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OPEN

No increased prevalence of malignancies among first-degree relatives of 800 patients with chronic myeloid leukemia: a population-based study in Sweden

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The etiology of chronic myeloid leukemia (CML) remains essentially unknown with exposure to high doses of ionizing radiation being the only well-established risk factor.¹ We have recently published two large population-based studies showing an increased prevalence of other malignancies in patients with CML, both preceding and subsequent to their diagnosis, as compared with age- and gender-matched controls.^{2,3} One may therefore speculate whether CML patients have a congenital or acquired susceptibility to develop cancer. In the former case, one would expect an increased prevalence of malignancies among first-degree relatives (FDR) of CML patients.

In a previous report based on information in the Swedish Cancer Register, no increased aggregation of malignancies was detected among family members of CML patients diagnosed between 1958 and 2004.⁴ However, a more strict definition of CML (requiring, for example, the presence of a Philadelphia chromosome or the BCR/ABL fusion gene) was introduced with the updated WHO classification in 2002, making subsequent CML cohorts more stringently diagnosed.⁵

Aiming to examine the prevalence of malignancies among FDR of a large and well-defined contemporary CML cohort in Sweden compared with randomly selected population controls,

we used information retrieved from four Swedish population-based registers.

To identify patients with CML diagnosed between 2002 and 2013, we used the Swedish CML Register to which virtually all Swedish CML patients diagnosed January 1st 2002 and later are reported. The diagnosis of CML was generally based on a cytogenetic finding of the Philadelphia chromosome by karyotyping or by fluorescence *in situ* hybridization, and/or by detection of the BCR-ABL1 fusion transcript by reverse transcription-polymerase chain reaction, as well as a blood and bone marrow picture typical for this disease, morphologically verified by hematopathologists (that is, granulocytosis, hypercellular marrow with a left-shifted granulocytopenia and a varying degree of increase in percentage of immature cells). In a minute portion (3%) of patients included in the CML Register, cytogenetics had not been performed but were included based on their clinico-pathological picture compatible with typical CML.

Each CML patient was matched with five, age-, sex- and county of residence-matched controls, randomly selected from the Swedish Total Population Register. All controls had to be alive and free of CML at the time of diagnosis for the matching CML patient.

FDR of both CML patients and matched controls were identified by use of the Swedish Multi-Generation Register, which includes information about parent–sibling–offspring relationships of

persons born later than 1932 and registered in Sweden at some time since 1961. By means of record linkage to the Swedish Cancer Register, we retrieved information about malignancies diagnosed later than 1958.

To calculate odds ratio (OR) and 95% confidence intervals (CI), conditional logistic regression was used.

Using the Swedish CML Register, we identified 984 patients diagnosed as CML in chronic, accelerated or blastic phase. Among them, 184 patients were born before 1932 and were therefore excluded. For the 800 remaining CML patients, 4287 FDR were identified and included in the analysis (parents: 1346, siblings: 1497 and children: 1444). Correspondingly, 20 930 matched controls were included in the analysis.

In total, 611 malignancies were identified among the FDR of CML patients compared with 2844 in the control group, yielding an OR of 1.06 (95% CI: 0.96–1.16).

Neither hematological malignancies nor solid tumors were increased in the CML–FDR group (Table 1). Notably, none of the

FDRs in the CML–FDR group had a CML diagnosis. Results remained unchanged following exclusion of nine patients in the CML register with only a clinico-pathological picture typical of CML and their matched controls.

Thus, using population-based data based on the fate of more than 4000 FDR of 800 CML patients diagnosed in the modern era of cytogenetics and molecular assays, we found no evidence of familial aggregation of malignancies (including CML) of patients with CML. Taken together, our findings suggest that a hereditary predisposition to develop cancer is unlikely to be a part of the pathogenesis of CML.

CONFLICT OF INTEREST

FS has a consulting role for Biogen. JR has received honoraria from ARIAD, Bristol-Myers Squibb, Novartis and Pfizer and received research funding from Bristol-Myers Squibb and Novartis. MB has received research funding from Adolf H Lundin Charitable Foundation. MH has a consulting role for Akinion Pharmaceuticals and Janssen-Cilag. ML has stock ownership in AstraZeneca and Pfizer. UO-S received research funding and honoraria from Bristol-Myers Squibb. The remaining authors declare no competing financial interest.

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AUTHOR CONTRIBUTIONS

AS, MH, LS and JR provided conception and design, patient material, data collection, data interpretation and manuscript writing. NG provided conception and design, data collection, data interpretation and manuscript writing. FS provided data analysis and interpretation, statistical methodology and manuscript writing. MB, AD, ML, BM, UO-S and HW provided patient material, data collection and manuscript writing. All authors contributed with critical revision of the manuscript.

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Table 1. Odds ratio for malignancies among first-degree relatives of CML patients

Outcome in relatives	CML, n = 4287	Controls, n = 20 930	OR	95% CI
<i>Myeloid</i>				
AML	3	12	1.22	0.34–4.33
MDS	0	15	—	—
AML/MDS	3	26	0.54	0.17–1.86
CML	0	4	—	—
MPN	6	19	1.54	0.62–3.87
Any myeloid malignancy	8	46	0.85	0.40–1.80
<i>Lymphoid</i>				
NHL	12	64	0.92	0.49–1.70
HL	1	12	0.41	0.05–3.13
CLL	8	29	1.35	0.62–2.95
WM	0	0	—	—
MM	6	26	1.13	0.46–2.74
ALL	0	10	—	—
Any lymphoproliferative malignancy	32	163	0.96	0.66–1.40
Any hematologic malignancy	42	211	0.97	0.70–1.36
<i>Solid malignancies</i>				
Gynecological (females only)	134	617	1.56	0.87–1.28
Gastrointestinal	125	519	1.18	0.97–1.44
Breast (females only)	97	398	1.19	0.95–1.49
Breast (males only)	0	2	—	—
Prostate (males only)	76	393	0.95	0.74–1.22
Urinary tract	36	211	0.83	0.58–1.19
Lung	32	175	0.89	0.61–1.30
Endocrine	29	149	0.95	0.64–1.42
Malignant melanoma	27	150	0.88	0.58–1.32
CNS	26	94	1.35	0.88–2.09
ENT	19	81	1.15	0.70–1.89
Any solid malignancy	578	2673	1.06	0.97–1.17
Any malignancy	611	2844	1.06	0.96–1.16

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CI, confidence interval; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CNS, central nervous system; ENT, ear-nose-throat; HL, Hodgkin lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; MPN, myeloproliferative neoplasms; NHL, non-Hodgkin lymphoma; OR, odds ratio; WM, Waldenström macroglobulinemia.

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Germline *SAMD9* mutation in siblings with monosomy 7 and myelodysplastic syndrome

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Myelodysplastic syndromes (MDS) are poorly understood and rare hematologic malignancies in children. In recent years, several germline mutations have been implicated in a subset of familial cases of MDS, other myeloid neoplasms or bone marrow failure syndromes including mutations in *GATA2*, *ETV6* and *DDX41*.^{1,2} However, for some families, such as those first reported nearly 30 years ago,^{3,4} who present with MDS and monosomy 7 (OMIM 252270), the predisposing genetic alteration has remained elusive. Here we report our findings on a family without historical suggestions of MDS/AML predisposition but with three siblings with monosomy 7 and MDS.

A 4-year-old male (proband/SJ015856/Sibling 1) was found to have severe neutropenia, macrocytosis and thrombocytopenia (absolute neutrophil count=400/mm³, mean corpuscular volume = 101.7 fl, platelets = 111 × 10⁹/l). Bone marrow evaluation revealed hypocellularity with trilineage dysplasia and 1% blasts (Figure 1a). His 3-year-old sister (SJ015855/Sibling 2) also had low peripheral cell counts and bone marrow dysplasia, while his youngest brother (14-month-old/SJ018228/Sibling 3) had normal peripheral counts and a hypocellular bone marrow with megakaryocytic dysplasia. Cytogenetic analysis was significant for monosomy 7 in all siblings (Figure 2; Supplementary Figure 1) and thus all three were diagnosed with refractory cytopenia of childhood. All three siblings had a transient thrombocytopenic phase at birth requiring a platelet transfusion, but had otherwise normal developmental histories and stature-for-age (at or above the 75th percentile). The proband was born with severe hypospadias and a bifid scrotum requiring multiple corrective surgeries without any bleeding complications. A karyotype during the neonatal period demonstrated a normal male karyotype. No other congenital abnormalities were noted in the siblings. Chromosome breakage studies for Fanconi Anemia were negative. The two eldest siblings have been treated with matched, unrelated donor bone marrow transplants, while the youngest sibling remains asymptomatic with normal peripheral blood counts and is monitored annually.

Bone marrow samples from the three siblings were banked for research after informed consent was obtained. All studies were approved by the Institutional Review Board at St Jude Children's Research Hospital. Cryopreserved marrow samples were flow-sorted into lymphocyte (source of 'germline' DNA), as an alternate source of germline DNA was not available,⁵ and bulk myeloid populations. Whole exome sequencing was performed on paired tumor/normal samples from the three siblings. Whole

genome sequencing was also performed on the paired samples from Sibling 2 and only the normal samples from Siblings 1 and 3. Genomic data have been deposited in the European Genome-Phenome Archive (EGAS00001002202).

The loss of chromosome 7 was confirmed in the myeloid cells of all three siblings using Whole genome sequencing data,⁶ with a clear subclonal loss in Sibling 3 (Figure 1b). No copy number loss was observed in the lymphocyte samples; however, a copy number neutral loss of heterozygosity (CN-LOH) event was noted at 7q (chr7: 78–159 Mb, q21.11-q36.3) in Sibling 3 (Figure 1c). A total of 19 non-synonymous-shared high-confidence germline variants were present when intersecting the WES and WGS data from all 3 siblings, 10 of which were predicted to functionally impact the gene product by either SIFT or Polyphen2 (Supplementary Figure 2 and Supplementary Table 1). These analyses pointed to a heterozygous missense mutation (c.3406G>C) in *SAMD9* (NM_017654), resulting in a p.E1136Q mutation (NP_010124; CADD score:19.7), as a potential causal lesion for MDS and monosomy 7 in this family.⁷ This mutation affects an evolutionarily conserved position and is not present in the ExAC or in-house databases. Sanger sequencing of genomic DNA isolated from the peripheral blood of the parents observed this mutation in the clinically asymptomatic mother, confirming this as an inherited variant (Figures 2a and b). Furthermore, a CLIA-approved laboratory confirmed the presence of the *SAMD9* p.E1136Q in a buccal swab sample obtained from the proband. Similar *SAMD9* missense mutations have recently been reported in patients with MIRAGE syndrome, a multisystem disorder associated with MDS and monosomy 7.⁸

Although present in the patients' lymphocytes, the mutant allele was less common in the myeloid cell fraction (Figure 2b), suggesting a preferential loss of the copy of chromosome 7 that harbors the *SAMD9* variant. A similar decrease in variant allele frequency was reported in children with MIRAGE syndrome, presumably through a mechanism referred to as 'adaptation by aneuploidy,' to deal with the growth-restrictive properties of *SAMD9*-mutant proteins.⁸ Similar to those mutations present in MIRAGE syndrome, the p.E1136Q mutation results in decreased induction of ERK phosphorylation (Figure 2c) after serum stimulation. Additional sibling-specific alterations involving *SAMD9* (Supplementary Figures 3 and 4) were also observed in the lymphocyte fractions: a p.R221* (c.661C>T) in Sibling 1, p.F583I (c.1747C>A) in Sibling 2 and the aforementioned CN-LOH event in Sibling 3. The p.R221* and p.F583I were confirmed to be in *cis* with the p.E1136Q (Supplementary Figure 3C). These additional mutations are