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Genotype and phenotype of glutathione *S*-transferase μ in testicular cancer patients

Kirsten Vistisen[1*], Helene Priemé[1], Henrik Okkels[2], Susanne Vallentin[3], Steffen Loft[1], Jørgen H. Olsen[4] and Henrik E. Poulsen[1,5‡]

[1]*Department of Pharmacology, The Panum Institute, University of Copenhagen, Denmark*
[2]*Department of Environmental and Occupational Medicine, University of Aarhus, Denmark*
[3]*Department of Oncology, Herlev University Hospital, Copenhagen, Denmark*
[4]*Danish Cancer Society, Division for Cancer Epidemiology, Copenhagen, Denmark*
[5]*Department of Clinical Pharmacology Q, Rigshospitalet, Copenhagen, Denmark*

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The incidence rate of testicular cancer has been steadily increasing during the last 50 years, and only cryptorchidism, i.e. undescended testes, has been identified as an important risk factor. An interplay between changing environmental factors and genetic susceptibility e.g. in foreign compound metabolizing enzymes, may have important influences on the risk.

The aim of this study was to investigate if glutathione *S*-transferase μ (GSTμ) deficiency, which in previous studies has been associated with malignant melanoma and cancers of the lung and bladder, is a risk factor of testicular cancer. Three hundred and seventy-eight men participated (80 seminomas, 104 non-seminomas and 194 controls) in a population-based case-control study.

The phenotype of GSTμ was determined in 366 men by ELISA, the genotype was determined in 324 men by polymerase chain reaction. The concordance between geno- and phenotype was 94.4%. The odds ratio of having the GSTμ negative phenotype and testicular cancer was 1.08, (0.72–1.64; 95% confidence interval (CI)), and the odds ratio of having the *GSTM1* null genotype and testicular cancer was 1.10; $CI_{95\%}$ (0.71–1.70).

This study provides no evidence of an association between phenotypically determined GSTμ deficiency or *GSTM1* null genotype and testicular cancer. The narrow confidence intervals rule out GSTμ as a major single risk factor for testicular cancer.

Keywords: glutathione *S*-transferase μ, testicular cancer, population-based

Introduction

The incidence of testicular cancer shows a large international variation. The incidence rate in Denmark is one of the highest in the world being 8.9 per 100 000 per year in 1990 (Parkin *et al.*, 1992; Møller, 1993), and in general the highest incidences are found in the industrialized countries. In Denmark the incidence has been steadily increasing during the last 50 years, except for men born during World War II, who maintain a reduced lifetime risk (Møller, 1993). Despite numerous epidemiological studies in this field only cryptorchidism has been identified as an important risk factor, and it explains only a minor fraction of all testicular cancer cases (Forman *et al.*, 1990; Abratt *et al.*, 1992).

Brothers of patients with testicular cancer may have an up to 12-fold increased risk of developing the disease and the risk for bilateral cancer seems higher than expected by chance alone, suggesting that a genetic predisposition could be important (Forman *et al.*, 1992; Nicholson & Harland, 1995; Westergaard *et al.*, 1996). However, the rapid increase in incidence rate suggests that environmental factors also influence the

* To whom correspondence should be addressed at: Department of Pharmacology, University of Copenhagen, The Panum Institute 18-5-50, Blegdamsvej 3, DK-2200 Copenhagen N., Denmark. Tel: +45 35 32 76 55; Fax: +45 35 32 76 10.
‡ To whom reprint requests should be addressed at: Department of Clinical Pharmacology Q 76-4-2, Rigshospitalet, University of Copenhagen, Tagensvej 20, DK-2200 Copenhagen N., Denmark.

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risk for testicular cancer. An interplay between genetic and environmental factors could relate to the genetic control of the detoxification enzymes eliminating or deactivating carcinogens.

GSTμ belongs to a multi isoenzyme system with several genetic polymorphisms and importance for detoxification and deactivation of carcinogens (Hayes & Pulford, 1995). About 50% of Caucasians show a genetical deficiency in GSTμ activity due to a deletion in the *GSTM1* gene *(GSTM1*0/0)* (Board, 1981; Seidegaard *et al.*, 1988; Hayes & Pulford, 1995). This deficiency has been associated with an increased risk for cancer of a number of sites including the lung, bladder, colon, skin and stomach (Hayes & Pulford, 1995; Raunio *et al.*, 1995; Nebert *et al.*, 1996). To date no data have been published on associations between testicular cancer and GSTμ activity.

In this study we examined, if GSTμ deficiency relates to testicular cancer, since this might give some clue about the involvement of environmental carcinogens in testicular cancer.

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 informed consent.

A population-based case-control design was employed. Cases of histologically verified testicular cancer were identified from the files of the Danish Cancer Registry and from the in-patient files at 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 at the time of diagnosis, at least 6 months elapsed since any radio- or chemotherapy, Caucasian race, Denmark as the place of birth, alive and living in the Greater Copenhagen area at the time of participation.

Of 238 eligible cases identified, ten were not contacted: two had died in the intervening period, six were treated for other primary diseases, one had atrophy of the remaining testicle and one had requested not to be contacted by the hospital.

The cases were divided in two groups (seminoma and non-seminoma) according to the histological diagnosis of the tumours. For each group of cases, a group of controls was drawn at random from the Danish National Population Registry with the following eligibility criteria: male, born in Denmark between 1945 and 1973, alive and living in the Greater Copenhagen area at the time of participation. The controls were frequency matched to the cases for the year of birth.

Two hundred and twenty-eight 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. One hundred and eighty-four cases (80 (76.9%) seminomas, 104 (83.9%) non-seminomas) and 194 (59.3%) controls participated in the investigation. The 184 cases (80.7%) 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%) received chemotherapy, 11 (6%) received radiation and only four (2%) received both. At the time of participation all 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 delivered blood samples except four men (three cases and one control), who refused to deliver blood, or in whom a venepuncture was impossible due to previous chemotherapy. All participants gave information regarding height, weight, their consumption of alcoholic beverages, coffee, tea, cola, the duration of their exercise during the preceding two weeks, the ingestion of drugs, and whether they suffered from cryptorchidism in their childhood. All tobacco containing items smoked were counted as cigarette-equivalents (Prignot, 1987). The consumption of caffeine was estimated as the caffeine index calculated as the number of cups (150 ml) of coffee plus 0.3 times the number of cups of tea plus 0.18 times the number of cups of cola (Barone & Roberts, 1996).

Blood samples for determination of the pheno- and genotype of GSTμ

The phenotype of the enzyme GSTμ in blood cells was determined once by the commercial ELISA-kit, MUKIT ® (Biotrin International, Ireland). Blood samples were collected by venepuncture in heparinized tubes centrifugated at 350 ×**g** for 10 min and after removal of plasma stored at −20 °C until analysis. The GSTμ phenotypes were determined in 366 participants (80 seminomas, 98 non-seminomas and 188 controls), in eight participants the phenotypes were non-determinable (three cases and five controls). The *GSTM1* genotype was determined twice on the same sample by polymerase chain reaction (PCR) technique as described elsewhere (Autrup *et al.*, 1995). Blood samples were collected by venepuncture in heparinized tubes stored at −20 °C until analysis. The GSTμ genotypes were determined in 324 participants (79 seminomas, 97 non-seminomas and 148 controls).

Table 1. Demographic characteristics (median, 30 and 70% percentiles) of the study population

	Seminoma	*Controls of seminoma*	*Non-seminoma*	*Controls of non-seminoma*
No. of subjects	80	83	104	111
Age (years)	36.4 (33.4–40.8)	36.9 (33.5–40.8)	31.5 (28.9–35.5)	31.4 (28.6–35.5)
Height (cm)	182.0 (178.0–186.0)	179.0 (176.0–183.0)	183.0 (179.0–186.0)	182.0 (178.0–185.0)
Weight (kg)	80.5 (76.5–87.0)	80.0 (75.5–87.0)	77.5 (73.0–85.0)	79.0 (75.0–85.0)
Intake alcohol (units per week)	10.0 (6.3–20.0)	9.5 (6.0–16.0)	9.0 (4.0–17.0)	12.0 (7.0–20.0)
Index of caffeine daily	5.0 (3.3–7.2)	5.2 (4.0–7.0)	4.7 (2.4–7.4)	4.6 (2.5–6.6)
Exercise (hours per fortnight)	3.00 (0–6.0)	3.0 (0.0–7.0)	3.3 (0.0–7.0)	4.0 (0.0–8.0)
No. of smokers	36 (45.0%)	39 (47.0%)	48 (46.2%)	52 (46.9%)
No. of cigarettes smoked daily	16.3 (12.5–20.0)	20.0 (15.0–20.0)	17.5 (15.0–20.0)	20.0 (12.5–20.0)

* $p < 0.05$ Mann Whitney U test.

Table 2. The distribution of GSTμ phenotype in the study population

	GST−[a]		*GST+*[b]		*OR*	*95% CI*	p-*value*[c]
	n	%	n	%			
Seminoma ($n = 80$)	51	64	29	36	1.34	0.71–2.52	0.46
Seminoma controls ($n = 81$)	46	57	35	43			
Non-seminoma ($n = 98$)	50	51	48	49	0.91	0.53–1.58	0.86
Non-seminoma controls ($n = 107$)	57	53	50	47			
All testicular cancer patients ($n = 178$)	101	57	77	43	1.08	0.72–1.64	0.79
All controls ($n = 188$)	103	55	85	45			

Based on 178 cases and 188 controls.
[a] GSTμ phenotype negative.
[b] GSTμ phenotype positive.
[c] χ^2 test.

Statistical analysis

The demographic values (age, height, weight, consumption of alcohol, caffeine and tobacco and amount of exercising) of the groups were compared by means of the Mann-Whitney U test. The frequencies of the GSTμ phenotype and *GSTM1* genotype were compared between the groups by the χ^2 test. For all statistical evaluations, a two-sided p value of 0.05 was considered to be statistically significant. The odds ratios (OR) with 95% confidence intervals for each type of testicular cancer and the types combined were estimated for the GSTμ negative phenotype and the *GSTM1* null genotype by comparison with the respective control group.

Results

The cases and controls were similar with respect to age, height and weight, the daily consumption of beverages containing alcohol or caffeine and the duration of exercise during the preceding 2 weeks (Table 1).

Tables 2 and 3 show the frequencies of the GSTμ phenotypes and the *GSTM1* genotypes in controls and in the groups of seminomas, non-seminomas and all cases, no significant differences were found. The ORs of having the GSTμ negative phenotype and testicular cancer ranged from 0.91–1.34, no significant associations were found (Table 2). The ORs of having the *GSTM1* null genotype and testicular cancer ranged from 0.84–1.54, no significant associations were found (Table 3).

Among the controls 55% were phenotypically deficient in respect to the GSTμ isoenzyme, and the frequency of the *GSTM1* null genotype in the group of controls was 49%. In 319 men both the geno- and the phenotype were determined, and the concordance between the geno- and the phenotype was 94.4 % (14/319 had no *in vivo* enzyme activity and no mutation at the locus of *GSTM1*, and 4/319 had *in vivo* enzyme activity and the *GSTM1* null genotype).

When stratifying on smoking habits, the ORs of having testicular cancer and GSTμ negative phenotype

Table 3. The distribution of GSTμ genotype in the study population

	GST−[a]		*GST+*[b]		*OR*	*95% CI*	p-*value*[c]
	n	%	n	%			
Seminoma ($n = 79$)	49	62	30	38	1.54	0.79–2.98	0.27
Seminoma controls ($n = 66$)	34	52	32	48			
Non-seminoma ($n = 97$)	42	43	55	57	0.84	0.47–1.52	0.67
Non-seminoma controls ($n = 82$)	39	48	43	52			
All testicular cancer patients ($n = 176$)	91	52	85	48	1.10	0.71–1.70	0.75
All controls ($n = 148$)	73	49	75	51			

Based on 176 cases and 148 controls.
[a] GSTμ genotype null.
[b] GSTμ genotype not null.
[c] χ^2test.

Table 4. Frequencies of partcipant who reported cryptorchidism in their childhood

	n	*Cryptorchidism*	*Unknown*	*No cryptorchidism*
Seminoma	80	19 (24%)**	4 (5%)	57 (71%)
Seminoma controls	83	4 (5%)	1 (1%)	78 (94%)
Non-seminoma	104	21 (20%)*	5 (5%)	78 (75%)
Non-seminoma controls	111	10 (9%)	1 (1%)	100 (90%)

χ^2 test ,* $p < 0.05$, ** $p < 0.001$.

were 0.98 $CI_{95\%}$ (0.53–1.80) for smokers and 1.18 $CI_{95\%}$ (0.67–2.08) for non-smokers.

Among the cases with either seminoma or non-seminoma a significantly higher proportion reported suffering from cryptorchidism in their childhood, as compared to the corresponding control group (Table 4). The OR of having testicular cancer in men reporting cryptorchidism was significant 3.77 $CI_{95\%}$ (1.97–7.21).

Discussion

In this study we investigated a possible association between GSTμ deficiency and testicular cancer. To our knowledge, this is the first case-control study relating testicular cancer to the metabolic capacity for activation or inactivation of carcinogens. Although our finding is negative, it is still possible that other enzymes activating or inactivating carcinogens could be risk factors and thus might help understanding the familiar occurrence, the geographical variation, the rapidly increasing incidence and the relation to environmental chemicals.

The use of central population and cancer registries with complete registration and the high rate of participation minimize the potential risk of bias of selection. The validity of the study was further strengthened because all the participants were drawn from the racially homogeneous population of men born in Denmark. In this setting the narrow confidence intervals of the ORs overlapping one indicate, that GSTμ deficiency is not a risk factor for testicular cancer.

The results in the group of controls confirm previous studies, showing that 55% are phenotypically deficient in respect to the GSTμ isoenzyme (Brockmöller *et al.*, 1994). The frequency of the *GSTM1* null genotype in the controls was 49%, which agrees with the single previously published study in Denmark (Autrup *et al.*, 1995). Our study showed 94.4% concordance between the geno- and phenotype in agreement with previous studies showing 90–99% accordance (Brockmöller *et al.*, 1993). In the present study re-testing was not possible. Taking the genotypes as the 'true measure', 14 were false negative and four were false positive. This could be due to environmental factors not identified in this study. Some drugs such as ethacrynic acid and indomethacin are well-known inhibitors of glutathione *S*-transferases (Hansson *et al.*, 1991; Beckett & Hayes, 1993). In this study the participants did not use any of these drugs at the time of partcipation.

Cryptorchidism has been shown to be a risk factor

for testicular cancer (Forman *et al.*, 1990). The present study confirmed this showing that the study had sufficient statistical power to detect risk factors.

GSTμ is distributed in many tissues, mainly in the liver but also in the testicular tissue and testicular tumours (Klys *et al.*, 1992). GSTμ exists in five subclasses, GSTM1-5. GSTM3 and 4 have been shown in testicular tissue (Campbell *et al.*, 1990: Morel *et al.*, 1994). In our study we determined the null *GSTM1* genotype corresponding to the GSTμ phenotype in blood.

Exposure to specific chemical(s) in susceptible individuals could be responsible for the development of many cancer diseases, and the importance of investigating the distributions of the polymorphically activating and deactivating (defence) enzymes is obvious. With narrow confidence intervals this study did not show signs of any association between testicular cancer and the polymorphism of GSTμ. Specific compounds (carcinogens) inactivated by GSTμ are not likely to be involved in the development of testicular cancer.

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