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Angaben zur Veröffentlichung / Publication details:

Lumbreras, B., S. Garte, K. Overvad, A. Tjonneland, F. Clavel-Chapelon, Jakob Linseisen, H. Boeing, et al. 2008. "Meat intake and bladder cancer in a prospective study: a role for heterocyclic aromatic amines?" *Cancer Causes and Control* 19 (6): 649–56. https://doi.org/10.1007/s10552-008-9121-1.

Meat intake and bladder cancer in a prospective study: a role for heterocyclic aromatic amines?

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Abstract
Background The suspect carcinogens, heterocyclic amines (HAAs), found in well-done meat require host-mediated metabolic activation before inducing DNA mutations. The role of *SULT1A1* and of *NAT2* on the activation of HAAs suggests that *NAT2* rapid acetylator genotype and *SULT1A1* allele variants can have an effect on HAA carcinogenicity.
Methods Data were collected as part of a case–control study nested within the EPIC cohort, the Gen Air

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investigation. EPIC is a prospective study designed to investigate the relationship between nutrition and cancer. Information was collected through a non-dietary questionnaire on lifestyle variables and through a dietary questionnaire. The subjects were restricted to non-smokers. We calculated the matched odds ratio for bladder cancer risk using logistic regression, controlling for potential confounders.

Results There were 227 bladder cases and 612 controls matched 1:3. Meat intake and *NAT2* genotype were not independently associated with bladder cancer risk. A significant relationship was observed between bladder cancer risk and consumption of meat only among subjects with the rapid *NAT2* genotype (odds ratios [OR] 2.9, 95% CI 1.0–7.9 for the 2nd quartile of meat intake; 3.6, 95% CI 1.3–9.7 for the 3rd quartile; and 3.5, 95% CI 1.2–9.7 for the 4th quartile), and was not present among subjects with the slow genotype. An interaction between *NAT2* and meat intake was found in logistic regression ($P = 0.034$). No association was observed for *SULT1A *1/2* genotype (1.0; 95% CI 0.7–1.5) and for *SULT1A1 *2/2* genotype (0.9; 95% CI 0.5–1.7).

Conclusions These results are suggestive of a role of meat intake and *NAT2* on bladder cancer risk. They support the hypothesis that among subjects with the rapid *NAT2* acetylation genotype higher levels of HAAs exposure are a bladder cancer risk factor. We did not observe an effect of *SULT1A1* allele variants on this cancer. The present study adds new information on the possible long-term adverse effects of diets with high meat intake.

Keywords Bladder cancer · Meat intake · *N*-acetyltransferase · Gene-environment interaction

Introduction

Bladder cancer is the second most common genitourinary cancer [1]. Known risk factors for lower urinary tract cancer include cigarette smoke and occupational exposures to aromatic amines [2, 3].

Meat intake has been suspected of being a risk factor for bladder cancer, but the evidence is rather sparse [4]. In a cohort study conducted in Hawaii, elevated rates of bladder cancer were found among subjects who had the most frequent intake of meat compared to those with the least intake [5]. In contrast, in a case–control study in Spain [6] a lower risk of bladder cancer was related to the highest intakes of meat. However, one might argue that the effect of meat intake could be observed in subjects with particular genotypes. Heterocyclic amines (HAAs), which are suspected carcinogens formed when meat is cooked at high temperatures until well done [7], require enzymatic activation to achieve their biological effects. Bioactivation of HAAs to carcinogenic metabolites in vivo is initiated by N-oxidation of the compound. This reaction occurs primarily in the liver and is catalyzed by hepatic cytochrome P450 1A2 (*CYP1A2*) [8]. Additional metabolism by acetyltransferases (*NAT*) [9] generates acetoxy esters, electrophiles that are much more reactive with DNA, producing covalent DNA adducts. It is hypothesized that the genetic variations in the genes encoding these metabolic enzymes might contribute the inter-individual variations of genetic susceptibility to bladder cancer. *NAT2* polymorphisms are very common in human populations, and individuals can be subdivided into rapid/intermediate and slow acetylator phenotypes [10].

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Some evidence of a role of HAA in carcinogenesis has come from epidemiological studies that have observed that well-done meat increases the risk of colorectal cancer, especially in individuals with the rapid phenotype for *CYP1A2* and *NAT2*. In fact, the evidence is not totally consistent [11, 12]. Some studies have also suggested that the rapid *NAT2* acetylator phenotype modifies the risk of breast cancer from meat intake, but the evidence is inconsistent [13–15].

Sulfonation is an important detoxification pathway for numerous xenobiotics and endogenous compounds, however it also plays an important role in the bioactivation of many dietary and environmental mutagens, including heterocyclic amines (HAAs), to form more reactive DNA-damaging electrophiles [16]. Sulfonation is catalyzed by members of the sulfotransferase (SULT) enzyme family, and in humans there are at least seven human SULT isoforms. One of them, *SULT1A1*, appears to be one of the most important members in the family due to its abundance and distribution in a wide variety of tissues. The *SULT1A1* gene possesses three polymorphisms resulting in an amino acid change, Arg to His, which has been observed to have a functional impact in humans [17]. The risk of colorectal cancer was reduced in *SULT1A1*2/2* genotype-subjects who consumed high amounts of red meat [18]. However, other studies did not find an overall association between the *SULT1A1*1/2* and *SULT1A1*2/2* allele genotypes and colorectal cancer [19, 20]. A case–control study observed evidence of a reduced bladder cancer risk associated with the *SULT1A1*2/2*, but it did not take into account the possible effect of diet or HAAs on the bladder cancer risk [17]. Thus, due to complex and dual roles of this enzyme in both activation and detoxification pathways the direction of the effect of this polymorphism cannot be predicted.

No study has been done, to our knowledge, on meat intake, the *NAT2* genotype, and *SULT1A1* polymorphism and bladder cancer. We have tested the hypothesis that meat intake can increase the risk of bladder cancer among rapid acetylators (*NAT2* rapid genotype) and in subjects with the His^{213} *SULT1A1* genotype in a nested case–control design in the EPIC (European Prospective Investigation into Cancer and Nutrition) investigation. To avoid confounding from a strong risk factor such as smoking, we have used only never smokers and ex-smokers since at least 10 years.

Methods

Patients and procedures

EPIC is a multicenter study, coordinated by the International Agency for Research on Cancer (Lyon), in which more than 500,000 volunteers have been recruited in 10 European countries (Sweden, Denmark, Norway, Netherlands, UK, France, Germany, Spain, Italy, Greece) [21]. The cohort includes subjects of both genders, mostly in the age range 35–74 at recruitment. Recruitment took place between 1993 and 1998. The usual diet over the previous 12 months was measured at EPIC study recruitment with the use of country-specific validated questionnaires [22]. A detailed computerized 24-h diet recall (24HR) method was used to obtain a second dietary measurement (between 1995 and 1999) from a random sample of the cohort (7.1% of total cohort; $n = 36{,}994$ participants) to calibrate dietary measurements across countries and to correct for systematic over- or underestimation of dietary intakes [23]. Intakes of meat, red meat, poultry and processed meat, were estimated, in grams per day, from information reported in the dietary questionnaires. Red meat intake included pork, beef, veal, and lamb. Poultry intake included chicken, turkey, and duck. Processed meat intake included ham, bacon, sausages, processed meat cuts, hamburgers (i.e., beef burgers), meatballs, and pâtés [24]. Lifestyle questionnaires included questions on reproductive history, use of oral contraceptives, use of hormone replacement therapy, education, physical activity, history of previous illness or surgical operation, and history of consumption of tobacco and alcoholic beverages. The follow-up was based on population cancer registries in seven of the participating countries: Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. In France, Germany, and Greece, we used a combination of methods including health insurance records, cancer and pathology registries, and active follow-up through study participants and their next-of-kin. Mortality data were also obtained from either the cancer registry or mortality registries at the regional or national level. Follow-up was virtually 100% complete. We used the 10th Revision of the International Statistical Classification of Diseases, Injuries, and Causes of Death (ICD). The median follow-up time when the present cases and controls were identified was 7 years.

Gen Air is a case–control study nested within the EPIC cohort, aiming at studying the relationship between air pollution or ETS (Environmental Tobacco Smoke) and cancers of the bladder (transitional-cell carcinomas), lung, oral cavity, pharynx, larynx, or leukemia, all newly diagnosed after recruitment [25]. Only never smokers or ex-smokers since at least 10 years have been included. We have matched three controls per case for exposure assessment and the analysis of questionnaire data, and two controls per case for laboratory analyses. Matching was introduced to allow strict control of potentially confounding variables. In addition, matching was needed for laboratory analyses, to avoid differential sample

degradation between cases and controls. Matching criteria were gender, age (plus or minus 5 years), smoking status (never/former smoker), country of recruitment, and time elapsed since recruitment (months). Gen Air has been approved by the Ethical Committee of the International Agency for Research on Cancer, and by all the local Ethical Committees of the participating centers. Polymorphisms in two genes involved in metabolism of HAAs were analyzed by Taqman assays, using DNA extracted from white blood cells. Laboratory methods of blood collection and storage, and polymorphism analysis have been previously described [26].

Statistical analyses

We have computed odds ratios (OR) and 95% confidence intervals (CI) in conditional logistic regression models that included educational level, energy intake, fruit and vegetables consumption, and physical activity as further adjustment variables in addition to matching variables. We also performed unconditional logistic regression analyses, which were based on the whole set of controls. We assessed interaction by creating indicator variables for the combination of single variables, and by the Breslow-Day test for heterogeneity [27]. All statistical tests were two-sided and *P*-values less than 0.05 were considered statistically significant. To calculate *P* values for trends across quartiles, participants were assigned a score ranging from 1 to 4 according to their quartile of meat intake. Analyses were performed with the SAS package (Cary, NC, USA) for a personal computer.

Results

The distribution by *NAT2* genotype and meat intake is shown in Table 1. In model I, we included 118 bladder cancer cases and 213 controls for whom *NAT2* has been looked at (DNA available); we also included 1,063 controls matched to other cancer sites. In model II, we included 227 bladder cancer cases in Gen Air and their 612 controls matched 1:3; we also incorporated 3,010 Gen Air matched controls. Details on the distribution by demographic and other characteristics are given elsewhere [28]. The frequency of rapid *NAT2* genotype was similar for cases (56.8%) and controls (55.3%). We did not find an association between either *NAT2* genotype (rapid *NAT2* genotype compared to slow *NAT2* genotype) (model 1, Table 1) nor meat intake overall (model 2, Table 1) and bladder cancer.

When we stratified by *NAT2* (Table 2; based on unconditional regression for statistical instability of conditional models), a strong association became apparent between meat intake and bladder cancer in rapid acetylators, but not in slow acetylators. Among subjects with the rapid *NAT2* genotype, the 4th quartile of meat intake was associated with a 3-fold risk of bladder cancer compared with those in the 1st quartile of meat intake (odds ratio

Table 1 Odds ratios (OR) and 95% Confidence Intervals for bladder cancer and *NAT2* genotype (model I) and bladder cancer and quartiles of meat intake (model II)

			Controls		Cases
			(a)	(b)	
Model I	*NAT2* genotype	Slow	475	87	51
		Rapid	588	126	67
		Total	1,063	213	118
		OR and 95% CI 1.1, 0.7–1.6			
Model II	Quartiles of meat intake[1]	Controls		Cases	OR (95% CI)*
		(a)	(b)		
	1st	793	149	44	1.0
	2nd	736	138	61	1.4 (0.7–2.6)
	3rd	729	143	64	1.6 (0.8–3.0)
	4th	752	182	58	1.4 (0.7–2.7)
	Total	3,010	612	227	
	P value for trend = 0.11				

(a) All controls irrespective of matching (b) Matched controls

[1] Quartiles of meat intake (mean and difference between highest and lowest value in g/dl): quartile 1, 32 and 58; quartile 2, 75 and 53; quartile 3, 110 and 40; quartile 4, 180 and 472

*OR are all adjusted by gender, age (plus or minus 5 yrs), smoking habits (former or never smoker), country, school years, BMI, physical activity, intake of fruit, vegetables, meat, and energy (quartiles of each)

Table 2 Unadjusted and adjusted odds ratios (OR) and 95% Confidence Intervals for bladder cancer and quartiles of meat intake by slow and rapid acetylator status

Quartiles of meat intake[1]	Cases	Controls		ORcrude*	ORadj*	95% CI
Only slow acetylators						
		(a)	(b)			
1st	12	98	17	1.0	1.0	–
2nd	14	125	17	0.9	0.8	0.3–1.9
3rd	13	110	16	0.96	0.9	0.4–2.5
4th	12	142	37	0.69	0.7	0.3–1.9
P-trend = 0.41						
Only rapid acetylators						
		(a)	(b)			
1st	6	129	27	1.0	1.0	–
2nd	15	131	28	2.5	2.9	1.0–7.9
3rd	23	151	31	3.3	3.6	1.3–9.7
4th	23	177	40	2.8	3.5	1.2–9.7
P-trend = 0.037						

(a) All controls irrespective of matching (b) Matched controls

[1] Quartiles of meat intake (mean and difference between highest and lowest value in g/dl): quartile 1, 32 and 58; quartile 2, 75 and 53; quartile 3, 110 and 40; quartile 4, 180 and 472

*OR are all adjusted by gender, age (plus or minus 5 years), smoking habits (former or never smoker), country, school years, BMI, physical activity, intake of fruit, vegetables, and energy (quartiles)

Table 3 Adjusted risk for bladder cancer and SULT1A1 genotype

		Cases	Controls	Adj OR*	95% CI
SULT1A1 genotype	1/1	57	513	1.0	
	1/2	54	451	1.0	0.7–1.5
	2/2	13	125	0.9	0.5–1.7
	Total	124	1,089		

*OR are all adjusted by gender, age (plus or minus 5 years), smoking habits (former or never smoker), country, school years, BMI, physical activity, intake of fruit, vegetables, and energy (quartiles)

[OR] 3.4; 95% CI 1.2–9.7). An interaction between *NAT2* and meat intake (quartiles) was found in logistic regression ($P = 0.034$).

There was no association between *SULT1A1* allele variants and bladder cancer when we compared the *SULT1A1*1/2* genotype (odds ratio [OR] 1.0; 95% CI 0.7–1.5) and the *SULT1A1*2/2* genotype (odds ratio [OR] 0.9; 95% CI 0.5–1.7) to the *SULT1A1*1/1* genotype (Table 3). There was no interaction between *SULT1A1* polymorphism and meat intake ($P = 0.347$).

Discussion

Metabolic activation of carcinogenic HAAs is a multistep process catalyzed by both phase I and phase II enzymes. One working hypothesis suggests initial *N*-oxidation by hepatic *CYP1A2*, followed by transport of the *N*-hydroxy-heterocyclic amine to tumor target organs, where it undergoes acetylation catalyzed by *NAT* (s) and sulfonation by *SULT1A1*, which ultimately leads to DNA adducts and possibly mutations [9, 29]. This hypothesis suggests that, compared with subjects with the slow *NAT2* phenotype, subjects with the rapid/intermediate *NAT2* phenotype may more readily activate HAAs present in meat to reactive metabolites that form DNA adducts. Sulfonation, as an activation pathway of HAAs, is substantially reduced in carriers of the *SULT1A1*1/2* and *SULT1A1*2/2* allelic variants. The present study is the first to examine the role of genotype on the association of meat intake with bladder cancer risk. No effect of *SULT1A1*1/2* and *SULT1A1*2/2* allelic variants on bladder cancer was observed compared to the reference category *SULT1A1*1/1*. There was no interaction between *SULT1A1* genotypes and meat intake. Neither *NAT2* genotype nor meat intake alone was associated with bladder cancer risk in our nested case–control study. However, consistent with the increased risk for colorectal cancer [14] and for breast cancer [15] conferred by the rapid *NAT2* phenotype, bladder cancer risk was more than three times greater among the rapid acetylators who consumed more meat (within the 4th quartile of meat intake), relative to the slow *NAT2* acetylators with low meat consumption. Thus, this study implies that exposure to HAAs that are substrate for *NAT2*, may increase the risk of bladder cancer. The association was greatest in higher

quartiles of meat intake. This is consistent with previous studies in which the amount of meat influenced the risk of human cancer [30–32]. Other investigations found only slightly increased risks of bladder cancer in the highest versus the lowest quintile of meat intake [33], but they did not consider the role of *NAT2*.

Chemical and mutagenicity analyses of meat have suggested that HAAs are the primary chemical class of compounds responsible for the observed mutagenic activity of cooked meat [34]. About 5 years after the discovery that mutagens could be formed in meat while cooking at high temperature, Baker et al. [35] also demonstrated that consumption of cooked meat resulted in mutagenic urine in humans. This observation has been confirmed and extended by others [36, 37]. Urinary excretion of mutagens can directly affect the urinary mucosa. However, data in two recent prospective cohort studies observed a positive association for bladder cancer risk and intake of chicken without skin, but not for chicken with skin or for others meats, including processed meats, hot dogs, and hamburgers [38].

Epidemiologic studies of the potential role of HAAs in human carcinogenesis have mostly focused on colorectal cancer. Several investigators have studied the interaction of meat intake with acetylator status and found positive associations, although not all have been statistically significant [11, 12]. Concerning bladder cancer, human epidemiological studies associated the slow acetylator phenotype with a higher incidence of urinary bladder cancer after aromatic amine exposure [39], for which *N*-acetylation is a detoxification step [10]. The first association between slow acetylator phenotype and urinary bladder cancer was reported many years ago [40]. The mechanism for this association suggests that slow *NAT2* acetylation of aromatic amine carcinogens competes poorly with metabolic activation via cytochrome P450(s) and/or prostaglandin H-synthases, thus accounting for the higher risk in the slow *NAT2* phenotype(s). However, for substances for which *N*-acetylation is an activating step, such as HAAs, rapid acetylators are expected to be at higher risk. This hypothesis has been tested for breast and colorectal cancer, but not yet for bladder cancer. In the present study, an association between HAAs, rapid *NAT2* genotype and bladder cancer risk has been observed, but only in people with high intake of meat.

Epidemiologic studies have observed a range of effects associated with the *SULT1A1* polymorphisms, including both increased and decreased risks of cancer [18–20]. In a previous study [17], *SULT1A1* polymorphism was examined in the context of bladder cancer observing a statistically significantly reduced risk for *SULT1A1*1/2* and *SULT1A1*2/2* compared to *SULT1A1*1/1*. However, we did not observe any effect. *SULT1A1* is important in both the bioactivation and detoxification of various dietary and environmental mutagens and its role may depend on the tissue or organ [40, 41]. Thus, further studies with a higher number of samples are needed to examine the possible role of the *SULT1A1*1/2* and *SULT1A1*2/2* alleles in bladder cancer risk.

We have to consider the limitations of our study. First, we were not able to obtain DNA for all the eligible subjects, but from 68% on average. In particular, we obtained DNA for only 10% subjects from France and for 50% of the eligible UK subjects. After exclusion of these countries, the distribution by relevant variables did not differ between subjects with DNA and subjects without DNA (data not shown). However, this smaller number of subjects with available DNA data has affected the precision of our results, particularly in the study by acetylator status. Second, our results could also be affected by measurement error in dietary intake, a common limitation of epidemiologic studies. The wide range of intakes of meat and processed meat reported in the EPIC study reduced, but did not eliminate, potential effects of measurement error. Because the magnitude of the distortion in the estimated relative risk depends on the ratio of the inter-individual variation to the intra-individual measurement error [42], regression dilution should have been lessened by the inclusion of a diverse range of intakes. Finally, accurate assessment of exposure to HAAs, through parameters such as cooking methods, is essential for the evaluation of human cancer risks [43]. Unfortunately, only some of the EPIC dietary questionnaires recorded detailed information about the method used to cook meat or the frequency of intake of more-well-done versus less-well-done meat. The variation between regions is very high: the frequency of use of high-temperature cooking methods (i.e., grilling, frying, or barbecuing) varied from 15% in the EPIC cohort of Italy to 49% in the EPIC cohort of The Netherlands [44].

The bladder cancer risk could be associated not only with exposure to HAAs but also with the presence of other risk factors in the group studied, such as diet and lifestyle characteristics. In our study, the presence of other risk factors was determined through questionnaires and they were included in the final model.

It is well established that smoking increases the risk of bladder cancer, probably because tobacco smoking is a primary source of exposure to aromatic amines in the general population, and aromatic amines are suspected of being the primary bladder carcinogen in tobacco smoking [45]. Previous analyses have suggested that the relative risk for smoking is stronger for *NAT2* slow than for rapid/intermediate acetylators [46] and for *SULT1A1*1/1* than *SULT1A1*1/2* and *SULT1A1*2/2* [17]. However, in this study confounding by smoking was avoided restricting the subjects to non-smokers.

In summary, our results suggest that exposure to HAAs through the consumption of meat may be a bladder cancer risk factor among subjects with the *NAT2* rapid genotype. The ubiquitous exposure to HAAs together with high frequency of the *NAT2* acetylation polymorphism in human populations suggests that exposure to HAAs could be an important risk factor for human cancer. The present study adds new information on the possible long-term adverse effects of diets with high protein content, particularly from meat.

Acknowledgments This paper was made possible by a grant of the European Community (5th Framework Program) to PV (GENAIR investigation, grant QLK4-CT-1999–00927) and a grant of the Compagnia di San Paolo to the ISI Foundation. All authors are independent from funders.

References

1. World Health Organization. The world health report. Geneva, Switzerland: World Health Organization, 2003
2. Vineis P, Simonato L (1991) Proportion of lung and bladder cancers in males resulting from occupation: a systematic approach. Arch Environ Health 46:6–15
3. Vineis P, Talaska G, Malaveille C et al (1996) DNA adducts in urothelial cells: relationship with biomarkers of exposure to arylamines and polycyclic aromatic hydrocarbons from tobacco smoke. Int J Cancer 65:314–316
4. Steinmaus CM, Nunez S, Smith AH (2000) Diet and bladder cancer: a meta-analysis of six dietary variables. Am J Epidemiol 151:693–702
5. Chyou PH, Nomura AM, Stemmermann GN (1993) A prospective study of diet, smoking, and lower urinary tract cancer. Ann Epidemiol 3:211–216
6. Riboli E, Gonzalez CA, Lopez-Abente G et al (1991) Diet and bladder cancer in Spain: a multi-centre case–control study. Int J Cancer 49:214–219
7. Gross GA, Gruter A (1992) Quantitation of mutagenic/carcinogenic heterocyclic aromatic amines in food products. J Chromatogr 592:271–278
8. Boobis AR, Lynch AM, Murray S et al (1994) CYP1A2-catalyzed conversion of dietary heterocyclic amines to their major route of metabolism in humans. Cancer Res 54:89–94
9. Minchin RF, Reeves PT, Teitel CH et al (1992) *N*-and *O*-acetylation of aromatic and heterocyclic amine carcinogens by human monomorphic and polymorphic acetyltransferases expressed in COS-1 cells. Biochem Biophys Res Commun 185:839–844
10. Hein DW, Doll MA, Fretland AJ et al (2000) Molecular genetics and epidemiology of the *NAT1* and *NAT2* acetylation polymorphisms. Cancer Epidemiol Biomarkers Prev 9:29–42
11. Berlau J, Glei M, Pool-Zobel BL (2004) Colon cancer risk factors from nutrition. Anal Bioanal Chem 378:737–743
12. Butler LM, Sinha R, Millikan RC et al (2003) Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. Am J Epidemiol 157:434–445
13. Webster DJ, Flook D, Jenkins J, Hutchings A, Routledge PA (1992) Drug acetylation in breast cancer. Br J Cancer 60: 236–237
14. Ilett KF, Detchon P, Ingram DM, Castleden WM (1990) Acetylation phenotype is not associated with breast cancer. Cancer Res 50:6649–6651
15. Sardas S, Cok I, Sardas OS, Ilhan O, Karakaya AE (1990) Polymorphic *N*-acetylation capacity in breast cancer patients. Int J Cancer 46:1138–1139
16. Ward MH, Sinha R, Heineman EF, Rothman N, Markin R, Weisenburger DD et al (1997) Risk of adenocarcinoma of the stomach and esophagus with meat cooking method and doneness preference. Int J Cancer 71:14–19
17. Zheng L, Wang Y, Schabath MB, Grossman HB, Wu X (2003) Sulfotransferase 1A1 (SULT1A1) polymorphism and bladder cancer risk: a case–control study. Cancer Lett 202:61–69
18. Nowell S, Coles B, Sinha R et al (2002) Analysis of total meat intake and exposure to individual heterocyclic amines in a case–control study of colorectal cancer: contribution of metabolic variation to risk. Mutat Res 506–507:175–185
19. Wong CF, Liyou N, Leggett B, Young J, Johnson A, McManus ME (2002) Association of the SULT1A1 R213H polymorphism with colorectal cancer. Clin Exp Pharmacol Physiol 29:754–758
20. Sachse C, Smith G, Wilkie MJ et al Colorectal Cancer Study Group (2002) A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. Carcinogenesis 23:1839–1849
21. Riboli E, Hunt KJ, Slimani N et al (2002) European prospective investigation into cancer and nutrition (EPIC): study populations and data collection. Public Health Nutr 5:1113–1124
22. Margetts BM, Pietinen P (1997) European prospective investigation into cancer and nutrition: validity studies on dietary assessment methods. Int J Epidemiol 26 Suppl 1:S1–S5
23. Rosner B, Gore R (2001) Measurement error correction in nutritional epidemiology based on individual foods, with application to the relation of diet to breast cancer. Am J Epidemiol 154:827–835
24. Linseisen J, Kesse E, Slimani N et al (2002) Meat consumption in the European prospective investigation into cancer and nutrition (EPIC) cohorts: results from 24-hour dietary recalls. Public Health Nutr 5:1243–1258
25. Peluso M, Hainaut P, Airoldi L et al EPIC investigators (2005) Methodology of laboratory measurements in prospective studies on gene-environment interactions: the experience of GenAir. Mutat Res 574:92–104
26. Manuguerra M, Matullo G, Veglia F et al (2007) Multi-factor dimensionality reduction applied to a large prospective investigation on gene-gene and gene-environment interactions. Carcinogenesis 28:414–422
27. Reis IM, Hirji KF, Afifi AA (1999) Exact and asymptotic tests for homogeneity in several 2 x 2 tables. Stat Med 18:893–906
28. Vineis P, Veglia F, Garte S, Malaveille C, Matullo G, Dunning A et al (2007) Genetic susceptibility according to three metabolic pathways in cancers of the lung and bladder and in myeloid leukemias in nonsmokers. Ann Oncol 18:1230–1242
29. Chou HC, Ozawa S, Fu PP, Lang NP, Kadlubar FF (1998) Metabolic activation of methyl-hydroxylated derivatives of 7,12-dimethylbenz[a]anthracene by human liver dehydroepiandrosterone-steroid sulfotransferase. Carcinogenesis 19:1071–1076
30. Sandhu MS, White IR, McPherson K (2001) Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. Cancer Epidemiol Biomarkers Prev 10:439–446
31. Norat T, Riboli E (2001) Meat consumption and colorectal cancer: a review of epidemiologic evidence. Nutr Rev 59:37–47
32. Norat T, Lukanova A, Ferrari P, Riboli E (2002) Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiological studies. Int J Cancer 98:241–256
33. Augustsson K, Skog K, Jägerstad M, Dickman PW, Steineck G (1999) Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: a population-based study. Lancet 353:703–707

34. Dolara P, Commoner B, Vithayathil A et al (1979) The effect of temperature on the formation of mutagens in heated beef stock and cooked ground beef. Mutat Res 60:231–237
35. Baker R, Arlauskas A, Bonin A, Angus D (1982) Detection of mutagenic activity in human urine following fried pork or bacon meals. Cancer Lett 16:81–89
36. Murray S, Lake BG, Gray S et al (2001) Effect of cruciferous vegetable consumption on heterocyclic aromatic amine metabolism in man. Carcinogenesis 22:1413–1420
37. Pavanello S, Simioli P, Mastrangelo G et al (2002) Role of metabolic polymorphisms *NAT2* and CYP1A2 on urinary mutagenicity after a pan-fried hamburger meal. Food Chem Toxicol 40:1139–1144
38. Michaud DS, Holick CN, Giovannucci E, Stampfer MJ (2006) Meat intake and bladder cancer risk in 2 prospective cohort studies. Am J Clin Nutr 84:1177–1183
39. Garcia-Closas M, Malats N, Silverman D et al (2005) *NAT2* slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish bladder cancer study and meta-analyses. Lancet 366:649–659
40. Lower GM Jr, Nilsson T, Nelson CE, Wolf H, Gamsky TE, Bryan GT (1979) N-Acetyltransferase phenotype and risk in urinary bladder cancer: approaches in molecular epidemiology. Preliminary results in Sweden and Denmark. Environ Health Perspect 29:71–79
41. Glatt H, Boeing H, Engelke CE et al (2001) Human cytosolic sulphotransferases: genetics, characteristics, toxicological aspects. Mutat Res 482:27–40
42. Day N, McKeown N, Wong M, Welch A, Bingham S (2001) Epidemiological assessment of diet: a comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium. Int J Epidemiol 30:309–317
43. Skog KI, Johansson MA, Jagerstad MI (1998) Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. Food Chem Toxicol 36:879–896
44. Rohrmann S, Linseisen J, Becker N, Sinha R EPIC Working Group on Dietary Patterns (2002) Use of high-temperature cooking methods in preparation of meat and fish in European countries. IARC Sci Publ 156:219–220
45. Vineis P, Pirastu R (1997) Aromatic amines and cancer. Cancer Causes Control 8:346–355
46. Vineis P, Marinelli D, Autrup H et al (2001) Current smoking, occupation, *N*-acetyltransferase-2 and bladder cancer: a pooled analysis of genotype-based studies. Cancer Epidemiol Biomarkers Prev 10:1249–1252