ORIGINAL ARTICLE

Germline mutations in the DNA damage response genes *BRCA1*, *BRCA2*, *BARD1* and *TP53* in patients with therapy related myeloid neoplasms

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ABSTRACT

Background Therapy related myeloid neoplasms (t-MNs) are complex diseases originating from an interplay between exogenous toxicities and a susceptible organism. It has been hypothesised that in a subset of cases t-MNs develop in the context of hereditary cancer predisposition syndromes.

Methods The study systematically evaluated pedigrees of patients with t-MNs for cancer incidences and the possibility of a hereditary cancer predisposition syndrome. In addition, mutational analyses were performed using constitutional DNA from index patients, and deleterious heterozygous germline mutations were assessed for loss of heterozygosity in sorted leukaemic cells by single nucleotide polymorphism array.

Results A nuclear pedigree was obtained in 51/53 patients with t-MNs resulting in a total of 828 individuals analysed. With a standardised incidence ratio of 1.03 (95% CI 0.74 to 1.39), the tumour incidence of first-degree relatives was not increased. However, six pedigrees were suggestive for a hereditary breast and ovarian cancer syndrome, three of a Li-Fraumeni like syndrome, and three index patients showed multiple primary neoplasms. Mutational analysis revealed two *BRCA1* (c.3112G→T, c.5251C→T), one *BRCA2* (c.4027A→G), two *BARD1* (C557S) and four *TP53* germline mutations (g.18508_18761delinsGCC, c.847C→T, c.845_848dupGGCG, c.1146delA) in nine of 53 (17%) index patients with t-MNs. Loss of heterozygosity in leukaemic cells was demonstrated for the *BRCA1*c.3112G→T and *TP53*c.845_848dupGGCG mutations, respectively.

Conclusion It is concluded that a proportion of patients with t-MNs carry cancer susceptibility mutations which are likely to contribute to therapy related leukaemogenesis.

INTRODUCTION

Therapy related myeloid neoplasms (t-MNs) constitute a distinct clinical syndrome including therapy related acute myeloid leukaemia (t-AML), myelodysplastic syndrome (t-MDS), and myelodysplastic/myeloproliferative neoplasm (t-MDS/MPN).[1 2] These secondary diseases are mainly observed after cytotoxic treatments for a malignancy, but occur also after immunosuppressive medication for autoimmune disorders or following solid organ transplantation.[3 4] The prognosis of patients with t-MNs is dismal and allogeneic haematopoietic stem cell transplantation (HSCT) remains the only curative approach for the majority of them.[5 6] t-MNs are regarded as complex diseases and previously published data on genetic predisposition to t-MNs pointed to an interactive effect of different single nucleotide polymorphisms (SNPs) in pathways that mediate carcinogen detoxification, proliferation, DNA repair, and apoptosis.[7 8] The demonstration of increased familial tumour risk in patients with AML following a primary neoplasm further supported the concept of genetic susceptibility to t-MNs.[9 10] Nevertheless, so far the prevalence of dominant cancer susceptibility gene mutations in patients with t-MNs has not been systematically assessed. We therefore addressed the question of whether t-MNs may also develop in the context of hereditary cancer predisposition syndromes.

METHODS

Patients

From February 1997 to December 2010, 69 patients with t-MNs were treated at the Divisions of Hematology and Paediatric Hematology and Oncology, Medical University of Graz, Austria, which are tertiary cancer centres for a population of about 1.5 million people. Of those, 53 patients developed t-MNs after a primary malignant disorder and were included in this study. Diagnosis and classification of t-MNs were made according to standard criteria.[1 11]

Pedigree analyses

Personal and telephone interviews were conducted with index patients and/or their relatives to retrieve the following information: (1) history of previous illnesses of the index patient including cancer, age at cancer diagnosis, date of diagnosis, and institution at which the diagnosis and cancer treatment were performed; (2) cancer history of family members. If a malignancy was identified in a relative, the same information was sought as for the index patient. To allow standardised evaluation, a nuclear pedigree according to Lynch *et al* was obtained that included information on the patient's siblings, progeny and both parents (first-degree relatives) as well as maternal and paternal aunts and uncles and both sets of grandparents (second-degree relatives).[12] We

attempted to confirm each cancer diagnosis through pathology reports from a medical database linking the university clinic with referral hospitals, as well as medical reports requested from non-affiliated hospitals and family physicians. In ambiguous cases, tissue specimens were re-evaluated by pathologists experienced in the diagnosis of neoplastic diseases. The accuracy of correctly reporting cancer diagnoses by family members was evaluated according to previous reports.[13]

Cancer incidence in first-degree relatives of patients with therapy related myeloid neoplasms

To explore whether the incidence of malignancies was increased in families of t-MN patients, we calculated the expected number of cancer cases among first-degree relatives.[14] This number was obtained by multiplying the person-years for sex and 5-year-age groups by corresponding age and sex specific incidence rates using data from the tumour registry of the Austrian province Tyrol as standard.[15] Person-years at risk were accumulated for each first-degree relative, beginning with the date of birth and ending either with the date of diagnosis of the first malignancy, date of death, or 31 December 2010. The standardised incidence ratio (SIR) was calculated as the ratio of observed to expected number of cases, a 95% CI assuming a Poisson distribution is given.

Mutational analyses

All index patients and pedigrees fulfilling one of the current clinical classification criteria for the Li-Fraumeni/Li-Fraumeni-like syndrome (LFS/LFL)[16–19] as well as index patients with multiple primary tumours were tested for *TP53* germline mutations. To identify patients suspicious of hereditary breast and ovarian cancer syndrome (HBOCS), guidelines of the US Preventive Services Task Force were implemented.[20] We tested every patient with a >10% probability of carrying germline mutations in the BRCA genes.[21 22] Following a hierarchical order, patients tested negative for *BRCA1/2* germline mutations were evaluated for mutations in *TP53* as well as for the *BARD1* C557S and *CHEK2* 1100delC alleles.

Genomic DNA was isolated from cultured fibroblasts from index patients using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany).[23] Mutations were confirmed with DNA from different tissues of the same individual. In patients harbouring heterozygous germline mutations, leukaemic cells were analysed for loss of the wild-type allele. CD34+ cells were separated from diagnostic blood or bone marrow specimens using the magnetic activated cell sorting CD34 MicroBead Kit together with MS Columns and MiniMACS Separator (all Miltenyi Biotec, Bergisch Gladbach, Germany). Purity of CD34+ cells was assessed by flow cytometry with a FACSCalibur (BD, FranklinLakes, New Jersey, USA). In case of homozygosity of the mutant allele, copy number variation analysis and heterozygosity mapping were performed using Affymetrix human genome-wide SNP 6.0 arrays (Affymetrix, High Wycombe, UK) to distinguish between loss of heterozygosity (LOH) and uniparental disomy as previously described.[24]

Screening for *BRCA1/2* germline mutations in HBOCS patients was performed by Sanger sequencing as previously described.[25] Briefly, all *BRCA1/2* exons as well as surrounding intronic sequences were directly sequenced using an ABI3100 automated sequencer (Applied Biosystems, Foster City, California, USA). Genetic variants were designated in accordance with the BIC database (http://research.nhgri.nih.gov/bic/). To screen for large genomic rearrangements (LGRs), multiplex ligation dependent probe amplification (MLPA) analysis was performed using the Salsa MLPA P002-B1 and P045-B1 kits, respectively, according to the manufacturer's instructions (MRC Holland, Amsterdam, The Netherlands). With respect to the *TP53* gene, coding exons 2 to 11 including splice sites were amplified by PCR and analysed by direct Sanger sequencing on an ABI3730 (Applied Biosystems) using oligonucleotides previously described by the IARC TP53 database (http://www-p53.iarc.fr/). MLPA for detection of LGRs was conducted using the Salsa MLPA kit P056 TP53 (MRC Holland). This kit also incorporates a specific probe for detection of the *CHEK2* 1100delC mutation. In the case of *BARD1*, mutational analysis was focused on the C557S allele by PCR and direct sequencing. All primer sequences are available upon request. All variants were also compared with the data from COSMIC (http://www.sanger.ac.uk/genetics/CGP/cosmic), HGDM (http://www.hgmd.cf.ac.uk), the 1000 Genomes Project (http://www.1000genomes.org), and dbSNP131 to determine their pathogenic relevance. Nonsense mutations were regarded as potentially pathogenic but additionally tested for functional consequences using MutationTaster (http://www.mutationtaster.org). Non-synonymous variations were also tested with SIFT (http://sift.jcvi.org/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/).

To assess the validity of this clinical approach described above, all remaining index patients with available DNA specimens were

Table 1 Characteristics of 53 patients with therapy related myeloid neoplasms (t-MNs) studied

Characteristic	Number (%)
Age, years	
Median	62
Mean	56
Range	2–85
Female	35 (66)
Male	18 (34)
Primary neoplasms	63 (100)
Solid tumours	37 (59)
Breast cancer	18 (29)
Sarcoma	6 (10)
Colon cancer	2 (3)
Other	11 (17)
Haematological malignancies	26 (41)
Non-Hodgkin's lymphoma	16 (25)
Acute myeloid leukaemia	4 (6)
Acute lymphoblastic leukaemia	2 (3)
Hodgkin's disease	2 (3)
Plasma cell myeloma	2 (3)
Primary cytotoxic treatments	
Alkylating agents	29 (55)
Topoisomerase II inhibitors	12 (23)
Antimetabolites	20 (38)
Ionising radiation	34 (64)
ASCT	5 (9)
Others	35 (66)
t-MNs at presentation	
t-MDS	23 (43)
t-AML	28 (53)
t-MDS/MPN	2 (4)
Latency period, months	
Median	53
Range	6–420
Progression t-MDS → t-AML	14 (61)
Median time, months	3
Range, months	1–31

ASCT, autologous haematopoietic stem cell transplantation; t-AML, therapy related acute myeloid leukaemia; t-MDS, therapy related myelodysplastic syndrome; MPN, myeloproliferative neoplasm.

screened for *TP53* germline mutations as described above. Furthermore, all remaining t-MN patients with breast cancer as primary malignancy—irrespective of their family history—were tested for *BRCA1/2* germline mutations. Therefore, next generation sequencing was applied using the HaloPlex PCR target enrichment system (Halo Genomics, Uppsala, Sweden). In brief, patient DNAs were digested by a cocktail of restriction enzymes and hybridised to *BRCA1/2* specific probes incorporating Illumina specific sequence motifs. Hybridised molecules were captured with magnetic beads, subsequently amplified using indexed primers, and finally sequenced with the MiSeq system (Illumina, San Diego, California, USA). Unknown or pathogenic variants were further verified by conventional Sanger sequencing.

Immunohistochemical analysis of p53

Immunohistochemistry of p53 was performed on paraffin embedded tissue with the IgG2b monoclonal mouse anti-human antibody clone (M7001, DakoCytomation, Glostrup, Denmark) according to the supplier's recommendations. The epitope recognised by the antibody is located between N-terminal amino acids 1 and 45 of the human p53 protein.[26]

RESULTS

Of the 53 t-MN patients studied, 49 were adults and four were minors (table 1). A total of 63 primary malignancies were diagnosed in these individuals with breast cancer (18/63, 29%), and non-Hodgkin's lymphoma (16/63, 25%) being the most common. The 18 breast cancers were observed in 14 t-MN patients; multiple primary malignancies were recorded in 10/53 (19%) individuals. Cytogenetic analysis of leukaemic cells obtained at t-MN diagnosis revealed clonal aberrations in 42/50 available specimens (84%) (supplementary table 1).

A nuclear pedigree was obtained in 51/53 patients with t-MNs. These pedigrees consisted of a total of 828 individuals with 327 first- and 450 second-degree relatives. The median number of family members per pedigree was 16 (range 10–25). Personal interviews revealed a total of 218 malignancies: 111 in index patients, 46 in first- and 61 in second-degree relatives. One hundred and sixty malignancies could be confirmed by medical reports, death certificates or re-evaluation, resulting in a 73% accuracy of correctly reported cancer diagnoses. For the calculation of SIR, data from 41 first-degree relatives with a neoplastic disease were used. With 39.9 expected cases, a SIR of 1.03 (95% CI 0.74 to 1.39) was obtained based on data from the tumour registry Tyrol.

Among 51 patients with known pedigrees, nine (18%) were suspicious of a hereditary cancer predisposition syndrome: six (12%) of an HBOCS and three (6%) of an LFS/LFL. Moreover, three index patients (6%) showed at least two primary benign and/or malignant tumours in addition to breast cancer raising the suspicion of LFL. Therefore, a total of 12 patients (24%) were initially tested for germline mutations in cancer associated genes. Germline mutations were found in six patients with t-MNs (12%) by this clinically oriented approach: two with a *BRCA1* and three with a *TP53* mutation as well as one with the *BARD1* C557S variant. To assess the validity of this approach, *TP53* mutation screening was extended to 37 remaining t-MN index patients with available DNA samples. Additionally, all remaining untested breast cancer index patients were analysed for *BRCA1/2* germline mutations as well as the *BARD1* C577S variant. We also included two more t-MN patients with unknown family history, one with breast cancer and one minor with a sarcoma. Three additional germline mutations could be identified, one each in *BRCA2, BARD1*, and *TP53*. Overall, we tested 13 patients for *BRCA1*/2, 49 patients for *TP53* as well as *CHEK2* 1100delC, and 11 patients for the *BARD1* C577S variant, and identified a total of two *BRCA1*, one *BRCA2*, two *BARD1*, and four *TP53* germline mutations (9/53, 17%) (table 2).

Patient UPN 5869 developed t-MDS with a complex karyotype 5 years after chemotherapy for ovarian cancer at the age of 56 years. Her pedigree showed multiple breast and gastric cancers. A heterozygous, nonsense c.3112G→T *BRCA1* mutation was detected in constitutional DNA. Leukaemic cells revealed hemizygosity of the mutation due to loss of the wild-type allele (figure 1A). In patient UPN 5824, t-MDS with monosomy 7 was diagnosed at the age of 35 years. She suffered from bilateral breast cancer at the age of 28 and 33 years, respectively, and was treated with surgery, ionising radiation, and multiple cycles of cytotoxic

Table 2 Germline mutations identified in patients with therapy related myeloid neoplasms

UPN	Gene	Mutation	Predicted effect	Pedigree	Previous description
5869	*BRCA1*	c.3112G>T p.E1038* rs80357161	Truncating mutation (NMD), clinically important (BIC)	HBOCS	One entry in BIC
5824	*BRCA1*	c.5251C>T p.R1751* rs80357123	Truncating mutation (NMD), clinically important (BIC)	HBOCS	Multiple entries in BIC
5298	*BRCA2*	c.4027A>G p.K1343E	Benign (PP2); tolerant (SIFT); polymorphism, protein features (might be) affected, splice site changes (MT)	Inconspicuous, identified by systematic screening	None
6387	*BARD1*	c.1670G>C	Reduced capability of growth suppression and apoptosis	HBOCS	Sauer *et al*[27]
4353		p.C557S rs28997576		Unknown	
5650	*TP53*	g.18508_18761delinsGCC c.782+143_835delinsGCC	Pathogenic; mutation involves splice site, protein structure unknown	Inconspicuous, but patient fulfils LFL criteria	None
6371	*TP53*	c.845_848dupGGCG p.R283fs*22	Truncating mutation (NMD), predicted disease causing (MT)	Multiple diverse neoplasms	None
6144	*TP53*	c.1146delA p.K382fs*40	Prolonged p53 (+27 AA), predicted disease causing (MT)	Inconspicuous	Somatic mutation in a gastric cancer cell line (COSMIC) and EBV transformed lymphoblasts[28]
7047	*TP53*	c.847C>T p.R283C	Deleterious (TMD)	Inconspicuous, identified by comprehensive screening	Multiple entries in TMD and COSMIC

dbSNP build 131; NM_007294.3 (*BRCA2*); NG_017013.1, NM_000546.4 (*TP53*).
BIC, breast cancer information core database; EBV, Epstein-Barr virus; HBOCS, hereditary breast and ovarian cancer syndrome; LFL, Li-Fraumeni like syndrome; MT, MutationTaster; NMD, nonsense mediated mRNA decay; PP2, PolyPhen-2; SIFT, Sorting Tolerant From Intolerant algorithm; TMD, IARC TP53 Mutation Database; UPN, unique patient number.

drugs. Pedigree analysis demonstrated multiple neoplasms including breast, ovarian, and lung cancer. A heterozygous, nonsense c.5251C→T *BRCA1* mutation was identified in both, constitutional and leukaemic DNA (supplementary figure 1). Patient UPN 5298 who also had a history of thyroid adenoma received combined chemo- and radiotherapy for breast cancer at the age of 62 and developed t-AML with trisomy 8 two years later. Her family history included malignant melanoma and metastatic cancer of unknown origin. Constitutional DNA and DNA from unsorted leukaemic cells showed a *BRCA2* c.4027A→G variant (supplementary figure 2). t-AML with trisomy 8 developed in patient UPN 6387 following ionising radiation for breast cancer. Pedigree analysis revealed HBOCS and a heterozygous missense *BARD1* C557S variant was identified in constitutional and leukaemic DNA (supplementary figure 3).

A second patient (UPN 4353) with the same mutation was identified by screening of those breast cancer patients not included in the first approach. This individual received radiotherapy for breast cancer at the age of 63 years and developed t-AML at age 72. UPN 6371 was diagnosed with t-MDS with a complex karyotype at the age of 54 years. Her history included angiomyolipoma, enchondroma and breast cancer, the latter treated with surgery and ionising radiation at the age of 50 years. Pedigree analysis showed multiple diverse neoplasms in first- and second-degree relatives. Constitutional DNA revealed a heterozygous c.845_848dupGGCG *TP53* mutation and leukaemic DNA, a hemizygous state due to loss of the wild-type allele (figure 1B).

A heterozygous LGR encompassing parts of *TP53* intron 7 and exon 8 was identified in the paediatric patient UPN 5650 by MLPA and confirmed as g.18508_18761delinsGCC by direct

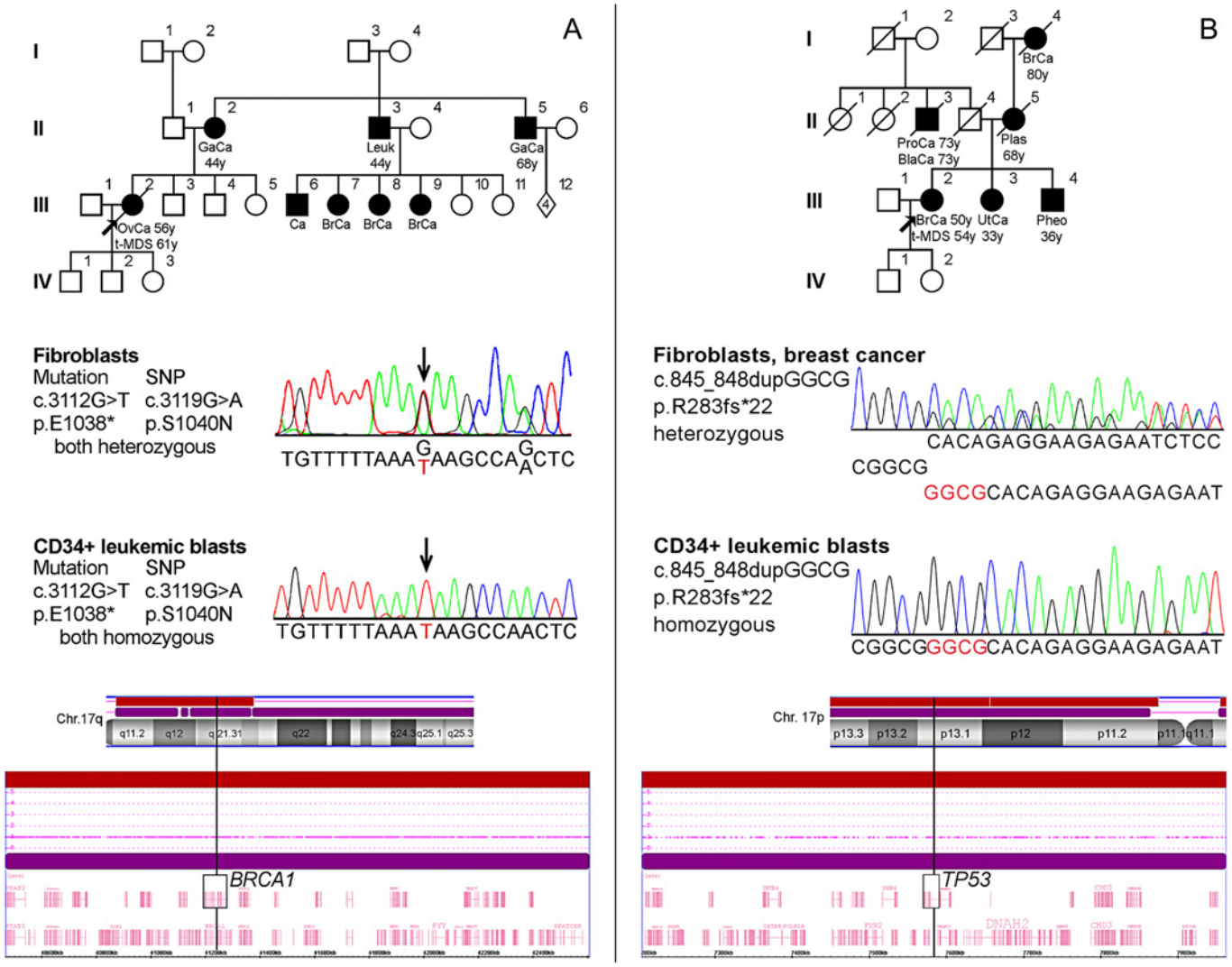

Figure 1 (A) Pedigree and mutational analysis of index patient UPN 5869. The pedigree indicates a hereditary breast and ovarian cancer syndrome. Electropherograms illustrate the BRCA1 c.3112G→T mutation (arrow) as well as a nearby single nucleotide polymorphism (SNP) in DNA from fibroblasts and CD34+ leukaemic cells. Both mutation and SNP became homozygous in the leukaemic clone. The SNP array reveals a copy number state 1 and loss of heterozygosity (LOH) at the BRCA1 locus on chromosome 17q of CD34+ leukaemic blasts. (B) Pedigree and mutational analysis of index patient UPN 6371. The pedigree is not specific for a certain syndrome. Electropherograms illustrate the *TP53* c.845_848dupGGCG mutation in germline as well as somatic DNA. SNP array shows copy number state 1 and LOH at the *TP53* locus on chromosome 17p of CD34+ leukaemic cells. Filled symbols, subjects with malignancies; open symbols, asymptomatic subjects; the arrow indicates the index patient; number within symbol corresponds to the number of asymptomatic individuals. Red regions represent losses and violet regions LOH. BlaCa, bladder cancer; BrCa, breast cancer; Ca, cancer of unknown origin; GaCa, gastric cancer; Leuk, leukaemia of unknown type; Pheo, pheochromocytoma; Plas, plasmacytoma; ProCa, prostate cancer; UtCa, uterine cancer; y, age in years at diagnosis.

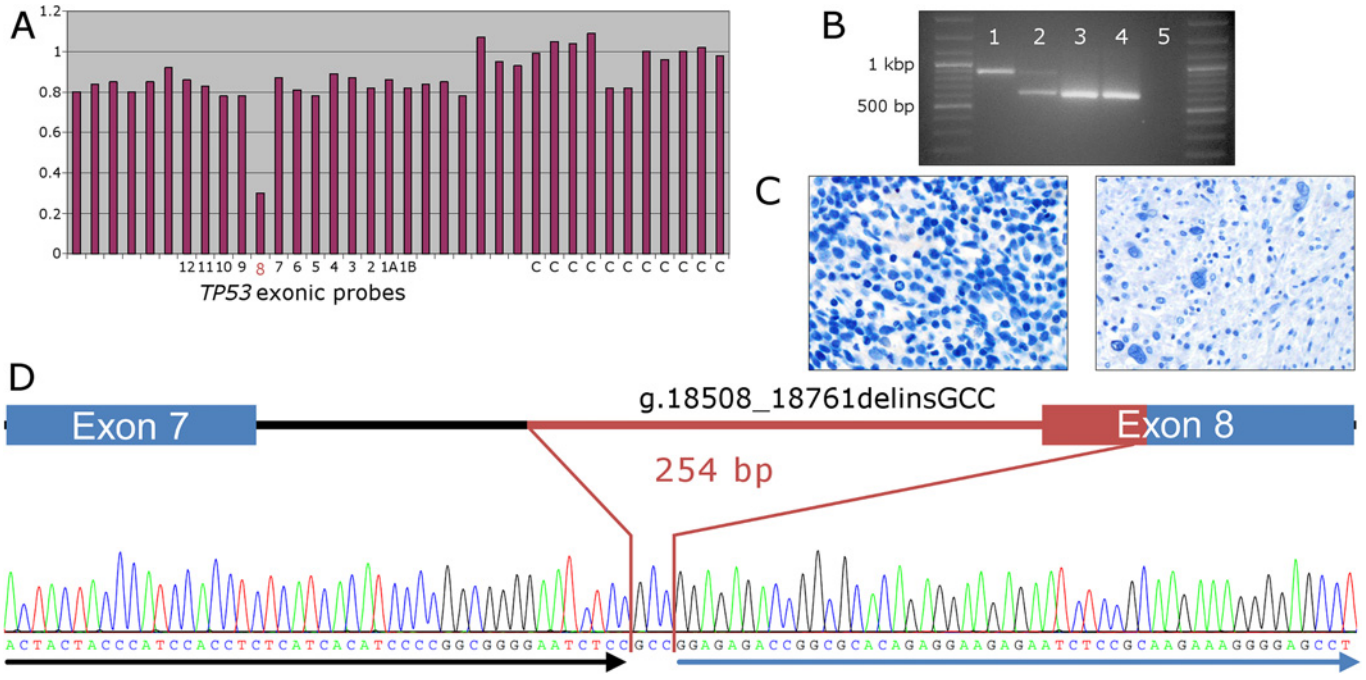

Figure 2 Mutational analysis of index patient UPN 5650 with a TP53 g.18508_18761delinsGCC germline mutation. (A) Multiplex ligation dependent probe amplification (MLPA) analysis showing a reduced amplification signal of the *TP53* exon 8 probe as compared with the other *TP53* and control probes. (B) Agarose gel electrophoresis of PCR products obtained from TP53 amplicons encompassing exons 7 to 9. Lane 1, wild-type amplicon whose calculated size is 866 bp. Lane 2, whole blood DNA collected during remission showing a heterozygous pattern with an additional shorter PCR product. Lane 3, DNA from embryonal rhabdomyosarcoma. Lane 4, DNA from fibrosarcoma showing the mutated amplicon only, indicating a homozygous state and loss of the wild-type TP53 allele. Lane 5, negative control. (C) Immunohistochemistry of p53. Histologic specimens of therapy related acute myeloid leukaemia (left) and fibrosarcoma (right) showing negative staining. (D) Direct sequencing of the mutated amplicon demonstrating the genomic breakpoints of the TP53 LGR. TP53 reference: NG_017013.1.

sequencing (figure 2). She suffered from t-AML at the age of 7 years, 2.5 years after the commencement of combined chemo- and radiotherapy for embryonal rhabdomyosarcoma. Her leukaemia was cured by allogeneic HSCT but she eventually developed a fibrosarcoma in the former radiation field at the age of 10 years. Somatic homozygosity of the mutated allele could be demonstrated in DNA from both sarcomas by PCR and these neoplastic tissues were also negative for p53 immunostaining. Her family history was negative for any cancer, suggesting a de novo germline mutation.

Patient UPN 6144 suffered from t-AML following chemotherapy for breast cancer at the age of 78 years. She had a history of cystadenoma of the ovary, uterine leiomyoma and renal cancer; however, her pedigree was inconspicuous. DNA from fibroblasts and leukaemic cells showed a heterozygous c.1146delA *TP53* mutation (supplementary figure 4).

Finally, patient UPN 7047 was identified as carrying the deleterious R283C *TP53* germline mutation which remained heterozygous in DNA from blast cells (supplementary figure 5). p53 immunohistochemistry was strongly positive in over 80% of leukaemic cells. He developed t-AML with a 7q deletion as well as a colonic adenoma at the age of 67, both 2 years after radiation for primary prostate cancer. His family history revealed a grandfather suffering from prostate cancer as well.

In addition, 10 different SNPs in *BRCA1* (seven non-synonymous) and 15 in *BRCA2* (six non-synonymous) were detected in the 13 patients tested for *BRCA1/2* mutations. Forty-two of 49 patients tested had the known *TP53* P72R SNP, of whom 20 (41%) were homozygous. No *CHEK2* 1100delC mutation was found. Results are summarised in supplementary tables 2–4. Surprisingly, the possibility of a hereditary cancer predisposition syndrome was recognised by the caring physicians in only one of the suspected 12 index patients at the time of the primary malignancy. In this individual (UPN 5294), a screen for germline mutations in the *BRCA* genes was negative.

DISCUSSION

In this study, we performed systematic pedigree and mutational analyses on patients with t-MNs seen at a tertiary cancer centre. A major goal was the identification of hereditary cancer predisposition syndromes; we therefore focused on patients who developed t-MNs after a primary malignant disorder. Importantly, this cohort is representative of t-MNs since demographic data were comparable to published studies.[3 4]

Previous publications on pedigree analyses of patients with secondary myeloid neoplasms pointed to an increased familial risk. Although Ben-Yehuda *et al* reported a family history of cancer in 55% of patients with t-MNs, no details are given on exact incidence rates as the authors primarily focused on molecular aspects of t-MNs.[9] Pagana *et al* described a significant increase of neoplastic diseases among first-degree relatives of patients with secondary AML as compared to those with de novo AML.[10] The authors, however, did not restrict their analysis to patients with AML following cytotoxic treatments but also included patients whose primary malignancies were treated with surgery only. In the cohort analysed here, the SIR of cancers among first-degree relatives was not significantly increased when compared to population based data from the tumour registry Tyrol, an Austrian province with an almost identical ethnic background. One explanation might be that an analysis of SIR focuses on first-degree relatives. Our cohort, however, is

characterised by a high incidence of neoplasms in second- and third-degree relatives as is shown for both *BRCA1* mutation carriers (figure 1A, supplementary figure 1). Another reason might be the relatively small number of family members analysed here. A more accurate evaluation of the familial cancer risk of patients with t-MNs using registry data, however, is impossible at present as t-MNs are not coded as a separate disease entity.

Based on pedigree data, we suspected a hereditary cancer predisposition syndrome in 12 index patients and eventually identified six clinically relevant germline mutations in genes associated with DNA damage response. Interestingly, through comprehensive screening, another three germline mutations could be detected. The majority of mutations were found in patients with breast cancer as the primary malignancy (6/14, 43%). Both *BRCA1* mutations identified are pathogenic and have been described previously in the BIC database. Although they have not been associated with the development of myeloid malignancies so far, deregulated BRCA function is increasingly recognised as contributing to myeloid leukaemogenesis. Germline mutations in *BRCA2*—also known as *FANCD1*—cause Fanconi anaemia, a congenital disorder with high propensity for the development of AML.[29] Furthermore, reduced expression of BRCA1 was reported in leukaemic specimens of cases with t-AML.[30] We identified the new *BRCA2* K1343E variation by the comprehensive mutational screening approach. This non-synonymous variation was rated as SNP by computational functional significance predictors; however, it is located between the highly conserved domains BRC1 and BRC2 of BRCA2 which are involved in RAD51 binding and essential for initiation of homologous recombination at sites of DNA double-strand breaks.[31] SNPs and non-synonymous variants are common in *BRCA2* (supplementary table 3), but without further functional evaluation it is difficult to distinguish between truly pathogenic mutations and rare but harmless variants. Given the inconspicuous pedigree of patient UPN 5298, mediating predisposition to HBOCS by K1343E seems unlikely. It nevertheless might contribute to leukaemogenesis following cytotoxic stress, which applies to all mutations found in this study.

BARD1 is a tumour suppressor which acts together or independently of BRCA1 in mediating cell cycle regulation and DNA repair in response to genotoxic stress.[32] Repression of BARD1 decreases sensitivity to doxorubicin induced apoptosis, and concomitant depletion of BRCA1 and BARD1 disturbs the G1/S checkpoint arrest through lack of p53 phosphorylation following irradiation.[33 34] The *BARD1* C557S variant has been described as a susceptibility allele for *BRCA1/2* negative hereditary breast cancer, and clinical data are supported by functional studies showing that this mutation mediates loss of growth suppression and reduced apoptosis in vitro.[27 35] To date, however, no association of *BARD1* mutations with myeloid leukaemias has been reported. *TP53* germline mutations have been described before in single cases of t-MNs. In the cohort studied here, *TP53* germline mutations are highly enriched (4/53, 8%), given an estimated prevalence of 1:20000 in the general population.[36] Three of these mutations have not yet been reported in primary human malignancies. The *TP53* c.1146delA variant was described in two cancer cell lines suggesting a possible role in immortalisation.[28] This mutation elongates the p53 protein, leaving the transactivation domain intact, but results in a lysine to alanine substitution at residue 382 and loss of the phosphorylation site at serine 392—both sites important for stabilisation of p53 following irradiation and other stimuli.[37 38] Interestingly, patient UPN 5650 with a *TP53* germline LGR developed her second sarcoma in a former radiation field, emphasising the risk of therapy related oncogenesis in patients with the LFS.[39] The detection of LOH for the *BRCA1* c.3112G→T and *TP53* c.845_848dupGGCG mutations in leukaemic cells as well as overexpression of p53 in blasts with heterozygous R283C suggest that they contribute to leukaemogenesis. However, functional studies which are underway will conclusively determine their exact role in the pathogenesis of these secondary malignancies.

In conclusion, our study demonstrates that cancer predisposition syndromes are prevalent in patients with t-MNs and can be identified by conventional pedigree analysis as well as systematic mutational screens. With respect to therapy related myeloid leukaemogenesis, they may extend current knowledge as germline mutations in cancer predisposition genes may also contribute to these secondary disorders. Clinically, the detection of such mutations has far reaching consequences for the surveillance of both patients and their family members.

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Competing interests None.

Ethics approval The study was performed according to the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Medical University of Graz, Austria, and written informed consent was obtained from all individuals.

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