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SHORT REPORT

Inherited cancer predisposing mutations in patients with therapy-related myeloid neoplasms

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Summary

Some patients with therapy-related myeloid neoplasms (t-MN) may have unsuspected inherited cancer predisposition syndrome (CPS). We propose a set of clinical criteria to identify t-MN patients with high risk of CPS (HR-CPS). Among 225 t-MN patients with an antecedent non-myeloid malignancy, our clinical criteria identified 52 (23%) HR-CPS patients. Germline whole-exome sequencing identified pathogenic or likely pathogenic variants in 10 of 27 HR-CPS patients compared to 0 of 9 low-risk CPS patients (37% vs. 0%, $p = 0.04$). These simple clinical criteria identify t-MN patients most likely to benefit from genetic testing for inherited CPS.

KEYWORDS
cancer predisposition, germline, leukaemia, therapy-related myeloid neoplasm, whole-exome sequencing

INTRODUCTION

Therapy-related acute myeloid leukaemia (AML) and therapy-related myelodysplastic syndrome [MDS; collectively referred to as therapy-related myeloid neoplasms (t-MN)] are frequently fatal conditions resulting from prior exposure to chemotherapy, radiation therapy, and/or immunosuppressive agents. The incidence of t-MN is influenced by a number of factors, but is most affected by the agent(s) to which a patient is exposed, and the dosage and schedule of treatment.[1] Two mechanisms have been proposed for the aetiology of t-MN: (1) direct damage to DNA in normal haematopoietic stem cells

by mutagenic agents; and (2) selection and expansion of pre-existing mutant haematopoietic clones.

As many t-MN patients have had more than one primary cancer, some may have an unsuspected cancer predisposition syndrome (CPS). Indeed, one of the very first successes of whole-genome sequencing identified a deleterious germline *TP53* deletion in a patient with t-MN.[2] Furthermore, germline mutations in CPS genes such as *BRCA1*, *BRCA2*, and *BARD1*, as well as *TP53*, have been reported in t-MN patients.[3,4]

To investigate the occurrence of CPS in t-MN patients, we analysed an unselected series of 226 patients who developed t-MN following exposure to chemotherapy and/or radiation for an antecedent non-myeloid malignancy (Table 1; see Appendix S1 for details on the analysis). The most common primary malignancies were: breast cancer ($n = 72$; 32%), non-Hodgkin-lymphoma (NHL; $n = 38$; 17%), Hodgkin lymphoma (HL; $n = 26$; 12%), and acute and chronic lymphoid leukaemias ($n = 17$; 8%).

Because family history data were missing or incomplete for almost all t-MN patients, we employed criteria used routinely by clinical cancer geneticists to identify high-risk CPS (HR-CPS) patients[5]: (1) the diagnosis of breast or colon cancer under age 50; (2) the diagnosis of sarcoma under age 46 (meeting revised Chompret criteria for Li–Fraumeni syndrome[6]); and (3) the diagnosis of two or more primary cancers other than non-melanoma skin cancer in addition to the diagnosis of a t-MN. Because of the known biology of t-MN, we also included the following two criteria: (4) latency to t-MN of more than 15 years following initial exposure to cytotoxic therapy—since most t-MN cases occur with a latency of 10 years or less,[7] long-latency t-MN may in fact represent a second primary cancer; and (5) a latency to t-MN diagnosis of no more than one year following exposure to cytotoxic therapy and a t-MN karyotype with either abnormalities of chromosome 5 and/or 7 or a complex tumour karyotype (≥3 unrelated cytogenetic abnormalities), since this subset of t-MN very rarely occurs with a latency of less than three

TABLE 1 Demographic and clinical characteristics of all 226 t-MN patients stratified as high-risk or low-risk cancer predisposition syndromes (HR-CPS or LR-CPS), and of the 27 HR-CPS patients and nine LR-CPS patients analysed via whole-exome sequencing (WES). For individual-level data on these patients, see Table S3. Note that some HR-CPS patients met multiple HR-CPS criteria

	All patients				Patients whole exome sequenced		
Clinical characteristics	**HR-CPS patients**	**LR-CPS patients**	**All t-MN patients**	***p*-value**	**HR-CPS patients**	**LR-CPS patients**	***p*-value**
Patients (%)	52 (23.0)	174 (77.0)	226		27	9	
Males (%)	12 (23.1)	85 (48.6)	97 (42.9)	0.001	7 (25.9)	4 (44.4)	0.409
Median age at primary cancer diagnosis (range)	43 (12–80)	53 (13–83)	51 (12–83)	0.003	45 (16–80)	40 (18–69)	0.497
Median age at therapy-related myeloid neoplasm diagnosis (range)	50 (17–85)	59 (18–87)	58 (17–87)	0.039	51 (17–85)	43 (18–73)	0.257
Primary malignancy (%)							
Breast cancer	31 (59.6)	41 (23.6)	72 (31.8)	0.000	13 (48.1)	3 (33.3)	0.700
NHL	4 (7.7)	34 (19.5)	38 (16.8)	0.056	2 (7.41)	0 (0)	1.000
HL	4 (7.7)	22 (12.6)	26 (11.5)	0.459	2 (7.41)	2 (22.2)	0.255
Leukaemia	3 (5.8)	14 (8.0)	17 (7.5)	0.768	1 (3.76)	1 (11.1)	0.443
Other	12 (23.1)	63 (36.2)	75 (33.2)	0.094	10 (37.0)	3 (33.3)	1.000
Cytogenetics (%)							
Abnormalities of chr 5 and/or 7	5 (9.6)	31 (17.8)	36 (15.9)	0.197	4 (14.8)	4 (44.4)	0.086
Complex	21 (40.3)	56 (32.2)	77 (34.1)	0.318	10 (37.0)	4(44.4)	0.712
Translocation	10 (19.2)	26 (14.9)	36 (15.9)	0.517	4 (14.8)	0 (0)	0.553
Inversion	4 (7.7)	6 (3.4)	10 (4.4)	0.244	4 (14.8)	0 (0)	0.553
Normal	6 (11.5)	26 (14.9)	32 (14.2)	0.654	3 (11.1)	0 (0)	0.558
Other	1 (1.9)	8 (4.6)	9 (4.0)	0.688	0 (0)	1 (11.1)	0.250
Unknown	5 (9.6)	21 (12.1)	26 (11.5)	0.806	2 (7.4)	0 (0)	1.000
HR-CPS criteria							
Breast cancer <50 yrs	25				9		
Multiple primaries	11				8		
Latency >15 yr	8				5		
Latency >1 yr and abnormalities of chr 5/7 or complex karyotype	7				4		
Sarcoma	3				3		

years.[7] All other patients were considered low-risk for CPS (LR-CPS).

We identified 52/226 patients (23%) meeting one or more HR-CPS criteria, suggesting that an unexpectedly high proportion of t-MN patients may have an undiagnosed CPS (Table 1). Of these, 25 were diagnosed with breast cancer at less than 50 years of age; 11 had multiple primary cancers; eight had latencies longer than 15 years; seven had cytogenetic abnormalities of chromosomes 5/7 or complex karyotypes with latencies of no more than one year; and three had sarcomas. Two patients met more than one criterion. HR-CPS patients had an earlier median age of diagnosis for both their primary malignancy and t-MN as compared to LR-CPS patients [43 versus 53.2 years of age for primary malignancy ($p = 3.4 \times 10^{-5}$), and 49.5 versus 59 years of age for t-MN ($p = 0.011$)].

We performed whole-exome sequencing (WES) on all HR-CPS patients with available germline DNA (27/52), as well as nine LR-CPS patients with available germline DNA (see Table 1 and Table S1). Following WES and quality control, the average exome coverage at 10× for the HR-CPS and LR-CPS patients was 95% and 85% respectively, with an average read depth of 70× and 101× respectively (Table S2). After filtering out non-coding variants and those leading to synonymous amino acid changes, an average of 8767 rare germline variants [minor allele frequency (MAF) < 0.01] were identified in each sample.

To identify high/moderate penetrance CPS variants in t-MN patients, we searched Ambry Genetics' (Aliso Viejo, CA, USA) clinical database for variants in the 52 genes associated with hereditary cancer on the CancerNext-Expanded hereditary cancer gene panel (Table S3). Ambry Genetics is a genetic testing laboratory, and the clinical validity of all gene–disease associations are thoroughly assessed using a weighted scoring system guided by ClinGen gene curation criteria (https://clinicalgenome.org/curation-activities/gene-disease-validity/).

We identified eight t-MN patients with a germline pathogenic variant or likely pathogenic variant (VLP) in two tumour suppressor genes: *TP53* ($n = 6$), and *CHEK2* ($n = 2$). We also identified one t-MN patient (2A) with a variant of uncertain significance (VUS) in *TP53* who also had a co-occurring pathogenic *TP53* variant (Table 2). All variants were heterozygous with allele fractions of approximately 50%. One identical *TP53* pathogenic variant was identified in patients 2A and 15A. Variants were confirmed by Sanger sequencing, based on DNA availability (Table S4). See supporting evidence for each variant in the Appendix S1.

Because criteria for clinically actionable CPS variants are very stringent, we hypothesized that there may be other patients in our series with CPS variants not identified by our clinical pipeline. Consequently, we performed an *in-silico* bioinformatics analysis to identify rare potentially deleterious variants in the CPS genes identified by our clinical analysis, *TP53* and *CHEK2*. Variants meeting the following criteria were characterized as bioinformatically defined CPS variants: (1) MAF less than 0.01 in both the 1000 Genomes Project[8] and the NHLBI Exome Sequencing Project[9]; (2) CADD Phred[10] scores of 20 or higher or GERP RS[11] scores of 4 or higher, both of which are highly sensitive for identifying pathogenic variants in the ClinVar database[12]; and (3) not classified as benign or very likely benign by Ambry. We successfully rediscovered 8/9 variants in HR-CPS patients (Table 2); only patient 104A (*TP53* pathogenic variant) was not identified because his variant did not meet CADD Phred or GERP RS score thresholds (16.5/3.5). Additionally, we identified two patients with variants predicted to be deleterious, both of whom are HR-CPS patients, and both of whom

TABLE 2 All patients with putative cancer predisposition syndrome (CPS) variants identified by either the clinical or bioinformatics analysis. No variants were identified in the low-risk (LR)-CPS patient set. High-risk (HR)-CPS patient 104A was identified only by the clinical analysis, whereas patients 5A and 163A were identified only by the bioinformatics analysis. Patient 21A was diagnosed with t-MN at age 45, and so meets HR-CPS criteria by virtue of the diagnosis of breast cancer under age 50. For individual-level data, see Table S3

	Patient ID	Sex	Primary malignancy	Age at primary cancer diagnosis (yrs)	Latency to t-MN (yrs)	Gene	Variant	Variant classification	Discovered by clinical analysis	Discovered by bioinformatics analysis
HR-CPS	2A	F	Breast	35	11	*TP53*	p.V143M; p.Y205N	V134M – Pathogenic Y205N - VUS	+ (2)	+ (2)
	15A	F	Breast	56	20	*TP53*	p.V143M	Pathogenic	+	+
	19A	F	Breast	66	16	*TP53*	p.K132R	Pathogenic	+	+
	21A	F	Breast	Unk	Unk	*TP53*	p.C277Y	VLP	+	+
	191A	F	Cervical Ovarian	66	2	*TP53*	p.H179Y	Pathogenic	+	+
	104A	M	NHL	50	20	*TP53*	p.Y234C	Pathogenic	+	–
	189A	F	Thyroid	27	32	*CHEK2*	p.R117G	VLP	+	+
	267A	M	Mel Sarc Prostate	50	2	*CHEK2*	p.I157T	Pathogenic	+	+
	5A	F	Breast	42	3	*CHEK2*	p.L338V	–	–	+
	163A	M	HL	35	0	*CHEK2*	p.F310S	–	–	+

have variants in *CHEK2*. This *in-silico* analysis replicates and potentially extends the results of our clinical analysis, though the pathogenicity of these bioinformatically predicted deleterious variants should be validated in additional studies. Combining our clinical and bioinformatic analyses, all individuals with pathogenic variants, VLPs, or VUS were HR-CPS patients (10/27, 37%), versus 0/9 LR-CPS patients (Fisher's exact $p = 0.04$).

In conclusion, whereas only 5%–10% of all cancers are caused by deleterious germline mutations in CPS genes,[13,14] we found evidence for CPS mutations in a large proportion of t-MN patients, all of whom met HR-CPS criteria. Based upon the combined results of our clinical and bioinformatics analysis, we identified putative CPS variants in 10/27 HR-CPS patients, and 0/9 LR-CPS patients, with all variants identified in either *TP53* or *CHEK2*. However, given the small sample size here, the HR-CPS criteria should be tested in a larger cohort to determine their sensitivity and specificity for identifying patients with CPS. Another possible limitation of our results is that the CancerNext-Expanded gene panel does not include genes associated with predisposition to 'pure' haematological malignancy syndromes such as *RUNX1* or *CEBPA*.

Wong and colleagues demonstrated that haematopoietic stem cell clones with somatic *TP53* mutations can be selected for by exposure to chemotherapy and outcompete clones with wild-type *TP53*, thereby giving rise to t-MN.[15] Our results complement these observations by suggesting that individuals with germline *TP53* mutations are similarly at increased risk for t-MN.

Moreover, our results align with work by Singhal and colleagues suggesting that CPS are likely to be more common among t-MN patients and that clinical criteria are needed to guide CPS screening in t-MN patients.[4] The simple HR-CPS criteria we employed, which do not require a family history, may provide a simple and effective means of identifying t-MN patients for CPS screening and should be studied further.

Currently, the vast majority of patients who could benefit from genetic testing for inherited CPS are not being offered genetic testing. Our findings provide both a powerful rationale for the importance of genetic testing in t-MN patients as well as a simple clinical algorithm to identify those t-MN patients who are most likely to benefit. These results may have significant diagnostic and clinical implications for both these patients and their family members.

AUTHOR CONTRIBUTIONS

Andrew J. Shih, Andrew D. Skol, Felicia Hernandez, Friedrich Stölzel, James M. Allan, and Kenan Onel designed the research; Andrew J. Shih, Andrew D. Skol, Friedrich Stölzel, James M. Allan, and Kenan Onel wrote the manuscript with substantial input from Felicia Hernandez; Andrew J. Shih, Tomi Jun, Andrew D. Skol, Riyue Bao, Lei Huang, Sapana Vora, Megan E. McNerney, Eric A. Hungate, Aaron Elliott, Robert Huether, and Felicia Hernandez performed the research and statistical analysis; Megan E. McNerney, Michelle M. Le Beau, and Richard A. Larson contributed critical reagents and clinical data; Kenan Onel directed the research.

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CONFLICT OF INTERESTS

All authors declare no conflicts of interest or competing financial interests.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

1. Leone G, Fianchi L, Pagano L, Voso MT. Incidence and susceptibility to therapy-related myeloid neoplasms. Chem Biol Interact. 2010;184(1–2):39–45.
2. Link DC, Schuettpelz LG, Shen D, Wang J, Walter MJ, Kulkarni S, et al. Identification of a novel TP53 cancer susceptibility mutation through whole-genome sequencing of a patient with therapy-related AML. JAMA. 2011;305(15):1568–76.
3. Churpek JE, Marquez R, Neistadt B, Claussen K, Lee MK, Churpek MM, et al. Inherited mutations in cancer susceptibility genes are common among survivors of breast cancer who develop therapy-related leukemia. Cancer. 2016;122(2):304–11.
4. Singhal D, Hahn CN, Feurstein S, Wee LYA, Moma L, Kutyna MM, et al. Targeted gene panels identify a high frequency of pathogenic germline variants in patients diagnosed with a hematological malignancy and at least one other independent cancer. Leukemia. 2021;35(11):3245–56.
5. Hampel H, Bennett RL, Buchanan A, Pearlman R, Wiesner GL, Guideline development group, American College of Medical Genetics and Genomics Professional Practice and guidelines committee and National Society of genetic counselors practice guidelines committee. A practice guideline from the American College of Medical Genetics and Genomics and the National Society of genetic counselors: referral indications for cancer predisposition assessment. Genet Med. 2015;17(1):70–87.
6. Tinat J, Bougeard G, Baert-Desurmont S, Vasseur S, Martin C, Bouvignies E, et al. Version of the Chompret criteria for Li Fraumeni syndrome. J Clin Oncol. 2009;27(26):e108–9. author reply e110.
7. Smith SM, Le Beau MM, Huo D, Karrison T, Sobecks RM, Anastasi J, et al. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. Blood. 2003;102(1):43–52.
8. 1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, et al. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012;491(7422):56–65.

9. Fu W, O'Connor TD, Jun G, Kang HM, Abecasis G, Leal SM, et al. Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants. Nature. 2013;493(7431):216–20.
10. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014;46(3):310–5.
11. Cooper GM, Stone EA, Asimenos G, NISC Comparative Sequencing Program, Green ED, Batzoglou S, et al. Distribution and intensity of constraint in mammalian genomic sequence. Genome Res. 2005;15(7):901–13.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24.
13. Garber JE, Offit K. Hereditary cancer predisposition syndromes. J Clin Oncol. 2005;23(2):276–92.
14. Zhang J, Walsh MF, Wu G, Edmonson MN, Gruber TA, Easton J, et al. Germline mutations in predisposition genes in pediatric cancer. N Engl J Med. 2015;373(24):2336–46.
15. Wong TN, Ramsingh G, Young AL, Miller CA, Touma W, Welch JS, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature. 2015;518(7540):552–5.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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