Human Genome Epidemiology (HuGE) Review

XRCC3 and XPD/ERCC2 Single Nucleotide Polymorphisms and the Risk of Cancer: A HuGE Review

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Hundreds of polymorphisms in DNA repair genes have been identified; however, for many of these polymorphisms, the impact on repair phenotype and cancer susceptibility remains uncertain. In this review, the authors focused on the x-ray repair cross-complementing protein group 3 (XRCC3) and xeroderma pigmentosum group D (XPD)/excision repair cross-complementing rodent repair deficiency (ERCC2) genes, because they are among the most extensively studied but no final conclusion has yet been drawn about their role in cancer occurrence. XRCC3 participates in DNA double-strand break/recombinational repair through homologous recombination to maintain chromosome stability. XPD/ERCC2 is a helicase involved in the nucleotide excision repair pathway, which recognizes and repairs many structurally unrelated lesions, such as bulky adducts and thymidine dimers. The authors identified a sufficient number of epidemiologic studies on cancer to perform meta-analyses for XPD/ERCC2 variants in codons 156, 312, and 751 and XRCC3 variants in codon 241. The authors evaluated all cancer sites to investigate whether DNA repair is likely to take place in a rather nonspecific manner for different carcinogens and different cancers. For the most part, the authors found no association between these genes and the cancer sites investigated, except for some statistically significant associations between XPD/ERCC2 single nucleotide polymorphisms and skin, breast, and lung cancers.

ERCC2; ERCC2 protein, human; genetics; meta-analysis; neoplasms; XPD; XRCC3; x-ray repair cross complementing protein 3

Abbreviations: ERCC, excision repair cross-complementing rodent repair deficiency; HuGE, Human Genome Epidemiology; SNP, single nucleotide polymorphism; XPD, xeroderma pigmentosum group D; XRCC, x-ray repair cross-complementing protein.

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condition in which even short exposure to ultraviolet light can lead to early death from cancer). These genes also show common polymorphisms, whose effects on DNA repair enzymes are milder. A number of studies suggest that such mild defects in DNA repair may predispose to cancer (1\textendash}3). Environmental and occupational chemical carcinogens, such as polycyclic aromatic hydrocarbons, aromatic amines, and N-nitroso compounds, form bulky DNA adducts that are repaired mostly through the nucleotide excision repair pathway (e.g., the xeroderma pigmentosum group D (XPD) gene, also called the excision repair cross-complementing rodent repair deficiency group 2 (ERCC2) gene). These agents can also produce interstrand cross-links that are repaired by genes involved in both nucleotide excision repair pathways (e.g., the excision repair cross-complementing rodent repair deficiency group 1 (ERCC1) and group 4 (ERCC4) genes) and homologous recombinational repair pathways (e.g., x-ray repair cross-complementing protein group 2 or 3 (XRC2-3)). Reactive oxygen species also can induce base damage, abasic sites, single strand breaks, and double strand breaks. Single strand breaks are repaired through the basic excision repair pathway (e.g., x-ray repair cross-complementing protein group 1 (XRC1), proliferating cell nuclear antigen (PCNA)), while double strand breaks are corrected by either homologous recombination (e.g., XRC2-3) or nonhomologous end-joining pathways. Hundreds of polymorphisms in DNA repair genes have been identified; however, for many of these polymorphisms, the impact on repair phenotype and cancer susceptibility remains uncertain (1, 3).

Among the different DNA repair pathways, we focused in this Human Genome Epidemiology (HuGE) review on XRCC3 and XPD because, besides the genes already discussed by Hung et al. (4) in a recent HuGE review, these genes are among the most extensively studied for their potential implication in cancer risk. Although XPD and XRCC3 belong to two different repair pathways (nucleotide excision repair and homologous recombination, respectively), there is evidence that for some important exposures (e.g., smoking), both genes could be involved in repairing the relevant DNA damage (5). However, no final conclusion has yet been drawn about their role in cancer occurrence.

The XRCC3 gene is located in the 14q32.3 region. The XRCC3 protein participates in DNA double-strand break/recombinational repair and is a member of a family of Rad-51-related proteins that probably participate in homologous recombination to maintain chromosome stability and repair DNA damage (6). XPD is located at chromosome 19q13.3 and is involved in the nucleotide excision repair pathway, which recognizes and repairs many structurally unrelated lesions, such as bulky adducts and thymidine dimers (7). XPD functions as an adenosine triphosphate-dependent 5'-3'-helicase joint to the basal transcription factor IIH complex. Its protein has a role in the initiation of RNA transcription by RNA polymerase II (8).

No meta-analysis of data on the XRCC3 gene has been published, whereas for XPD, only lung cancer risk has been evaluated by meta-analysis (9, 10).

GENE VARIANTS

For the XPD gene, eight coding single nucleotide polymorphisms (SNPs) (four synonymous and four amino acid substitutions) and 138 intronic SNPs have so far been included in the National Center for Biotechnology Information’s SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). For the XRCC3 gene, four coding SNPs (one synonymous and three amino acid substitutions) and 109 intronic SNPs have been described, but most of them have not been studied in relation to cancer risk and thus were not considered in this review. We identified a sufficient number of epidemiologic studies on cancer to perform meta-analyses only for the XPD variants Arg156Arg (C/A), Asp312Asn (G/A), and Lys751Gln (A/C) and for the XRCC3 variant Thr241Met (C/T). Sparse data are available for XPD-Asp711Asp (C/T), -His201Tyr (C/T), -Ile199Met (C/G), and -IVS4-A/G and for XRCC3-IVS6 1571, -5'-UTR 4541, -A4552C, and -IVS5-14; those polymorphisms were not considered in this review. Allele and genotype frequencies for all of the polymorphisms are reported in Web table 1 (posted on the Journal’s website (http://www.aje.oxfordjournals.org)) by study and ethnicity. Genotype frequencies among controls were in agreement with those predicted under Hardy-Weinberg equilibrium in almost all populations (Web table 1).

GENOTYPE-PHENOTYPE CORRELATIONS

A variety of studies have been conducted to investigate the functional effects of variant DNA repair genes through the use of various biomarkers (3). However, these biomarker investigations did not provide consistent observations on genotype-phenotype correlations. This is probably due to the small sample sizes used and inappropriate biomarkers investigated, such as sister chromatid exchanges, because the mechanisms for formation of such changes and their biologic significance are unknown. Below we summarize the evidence on the relation between the XRCC3 and XPD/ERCC2 genotypes and the functional biomarkers that have been investigated to date (3, 11–16).

XRCC3

Allele and genotype frequencies of XRCC3 polymorphisms considered in the present study are shown by ethnic group in Web table 1. Variant allele frequencies ranged from 5 percent to 45 percent, with a statistically significant difference in the prevalence of the XRCC3-241 polymorphism between different ethnic groups (the prevalence of Met/Met homozygosity was 4.6 percent in African Americans, 0.2 percent in Asians, and 12.4 percent in Caucasians; $p < 0.001$). An opposite allele frequency distribution was observed for the ’XRCC3-5'-UTR 4541 polymorphism in the study by Winsey et al. (17) as compared with other studies, indicating a possible inversion in the assignment of the alleles (Web table 1).

The XRCC3-241Met variation is a nonconservative change, but it does not reside in the adenosine triphosphate-binding domain, the only functional domain identified in the
protein. The impact of this polymorphism on repair phenotype was studied in 80 healthy subjects (18); the XRCC3 241Met allele was associated with significant increases in chromosome deletions in x-ray-challenged blood lymphocytes ($p = 0.05$). Chromosome deletion is specific for abnormal repair of x-ray-induced DNA strand breakage. The overall frequency of aberrant cells associated with the variant was nonsignificantly higher than that in the wild-type genotype. On the other hand, the variant genotype had no effect on the repair of ultraviolet light-induced DNA damage in comparison with the wild-type genotype. These results suggest that the XRCC3 241Met allele might be defective in repairing double strand breaks but not in nucleotide excision repair.

In a study of 133 nonsmokers, 93 former smokers, and 82 current smokers, the XRCC3 241Met variant was significantly associated with increased bulky DNA adduct levels among all volunteers as a group and among the nonsmokers (14).

In blood samples taken from 435 newborns, the variant gene was not associated with an increase in the frequency of glycoporphin A NN or NO mutations (16).

In the one study that investigated the XRCC3-241Met variant using a specific functional assay (19), the findings suggested that the increased cancer risk associated with the XRCC3-241 variant may not be attributable to an intrinsic homology-directed repair. However, such experiments cannot definitely rule out the involvement of other XRCC3 variants in linkage disequilibrium or possible genetic interactions between the XRCC3-241 variant and polymorphic alleles of other DNA repair genes that may lead to a homology-directed repair defect. It is still possible that an extremely mild homology-directed repair defect would not be detectable in the assay or that XRCC3 acts within other cellular pathways not assayed in this in vitro model.

**XPD/ERCC2**

A number of SNPs in the XPD gene have been reported. Among these SNPs, common polymorphisms have been observed at codons 312 and 751, with allelic frequencies ranging from 6 percent to 34 percent and from 9 percent to 37 percent, respectively. A statistically significant difference between different ethnic groups has been observed for XPD/ERCC2-751 (the prevalence of Gln/Gln homozygosity was 6.9 percent in African Americans, 1.1 percent in Asians, and 13.4 percent in Caucasians; $p < 0.001$) and XPD/ERCC2-312 (Asn/Asn homozygosity was absent in Asians and prevalence was 11.1 percent in Caucasians; $p < 0.001$) (Web table 1). The pattern of allele and genotype frequencies was very different in the study by Chen et al. (20) as compared with the other Asian populations, with approximately 18 percent of subjects carrying the homozygous Gln/Gln genotype. This could have been due to errors in genotyping, since it seems unlikely that such great variation would exist in a population where all persons were of the same ethnicity.

The above polymorphisms result, respectively, in amino acid changes of aspartic acid to asparagine (Asp/Asn) in codon 312 and lysine to glutamine (Lys/Gln) in codon 751. Studies of the functional significance of these XPD variants include studies of chromosome aberrations, p53 mutations, changes in DNA repair capacity, and formation of DNA adducts. Expression of induced chromosome damage in relation to polymorphisms in XPD codon 312 was investigated by Lunn et al. (13), Au et al. (18), and Gao et al. (12). Lunn et al. (13) studied blood samples from 31 female donors who had various risk factors for breast cancer. Lymphocytes were irradiated with x-rays, allowed to repair the damage for 1.5 hours, and then harvested for analyses of chromatid-type aberrations. No association between the variant genotype and aberrations was observed, supporting the suggestion that the XPD gene is not commonly involved in base excision repair, the primary repair pathway for damage induced by x-rays. In contrast, in another cytogenetic study, Au et al. (18) showed that XPD 312Asn is associated with defective repair of ultraviolet light-induced DNA damage. The observed damage consisted of chromatid-type aberrations that are derived specifically from insufficient repair of ultraviolet-induced DNA damage, that is, nucleotide excision repair deficiency. Consistent with the study by Lunn et al. (13), the variant genotype had no significant effect on chromosome damage following exposure to x-rays, again confirming that the variant genotype is not involved in base excision repair.

The cytogenetic observation, however, is different from the p53 gene mutation data from Gao et al. (12) among lung cancer patients. In that study, the wild-type XPD codon 312 Asp allele was significantly associated with the presence of mutations in p53 exons 5–8. This observation by Gao et al. (12) may have been influenced by the small number of patients with the p53 gene mutation ($n = 40$) and/or the low frequency of the mutation among lung cancer patients (20 percent) in that study population. The function of the XPD codon 751 polymorphism has been extensively investigated, but again the suitability of the biomarker for XPD can be brought into question in some of the studies. In a study of 308 healthy people by Matullo et al. (14), the variant 751Gln genotype was not associated with a significant increase in bulky DNA adducts. In addition, it was not correlated with sister chromatid exchange frequencies or with polyphenol DNA adducts among 76 normal volunteers (11). The lack of association may indicate that sister chromatid exchange and polyphenol DNA adducts are not relevant biomarkers for XPD variant genotypes in the nucleotide excision repair pathway.

In the study of 31 subjects mentioned above, Lunn et al. (13) reported that having the wild-type XPD codon 751 genotype was associated with a significant increase in x-ray-induced chromosome aberrations compared with the variant genotypes. However, the significant association was with the combined chromatid breaks and gaps, not with breaks alone. In addition, the XPD gene may not be involved in the repair of x-ray-induced damage that appears to predominantly require the base excision repair mechanism.

Data from a study conducted by Qiao et al. (15) indicated that post-ultraviolet defective repair capacity for nucleotide excision repair using the host cell reactivation assay can be modulated by genetic polymorphisms of XPD in healthy subjects. The homozygous forms of two XPD variant alleles, XPD 312Asn and XPD 751Gln, were associated with lower defective repair capacity of ultraviolet-induced DNA
DNA repair affects multiple diseases, particularly different types of cancer. Therefore, we included in the present meta-analyses studies that considered any type of cancer as the outcome. XP/D/ERCC2 was investigated in studies of lung (n = 13), breast (n = 4), bladder (n = 4), skin (n = 7), head and neck (n = 2), esophageal (n = 2), colorectal (n = 1), and prostate (n = 1) cancer, as well as glioma (n = 1) and leukemia (n = 2). XRCC3 was investigated in studies of lung (n = 7), breast (n = 5), bladder (n = 4), and skin (n = 5) cancer and in single studies for each of the following types of cancer: leukemia, colorectal, endometrial, gastric, glioma, head and neck, and oral-larynx-pharynx. Many studies included evaluations of both genes. Occasionally, associations with cancers of the head and neck, prostate, endometrium, colon/rectum, or stomach or gliomas were described, but those studies were not included in the present meta-analysis. The association between lung cancer and XP/D was considered in a previous HuGE review (9), but the current review has been updated (four more studies were included) and includes other cancers, as well as XRCC3.

META-ANALYSIS

We conducted a search of the English literature using the National Library of Medicine’s MEDLINE system and essential search terms for the years 1985 (January) to 2005 (March) to identify all published articles or abstracts in which the frequencies of XP/D and XRCC3 were determined for human cancer. (All of the Web tables and references to original papers are available on the ISI Foundation’s Human Molecular Epidemiology website (http://www.hume.unito.it).) The search was organized by genetic polymorphism, organ site, histologic type, and any exposures evaluated as potential effect modifiers (i.e., exposures that may interact with genotype). We identified additional articles by searching through references cited in the first series of articles found in PubMed. Articles selected for meta-analysis were all case-control in design, had been published in the primary literature, and had no obvious overlap with each other in terms of subject. Heterogeneity among the studies was evaluated by means of Cochran’s Q test (23) and was considered significant if p < 0.05. If the test result was negative, a fixed-effects model (Mantel-Haenszel method) was used. This model assumes a common genotype effect between the studies. On the contrary, if the test result was positive, we used a random-effects model (24) to take the heterogeneity into account. This model assumes that the studies are a random sample of a hypothetical population of studies taking into account within- and between-study variability. All of the calculations were performed with the computer program R, version 2.0.1 (R Foundation for Statistical Computing, Vienna, Austria).

Because heterogeneity of allele frequencies in different populations could have introduced bias into the odds ratio estimates if different ethnic groups had not been well-matched within studies, the quality of the studies used in our meta-analysis was carefully checked, as well as control for potential bias. Nevertheless, ethnicity could have acted as an
effect modifier if the odds ratios were significantly different in different populations. Thus, we repeated the meta-analysis, whenever possible, stratifying by population.

In order to include all possible studies (i.e., to increase the statistical power of the meta-analyses), we also used the absolute numbers calculated from published genotype frequencies in these studies. Thus, we performed the meta-analysis in two ways: first, based on the original odds ratios published in the papers (indicated as “adjusted odds ratios”), and second, based on the absolute numbers reported in the papers or calculated from genotype frequencies and sample sizes (indicated as “crude odds ratios”).

The wild type was defined on the basis of genotype frequencies (most common allele) unless functional information was available. When the analyses were both stratified (i.e., by another factor) and unstratified (i.e., considered the main genotype effect) in the same paper, we used the odds ratio based on the latter. We also used the crude odds ratio when ethnicity was not specified.

ASSOCIATIONS AND INTERACTIONS

XPD/ERCC2

Web table 4 shows the results of the meta-analyses for XPD/ERCC2. The study by Chen et al. (20) was excluded from the XPD/ERCC2-751 lung cancer meta-analysis because of the large difference in allele/genotype frequency between that population and other populations of the same ethnicity. A few statistically significant odds ratios were found. Codon 156 was important in skin cancer, and codons 312 and 751 were important in breast cancer and lung cancer. Codon 751 was also significant in esophageal squamous cell carcinoma, but only two studies were included in the meta-analysis, which produced a relatively wide 95 percent confidence interval. Tests for interstudy heterogeneity were not statistically significant for these associations; that is, results were consistent across studies. No significant associations were found for bladder cancer or leukemia. To test whether the heterogeneity of allele frequencies observed in different populations could have introduced bias into the odds ratio estimates for different ethnic groups, we performed meta-analyses by Asian and Caucasian ethnicity for XPD/ERCC2-751 and -312 in lung cancer (the only possible stratifications). The results showed that, for the above SNPs, there was no statistically significant difference in odds ratios between Asian and Caucasian populations, in spite of the different allele frequencies (Web table 1).

XRCC3

None of the odds ratios in meta-analyses of XRCC3 were statistically significant (Web table 5 (http://www.aje. oxfordjournals.org)). However, the comparison between the TT and CC genotypes was close to statistical significance for lung cancer when the adjusted odds ratios were used (odds ratio = 1.25, 95 percent confidence interval: 0.97, 1.60). As for XPD/ERCC2 meta-analyses, the interstudy heterogeneity test was negative. No stratification by ethnic group was possible for XRCC3 polymorphisms.

DISCUSSION AND POPULATION TESTING

In spite of good biologic reasons for a role of DNA repair genetic polymorphisms in cancer risk modulation, the literature on the functional significance of the XPD/ERCC2 and XRCC3 genotypes considered remains relatively scanty (3). We chose two genes for which a reasonably large number of papers have been published and that are likely to be actively involved in both the repair of carcinogen adducts and the risk of cancer. We evaluated all cancer sites, because DNA repair is likely to take place in a rather nonspecific manner for different carcinogens and different cancers. However, with the accumulation of data on DNA repair gene polymorphisms, some SNPs seem to have opposite risk trends at different cancer sites. These results could simply reflect chance associations, although a possible explanation could be the tissue-specific balance between apoptotic signals and repair effects in the different tissues. Less efficient repair variants of specific repair pathways can result in a protective signal (accumulation of damage, cell-cycle block, apoptosis) in some tissues, whereas in others they could be risk factors (unrepaired or abortive attempt to repair damage and subsequent mutation).

For the most part, we found no association between the cancer sites we investigated and XRCC3. We detected some statistically significant associations between skin, breast, and lung cancers and XPD/ERCC2 SNPs. These observations are not surprising, because XPD/ERCC2 is known to play a key role in nucleotide excision repair, which in turn is crucial in, for example, the elimination of bulky DNA adducts. Less surprising is the lack of association with XRCC3. Potential explanations are both methodological (i.e., low study power to demonstrate small effects and too few cases to investigate disease heterogeneity (e.g., by tumor histology)) and substantive (i.e., the existence of multiple repair pathways that can compensate for each other). Moreover, our analysis did not consider the possibility of gene-gene or SNP-SNP interactions or the possibility of linkage disequilibrium between polymorphisms. Further investigations of the haplotypic effect of a gene and the study of multiple polymorphisms in different genes within the same pathway and different pathways are needed.

On the basis of our meta-analyses, there is no strong indication for testing populations, or subgroups with exposure to carcinogens, for the XPD/ERCC2 or XRCC3 genotype. However, given the many limitations of the existing literature mentioned above, more complete analyses of these genes are warranted. Although the evidence suggests that XPD/ERCC2 could play a role in individual susceptibility to lung, breast, and skin cancer, the associations are weak and presently do not justify screening.

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