ARTICLE

Cancer risk in patients with Noonan syndrome carrying a *PTPN11* mutation

Marjolijn CJ Jongmans*,[1], Ineke van der Burgt[1], Peter M Hoogerbrugge[2], Kees Noordam[3], Helger G Yntema[1], Willy M Nillesen[1], Roland P Kuiper[1], Marjolijn JL Ligtenberg[1], Ad Geurts van Kessel[1], J Han JM van Krieken[4], Lambertus ALM Kiemeney[5,6] and Nicoline Hoogerbrugge[1]

Noonan syndrome (NS) is characterized by short stature, facial dysmorphisms and congenital heart defects. *PTPN11* mutations are the most common cause of NS. Patients with NS have a predisposition for leukemia and certain solid tumors. Data on the incidence of malignancies in NS are lacking. Our objective was to estimate the cancer risk and spectrum in patients with NS carrying a *PTPN11* mutation. In addition, we have investigated whether specific *PTPN11* mutations result in an increased malignancy risk. We have performed a cohort study among 297 Dutch NS patients with a *PTPN11* mutation (mean age 18 years). The cancer histories were collected from the referral forms for DNA diagnostics, and by consulting the Dutch national registry of pathology and the Netherlands Cancer Registry. The reported frequencies of cancer among NS patients were compared with the expected frequencies using population-based incidence rates. In total, 12 patients with NS developed a malignancy, providing a cumulative risk for developing cancer of 23% (95% confidence interval (CI), 8–38%) up to age 55 years, which represents a 3.5-fold (95% CI, 2.0–5.9) increased risk compared with that in the general population. Hematological malignancies occurred most frequently. Two malignancies, not previously observed in NS, were found: a malignant mastocytosis and malignant epithelioid angiosarcoma. No correlation was found between specific *PTPN11* mutations and cancer occurrence. In conclusion, this study provides first evidence of an increased risk of cancer in patients with NS and a *PTPN11* mutation, compared with that in the general population. Our data do not warrant specific cancer surveillance.
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INTRODUCTION

Noonan syndrome (NS, OMIM 163950) is an autosomal dominant developmental disorder characterized by short stature, facial dysmorphisms and congenital heart defects (reviewed in Tartaglia *et al*[1]). It is a relatively common syndrome with an estimated incidence of 1 in 2500 births. NS is associated with a higher risk for benign and malignant proliferative disorders. Approximately 10% of the children with NS are affected by a mild myeloproliferative disorder, which is usually self-limiting.[2] A smaller percentage of patients develops juvenile myelomonocytic leukemia (JMML)[3,4] and/or other hematological malignancies.[5–11] In addition, several case reports have suggested a possible increased incidence of solid tumors in NS patients, specifically neuroblastoma and embryonal rhabdomyosarcoma.[12–19] Benign proliferative conditions reported include multiple giant cell lesions and granular cell tumors.[20,21] Until now, heterozygous germline mutations in six genes encoding components of the RAS/MAPK pathway, that is, *PTPN11, KRAS, SOS1, RAF1, NRAS* and *SHOC2* have been identified in NS.[1] The RAS/MAPK pathway represents a signal transduction cascade involved in processes of cell proliferation, differentiation, survival and death. Mutations in *PTPN11,* which encodes the non-receptor protein tyrosine phosphatase SHP-2, are most frequently observed and account for ~50% of NS cases. SHP-2 has two Src homology 2 domains

(N-SH2 and C-SH2), a catalytic protein tyrosine phosphatase (PTP) domain and a COOH terminus containing tyrosine phosphorylation sites. Most *PTPN11* mutations involved in NS disrupt the autoinhibitory interaction between the N-SH2 and the PTP domain, resulting in enhanced phosphatase activity and increased signaling through the RAS–MAPK pathway.[22]

The observed increase in JMML incidence in NS and the important role of SHP-2 in RAS signaling, suggest a role of mutant SHP-2 in cancer development. Indeed, somatic mutations in *PTPN11* occur in ~35% of sporadic JMML and in a small percentage of other hematological malignancies.[23–25] Similarly, solid tumors have been screened for somatic *PTPN11* mutations. In some of them, such as lung-, liver- and colorectal cancer, *PTPN11* mutations have been identified, be it infrequent.[19,26] On the basis of these findings, and considering the universal role of increased RAS–MAPK signaling in oncogenesis, we hypothesized that patients with NS might have an increased risk for developing a broad range of malignancies, in addition to leukemia. So far, no quantitative data are available on the risk of cancer in NS caused by *PTPN11* mutations. Therefore, guidelines for cancer prevention in patients with NS are yet to be developed. We have performed a first cohort study to explore the cumulative risk of developing cancer in patients with NS carrying a

[1]Department of Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; [2]Departments of Pediatric Hemato-oncology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; [3]Department of Pediatric Endocrinology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; [4]Department of Pathology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; [5]Departments of Epidemiology, Biostatistics and HTA, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; [6]Department of Urology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
*Correspondence: Dr MCJ Jongmans, Department of Human Genetics, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB Nijmegen, The Netherlands. Tel: +31 24 3613946; Fax: +31 24 3668774; E-mail: m.jongmans@antrg.umcn.nl
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germline *PTPN11* mutation. In addition, we investigated whether specific *PTPN11* mutations result in an increased cancer risk and whether specific malignancies are particularly associated with NS. For the latter purpose, we have reviewed all NS patients with a *PTPN11* mutation and a malignancy reported in the literature.

PATIENTS AND METHODS

Study population
The study population consisted of 318 Dutch patients with NS in whom a germline *PTPN11* mutation was identified in the laboratory for DNA diagnostics of the Department of Human Genetics, Radboud University Nijmegen Medical Center, the Netherlands. To obtain a genetically uniform cohort of patients, we exclusively included patients with a *PTPN11* mutation. This gene is most commonly involved in NS, accounting for ~50% of patients. The mutations were identified between January 2002 and December 2008. Patients with a mutation related to LEOPARD syndrome (*n*=17) and patients who died prenatally or in the first 3 months of life (*n*=4) were excluded. After this exclusion, the cohort consisted of 235 index patients and 62 affected family members, of which 143 (48%) were males and 154 (52%) were females. At the end of the follow-up period, the patients had a mean age of 18 years and a median age of 13 years (range 0.6–95 years).

Cancer follow-up
Diagnoses of cancer were retrieved using three different sources of information. First, we screened the medical history of the patients using the referral forms for *PTPN11* analysis. Next, we performed a search in the National Automated Archive of Pathology (PALGA). All histology and cytology reports in the Netherlands are collected in this national database, which was founded in 1971 and achieved virtually complete national coverage in 1980.[27] As PALGA is an incomplete database for hematological malignancies and lacks malignancies without histological confirmation, we also consulted The Netherlands Cancer Registry (NCR). The NCR has a complete national coverage since 1989.

Statistical methods
Time at risk started on date of birth of the patient and ended on the date of cancer diagnosis, emigration, the date of death or the closing date of the study (5 December 2008), whichever occurred first. Cumulative risks of cancer were calculated by standard Kaplan–Meier analysis in which the end point was defined as a diagnosis of any cancer and follow-up was defined as age at diagnosis. Patients who died before a cancer diagnosis, were lost to follow-up or reached the end of the study period without a cancer diagnosis, were censored at the corresponding age.

To be able to compare the observed cumulative risk of cancer among the study population with the average cumulative risk in the Dutch population, we calculated the expected frequency of cancer in the study population based on the Dutch population-based incidence rates in 2002–2006 http://www.ikcnet.nl). Because the Netherlands has a nationwide population-based cancer registry, reference values can be taken efficiently from the registry and can be considered more valid and stable than estimates taken from a specific (smaller) control group. Basal cell carcinoma of the skin was excluded from the analysis, as this neoplasm is not registered in the NCR. For the calculation of the expected frequency, we multiplied the gender and 5-year age-specific population years in the study population with the gender and 5-year age-specific incidence rates in the Dutch population. Subsequently, the 95% confidence intervals (CIs) of the observed *versus* expected ratios were calculated using the open source program OpenEpi version 2.3 (http://www.openepi.com).

Sequence analyses
Sequencing of *PTPN11* exons 1–4, 7, 8 and 11–14 and surrounding splice donor and acceptor sites was performed in a routine DNA diagnostic setting (www.dnadiagnostieknijmegen.nl) on DNA isolated from peripheral blood cells. Primer sequences and PCR conditions are available upon request. In one patient who was diagnosed with acute lymphocytic leukemia (ALL), mutation analysis was repeated on DNA isolated from gastric antrum tissue, to confirm the germline status of the mutation.

Except for one JMML and one malignant mastocytosis, all malignancies were available for sequence analysis. In the patient with ALL, DNA was extracted from bone marrow cells, in all other cases DNA was extracted from paraffin-embedded tumor tissue. The sample derived from the patient with basal cell carcinoma, had a tumor cell percentage of less than 60% and was, therefore, excluded from further analysis.

Primer pairs were developed that covered a region of approximately 150 base pairs, including the *PTPN11* mutation identified in the germline of the patient. Primer sequences and PCR conditions are available upon request.

RESULTS

The median follow-up time for cancer in our cohort of 235 index patients and 62 affected family members was 13 years (range 0.6–95 years). Approximately 18.5% of the patients were followed for less than 5 years, 36.4% for 5–15 years and 45.1% for more than 15 years. In total, 12 malignancies were observed: three in children (≤15 years), three in adolescents and young adults (15–29 years) and six in adults (≥30 years) (Table 1). The median age at diagnosis was 25 years (range: 0–57). One patient developed a basal cell carcinoma that was not included in our statistical analyses. In one case, the definitive cancer diagnosis was unclear and, as a consequence, this patient was censored at the date of that arbitrary diagnosis. This latter patient, a 5-year-old child, carried a c.188A>G (p.Tyr63Cys) *PTPN11* mutation and was diagnosed with nephroblastomatosis and, possibly, a small nephroblastoma. The germline status of the c.188A>G mutation in the patient with ALL was confirmed through the analysis of DNA isolated from previously obtained stomach (gastric antrum) tissue from this patient. Out of 10, 5 *PTPN11* mutations found in our NS patients with a malignancy were previously reported as somatic mutations in sporadic malignancies (Table 2). The frequency of malignancies among carriers of the same germline mutation in our cohort is also displayed in Table 2.

Assessment of the occurrence of cancer in our study cohort (Figure 1) provided a cumulative risk of 23% (95% CI, 8–38%) up to the age of 55 years, which represents a 3.5-fold increased risk (95% CI, 2.0–5.9) compared with that in the general population.

Breast cancer was observed twice, whereas all other malignancies were observed only once. Six cancer types have previously been reported in the literature in patients with NS and a *PTPN11* mutation, that is, JMML, neuroblastoma, ALL, non-Hodgkin lymphoma, glioma and breast cancer (Table 1).

The child with JMML harbored a *de novo* p.Gly503Ala mutation. To our knowledge, this mutation has not yet been reported as a germline mutation in a patient with NS, but only as a somatic event in JMML.[23,24,28,29] Other tissues of this patient were unavailable to confirm the germline status of the mutation. However, the child was reported to have a valvular pulmonic stenosis, an atrial septum defect, hypertelorism and low-set ears with a thick helix, and, therefore, likely had NS. Photographs of the patient were seen by Dr I van der Burgt (coauthor), and she considered the patient's facial features as suggestive for NS. It can be difficult to distinguish a benign myeloproliferative disorder, which is usually self-limiting and frequently observed in NS, from the rare aggressive condition JMML.[28,30,31] Assessment of the clonal status of the monocytic population can be helpful in this respect,[32] but we were unable to infer whether such a diagnostic test was performed in this patient. The patient underwent a bone marrow transplantation, indicating that the course of the disease was aggressive.

In a search for copy number alterations of *PTPN11* mutant alleles, we performed sequence analysis in tumor DNA of 9 out of 12 patients diagnosed with a malignancy. None of the sequences showed an overrepresentation of the mutated allele compared with the wild-type allele.

Table 1 Patients with Noonan syndrome carrying a germline *PTPN11* mutation and diagnosed with a malignancy reported here (1–12) and from the literature

No./Ref.	Malignancy	Age at diagnosis	PTPN11 mutation
1	JMML	1 year	c.1508G>C (p.Gly503Ala)
2	Neuroblastoma (adrenal gland)	1 year	c.844A>G (p.Ile282Val)
Mutesa et al[18]	Neuroblastoma	5 months	c.179G>C (p.Gly60Ala)
Bentires-Alj et al[19]	Neuroblastoma	?	c.185A>C (p.Tyr62Cys)
Kondoh et al[16]	Neuroblastoma	6 months	c.1504T>A (p.Ser502Thr)
Chantrain et al[5]	Neuroblastoma[a]	4 years	c.922A>G (p.Asn308Asp)
3	Dysembryoplastic neuro-epithelial tumor (temporal lobe)	10 years	c.179G>C (p.Gly60Ala)
4	Oligodendroglioma (hypothalamus)[b]	17 years	c.417G>C (p.Glu139Asp)
Sherman et al[33]	Low grade glioneural tumor	6 years	c.172A>G (p.Asn58Asp)
Martinelli et al[34]	Oligodendroglioma[c]	24 years	c. 64A>G (p.Thr22Ala)
Schuettpelz et al[35]	Pilocytic astrocytoma	8 years	c.1471_1472delinsTT (p.Pro491Phe)
5	Precursor B-ALL	23 years	c.188A>G (p.Tyr63Cys)
Roti et al[11]	Common B ALL	17 years	c.179G>C (p.Gly60Ala)
Karow et al[7]	Pre-B cell ALL	2 years	c.1510A>G (p.Met504Val)
Karow et al[7]	Pre-B cell ALL	8 years	c.417G>C (p.Glu139Asp)
Chantrain et al[5]	AML-M4[a]	5 years	c.922A>G (p.Asn308Asp)
La Starza et al[8]	AML-M4	34 years	c.178G>T (p.Gly60Cys)
Matsubara et al[10]	CMML	35 years	c.922A>G (p.Asn308Asp)
6	Non-Hodgkin lymphoma (jejunum)	25 years	c.1472C>T (p.Pro491Leu)
Ferrero et al[6]	Non-Hodgkin lymphoma[d]	?	c.1508G>A (p.Gly503Glu)
Lo et al[9]	Hodgkin's lymphoma	35 years	c.1507G>C (p.Gly503Arg)
7	Multifocal ductal breast cancer	48 years	c.774G>T (p.Glu258Asp)
8	Ductal breast cancer	52 years	c.925A>G (p.Ile309Val)
Shaw et al[36]	Breast cancer[e]	45 years	c.317A>C (p.Asp106Ala)
9	Malignant epithelioid angiosarcoma (mediastinum)	38 years	c.1510A>G (p.Met504Val)
10	Colorectal cancer (sigmoid)	44 years	c.1510A>G (p.Met504Val)
11	Malignant mastocytosis	46 years	c.922A>G (p.Asn308Asp)
12	Basal cell carcinoma	57 years	c.188A>G (p.Tyr63Cys)
Yoshida et al[37]	Hepatoblastoma	1 months	c.922A>G (p.Asn308Asp)

The lines in white are patients from our study cohort, the lines in gray are patients reported in the literature. Reports of children with NS who developed JMML are not included, these are summarized in the report of Aoki et al.[38]
[a]Same patient.
[b]This patient has previously been published by us.[39]
[c]The p.Thr22Ala change was found in a brain tumor and subsequently in peripheral blood cells. The patient had no features of NS. This molecular lesion results in an increased basal phosphatase activity of *PTPN11* and was not observed in over 300 control individuals.[34]
[d]The mutation was identified in the son of this patient. The mother had short stature and a facial appearance typical of NS. DNA was not available for segregation analysis.
[e]Mutation details derived by personal communication with the author.

DISCUSSION

Our cohort study shows a 3.5-fold (95% CI, 2.0–5.9) increase in the overall cancer risk up to the age of 55 years in NS patients with a *PTPN11* mutation, compared with that in the general population. We encountered six patients who developed cancer at adult age, whereas until now only four adult patients with a germline *PTPN11* mutation and a malignancy have been reported in the literature. The overall risk of cancer up to the age of 70 years cannot be estimated because of the age distribution in our cohort, with a majority of patients below 30 years of age.

As expected, hematological malignancies were most frequent (4/12, 33%). In one patient, this malignancy was specified to be a malignant mastocytosis, which so far has not been reported in NS. The non-hematological malignancies observed included three novel cancers in NS: a malignant epithelioid angiosarcoma, a colon cancer of the sigmoid and a basal cell carcinoma.

One way to explore potential correlations between germline *PTPN11* mutations and the development of a specific malignancy is screening for loss of heterozygosity and/or uniparental disomy, in conjunction with duplication or amplification of a heterozygous germline *PTPN11* mutation in the tumors of patients. This two-hit scenario was previously observed in NS, but is not a consistent finding in all NS-associated malignancies.[7,40] In our cohort, we did not observe multiple copies of the mutant *PTPN11* allele in nine tumors tested. As a consequence, we can only speculate about the influence of *PTPN11* mutations on the development of these malignancies. For instance, we found one patient with colorectal cancer diagnosed at an unusually young age of 44 years. This early onset may be related to the *PTPN11* mutation. In sporadic colorectal cancer an association has been found with the RAS–MAPK pathway and activating mutations in the *KRAS* oncogene are known to have a role in the progression from a benign adenoma to a dysplastic adenocarcinoma.[41] Nearly 50% of colon cancers harbor activating mutations in *KRAS*.[41] Therefore, the germline *PTPN11* mutation in the patient with colorectal cancer may have played a role in tumor progression.

Similarly, a relation between *PTPN11* and breast cancer has not been noted before. Remarkably, however, two patients in our cohort (48 and 52 years of age) developed breast cancer, which has a mean age at diagnosis of 60 years in the general population. Interestingly, several studies have shown a role for RAS–MAPK pathway activation in breast cancer development.[42] Furthermore, women with Neurofibromatosis type1, another syndrome associated with aberrant RAS

Table 2 Frequency of *PTPN11* mutations associated with cancer in our cohort and literature data on its somatic occurrence

PTPN11 mutation	Cancer related frequency[a]	Previously reported as somatic mutation in cancer[b]
c.179G>C (p.Gly60Ala)	1 of 8	+
c.188A>G (p.Tyr63Cys)	2 of 41	+
c.417G>C (p.Glu139Asp)	1 of 17	+
c.774G>T (p.Glu258Asp)	1 of 3	−
c.844A>G (p.Ile282Val)	1 of 14	−
c.922A>G (p.Asn308Asp)	1 of 62	−
c.925A>G (p.Ile309Val)	1 of 3	−
c.1472C>T (p.Pro491Leu)	1 of 4	+
c.1508G>C (p.Gly503Ala)	1 of 1	+
c.1510A>G (p.Met504Val)	2 of 17	−

[a]The second number represents how often the mutation has been identified in our cohort, whereas the first number displays the number of patients in the cohort with this mutation who developed a malignancy.
[b]The data in this column are derived from Aoki *et al*.[38]
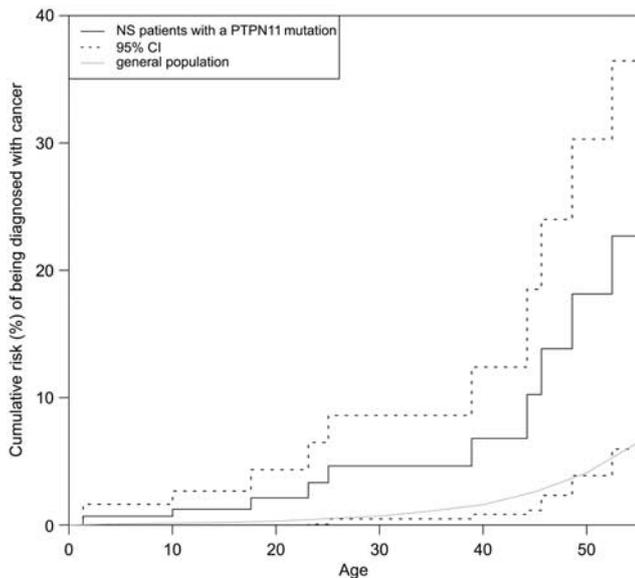
Figure 1 Age-specific cumulative risk of cancer in patients with Noonan syndrome and a mutation in *PTPN11*. We found a cumulative risk of 23% (95% CI, 7.6–38.4%) up to age of 55 years, which represents a 3.5-fold increased risk (95% CI, 2.0–5.9) compared with the general population.

signaling, are at a moderately increased risk of developing breast cancer.[43] However, in a large panel of human breast cancers, no somatic mutations in *PTPN11* were detected.[19]

It is known that somatic *PTPN11* mutations associated with cancer generally confer a higher activation of SHP-2 function than those associated with NS. Likewise, SHP-2 mutants related to NS-associated leukemia exhibit higher PTP activity than do those related to NS alone.[22,29,44] On the basis of these findings we hypothesized that the *PTPN11* mutations found in our cohort of NS patients with a malignancy might show overlap with mutations detected somatically in cancer. Indeed, half of the NS patients with cancer are carriers of a mutation that has previously been reported as a somatic mutation in sporadic malignancies (Table 2).[38] However, also 34% of the NS patients without cancer diagnosis in our cohort carried a mutation

that has previously been detected somatically in cancer. These data suggest that assessment of the cancer risk based on the type of mutation detected is not feasible.

Remarkably, the p.Gly503Ala change identified in a patient with NS and JMML, has so far only been observed as a somatic mutation in JMML. For such mutations, which only seem to have a role as an acquired anomaly in leukemia, an association with embryonic lethality has been suggested.[24] A mosaicism for the p.Gly503Ala mutation could explain our finding of this aberration in a life-born child. Unfortunately, no other tissues of this patient were available for further analysis.

As 65 (22%) of the patients in the study cohort were born before PALGA and NCR had an almost complete coverage in the Netherlands, relevant data may be lacking for the first years of life of those patients. This may have resulted in an underestimation of pediatric cancers. On the other hand, as a result of the awareness of a tumor predisposition in NS among physicians, patients with NS and a malignancy are probably more likely to be referred for *PTPN11* analysis than those with NS alone. This may have resulted in an overestimation of the life-time risk of cancer. Other constraints of our study are its retrospective nature and the young age distribution of the study cohort, with a median age of 13 years. Clearly, multicenter life-long follow-up studies are required to evaluate the full spectrum of cancer risk in NS patients with a *PTPN11* mutation. Similarly, such studies may be required to monitor the effect of growth hormone (GH) therapy for short stature in children with NS. As GH can cause proliferation of normal and malignant cells, the possibility that GH can induce cancer, specifically in persons with a tumor predisposition, has resulted in concerns over its use. Until now, however, follow-up studies in large cohorts of children and in smaller cohorts of adults have indicated that GH is not associated with an increased risk of tumor occurrence or recurrence (reviewed in refs Jenkins *et al*[45]; Ogilvy-Stuart and Gleeson[46]). But, as these studies have been performed in recipients of GH therapy who do not have a cancer predisposing genetic condition and the average duration of follow-up is only 4 years, we still recommend that all NS patients who have received GH should be included in follow-up programs to assess a possible relation between cancer risk and GH therapy in this specific condition.

A surveillance program for patients with a tumor predisposition syndrome is indicated when the benefits of such a strategy outweigh the disadvantages. The increased risk for developing a malignancy should be evident and early detection of cancer should positively influence patient outcome. In this study we showed that, although there is an increased risk of developing cancer in patients with NS with a *PTPN11* mutation, the malignancies involve multiple sites and develop throughout life, making a straightforward screening protocol ineffective. Moreover, for hematological malignancies, most frequently occurring in NS, benefit of monitoring is unclear. Therefore, we conclude that at present a tumor surveillance program is not warranted for patients with NS carrying a *PTPN11* mutation.

In conclusion, we have found an excess risk of cancer in patients carrying a mutation in *PTPN11*, compared with the risk of cancer in the general population. In addition to several malignancies that one would expect in a cohort of *PTPN11* mutation carriers, some cancers were encountered that so far were not associated with NS. The initiation and/or progression of these tumors may very well be because of activating mutations in the *PTPN11* gene. Both, on the basis of literature data and those presented here, however, no clear correlation has emerged between specific mutations in *PTPN11* and the occurrence of cancer. Our data do not warrant a tumor surveillance program for patients with NS and a mutation in *PTPN11*.

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