LJMU Research Online

De Ruyck, K, Wilding, CS, Van Eijkeren, M, Morthier, R, Tawn, EJ and Thierens, H

Microsatellite polymorphisms in DNA repair genes XRCC1, XRCC3 and XRCC5 in patients with gynecological tumors: Association with late clinical radiosensitivity and cancer incidence

http://researchonline.ljmu.ac.uk/2706/

Article

For more information please contact researchonline@ljmu.ac.uk

http://researchonline.ljmu.ac.uk/

Microsatellite Polymorphisms in DNA Repair Genes *XRCC1, XRCC3* and *XRCC5* in Patients with Gynecological Tumors: Association with Late Clinical Radiosensitivity and Cancer Incidence

K. De Ruyck,[a,1] C. S. Wilding,[b] M. Van Eijkeren,[c] R. Morthier,[d] E. J. Tawn[b] and H. Thierens[a]

[a] *Department of Anatomy, Embryology, Histology and Medical Physics, Ghent University, Gent, Belgium;* [b] *Genetics Department, Westlakes Research Institute, Moor Row, Cumbria CA24 3JY, United Kingdom;* [c] *Department of Radiation Oncology, Ghent University Hospital, Gent, Belgium; and* [d] *Occupational Medicine Service IDEWE, Ghent University Hospital, Gent, Belgium*

De Ruyck, K., Wilding, C. S., Van Eijkeren, M., Morthier, R., Tawn, E. J. and Thierens, H. Microsatellite Polymorphisms in DNA Repair Genes *XRCC1, XRCC3* and *XRCC5* in Patients with Gynecological Tumors: Association with Late Clinical Radiosensitivity and Cancer Incidence. *Radiat. Res.* 164, 237–244 (2005).

This study investigates the association of microsatellite polymorphisms in *XRCC1, XRCC3* and *XRCC5* with the development of late radiation-induced radiotherapy reactions and examines the correlation between these microsatellites and cancer incidence. Sixty-two women with cervical or endometrial cancer treated with radiotherapy were included in the study. According to the CTCAEv3.0 scale, 22 patients showed late adverse radiotherapy reactions (grade 2 or more). PCR on lymphocyte DNA followed by automated fragment analysis was performed to examine the number of tandem repeat units at each locus. No significant association was found between the repeat length at any of the microsatellites in *XRCC1, XRCC3* or *XRCC5* and the incidence of late radiotherapy complications. Since higher odds ratios (ORs) were found for the rare *XRCC1* $[AC]_{11}$ and $[AC]_{21}$ repeats (OR = 2.65, $P = 0.325$ and OR = 8.67, $P = 0.093$, respectively), the possible involvement of these small and large repeats in clinical radiosensitivity cannot be completely ruled out. When specific numbers of repeats were examined, no significant correlation was found between the microsatellite repeat length in *XRCC1* and *XRCC5* and cancer incidence. A weak correlation between *XRCC3* $[AC]_{16}$ homozygotes and cancer incidence was found (OR = 2.56, $P = 0.055$). A large-scale multicenter study of cancer patients with a high number of radiosensitive individuals is needed to clarify the value of rare polymorphic microsatellite repeats in *XRCC1* and *XRCC3* as a biomarker of clinical radiosensitivity or increased cancer risk.   © 2005 by Radiation Research Society

INTRODUCTION

In radiation oncology, radiation dose protocols are dependent on both the tolerance of healthy tissue and the

tumor control probability. In a small percentage of patients, radiation doses that are usually well tolerated by the healthy tissues within the irradiation field result in unexpected acute and/or late radiotoxic effects. The development of predictive methods to determine the degree of radiosensitivity of both tumor and healthy tissue has become of major interest in radiobiological research (*1*).

Several observations indicate that normal tissue hypersensitivity may be related to genetic factors (*2, 3*). Several studies have reported a possible correlation between genetic polymorphisms and adverse radiotherapy reactions in patients (*4–12*). Although most studies have not detected a conclusive correlation between genotype and clinical radiosensitivity, Price *et al.* have reported a highly significant association between clinical radiosensitivity and rare microsatellites (unusually large or unusually small alleles) in the DNA repair genes *XRCC1* and *XRCC3* (*13*).

Microsatellites are tandemly repeated highly polymorphic sequences and are common throughout the human genome. Repeat units are gained and lost by DNA replication slippage, a mutation mechanism that results from the transient dissociation of the replicating DNA strands followed by misaligned reassociation (*14, 15*). Expansions of triplet repeats are the underlying cause of several genetic diseases such as myotonic dystrophy, Huntington's disease and fragile X syndrome (*16–18*). Furthermore, microsatellites are the molecular targets for malfunctioning repair and replication proteins in diseases such as hereditary non-polyposis colorectal carcinoma (HNPCC), where there is a defect in mismatch repair, and Bloom's syndrome, where a DNA helicase homologue is defective (*19, 20*).

XRCC1 plays an important role in the base excision repair pathway (BER) and participates as a scaffolding intermediate by interacting with ligase III, DNA polymerase β and poly(ADP-ribose) polymerase (*21*). XRCC3 functions in the homologous recombination repair pathway (HHR) by repairing double-strand breaks. XRCC5 or Ku80 is involved in the non-homologous end-joining repair pathway (NHEJ), and encodes, together with the *G22P1 (KU70)* and

[1] Address for correspondence: Department of Anatomy, Embryology, Histology and Medical Physics, Proeftuinstraat 86, B-9000 Gent, Belgium; e-mail: kim.deruyck@UGent.be.

PRKDC genes, components of a DNA-dependent protein kinase (DNA-PK) (*22*).

In present study we investigated whether polymorphic microsatellites in three DNA repair genes, *XRCC1, XRCC3* and *XRCC5,* are associated with clinical radiosensitivity and cancer incidence. To this end we have screened for these microsatellites in patients with cervical or endometrial cancer who received radiotherapy treatment and in a control population of healthy individuals.

MATERIALS AND METHODS

Participants

The patient group has been described previously (*12*). Sixty-two women with cancer of the cervix (*n* = 30) or endometrium (*n* = 32) were treated with fractionated external-beam radiotherapy to the pelvis (one anterior and two lateral fields, 25 MV photons) followed by a brachytherapy boost at the Ghent University Hospital. Fifteen patients received a tumor dose of 45 Gy (25 × 1.8 Gy), 22 patients received a tumor dose of 46 Gy (23 × 2 Gy), and 22 patients received a tumor dose of 50 Gy (25 × 2 Gy). Three patients received 46 Gy supplemented with a parametrial boost up to 60 Gy. Except for two patients, all patients were additionally treated by brachytherapy using either vaginal ovoids, Fletcher-type applications, or perineal implants. All brachytherapy was performed using a pulsed dose-rate technique with iridium-192. Total doses from brachytherapy ranged from 15 to 35 Gy (dose rate 0.5 to 0.65 Gy/h). Eighteen patients with cancer of the cervix received combined radiochemotherapy and were treated with 40 mg/m^2 cisplatinum per week during the period of the external radiotherapy. Forty-six patients were operatively treated with a Wertheim Meigs hysterectomy. Nine premenopausal patients received hormone replacement therapy (estrogen). The mean age of the patients at the time of treatment was 59 years (range 24–80 years).

All patients have been scored with respect to several different normal tissue reactions by the same oncologist according to the Common Terminology Criteria for Adverse Events (CTCAE) scale version3.0 of the National Cancer Institute (*23*). Forty patients showed no or very light reactions to radiotherapy (CTC0–1), 14 patients experienced intermediate but distinct radiotherapy reactions (CTC2), six patients showed severe radiotherapy reactions (CTC3), one patient experienced life-threatening radiotherapy reactions (CTC4), and one patient died as a consequence of the radiotherapy (CTC5). Complication specifications are described in more detail by De Ruyck *et al.* (*12*). All normal-tissue reactions appeared 6 months up to 5.7 years after radiotherapy and can be considered as late reactions. The mean time of follow-up was 4.8 years (range 0.7–10.6 years). Patients classified in CTC0–1 are indicated as nonradiosensitive patients, while patients classified in CTC2, CTC3, CTC4 and CTC5 (CTC2+) are indicated as radiosensitive patients. For these two patient groups under study, age at time of treatment and follow-up period were very similar. Mean age at the end of the last radiation treatment for the patients without adverse reactions (CTC0–1) was 57 years and for the patients with adverse reactions (CTC2+) 62 years, while the mean follow-up time for the two groups was 5.0 and 4.5 years, respectively.

A Caucasian control population of 118 cancer-free individuals was used to determine the overall population microsatellite frequency and allows association analysis of microsatellite genotype with cancer incidence. The control individuals were employees of the Ghent University Hospital and were recruited during the annual occupational medical examination. The mean age of controls was 38 years (range 22–62 years). The patient and the control populations were ethnically matched. All individuals were Belgian. The mean age of the patients was higher in comparison with the healthy controls, 59 years and 38 years, respectively. However, there are no indications that microsatellite frequencies at the loci considered vary with age. The healthy control population consisted of 53 men and 65 women, while the patients are all women. This lack of sex matching should not cause a problem since the loci studied are located on autosomes. Moreover, χ^2 tests on the control population verified that there are no differences between microsatellite frequencies in men and women ($0.16 < P < 0.96$ for all repeats tested separately in the three *XRCC* genes).

A heparinized blood sample was taken from each individual in the study, and lymphocytes were isolated and frozen for genotyping analysis. The study was approved by the Ghent University Hospital Ethical Committee. All participants received oral and written information concerning the study and signed the informed consent form.

Genotyping Analysis

Genomic DNA was extracted from isolated lymphocytes, and DNA analysis was successful on all samples. A $[AC]_n$ microsatellite repeat region in the 3′ untranslated region (3′ UTR) of *XRCC1* (accession number L34079), a $[AC]_n$ microsatellite repeat located in intron 3 of *XRCC3* (accession number AF000735), and a $[GAPyA]_n$ repeat located 120kb 5′ of *XRCC5* (accession number AF000736) were analyzed. The repeat regions were amplified by PCR, and sizes were analyzed using an ABI Prism 310 Genetic Analyser (PE Applied Biosystems). The *XRCC1* 3′ UTR microsatellite tandem $[AC]_n$ repeat region was amplified using the following primers (MWG Biotech): XRCC1F 5′-CCC GAT GGA TCT ACA GTT GC-3′ and XRCC1R 5′-CCC AGG GAG CCT CTT AGA GT-3′. The forward primer was labeled with the fluorophore FAM-6. The intron tandem $[AC]_n$ repeat region in *XRCC3* was amplified with XRCC3F 5′-GAC AAT ATG CAT GTA TTA CTT TG-3′ and XRCC3R 5′-GTG TGC AGT TTA TAT AAG GCA GG-3′. The *XRCC5* $[GAPyA]_n$ repeat region was amplified using XRCC5F 5′-TGT TGC TAT TGT TGT CTA GC-3′ and XRCC5R 5′-AAG TCA CTC ACA TGT AAT CC-3′. Both XRCC3R and XRCC5R were labeled with the fluorophore TET. Multiplex PCR was undertaken in 12.5-μl volumes on an ABI9700 thermal cycler with conditions of 95°C for 15 min followed by 25 cycles of 94°C for 30 s, 57°C for 90 s and 72°C for 60 s, with a final 60°C hold for 30 min. PCR was undertaken using a multiplex PCR mix (Qiagen). Each reaction contained 1× Qiagen Multiplex PCR mix, 0.2 μ*M* of each primer, and 0.5 μl template DNA. After PCR, fragment analysis was undertaken on an ABI Prism 310 Genetic Analyser. One microliter of PCR sample was mixed with 12 μl of deionized formamide and 0.5 μl of Genescan-500 TAMRA size standard (Applied Biosystems) and denatured for 3 min at 94°C. Capillary electrophoresis used POP-4 polymer with a 5-s injection time and 27 min electrophoresis at 60°C. Microsatellite allele sizes were converted to repeat lengths based on allele size as described by Price *et al.* (*13*). All genotyping was performed in duplicate.

Statistical Analysis

Statistical analysis was performed by MedCalc 4.0. Allele frequencies of the different patient groups and the control population were determined and displayed graphically. Heterozygosities were calculated by dividing the number of heterozygotes by the total number of individuals. Odds ratios (ORs) with 95% confidence intervals (95% CI) were calculated for each microsatellite repeat length to evaluate the association of *XRCC1, XRCC3* and *XRCC5* microsatellite genotypes with both clinical radiosensitivity and cancer incidence. Corresponding *P* values were obtained using the χ^2 test. The reference genotype was a pooled sample of individuals with all repeat numbers, except the one examined. For clinical radiosensitivity, genotypes were compared between radiosensitive patients (CTC2+) and nonradiosensitive patients (CTC0–1). For cancer incidence, genotypes were compared between patients (total population) and control individuals. Impact of the different external radiotherapy doses, brachytherapy doses and total doses was evaluated with the Mann-Whitney test. Influence of chemotherapy, surgery and hormone therapy in the patient population and influence of gender in the control population was tested using the χ^2 test.

TABLE 1
Overview of Cancer Type, Treatment Protocols and Clinical Radiosensitivity according to the CTCAE Scale

Patient no.	Cancer type	Hormone therapy	External radio-therapy dose (Gy)	Brachy-therapy dose (Gy)	Clinical radio-sensitivity CTC	Patient no.	Cancer type	Hormone therapy	External radio-therapy dose (Gy)	Brachy-therapy dose (Gy)	Clinical radio-sensitivity CTC
1	Cervix	yes	45	15	0	32	Cervix	no	46	35	3
2	Cervix	yes	45	30	0	33	Cervix	yes	46	19	0
3	Cervix	no	45	20	0	34	Endometrium	no	46	19	0
4	Cervix	yes	45	19	0	35	Endometrium	no	46	19	0
5	Cervix	no	45	20	0	36	Endometrium	no	46	24	2
6	Cervix	no	45	30	0	37	Endometrium	no	46	19	2
7	Endometrium	no	45	30	0	38	Endometrium	no	50	/	5
8	Cervix	no	45	20	0	39	Endometrium	no	50	15	2
9	Cervix	no	45	20	0	40	Cervix	yes	50	19	4
10	Endometrium	no	45	27	0	41	Endometrium	no	50	15	2
11	Endometrium	no	45	22	0	42	Endometrium	no	50	15	1
12	Cervix	no	45	15	2	43	Endometrium	no	50	15	0
13	Cervix	no	45	25	0	44	Endometrium	no	50	15	0
14	Endometrium	no	45	20	0	45	Cervix	no	50	19	0
15	Cervix	no	45	35	2	46	Cervix	no	50	15	0
16	Cervix	no	46	30	3	47	Endometrium	no	50	15	2
17	Cervix	no	46	19	0	48	Endometrium	no	50	15	2
18	Cervix	no	46	15	3	49	Cervix	no	50	15	2
19	Cervix	no	46	34	3	50	Endometrium	no	50	/	0
20	Endometrium	no	46	19	2	51	Endometrium	no	50	30	1
21	Cervix	no	46	24	0	52	Endometrium	no	50	15	2
22	Endometrium	no	46	34	3	53	Cervix	yes	50	14	0
23	Cervix	yes	46	30	0	54	Endometrium	no	50	19	0
24	Cervix	yes	46	20	0	55	Endometrium	no	50	15	0
25	Endometrium	no	46	19	0	56	Endometrium	no	50	15	0
26	Endometrium	no	46	19	1	57	Endometrium	no	50	15	2
27	Cervix	no	46	34	0	58	Endometrium	no	50	20	2
28	Endometrium	no	46	19	0	59	Endometrium	no	50	15	0
29	Endometrium	no	46	19	0	60	Cervix	no	60	19	0
30	Cervix	no	46	19	3	61	Cervix	no	60	20	2
31	Endometrium	yes	46	19	0	62	Cervix	no	60	15	0

RESULTS

An overview of the radiotherapy treatment protocols and the radiotherapy reactions is given in Table 1. To investigate a possible bias of the treatment protocols, the delivered radiation doses of both the CTC2+ and the CTC3+ groups were compared with the CTC0–1 patient group using a two-tailed Mann-Whitney test. For the CTC0–1 and CTC2+ groups, no significant difference could be shown with respect to the external radiotherapy dose ($P = 0.13$), the dose delivered by brachytherapy ($P = 0.74$), and the summation of both doses ($P = 0.72$). The comparison of the CTC0–1 and CTC3+ groups resulted in nonsignificant P values of 0.58, 0.10 and 0.28, respectively. The impact of the chemotherapy treatment, surgery and hormone therapy on the clinical radiosensitivity in the population was evaluated by χ^2 analysis. Therefore, the patient population was sorted according to the treatment and the CTC grading. This analysis showed no significant differences in CTC classification between patients with or without chemotherapy ($P = 0.27$), with or without surgery ($P = 0.91$), and with or without hormone therapy ($P = 0.20$).

Figures 1, 2 and 3 show the allele frequencies of the different microsatellite repeats in *XRCC1*, *XRCC3* and *XRCC5*, respectively, for healthy controls, all cancer patients, nonradiosensitive cancer patients (CTC0–1), and radiosensitive cancer patients (CTC2+). The data on the association between the number of microsatellite repeats in *XRCC1, XRCC3* and *XRCC5* and clinical radiosensitivity are given in Table 2, while the data on the association between the microsatellites and cancer incidence are given in Table 3.

The *XRCC1* $[AC]_n$ microsatellite was highly polymorphic, with between 11 and 22 repeat units and an observed heterozygosity of 0.74. The most frequently occurring alleles were in the range $[AC]_{17}$ to $[AC]_{19}$. Allele frequencies for these repeats were similar in all groups considered. The smallest observed allele size ($[AC]_{11}$) had a frequency of 0.047 in healthy controls, 0.073 in all patients, 0.050 in nonradiosensitive patients, and 0.114 in radiosensitive patients. Patients with one $[AC]_{11}$ repeat had a 2.65 times higher risk of developing adverse radiotherapy reactions. This result, however, is not statistically significant ($P = 0.325$). With this obtained OR of 2.65, a sample size of 160 individuals is needed to reach statistical significance. Allele
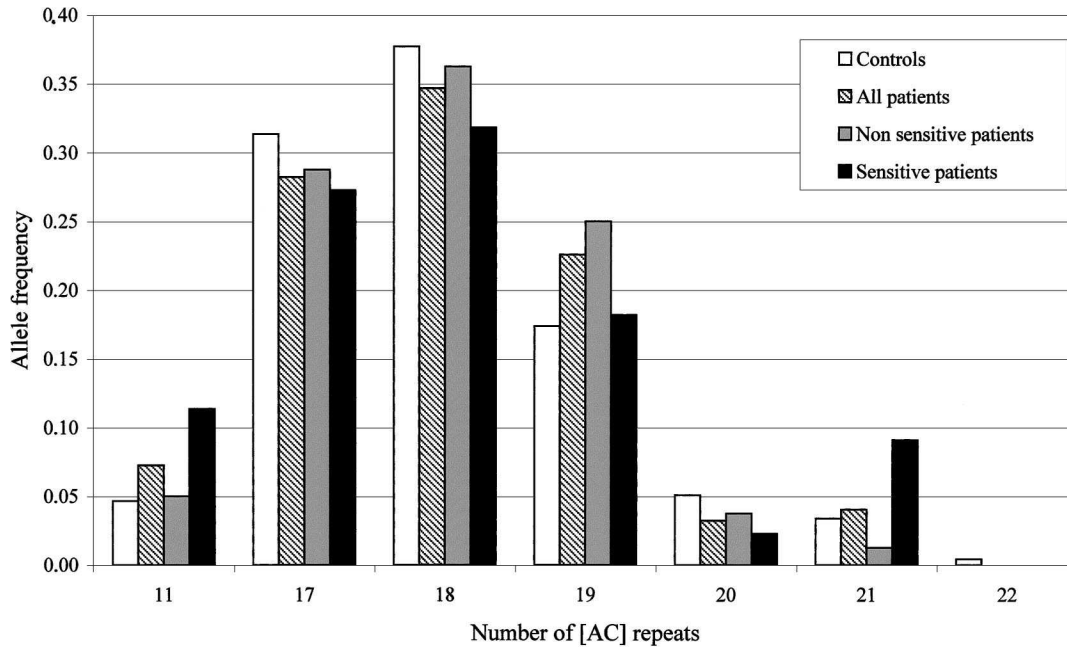
FIG. 1. Allele frequencies of the [AC]$_n$ microsatellite in the 3' untranslated region of *XRCC1*. Number of healthy controls, 118; number of nonradiosensitive cancer patients, 40; number of clinically radiosensitive cancer patients, 22.

frequencies for [AC]$_{21}$ repeats were 0.034, 0.040, 0.013 and 0.091 for healthy controls, all cancer patients, nonradiosensitive cancer patients, and radiosensitive cancer patients, respectively. Four patients with one [AC]$_{21}$ repeat were found among the 22 clinically radiosensitive patients, while only one [AC]$_{21}$ heterozygote was found in the 40 nonradiosensitive patients (OR = 8.67, *P* = 0.093). With this very high OR of 8.67, an increase of the sample size to 80 individuals would be needed to reach statistical signifi-

cance. None of the patients were homozygous for [AC]$_{11}$ and [AC]$_{21}$ microsatellite copy numbers; thus we were unable to determine whether these alleles act in a recessive fashion. Comparison of cancer incidence with the presence of any number of microsatellite repeats in *XRCC1* did not show any distinct association (Table 3).

The *XRCC3* [AC]$_n$ microsatellite exhibited between 9 and 21 repeat units. The observed heterozygosity for this repeat was 0.63. The most frequently occurring alleles were
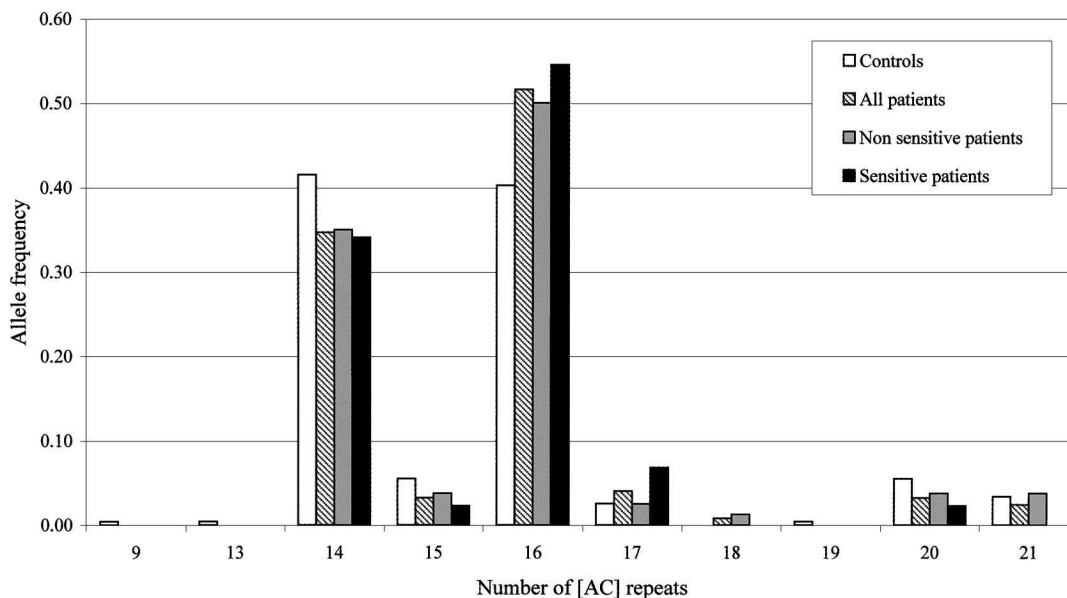

FIG. 2. Allele frequencies of the [AC]$_n$ microsatellite in intron 3 of the *XRCC3* gene. Number of healthy controls, 118; number of nonradiosensitive cancer patients, 40; number of clinically radiosensitive cancer patients, 22.
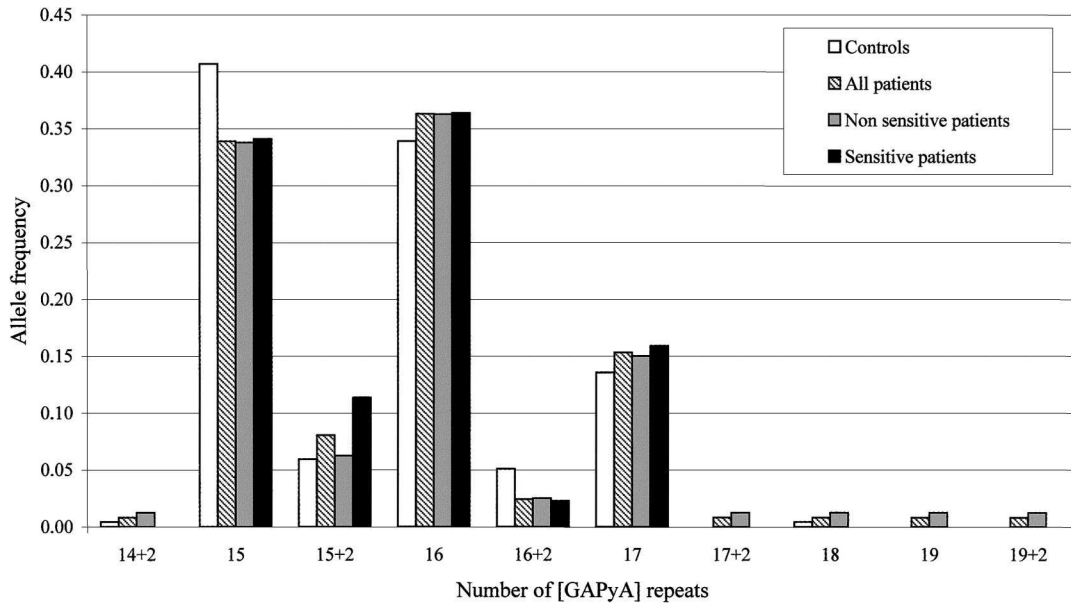
FIG. 3. Allele frequencies of the $[GAPyA]_n$ microsatellite 120 kb from the *XRCC5* gene. A small proportion of the alleles were extended by two nucleotides, resulting in $[GAPyA]_{14+2}$, $[GAPyA]_{15+2}$, $[GAPyA]_{16+2}$, $[GAPyA]_{17+2}$ and $[GAPyA]_{19+2}$ repeats. Number of healthy controls, 118; number of nonradiosensitive cancer patients, 40; number of clinically radiosensitive cancer patients, 22.

$[AC]_{14}$ and $[AC]_{16}$. The largest allele, $[AC]_{21}$, was found at a frequency of 0.034 in controls, 0.024 in all cancer patients, 0.038 in nonradiosensitive cancer patients, and 0 in clinically radiosensitive cancer patients. Accordingly, no measure of association with radiosensitivity could be undertaken. Patients carrying one $[AC]_{17}$ repeat had a three times higher risk of developing normal-tissue reactions after radiotherapy in comparison with patients having any other number of repeats, although this is not statistically significant ($P = 0.479$). None of the patients were homozygous for the $[AC]_{17}$ repeat (Table 2). A borderline significant positive association was found between the pres-

TABLE 2
Association between Microsatellite Repeat Number in *XRCC1*, *XRCC3* and *XRCC5* and Clinical Radiosensitivity

	Number of repeats					
XRCC1[a]	11	17	18	19	20	21
Nonradiosensitive patients	4 (4/0)	18 (13/5)	20 (11/9)	17 (14/3)	3 (3/0)	1 (1/0)
Radiosensitive patients	5 (5/0)	11 (10/1)	12 (10/2)	8 (8/0)	1 (1/0)	4 (4/0)
Odds ratio	2.65	1.22	1.20	0.77	0.59	8.67
95% CI	0.63–11.13	0.43–3.47	0.42–3.41	0.26–2.26	0.06–6.01	0.90–83.17
XRCC3[b]	14	15	16	17	20	21
Nonradiosensitive patients	24 (20/4)	3 (3/0)	29 (18/11)	2 (2/0)	3 (3/0)	3 (3/0)
Radiosensitive patients	13 (11/2)	1 (1/0)	17 (10/7)	3 (3/0)	1 (1/0)	0
Odds ratio	0.96	0.59	1.29	3.00	0.59	—
95% CI	0.33–2.78	0.06–6.01	0.38–4.35	0.46–19.51	0.06–6.01	—
XRCC5[c]	14 + 2	15	15 + 2	16	16 + 2	17
Nonradiosensitive patients	1 (1/0)	22 (17/5)	4 (3/1)	24 (19/5)	1 (0/1)	12 (12/0)
Radiosensitive patients	0	12 (9/3)	4 (3/1)	12 (8/4)	1 (1/0)	6 (5/1)
Odds ratio	—	0.98	2.00	0.80	1.86	0.88
95% CI	—	0.35–2.79	0.45–8.94	0.28–2.29	0.11–31.22	0.28–2.78

Notes. The number of individuals carrying at least one allele is given (with number of heterozygous/homozygous individuals indicated in parentheses). Odds ratios, with 95% CI, for risk of radiosensitivity are shown for nonradiosensitive patients ($n = 40$) compared to radiosensitive patients ($n = 22$).

[a] Does not include $[AC]_{22}$ repeats for which only one individual was seen.

[b] Does not include $[AC]_9$, $[AC]_{13}$, $[AC]_{18}$, $[AC]_{19}$ repeats for which only one individual each was seen.

[c] Does not include $[GAPyA]_{17+2}$, $[GAPyA]_{19}$, $[GAPyA]_{19+2}$ for which only one individual each was seen and $[GAPyA]_{18}$ for which only two individuals were seen.

TABLE 3
Association between Microsatellite Repeat Number in *XRCC1*, *XRCC3* and *XRCC5* and Cancer Incidence

	Number of repeats					
XRCC1[a]	11	17	18	19	20	21
Controls	11 (11/0)	67 (60/7)	74 (59/15)	36 (31/5)	12 (12/0)	8 (8/0)
Patients	9 (9/0)	29 (23/6)	32 (21/11)	25 (22/3)	4 (4/0)	5 (5/0)
Odds ratio	1.65	0.67	0.63	1.54	0.61	1.21
95% CI	0.64–4.23	0.36–1.24	0.34–1.18	0.81–2.92	0.19–1.97	0.38–3.86
XRCC3[b]	14	15	16	17	20	21
Controls	76 (54/22)	11 (9/2)	77 (59/18)	5 (4/1)	13 (13/0)	8 (8/0)
Patients	37 (31/6)	4 (4/0)	46 (28/18)	5 (5/0)	4 (4/0)	3 (3/0)
Odds ratio	0.82	0.67	1.53	1.98	0.56	0.70
95% CI	0.43–1.54	0.20–2.20	0.77–3.03	0.55–7.13	0.17–1.79	0.18–2.74
XRCC5[c]	14 + 2	15	15 + 2	16	16 + 2	17
Controls	1 (1/0)	80 (64/16)	10 (6/4)	72 (64/8)	12 (12/0)	31 (30/1)
Patients	1 (1/0)	34 (26/8)	8 (6/2)	36 (27/9)	2 (1/1)	18 (17/1)
Odds ratio	1.92	0.58	1.60	0.88	0.29	1.17
95% CI	0.12–31.20	0.31–1.09	0.60–4.29	0.47–1.65	0.06–1.36	0.59–2.33

Notes. The number of individuals carrying at least one allele is given (with number of heterozygous/homozygous individuals indicated in parentheses). Odds ratios, with 95% CI, for risk of cancer are shown for healthy controls ($n = 118$) compared to cancer patients ($n = 62$).

[a] Does not include $[AC]_{22}$ repeats for which only one individual was seen.

[b] Does not include $[AC]_9$, $[AC]_{13}$, $[AC]_{18}$, $[AC]_{19}$ repeats for which only one individual each was seen.

[c] Does not include $[GAPyA]_{17 + 2}$, $[GAPyA]_{19}$, $[GAPyA]_{19 + 2}$ for which only one individual each was seen and $[GAPyA]_{18}$ for which only two individuals were seen.

ence of two $[AC]_{16}$ repeats and cancer incidence (OR = 2.56, $P = 0.055$). Other $[AC]$ repeats did not show any significant association with cancer incidence.

The *XRCC5* $[GAPyA]_n$ tetranucleotide microsatellite was polymorphic, with repeat units from 14 to 19. The observed heterozygosity for this repeat was 0.63. A small proportion of the alleles were extended by two nucleotides, resulting in $[GAPyA]_{14+2}$, $[GAPyA]_{15+2}$, $[GAPyA]_{16+2}$, $[GAPyA]_{17+2}$ and $[GAPyA]_{19+2}$ repeats. The most frequent alleles were $[GAPyA]_{15}$ and $[GAPyA]_{16}$. The rarest alleles were the smallest and the largest ($[GAPyA]_{14+2}$, $[GAPyA]_{17+2}$, $[GAPyA]_{18}$, $[GAPyA]_{19}$, $[GAPyA]_{19+2}$), representing together 0.8, 4, 6.3 and 0% in healthy controls, all cancer patients, nonradiosensitive cancer patients, and radiosensitive cancer patients, respectively (Fig. 3). Statistical analysis of clinical radiosensitivity or cancer incidence with the presence of any number of microsatellite repeats in *XRCC5* did not show any significant association.

DISCUSSION

This study investigated the association between polymorphic microsatellites in the DNA repair genes *XRCC1*, *XRCC3* and *XRCC5* and the risk of developing normal-tissue reactions after radiotherapy treatment. Therefore, we have screened for these microsatellites in patients with cancer of the cervix or endometrium who received radiotherapy. To determine the overall microsatellite frequency in a Belgian population and to assess the correlation of these microsatellites with gynecological tumors, a control population consisting of healthy individuals was also screened.

The microsatellites examined are located in different genomic contexts: The *XRCC1* microsatellite occurs in the 3′UTR of the gene, the *XRCC3* microsatellite is intronic, and the *XRCC5* microsatellite is located 120 kb from the gene. The microsatellites within *XRCC1* and *XRCC3* have previously been suggested to be associated with radiosensitivity and cancer incidence (13). The microsatellite in *XRCC5* was included based on the involvement of the *XRCC5* gene in the NHEJ repair pathway.

The overall microsatellite frequencies and distribution of repeat lengths in a healthy Belgian population were similar to those reported previously in studies of UK newborns and retired UK radiation workers (25, 26) and in an Australian twin study for *XRCC3* and *XRCC5* (27).

In 1997, Price *et al.* (13) reported a highly significant association between clinical radiosensitivity and rare microsatellites in *XRCC1* and *XRCC3* in a population of 19 cancer patients. In their study, rare microsatellites were alleles with less than 12 or more than 23 repeats for *XRCC1*, alleles with more than 20 repeats for *XRCC3*, and alleles with less than 14 or more than 18 repeats for *XRCC5*. All other microsatellite allele sizes were classified as common repeats. In this study we found a positive correlation between patients with $[AC]_{11}$ repeats and patients with $[AC]_{21}$ repeats in *XRCC1* and the risk of developing adverse radiotherapy reactions, but these results were not statistically significant for both repeat numbers ($P = 0.325$ and $P = 0.093$, respectively). Alleles with more than 23 $[AC]$ repeats were not present in the patient population or in the control population. Large *XRCC3* alleles ($[AC]_{20}$ and

[AC]$_{21}$ repeats) did not correlate with clinical radiosensitivity, while XRCC3 [AC]$_{17}$ repeats were slightly more common in patients with adverse radiotherapy reactions (P = 0.479). For XRCC5, no examples of rare ($\leq$14 or $\geq$18) repeat lengths were identified in the clinically radiosensitive patient group. Accordingly, no measure of association with radiosensitivity could be undertaken. For the other more common repeat lengths, we found no association with clinical radiosensitivity.

Furthermore, we could not demonstrate an association between the rare microsatellite alleles considered and cancer incidence. However, a weak correlation was found between XRCC3 [AC]$_{16}$ homozygotes and cancer incidence (P = 0.055). Subdividing the patient group into cervical and endometrial cancer cases, the positive association with the XRCC3 [AC]$_{16}$ repeat is retrieved only for endometrial cancer (P = 0.057).

Although we found no significant association between the rare microsatellite alleles considered and clinical radiosensitivity or cancer incidence, the microsatellites could affect radiosensitivity or cancer in a recessive manner. Because of the small numbers of individuals homozygous for the rare microsatellite repeats, we are unable to test this hypothesis. For other microsatellite repeat lengths where sufficient variant homozygotes were detected, we found (XRCC3 [AC]$_{16}$ excluded) no evidence for a recessive action of these polymorphisms. We have consequently assumed dominance and treated the data accordingly.

For this study, radiosensitivity classification of the patients is based on grading of the normal-tissue reactions to the radiotherapy according to the CTCAE scale (*23*). Patients with intermediate but distinct radiotherapy reactions (CTC2) were pooled with patients with severe (CTC3) to life-threatening (CTC4/5) radiotherapy reactions. To assess possible differences in genotypes between the more severe radiosensitive individuals (CTC3/4/5), the intermediate radiosensitive patients (CTC2) and the nonradiosensitive patients (CTC0–1), we performed analyses of CTC3/4/5 (n = 8) compared to CTC0–1 (n = 40) and CTC2 (n = 14) compared to CTC0–1 (n = 40) analysis. The outcome of these analyses showed similar associations with the initial analysis (results not shown). Furthermore, the supplemental analysis showed that the XRCC1 [AC]$_{11}$ repeat occurred 2.3 times more frequently in the CTC2 patient group than in the CTC3/4/5 patient group. On the other hand, the XRCC1 [AC]$_{21}$ repeat was present more often in the CTC3/4/5 patient group, resulting in an odds ratio of 13 compared the nonradiosensitive patient group (CTC0–1). Due to the low frequency of this microsatellite repeat and the small number of patients with very severe radiotherapy reactions, no statistical significance for this effect was reached (P = 0.110).

Although not statistically significant, higher odds ratios were obtained for XRCC1 [AC]$_{11}$ and XRCC1 [AC]$_{21}$ alleles in radiosensitive patients. Therefore, we are not able to completely reject the hypothesis that these small and large rare alleles may be associated with adverse radiotherapy outcome. The strongly positive associations found by Price *et al.* between XRCC1 [AC]$_{24}$ and XRCC3 [AC]$_{20}$ repeats and clinical radiosensitivity were not found in this study, nor could we show significant associations between rare microsatellite repeats and cancer incidence. These discrepancies could be due to the fact that the two studies are based on different cancer populations and studied different radiosensitivity end points. The fact that both studies could not demonstrate an association between the XRCC5 microsatellite, clinical radiosensitivity and cancer incidence could be explained by the distant location of the microsatellite considered from the gene.

Due to the highly polymorphic nature of the loci considered, in the future, larger studies with larger numbers radiosensitive cases in a multicenter setting are needed to clarify the involvement of rare polymorphic microsatellites in XRCC1 and XRCC3 DNA repair genes in either clinical radiosensitivity or cancer incidence.

REFERENCES

1. B. Emami, J. Lyman, A. Brown, L. Coia, M. Goitein, J. E. Munzenrider, B. Shank, L. J. Solin and M. Wesson, Tolerance of normal tissue to therapeutic irradiation. *Int. J. Radiat. Oncol. Biol. Phys.* **21**, 109–122 (1991).

2. N. G. Burnet and J. H. Peacock, Normal cellular radiosensitivity in an adult Fanconi anemia patient with marked clinical radiosensitivity. *Radiother. Oncol.* **62**, 350–351 (2002).

3. P. B. Rogers, P. N. Plowman, S. J. Harris and C. F. Arlett, Four radiation hypersensitivity cases and their implications for clinical radiotherapy. *Radiother. Oncol.* **57**, 143–154 (2000).

4. U. Oppitz, U. Bernthaler, D. Schindler, A. Sobeck, H. Hoehn, M. Platzer, A. Rosenthal and M. Flentje, Sequence analysis of the ATM gene in 20 patients with RTOG grade 3 or 4 acute and/or late tissue radiation side effects. *Int. J. Radiat. Oncol. Biol. Phys.* **44**, 981–988 (1999).

5. D. M. Severin, T. Leong, B. Cassidy, H. Elsaleh, L. Peters, D. Venter, M. Southey and M. McKay, Novel DNA sequence variants in the hHR21 DNA repair gene in radiosensitive cancer patients. *Int. J. Radiat. Oncol. Biol. Phys.* **50**, 1323–1331 (2001).

6. C. M. Ianuzzi, D. P. Atencio, S. Green, R. G. Stock and B. S. Rosenstein, ATM mutations in female breast cancer patients predict for an increase in radiation-induced late effects. *Int. J. Radiat. Oncol. Biol. Phys.* **52**, 606–613 (2002).

7. K. Borgman, B. Röper, R. Abd El-Awady, S. Brackrock, M. Bigalke, T. Dörk, W. Alberti, E. Dikomey and J. Dahm-Daphi, Indicators of late normal tissue response after radiotherapy for head and neck cancer: fibroblasts, lymphocytes, genetics, DNA repair, and chromosome aberrations. *Radiother. Oncol.* **64**, 141–152 (2002).

8. H. Green, G. Ross, J. Peacock, R. Owen, J. Yarnold and R. Houlston, Variation in manganese superoxide dismutase gene (SOD$_2$) is not a major cause of radiotherapy complications in breast cancer patients. *Radiother. Oncol.* **63**, 213–216 (2002).

9. C. N. Andreassen, J. Alsner, M. Overgaard and J. Overgaard, Prediction of normal tissue radiosensitivity from polymorphisms in candidate genes. *Radiother. Oncol.* **69**, 127–135 (2003).

10. S. Quarmby, H. Fakhoury, E. Levine, J. Barber, J. Wylie, A. H. Hajeer, C. West, A. Stewart, B. Magee and S. Kumar, Association of transforming growth factor beta-1 single nucleotide polymorphisms with radiation-induced damage to normal tissues in breast cancer patients. *Int. J. Radiat. Biol.* **79**, 137–143 (2003).

11. N. Moulan, D. G. Cox, S. Angèle, P. Romestaing, J. P. Gérard and J. Hall, Polymorphisms in the DNA repair gene XRCC1, breast cancer risk, and response to radiotherapy. *Cancer Epidemiol. Biom.* **12**, 1168–1174 (2003).

12. K. De Ruyck, M. Van Eijkeren, K. Claes, R. Morthier, A. De Paepe, A. Vral, L. De Ridder and H. Thierens, Radiation induced damage to normal tissues following radiotherapy in patients treated for gynecological tumors: association with single nucleotide polymorphisms in XRCC1, XRCC3 and OGG1 genes and *in vitro* chromosomal radiosensitivity in lymphocytes. *Int. J. Radiat Oncol. Biol. Phys.,* **62**, 1140–1149 (2005).

13. E. A. Price, S. L. Bourne, R. Radbourne, P. A. Lawton, J. Lamerdin, L. H. Thompson and J. E. Arrand, Rare microsatellite polymorphisms in the DNA repair genes XRCC1, XRCC3 and XRCC5 associated with cancer patients of varying radiosensitivity. *Somat. Cell. Mol. Genet.* **23**, 237–247 (1997).

14. C. Schlötterer, The evolution of molecular markers—just a matter of fashion? *Nat. Rev.* **5**, 63–69 (2004).

15. H. Ellegren, Microsatellites: Simple sequences with complex evolution. *Nat. Rev.* **5**, 435–445 (2004).

16. N. De Temmerman, K. Sermon, S. Seneca, M. De Rycke, P. Hilven, W. Lissens, A. Van Steirteghem and I. Liebaers, Intergenerational instability of the expanded CTG repeat in the DMPK gene: Studies in human gametes and preimplantation embryos. *Am. J. Hum. Genet.* **75**, 325–329 (2004).

17. M. E. MacDonald, S. Gines, J. F. Gusella and V. C. Wheeler, Huntington's disease. *Neuromol. Med.* **4**, 7–20 (2003).

18. J. L. Mandel and V. Biancalana, Fragile X mental retardation syndrome: From pathogenesis to diagnostic issues. *Growth Horm. IGF Res.* **14** (Suppl. A), 158–165 (2004).

19. G. S. Charames and B. Bapat, Genomic instability and cancer. *Curr. Mol. Med.* **3**, 589–596 (2003).

20. H. Kaneko, R. Inoue, Y. Yamada, K. Sukegawa, T. Fukao, H. Tashita, T. Teramoto, K. Kasahara, T. Takami and N. Kondo, Microsatellite instability in B-cell lymphoma originating from Bloom syndrome. *Int. J. Cancer* **69**, 480–483 (1996).

21. G. L. Dianov, K. M. Sleeth, I. I. Dianova and S. L. Allinson, Repair of abasic sites in DNA. *Mutat. Res.* **531**, 157–163 (2003).

22. J. Thacker and M. Z. Zdzienicka, The XRCC genes: Expanding roles in DNA double-strand break repair. *DNA Repair* **3**, 1081–1090 (2004).

23. *Common Terminology Criteria for Adverse Events v3.0 (CTCAE).* National Cancer Institute, Bethesda, MD, 2003. [available online at http://ctep.cancer.gov/reporting/ctc.html]

24. C. N. Andreassen, J. Alsner and J. Overgaard, Does variability in normal tissue reactions after radiotherapy have a genetic basis—where and how to look for it? *Radiother. Oncol.* **64**, 131–140 (2002).

25. C. L. Relton, C. P. Daniel, A. Fisher, D. S. Chase, J. Burn and E. J. Tawn, Polymorphisms of the DNA repair gene XRCC1 and the frequency of somatic mutations at the glycophorin A locus in newborns. *Mutat. Res.* **502**, 61–68 (2002).

26. C. S. Wilding, C. L. Relton, G. S. Rees, R. E. Tarone, C. A. Whitehouse and E. J. Tawn, DNA repair gene polymorphisms in relation to chromosome aberration frequencies in retired radiation workers. *Mutat. Res.* **570**, 137–145 (2005).

27. G. Zhu, D. L. Duffy, D. R. Turner, K. R. Ewen, G. W. Montgomery and N. G. Martin, Linkage and association analysis of radiation damage repair genes XRCC3 and XRCC5 with nevus density in adolescent twins. *Twin Res.* **6**, 315–321 (2003).