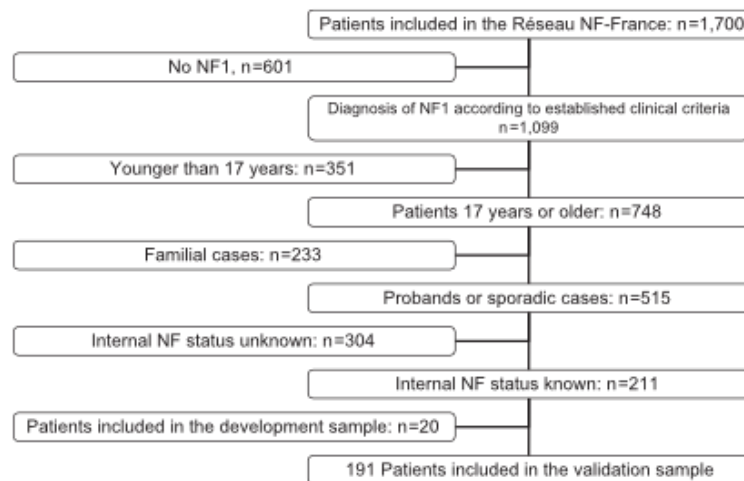


Figure 1. Flow chart of the validation sample.

0.10 were considered for prognostic modeling (Steyerberg et al., 2000). The characteristics of the development and validation samples were described. The characteristics of the patients who were not included in the validation sample because their internal neurofibroma status was unknown, were compared to those of the patients included in the validation sample. Quantitative variables are either reported as median  $\pm$  SD or converted to categorical variables. Thus, age was dichotomized according to the peak MPNST incidence in NF-1 patients ( $< 30$  or  $\geq 30$  years) (Evans et al., 2002). Qualitative variables are reported as number (%).

#### Model development

The characteristics of patients with and without internal NF were compared in univariate analyses using the  $\chi^2$ -test or Fisher's exact test, as appropriate. Odds ratios (ORs) were estimated with their 95% CIs, using logistic regression models. Two-by-two analyses were also performed to assess potential interactions and confounding by fitting multiplicative models. Variables yielding P-values less than 0.15 in the univariate analyses were entered into a multiple logistic regression model. The final model included the variables independently associated with the presence of internal neurofibromas. Performance of the model, including calibration and discrimination, was evaluated by computing the Hosmer-Lemeshow statistic (Hosmer and Lemeshow, 1989) and the AUC-ROC (Hanley and McNeil, 1982), respectively. The Hosmer-Lemeshow test evaluates whether the predicted probabilities agree with the observed probabilities. Discrimination is the ability to distinguish patients with internal neurofibromas from those without internal neurofibromas.

Then, we used the variables identified by the multivariate analysis to build a simple-to-use score for predicting the presence of internal neurofibromas. Points were assigned to each variable on the basis of the regression coefficients in the final model: the b-coefficient was multiplied by 10 and the result was rounded to the nearest integer (Le Gall et al., 1993). We checked whether the predictive performance of these rounded coefficients was similar to that of the original coefficients. The score was calculated for each patient and a multiple logistic regression equation was used to convert the score into a probability of having internal neurofibromas: the logit ( $a + b \times \text{Score}$ ) was computed and the probability was then estimated as  $P = \frac{e^{\text{logit}}}{1 + e^{\text{logit}}}$ .

#### Model assessments

**Internal validation.** As the development sample comprised only 208 patients, we used bootstrapping to estimate shrinkage coefficients (van Houwelingen and Le Cessie, 1990) to avoid over-optimism (Miller and Hui, 1991; Steyerberg et al., 2004) and to obtain nearly unbiased estimates of the predictive accuracy of the model (Harrell et al., 1996). We drew 1,000 samples at random. The logistic regression coefficients were re-estimated in the bootstrap samples.

**External validation.** Performance of the model, including calibration and discrimination, was assessed in the validation sample.

#### CONFLICT OF INTEREST

The authors state no conflict of interest.

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## Title page

### Clinical characteristics Predicting Internal Neurofibromas in 357 Children with Neurofibromatosis-1: results from a cross-sectional study

Emilie Sbidian, MD,<sup>1,2,3</sup> Smaïl Hadj-Rabia, MD PhD,<sup>5</sup> Vincent M. Riccardi, MD PhD,<sup>6</sup> Laurence Valeyrie-Allanore L, MD,<sup>1,2,4</sup> Sébastien Barbarot, MD PhD,<sup>7</sup> Olivier Chosidow, MD PhD,<sup>2,8</sup> Salah Ferkal, MD,<sup>4,9</sup> Diana Rodriguez, MD PhD,<sup>10</sup> Pierre Wolkenstein\*, MD PhD<sup>1,2,4</sup> and Sylvie Bastuji-Garin\*, MD PhD,<sup>1,3,4,11</sup> on behalf of the NF France Network

\*Sylvie Bastuji-Garin and Pierre Wolkenstein contributed equally to this work

Emilie Sbidian, MD : [emilie.sbidian@hmn.aphp.fr](mailto:emilie.sbidian@hmn.aphp.fr)<sup>1,2,3</sup>

Smaïl Hadj-Rabia, MD PhD : [hadj@necker.fr](mailto:hadj@necker.fr)<sup>5</sup>

Vincent M. Riccardi, MD PhD: [vmriccardi@charter.net](mailto:vmriccardi@charter.net)<sup>6</sup>

Laurence Valeyrie-Allanore, MD : [laurence.allanore@hmn.aphp.fr](mailto:laurence.allanore@hmn.aphp.fr)<sup>1,2,4</sup>

Sébastien Barbarot, MD PhD : [sebastien.barbarot@chu-nantes.fr](mailto:sebastien.barbarot@chu-nantes.fr)<sup>7</sup>

Olivier Chosidow, MD PhD : [olivier.chosidow@hmn.aphp.fr](mailto:olivier.chosidow@hmn.aphp.fr)<sup>2,8</sup>

Salah Ferkal, MD : [salah.ferkal@hmn.aphp.fr](mailto:salah.ferkal@hmn.aphp.fr)<sup>4,9</sup>

Diana Rodriguez, MD PhD, [diana.rodriguez@trs.aphp.fr](mailto:diana.rodriguez@trs.aphp.fr)<sup>10</sup>

Pierre Wolkenstein, MD PhD : [pierre.wolkenstein@hmn.aphp.fr](mailto:pierre.wolkenstein@hmn.aphp.fr)<sup>1,2,4</sup>

Sylvie Bastuji-Garin, MD PhD: [sylvie.bastuji-garin@hmn.aphp.fr](mailto:sylvie.bastuji-garin@hmn.aphp.fr)<sup>1,3,4</sup>

<sup>1</sup>Université Paris Est (UPEC), LIC EA4393 (Laboratoire d'Investigation Clinique), F-94010 Créteil, France;

<sup>2</sup>Assistance Publique-Hôpital Paris (AP-HP), Hôpital Henri-Mondor, Service de Dermatologie, F-94010 Créteil, France;

<sup>3</sup>Assistance Publique-Hôpital Paris (AP-HP), Hôpital Henri-Mondor, Pôle Recherche Clinique-Santé Publique, F-94010 Créteil, France;

<sup>4</sup> Assistance Publique-Hôpital Paris (AP-HP), Hôpital Henri-Mondor, Centre de référence des Neurofibromatoses, F-94010 Créteil, France;

<sup>5</sup>Assistance Publique-Hôpital Paris (AP-HP), Hôpital Necker-Enfants malades, Centre de Référence des Maladies Génétiques à Expression Cutanée (MAGEC) et Service de Dermatologie, Université Paris V Descartes, F-75015 Paris, France;

<sup>6</sup>The Neurofibromatosis Institute, La Crescenta, California, USA;

<sup>7</sup>Service de Dermatologie, CHU Hôtel Dieu, Nantes, F-44200, France;

<sup>8</sup>Université Paris Est (UPEC), F-94010 Créteil, France;

<sup>9</sup>INSERM, Centre d'Investigation Clinique 006, F-94010 Créteil, France;

<sup>10</sup> Assistance Publique-Hôpital Paris (AP-HP), Hôpital Trousseau, Service de Neuropédiatrie, Université Pierre et Marie Curie, Paris F-75571, France

<sup>11</sup> Assistance Publique-Hôpital Paris (AP-HP), Hôpital Henri-Mondor, Unité de Recherche Clinique (URC), F-94010 Créteil, France;

**Corresponding author:** Sylvie Bastuji-Garin, Service de Santé Publique, Hôpital Henri-Mondor, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Creteil Cedex, France

[sylvie.bastuji-garin@hmn.aphp.fr](mailto:sylvie.bastuji-garin@hmn.aphp.fr)

Tel: +33 149 813 706; Fax: +33 149 813 697

## Abstract

**Objective.** To identify clinical characteristics associated with internal neurofibromas in children with NF1, as a means of ensuring the early identification of patients at high risk for malignant peripheral nerve-sheath tumors developed from preexisting internal neurofibromas.

**Patients and methods.** We used data from two NF1 populations, in France and North America, respectively. The French database comprised 1083 patients meeting NIH diagnostic criteria for NF1 and the Neurofibromatosis Institute Database of North America comprised 703 patients. Patients younger than 17 years of age were eligible for our study if they had been evaluated for internal neurofibromas using computed tomography and/or magnetic resonance imaging. Clinical characteristics associated with internal neurofibromas by univariate analysis ( $P \leq 0.15$ ) were entered into a multiple logistic regression model after checking for potential interactions and confounding. Multiple imputation was used for missing values.

**Results.** Among the 746 children in the two databases, 357 (48%) met our inclusion criteria. Their mean age was  $7.7 \pm 5.0$  years and there were 192 (53.8%) males. Internal neurofibromas were present in 35 (9.8%) patients. Internal neurofibromas developed earlier in females than in males and their prevalence increased during adolescence. Factors independently associated with internal neurofibromas were age (OR=1.16 [1.07-1.27]), xanthogranulomas (OR=5.85 [2.18-15.89]) and presence of both subcutaneous and plexiform neurofibromas (OR=6.80 [1.52-30.44]).

**Conclusions.** Several easily recognizable clinical characteristics indicate a high risk of internal neurofibromas in children with NF1 and, therefore, a need for very close monitoring.

## Key Words

Neurofibromatosis 1, Internal neurofibromas, Subcutaneous neurofibromas, Children, Cross-sectional study

## Words count

Abstract word count: 232

Manuscript word count (without references): 2444

**Number of references: 34**

**Number of tables: 3**

**Number of figures: 3**

## INTRODUCTION

Neurofibromatosis-1 (NF1 [MIM 162200]) is a common autosomal dominant disorder with an incidence of 1 in 2500-3000 births and a prevalence of 1 in 4000 [(15)]. NF1 is associated with increased morbidity and mortality rates (23, 84). In spite of tremendous progress in understanding and treating NF1, there continues to be inconsistency, if not confusion about the various types of neurofibromas. Our interest in this article is to sensitize clinicians to NF1 neurofibromas that are not obvious by inspection and/or palpation of the skin. That is, the focus is on internal neurofibromas. An internal neurofibroma simply is a neurofibroma that is not appreciated by physical examination. Part of the problem is that some neurofibromas that may have internal components may also be apparent externally. In particular, both the large *diffuse plexiform neurofibroma* and the large *nodular plexiform neurofibroma*, as described by Riccardi (47)], corresponding to Masson's *diffuse neurofibroma* and *encapsulated neurofibroma*, respectively, as utilized by Tucker and coworkers (85-86)], can have both external and internal components. In the present article we are reserving the term, internal neurofibroma, for those neurofibromas not having an apparent external component. By definition, both cutaneous neurofibromas and subcutaneous neurofibromas are excluded from being internal neurofibromas. Most specifically, the term, plexiform neurofibroma, does not on its own afford distinction from or inclusion in what we are specifying here as an internal neurofibroma.

Internal neurofibromas are among the main causes of life-threatening events in patients with NF1 (25, 47)]. Ten percent of internal neurofibromas undergo transformation to malignant peripheral nerve-sheath tumors (MPNSTs) (21), a leading cause of death in adults with NF1 (23, 84)]. Clinical indicators of MPNST are persistent or increasing pain, enlargement of the tumor, and neurological deficiencies (27)]. The diagnosis is often delayed, especially for deep diffuse plexiform or internal neurofibromas as imaging studies are not performed routinely as part of the follow-up of patients with NF1 (14)] but instead are ordered only as clinically indicated (31)]. The mean age at diagnosis of MPNST is younger in patients with NF1 than in unaffected individuals (21, 60-62). MPNSTs may develop before 30 years of age (21, 60) and even in childhood (61). Moreover internal neurofibromas grow faster in young patients (63). In previous work, we developed a simple scoring system (the NF1Score) that accurately predicted the presence of internal neurofibromas at risk for



transformation to MPNST in adults with NF1 (87). Four characteristics were independently associated with internal neurofibromas: presence of subcutaneous neurofibromas, fewer than 6 café-au-lait spots, absence of cutaneous neurofibromas, and age  $\leq 30$  years. However, the NF1Score was developed in patients aged 17 years or older. Consequently, a specific study was needed in pediatric patients. The clinical expression of NF1 varies across the life span in a given patient (31, 59)]. For instance, café-au-lait spots are present within the first year of life and cutaneous or subcutaneous neurofibromas are usually present by adolescence (15-16, 59)].

The purpose of this study was to identify clinical characteristics associated with internal neurofibromas in children with NF1. Such clinical characteristics could serve to ensure the early identification of children with NF1 who require particularly close monitoring for internal neurofibromas.

## **PATIENTS AND METHODS**

### **Population and study samples**

The study was performed using data from two NF1 populations, one in France and the other in North America. Patients meeting the diagnostic criteria for NF1 established at the National Institutes of Health Consensus Development Conference (88)] were included prospectively by the NF-France network (Réseau NF-France) from 2002 to 2005 (83)] and the Neurofibromatosis Institute Database (NFID) from 1977 to 1996 (47)]. The NF-France database was a French Clinical Research Program entitled “Study of expressivity of neurofibromatosis-1: constitution of a phenotype-genotype database” (83)]. The NFID is a collaborative system for collecting demographic information, clinical signs and symptoms, basic measurements, and psychosocial assessments of individuals and families with neurofibromatosis (47)]. The NF-France database included 1083 patients and the NFID 703 patients. For the present study, patients were eligible if they were younger than 17 years of age and had undergone imaging studies to look for internal neurofibromas because of symptoms such as pain or neurologic deficits or when there was evidence of internal neurofibromas on other imaging studies (Figure 1). In all, 357 children met these criteria.

The study was approved by the Île-de-France IX Ethics committee Paris, France. Informed consent was obtained from all patients. The study adhered to Declaration of Helsinki guidelines.

## Data collection

The demographic information (age, sex, and whether the NF1 was familial or sporadic) and clinical characteristics recorded in the databases were collected during routine clinical assessments at neurofibromatosis clinics (Table 1). Among the clinical characteristics, we selected those known to be associated with mortality in children with NF1 (plexiform neurofibromas) or showing trends toward an association with mortality in NF1 patients (subcutaneous neurofibromas, absence of cutaneous neurofibromas, and short stature) (33)]. Special attention was also given to clinical characteristics independently associated with internal neurofibromas in adult NF1 patients, namely, fewer than 6 café-au-lait spots, age  $\leq 30$  years (a criterion met by all our patients), and subcutaneous and cutaneous neurofibromas (87)]. Most of the other clinical characteristics selected for our study were easily identified by physical examination, with the exception of Lisch nodules, which were evaluated by slit lamp examination. Detailed information was available on the dermatological characteristics, namely, freckles, plexiform neurofibromas, and xanthogranulomas (previous or current diagnosis). We recorded the following characteristics as present or absent: facial asymmetry, orthopedic complications (nonunion, dysplasia), hypertension, and macrocephaly.

## Study definitions

Cutaneous neurofibromas are exophytic tumors that move with the skin on examination (47)]. Subcutaneous neurofibromas lie deeper in the skin, do not move with the skin, and are firm and sometimes tender to palpation (47)]. A plexiform neurofibroma is an area of thick hypertrophic skin with tissue hyperpigmentation overlying a subcutaneous tumor (47)]. Xanthogranulomas are soft, flat, yellow-to-pink papules. Macrocephaly was defined as a head circumference 2 SDs or more above the age- and sex-matched population mean. Short stature was defined as a height 2 SDs or more below the age- and sex-matched population mean.

## **Classification of patients: identification of internal neurofibromas**

All the study patients had been evaluated for internal neurofibromas using computed tomography (CT) and/or magnetic resonance imaging (MRI). The presence of internal neurofibromas was coded 1 in the databases.

## **Statistical analysis**

Quantitative variables are described as mean±standard deviation (SD). Age was not converted to a categorical variable. Qualitative variables are described as number (%). All tests were two-tailed and *P* values <0.05 were considered statistically significant.

The characteristics of patients with and without internal neurofibromas were compared in univariate analyses. Odds ratios (ORs) were estimated with their 95% confidence intervals (95% CIs), using logistic regression models. ORs were adjusted for age because the prevalence of several NF1 characteristics varies with age (number of café-au-lait spots and cutaneous and subcutaneous neurofibromas). Potential interactions were assessed by pairwise analyses and confounding by fitting multiplicative models. Variables yielding *P* values smaller than 0.15 in the univariate analyses were entered into a multiple logistic regression model. The final model included the variables independently associated with the presence of internal neurofibromas.

We first conducted an analysis without the individuals who had missing data (complete-case analysis). We then estimated the missing values for the co-variables independently associated with the internal neurofibromas in the final model, using the multiple-multivariate-imputations-by-chained-equations procedure in STATA (69)], with the missing-at-random assumption. We used all predictors together to impute the missing data values, and we independently analyzed 10 copies of the data using 10 cycles of regression. Logistic regression for binary variables and multinomial logistic regression for categorical variables with  $k > 2$  classes were used to impute missing values.

We conducted all statistical analysis using STATA Statistical Software (version 11.0, StataCorp LP, College Station, TX, USA) and LogXact-8 software (2007, CYTEL Inc. Cambridge, MA, USA).

## **RESULTS**

### **Study population**

Table 1 reports the main characteristics of the 357 children with NF1 included in our study. Mean age was 7.7 ( $\pm 5.0$ ) years (range, 0.01-16.9), and there were 192 (53.8%) males. Internal neurofibromas were present in 35 (9.8%) patients. The prevalence of internal neurofibromas increased during adolescence (Figure 2). Internal neurofibromas developed earlier in females than in males (Figures 3).

### **Characteristics associated with internal neurofibromas**

Table 2 compares the clinical characteristics in the patients with and without internal neurofibromas. By univariate analysis, five variables were associated or nearly associated with internal neurofibromas: age (continuous variable), subcutaneous neurofibromas, plexiform neurofibromas, Lisch nodules, and xanthogranulomas.

The presence of Lisch nodules was strongly associated with the other variables and was not independently associated with internal neurofibromas in the multivariate analysis. The study of potential interactions between variables showed a effect modification between plexiform neurofibromas and subcutaneous neurofibromas ( $P=0.10$ ). To facilitate the interpretation of the model, the usual assessment of interaction effects is done by entering an additional variable composed of the product of the two variables (76)]. So, we replaced the interaction term by a composite variable: no subcutaneous and no plexiform neurofibromas (reference category), either subcutaneous or plexiform neurofibromas, and both subcutaneous and plexiform neurofibromas. We chose these three categories because subcutaneous and plexiform neurofibromas had closely similar OR values by univariate analysis (Table 2).

By multivariate analysis, three characteristics were independently associated with internal neurofibromas: age, presence of xanthogranulomas and presence of both subcutaneous and plexiform neurofibromas (Table 3).

The general pattern of the results after multiple imputation was similar to that obtained in the patient subset with complete data (Table 3). *P* values were smaller for all three variables independently associated with internal neurofibromas. The OR for the presence of subcutaneous or plexiform neurofibromas was similar between the complete-case and imputed models. The bound categories were thinner as the multivariate analysis included all cases after multiple imputation.

## DISCUSSION

In this study, we identified easily recognizable clinical characteristics associated with internal neurofibromas in children with NF1. By multivariate analysis, age, xanthogranulomas, and presence of both subcutaneous and plexiform neurofibromas were independently associated with internal neurofibromas.

NF1 has been reported to be associated with a 15-year decrease in life expectancy (23)]. We recently reported overall excess mortality in a cohort of 1895 patients with NF1 compared to the general population in France (84)]. Excess mortality occurred among NF1 patients aged 10 to 20 years (Standard Mortality Ratio, SMR, 5.2; 95%CI, 2.6-9.3;  $P < 10^{-4}$ ) and 20 to 40 years (SMR, 4.1; 95%CI, 2.8-5.8;  $P < 10^{-4}$ ). MPNSTs were the main cause of death (60%) (84). Thus, the main concern in the long-term clinical management of patients with NF-1 is the identification of patients at high risk for MPNSTs developed from preexisting internal neurofibromas (23)]. Although no effective treatment is available for inoperable MPNSTs, several novel targeted treatments may hold promise [20]. We therefore developed a clinical score, the NF1Score, for predicting the presence of internal neurofibromas in adults with NF1 (87)].

Internal neurofibromas grow faster in young children than in adults (63)]. Volumetric MRI has been used to assess the growth rate of internal neurofibromas in 49 NF1 patients (median age, 8.3 years; range, 3.3-25) with a median follow up of 34 months. The growth rate

per year of internal neurofibromas was significantly greater in the younger patients (<8.3 years) than in the older patients (21.1% versus 8.4%,  $P=0.001$ ). In addition, if effective treatments are found, they will be more likely to prevent the growth of internal neurofibromas than to reduce their size (81)]. Therefore, the benefits will probably be greatest if the treatments are given at the time of most rapid internal neurofibroma growth, that is, in childhood or adolescence. Therefore, the risk factors identified in our study will help to improve the clinical management of children with NF1.

Many factors support the internal validity of our study. First, all patients had a definitive diagnosis of NF1. Selection bias seems unlikely, as the prevalence of NF1 characteristics were consistent with those reported previously in other populations of NF1 patients (89-90)]. Our study provided a good external validation as it was performed using two NF1 populations, from France and North America, respectively. However, our patients were recruited at hospitals and may therefore have had greater disease severity compared to the overall NF1 population. An important strength of our study is the accuracy of the information on internal neurofibroma status obtained by using MRI or CT. However, the absence of routine MRI or CT in the study population may constitute a limitation of our study. MRI or CT was performed only when routine imaging studies (chest radiograph and abdominal sonogram) or clinical symptoms suggested the presence of internal neurofibromas. Half our patients did not undergo MRI or CT. This may have resulted in verification bias. Finally, to validate the final model, we used multiple imputation analysis to deal with the missing values. Multiple imputations allow individuals with incomplete data to be included in analyses and improve the validity of the results. The empirical rule of entering only one variable per 10 events in a multivariate analysis model was followed (53)].

Internal neurofibromas were present in 35 (9.8%) patients, in keeping with previous data. In a study of 53 children with NF1 who underwent MRI of the entire spine, 7 (13.2%) patients had internal spinal neurofibromas (80)]. In our study, older age was independently associated with internal neurofibromas. Excess risk of developing internal neurofibromas seems to occur between the adolescence and the age of to 30 in NF1 patients. Moreover internal neurofibromas increased during adolescence and developed earlier in females, in agreement with previous data (82)]. Changes in steroid hormone production may affect the NF1 phenotype. Immunostaining studies have provided support for this hypothesis by identifying the progesterone receptor in neurofibromas (41)]. Estrogen and progesterone

increased the growth rate of MPNSTs xenografts in mice (43)]. Plexiform and subcutaneous neurofibromas were also independently associated with internal neurofibromas in our study, in accordance with earlier work in both adults and children (25, 91)]. In the MRI study of the spine in children with NF1, characteristics found more often in patients with than without internal neurofibromas included scoliosis (71.4% vs. 30.4%), subcutaneous neurofibromas (71.4% vs. 39.1% ), and plexiform neurofibromas (28.6% vs. 8.7%); none of these differences was statistically significant, ( $P=0.08$ ,  $P=0.22$ , and  $P=0.17$ , respectively), possibly because statistical power was limited (80)]. Furthermore, patients with subcutaneous neurofibromas were at higher risk for mortality in two different NF1 populations, from France (34)] and North America (33)], respectively. Finally, xanthogranulomas were independently associated with internal neurofibromas in our study. NF1 children with xanthogranulomas are at increased risk for juvenile chronic myelogenous leukemia (92)]. Although it should be noted that this study included a small number of patients and has not been confirmed since. To our knowledge, xanthogranulomas have not been previously reported to be associated with internal neurofibromas.

In sum, we identified easily recognizable clinical characteristics that were associated with internal neurofibromas in children with NF1. These risk factors could be used to ensure the early identification of NF1 patients who require particularly close monitoring for internal neurofibromas. In the future, this approach may allow the initiation of treatments at the time of fastest growth of internal neurofibromas, when they are most likely to be effective.

## **List of Abbreviations**

aOR: adjusted odds ratio

CI: confidence interval

CT: computed tomography

MPNST: malignant peripheral nerve sheath tumor

MRI: magnetic resonance imaging

NF-1: neurofibromatosis 1

SC-NF(s): subcutaneous neurofibroma(s)

SD: standard deviation

**Conflict of interest:** SBG, PW, DR, SF, OC, SB, LVA, VMR, SHR, ES have non-financial interests that may be relevant to the submitted work.

## **Contributors' statement**

SBG and PW made substantial contributions to the study conception and design. SBG, PW, SF, SHR, VMR, LVA, ES, DR, and SB made substantial contributions to data acquisition or analysis and to interpretation of the data. ES performed the data analysis under the supervision of SBG. SBG, PW, and ES were involved in drafting the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version submitted for publication

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**Table 1. General characteristics of the 357 children with neurofibromatosis-1 included in the study. The data are number (%) unless otherwise specified.**

<b>Clinical characteristics (n=missing data)</b>	<b>n (%)</b>
Male gender	192 (53.8)
Age at the first visit in years, mean±SD (range)	7.7±5.0 (0.01-16.9)
Familial case	183 (51.3)
<b>Internal neurofibromas</b>	<b>35 (9.8)</b>
Subcutaneous neurofibromas (n=17)	72 (20.2)
Cutaneous neurofibromas	153 (42.9)
Plexiform neurofibromas (n=184)	83 (23.3)
Café-au-lait spots	354 (99.2)
Freckles (n=11)	219 (61.3)
Lisch nodules (n=84)	160 (44.8)
Xanthogranulomas (n=68)	14 (3.9)
Short stature (n=32)	36 (10.1)
Macrocephaly (n=21)	85 (23.8)
Nonunion	13 (3.6)
Dysplasia (n=8)	31 (8.7)
Facial asymmetry (n=72)	23 (6.4)
Hypertension (n=28)	3 (0.8)

**Table 2: Univariate analysis of selected characteristics for associations with the presence of internal neurofibromas in 357 children with neurofibromatosis-1**

	Internal neurofibromas		Odds Ratio (95%CI)†	P value‡
	No n=322	Yes n=35		
Male gender	148 (46)	16 (46)	1.01 (0.49-2.07)	0.977
<b>Age (years)</b>	7.35 ± 4.87	10.67 ± 4.81	<b>1.14* (1.07-1.23)</b>	<b>&lt;10<sup>-4</sup></b>
Familial cases	166 (51)	17 (49)	0.61 (0.29-1.27)	0.184
<b>Subcutaneous neurofibromas (n=305/35)</b>	53 (17)	19 (54)	<b>4.51 (2.13-9.55)</b>	<b>&lt;10<sup>-4</sup></b>
Cutaneous neurofibromas	139 (43)	14 (40)	0.78 (0.37-1.64)	0.512
<b>Plexiform neurofibromas (n=140/33)</b>	<b>60 (43)</b>	<b>19 (70)</b>	<b>3.60 (1.55-8.34)</b>	<b>0.003</b>
Café-au-lait spots	319 (99)	35 (100)	0.81 (0.04 - ∞)	1.000
Freckles (n=312/34)	194 (56)	13 (28)	1.09 (0.47-2.52)	0.840
<b>Lisch nodules (n=250/23)</b>	<b>151 (60)</b>	<b>9 (39)</b>	<b>0.35 (0.14-0.86)</b>	<b>0.05</b>
<b>Xanthogranulomas (n=257/32)</b>	<b>10 (4)</b>	<b>4 (13)</b>	<b>6.60 (1.69-25.74)</b>	<b>0.007</b>
Short stature (n=293/32)	32 (11)	4 (13)	1.00 (0.32-3.13)	0.996
Macrocephaly (n=302/34)	12 (35)	73 (24)	1.65 (0.76-3.54)	0.203
Nonunion	1 (3)	12 (4)	1.8 (0.3-10.2)	0.684
Dysplasia (=314/35)	27 (9)	4 (11)	1.06 (0.34-3.35)	0.914
Facial asymmetry (n=261/24)	20 (8)	3 (12)	1.23 (0.45-6.93)	0.419
Hypertension (n=298/31)	2 (1)	1 (3)	5.41 (0.40-73.93)	0.205

†Odds ratio with 95% confidence interval (95% CI) by logistic regression adjusted for age;

\*OR with 95%CI giving the risk increase for a 1-year increase in age; ‡ P value by logistic regression, Data are numbers (%) or means±1 standard deviation.

**Table 3: Characteristics independently associated with internal neurofibromas in the multivariate analysis on complete cases (n=184) and after multiple imputation (n=357).**

	Complete-case analysis <sup>a</sup> , n=184			Imputed data <sup>b</sup> , n=357		
	Odds Ratios	95% CI <sup>†</sup>	<i>P</i> value <sup>‡</sup>	Odds Ratios	95% CI*	<i>P</i> value**
<b>Age</b>	<b>1.12<sup>x</sup></b>	1.02-1.23	0.012	<b>1.16<sup>x</sup></b>	1.07-1.27	0.001
<b>Subcutaneous/plexiform NFs</b>						
- None	<b>1</b>	-	-	<b>1</b>	-	-
- Either subcutaneous or plexiform NFs	<b>1.15</b>	0.42-3.15	0.781	<b>1.06</b>	0.40-2.80	0.901
- Both subcutaneous and plexiform NFs	<b>4.95</b>	1.80-13.64	0.002	<b>5.89</b>	2.18-15.89	<10 <sup>-4</sup>
<b>Xanthogranuloma</b>	<b>4.51</b>	0.94-21.73	0.06	<b>6.80</b>	1.52-30.44	0.012

<sup>a</sup>Excluding individuals with missing values

<sup>b</sup>Missing data imputed using imputation with chain equations

<sup>†</sup>Odds ratio with 95% confidence interval (95% CI) by logistic regression

<sup>‡</sup>*P* value by logistic regression

<sup>x</sup>OR with 95% CI giving the risk increase for a 1-year increase in age

\*Odds ratio with 95% confidence interval (95% CI) by logistic regression with multiple imputation

\*\* *P* value by logistic regression with multiple imputation

NF, neurofibroma

## **Figure Legends**

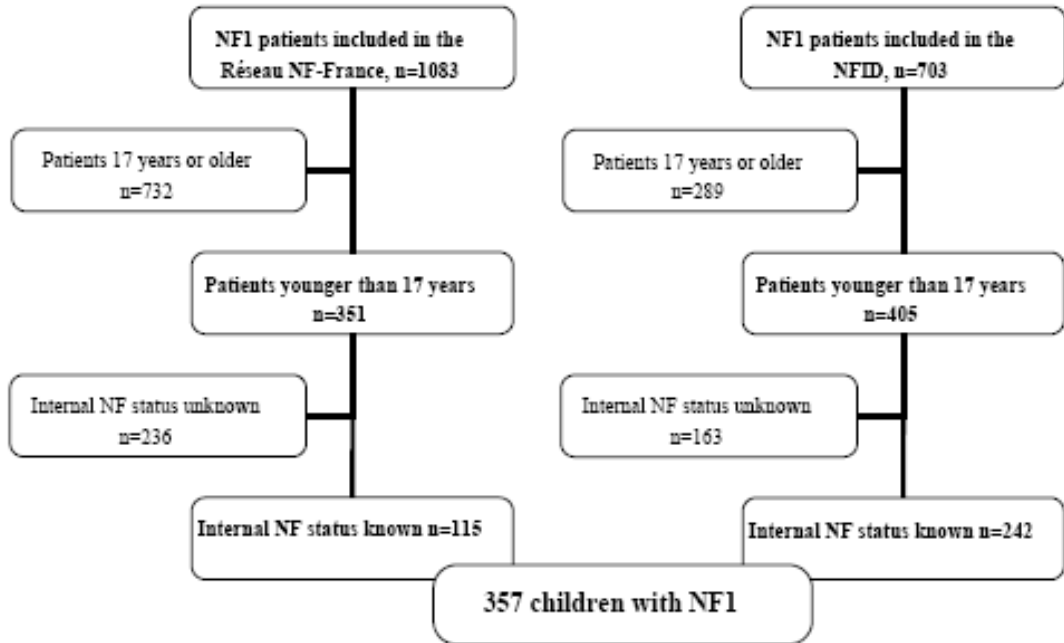
**Figure 1.** Flow chart

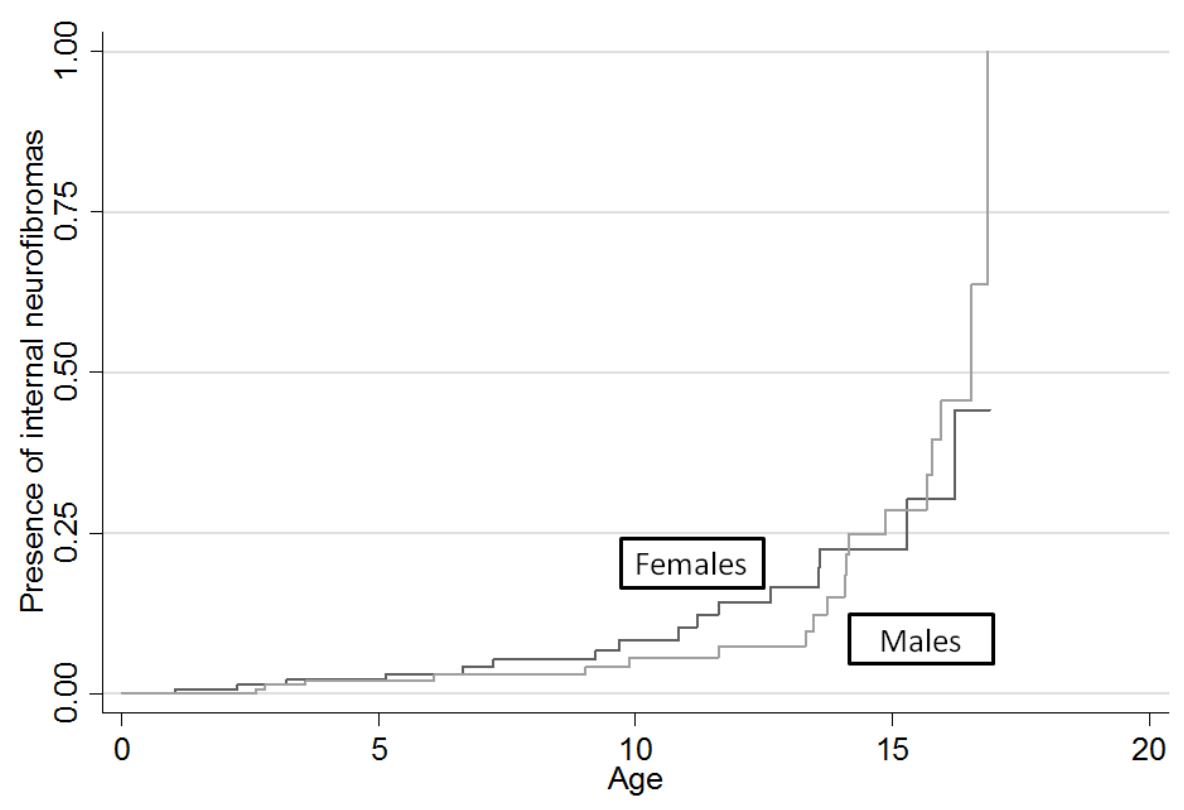
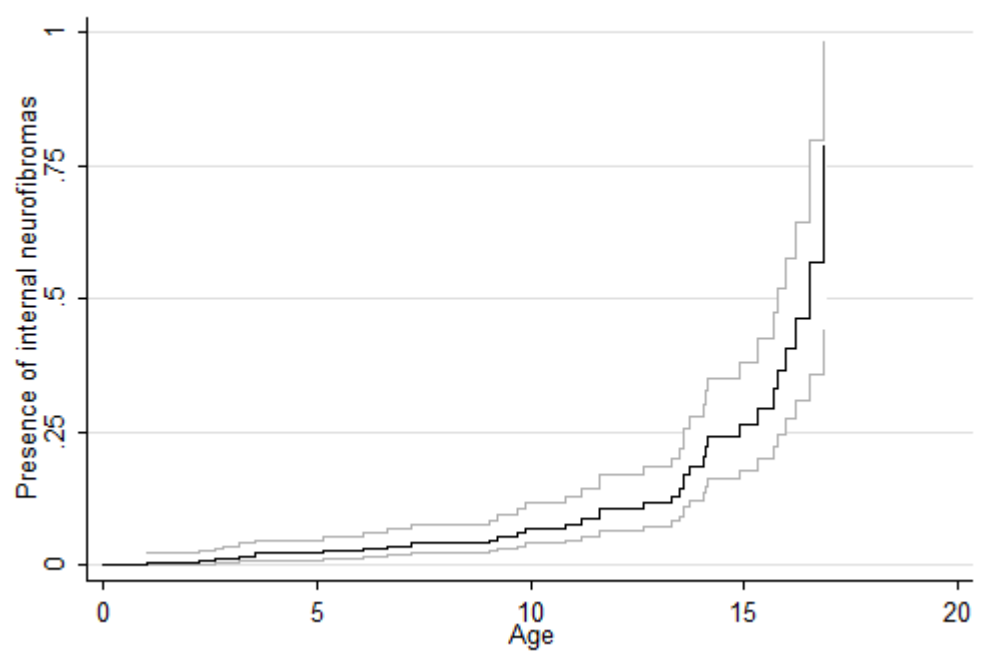
**Figure 2.** Probability of having internal neurofibromas with the 95% Confidence Interval

**Figure 3.** Probability of having internal neurofibromas stratified by sex (females black line and males grey line)



Figure 1





## **ANNEXE 5**

**Cahier d'observation** « *Analyse de la mortalité au cours de la Neurofibromatose 1*  
*Cohortehistorique française de 1895 patients* »

### Identité Patient

Numéro du patient : Cohorte I\_\_I\_\_I\_\_I  
Site :  Necker  Trousseau  Henri Mondor  
NIP : .....  
NDA : .....  
Nom  
Prénom  
Date de naissance : I\_\_I\_\_I / I\_\_I\_\_I / I\_\_I\_\_I\_\_I (jour / mois / année)  
Sexe :  Féminin  Masculin  
Décédé :  Oui  Non Date de décès : I\_\_I\_\_I / I\_\_I\_\_I / I\_\_I\_\_I\_\_I  
Lieu de naissance : ..... CP : I\_\_I\_\_I\_\_I

### Information patient

Médecin Responsable : .....  
Autre Médecin : .....  
Adresse : .....  
.....  
Ville : ..... Code Postal : I\_\_I\_\_I\_\_I  
Téléphone 1 : ..... Téléphone 2 : .....  
Date de la première visite : I\_\_I\_\_I / I\_\_I\_\_I / I\_\_I\_\_I\_\_I  
Date de la dernière visite : I\_\_I\_\_I / I\_\_I\_\_I / I\_\_I\_\_I\_\_I  
Forme Familiale :  Oui  Non

### Critères de diagnostics NF1

Plus de 6 taches café au lait significative  Oui  Non  
Deux neurofibromes ou plus de n'importe quel type  
ou un neurofibrome plexiforme  Oui  Non  
Ephélides" axillaires ou inguinales.  Oui  Non  
Gliome des voies optiques  Oui  Non  
Deux nodules de Lisch ou plus  Oui  Non  
Une lésion osseuse caractéristique  Oui  Non  
Un parent du premier degré atteint de NF1  Oui  Non

### Complications

Neurofibrome(s) plexiforme(s)  Oui  Non  
Nombre : I\_\_I\_\_I  
Localisations :  Tronc..  Tête  Membres  
Gliome des voies optiques :  Non  Symptomatique  Asymptomatique  
Objets brillants non identifiés à l'IRM :  Oui  Non  
Scoliose nécessitant chirurgie :  Oui  Non  
Difficultés d'apprentissage :  Oui  Non  
Hydrocéphalie :  Oui  Non  
Tumeurs malignes des gaines nerveuses :  Oui  Non  
Phéochromocytome :  Oui  Non  
Sténose de l'artère rénale :  Oui  Non