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Enzyme polymorphisms influencing the metabolism of heterocyclic aromatic amines

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Abstract

Heterocyclic aromatic amines are dietary carcinogens possibly involved in human carcinogenesis, DNA-adduct formation being an obligatory step in this multistage process. Heterocyclic amine binding to DNA largely depends on the balance between metabolic activation and detoxification pathways and DNA repair efficiency. Several genes coding for metabolic enzymes are polymorphic, which affects gene expression and/or enzyme activity. This paper briefly reviews the effect of polymorphisms of activating/detoxifying enzymes on the metabolism of heterocyclic amines. Despite some epidemiological evidence of an association between genetic polymorphisms and susceptibility to cancer possibly resulting from dietary exposure to heterocyclic aromatic amines (HA), the genetic polymorphisms had only slight effects on biomarker levels, suggesting the existence of further unknown factors.

Keywords: Heterocyclic aromatic amines metabolism; Enzyme polymorphisms

1. Introduction

Common cooking procedures for meat and fish produce a family of compounds known as heterocyclic aromatic amines (HA). All HA are structurally related, having heterocyclic aromatic rings and an exocyclic amine function, the latter being essential for DNA binding [1]. The most frequent HA found in cooked meats have a quinoline, quinoxaline or pyridine moiety [1]. These compounds are formed in ppb amounts, depending on the type of meat, cooking temperature, and how well done it is [2]. HA have proved to be mutagenic in vitro and carcinogenic in animals [1,3]. The liver was the major target organ in the animal models used for the majority of HA examined to date, with the exception of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), the predominant HA formed [2], which induces colon, mammary gland and prostate tumors in rats, and lymphomas in mice [3]. HA metabolites have been detected in human urine after eating cooked meats or oral administration of HA, indicating that these compounds are readily bioavailable and may have some role in human carcinogenesis [4–7].

Epidemiological evidence suggests that consumption of red meat is not only an important risk factor for colorectal and other cancers, including breast, pancreatic and prostate cancers, but the relative risk increases with cooking temperature and duration [8–13]. However, the results are controversial [14,15].

HA themselves are not genotoxic but, like most chemical carcinogens, require metabolic activation, DNA-adduct formation in target tissues being an obligatory step in chemical carcinogenesis [16]. The metabolism of HA to reactive species that bind to DNA involves *N*-oxidation to the *N*-hydroxyl (*N*-OH–) derivative. This reaction occurs primarily in the liver and is catalyzed mainly by the inducible cytochrome P4501A2 (CYP1A2) enzyme, though other P450 enzymes might be involved too [17–20]. *N*-OH-HA themselves can react with DNA, but the subsequent *O*-esterification catalyzed by phase II enzymes, mainly acetyltransferases and sulfotransferases, leads to the formation of *O*-derivatives that are more reactive electrophiles [16].

Besides the mentioned enzymes, *N*-OH-HA or *N*-acetoxy-HA may become substrates for other phase II enzymes, including those belonging to the glutathione *S*-transferase

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(GST) and UDP-glucuronosyl transferase (UGT) superfamilies [21,22]. Glutathione and/or glucuronic acid conjugation participates in cellular trapping and elimination of reactive species permitting their elimination in urine or bile.

HA binding to DNA largely depends on the balance between metabolic activation and detoxification pathways and DNA repair efficiency. The activity of most enzymes involved in HA metabolism can be modified by lifestyle or other environmental factors and by inherited genetic traits. Several genes coding for metabolic enzymes are polymorphic, which affects gene expression and/or enzyme activity. Variants of these genes have high frequency and low penetrance, but high relative and absolute risk, and potentially high population attributable risk.

This paper briefly reviews the effect of polymorphisms of activating/detoxifying enzymes on the metabolism of HA.

1.1. Heterocyclic amines, metabolic polymorphisms and cancer risk

Considerable data supports the view that polymorphism at different genes encoding for enzymes involved in the metabolism of chemical carcinogens influences susceptibility to cancer, though it is not clear why any particular genotype is associated with an increased risk of a particular cancer but not another [23].

1.1.1. HA activation pathways

The metabolic activation and detoxification pathways of HA are known. CYP1A2 is first involved in the metabolic activation of these compounds [16]. In human, the expression of CYP1A2 was found to be confined to the liver, and only negligible levels have been detected in extrahepatic tissues [24]. In vivo phenotyping assays indicate that CYP1A2 activity is bimodally or trimodally distributed in several populations, suggesting, among other factors, a polymorphic control of enzyme activity [24]. Genetic polymorphisms affecting CYP1A2 inducibility may have a role in carcinogen metabolism [25,26]. Using phenotypic assays to assess CYP1A2 and NAT2 polymorphism, an increased risk of colon cancer has been reported in persons who consumed red meat and were rapid metabolizers for both CYP1A2 and NAT2 [27,28].

In extrahepatic tissues, including human mammary epithelial cells, where the expression of CYP1A2 is barely detectable, CYP1A1 may have a greater role in HA *N*-hydroxylation [19,29]. *CYP1A1* variant alleles have been described which may affect enzyme inducibility, i.e. the A to G transition at base position 4889 in exon 7 results in the replacement of Ile by Val at the residue 462 which was significantly associated with gene inducibility and increased enzyme activity [30,31]. Variant genotypes at the *Msp1* site had no effect on *CYP1A1* gene induction [31]. Moreover, polymorphism of CYP1A1 may influence the in vivo functions of CYP1A2: individuals with the *CYP1A1* exon 7 common allele (Ile/Ile) had greater CYP1A2 activity than those with the heterozygous variant Ile/Val [32]. However, exposure to cigarette smoke or intake of high-temperature cooked meat resulted in an increased CYP1A2 activity in individual with the *CYP1A1* variant alleles compared to those with the common allele. This suggest that a product of CYP1A1 metabolism may increase CYP1A2 activity or, alternatively, that evolutionary determinants have provided advantages for gene-gene co-inducibility [32].

Another cytochrome P450 enzyme, CYP1B1, also produces *N*-OH-HA, though to a lesser extent [18,19]. The presence of variant alleles in the *CYP1B1* gene has been associated with an increased risk of breast and prostate cancer [33–35].

Two different genes, *NAT1* and *NAT2* encode for *N*-acetyltransferase activity in humans. Because both NAT2 and NAT1 catalyze the *O*-acetylation of HA, tissue specific expression of these enzymes is important for the polymorphism effect [36]. NAT2 activity is highest in the liver and gastrointestinal tract, whereas NAT1 activity is expressed in the liver and many extrahepatic tissues [37]. Both enzymes are polymorphic and the *NAT2* genotype segregates the human population into rapid and slow acetylators; *NAT1* genotypes seem to have little effect on enzyme activity [38,39].

The rapid acetylator phenotype appears to be associated with an increased risk of colon cancer, but it is still controversial [27,37,40–44]. An interaction between rapid/intermediate *NAT2* genotype and consumption of well-done meat was associated with an elevated risk of breast cancer, but not all studies support this finding [45–47]. No association between *NAT2* genotype and prostate cancer risk has been observed, though this association was reported with the *NAT1* genotype [37]. *NAT1* genotype was also reported to modify colorectal cancer risk [48], but not colorectal adenomas [49].

The members of the soluble sulfotransferase (SULT) enzyme superfamily catalyze the sulfation of xenobiotics. Humans have at least eleven SULT isoforms [50,51]. The major SULT form in human liver is encoded by the gene *SULT1A1* which is polymorphic, the mutation being associated with low enzyme activity [50,52–54]. SULT1A1 and SUL1A2 efficiently transfer a sulfonate moiety to *N*-OH-PhIP [55,56]. Several SULT1A2 alloenzymes were shown to activate to mutagenic derivatives different compounds, including a heterocyclic amine [51]. SULT1A1 activity is expressed in the liver and various human extrahepatic tissues, including breast and intestine, where the enzyme might play a role in the activation of HA [57]. The frequency of *SULT1A1* alleles was not different in control and colorectal cancer patients, but in patients diagnosed before 80 years of age the common allele was associated with a reduced risk of colorectal cancer [58]. Other studies, however, do not confirm this [41,59]. The risk of breast cancer was associated with *SULT1A1* genotype and the intake of well-done red meat [60]. *SULT1A1* genotype did not modify the risk of prostate cancer [61].

1.1.2. HA detoxification pathways

Acetylation and sulfation are activation pathways for HA, but several detoxification pathways exist too, including conjugation of reactive species with glutathione [62,63]. Several members of the GST superfamily are polymorphic. Most important are the polymorphisms of the *GSTM1* and *GSTT1* genes: null gene deletions result in the lack of the expression of the respective proteins in individuals who are homozygous-null [64]. *GSTA1* and *GSTA2* also show highly variable expression in human liver. This may depend on the presence of variant alleles in the *GSTA1* promoter [65]. *GSTA1-1* and, to a lesser extent, *GSTA2-2* catalyze the detoxification of *N*-acetoxy-PhIP by reducing the *N*-acetoxy derivative back to the parent amine [62,66].

The role of GST in cancer risk attributable to dietary HA is debated. A number of studies found no association between *GSTM1* polymorphism and the risk of colon or breast cancer [29,67,68], whereas others did [29,41,69]. Polymorphism of another member of the GST superfamily, *GSTA1*, affected colon cancer risk [66].

Glucuronidation is a major pathway of detoxification for a variety of chemical carcinogens. UGT-mediated glucuronidation might play a role in the detoxification of food-borne carcinogenic HA. In humans, detoxification of the reactive *N*-OH-PhIP to *N*-OH-PhIP-glucuronide appears to be catalyzed by members of the *UGT1A* gene family [70–73]. Glucuronidation of *N*-OH-PhIP has been observed in the liver and in extrahepatic tissues, particularly the intestine [70,72,73]. There is considerable inter-individual variability in the urinary excretion of *N*-OH-PhIP glucuronides, suggesting differences in the expression of the enzymes or genetic polymorphisms of the UGT isoforms [7]. In vitro, human UGT1A1 expressing microsomes had the highest capacity to conjugate *N*-OH-PhIP to glucuronic acid when compared with UGT1A4, UGT1A6, or UGT1A9 [72]. The *UGT1A1* gene's promoter containing seven thymine adenine repeats (TA) was less active than the wild-type six repeats [71]. Moreover, *UGT1A7*, which is expressed in gastrointestinal tissues but not in the liver, has been reported to be polymorphic with alleles having reduced UGT activity [74]. Polymorphism of the *UGT1A7* gene may modify colorectal cancer risk [74].

1.2. Effect of genetic polymorphisms on the metabolism of HA

One way of assessing the effect of genetic polymorphisms on the activation/detoxification of HA is to measure DNA-adducts in experimental and human studies.

1.2.1. In vitro studies

Using cell-free systems, different rat and human GST isozymes inhibited the binding of *N*-acetoxy-PhIP to calf thymus DNA to different extents, with GST of the alpha class being most effective [62]. The same study also evaluated GST activity in the cytosol from human liver and colon mucosa and found an enzymatic activity affecting DNA-adduct formation in liver but not colon mucosa samples [62]. The finding was in agreement with the different isozyme expression in different tissues [75]. DNA-adduct formation was increased when human hepatocytes were incubated with PhIP in the presence of GST or UGT inhibitors, suggesting these enzymes are involved in the detoxification pathways of HA [63].

Incubation of primary cultures of human mammary epithelial cells with 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-methylimidazo[4,5-*f*]quinoline (MeIQ), or PhIP, or with *N*-OH-PhIP or *N*-OH-IQ led to the formation of DNA-adducts, detected by ^{32}P-postlabelling. DNA-adduct levels were higher in cells from *NAT2* fast acetylators than slow acetylators, though the difference was not significant [76].

DNA-adduct formation in human prostate adenocarcinoma cell lines containing a silenced *GSTP1* gene or genetically modified to express GSTP1 was reduced by the presence of GSTP1 activity [77].

1.2.2. Animal studies

Studies with rat and human liver microsomes have suggested that CYP1A2 is critical for HA–DNA-adduct formation [78]. To assess how CYP1A2 contributes to HA metabolic activation to DNA binding species Snyderwine et al. measured the formation of IQ and PhIP–DNA-adducts in CYP1A2-null and wild-type mice [79]. After administration of PhIP, PhIP–DNA-adducts in liver, kidney, mammary gland and colon were significantly fewer in CYP1A2-null than in wild-type mice [79]. There was no difference in DNA-adducts in the mammary gland after administration of IQ, and a difference in IQ–DNA-adducts in the colon only at a low IQ dose, suggesting that the deficiency of CYP1A2 may have different effects for different HA [79]. The formation of DNA-adducts even in CYP1A2-null mice supports the notion that other cytochromes P450 are involved in HA activation.

Syrian hamsters congenic strains differing in the *NAT2* allele are a useful model for investigating the relationship between acetylation genotype and HA carcinogenesis [80]. Rapid and slow acetylator congenic hamsters are genetically identical, differing only at the *NAT2* locus. Using this animal model Fretland et al. showed that similar levels of PhIP–DNA-adducts were formed in a variety of tissues after PhIP administration, irrespective of the acetylation genotype [81]. However, despite the DNA-adduct formation, no tumors were observed in these animals given repeated oral doses of PhIP and fed a high fat diet for 1 year, suggesting that the Syrian hamster is relatively resistant to PhIP-induced cancer [81]. In agreement with this, Muckel et al. using *S. typhimurium* strains expressing human NAT1, NAT2, or SULT1A1, showed that *N*-OH-HA are activated to mutagenic derivatives by different human enzymes. In particular, *N*-OH-PhIP was activated by SULT1A1, but not by NAT1 or NAT2 [82]. No preneoplastic lesions or tumors

Table 1
Effect of genetic polymorphisms on biomarker levels in humans possibly exposed to HA

Heterocyclic amine	Biomarker	Polymorphism	Effect on biomarker	Reference
Unknown	DNA-adducts	NAT1 NAT2 slow	No effect Increased	[86]
Unknown	DNA-adducts	MPO decreased expression CYP1A1, CYP1A2, GSTT1, GSTM1, GSTP1, NQO1	Increased No effect	[87]
PhIP	PhIP–DNA-adduct	GSTA1 (low activity) NAT1, NAT2, SULT1A1, GSTM1	Increased No effect	[88]
MeIQx	MeIQx + phase II conjugated metabolites	CYP1A2 high activity NAT2	Decreased No effect	[6,93]
MeIQx	*N*-OH-MeIQx-N^2-glucuronide	CYP1A2 NAT2	No effect No effect	[95]
PhIP	PhIP + phase II conjugated metabolites	CYP1A2 high activity NAT2	Decreased No effect	[6,94]
PhIP	*N*-OH-PhIP-N^2-glucuronide	CYP1A2 high activity NAT2	Slightly increased No effect	[4,95]

were observed in the gastrointestinal tract of congenic rapid and slow acetylator Syrian hamsters given PhIP or IQ [83]. This is in contrast with reports of higher levels of PhIP–DNA-adducts in the colon and prostate of Fischer 344 rats (rapid acetylators) than in the same tissues of Wistar Kyoto rats (slow acetylators) [84]. PhIP induced a higher number of aberrant crypt foci in rapid than slow acetylator rats [85].

1.2.3. Human studies

A limited number of studies, summarized in Table 1, has dealt with the effect of genetic polymorphisms on DNA-adduct formation in humans likely to be exposed to HA. One reported a significant association between *NAT2* genetic polymorphism and DNA-adduct levels in breast tissue [86]. Women carrying the *NAT2* slow genotype had higher DNA-adduct levels in their breast tissue than those with the *NAT2* rapid genotype, but the adduct, which was analyzed by ^{32}P-postlabelling followed by HPLC, could not be identified as a HA-adduct [86]. In the same study *NAT1* genotypes were not associated with DNA-adduct levels [86].

Myeloperoxidases (MPO) can activate chemical carcinogens, including aromatic amines, to reactive species, and catalyze the endogenous formation of free radical-induced DNA damage. MPO have been detected in breast milk and are involved in DNA-adduct formation by activating HA in human mammary epithelial cells [29]. Human breast tissue samples from women carrying at least one variant *MPO* allele, implying decreased gene transcriptional activity, had significantly higher DNA-adduct levels than samples from individuals with the common allele [87]. This is contrary to expectations and suggests a detoxification role for MPO. The same study also reported higher DNA-adducts in individuals carrying at least one variant *GSTP1* allele compared to subjects homozygous for the common allele [87]. Polymorphisms of CYP1A1, CYP1A2, GSTT1, GSTM1, and NAD(P)H-quinone oxidoreductase did not affect DNA-adducts [87].

To address the role of the genetic polymorphism of *NAT1*, *NAT2*, *SULT1A1*, *GSTM1*, and *GSTA1* in the metabolism of PhIP, we investigated whether these polymorphisms are determinants of the formation of PhIP–DNA-adducts in the lymphocytes of colorectal cancer patients likely to be exposed to dietary PhIP. PhIP released from adducted DNA after hydrolysis was quantitated by liquid chromatography–tandem mass spectrometry after immunoaffinity purification [88]. PhIP–DNA-adducts varied widely, with a factor of more than 10 between the lowest and the highest level, suggesting variable PhIP intake and/or differences in activation, deactivation, and DNA repair.

In a previous study, we observed that meat consumers had higher PhIP–blood protein adducts than vegetarians [89]. Our more recent study confirmed that dietary habits are important determinants of PhIP–DNA-adduct formation [88]. None of the genetic polymorphisms of PhIP activating or detoxifying enzymes studied significantly affected PhIP–DNA-adducts. However, individuals carrying two mutated *GSTA1* alleles and younger than the median age had higher adduct levels than homozygous wild-type and heterozygous ones, suggesting that young people with lower detoxification capacity may form a subgroup particularly susceptible to dietary carcinogen [88].

Measuring the urinary excretion of HA metabolites is another way of monitoring human metabolic activation/detoxification patterns of HA. HA are readily bioavailable after a meat meal [4–6,90]. After administration of ^{14}C-PhIP to colon cancer patients, or after a cooked meat meal, a number of PhIP urinary metabolites have been identified, *N*-OH-PhIP-N^2-glucuronide being the most abundant [7,91,92]. All these studies showed large inter-individual

differences, suggesting that host factors may be involved, including polymorphisms of HA metabolizing enzymes.

The effect of metabolic polymorphisms on the urinary excretion of HA has been evaluated in several studies. The urinary excretion of unmodified 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) was reported to be lower in individuals with high CYP1A2 activity, suggesting that greater CYP1A2 activity may produce more HA reactive metabolites with consequent greater DNA damage [93]. Subsequent studies showed that total MeIQx (unmetabolized plus acid labile conjugates, including N^2-glucuronide and sulfamate metabolites) were higher in individuals with higher CYP1A2 activity, as detected by measuring the ratio of urinary caffeine metabolites, whereas total PhIP (unmetabolized plus acid labile conjugates) was not affected [6]. No association was observed between the NAT2 acetylation phenotype and MeIQx or PhIP total urinary excretion [6,94]. The urinary excretion of *N*-OH-MeIQx-N^2-glucuronide or *N*-OH-PhIP-N^2-glucuronide was not influenced by the NAT2 phenotype, indicating that this metabolic pathway has limited effect on HA clearance [4,95]. Urinary *N*-OH-PhIP-N^2-glucuronide, but not *N*-OH-MeIQx-N^2-glucuronide, was associated with the activity of CYP1A2, though the association was of borderline significance [4,95]. Two different oxidation pathways for MeIQx and PhIP explained this difference mediated by CYP1A2. Thus, the main PhIP urinary metabolite was *N*-OH-PhIP-N^2-glucuronide, whereas MeIQx was excreted most as MeIQx-8-carboxylic acid [96].

2. Concluding remarks

Despite some epidemiological evidence of an association between genetic polymorphisms and susceptibility to cancer possibly resulting from dietary exposure to HA, and the results of experimental studies on the effect of metabolic polymorphisms on the formation of a given biomarker, human studies have not given firm results.

We have a number of possible explanations for this. One might be the limited size of most molecular epidemiology studies so far, which lowers their statistical power. Often the laborious and time consuming analytical methods applied for the detection and quantitation of biomarkers limit the number of individuals in a study. In some instances, when ^{32}P-postlabelling is used for the quantitation of DNA-adducts, the lack of the chemical identity of the adduct(s) measured makes it difficult to establish an association between genetic polymorphisms and DNA-adduct formation.

Overall, the genetic polymorphisms had only slight effects on biomarker levels and do not explain the inter-individual variability, suggesting that additional factors such as smoke-induced enzyme activity or the effects of high vegetable intake on activating/detoxifying enzymes might prevail over or interact with the effect of genetic polymorphisms.

With cancer as an end-point to exposure to HA more investigations are needed to clarify gene–environment interactions. These studies should involve larger numbers of subjects, evaluate the exposure to HA better, and identify factors that might affect HA metabolism and hence cancer risk.

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