Gender-related differences in response to mutagens and carcinogens

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The incidences of many cancers can be very different in men and women. Besides differences in exposures to putative causative agents, it is plausible that both genetic and epigenetic effects play roles in these differences. In addition, gender-specific lifestyle and behavioural factors may modulate the effects of exposure to genotoxins. This commentary focuses on several aspects of gender-related differences in responses to mutagens and carcinogens, including sensitivity to chromosome damage, the contribution of genotypic variation and the role of DNA methylation. It is concluded that the reasons for gender differences in cancer susceptibility remain largely unknown in many cases, and the subject deserves more attention and study.

Introduction: X and Y chromosomes

It can be postulated that gender-related differential responses to mutagens/carcinogens, where they exist, result from the genotype and/or from differences in the cultural and lifestyle environment. Sex chromosome dimorphism in mammals may be an important cause of gender-related differences. Women are 46 XX and men 46 XY, the mammalian sex chromosomes having arisen from autosomal progenitors ~300 million years ago. Before then, sex determination probably relied on environmental cues such as egg incubation temperature, as this is still the case for many extant reptiles. After the sex chromosomes were established, recombination between X and Y was suppressed progressively over time in a block-by-block manner along the chromosomes. As the sex chromosomes diverged, they developed their own identities. The Y chromosome underwent massive genic atrophy and a corresponding size reduction (and also accumulated male-beneficial genes) (for review see ref. 1). The genes expressed on the Y chromosome are essentially responsible for the induction of testis differentiation, which in turn will produce testosterone and the secondary male sex characteristics. In the absence of these genes, the gonads develop into ovaries producing oestrogens (and progesterone).

X inactivation in females is the classical form of dosage compensation that equalizes gene expression between the sexes. It occurs at random in the blastocyst stage and implies that women are mosaics for the X-linked genes. Although most genes on the X chromosome are subject to this haplo-inactivation, ~15% of X-linked genes escape inactivation to some degree (2). Typically, these escapees have functional (or very recently decayed) Y homologues. This is consistent with the notion that X inactivation evolved gene by gene and, in each case, as a delayed response to the degeneration of a corresponding Y homologue. The mechanisms by which X inactivation occurs are only partly understood (for review see ref. 3). In particular, it is uncertain how X inactivation spreads from the X inactivation centre (where the gene XIST resides) across the rest of the chromosome. One hypothesis holds that LINE1 (L1) repetitive elements may act as ‘way stations’ to facilitate the spread of X inactivation (for review see ref. 1).

Extending previous findings (4–6), Ross et al. (7) showed that the X chromosome is enriched for cancer-testis antigen genes, a subset of testis genes that are also expressed in cancer cells. Of note, a recent study (8) confirmed that the mammalian X chromosome is enriched for genes involved in early stages of spermatogenesis, consistent with the influence of hemizygous exposure. But the same study also showed a deficit of genes implicated in late stages of spermatogenesis, which was hypothesized to be an evolutionary response to male germ line X inactivation that occurs at the onset of male meiosis. Thus, the cancer-testis antigen genes shown by Ross et al. (7) to be enriched on the X chromosome are probably early spermatogenesis genes. The human X chromosome is also mildly enriched for brain and skeletal muscle genes, which could potentially be explained by hemizygous exposure (9). In addition to the accretion of certain male-beneficial genes, the mammalian X chromosome also seems to be enriched for female-beneficial genes such as those expressed in ovary and placenta.

Gene expression from the mammalian X chromosome is upregulated in somatic cells of males and females, a process that achieves dosage compensation by a doubling of the X transcriptional output (10). The same authors confirmed earlier findings (11) showing that X-linked genes are highly expressed in brain tissues, consistent with a role in cognitive functions. Furthermore, the X chromosome is expressed but not upregulated in spermatids and secondary oocytes, preserving balanced expression of the genome in these haploid cells. Upon fertilization, upregulation of the active X must occur to achieve the observed dosage compensation in early embryos.

When considering the possibility that genetic differences might be responsible for sex-related differences in sensitivity to
mutagens/carcinogens, one expects at first approach that they should result from different hormonal environments and/or be evident in sex-specific organs. In addition, as far as chromosomal changes are concerned, it is known that X chromosome loss occurs with age in women, as was demonstrated in several epidemiological studies assessing micronucleus (MN) frequencies in women versus men (12–14). It is also known that this (almost) inactive heterochromatic X is lost preferentially, and in this case, the few genes transcribed from this heterochromatic X may contribute to gender effects.

**Differences in cancer incidence between men and women**

The wide disparity between males and females in the incidence of many cancers is clearly illustrated by a recent analysis of cases in the USA from 1975 to 2004 based on the Surveillance, Epidemiology and End Results (SEER) programme (15). The 10 cancers with the largest male-to-female incidence rate ratios (IRR) were Kaposi sarcoma (28.73), lip (7.16), larynx (5.17), mesothelioma (4.88), hypopharynx (4.13), urinary bladder (3.92), oesophagus (3.49), tonsil (3.07), oophorix (3.06) and other urinary organs (2.92). Only five cancers had a higher incidence in females compared with males: breast (0.01); peritoneum, omentum and mesentery (0.18); thyroid (0.39); gallbladder (0.57) and anus, anal canal and anorectum (0.81).

Between 1975 and 2004, the largest consistent increases in male-to-female IRR were for cancers of the tonsil, hypopharynx, skin excluding basal and squamous and oesophagus, whereas the largest consistent decreases in IRR were for cancers of the lip and lung and bronchus. Male-to-female IRRs varied considerably by age, the largest increases of which were for ages 40–59 years for tonsil cancer and hepatocellular carcinoma. The largest decreases in male-to-female IRR by age, meanwhile, were for ages 30–49 years for thyroid cancer, ages >70 years for oesophageal squamous cell carcinoma and ages >30 years for lung and bronchus cancer. These observations emphasize the importance of sex in cancer aetiopathogenesis and may suggest the need of novel avenues of investigation.

Taking papillary thyroid cancer as a specific example of a cancer for which risk factors are largely unknown (except for radiation and being female), Kilfoy et al. (16) analysed the SEER 9 Registries Database for cases from 1976 to 2005 for aetiological clues. Standard descriptive epidemiology was supplemented with age–period–cohort (APC) models, simultaneously adjusted for age, calendar period and birth cohort effects. The papillary thyroid cancer incidence rate among females was 2.6 times that among males (9.2 versus 3.6 per 100 000 person-years, respectively), with a widening gender gap over time. Age-specific rates were higher among women than men across all age groups, and the female-to-male rate ratio declined quite consistently from >5 at ages 20–24 years to 3.4 at ages 35–44 years and approached 1 at ages 80+ years. APC models for papillary thyroid cancers confirmed statistically different age-specific effects among women and men ($P < 0.001$ for the null hypothesis of no difference by gender), adjusted for calendar-period and birth-cohort effects. The study illustrates the need for future attempts to identify risk factors to have adequate power to assess age-specific interaction among males and females.

Some differences in cancer incidence undoubtedly result from differences in ‘exposure’ to putative aetiological agents, which may result, for example, from differences between the sexes in time spent outdoors (exposure to ultraviolet or air pollution as risk factors; vitamin D production as a protective factor) or indoors (e.g. exposure to indoor air pollution arising from cooking and heating in dwellings with limited ventilation). While the current article is focused more on differences in ‘response’ to mutagens and carcinogens, it is worthwhile to give brief consideration to some general differences that may also account for differences in susceptibility in terms of gender-related protective factors, before going on to consider other differences in responses to mutagens/carcinogens, including cytogenetic effects, interaction between gender and genotypes relevant for the mutagenic/carcinogenic response, response to a known carcinogetic exposure (in this case smoking) and epigenetic effects.

In most, if not all, societies, women live longer than men, and most cancers increase in incidence with age. On the other hand, women have a more efficient immune system, which may give greater protection against some cancers (although making women more prone to autoimmune diseases such as rheumatoid arthritis and multiple sclerosis). Differences between the sexes in hormonal status, reproductive factors and tobacco smoking have well established influences on some cancers (17). In the recent study (15) on sex disparities in cancer incidence by period and age, a hormonal aetiology was suggested for skin and thyroid tumours but an anti-carcinogenic influence of oestrogen in hepatocellular carcinoma. As far as tobacco and alcohol are concerned, these were not found to be gender-related risk factors for tonsil and oropharyngeal cancer or for oesophageal cancer. The male-to-female IRRs for lung cancer reflect historical exposure to tobacco smoking, as is described in detail later in this article. The impact of viral infection on sex disparities in cancer should also be highlighted. Oral human papillomavirus (HPV) infections are considered to drive the increases in male-to-female IRRs for tonsil and oropharyngeal cancer, and this family of viruses may also be responsible for the higher incidence of anus, anal canal and anorectum cancer in females. Infection with hepatitis B and C viruses (as well as alcoholic liver disease) might explain the higher incidence of hepatocellular carcinoma among males <60 years (15). Another example is the higher rates of both human immunodeficiency virus infection and HPV-8 seropositivity in men, which are likely to contribute to the higher IRR for Kaposi sarcoma (18). As a protective factor, the use by women of lipstick and lip balm with sunscreen is thought to contribute to the lower incidence of lip cancer in women (19).

Dietary differences, both with regards to protective factors and those thought to be potential risk factors, have been documented. For example, in Sweden, females eat more fruit and berries, but there is also an age dimension with lower intake among young people for both males and females (20). Probiotics are also consumed more by women than men, whereas young males have a significantly higher consumption of sugary soft drinks than young females. These and/or other differences in dietary habits between men and women likely have a large impact on global cancer incidence (21).

**Gender-specific sensitivity to chromosome damage**

In the past, several investigations evaluated the presence of association between the level of chromosome damage and...
gender (22–24). Those studies were mostly driven by the evidence that in some cases a different susceptibility to genotoxic exposure could be mediated by gender, or gender-related features, and by interest in the mechanism behind this hypothesis. A significant difference between sexes was consistently found as regards the frequency of sister chromatid exchanges (SCEs), with a higher frequency in women accounting for 2–5% of the total observed variation in the baseline rate of this biomarker. The role of genetic factors on the observed sex difference in SCE frequency was then investigated in classic twin studies with contrasting results, mostly due to the small sample size of most studies (22). The difference in the genomic length of the X and Y chromosomes was considered the most likely cause of the different extent of chromosome damage between genders. However, the baseline heterogeneity in the occurrence of chromatid exchanges did not reflect a different susceptibility to genotoxic exposures (22).

In the same period, results from pooled analyses of chromosomal aberrations (CAs), as well as from *ad hoc* studies, did not find major differences between genders (22,25). Much more interesting findings came from the MN assay, with positive evidence from pooled studies based on the cytokinesis-block assay that consistently showed a 32% higher MN frequency in women (23,24). To investigate the segregation of sex chromosomes in human lymphocytes, centromeric fluorescence in situ hybridisation was used. The lack of gender-related difference in X chromosome reciprocal gain and loss suggested that the high loss of the X chromosome in women might be due to micronucleation (26).

The recent availability of large pooled datasets coming from international collaborative studies on biomarkers has allowed evaluation of the role of gender in a more systematic way. With these larger datasets, a number of hypotheses, including the presence of interaction between host factors, have been tested. The most extensive effort in the field of chromosome damage is represented by the Human MicroNucleus (HUMN) project, which started in 1997 to collect data worldwide from leading laboratories that use the cytokinesis-block MN assay. The analysis of these data, contributed by 25 laboratories in 16 countries, clearly showed a remarkable separation of MN frequency curves between the two genders, with a higher frequency in females starting in the age class 20–29 and increasing progressively (23) (see Figure 1). The presence of a gender effect in younger age groups was subsequently evaluated in a subset of 12 HUMN laboratories that measured MN frequency in children and supplemented by a meta-analysis of 13 studies selected from the literature. A clear effect of age was detected, even within the restricted range of paediatric age classes considered, while no effect of gender could be demonstrated (27). The lack of a sex difference in MN frequency in children, compared with the marked sex difference observed in adults, seems to support a role for female sex hormones in modulating MN formation. However, although the epidemiological evidence is suggestive of an association, and mechanistic hypotheses linking sex hormones to genetic instability are available (see the section on DNA methylation for some examples), human studies have provided little or no evidence of association (28,29). More thoroughly designed population studies, taking into consideration the effect of different hormones, evaluating different concentrations and measuring damage in different tissues, may address more properly the question about the role of sex hormones as determinants of MN formation.

The recent evolution of the MN assay into what has been defined as the cytokome assay has allowed a deeper insight into mechanisms. This new approach has been developed as an improvement of the MN assay not only in lymphocytes (30) but also in exfoliated buccal cells (31). Extensive knowledge gaps in the MN assay of exfoliated cells have been discussed in a review recently published by the HUMN steering committee, which has launched a new programme, called the HUMNXL project (XL designating eXfoLiated cells), specifically addressing chromosome damage in exfoliated buccal cells (32). The review traced 15 studies that evaluated the role of gender as a determinant of MN frequency. Results were conflicting, with only three studies reporting significant differences (32). Interestingly, two of these studies found a higher frequency in males, in evident contrast to data from the MN assay in lymphocytes. Assessing the role of gender and other host factors in the occurrence of MN and other endpoints of the cytokome assay in buccal cells has been prioritized within the aims of the new project (33,34).

A different susceptibility of males and females to diseases associated with chromosome breakage and rejoining might have evident implications for the study of mechanisms as well as for public health. Not many studies have addressed this topic, but interesting results can be obtained from cohort studies that have associated the levels of CA and MN in peripheral lymphocytes of healthy subjects with the risk of cancer. All details about the study design and the statistical methods can be found in the original papers (35,36). Table I summarizes the risk of cancer associated with CA and MN frequency stratified by gender in these studies. Results are quite heterogeneous, reflecting the large amount of variability introduced by the pooled nature of data, which were collected from several laboratories, at different times, and from populations exposed to different genotoxic agents. The association between CA frequency and risk of cancer was more evident in males, with a significantly increasing trend and
highly significant $P$-values. An incomplete correction for confounding due to occupational exposure and smoking habit may have played a role in the discrepancy between sexes observed in this cohort. On the other hand, the higher risks observed in the dataset of MN in males in the middle tertile with respect to those in the highest tertile seem more likely to reflect the effect of an incomplete adjustment for confounding than a real quantitative interaction. In conclusion, testing gender as a potential effect modifier of the association between CA, MN and cancer risk has not provided sound evidence of interaction. Similarly, contrasting results were found when specific types of cancer were tested instead of total cancer (data not shown).

The availability of new technologies that allow the evaluation of gene expression patterns resulting from exposure to genotoxic agents may provide further insight into the mechanisms linking chromosome damage to gender. So far, only a few studies have evaluated the link between biomarkers of chromosome damage and gene expression. Among them, a study by van Leeuwen et al. (37) compared gene expression and MN frequency in two groups of children living in two areas of the Czech Republic with different degrees of air pollution. Since these data were made available through a public database, we planned a new evaluation according to a candidate gene approach. Candidate genes that were reported in the literature to be associated with chromosome malsegregation were specifically associated with MN frequency and with all available covariates. The role of major covariates such as area, gender and age, as effect modifiers of this association was evaluated. This study did not show an association between the frequency of MN in peripheral blood lymphocytes and the expression level of the list of genes. However, an effect of area and age on MN frequency and a weak interaction between the expression of TP53 gene and gender were found (F. Gallo, S. Moretti and S. Bonassi, submitted for publication).

The relationship between chromosome damage and gender is well substantiated by extensive evidence. In particular, this association is more specifically linked with the induction of chromosome loss and is strongly modified by age class. On the other hand, no clear evidence is available that differences exist between females and males in the susceptibility to genotoxic exposure or in the mechanisms of disease aetiology and pathogenesis. The increased availability of data from studies using high-throughput techniques will offer an obvious tool to explore this field further.

### Risk of lung cancer from smoking in men and women

The relative susceptibilities of men and women to tobacco-related lung cancer have been a contentious issue for some time. Up until about 1970, a much greater proportion of the male population smoked than did females, but the age-specific mortality from lung cancer in males has been declining in most age groups for >20 years because the number of male smokers has declined. Today, the proportion of men and women who smoke in Western countries is very similar, but mortality from lung cancer in women is still rising, having surpassed mortality from breast cancer in the USA in 1987 (38) and in Canada in 1993 (39). At what level will lung cancer in women peak in developed countries? The answer to this important question, and the consequences for tobacco-induced lung cancer worldwide, depend on whether women are more at risk from smoking or not.

Evidence that women were at 1.5- to 2-fold higher risk than male smokers of developing lung cancer came initially from case–control (40–42) and cohort studies (43) in the 1990s. A subsequent prospective study (44) calculated an odds ratio (OR) of 1.9 [95% confidence interval (CI) 1.5–2.5] and observations on trends in smoking-associated cancers were interpreted as suggesting increased risk to females than males, given similar smoking exposure (45).

However, there have been a number of other studies that have not found a difference in susceptibility between men and women (46–48). It has also been suggested that at least some of the apparent higher risk to women was, in fact, a consequence of the lower baseline absolute risk of lung cancer for never-smoking women (49). Possible under-reporting of smoking habits by women has also been suggested, as well as a potentially greater exposure to passive smoking (38,50).

A number of experimental studies have investigated sex differences in biomarkers of tobacco smoke exposure and cancer risk. The levels of smoking-related DNA adducts, which provide an integrated dosimeter of carcinogen exposure, metabolic activation and target dose, were found at significantly higher levels in lungs of female smokers than in males, after adjustment for smoking levels (51). Moreover, CYP1A1 mRNA levels, representing an indicator of expression of a key enzyme involved in carcinogen activation, were also higher in the women’s lungs (51). In fact, both CYP1A1 and CYP1B1 mRNA levels are elevated in smokers’ lungs but only CYP1A1 showed higher levels in women than in men (51). CYP1A1 expression was also higher in adenocarcinoma cell lines derived from female patients, and DNA adduct formation by benzo[a]pyrene was also higher than in the male cell lines (H. Uppstad, G. H. Osnes, K. J. Cole, D. H. Phillips, A. Haugen and S. Mollerup, unpublished results).

Hormonal differences have been postulated to play a role in lung cancer susceptibility (38). These could include an influence of oestrogen receptor (ER) on aryl hydrocarbon receptor-mediated expression of a number of enzymes involved in carcinogen metabolism. However, in one study in human bronchial epithelial cells lines, ER did not influence expression of either CYP1A1 or CYP1B1, suggesting that the metabolic activation of polycyclic aromatic hydrocarbons (PAHs) (e.g. benzo[a]pyrene) would not be influenced by oestrogen levels (52). Nevertheless, other activating or detoxifying enzymes might be regulated by hormonal response (53), and more research is needed in this area.
The ability to repair smoking-induced DNA damage could be another factor. In a case–control study investigating the DNA repair capacity (DRC) in cultured lymphocytes in a host-cell reactivation assay (54), in which the reporter gene was modified by reaction with benzo[a]pyrene diol-epoxide, DRC was significantly \( P < 0.001 \) lower in cases compared with controls overall and lower \( P < 0.001 \) in female cases than male cases. DCR was also marginally significantly lower \( P = 0.058 \) in female controls than in male controls. Another element that might contribute to the greater susceptibility in women is the role of the GSTM1 null genotype, which has been implicated as a risk factor for several cancers. In a case–control study, GSTM1 null was significantly associated with lung cancer \( \text{OR} = 2.04, 1.13–3.68 \) at 95% CI, but the OR for women \( 3.03 \) was greater than for men \( 1.42 \), indicating a greater risk consequence of this polymorphism for women (55) (see also Contribution of genotypes to gender-related differences in responses to mutagens/carcinogens and in cancer frequencies).

Another potential mechanism relates to GRPR, a gene on the X chromosome that escapes X inactivation, such that women have two actively transcribed alleles. The gene appears to be induced by nicotine and is expressed, in descending frequency, in male smokers > female non-smokers > male smokers > male smokers (59). The gene encodes gastrin-releasing peptide receptor, which mediates a number of bombesin-like peptides that induce cell proliferation in bronchial epithelial cells, thereby potentially promoting lung carcinogenesis.

Thus, although lung cancer rates for men are declining in the developed world, they have not yet peaked for women; smoking rates are increasing in both sexes in developing countries, where lung cancer rates will increase dramatically in the 21st century. The epidemiological evidence for women being at greater risk of lung cancer from smoking is probably best described as inconclusive at present, but the inevitable future cases will undoubtedly resolve the matter. Experimental studies have certainly lent some credence to the hypothesis that there is a sex difference in susceptibility, although there is still little known about possible mechanisms for these differences.

### Contribution of genotypes to gender-related differences in responses to mutagens/carcinogens and in cancer frequencies

Males and females may have significant differences in their response to mutagens/carcinogens, but gender effects have received relatively little attention, often viewed as confounders rather than of primary importance. Moreover, although a literature search revealed that some in vitro studies have shown either gender-related differences or genotype-related effects in response to these toxicants (for reviews see refs. 60–62), no study could be identified with convincing evidence of statistically significant concurrent effects of both gender and genotype in individual responses to environmental insults. Thus, this issue remains to be resolved in the future studies.

The literature on contribution of genotypes to gender-related differences in cancer frequencies is similarly rather sparse. However, a few examples exist of males and females with the same at-risk genotype for a given relevant polymorphism having a different risk for cancer. For instance, in a pooled analysis (63), the high-inducibility-associated cytochrome P450 1A1 (CYP1A1) genotype (homozygous variant) posed a highly elevated risk of lung cancer among Caucasian males \( \text{OR} = 3.1, 95\% \text{ CI} 1.6–14.2 \), whereas no effect was observed among females \( \text{OR} = 1.1, 95\% \text{ CI} 0.2–4.6 \) (Table II).

Some evidence also exists on the role of glutathione S-transferase (GST) genotypes in gender-related differences in cancer proneness. Several GST genes are polymorphic, and some allelic variants causing impaired enzyme activity are suspected to increase susceptibility to malignancies associated with environmental PAH exposure. For instance, association between GSTM1 polymorphism and lung cancer has been the subject of numerous studies. In one study (55) (also discussed above in Contribution of genotypes to gender-related differences in responses to mutagens/carcinogens and in cancer frequencies), the GSTM1 null genotype posed a significantly elevated risk of lung cancer among smoking Caucasian females \( \text{OR} = 4.5, 95\% \text{ CI} 1.1–19.2 \), whereas no significant effect was observed among the smoking males \( \text{OR} = 1.3, 95\% \text{ CI} 0.3–5.6 \) (Table II).

In one study (64), the Caucasian males concurrently carrying at-risk alleles of GSTM1 and GST1 were at higher risk of colorectal cancer \( \text{OR} = 2.6, 95\% \text{ CI} 1.3–5.2 \) especially of distal tumours \( \text{OR} = 3.6, 95\% \text{ CI} 1.7–7.4 \), whereas no significant association was seen for females (Table II).

The association between N-acetyltransferase 2 (NAT2) polymorphism and bladder cancer is well established and the risk has been suggested to be higher in male smokers than female smokers. However, no data have yet been reported

<table>
<thead>
<tr>
<th>Cancer site (reference)</th>
<th>Genotype</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder (64)</td>
<td>SULT1A1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wild-type genotype</td>
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</tr>
<tr>
<td></td>
<td>Variant allele carrying</td>
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<tr>
<td>Colorectal (65)</td>
<td>GSTM1/GSTM3</td>
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</tr>
<tr>
<td>Total</td>
<td>Both at-risk genotypes</td>
<td>1.5 (0.6–3.5)</td>
</tr>
<tr>
<td>Proximal</td>
<td>Both wild-type genotypes</td>
<td>0.7 (0.3–1.5)</td>
</tr>
<tr>
<td>Distal</td>
<td>Both at-risk genotypes</td>
<td>0.7 (0.2–2.4)</td>
</tr>
<tr>
<td></td>
<td>Both wild-type genotypes</td>
<td>1.0 (0.7–4.3)</td>
</tr>
<tr>
<td>Colorectal (66)</td>
<td>NAT2</td>
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<td>Slow acetylator</td>
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<td>Fast acetylator</td>
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<tr>
<td>Lung (63)</td>
<td>CYP1A1 MspI</td>
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<td>Wild-type genotype</td>
<td>Variant genotype</td>
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<tr>
<td></td>
<td>Heterozygous variant genotype</td>
<td>1.0 (0.7–1.4)</td>
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<td>Lung (55)</td>
<td>GSTM1</td>
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</tr>
<tr>
<td>Total</td>
<td>Null</td>
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<tr>
<td></td>
<td>Null</td>
<td>1.5 (0.9–2.6)</td>
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</tbody>
</table>
supporting the existence of any NAT2 genotype effect in gender-related differences in the bladder cancer susceptibility. In contrast, the NAT2 fast acetylator genotype has been associated with increased risk of colorectal cancer, especially in females (Table II) (65).

This finding is supported by the observed significantly higher proportion (50.0%) of female colon cancer patients with the NAT2 fast acetylator genotype exhibiting K-RAS mutations compared with the fast acetylator males (26.5%) (Table III) (66).

The Arg213His polymorphism in sulfotransferase 1A1 (SULT1A1) gene has been suggested as modifier of individual susceptibility to several forms of environmentally induced cancers. In one study (67), American females carrying the SULT1A1 allele (213His) associated with low activity and low thermal stability were reported to be at significantly lower risk of bladder cancer (OR = 0.4, 95% CI 0.2–0.8) compared with the non-carriers of the allele, whereas no significant effect was observed among the males (OR = 0.8, 95% CI 0.6–1.2) (Table II).

To conclude, although there are only limited data available on the contribution of genotypes to gender-related differences in response to mutagens/carcinogens, the existing evidence suggests a clear modulating role of genotypes. One of the most obvious explanations for these phenomena is that elimination and/or toxification reactions of toxicants like cigarette smoke products are subject to competing metabolic pathways, some of which may be regulated by hormonal response.

The examples presented here together with the consistent observations of a difference between males and females in cancer risk, after allowing for known risk factors, strongly suggest consideration of genotype–gender interaction data in future molecular epidemiological studies of environmentally induced diseases. Furthermore, existing datasets in which sex was adjusted for as a potential confounder could or should be revisited to investigate evidence for interactions between gender and genotype.

DNA methylation and gender-related effects

The epigenetic interpretation of sex dimorphism may explain gender differences in susceptibility to mutagens/carcinogens. Epigenetic mechanisms may also be a basis for differential susceptibility to complex diseases (most notably cancer) between men and women (68). The term ‘epigenetics’ defines heritable changes in gene expression and chromatin organization that are not encoded in the genomic DNA itself. Epigenetic mechanisms can be classified into three distinct types: DNA methylation, histone modifications and non-coding RNAs. DNA methylation refers to covalent modification of the cytosine base that is located 5' to a guanine base in a CpG dinucleotide. The methylation of DNA has multiple roles in cellular processes, although its role in the regulation of gene expression has been most widely studied. Aberrant DNA methylation is universally present in human malignancies and is associated with inappropriate gene expression. Histone modifications refer to covalent post-translational modifications of histone proteins that are subject to different modifications, including acetylation, methylation, phosphorylation and ubiquitination. Different histone modifications appear to act in a combinatorial and consistent fashion, generating a code (‘histone code’) that is read by ‘cellular machineries’ to dictate different functional outcomes. RNA-mediated gene silencing, in the form of non-coding RNAs, is also an important epigenetic mechanisms that has been associated with the maintenance of gene transcription in a heritable manner. Different epigenetic mechanisms appear to cross-influence and reinforce each other in the orchestration of cellular response to environmental stimuli and endogenous cues (69,70).

While it is now widely accepted that epigenetic inheritance is essential in the regulation of critical cellular processes such as gene transcription, cell differentiation and protection against viral genomes, accumulating evidence suggests that epigenetic mechanisms may play important roles in DNA repair and cellular response to mutagen/carcinogen exposure and that their epigenetic deregulation may induce genetic changes and promote tumour development (69,71). For example, there are several distinct mechanisms by which aberrant levels and patterns of DNA methylation may trigger mutational events. These include the enhanced binding of carcinogens, increased mutability of methylated cytosines and silencing of tumour suppressors, DNA repair genes and carcinogen-detoxifying genes (69).

The presence of methylated CpG sequences per se is considered as the major cause of mutability in mammalian genomes. Methylated cytosine constitutes a threat to the genome due to its intrinsic instability (72,73). Different factors that confer enhanced susceptibility of 5-methylcytosine (5-mC) to mutational events in comparison to unmethylated cytosine include altered repair efficiency and differential rate of spontaneous deamination (69,74). This supports the notion that CpG sites have been lost during evolution due to increased mutability of 5-mC in CpG dinucleotides. Because it is prone to spontaneous hydrolytic deamination under physiological conditions, 5-mC is considered a potent endogenous promutagenic lesion and environmental exposure may further enhance its mutagenic effect. Therefore, differences in DNA methylation states between the sexes may constitute the basis for gender-dependent susceptibility to exposures to environmental mutagens or endogenous toxic agents and ultimately to differential susceptibility to diseases.

Although the methylation difference of a large number of CpGs analysed on three human chromosomes identified a relatively small mean methylation difference (0.1%) between males and females (75), these small differences in methylation patterns, if present at critical regulatory genes, may have significant impact on cellular response to mutagen exposure. While the identity of such genes or genomic sequences remains to be uncovered, DNA methylation changes in sex hormone genes and/or the gene targets of sex hormones may be an

| Table III. The effects of NAT2 genotypes on the occurrence of KRAS mutations in colorectal cancer patients of different genders (67) |
|-----------------|-----------------|-----------------|
| NAT2 genotypes | Absence (%)      | Presence (%)    | OR (95% CI) |
| Females        |                 |                 |             |
| Slow           | 11 (78.6)       | 3 (25.0)        | 1.0         |
| Fast           | 29 (50.0)       | 29 (50.0)       | 4.8 (1.1–20.9)|
| Males          |                 |                 |             |
| Slow           | 9 (81.8)        | 2 (18.2)        | 1.0         |
| Fast           | 50 (73.5)       | 18 (26.5)       | 2.2 (0.4–12.0) |

ORs were adjusted for age, smoking, tumour sites and tumour stage.
Possible mechanisms underlying gender-specific response to mutagens/carcinogens. In addition, epigenetic changes induced by sex hormones may be responsible for gender effects on cellular response to mutagens. Sex hormones are known to be potent modulators of specific genes and genomic functions, and increasing evidence suggests that sex hormones can modify gene expression through local reconfiguration of epigenetic states (68).

Numerous studies in different model systems showed that sex hormones may exert dramatic changes in epigenetic patterns. For example, administration of oestradiol in animal models triggers DNA methylation changes in the oestradiol regulatory region of specific genes (76). Histone modification states can also be altered by the nuclear hormone receptors and the steroid receptors (77). These changes in epigenetic states have either activating or repressive transcriptional consequences. Finally, it is possible that sex hormones exhibit their biological effect through their action on microRNA expression patterns, although further studies are needed to test this possibility.

Beside the differences in levels of specific sex hormones, tissue-specific expression of sex hormone receptors varies between males and females. In addition, gender-specific and tissue-specific expression patterns of different transcriptional co-regulators and epigenetic modifiers may be the cause of differences in quality and magnitude of response not only to sex hormones but also to mutagen exposures. Together, epigenetic interpretation of gender effects on differential response to mutagens/carcinogens and susceptibility to complex diseases appears to be a plausible biological hypothesis that warrants further study.

**Conclusion**

The wide disparity between males and females in the incidence of cancers is an established fact. A broad range of mechanisms may contribute to this difference (Figure 2) and their understanding is critical to a better primary prevention strategy and possibly to more adequate therapy. Besides differences in exposures to putative causative agents, it is considered that both genetic and epigenetic effects play roles in these differences. In addition, gender-specific lifestyle and behavioural factors may modulate the effects of exposure to genotoxins.

In conclusion, there are mechanistic, experimental and epidemiological data indicating that the known differences in cancer frequencies between men and women might be due not only to different exposures but also to gender-related responses to mutagens. In particular, some chromosomal changes, specific genetic polymorphisms and epigenetic effects are plausible mechanisms explaining the gender-related differences. However, in general, too few studies are available to draw firm conclusions and, therefore, the reasons for gender differences in cancer susceptibility remain largely unknown in many cases. New molecular and cellular approaches using high-throughput techniques will offer obvious tools to explore this question further, and there is a compelling need to consider sex as an important variable in future studies of the aetiology of cancer.

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**References**


