Low CYP1A2 activity associated with testicular cancer

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The incidence rate of testicular cancer has increased during the last 50 years. An interplay between changing environmental factors and individual susceptibility, e.g. in foreign compound metabolizing enzymes, may have important influences on the risk of testicular cancer. The cytochrome P4501A2 (CYP1A2) enzyme and the bimodally expressed enzyme N-acetyltransferase2 (NAT2) metabolize many procarcinogens/carcinogens. The aim of this population-based case-control study was to investigate if CYP1A2 or NAT2 activity measured as a ratio of urinary metabolites of dietary caffeine is a risk factor in testicular cancer. 378 men participated (80 seminomas, 104 non-seminomas and 194 controls). The CYP1A2 activity was lower in the cases than in the controls [median and 30–70% percentiles: 4.7 (3.9–5.7) and 5.2 (4.4–6.4), respectively]. The subjects were classified in tertiles with low, medium or high CYP1A2 activity. A low CYP1A2 activity was associated with the highest risk of testicular cancer. Including all participants except men using drugs suspected to influence CYP1A2 activity ($n = 15$), medium and low activity conferred odds ratios (ORs) of 1.54 [confidence intervals of 95% ($CI_{95\%}$) 0.93–2.55] and 2.11; $CI_{95\%}$ (1.23–3.62), respectively, of having testicular cancer. Excluding smokers ($n = 157$) the ORs of medium and low activity were 3.63; $CI_{95\%}$ (1.53–8.60) and 4.70; $CI_{95\%}$ (2.03–10.89), respectively. After further exclusion of cases that had received chemotherapy or radiation ($n = 47$), similar significant results were achieved. In the groups with the lowest CYP1A2 activity the ORs for seminoma and non-seminoma were 2.12; $CI_{95\%}$ (0.93–4.81) and 2.10; $CI_{95\%}$ (1.02–4.32). The phenotype of NAT2 was not associated with testicular cancer. In conclusion, we found no association of NAT2 phenotype to testicular cancer, whereas significant associations between CYP1A2 activity and testicular cancer were shown.

Introduction

Large geographical and temporal variation in the incidence rates of testicular cancer has been shown, with Denmark having one of the highest rates in the world and a steady increase during the last 50 years (1,2). The only major predisposing factor of testicular cancer identified so far is cryptorchidism, i.e. undescended testicle, with an increased risk of about 4 (3,4). Furthermore, it has been shown that first-degree relatives have an increased risk of testicular cancer (5,6).

These observations support the hypothesis that interaction between exposure to unidentified environmental factor(s) and genetic susceptibility may influence the risk of testicular cancer.

The predominant aetiological hypothesis suggests that prenatal exposure to hormone disruptors, including oestrogens, play a major role in testicular cancer as well as in cryptorchidism (7,8). This is supported by higher incidence of testicular cancer in firstborns where the mothers have higher levels of oestrogens (9,10).

Most cancers are believed to be related to activation of procarcinogens by the body's own enzymes. The cytochrome P450 isozyme system is important in this aspect and can metabolize a huge number of foreign compounds including drugs and hormones (11–16). Enzymes of this system, exemplified by cytochrome P4501A2 (CYP1A2), may be inhibited or induced by a variety of substances, e.g. cruciferous vegetables and contraceptives, respectively (17–21).

Large inter-individual differences in the activity of CYP1A2 are found indicating that genetic factors are supposed to influence, although the significance is controversial (11,21). A unimodal distribution of a urinary metabolite ratio of an *in vivo* marker of CYP1A2 activity (caffeine) has been shown by several groups (21–23). Other investigators describe trimodal distribution of an alternative urinary metabolite ratio of caffeine as an *in vivo* marker of CYP1A2 (24). Genetic polymorphisms in CYP1A2 have not been convincingly shown to relate to activity, although a significantly lower activity was shown in smokers having a point mutation compared with smokers with the wild-type (25–28). High CYP1A2 activity has been associated with polyps in colon or colorectal cancer (29,30). Recently, a large twin study showed a heritability estimate of CYP1A2 activity assessed as the caffeine metabolite ratio of 0.725, whereas unique environmental factors (excluding smoking and oral contraceptives) appeared to account for the remainder 0.275 (31).

The enzyme N-acetyltransferase 2 (NAT2) activates (by O-acetylation) and inactivates (by N-acetylation) several carcinogens (32). The expression of NAT2 is genetically determined by a polymorphism. The phenotypically determined slow acetylators are homozygous for the slow acetylator gene, and the fast acetylators are heterozygous or homozygous for the fast acetylator gene (33). The acetylator phenotype and/or genotype have/has been associated with increased risk of

cancer of the urinary bladder, breast, colorectal, lung and liver, although conflicting results have been shown (34,35).

Only a few case-control studies on the possible association between xenobiotic metabolizing enzymes and testicular cancer have been published. Comparison of the metabolism of antipyrine has been performed between eight men with disseminated non-seminomatous testicular cancer and 14 controls (36). Antipyrine is partially metabolized by CYP1A2 with preferential formation of the metabolite 3-hydroxymethylantipyrine (HMA) (37). The formation of the HMA metabolite was shown to be significantly increased in cases compared with controls both before and after cytostatic treatment (36). We have investigated previously the possibility of deficiency of the detoxifying enzyme glutathione *S*-transferase M1 (GSTM1) in men with testicular cancer (38). No evidence of an association between GSTM1 deficiency and testicular cancer was found.

We investigated the hypothesis: men with a deviating xenobiotic metabolizing enzyme activity have an increased risk for testicular cancer We conducted a population-based case-control study of testicular cancer in Denmark with estimations of the activities of CYP1A2 and NAT2, enzymes that are involved in activation and elimination of a variety of endogenous and exogenous substances.

Materials and methods

Study design

The protocol was approved by the local ethics committees, and the study was conducted in accordance with the Declaration of Helsinki. All participants signed written informed consent. The basic design of the study and the results regarding GSTM1 genotypes and phenotypes have been published previously (38).

A population-based case-control design was used. Cases of histologically verified testicular cancer were identified from the files of the Danish Cancer Registry and from the in-patient files of the oncological departments in the Greater Copenhagen Area. Additional eligibility criteria included: unilateral testicular cancer (seminoma or non-seminoma), time of diagnosis January 1, 1989 to June 30, 1993, age 18–45 years at the time of diagnosis, at least 6 months elapsed since any radiation or chemotherapy, Caucasian race, Denmark as the place of birth, alive and living in the Greater Copenhagen area at the time of participation.

The cases were classified as seminoma or non-seminoma according to the histological diagnosis of the tumours. For each group of cases, a group of male controls matched for the year of birth was drawn at random from the Danish National Population Registry. The eligibility criteria of the controls included: Caucasian race, Denmark as the place of birth, alive and living in the Greater Copenhagen area at the time of participation.

228 cases and 327 controls were asked by letter to participate in the investigation with one visit at a hospital for interview and collection of blood and urine samples. 184 cases [80 (76.9%) seminomas, 104 (83.9%) non-seminomas] and 194 (59.3%) controls participated in the investigation. The 184 cases had had unilateral orchidectomy 1.0–6.3 years (median = 3.4) before participating. Ninety-two cases (50%) did not receive further treatment. Seventy-seven cases (42%) had received chemotherapy, 11 (6%) had received radiation and only four (2%) had received both. At the time of participation all patients were clinically cured, and a period of 0.5–5.9 years (median 2.3) had passed since termination of treatment with chemotherapy or radiation.

All participants gave information regarding height, weight, their consumption of alcoholic beverages, coffee, tea, cola, the duration of exercise during the preceding 2 weeks, the ingestion of drugs and whether they suffered from cryptorchidism in their childhood.

Determination of CYP1A2 and NAT2 activity

Dietary caffeine was used as a probe and some selected metabolites of caffeine were measured in the urine. The participants consumed three cups of coffee, tea or cola 2–6 h before sampling. A urine sample of 5 ml was immediately acidified with 100 μl 2 M HCl and stored at −20°C. As soon as possible (most often the day after sampling) acidification to pH 3.5 was ensured.
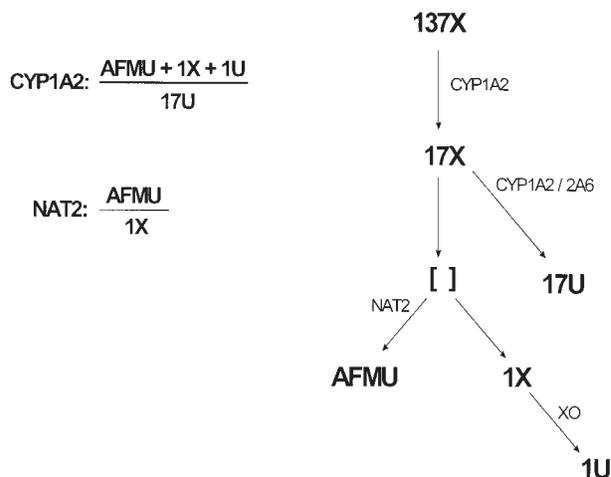

Fig. 1. Caffeine metabolism and ratios reflecting CYP1A2 and NAT2. 137X, 1,3,7-trimethylxanthine (caffeine). 17X, 1,7-dimethylxanthine; 17 U, 1,7-dimethyl uric acid.

The five major metabolites of caffeine 5-acetyl-6-formylamino-3-uracil (AFMU), 1-methylxanthine (1X), 1-methyluric acid (1 U), 1,7-dimethylxanthine (17X) and 1,7-dimethyl uric acid (17 U) were determined by a standard HPLC method modified by using gradient control (Figure 1) (21,39). The solvents used for elution were A: 0.05% acetic acid containing 6.5% methanol (v/v), pH 3.2; B: 0.05% acetic acid containing 20.0% methanol (v/v), pH 7.0. Typical conditions for elution were 0% B (0–10.5 min), 0–40% B (10.5–19 min), 40–100% B (19–24 min), 100% B (24–27 min) and 100–0% B (27–29 min), 0% B (29–35 min). The analytical inter-day coefficient of variation of the caffeine metabolic ratio was 6.6%. This is in accordance with other studies in which similar methods were used (20,40). The limit of detection was ~10 μmol/l for each metabolite.

The caffeine metabolic ratio (AFMU + 1X + 1 U)/17 U was used to estimate the CYP1A2 activity. Campbell *et al*. have shown previously that the caffeine metabolite ratios (AFMU + 1 U + 1X)/17 U after multiple caffeine administrations were similar to the ratios obtained after a pooled 24-h urine sample obtained after ingestion of one single caffeine dose. Furthermore, they showed that the mean coefficient of variation among (AFMU + 1 U + 1X)/17 U) ratios obtained from three randomly collected urine samples after chronic dietary caffeine intake and one sample obtained from a 24-h pooled urine after a single dose of caffeine was 12% (range 4–21%) (39). This supports the relevance of using the caffeine metabolic ratio in population studies.

The metabolic ratio AFMU/1X was used to determine the NAT2 phenotype. The antimode that separates slow acetylators from fast and intermediate acetylators was 0.55.

The CYP1A2 and NAT2 phenotypes were determined in 357 participants (77 seminomas, 97 non-seminomas and 183 controls), in 21 participants the phenotype was not determinable (10 cases and 11 controls) due to low levels of caffeine metabolites in the urine.

Hormonal analyses

Hormone analyses were performed at The Department of Growth and Reproduction, Copenhagen University Hospital Rigshospitalet. Commercial kits were used. Testosterone was analysed by radioimmuno-assay (Coat-a-count®, DPC, Los Angeles, CA). [Lowest detectable dose 0.23 nmol/l, intra-assay coefficients of variation (CV) 17%, inter-assay CV 16.7%.] Oestradiol was analysed by radioimmuno-assay (Pantex, Santa Monica, CA). (Lowest detectable dose 18 pmol/l, intra-assay CV 7.5%, inter-assay CV 12.9%.) SHBG was analysed by time-resolved fluoroimmunoassay (DELFIA, Wallac, Turku, Finland). (Lowest detectable dose 0.23 nmol/l, intra-assay CV 5.1%, inter-assay CV 4.8%.)

Sample size calculation

Estimation of the CYP1A2 activity was the primary efficacy variable. Pre-study sample size calculations were performed with the following assumptions: the risk of type one error, 5%; the risk of type two error, 20%; the standard deviation, 2.59 and a difference in the CYP1A2 activity between cases and control of minimum, 1.0. A group size of 105 was estimated.

Statistical evaluation of data

A group was matched by age and sex to each of the histological subgroups, seminoma and non-seminoma.

The statistical evaluations of the demographic values and the frequencies of having cryptorchidism in childhood have been published elsewhere (38).

The values of the caffeine metabolic ratios (CYP1A2 activity) were compared for cases and controls as well as for smoking controls and non-smoking controls, respectively, by means of the Mann–Whitney U test. CYP1A2 activities of the controls (after excluding all current smokers and men who used drugs suspected to affect the CYP1A2 activity) were divided in tertiles, and according to this the population was categorized in groups with low, medium or high CYP1A2 activity. The crude odds ratios (ORs) with 95% confidence intervals for testicular cancer (seminomas and non-seminomas combined and each type separately) were estimated as the odds of testicular cancer patients having low or medium levels of CYP1A2 activity, respectively, divided by the odds of control subjects having low or medium levels of CYP1A2 activity, respectively.

In order to control for confounding analyses a logistic regression was performed. The potential confounders were the factors that may affect the level of CYP1A2, such as tobacco smoking, consumption of alcoholic beverages, coffee, tea, cola, cabbage and charcoal-grilled meat, the duration of exercise during the preceding 2 weeks, age, height, weight and the plasma level of total oestradiols. All potential confounders were tested for correlation with CYP1A2 by Spearman rank correlation or Mann–Whitney U test (only cryptorchidism). All potential confounders were tested separately by logistic regression using the status of case/control as the dependent variable. A potential confounder was included in a logistic regression model if the *P*-value was ≤0.10 in both tests. None of the potential confounders were found to be confounders in this material. Cryptorchidism and age were included as obligatory confounders in the analyses.

The OR with 95% confidence intervals for each type of testicular cancer and the types combined were estimated for the NAT2 phenotype (slow acetylator) by comparison with the respective control group.

The statistics were handled by using STATISTICA® 5.0.

Results

Demographic values

The cases and controls were similar with respect to age, height and weight, the daily consumption of beverages containing alcohol or caffeine, smoking habits and the duration of exercise during the preceding 2 weeks (analysed in ref. 38). Cryptorchidism was shown to be a significant risk factor for testicular cancer [OR = 3.77; CI$_{95\%}$ (1.97–7.21)], indicating that this study has statistical power to detect risk factors (38).

CYP1A2

The distribution of the caffeine metabolic ratios was unimodal but not normal. The medians, 30 and 70% percentiles were 4.7 (3.9–5.7) and 5.2 (4.4–6.4) for cases and controls, respectively (*P* = 0.01) (Figure 2). The medians, 30 and 70% percentiles of the controls were for non-smokers 4.5 (3.8–5.1) and for smokers 6.6 (5.5–7.8), respectively (*P* = 0.00).
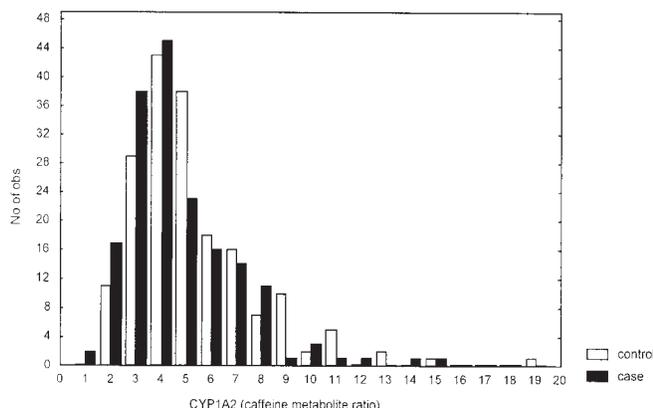

Fig. 2. Distribution of CYP1A2 of the cases and controls.

The association between testicular cancer and CYP1A2 was estimated in different groups/subgroups, with elimination of factors with potential influence on CYP activity. Group 1 included all participants except men using drugs known to influence the activity of CYP1A2 (*n* = 15). Group 2 was derived from group 1 after the exclusion of all current smokers. Group 3 was derived from group 2 after a further exclusion of cases formerly treated with chemotherapy or radiation. The crude OR were determined in each group (Table I).

A low CYP1A2 ratio was associated with the highest risk of testicular cancer (Table I). Including all participants except men using drugs suspected to affect the activity of CYP1A2 (group 1), medium and low activity conferred the ORs 1.54 and 2.11 of which only the low activity was significantly associated to testicular cancer. After exclusion of smokers [(*n* = 157), group 2], in order to reduce variation, the ORs of medium and low activity 3.63 and 4.70, respectively, were still associated with testicular cancer. After elimination of cases, who had received chemotherapy or radiation [(*n* = 47), group 3], which hypothetically could affect the CYP1A2 activity, similar significant results were achieved.

Analyses of associations of testicular cancer and CYP1A2 were further performed in the histological subgroups seminoma and non-seminoma. All participants were included except men using drugs known to influence the activity of CYP1A2. Because of the smaller number of participants the data were only analysed for group 1 (including all participants; Tables II–III). In the group of non-seminoma a significant association with testicular cancer and CYP1A2 activity was shown in the group with lowest CYP1A2 activity. In the group with medium CYP1A2 activity an insignificant increased OR was found. In the group of seminoma insignificant increased ORs were found.

To control for confounding, logistic regressions were performed (Table IV). Model 1 included all participants. Model 2 included all participants except smokers and men using drugs suspected to influence the activity of CYP1A2. Model 3 was further restricted since all cases who had received chemotherapy or radiation were excluded. The increased risk was associated with a low CYP1A2 activity. In Table IV the OR of the risk for testicular cancer has been estimated for a man with a caffeine metabolic ratio 1 lower than another man. Testicular cancer was shown to be significantly associated with CYP1A2 activity.

Table I. Testicular cancer and CYP1A2

	Cases	Controls	OR	CI
Group 1				
Lowest tertile	49	33	2.11	(1.23–3.62)
Middle tertile	51	47	1.54	(0.93–2.55)
Highest tertile	67	95	1	–
Group 2				
Lowest tertile	47	32	4.70	(2.03–10.89)
Middle tertile	34	30	3.63	(1.53–8.60)
Highest tertile	10	32	1	–
Group 3				
Lowest tertile	21	32	3.50	(1.25–9.82)
Middle tertile	17	30	3.02	(1.05–8.69)
Highest tertile	6	32	1	–

The crude OR and 95% confidence intervals of testicular cancer for low or medium CYP1A2 activity. The tertiles of high CYP1A2 activity were used as references.

Table II. The crude OR and 95% confidence intervals of testicular cancer (seminoma) for low or medium CYP1A2 activity

CYP1A2	Seminoma	Seminoma controls	OR	CI
Low	22	15	2.12	(0.93–4.81)
Medium	24	19	1.82	(0.84–3.97)
High	27	39	1	–

All seminomas and controls of seminomas except men using drugs suspected to influence the CYP1A2 activity are included.

Table III. The crude OR and 95% confidence intervals of testicular cancer (non-seminoma) for low or medium CYP1A2 activity

CYP1A2	Non-seminoma	Non-seminoma controls	OR	CI
Low	27	18	2.10	(1.02–4.32)
Medium	27	28	1.35	(0.69–2.63)
High	40	56	1	–

All non-seminomas and controls of non-seminomas except men using drugs suspected to influence the CYP1A2 activity are included.

Table IV. Associations between CYP1A2 activity and testicular cancer

Model[a]	Number of cases/controls[b]	OR (CI$_{95\%}$)[c]	
		Unadjusted	Adjusted[d]
1	164/181	1.11 (1.02–1.22)	1.11 (1.01–1.22)
2	84/92	1.45 (1.14–1.83)	1.42 (1.12–1.81)
3	38/92	1.58 (1.14–2.20)	1.51 (1.08–2.12)

[a]Models explained in the text.
[b]Men with unknown status of cryptorchidism were excluded in these analyses.
[c]OR and 95% confidence interval. The OR of the risk for testicular cancer has been estimated for a man with a caffeine metabolic ratio 1 lower than another man.
[d]Adjusted for cryptorchidism and age.

Logistic regression analyses of model 1 were performed for the histological subgroups seminoma and non-seminoma. The unadjusted OR of the association between seminoma and CYP1A2 activity was 1.21 CI$_{95\%}$ (1.04–1.41), and this was unchanged after adjustment for cryptorchidism and age. A model of logistic regression did not fit the data found in the group of non-seminoma.

The levels of total oestradiols were not statistically different between cases and controls [median and 30–70% percentiles: 78.0 pmol/l (65.5–88.0) and 82.0 pmol/l (67.0–97.0), $P = 0.13$ Mann-Whitney U test]. With regard to the criteria of inclusion in the statistical analyses, the level of total oestradiols was rejected as a confounder and therefore not included in the logistic regression analyses.

Testosterone is not known to influence the activity of CYP1A2 and therefore not primarily included in the logistic regression analyses of CYP1A2. The levels of free testosterone [testosterone index (testosterone/sex hormone-binding globulin (SHBG) * 100] were not statistically different between cases and controls [median and 30–70% percentiles: 44.0 (36.2–54.6) and 44.6 (37.2–52.9), $P = 0.90$ Mann–Whitney U test]. However, the level of total testosterone was significantly lower in cases than in controls [median and 30–70% percentiles: 14.7 nmol/l (11.9–17.6) and 16.3 nmol/l (13.6–19.1), $P = 0.004$ Mann–Whitney U test). Therefore, we tested if testosterone was a confounder in the analyses of CYP1A2 activity.

Table V. The distribution of NAT2 phenotype in the study population

NAT2 phenotype	NAT2 slow[a]	NAT2 fast[b]	OR (CI$_{95\%}$)
All cases	105 (60%)	69 (40%)	1.08 (0.71–1.65)
All controls	107 (59%)	76 (41%)	
Seminoma	42 (55%)	35 (45%)	0.82 (0.43–1.54)
Seminoma controls	47 (59%)	32 (41%)	
Non-seminoma	63 (65%)	34 (35%)	1.36 (0.77–2.40)
Non-seminoma controls	60 (58%)	44 (42%)	

The association of having testicular cancer and NAT2 slow was estimated as OR with 95% confidence interval (CI$_{95\%}$).
[a]NAT2 slow acetylator.
[b]NAT2 fast acetylator.
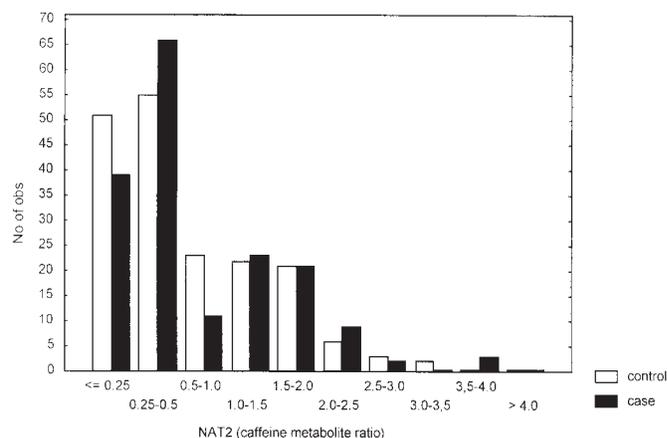
Fig. 3. Distribution of NAT2 of the cases and controls.

In respect to the criteria of inclusion (see Statistics) total testosterone fitted into the logistic regression model. In a logistic regression model including all cases and controls, total testosterone was found to be a confounder, and testicular cancer was no longer significantly associated with CYP1A2 activity [OR = 1.09; CI$_{95\%}$ (0.99–1.20)]. However, excluding all smokers and men using drugs suspected to influence CYP1A2, total testosterone was found not to be a confounder.

No significant differences in the caffeine metabolic ratios were found between the group treated with chemotherapy and orchiectomy compared with the group, which had had orchiectomy (Mann–Whitney U test; $P = 0.95$ and $P = 0.90$ for all patients/cases and for non-smokers, respectively).

NAT2

A bimodal distribution of NAT2 was shown. The cut-off was 0.55. The frequencies of the NAT2 slow acetylators ranged from 55 to 65% between the groups (Table V and Figure 3). In the group of controls 59% were slow acetylators.

The NAT2 phenotype was not associated with seminoma, non-seminoma or the combined types (Table V). The proportion of slow acetylators in the group of cases solely treated with orchiectomy ($n = 85$) was 56%, and it was not significantly different from the proportion in the controls (χ^2-test, $P = 0.86$).

Discussion

The present study identified CYP1A2 activity assessed as a metabolic ratio of caffeine as a risk factor of testicular cancer, whereas the NAT2 phenotype was not a risk factor.

CYP1A2 activity was significantly associated with testicular cancer with the lowest activity of CYP1A2 having the highest

risk of testicular cancer. A similar association was also significant in the subgroup of cases with non-seminoma. The association between testicular cancer and CYP1A2 was consistent and even stronger after elimination of ~50% of the cases, who were smokers, used drugs suspected to affect the activity of CYP1A2 or had been treated with radiation and/or chemotherapy. This indicates that the differences between cases and controls found in this material were not due to the treatment of the cases or differences in smoking habits between cases and controls. Moreover, in non-smokers the CYP1A2 activity has been shown to be determined mainly by heritable factors (31). Furthermore, the activity of CYP1A2 appears to be relatively constant in persons examined several times (21).

The metabolism of antipyrine has been studied in men with testicular cancer (36). Antipyrine is partially metabolized by CYP1A2 with preferential formation of the metabolite HMA. The study included eight cases and 14 controls shortly after diagnosis and weeks after the start of the last chemotherapy treatment cycle (36). In that study the formation of the HMA in the group of cases was significantly increased compared with the group of controls both before and after treatment with cytostatics (cisplatin, vinblatin and bleomycin). These results appear to be in contrast to the present data. However, these cases could be affected by the disease itself and/or by treatment with chemotherapy. No data of the long-term effects of chemotherapy or radiation on CYP1A2 activity in humans is available. In the present study, the CYP1A2 activity in cases that, in addition to orchiectomy, received chemotherapy did not differ significantly from the CYP1A2 activity in cases that solely received orchiectomy. The validity of the results in the present study are supposed to be higher, as the case-control study is population-based, the number of participants is higher, the rate of participation is satisfactory, at least 6 months elapsed since any treatment of testicular cancer and the fact that caffeine is a more specific probe of the CYP1A2 activity than antipyrine used in the study of Teunissen et al. (36). Furthermore, the Dutch study was not designed to identify metabolic risk factors of testicular cancer, but rather to quantify drug elimination capacity in these patients in the acute phase.

In the present study the cases had a lower CYP1A2 activity than the controls.

CYP1A2 has the ability to activate and inactivate carcinogens and the logical conclusion of this result would be, that the testicular cancer patients are deficient in respect to inactivate carcinogens. However, many carcinogens are activated and inactivated by complicated pathways that are far from totally described. Estrogens have been suggested to be involved in the development of testicular cancer and CYP1A2 is involved in the 2-hydroxylation of estradiol with formation of 2-hydroxyestrone (2-OHE1) (15,16,41). In experimental studies, decreased formation of 2-OHE1 seem to be associated with tumor formation (42) and a low 2-OHE1/16α-OHE1 ratio has been found in postmenopausal breast cancer patients (44) although this ratio did not convincingly predict the risk of breast cancer in prospective studies (45,46).

Whereas estrogens have been implicated in cancer development, the complicated oestrogen metabolism by CYP1A2 and other enzymes, and the possible interactions with testosterone, make it difficult to evaluate if the low CYP1A2 indicates a change in oestrogen metabolism that favours development of testicular cancer, or if it is related to decreased detoxification of (other) environmental carcinogens.

In this study the phenotype of NAT2 was not associated with testicular cancer. The narrow confidence intervals exclude the phenotypically determined NAT2 as a major risk factor of testicular cancer.

Fifty-nine per cent of the controls were slow acetylators, which was in agreement with previously published results in Danes (21,47,48). The phenotypical acetylator status has been claimed to change in breast cancer patients before and after treatment with chemotherapy (49). In our laboratory we have shown previously reproducibility in healthy subjects of the phenotypically determined acetylator status, however, we have never tested men treated previously with chemotherapy (21). The subjects in the present study were tested at least 6 months after treatment with chemotherapy or radiation and/ or orchiectomy and further, the subgroup of cases that was not treated with chemotherapy or radiation did not differ from the group of controls.

A satisfying concordance has been shown between simultaneous determination of geno- and phenotype by which only 2–7% was misclassified, when caffeine was used as a probe (50,51). However, this study could have been extended with estimation of the distribution of the mutations responsible for the slow phenotypes, since the activities of hetero- and homozygotic rapid acetylators had been shown to differ significantly, and therefore hypothetically could give rise to different risks (51).

The use of central population and cancer registers with complete registration and the high rate of participation minimize the potential risk of bias of selection. The validity of the study was furthermore strengthened because all the participants were drawn from the racially homogeneous population of men born in Denmark.

This study supports the hypothesis that the risk of testicular cancer could be modified by susceptibility factors. However, the mechanisms of the CYP1A2 enzymes ability to modify the risk of testicular cancer are still uncovered.

Acknowledgements

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