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# Cancer spectrum and frequency among children with Noonan, Costello, and cardio-facio-cutaneous syndromes

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**Background:** Somatic mutations affecting components of the Ras-MAPK pathway are a common feature of cancer, whereas germline Ras pathway mutations cause developmental disorders including Noonan, Costello, and cardio-facio-cutaneous syndromes. These 'RASopathies' also represent cancer-prone syndromes, but the quantitative cancer risks remain unknown.

**Methods:** We investigated the occurrence of childhood cancer including benign and malignant tumours of the central nervous system in a group of 735 individuals with germline mutations in Ras signalling pathway genes by matching their information with the German Childhood Cancer Registry.

**Results:** We observed 12 cases of cancer in the entire RASopathy cohort vs 1.12 expected (based on German population-based incidence rates). This corresponds to a 10.5-fold increased risk of all childhood cancers combined (standardised incidence ratio (SIR) = 10.5, 95% confidence interval = 5.4–18.3). The specific cancers included juvenile myelomonocytic leukaemia = 4; brain tumour = 3; acute lymphoblastic leukaemia = 2; rhabdomyosarcoma = 2; and neuroblastoma = 1. The childhood cancer SIR in Noonan syndrome patients was 8.1, whereas that for Costello syndrome patients was 42.4.

**Conclusions:** These data comprise the first quantitative evidence documenting that the germline mutations in Ras signalling pathway genes are associated with increased risks of both childhood leukaemia and solid tumours.

Noonan syndrome (NS; OMIM 163950), Costello syndrome (CS; OMIM 218040), and cardio-facio-cutaneous syndrome (CFCS; OMIM 115150) are a clinically related group of developmental syndromes caused by germline RAS mutations or by mutations in other genes from the Ras signalling pathway (Roberts *et al*, 2013). The Ras pathway is frequently somatically altered in a broad spectrum of neoplasms (Schubert *et al*, 2007). Thus, the hypothesis that these 'RASopathies' are cancer-prone syndromes is biologically plausible. Indeed, it is widely accepted that NS is associated with a myeloproliferative disease resembling juvenile myelomonocytic leukaemia (JMML), and that individuals with CS are predisposed to embryonal rhabdomyosarcoma (ERMS), neuroblastoma (NBL), and bladder cancer (Tartaglia *et al*, 2003; Gripp, 2005; Kratz *et al*, 2005; Niemeyer *et al*, 2010; Kratz *et al*, 2011; Strullu *et al*, 2014). However, the childhood cancer spectrum and cancer risk in individuals with these RASopathies have not been quantified. Therefore, we investigated the occurrence of childhood cancer in mutation-positive individuals with NS, CS, or CFCS by matching genetic laboratory data with the German Childhood Cancer Registry (GCCR).

The personal identifiers from individuals diagnosed in the laboratories were encrypted, as were the corresponding identification data from the GCCR, using the same asymmetric key. A matching procedure identified individuals contained in both data sets, as described previously (Hammer *et al*, 2009). Person-years of observation were accumulated from birth to date of last follow-up and were left censored before 1 January 1980. They were observed through 31 December 2012, or right censored at cancer diagnosis, or their 15th birthday, whichever occurred first. Vital status information in the laboratory data was incomplete; individuals without a specified status were assumed to be alive at the cutoff (mostly 15th birthday) by default. All cancer cases with an encrypted name younger than 15 years between 1980 and 2012 were included in the matching procedure. Comparisons are presented as standardised incidence ratios (SIRs) with an exact 95% confidence interval (CI). Expected values were derived from the same subset of the GCCR data, which was used for the matching procedure and where a name for encryption was available. All mutations were reviewed by a RASopathy expert and classified as disease-causative variants.

## MATERIALS AND METHODS

We investigated the occurrence of childhood cancer in a group of 735 individuals with germline mutations in Ras signalling pathway genes by matching their information with the GCCR. We also included an analysis of both benign and malignant tumours of the central nervous system. The study was approved by the institutional review board at the University in Magdeburg. Mutation-positive cases of NS, CS, and CFCS tested between 1 January 2002 and 31 December 2012 were identified at 19 private and 6 academic German laboratories that offer quality-controlled testing for the genes known to be mutated in these three syndromes. To reduce bias, we excluded one laboratory at the German study center for children with JMML, where all patients with JMML are genetically classified as syndrome-associated vs sporadic JMML. Using names and dates of birth, we matched laboratory-diagnosed cases of mutation-positive NS, CS, and CFCS with the database of the GCCR (51 883 childhood cancer patients at cutoff date). The GCCR registers ~97% of all German childhood malignancies diagnosed at an age of <15 years in Germany since 1980 (Kaatsch, 2004). All diagnoses defined in the International Classification of Childhood Cancer, Third edition (Steliarova-Foucher *et al*, 2005) are registered systematically, that is, all malignant diseases and both benign and malignant tumours of the central nervous system.

## RESULTS

We identified 784 individuals with a mutation-positive RASopathy, of whom 28 were born before 1965 and not in the 0–14 year age range between 1980 and 2012. Hence, their childhood period did not overlap with the activity of the GCCR. Twenty-one additional individuals, who were clearly close relatives, parents or twins of the index person, were also excluded from the analysis. Seven hundred and thirty-five presumably unrelated individuals with a disease-related mutation in one of the Ras pathway genes and whose childhood period overlapped with the activity of the GCCR remained. Testing was performed between 2002 and 2012. The observed distribution of mutated genes in this study population deviates from the true distribution of mutated genes in all RASopathy patients because it is influenced by multiple factors, such as (1) several new genes have been discovered during the observation period 2002–2012 potentially leading to an under-representation of newer genes; (2) patients with mutations in genes giving rise to mild RASopathy phenotypes were less likely to be tested when compared with patients with mutations leading to obvious RASopathy phenotypes. Pathologic germline mutations were detected in *PTPN11* ( $n = 481$ ), *SOS1* ( $n = 81$ ), *RAF1* ( $n = 50$ ), *BRAF* ( $n = 41$ ), *HRAS* ( $n = 32$ ), *KRAS* ( $n = 17$ ), *SHOC2* ( $n = 17$ ), *MEK1* ( $n = 8$ ), *MEK2* ( $n = 4$ ), *NRAS* ( $n = 3$ ), and *CBL* ( $n = 1$ ). As

the clinical syndrome diagnosis was not available for all patients, we used the genetic test results to categorise patients into different syndrome groups (Table 1). Using this strategy, we classified 632 patients with germline mutations of *PTPN11*, *NRAS*, *SOS1*, *RAF1*, or *SHOC2* as having NS. Forty-four of these subjects harboured one of the known recurrent *PTPN11* mutations (p.Y279C; p.T468M) that are typically associated with NS with multiple lentiginos (LEOPARD syndrome; OMIM 151100), and 17 had a *SHOC2* mutation, which causes a clinical variant of NS termed 'NS-like disorder with loose anagen hair' (OMIM 607721). Thirty-two patients had CS defined by the presence of a germline mutation in *HRAS*, and 53 were classified as having CFCS based on a mutation in *BRAF*, *MEK1*, or *MEK2*. At last, 17 *KRAS* and one *CBL* mutation carriers were categorised as *KRAS* syndrome and *CBL* syndrome after taking into account the known variability of the *KRAS* mutation-associated phenotypes and the sometimes mild NS-like phenotype associated with *CBL* mutations (Table 1) (Zenker *et al*, 2007; Martinelli *et al*, 2010; Niemeyer *et al*, 2010).

The 735 individuals included in the final analytic data file contributed 7489.9 person-years of observation. Birth years ranged from 1965 to 2012. Age at genetic testing ranged from 0 to 45 years. The male-to-female ratio was 0.98. Twelve patients with

cancer, diagnosed between 2002 and 2012 and diagnosed with a mutation in the years 2003–2012 were identified in this laboratory population (Table 2). To our knowledge, patient 4 is the only patient included in a previous report (Laux *et al*, 2008).

On the basis of all person-years and the age distribution of the studied population, 1.14 cases of childhood cancer, all sites combined, would be expected *vs* 12 observed, a 10.5-fold increase (SIR = 10.5, 95% CI = 5.4–18.3) (Table 1). The childhood cancer risk in patients with NS was 8.1-fold increased (95% CI = 3.5–16.0), whereas patients with CS had a 42.4-fold (95% CI = 5.1–153.2) increased risk. A sensitivity analysis, excluding seven cases in whom the cancer and the syndrome diagnosis were made within 1 year of one another demonstrated a cancer risk of SIR 4.4 (SIR = 4.4, 95% CI = 1.4–10.2) for all RASopathies combined. The 17 *KRAS* syndrome subjects developed two cancers (SIR = 75.8, 95% CI = 9–273.7). There were no cancers observed either among the 53 CFCS patients (495.9 pyo; 0.08 cases expected) or the one patient with *CBL* syndrome.

SIRs of selected cancers in individuals with NS, CS, and patients with a germline *KRAS* mutation by cancer type are given in Table 3. High SIRs were observed for JMML in patients with NS (SIR = 717, 95% CI = 148–2094) and in patients with a RASopathy

**Table 1. Genotype-dependent categorisation of RASopathies identified in 25 genetic laboratories in Germany in 2002–2012**

Syndrome	Mutated gene (n)	n	Cases of cancer <sup>a</sup>			
			Observed	Expected	PY	SIR, 95% CI
All RASopathies combined		735	12	1.14	7489.9	10.5 (5.4–18.3)
NS, all subtypes combined		632	8	0.99	6535.6	8.1 (3.5–16.0)
Classic NS	<i>PTPN11</i> (437), <i>NRAS</i> (3), <i>SOS1</i> (81), <i>RAF1</i> (50)	571	7	0.89	5900.6	7.9 (3.2–16.2)
NSLAH	<i>SHOC2</i> (17)	17	0	0.02	138.9	0.0 (0.0–159.0)
NSML	<i>PTPN11</i> (44)	44	1	0.08	496.2	13.1 (0.3–72.9)
CS	<i>HRAS</i> (32)	32	2	0.05	278.2	42.4 (5.1–153.2)
CFCS	<i>BRAF</i> (41), <i>MEK1</i> (8), <i>MEK2</i> (4)	53	0	0.08	495.9	0.0 (0.0–45.3)
<i>KRAS</i> <sup>b</sup>	<i>KRAS</i> (17)	17	2	0.03	175.2	75.8 (9.2–273.7)
<i>CBL</i> <sup>c</sup>	<i>CBL</i> (1)	1	0	–	–	–

Abbreviations: CS = Costello syndrome; CFCS = cardio-facio-cutaneous syndrome; CI = confidence interval; *KRAS* = RASopathy with a germline mutation of *KRAS*; NS = Noonan Syndrome; NSLAH = NS-like disorder with loose anagen hair; NSML = NS with multiple lentiginos; PY = person-years; SIR = standardised incidence ratio.

<sup>a</sup>Data from the German Childhood Cancer Registry (see Materials and Methods for details).

<sup>b</sup>RASopathy with a germline mutation of *KRAS*.

<sup>c</sup>RASopathy with a germline mutation of *CBL*.

**Table 2. Description of 12 individuals with a RASopathy who developed cancer**

Patient (syndrome)	Sex	Age (years) at genetic testing	Amino-acid change (number of cases with this specific mutation in entire cohort)	Neoplasm (age in years)	Mutation previously associated with cancer
<b>PTPN11</b>					
1 (NS)	F	0.2	A72G (8)	JMML (0.1)	(Strullu <i>et al</i> , 2014)
2 (NS)	M	0.4	G503R (15)	JMML (0.2)	(Strullu <i>et al</i> , 2014)
3 (NS)	M	0.4	E139D (20)	JMML (0.3)	(Strullu <i>et al</i> , 2014)
4 (NSML)	F	4	Y279C (17)	ALL (8)	(Ucar <i>et al</i> , 2006)
5 (NS)	M	0.8	M504V (25)	ALL (4)	(Karow <i>et al</i> , 2007)
6 (NS)	F	13	G60A (9)	Pilocytic astrocytoma (7)	(Strullu <i>et al</i> , 2014)
7 (NS)	F	–	N308D (107)	Dysembryoplastic neuroendothelial tumour (6)	(Strullu <i>et al</i> , 2014)
8 (NS)	F	3	I282M (1)	NBL (3)	Cosmic database
<b>HRAS</b>					
9 (CS)	M	1	G12S (24)	ERMS (1)	(Kerr <i>et al</i> , 2006)
10 (CS)	F	0.5	G12C (2)	ERMS (3)	(Kerr <i>et al</i> , 2006)
<b>KRAS</b>					
11 (KRAS)	M	2	D153V (4)	Astrocytoma (2)	–
12 (KRAS)	F	1	T58I (1)	JMML (0.5)	(Schubbert <i>et al</i> , 2006)

Abbreviations: ALL = acute lymphoblastic leukaemia; CS = Costello syndrome; ERMS = embryonal rhabdomyosarcoma; F = female; JMML = juvenile myelomonocytic leukaemia; *KRAS* = RASopathy with a germline mutation of *KRAS*; NBL = neuroblastoma; M = male; NS = Noonan Syndrome; NSML = NS with multiple lentiginos.

**Table 3. Standardised incidence ratios for specific cancers in patients with Noonan syndrome, Costello syndrome, and KRAS syndrome**

Syndrome	Cancer type	n	Cases of cancer		PY	SIR, 95% CI
			Observed	Expected		
NS combined	JMML	632	3	0.004	6535.6	717 (148–2094)
	ALL	632	2	0.282	6535.6	7.1 (0.9–25.6)
	NBL	632	1	0.093	6535.6	10.8 (0.3–59.9)
CS	ERMS	32	2	0.001	278.2	1630 (197–5887)
KRAS	Astrocytoma	17	1	0.002	175.2	410 (10–2287)
	JMML	17	1	0.000	175.2	10172 (258–56672)

Abbreviations: ALL = acute lymphoblastic leukaemia; CI = confidence interval; CS = Costello syndrome; ERMS = embryonal rhabdomyosarcoma; JMML = juvenile myelomonocytic leukaemia; KRAS = RASopathy with a germline mutation of KRAS; NBL = neuroblastoma; NS = Noonan Syndrome; PY = person-years.

because of a KRAS mutation (SIR = 10,172, 95% CI = 258–56672) and for ERMS in patients with CS (SIR = 1630, 95% CI = 197–5887).

## DISCUSSION

Our study is the first to quantify cancer risk in children with NS, CS, and CFCS. In this population-based study, we observed a significant excess risk for all childhood cancers combined compared with the general population. The elevated overall cancer risk was primarily due to significant site-specific excesses of JMML, ERMS, and brain tumours.

The Ras signalling pathway is frequently activated somatically in a broad spectrum of malignancies (Schubbert *et al*, 2007). Therefore, it is biologically plausible that individuals with RASopathies who display germline mutations in various Ras pathway genes might be at increased risk of developing cancer. Although a number of case reports and case series have qualitatively suggested an important link between cancer and RASopathies, as recently documented in an extensive descriptive literature review (Kratz *et al*, 2011), few epidemiologic studies have investigated this question quantitatively. A recent French study addressed the association between JMML and NS in a large cohort of 641 patients with germline *PTPN11* mutations. Twenty patients developed JMML and these patients carried specific *PTPN11* alleles, suggesting a genotype/phenotype correlation (Strullu *et al*, 2014). However, these authors included patients that were referred because of the presence of JMML. This approach differed from ours, owing to our efforts aimed at minimising selection bias. Another report from the Netherlands found a 3.5-fold increased risk of all cancers combined in a cohort of 297 individuals with germline *PTPN11* mutations (Jongmans *et al*, 2011). This study that also included adult cancer cases is quantitatively limited by having estimated only risk information for all cancers combined. In addition, this patient series only included patients with a mutation in *PTPN11*.

We observed three cases of JMML among 519 patients with a germline *PTPN11* mutation and one case among 17 patients with a KRAS germline mutation. We observed considerably fewer JMML cases than that observed in the recent French study that reported 20 JMML cases among 641 patients with a *PTPN11* mutation (Strullu *et al*, 2014). However, important methodological differences in study design prevent a direct comparison of these two studies. To reduce the possibility of including individuals with a RASopathy who were diagnosed because of their malignancy, we purposefully excluded one paediatric hematology/oncology laboratory in Germany that focuses specifically on and collects specimens from patients with NS-associated and non-syndromic JMML. This strategy may explain the fact that we found 11 additional cases of NS-associated JMML registered at the GCCR 2002–2012 that were not ascertained in our study population; most of the cases missing from our series were diagnosed by the aforementioned specialised

laboratory. Consequently, our JMML-related SIR, while statistically significant, clearly underestimates the actual JMML risk in our population, although it nonetheless provides statistically significant evidence in support of the JMML-RASopathy association.

The mutation spectrum that we identified in the four RASopathy-associated JMML patients (Table 2) overlapped completely with the NS-associated JMML literature (Schubbert *et al*, 2006; Strullu *et al*, 2014). We detected no novel mutations in our series, confirming earlier conclusions that specific mutations tend to be associated with JMML, that is, that there is a strong correlation between genotype and phenotype in this group of patients. We also confirmed the previously described association between JMML and the rare KRAS p.T58I germline mutation (Schubbert *et al*, 2006) by identifying another patient with this mutation and JMML among our 17 KRAS subjects, an excess that is statistically significant despite the very small numbers (SIR = 10172; 95% CI = 258–56672) (Table 2).

In agreement with previous case reports, our data suggested an association between *PTPN11* germline mutations and ALL (Observed = 2, SIR = 7.1, 95% CI = 0.9–25.6), which did not reach statistical significance. Interestingly, we have previously described another patient from Switzerland with NS and ALL (not included in the current case series) who carried the same *PTPN11* M504V germline mutation (Karow *et al*, 2007) that was also present in one of our two NS/ALL patients (Table 2).

We found three patients with brain tumours in our cohort, consistent with prior reports of somatic mutations in Ras pathway genes in glioma tumour tissue. One patient had a dysembryoplastic neuroepithelial tumour, a rare central nervous system neoplasm that has previously been described in several other patients with a *PTPN11* mutation (Selter *et al*, 2010; Jongmans *et al*, 2011), suggesting that these tumours are associated with NS. At last, 2 of our 32 patients with a germline *HRAS* mutation developed ERMS (SIR = 1630, 95% CI = 197–5887), confirming the strong association between CS and ERMS (Gripp, 2005).

**Our study has several limitations.** (1) We were unable to ascertain cancers in patients older than 14 years, as the case-identifying resource was a childhood cancer registry. Germany does not have an equivalent cancer registry for adults. Thus, the risk of adult-onset cancers in NS, CS, and CFCS cannot be defined here and would comprise an important future analysis if the proper study populations and registries can be identified. (2) Patients carrying mutations in *RIT1* and *RRAS* were not included in this study, as those NS genes were discovered after the current data collection had been completed (Aoki *et al*, 2013; Flex *et al*, 2014). (3) We did not have access to patient medical records, and thus could not determine each subject's age or date at syndrome diagnosis. Consequently, we cannot adequately determine whether the cancers occurred prior to, at the time of, or subsequent to the date of syndrome diagnosis. In standard prospective epidemiologic studies, the analysis often excludes study end points that occur

before disease diagnosis. In the case of genetic diseases, it is a reasonable analytic option to begin observation at birth, as affected individuals are truly at risk of disease-related complications before the diagnosis is appreciated. It is likely that, in some instances, the RASopathy diagnosis was prompted by the development of an unusual childhood cancer, particularly for JMML, which is widely understood to be an important RASopathy syndrome manifestation. Of note, in seven patients, the cancer and the RASopathy were diagnosed within 1 year of each other (Table 2). If we exclude these seven cases from our analysis, the remaining cancer risk ratio for all cancers among all RASopathies combined equals 4.4 (observed = 5, 95% CI = 1.4–10.2). (4) As a consequence of identifying susceptible individuals through genetic testing labs, our analytic cohort excludes RASopathy patients who have never undergone genetic testing (i.e., who were diagnosed clinically). There is no way to evaluate the impact of this subgroup's absence on our analysis. (5) We excluded the major JMML reference laboratory from this study because it receives samples from children with suspected JMML and did not routinely provide comprehensive RASopathy gene mutation testing, for example, it is likely that the 11 JMML cases identified from the GCCR with a concurrent RASopathy syndrome diagnosis, which did not appear in our cohort, were gene-tested at that institution. Excluding them from our analysis results in a significant underestimation of the JMML risk in this analysis, as noted above. (6) The observed frequency of mutations in the various genes does not represent the true distribution of mutated genes. The observed distribution is influenced by the year of gene discovery.

RASopathies represent monogenic traits, and the underlying rare disease-causing mutations have a high penetrance for the syndrome-defining phenotypic features. However, our data suggest that cancer risks are not markedly elevated in these syndromes. Rather, germline Ras pathway mutations are associated with risks that are significantly greater than those expected in the general population, but which are meaningfully lower than those seen in the more familiar adult-onset cancer susceptibility disorders such as hereditary breast/ovarian cancer and hereditary colorectal cancer. Thus, it appears that germline Ras pathway mutations represent intermediate cancer risk variants, leading to significantly but moderately increased cancer risk. Such rare, intermediate-risk variants are thought to contribute significantly to the pathogenesis of many cancers and other complex diseases (Saint Pierre & Genin, 2014), but they are difficult to uncover by the use of genetic risk variant discovery strategies such as genome-wide association studies (Yokoyama *et al.*, 2011). Our study is one of the few studies demonstrating the existence of such rare intermediate-risk variants.

Our study provides quantitative epidemiologic evidence of an increased risk of childhood cancer in RASopathy patients. The childhood cancer risk is dependent on distinct underlying genetic defects. These conclusions are supported by the findings that (1) most cancer patients from our cohort harboured germline mutations previously associated with the same cancer type, and that (2) most observed cancers have occurred in several other RASopathy patients (within this study and/or the published literature), and that (3) all cancers occurring in excess in our cohort are biologically plausible because these cancers are known to be associated with somatic Ras pathway activation. Notably, our study does not imply that specific clinical cancer-screening measures are beneficial for patients with RASopathies.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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