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Germline variants of the promyelocytic leukemia tumor suppressor gene in patients with familial cancer

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The promyelocytic leukemia *(PML)* gene is an important tumor suppressor gene. We tested the hypothesis that germline disruption of the *PML* gene may be associated with a cancer predisposition syndrome. Mutation analysis of the *PML* gene was performed in 111 patients with familial adult cancer or young age-onset adult cancer. These were mostly breast and colon cancer, or colon polyposis patients in whom mutation analyses of the *BRCA1, BRCA2, MLH1, MSH2, APC* or *TP53* genes did not detect a pathogenic germline mutation. Heteroduplex analysis and direct sequencing were used for mutation screening. Mutation-specific methods were designed for frequency determination of novel variants in the general population. No deleterious nonsense or frameshift germline mutations were detected. Several missense single-nucleotide substitutions were found, including two novel missense variants, c.83C>T (p.Thr28Ile) in exon 1 in a 42-year-old breast cancer patient and c.1558C>T (p.Pro520Ser) in exon 6 in a 32-year-old colon cancer patient, that were not detected in 100 and 214 non-cancer persons, respectively. Frequency of the c.2260G>C (p.Ala754Pro) variant in isoform IV of the *PML* gene was higher in patients with colon polyposis and cancer than in the control group (P = 0.029). In conclusion, germline disruption of the *PML* gene is probably not associated with a highly penetrant susceptibility to adult-onset breast and colon cancer. Pathogenicity of c.83C>T and c.1558C>T variants in the *PML* gene is uncertain. Carriers of the c.2260 G>C variant in *PMLIV* isoform may be at an increased risk of colon polyposis and cancer.

Key Words: breast cancer; cancer risk; colon cancer; colon polyposis; germline mutation; PML gene

The promyelocytic leukemia (*PML*) gene encodes the PML protein, that is concentrated in special subnuclear structures, the so-called PML nuclear domains, and it plays a role in the formation and stability of these domains. PML domains represent nuclear depots of more than 50 proteins [1–3]. Through interactions of these proteins on PML domains, as well as the apparently regulated recruitment and release of proteins upon some signaling pathways induced by external insults (e.g. heat, stress or interferon treatment), PML harmonically coordinates and controls transcription, DNA damage repair, antiviral response and tumor suppressive functions, such as induction of apoptosis, growth arrest and senescence [1, 4–7].

The only clearly defined human pathological condition involving the *PML* gene is acute promyelocytic leukemia. The t(15;17) gives rise to the *PML/RARα* (retinoic acid receptor *α)* fusion gene. The PML/RARα fusion protein interferes with the normal functions of PML (causing resistance to cell death) and RARα (causing differentiation block and aberrant self-renewal), both of which contribute to the disease [8]. *PML* gene disruption due to t(15;17) in acute promyelocytic leukemia is a somatic event. Variants of the *PML* gene have been described in solid tumors and in patients with acute promyelocytic leukemia [9, 10]. However, there are so far no human conditions known to be associated with germline mutations of the *PML* gene. Although *PML*$^{-/-}$ mice are viable, they develop a larger number and a different spectrum of tumors upon carcinogenic treatment and are more susceptible to PML-RARα-mediated leukaemogenesis [11, 12].

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With respect to the important tumor suppressive functions of the *PML* gene, we tested the hypothesis that germline disruption of the *PML* gene may predispose one to an increased susceptibility to cancer development and may be associated with a cancer predisposition syndrome with an autosomal dominant inheritance, along with other tumor suppressor genes including *BRCA1* and *BRCA2* in hereditary breast and ovarian cancer, mismatch repair genes (*MLH1, MSH2, MSH6* and *PMS2*) in hereditary nonpolyposis colon cancer (HNPCC), *APC* in familial adenomatous polyposis, *TP53* in Li-Fraumeni syndrome, and others.

Patients and methods

Patients. The *PML* gene was analyzed in 111 patients with solid tumors. These included 49 patients with breast cancer who fullfilled the criteria for *BRCA1* and *BRCA2* mutation analysis [13] but in whom *BRCA1* or *BRCA2* mutation analysis did not reveal a germline mutation. There were four patients with breast cancer and one with breast and ovarian cancer included in whom no *BRCA* gene mutation was initially found. After *PML* gene analysis was performed, a new *BRCA1* and *BRCA2* mutation screening method was introduced and a *BRCA1* mutation was found. There were 38 patients with colon cancer fullfilling the Amsterdam or Bethesda guidelines for hereditary non-polyposis colon cancer [14, 15] and who tested negative for *MLH1* and *MSH2* germline mutations, as well as 13 patients with multiple colon polyposis who tested negative for an *APC* germline mutation. There were 7 patients with other cancers (for details see Table 1). The patients were aged 24 to 73 years. The control groups for population frequency analysis of some novel single-nucleotide polymorphisms consisted of 100 and 214 non-cancer persons (male/female pairs of reproductive age). For the analysis of some *PML* variants, there was included an extended group of up to 330 cancer patients, mostly with gastrointestinal cancer. Written informed consent was obtained from each subject. The study was performed after approval by the local institutional ethics committee.

Methods. Genomic DNA was isolated from peripheral blood using the QIAamp DNA Blood Kit (Qiagen, the Netherlands)

Table 1. Characteristics of patients included into the study.

	No. of patients analysed by			
Type of cancer	Total no. of patients	Heteroduplex analysis	Direct sequencing	Previous gene analysis with a negative result
Breast cancer, non-*BRCA*	42	25	17	*BRCA1, BRCA2*
Breast and urinary bladder cancer, non-*BRCA*	1	1		*BRCA1, BRCA2*
Breast cancer and colon polyposis, non-*BRCA*	1	1		*BRCA1, BRCA2*
Breast and ovarian cancer, non-*BRCA*	3		3	*BRCA1, BRCA2*
Breast and colon cancer, non-*BRCA*	1		1	*BRCA1, BRCA2*
Breast cancer and uterine sarcoma, non-*BRCA*	1		1	*BRCA1, BRCA2*
Breast cancer, *BRCA*-associated	3	3		
Breast and ovarian cancer, *BRCA*-associated	1	1		
Colon cancer	35	20	15	*MLH1, MSH2*
Colon and ovarian cancer	1		1	*MLH1, MSH2*
Colon and endometrial cancer	1		1	*MLH1, MSH2*
Colon cancer and non-Hodgkin´s lymphoma	1	1		*MLH1, MSH2*
Colon polyposis	12	11	1	*APC*
Colon polyposis and Hodgkin´s lymphoma	1	1		*APC*
Stomach cancer	1	1		*MLH1, MSH2*
Ovarian cancer	1	1		*BRCA1, BRCA2*
Ovarian and thyroid cancer	1		1	*BRCA1, BRCA2*
Ovarian and endometrial cancer and carcinoid of the appendix	1	1		*BRCA1, BRCA2*
Meningeoma	1	1		-
Thyroid cancer	1	1		*PTEN*
Pleural sarkoma	1	1		*TP53*
Total number of patients	111	70	41	

Table 2. Primers, probes and RT-PCR conditions for the c.83C>T and c.1558C>T *PML* gene variant detection.

	c.83C>T	c.1558C>T
Forward primer	5´-CTTCTCTTCACGCACTCCAAGAT-3´	5'-CACTCACCCCTGCAGGCACCTGA-3'
Revers primer	5´-GGTACTTGACCAAATCCTCCGT-3´	5'-GCACCGCCCAGACCCTGGCGG-3',
Sensor probe	TTCAGAGGGGATCTCGGG-FL	TCCATCCAGGTGGGATGGTGA--FL
Anchor probe	LC640-GGAGGCATGGTGGGCTCCT-PH	LC-CTGCCTTGGAGGTGCTGGGCCT—PH

RT-PCR conditions

Step	No. of cycles	Temperature	Time	Ramp rate	Acquisition mode	Temperature	Time	Ramp rate	Acquisition mode
		(°C)	(s)	(°C)		(°C)	(s)	(°C)	
1. Denaturation	1	95	15 min	20	none	95	15 min	20	none
2. Amplification	45	95	5	20	none	95	0	20	none
		50	10	20	single	55	10	20	single
		72	10	20	none	72	10	20	none
3. Melting curve	1	95	1	20	none	95	1	20	none
		60	20	20	none	60	20	20	none
		40	20	20	none	40	20	20	none
		80	1	0.3	step	80	1	3	step
4. Cooling	1	40	20	20	none	40	20	20	none
Melting temperatures									
– wild type allele	53°C					53°C			
– mutated allele	62°C					59°C			

or Gentra Puregene Blood Kit (Qiagen, the Netherlands). Fourteen primer sets were used to amplify the entire PML coding sequence and intron-exon boundaries as previously published (10). Polymerase chain reaction (PCR) was performed in a volume of 25 µl containing 1 µl of 50-200 ng of genomic DNA, 12.5 pmol of each primer, 12.5 µl of 2x PCR Master Mix (Fermentas, Germany) and 9.5 µl of PCR water. PCR reaction conditions were 94°C for 5 min (94°C for 1 min, 60–66°C for 1 min, 72°C for 1 min)$_{30}$ and 72°C 7 min.

In 70 patients (see Table 1), heteroduplex analysis was used as a mutation screening method. Composition of the gel for heteroduplex analysis included 15.6 ml of dH_2O, 1.8 ml of 10xTBE (Tris, boric acid, EDTA disodium), 17.5 ml of MDE (Acrylamide Solution for Mutation Detection, Sigma, Germany), 30 µl of TEMED and 225 µl of 10% amonium persulfate. Heteroduplex formation was performed by denaturation of 10 µl of the PCR product at 95°C for 5 minutes, followed by 30 minutes of incubation at 60°C and 30 minutes of cooling to room temperature. Electrophoresis was performed at 100V for 15–17 hours according to each fragment mobility. Gels were silver-stained. PCR products displaying an altered electrophoretic mobility by heteroduplex analysis were reamplified and sequenced on an ABI PRISM 3130 genetic analyser (Applied Biosystems, USA).

In 41 patients (see Table 1), nine exons of the *PML* gene were analysed by bidirectional sequencing using the ABI Big Dye Terminator Cycle Sequencing Detection Kit v.3.1 (Applied Biosystems, USA) on an ABI 3130 genetic analyser (Applied Biosystems, USA) according to the manufacturer's instructions. The sequence of the gene was compared to the reference *PML* gene sequence available at the Ensembl gene database (http://www.ensembl.org; GenBank NM_033240). Changes in the *PML* gene were described according to the recommended mutation nomenclature [16]. If a previously undescribed change in the *PML* gene was found, a simple method was designed to screen a control population.

The c.83C>T (p.Thr28Ile) substitution in exon 1 and the c.1558C>T (p.Pro520Ser) substitution in exon 6 of the *PML* gene were detected using real-time PCR (RT-PCR) on the LightCycler 1.5 instrument (Roche, USA). Primers, probes (TIB MOLBIOL, Germany) and RT-PCR conditions are described in Table 2. PCR was performed in a volume of 10 µl containing 10 pmol of each primer, 2 pmol of each probe, 1 µl of dimethylsulfoxide, 4 µl of ABsolute QPCR Capillary Mix (AB gene, Great Britain) and 1 µl containing 50-200 ng of DNA. The method was used for the analysis of 100 non-cancer persons in the control group in case of c.83C>T and of 214 non-cancer persons in the control group and of an extended group of 80 colon cancer patients in case of c.1558C>T.

To detect the c.1902T>C (p.Pro634Pro) substitution in isoform IV of the *PML* gene, an allele-specific PCR method was designed. The sequences of the primers were the following: the common forward primer, 5'- CCGCTAC-CTGTTGTACAGAG-3'; the normal reverse primer, 5'- GGGAGACCAAGTCCGAAGA-3'; and the mutated reverse primer, 5'- GGGAGACCAAGTCCGAAGG-3'. PCR was

performed in a volume of 25 μl containing 1 μl of 50-200 ng of genomic DNA, 12.5 pmol of each primer, 12.5 μl of 2x PCR Master Mix (Fermentas, Germany) and 9.5 μl of PCR water. PCR conditions were the following: 95°C for 5 min (95°C for 1 min, 62°C for 1 min, 72°C for 40s)$_{30}$ and 72°C for 7 min. Electorophoresis was performed on a 5% nondenaturing polyacrylamide gel for 1 hour at 200 V. The fragments were visualized using silver staining. In samples without the substitution, a fragment of 100 base pair (bp) was present only in the tube with the normal reverse primer. If the patient was heterozygous for the substitution, fragments were present in both the tubes with the normal and mutated forward primers. This method was used to verify the result in 70 patients analysed with heteroduplex analysis as well as to detect the variant in the control group of 100 cancer-free persons and in an extended group of 78 other cancer patients.

The c.2170A>G (p.Ser724Gly) substitution in isoform IV of the *PML* gene was verified in 70 samples analysed by heteroduplex analysis using an allele-specific PCR. The sequences of the primers were the following: the common forward primer, 5'-AAGCCAGCACTCCTGCCATCACA -3'; the normal reverse primer, 5'-GCTCCAAGAGTCTGGGCTCTGTT -3'; and the mutated reverse primer, 5'-GCTCCAAGAGTCTGGGCTCTGTC -3'. Composition of the PCR master mix, PCR conditions, electrophoresis, visualization and assessment were similar as for the c.1902T>C variant, PCR annealing temperature being 64°C. The size of the PCR fragment was 262 bp.

The c.2260G>C (p.Ala754Pro) variant in isoform IV of the *PML* gene was detected using restriction analysis. The sequences of the primers were the following: forward primer, 5′-AGG CCT CTC AAG TCC AAG TG-3′; and reverse primer, 5′- CTGTAAGAGCATGGGCTGGAGGA -3′. Composition of the PCR master mix and PCR conditions were similar as for the c.1902T>C variant, annealing temperature being 60°C. CvikI-1 restriction enzyme was used. Electorophoresis was performed on an 11% nondenaturing polyacrylamide gel at 300 V for 72 min. The fragments were visualized using silver staining. The method was used to verify the variant in 70 patients analysed by heteroduplex analysis as well as to detect the variant in the control group of 100 cancer-free persons and in an extended group of 330 cancer patients.

*Statistical analysis***.** The difference in the frequency of the variants in cancer patients compared to the control group was analyzed using Fisher's exact test (two-sided) at the 0.05 significance level. Relative risks and corresponding confidence intervals were calculated where appropriate.

Results

We detected no deleterious nonsense or frameshift germline mutations in the studied patients. We found only single-nucleotide substitutions, either synonymous or nonsynonymous missense variants (Table 3). For the occurence of the detected variants in the studied and control groups, see Table 4.

Nonsynonymous (missense) substitution c.83C>T (p.Thr28Ile) in exon 1 of the *PML* gene was found only in a 42-year-old female breast cancer patient. Mother and father of the patient are healthy at the ages of 68 and 72 years, respectively. Her mother's mother and sister suffered from ovarian cancer at the ages of 70 and 42 years, respectively. Her father's mother suffered from bilateral breast cancer at the age of 60 and father from gastrointestinal cancer.

Nonsynonymous (missense) substitution c.1558C>T (p.Pro520Ser) in exon 6 was found only in a 32-year-old male colon cancer patient. His parents were healthy. His father's mother suffered from oesophageal cancer at the age of 53 years and father of stomach cancer at the age of 47. This variant was not found in any sample of 214 in the control group and in an extended group of 80 other colon cancer patients. Thus, the variant occurred in one patient of 98 colon cancer patients (counting only those who were sequenced or analyzed using RT-PCR).

As regards synonymous c.1902T>C (p.Pro634Pro) variant in the *PML IV* isoform, we performed an extended study in 78 other cancer patients and found this variant in another patient with colon cancer (Table 5). The differences in c.1902T>C occurence between both breast cancer and other cancer versus non-cancer groups were not statistically significant (P = 0.614

Table 3. Variants of the *PML* gene found in cancer patients.

Exon	Variant	Change at the protein level	Type of mutation
1	c.83C>T	p.Thr28Ile	missense
6	c.1558C>T	p.Pro520Ser	missense
7b	c.1710+977T>C* (*PML IV* c.1902T>C**)	p.Pro570+112>Pro570+112* (*PML IV* p.Pro634Pro**)	synonymous
7b	c.1710+1245A>G* (*PML IV* c. 2170A>G**)	p.Ser570+202>Gly570+202* (*PML IV* p.Ser724Gly**)	missense
7b	c.1710+1355G>C* (*PML IV* c.2260G>C**)	p.Ala570+232>Pro570+232* (*PML IV* p.Ala754Pro**)	missense
9	c.1933T>C	p.Phe645Leu	missense
9	c.1956C>T	p.Ala652Ala	synonymous

*variant description according to the nomenclature for alternatively spliced exons;
**variant description for isoform IV of the *PML* gene that includes the alternatively spliced exon 7b

Table 4. The *PML* gene variants and their frequencies in the studied groups of patients, in the reference gene database or in the control group.

Exon	Mutation	Allele	Breast cancer	Colon cancer or polyposis	Other cancer	Control group
1	c.83C>T	CT	1/53 (2%) (P = 0.351)	0/51 (P = 1.000)	0/7	0/100
6	c.1558C>T	CT	0/53 (P = 1.000)	1/51 (2%) (P = 0.189)	0/7	0/214
7b	*PML IV* c.1902T>C	TC	2/53 (4%) (P = 0.281)	0/51 (P = 1.000)	0/7	1/100 (1%)
7b	*PML IV* c.2170A>G	AG	24/53 (45%)	22/51 (43%)	3/7 (42%)	Ensembl: 46-50%
		GG	17/53 (32%)	17/51(33%)	2/7 (28%)	Ensembl: 27-31%
7b	*PML IV* c.2260G>C	GC	6/53 (11%) (P = 0.581)	12/51 (24%) (P = 0.024)	1/7 (14%)	9/100 (9%), Ensemb: l 5-25%
		CC	1/53 (2%)	0/51	0/7	0/100
9	c.1933T>C	TC	26/53 (49%)	25/51 (49%)	3/7 (42%)	Ensembl: 40-50%
		CC	13/53 (24%)	13/51 (25%)	2/7 (29%)	Ensembl: 20-30%
9	c.1956C>T	CT	1/53 (2%)	0/51	0/7	Ensembl: not given

Table 5. Resuts of c.1902T>C variant detection in extended cancer group and control group (TT, carriers of the wild type allele; TC, carriers of the variant in the heterozygous form).

	Study group		Extended cancer patient group		Total number	
Type of cancer	c.1902T>C		c.1902T>C		c.1902T>C	
	TT	TC	TT	TC	TT	TC
Breast cancer	47	2	42		89	2 (2.2%)
BRCA-associated breast cancer	4		5		9	
Breast and colon cancer			1		1	
Breast and duodenal cancer			1		1	
Breast and endometrial cancer			1		1	
Colon cancer	38		10	1	48	1 (2.1%)
Colon and urinary bladder cancer			1		1	
Lynch syndrome - *MLH1, MSH2*- associated colon or endometrial cancer			5		5	
Colon polyposis	13				13	
Stomach cancer	1		2		3	
Duodenal cancer			1		1	
Pancreatic cancer			1		1	
Ovarian cancer	2				2	
Endometrial cancer			3		3	
Ovarian and endometrial cancer and carcinoid of the appendix	1				1	
Lung cancer			2		2	
Prostate cancer			1		1	
Meningeoma	1				1	
Brain tumor			1		1	
Thyroid cancer	1				1	
Pleural sarcoma	1				1	
Total No.	109	2	77	1	186	3 (1.62%)
Non-cancer persons – control group					99	1 (1%)

for breast cancer; P = 1.000 for other cancer patients, Fisher's exact test).

Statistical analysis disclosed a significant increase in the frequency of the c.2260G>C (p.Ala754Pro) variant in the *PML IV* isoform in patients with colon cancer and colon polyposis (Table 3). Therefore, we analyzed an extended group of 330 cancer patients. For results, see tables 6 and 7. Again, the occurrence of this variant was significantly increased in the group of patients with colon polyposis. We also compared the frequency of the c.2260G>C variant in cancer patients with another control group composed of non-cancer persons older than 60 years. The c.2260 G>C variant was found in 44

Table 6. Results of the c.2260G>C variant detection in isoform IV of the *PML* gene in the study group, in the extended cancer group and in the control group (GG, carriers of the wild type allele; GC, carriers of the variant in the heterozygous form; CC, carriers of the variant in the homozygous form).

	Study group		Extended group		Total	
Type of cancer	c.2260G>C		c.2260G>C		c.2260G>C	
	GG	GC+ CC	GG	GC	GG	GC+ CC
Breast cancer, non-*BRCA*	36	5+1	36	4	72	9+1
Breast and urinary bladder cancer, non-*BRCA*	1				1	
Breast cancer and colon polyposis, non-*BRCA*		1				1
Breast and colon cancer, non- *BRCA*	1		1		2	
Breast and duodenal cancer, non-*BRCA*			1		1	
Breast and ovarian cancer, non-*BRCA*	3				3	
Breast and endometrial cancer, non-*BRCA*			1		1	
Breast cancer and uterine sarcoma, non-*BRCA*	1				1	
Breast cancer, *BRCA*- associated	3		12	1	15	1
Breast and ovarian cancer, *BRCA*-associated	1				1	
Colon cancer	27	8	185	14	211	22
Colon cancer and non-Hodgkin´s lymphoma	1				1	
Colon and ovarian cancer	1				1	
Colon and endometrial cancer	1				1	
Colon and urinary bladder cancer			1		1	
Colon cancer, HNPCC- associated			26	3	26	3
Colon polyposis	9	3	7	2	16	5
Colon polyposis and Hodgkin´s lymphoma		1				1
Familial adenomatous polyposis, *APC*-associated			1		1	
Stomach cancer		1	19	4	19	5
Ovarian cancer	1		2		3	
Ovarian and thyroid cancer	1				1	
Ovarian cancer, *BRCA*- associated				1		1
Endometrial cancer			3		3	
Ovarian and endometrial cancer and carcinoid of the appendix	1				1	
Thyroid cancer	1				1	
Prostate cancer			1		1	
Renal cancer			2		2	
Pancreatic cancer			1		1	
Skin cancer, multiple			1		1	
Pleural sarkoma	1				1	
Meningeoma		1				1
Brain hemangioblastoma and pheochromocytoma, no *VHL* mutation				1		1
Total No. of patients	90	21	300	30	390	51

Table 7. Statistical analysis of the c.2260G>C variant in isoform IV of the *PML* gene (for statistical analysis, some groups from Table 7 were joined; GG, carriers of the wild type allele; GC, carriers of the variant in the heterozygous form; CC, carriers of the variant in the homozygous form)

	No. of patients			No. of patients		
	Studied group			Studied and extended groups		
Type of cancer	c.2260G>C		Fisher's exact test	c.2260G>C		Fisher's exact test
	GG	GC+ CC	Significance (P =)	GG	GC+ CC	Significance (P =)
Breast cancer, non-*BRCA*	42	6+1	0.404	82	11	0.638
Breast cancer, *BRCA*-associated	4		1	16	1	1
Colon cancer, non-HNPCC and colon polyposis	39	12	0.024	233	28	0.702
Colon cancer, non-HNPCC	30	8	0.079	217	22	1
Colon polyposis	9	4	0.042	16	6	0.029
Stomach cancer		1	0.099	19	5	0.144
Colon cancer, HNPCC-associated				26	3	0.731
Cancer-free control group	91	9		91	9	

of 385 cancer or colon polyposis patients and in one of 54 control persons. Patients with cancer or colon polyposis were significantly more often carriers of this variant (P = 0.029). The estimated relative risk of colon polyposis in c.2260G>C carriers was 2.68 (95% confidence interval 1.24 to 5.76) compared to non-carriers and 3.70 (95% confidence interval 2.19 to 6.25) compared to non-carriers older than 60 years of age. The estimated relative risk of cancer in c.2260G>C carriers was 1.13 (95% confidence interval 1.06 to 1.20) compared to non-carriers older than 60 years of age.

Discussion

Those patients with familial cancer or young age-onset cancer were included into the study, in whom mutation analysis of appropriate genes associated with known cancer susceptibility syndromes did not reveal a pathogenic mutation. We found no deleterious mutation in the *PML* gene in any of the patients. These results suggest that germline disruption of the *PML* gene is not a major cause of an adult-onset cancer predisposition syndrome with an autosomal dominant or recessive inheritance. We found several variants, four of which had not previously been described.

Two of the novel variants in the coding region, c.83C>T (p.Thr28Ile) and c.1558C>T (p.Pro520Ser), seem to be very rare according to our results and were detected only in young-age cancer patients. They are both missense mutations leading to an amino acid change. Pathogenicity of these variants is uncertain, and functional studies would be necessary to determine if they may be associated with an increased risk of cancer. The novel synonymous c.1902T>C (p.Pro634Pro) variant in isoform IV of the *PML* gene was detected in both healthy and cancer patients. With respect to the fact that it does not lead to an amino acid change, it is highly probable that it is a nonpathogenic polymorphism with a population frequency of 1–2% in the Czech population. The c.2170A>G (p.Ser724Gly) variant in isoform IV of the *PML* gene has been already described and its frequency corresponds to that shown in the Ensembl database. It is a nonpathogenic polymorphism. Before our study was performed, the c.2260G>C (p.Ala754Pro) variant in isoform IV of the *PML* gene was not shown in the Ensembl database. It was detected in about 16% of breast cancer patients and 30% of colon cancer or polyposis patients. Its frequency in the non-cancer control group was 9%. This suggested that this variant may be associated with an increased risk of cancer. We therefore performed an extended study. That study showed a significant association of this variant with colon polyposis, although the number of polyposis patients was relatively small. The control group consisted of young persons, and whether or not they will suffer from cancer one day cannot be known. Therefore, we created another control group comprised of cancer-free patients aged 60 years and more. In comparison with this group, carriers of the c.2260G>C variant were at an increased risk of cancer.

Also indentified were two variants in exon 9, both of which are mentioned in the Ensembl database. The frequency of the c.1933T>C (p.Phe645Leu) variant corresponds to that shown in the Ensembl database, and this is a very frequent polymorphism. The c.1956C>T (p.Ala652Ala) variant was observed in only one patient. Its frequency is not shown in the Ensembl database, but it had already been described in human tumors and patients with haematologic malignancies [9–10]. Our results prove that this variant detected by Gurrieri et al. [9–10] in tumors and blood of patients with haematologic malignancies was germline. Gurrieri et al. [10] had detected also other variants, including two deleterious mutations in two patients with acute promyelocytic leukemia. In one of these, it was proved to be somatic as it was not detected during the disease remission; in the other patient, no remission sample was available to prove whether it was somatic or germline.

The PML protein is the most well characterized member of a class of proteins reffered to as tripartite motif (TRIM)

family of proteins. Its importance in cellular defense against viral infections is a property that appears common to other TRIM family members [17]. Interferon, a component of the antiviral immune response and having PML as its primary target, induces production of PML and other proteins bound in PML domains [12]. The importance of PML in viral response is highlighted by the fact that many viruses have evolved different strategies in order to disrupt PML-domains and thereby to prevent PML induced cellular senescence and cell death and enable viral spread [18]. The assorted variants in the *PML* gene may cause variation in functional effectivity of the PML protein. Some variants may decrease the antiviral defence mechanisms and enable chronic infections, e.g. in the colon, that may be involved in cancer development.

There is no human clinical entity described as caused by germline *PML* gene disruption. With respect to its extremely important function, which may be comparable to that of the *TP53* and *RB* genes, it is highly probable that its disruption causes some disease with a specific clinical phenotype. Our results suggest that disruption of the *PML* gene does not lead to a highly penetrant susceptibility to adult-onset breast and colon cancer. As already mentioned, many proteins are bound and regulated at PML nuclear domains (including e.g. NBS1, ATM, ATR, and BLM). Immunodeficiency and increased risk of haematologic malignancies are typical for children affected with biallelic inactivation of both alleles of these genes. Impaired cooperation of the particular mutated protein with PML, thus disabling the process of antiviral response, may be involved in the mechanism of the disease. Thus, we may speculate that the clinical phenotype of a germline *PML* gene disruption may be a rare autosomal recessive disorder also characterized by immunodeficiency, an increased risk of childhood haematologic malignancies and perhaps other childhood cancer.

In conclusion, our results suggest that germline disruption of the *PML* gene is probably not associated with a highly penetrant susceptibility to adult-onset breast and colon cancer. We have detected several rare novel missense variants in young patients with cancer, whose pathogenicity is uncertain. Carriers of the c.2260G>C (p.Ala754Pro) variant in isoform IV of the *PML* gene may be at an increased risk of colon polyposis and cancer.

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