Genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes: role in mutagen sensitivity

Jarno Tuimala1, Gabor Szekely2, Sarolta Gundy2, Ari Hirvonen1 and Hannu Norppa1,3

1Laboratory of Molecular and Cellular Toxicology, Department of Industrial Hygiene and Toxicology, Finnish Institute of Occupational Health, FIN-00250 Helsinki, Finland and 2National Institute of Oncology, H-1122 Budapest, Hungary

3To whom correspondence should be addressed Email: hannu.norppa@ttl.fi

Mutagen sensitivity, measuring the extent of chromosome damage induced by an in vitro treatment of peripheral lymphocytes with bleomycin, has been associated with an increased risk of various human cancers. Sensitivity to bleomycin appears to have high heritability and is usually considered to reflect individual capacity to repair DNA lesions. Another potential contributor to variation in bleomycin sensitivity could be inherited differences in the metabolism of bleomycin. We assessed whether genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes (XMEs) could explain bleomycin sensitivity. Frequencies of bleomycin-induced chromatid breaks per cell (b/c) were determined for 80 healthy Caucasians. Genotypes of DNA repair genes XRCC (X-ray repair cross-complementing) 1 and 3 and XME genes bleomycin hydrolase (BLHX), glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) and N-acetyltransferase 2 (NAT2) were analyzed from leukocyte DNA using methods based on polymerase chain reaction. The mean number of chromatid b/c was increased in individuals with XRCC1 codon 280 variant allele (P = 0.002; two-sided Mann–Whitney test). Smokers carrying BLHX codon 1450 variant allele showed a decrease in the mean number of chromatid b/c (P = 0.036). In multiple linear regression models including adjustment for age, sex, smoking and genotype, the adjusted relative risks (and 95% confidence intervals) were 1.18 (0.98–1.41) and 0.84 (0.69–1.00) for carriers of XRCC1 codon 280 and BLHX codon 1450 variant alleles, respectively. XRCC1 codon 280 polymorphism had a significant effect (P = 0.012) in predetermined whether the individual was classified as non-sensitive, sensitive or hypersensitive to bleomycin. Although based on relatively few individuals, our results suggest that bleomycin sensitivity is partially explained by genetic polymorphisms affecting DNA repair (XRCC1) and in vitro metabolism of bleomycin (BLHX).

Introduction

Mutagen sensitivity assay, measuring chromatid-type breaks induced by an in vitro treatment of peripheral lymphocytes with the antitumor radiomimetic drug bleomycin, has been utilized as an indirect indicator of individual DNA repair capacity (1,2). Depending on the level of chromatid breaks induced by the bleomycin treatment, individuals can be classified as non-sensitive, sensitive or hypersensitive, which is thought to reflect their ability to repair DNA lesions induced by genotoxic environmental carcinogens. Accordingly, bleomycin-sensitive individuals have been observed to be over-represented among patients suffering from various cancers that are thought to have environmental origin, such as head and neck cancer (1), hepatocellular carcinoma (3), lung cancer (4) and upper aerodigestive tract cancers (5). The reasons for increased individual mutagen sensitivity are presently unknown. The high heritability (75%) of the bleomycin sensitivity trait indicates a strong genetic background (6). The lack of a clear bimodality (or trimodality) would suggest that multiple genes are involved—those affecting DNA repair being the most probable candidates. As bleomycin is metabolized in human cells, by enzymes also processing environmental carcinogens, genetic polymorphisms of xenobiotic-metabolizing enzymes (XMEs) could offer another explanation. The cancer risk predictivity of bleomycin sensitivity could thus reflect increased cancer risk associated with such polymorphisms. On the other hand, the metabolism of bleomycin during the in vitro assay is, per se, an important issue, as individual variations in the metabolic activation or detoxification of bleomycin in the cultured blood cells, due to genetic polymorphisms of XMEs, could affect assay outcome compromising the accuracy of cancer risk estimation.

Bleomycin induces oxidative base damage and strand breaks in DNA (7). Single base damage and strand breaks are mostly repaired by excision repair, whereas several mechanisms can be involved in the processing and sealing of double strand breaks (8). Recently, three non-conservative amino acid changes (Arg194Trp, Arg280His and Arg399Gln) were identified in the coding region of the XRCC1 (X-ray repair cross-complementing 1) gene and one (Thr241Met) in the XRCC3 (X-ray repair cross-complementing 3) gene (9). XRCC1 plays a central role in base excision repair, probably by mediating several protein–protein interactions, while XRCC3 is mainly involved in recombination repair and appears to be required for the maintenance of chromosome stability in mammalian cells (9,10). It is presently unclear whether the newly described polymorphisms of XRCC1 and XRCC3 influence DNA repair capacity.

A specific enzyme detoxifying bleomycin, bleomycin hydrolase, was recently put forward as a plausible candidate for associations with mutagen sensitivity (11). Bleomycin hydrolase, a neutral cysteine protease with exopeptidase activity that detoxifies bleomycin through deamination of the beta-aminoalanine moiety (12), has been found in most human tissues, including peripheral blood leukocytes (13). In fact, resistance to bleomycin chemotherapy, observed in some cancers, has been postulated to result from overactive bleomycin hydrolase (14).

Abbreviations: b/c, breaks per cell; BLHX, bleomycin hydrolase gene; CI, confidence interval; GSTM1, glutathione S-transferase M1; GSTT1, glutathione S-transferase T1; NAT, N-acetyltransferase; PCR, polymerase chain reaction; RR, adjusted relative risk; XME, xenobiotic-metabolizing enzyme; XRCC, X-ray repair cross-complementing.
A common genetic polymorphism was recently detected in the human bleomycin hydrolase gene (BLHX), affecting the C-terminal of the enzyme. An A126G transition in the coding region of the gene results in a valine to isoleucine substitution (15) and may impose changes in the structure of the enzyme affecting its catalytic activity by interrupting the essential C-terminal amino acid sequence (16). As the C-terminus is required for protection from bleomycin-induced chromosome breakage in Chinese hamster ovary cells (17), the amino acid substitution may be expected to affect the frequency of bleomycin-induced chromosomal breaks in human lymphocytes.

Besides bleomycin hydrolase, other XMEs may also be involved in the inactivation of bleomycin. In Chinese hamster ovary cells, hypersensitivity to bleomycin was suggested to be due to the lack of functional glutathione S-transferase (GST) alpha (18). Several polymorphic GSTs exist in humans. A deletion polymorphism, resulting in the total lack of enzyme activity in the homozygous null genotype, affects both GST M1 (GSTM1) and GST T1 (GSTM1) (19,20). Subjects with the GSTM1 and GSTT1 positive genotypes have at least one undeleted allele and show the respective enzyme activity, e.g., in their peripheral blood cells. In earlier studies, subjects with either GSTM1 or GSTT1 null genotype have shown an increased sensitivity to cytogenetic effects of various genotoxins (21–29).

Bleomycin-detoxification may also involve N-acetyltransferases (NATs). In Streptomyces verticillus, the bleomycin-producing actinomycete bacteria, resistance to bleomycin is conferred by a NAT enzyme (30,31). Transfection of mouse NIH/3T3 cells with the bleomycin acetyltransferase gene blmB resulted in a specific resistance to the bleomycin family of antibiotics (32). In humans, polymorphic NAT1 and NAT2 enzymes catalyze the addition of an acetyl group to a highly versatile selection of molecules (see ref. 33). The correlation between genotype and phenotype has been shown to be very good for NAT2 but less clear for NAT1 (34).

In the present study, we determined mutagen sensitivity for 80 healthy volunteers. The subjects were genotyped for the X RCC1, X RCC3, BLHX, GSTM1, GSTT1 and NAT2 genes, to assess the possible effect of genetic polymorphisms on bleomycin sensitivity. The level of bleomycin-induced chromosome damage was observed to be affected by XRCC1 and BLHX genotypes.

**Materials and methods**

**Subjects**

The study population included 80 healthy Hungarian volunteers (53 males and 27 females) without a history of cancer, other chronic diseases, therapeutic radiation or chemotherapy. The mean age of the subjects was 41.7 (range 21–64) years. Sixty-one percent of the individuals smoked >10 cigarettes daily. The rest were current non-smokers. The participants completed a short questionnaire about their previous medical history and lifestyle, and written informed consent was obtained from each subject.

**Mutagen sensitivity assay**

Whole-blood lymphocyte cultures were established from heparinized blood in three parallel tubes according to methods described earlier (1). Briefly, the culture medium contained 0.95 ml heparinized whole blood, 7 ml RPMI-1640 medium with 2 mM t-glutamine (Life Technologies,Gibco-BRL, Paisley, UK), 1 ml fetal calf serum (Sebak GmbH, Aidenbach, Germany), and 0.2% (v/v) of phytomenadione (Life Technologies). The cultures were incubated for 67 h at 37 °C before treatment with 30 µg/ml bleomycin (Nippon Kayaku Co., Tokyo, Japan) for 5 h and were harvested at 72 h. Non-treated 48 and 72 h control cultures were also prepared for all subjects, in order to establish the spontaneous frequency of chromosomal aberrations.

The frequency of bleomycin-induced chromatin breaks was assessed microscopically from Giemsa-stained slides essentially as described previously (1). Three microscopists performed the analysis, with a total of 100 cells being scored per sample. The slides were coded so that the scorers did not know the identity of the samples. Lesions wider than one chromatin fragment were considered as chromatid breaks. Smaller lesions were regarded as gaps and were not included among the breaks. Chromatid exchanges were considered as two breaks. A metaphase with >12 breaks was counted to contain 12 breaks. The frequency of bleomycin-induced chromatid breaks per cell (b/c) was used in the statistical analyses.

**Genotype analyses**

DNA was isolated from frozen peripheral blood samples collected into EDTA tubes (Becton Dickinson Vacutainer, Plymouth, UK). Briefly, the buffy coat containing the white blood cells was isolated, cells were lysed by proteinase-K digestion. DNA was extracted by phenol-chloroform, precipitated by absolute ethanol and finally dissolved in distilled water. The DNA (100–400 ng) was used as a template in the polymerase chain reaction (PCR)-based genotyping analyses.

**XRCC1 and XRCC3 genotypes** were determined by a multiplex PCR restriction fragment length polymorphism (RFLP) technique. Codons 194 and 399 polymorphism of XRCC1 were assessed as described previously (35) with a slight modification in 5'-GCC CCG TCC CAG GTA AGC-3' 194F primer in which three bases were added in the 3' end to obtain uniform annealing temperatures in the multiplex PCR reaction.

For the genotype analysis of codon 280 of XRCC1 and codon 241 of XRCC3, a multiplex PCR method was constructed. Primer pairs, used to amplify 280 and 335 bp fragments of XRCC1 and XRCC3, respectively, were as follows: XRCC1-280F GTC GGC CTC GAT TGC TGG GTG GC and XRCC1-280R CAG CAC CAC TAC CAC ACC CTG AAG G, and XRCC3-241F GCT GGC CTG GTG GTG ATC GAC TGC TG and XRCC3-241R AAG AGC ACA GTC CAG GTG AGC TG. PCR was performed in a total volume of 30 µl consisting of 100–400 ng of genomic DNA, 1.67 µM MgCl2, 200 mM each dNTPs, 1 U Taq polymerase (Promega, Madison, WI) and 10 pmol of each primer in 1× PCR buffer (Promega).

After PCR a 10 µl aliquot of the amplification product was digested by 5 U Rsul (New England Biolabs, Beverly, MA) and NfIII (New England Biolabs) restriction enzymes for 4 h at 37 °C. The digestion products were resolved in 3% agarose. A G280A substitution in XRCC1 abolishes Rsul cleavage site, and a C241T substitution in XRCC3 creates NfIII restriction site. Consequently, a 140 bp digestion product revealed the XRCC1 G allele and a 280 bp product the A allele, whereas fragments of 335 and 233 bp revealed the XRCC3 C and T alleles, respectively. In addition, a 102 bp NfIII restriction product was seen in all samples.

The GSTM1, GSTT1 and NAT2 genotyping analyses were performed in a total volume of 30 µl as described earlier (36–38). Briefly, in the GSTM1 and GSTT1 genotyping gene-specific primer pairs were used together with a third pair for β-globin in a multiplex PCR reaction. The absence of the GSTM1- or GSTT1-specific PCR product indicated the corresponding null genotypes, whereas presence of β-globin-specific fragment confirmed the proper functioning of the reaction. In the NAT2 genotyping, the NAT2*4, *5, *6 and *7 alleles were differentiated by a PCR-based method as described earlier (38). A combination of two of any of these variant alleles revealed the "slow acetylator" genotypes. Combinations involving the wild-type allele (*4) were regarded as 'rapid acetylator' genotypes.

A novel method based on PCR and RFLP was developed for the determination of BLHX genotype for the nucleotide substitution (A126G). Together with the mismatch incorporated in the forward primer, this substitution creates a MspI digestion site. The forward 5'-GCT GTT TTA GAG CAG GAA CCC AAT T-3' and reverse 5'-CCT GGA TCT GTC CTT TGC AGC TAC G-3' primers were used to amplify a DNA fragment of 130 bp under the PCR conditions consisting of 6 pmol of primers (Institute of Biotechnology, Helsinki, Finland), 1×PCR buffer (Promega), 200 µM dNTPs (Promega), 3 mM MgCl2 (Promega) and 1 U Taq polymerase (Promega), with the following program: denaturing 94 °C 30 s, annealing 58 °C 30 s and elongation 72 °C 15 s, for 35 cycles. After the digestion and electrophoresis on 3% agarose gel, the BLHX A/A genotype was identified by a 130 bp fragment, the A/G genotype by 130, 106 and 24 bp fragments, and the G/G genotype by 106 and 24 bp fragments. The validity of the method was confirmed by sequencing.

**Statistical analysis**

The main task of the statistical analysis was to assess whether subjects with different genotypes differed from each other with respect to b/c. For XRCC1, XRCC3 and BLHX, the homozygous and heterozygous variant alleles were combined in the statistical analyses, due to the low number of variant homozygotes. The Mann–Whitney test was used for the comparison of different genotype groups for b/c frequencies and for differences in distribution of subjects among the three categories of bleomycin sensitivity.
Adjusted relative risks (RRs) and 95% confidence intervals (CIs) for different enzyme polymorphism, smoking, age and sex were calculated as described earlier (39,40), by multiple linear regression models. The b/c frequencies were mutually adjusted for the other variables in the models. Age was used as a continuous variable. SPSS 9.0 for Windows (SPSS, Chicago, IL) and StatXact4 (Cytel Software, Cambridge, MA) were used for the statistical analyses. All tests were two-sided.

The b/c values observed after treatment with bleomycin were 10–100 times higher than those observed in the untreated control cultures (data not shown). As the difference between the treated and untreated cultures was this large, there was no need to subtract the baseline level from the induced values, which were used as such in further processing of the data.

The mean b/c frequency deviated from normality (skewness, 0.66; kurtosis, 0.99; Kolmogorov–Smirnov, 0.11; P = 0.02) because of one influential observation (b/c = 1.90). After removal of that individual, the b/c frequency was normalized (skewness, 0.29; kurtosis, −0.15; Kolmogorov–Smirnov 0.09; P = 0.08). The final multiple regression models with and without the influential observation were compared and found not to differ materially. Therefore, we present results of all 80 individuals.

### Results

The distribution of individual mean frequencies of b/c after treatment of cultured lymphocytes with bleomycin is shown in Figure 1A. The individual b/c values ranged from 0.27 to 1.90, with a mean of 0.84. The mean frequencies of b/c after the bleomycin treatment, according to the genotype of DNA repair genes and XME genes, are shown in Tables I and II; the allele frequencies did not markedly deviate from those reported earlier for Caucasian populations (35,37,41).

As shown in Table I, carriers of XRCC1 codon 280 variant allele displayed a 28% higher mean b/c frequency than wild-type homozygotes (1.01 versus 0.81). This difference was statistically significant (P = 0.002; two-sided Mann–Whitney test). Adjustment for smoking, age, sex and other genotypes in the multiple linear regression model weakened but did not remove this effect (RR = 1.18, 95% CI = 0.98–1.41).

Conversely, individuals with the XRCC1 codon 399 variant allele had a 10% lower mean b/c frequency than those with genotypes not including the variant allele, but this difference was not statistically significant. It may not either be biologically meaningful, as the eight XRCC1 codon 399 Gln/Gln homozygotes had a mean b/c that did not differ much from that of Arg/Arg homozygotes (Table I). No influence on the b/c frequency was observed for XRCC1 codon 194 or XRCC3 codon 241 polymorphisms.

Subjects with the BLHX A/A genotype displayed higher mean levels of bleomycin-induced chromatid breaks than the A/G heterozygotes and G/G homozygotes combined (0.89 versus 0.80; P = 0.061; two-sided Mann–Whitney test). When the individuals were further stratified according to smoking and BLHX genotype (Table II), the BLHX genotype effect appeared to concern smokers (mean 0.91 and 0.78; P = 0.036; two-sided Mann–Whitney test) while no effect was seen for non-smokers (0.84 and 0.85). The lower b/c frequency of the variant allele carriers among smokers was also evident in the multiple linear regression model, after adjustment for smoking, age, sex and other genotypes (RR = 0.84, 95% CI = 0.69–1.00; Table II). Other XME genotypes did not have clear effects on the frequency of bleomycin-induced chromosomal breakage.

When cut-off points of 0.8 (50% percentile) and 1.0 (75% percentile) b/c were considered for the sensitive and hypersensitive (1), respectively, the XRCC1 codon 280 polymorphism had a statistically significant effect (P = 0.012, two-sided Mann–Whitney test) in predetermining individual bleomycin sensitivity status, the carriers of the variant allele (Arg/His or His/His) showing higher sensitivity ranking (Figure 1B). A shift to the right was also seen in the b/c distribution of BLHX wild-type homozygous (A/A) smokers (black columns); White columns in (B) and (C) represent the rest of the subjects. Hatched lines show the 50th percentile and 75th percentile cutting points for classifying individuals as non-sensitive, sensitive and hypersensitive to bleomycin (1).
Table I. Mean number of chromatid b/c in cultured lymphocytes treated with bleomycin and adjusted RR for XRCC1 and XRCC3 genotypes

<table>
<thead>
<tr>
<th>Classification</th>
<th>No. of subjects</th>
<th>Mean b/c (SD)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRCC1 codon 194</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>72</td>
<td>0.84 (0.29)</td>
<td>1.00</td>
</tr>
<tr>
<td>Arg/Trip</td>
<td>8</td>
<td>0.84 (0.31)</td>
<td>1.00 (0.74–1.19)</td>
</tr>
<tr>
<td>XRCC1 codon 280</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>69</td>
<td>0.81 (0.25)</td>
<td>1.00</td>
</tr>
<tr>
<td>Arg/His or His/Hisb</td>
<td>11</td>
<td>1.01 (0.31)</td>
<td>1.18 (0.98–1.41)</td>
</tr>
<tr>
<td>XRCC1 codon 399</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>33</td>
<td>0.89 (0.31)</td>
<td>1.00</td>
</tr>
<tr>
<td>Arg/Gln or Gln/Glna</td>
<td>47</td>
<td>0.80 (0.30)</td>
<td>0.90 (0.77–1.04)</td>
</tr>
<tr>
<td>XRCC3 codon 241</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>23</td>
<td>0.80 (0.27)</td>
<td>1.00</td>
</tr>
<tr>
<td>Thr/Met</td>
<td>43</td>
<td>0.86 (0.31)</td>
<td>1.04 (0.80–1.11)</td>
</tr>
<tr>
<td>Met/Met</td>
<td>14</td>
<td>0.86 (0.35)</td>
<td>1.08 (0.87–1.34)</td>
</tr>
</tbody>
</table>

aAccording to linear regression models including the variables shown, smoking, age (continuous variable), sex and BLHX genotype.
bIncludes one His/His subject.

P = 0.002, in comparison with Arg/His and His/His subjects combined; two-sided Mann–Whitney test.

Includes eight Gln/Gln subjects (mean b/c 0.87, SD 0.52) and 39 Arg/Gln subjects (mean b/c 0.80, SD 0.06).

Table II. Mean number of bleomycin-induced chromatid breaks per cell (b/c) and adjusted RR for sex, bleomycin hydrodase (BLHX) genotype (stratified for smoking) and glutathione S-transferase M1 (GSTM1), glutathione S-transferase T1 (GSTT1) and N-acetyltransferase 2 (NAT2) genotypes

<table>
<thead>
<tr>
<th>Classification</th>
<th>No. of subjects</th>
<th>Mean b/c (SD)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>53</td>
<td>0.83 (0.27)</td>
<td>1.00</td>
</tr>
<tr>
<td>Women</td>
<td>27</td>
<td>0.87 (0.37)</td>
<td>1.05 (0.90–1.22)</td>
</tr>
<tr>
<td>Smoking and BLHX</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>31</td>
<td>0.85 (0.33)</td>
<td>1.00</td>
</tr>
<tr>
<td>A/A</td>
<td>11</td>
<td>0.84 (0.33)</td>
<td>1.00</td>
</tr>
<tr>
<td>A/G + G/Gc</td>
<td>20</td>
<td>0.85 (0.35)</td>
<td>1.09 (0.83–1.44)</td>
</tr>
<tr>
<td>Smokers</td>
<td>49</td>
<td>0.84 (0.29)</td>
<td>1.00</td>
</tr>
<tr>
<td>A/A</td>
<td>24</td>
<td>0.91 (0.28)</td>
<td>1.00</td>
</tr>
<tr>
<td>A/G + G/Gd</td>
<td>25</td>
<td>0.78 (0.28)</td>
<td>0.84 (0.69–1.00)</td>
</tr>
<tr>
<td>GSTM1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>36</td>
<td>0.82 (0.29)</td>
<td>1.00</td>
</tr>
<tr>
<td>Positive</td>
<td>44</td>
<td>0.86 (0.32)</td>
<td>1.04 (0.90–1.13)</td>
</tr>
<tr>
<td>GSTT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>11</td>
<td>0.81 (0.26)</td>
<td>1.00</td>
</tr>
<tr>
<td>Positive</td>
<td>69</td>
<td>0.85 (0.31)</td>
<td>1.01 (0.81–1.25)</td>
</tr>
<tr>
<td>NAT2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast</td>
<td>35</td>
<td>0.82 (0.30)</td>
<td>1.00</td>
</tr>
<tr>
<td>Slow</td>
<td>45</td>
<td>0.86 (0.31)</td>
<td>1.04 (0.89–1.21)</td>
</tr>
</tbody>
</table>

aAccording to linear regression models including the variables shown, age (continuous variable) and XRCC1 genotype.
bIncludes two G/G subjects.

P = 0.036, in comparison with A/G and G/G subjects combined; two-sided Mann–Whitney test.

Includes four G/G subjects.

sensitive individuals. When the cut-off points of 0.8 and 1.0 b/c, as used by Hsu et al. (1), were applied for the distinction of the three classes, the XRCC1 codon 280 polymorphism had a significant role in determining the sensitivity category of the healthy individuals. Therefore, the XRCC1 codon 280 genotype could influence the outcome of case-control studies by affecting subject classification in the mutagen sensitivity assay. The BLHX genotype, which appeared to have a smaller effect on b/c levels than XRCC1 codon 280 genotype, may also be expected to have such an influence, although its impact on this classification was not statistically significant in the present study.

The XRCC1 codons 194 and 280 polymorphisms are located in the vicinity of two sequences mediating the protein–protein interactions with poly(ADP-ribose) polymerase (PARP) and polymerase beta (42). The codon 399 polymorphism is in the domain mediating the interaction between XRCC1 and PARP. Thus, the variant alleles may encode a twisted XRCC1 protein, resulting in increased or decreased affinity to other proteins and to altered repair capacity. Our findings may indicate that XRCC1 codon 280 variant allele is associated with a reduced capacity to repair DNA lesions (DNA single or double strand breaks or oxidative base damage) produced by bleomycin. Alternatively, it may be that bleomycin-induced damage in DNA is not readily repairable by base excision repair. In this case, the codon 280 variant allele might alter the affinity of the XRCC1 protein, facilitating better binding to oxidative base damage, and thereby possibly partly hindering the normal repair of bleomycin-induced DNA damage.

Thus far, the possible associations of XRCC1 or XRCC3 polymorphisms with cancer have not been widely studied. The XRCC1 codon 280 variant allele was associated with an increased risk of lung cancer (43), which agrees with our finding that the variant confers increased mutagen sensitivity. The XRCC1 codon 399 variant allele appeared to be a risk factor in head and neck cancer (44), adenocarcinoma of the lung (45) and (in African-Americans but not Caucasians) breast cancer (46), but protective in bladder cancer, where also a non-significant interaction with smoking was observed (47); one investigation suggested no influence of codon 399 polymorphism on lung cancer risk (48). In our study, no significant effect of the codon 399 variant allele on mutagen sensitivity could be demonstrated.

Recently, the XRCC1 codon 399 variant allele was reported to be associated with increased DNA adducts in leukocytes and placenta, glycoporphin A variant erythrocytes and sister chromatid exchanges (SCEs) in lymphocytes of smokers, and with elevated SCE response to an in vitro treatment of human lymphocytes with the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (35,40,49,50). The present study did not, however, indicate any interaction between smoking and the XRCC1 codon 399 genotype in determining sensitivity to bleomycin (data not shown). The XRCC3 codon 241 variant allele was associated with an increased level of DNA adducts in leukocytes of healthy adults (49).

The interpretation of the association of induced breaks and BLHX genotype is complicated by the fact that the phenotypic consequences of BLHX genotype are inadequately known. In our study, the wild-type homozygotes (A/A) were more sensitive to bleomycin than heterozygotes (A/G) or homozygotes (G/G) for the variant allele. The increased sensitivity was exclusively seen in smokers, which could reflect increased metabolic activation or reduced detoxification of bleomycin in smokers. However, smoking or BLHX genotype alone did not result in an increased bleomycin-induced chromosomal breakage. Thus, smoking might induce bleomycin hydrodase activity in lymphocytes, so that differences in bleomycin hydroxylation capacity between the genotypes become visible, or the genotypes might differ in their response to such enzyme induction.
Could the cancer risk predictivity of bleomycin sensitivity, observed in various studies (1,2–5), partly reflect cancer risk associated with BLHX genotype itself? The possible association of BLHX genotype with cancer risk cannot be ruled out, as no data exist on this topic. Even if the BLHX genotype explained none of the cancer risk predictivity of mutagen sensitivity, it could be a source of in vitro variation. If this were true, controlling for BLHX genotype would be expected to improve the use of mutagen sensitivity as an indicator of cancer risk.

At any rate, further studies are required to better understand the possible influence of polymorphisms of DNA repair and xenobiotic metabolism on individual bleomycin sensitivity. Overall, the genotype effects we observed were modest, as expected for single polymorphisms on the basis of the b/c distribution, which shows no clear bi- or trimodality. Although bleomycin sensitivity seems to have substantial heritability (6), one should not forget the possible influence of methodological issues, secondary effects of cancer, and, e.g., serum folate (7).

In conclusion, our findings indicate that bleomycin sensitivity is partly determined by a polymorphism at codon 280 of the b/c distribution, which shows no clear bi- or trimodality. Overall, the genotype effects we observed were modest, as expected for single polymorphisms on the basis of the b/c distribution, which shows no clear bi- or trimodality.

Acknowledgements
This work was partly supported by Commission of the European Communities Contract No. QLK4-CT-2000-00628, ‘Cytogenetic Biomarkers and Human Cancer Risk’ by the Finnish Work Environment Fund, and by the National Scientific Research Fund OTKA 034416 and NKFP/1/48.

References

Received November 22, 2001; revised January 4, 2002; accepted March 11, 2002