Genetic variability of the forkhead box O3 and prostate cancer risk in the European Prospective Investigation on Cancer

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Abstract. Forkhead box O3 (FOXO3) has a wide range of functions: it promotes tumor suppression, cell cycle arrest, repair of damaged DNA, detoxification of reactive oxygen species, apoptosis and plays a pivotal role in promoting longevity. FOXO3 is a key downstream target of the PI3K-Akt pathway in response to cellular stimulation by growth factors or insulin and has been proposed as a bridge between ageing and tumor

suppression. Three SNPs in the FOXO3 gene (rs3800231, rs9400239 and rs479744) that have been shown to be strongly and consistently associated with longevity, were examined in relation to PC risk in a case control study of 1571 incident PC cases and 1840 controls nested within the European Prospective Investigation into Cancer and Nutrition (EPIC). There was no statistically significant association between the SNPs and PC risk regardless of the model of inheritance (dominant, codominant and recessive). The associations were not modified by disease aggressiveness, circulating levels of steroid sex hormones, or IGFs or BMI. We conclude that polymorphisms in the *FOXO3* gene that are associated with longevity are not major risk factors for PC risk, in this population of Caucasian men.

Introduction

FOXO transcription factors belong to the large forkhead family of proteins, a group of transcriptional regulators characterized by a conserved DNA-binding domain termed the 'forkhead box' (1). Emerging evidence suggests that FOXO factors play a role as tumor suppressors in a variety of cancers, translating environmental stimuli into changes in gene expression programs that may coordinate organismal longevity and tumor suppression (2-5).

Forkhead box O3 (FOXO3) has a wide range of functions: it promotes tumor suppression, cell cycle arrest, repair of damaged DNA, detoxification of reactive oxygen species, apoptosis and autophagy, by upregulating specific gene-expression programs. In addition, it can also regulate energy metabolism and development of a number of tissues including the prostate (3-5). Consistent with a key role of FOXO3 in tumor suppression, this transcription factor is a key downstream target of the PI3K-Akt pathway in response to cellular stimulation by growth factors or insulin (6). Akt/PKB is often constitutively activated in prostate cancer (PC) (7,8). This results in the deactivation of FOXO factors (9), which in turn improves PC cells survival (10) and PC progression from androgen-dependence to androgen-independence (11). In contrast, overexpression of FOXO3 in PC cells causes apoptosis and induction of genes that inhibt cellular proliferation (6). Moreover, FOXO factors have been found to physically interact with several tumor suppressors, including p53 (2,12) and SMAD transcription factors (13).

The ability FOXO3 shows in controlling many critical cellular functions may be related to its demonstrated role in promoting longevity. It is possible that FOXO3 could mediate the effect of ageing on tumor suppression. The hortologue of FOXO3 in worms (DAF-16) controls the response to critical threats to the organism such as oxidative stress, heat stress, resistence to UV radiation, protein damage and pathogens assault, leading to an increase in lifespan. Many of the mechanisms against which FOXO3 provides protection are directly involved in carcinogenesis in humans (5).

Several studies have been conducted on single nucleotide polymorphism (SNPs) belonging to the *FOXO3* gene in relation to traits such as BMI (14) and ageing (15,16). In particular, three SNPs (rs3800231, rs9400239 and rs479744) were recently found to be strongly associated with longevity in Europeans and Asian populations (15,16).

Given the important role FOXO3 plays in PC, the strong effect that the three SNPs have on the longevity phenotype and the overlap between ageing and cancer processes, we hypothesized that they could confer altered PC susceptibility. We tested the association of rs3800231, rs9400239 and rs479744 with PC risk in a study of 1571 invasive PC cases and 1840 controls nested within the European Prospective Investigation into Cancer and Nutrition (EPIC). This is the first report on polymorphisms of *FOXO3* and PC risk.

Materials and methods

The EPIC cohort. A fully detailed description of the EPIC cohort has been published elsewhere (17). Briefly, EPIC consists of about 370,000 women and 150,000 men, aged 35-69, recruited between 1992 and 2005 in 10 Western European countries.

The vast majority (>97%) of subjects recruited in the EPIC cohort are of European ('Caucasian') origin. All EPIC study subjects provided anthropometric measurements (height, weight, and waist and hip circumferences) and extensive, standardized questionnaire information about medical history, diet, physical activity, smoking, and other lifestyle factors. About 260,000 women and 140,000 men provided a blood sample.

Cases of cancer occurring after recruitment into the cohort and blood donation are identified through local and national cancer registries in 7 of the 10 countries, and in France, Germany, and Greece by a combination of contacts with national health insurances and/or active follow-up through the study subjects or their next of kin. Follow-up on vital status is achieved through record linkage with mortality registries.

Selection of case and control subjects. Case subjects were selected among men who developed PC after blood collection. Control subjects were selected randomly matching the cases for center of recruitment, age at blood donation, time between consumption of food or drink and blood draw and time of day of blood draw and duration of follow-up. Each control should have been free of cancer up to the duration of follow-up of the index case. A total of 1571 incident PC cases and 1840 controls were included in the present study. The study was approved by the Ethics Review Boards of the International Agency for Research on Cancer, and of the collaborating institutions responsible for subject recruitment in each of the EPIC recruitment centers.

SNP selection. Three SNPs (rs3800231, rs9400239 and rs479744) in the *FOXO3* gene were chosen because of their strong association with longevity (15,16). rs479744 is also the best tagging SNP for the *FOXO3* gene. It is in high linkage disequilibrium (r^2>0.8) with 19 additional common (minor allele frequency MAF ≥5% in Caucasians) SNPs, and represents 27% of the genetic variability of the gene based on HapMap (release 22, dbSNP version 126 and NCBI genome build 36), and the Tagger algorithm (18) as implemented in the Haploview software.

Sample preparation and genotyping. DNA was extracted from blood samples on an Autopure instrument (Qiagen, Hilden,

Table I. Associations between SNPs in *FOXO3* and PC risk.

SNP	Cases[a]/controls[a] M/M[b]	M/m[b]	m/m[b]	OR Mm vs MM (95% CI)[c]	OR mm vs MM (95% CI)[c]	p2df	ptrend
rs3800231 G/A	756/893	614/761	136/173	0.94 (0.82-1.09)	0.91 (0.71-1.17)	0.649	0.357
rs479744 C/A	930/1166	492/567	76/93	1.09 (0.94-1.27)	1.04 (0.75-1.44)	0.493	0.336
rs9400239 C/T	734/885	639/772	139/183	0.99 (0.86-1.15)	0.91 (0.71-1.17)	0.759	0.559

[a]Numbers may not add up to 100% of subjects due to genotyping failure. All samples that did not give a reliable result in the first round of genotyping were resubmitted to up to two additional rounds of genotyping. Data points that were still not filled after this procedure were left. [b]M/M, homozygotes for the common allele; M/m, heterozygotes; m/m, homozygotes for the rare allele. [c]Results of conditional logistic regression. OR Mm vs MM, odds ratios for the heterozygotes vs the homozygotes for the common allele; OR mm vs MM, odds ratios for the homozygotes for the rare allele vs the homozygotes for the common allele; 95% CI, 95% confidence interval.

Germany) with Puregene chemistry (Qiagen). The order of DNAs from cases and controls was randomized on PCR plates in order to ensure that an equal number of cases and controls could be analyzed simultaneously.

All the genotyping was carried out using the TaqMan assay. The method has been extensively reported elsewhere (19,20).

All samples that did not give a reliable result in the first round of genotyping were resubmitted to up to two additional rounds of genotyping. Repeated quality control genotypes (5% of the total) showed a concordance of 100%. Any sample where greater than 25% of the SNPs failed had all of the SNPs set to missing and these subjects were dropped from analysis.

Hormone level measurement. Hormone measurements on serum insulin-like growth factor-I (IGF-I), IGF-binding protein-3 (IGFBP-3) androstenedione (Δ4), androstanediol glucuronide (ADIOL), testosterone (TESTO) and sex hormone-binding globulin (SHBG) were available for 589 cases and 614 controls.

All hormone assays were performed by the Laboratory of the Hormones and Cancer Team at the International Agency for Research on Cancer, Lyon, France, by using commercially available immunoassays as described previously (21,22). In brief, Δ4 and ADIOL were measured by radio-immunoassay (RIA) with a double antibody system for the separation of free and bound antigen (Diagnostic Systems Laboratory, Webster, TX, USA). Serum testosterone concentrations were measured by RIA (Immunotech, Marseilles, France). SHBG was measured by a solid phase 'sandwich' RIA (Cis-Bio International, Gif-sur-Yvette, France). Serum IGF-I and IGFBP-3 concentrations were measured with ELISA-based assays from Diagnostic Systems Laboratories. IGF-I assays included an acid-ethanol precipitation of IGF-I-binding proteins to avoid interference of IGFBPs with the IGF-I assay.

The laboratory personnel who conducted the assays were blinded to the case or control status of the participants providing the samples. Serum samples from each case-control set were assayed within the same batch, analyzed on the same day and with the same immunoassay kit. Three quality control serum samples, which were indistinguishable from the subject samples, were inserted into each assay batch.

Statistical analysis. The association between PC risk and genotypes for each SNP was analysed using conditional logistic regression. Genotypes were coded either as counts of minor alleles (trend test) or as two indicator variables, one for heterozygotes and one for minor-allele homozygotes (two degrees of freedom test).

Subgroup analyses were also performed dividing by disease aggressiveness (aggressive disease was defined as extraprostatic extension) or high histologic grade (Gleason score ≥8) and BMI (<25, 25-29, ≥30). Analyses were also perform stratifying by circulating levels of IGF-I, IGFBP-3 and sex steroid hormones (ADIOL, Δ4, SHBG and TESTO), divided into thirds based on the tertile cutpoints of the hormone values among the controls. The tertile cut-off points were 4.8 and 7.9 ng/ml for ADIOL, 1.2 and 1.6 ng/ml for Δ4, 133.9 and 194.2 ng/ml for IGF-I, 3.369 and 3.992 ng/ml for IGFBP-3, 36.3 and 51.4 nmol/l for SHBG and 3.9 and 5.4 ng/ml for TESTO. Tests of heterogeneity of the association of each genotype on PC risk were performed across strata. Since we interrogated the possible association of the *FOXO3* gene using three SNPs we used a study-wise threshold of 0.05/3=0.016. All statistical analyses were performed using SAS 9.11.

Results

Three SNPs belonging to the *FOXO3* gene were analysed in 3411 samples. Fifty-nine (less than 2%) samples were removed due to poor genotyping performance, leaving 1571 PC cases and 1840 controls available for analysis. In the remaining subjects the call rate for each polymorphism was higher than 99%. The population of cases included 227 aggressive, 1342 non-aggressive and 2 not specified PC. The median age was 60.1 for the cases and 60.2 for the controls.

Main effects of genotyped SNPs. The genotype distributions at all SNPs were in Hardy-Weinberg equilibrium in controls, with non-significant χ^2 values (data not shown).

The frequencies and distribution of the genotypes and the odds ratios for the association of each polymorphism with PC risk are described in Table I. Overall, there were no statistically significant associations between any of the SNPs and PC risk.

Table II. Effects of genotyped SNPs in different population strata.

		Cases[a]			Controls[a]			OR Mm vs MM (95% CI)[c]			OR mm vs MM (95% CI)[c]				
SNP	Stratum	MM[b]	Mm[b]	mm[b]	MM[b]	Mm[b]	mm[b]	OR	Low	Up	OR	Low	Up	phtr[d]	ptrend
	IGF-1														
rs3800231	All subjects	262	235	42	256	209	52	1.16	0.84	1.61	0.86	0.48	1.53	0.18	0.89
rs3800231	<133.936 ng/ml	64	70	14	84	67	14	1.57	0.80	3.08	1.76	0.51	6.01		0.17
rs3800231	>133.936 ng/ml; <194.15 ng/ml	104	93	14	78	74	16	1.23	0.65	2.31	0.87	0.30	2.49		0.89
rs3800231	>194.15 ng/ml	94	72	14	94	68	22	0.87	0.47	1.58	0.50	0.19	1.34		0.21
rs9400239	All subjects	250	253	49	243	223	56	1.03	0.74	1.43	0.82	0.48	1.40	0.13	0.66
rs9400239	<133.936 ng/ml	63	75	15	80	74	14	1.26	0.66	2.39	1.65	0.50	5.51		0.34
rs9400239	>133.936 ng/ml; <194.15 ng/ml	96	98	18	75	77	17	1.23	0.65	2.32	1.07	0.39	2.94		0.69
rs9400239	>194.15 ng/ml	91	80	16	88	72	25	0.89	0.49	1.61	0.38	0.15	0.95		0.08
rs479744	All subjects	335	192	26	334	155	28	1.11	0.80	1.55	1.01	0.49	2.06	0.17	0.65
rs479744	<133.936 ng/ml	88	53	11	108	51	7	1.41	0.73	2.76	3.31	0.59	18.55		0.12
rs479744	>133.936 ng/ml; <194.15 ng/ml	134	71	10	105	53	9	1.33	0.70	2.54	1.22	0.34	4.38		0.45
rs479744	>194.15 ng/ml	113	68	5	121	51	12	1.00	0.55	1.81	0.38	0.11	1.35		0.34
	IGFBP-3														
rs3800231	All subjects	262	235	42	256	210	52	1.16	0.84	1.61	0.86	0.48	1.53	0.35	0.89
rs3800231	<3368.5 ng/ml	76	81	9	88	62	19	2.33	1.17	4.63	0.63	0.16	2.44		0.27
rs3800231	>3368.5 ng/ml; <3991.7 ng/ml	79	77	18	74	76	18	0.68	0.35	1.31	0.77	0.24	2.50		0.34
rs3800231	>3991.7 ng/ml	107	77	15	94	72	15	1.29	0.72	2.30	0.76	0.29	1.99		0.96
rs9400239	All subjects	250	253	49	243	224	56	1.03	0.74	1.43	0.82	0.48	1.40	0.45	0.66
rs9400239	<3368.5 ng/ml	73	87	12	84	69	17	1.73	0.89	3.36	0.60	0.18	2.00		0.72
rs9400239	>3368.5 ng/ml; <3991.7 ng/ml	75	86	17	71	80	20	0.68	0.35	1.30	0.55	0.17	1.72		0.17
rs9400239	>3991.7 ng/ml	102	80	20	88	75	19	1.11	0.62	2.00	0.84	0.35	1.97		0.87
rs479744	All subjects	335	192	26	334	156	28	1.11	0.80	1.55	1.01	0.49	2.06	0.04	0.65
rs479744	<3368.5 ng/ml	100	64	7	112	50	6	1.63	0.84	3.16	5.88	0.40	85.78		0.08
rs479744	>3368.5 ng/ml; <3991.7 ng/ml	108	58	12	100	54	13	0.54	0.28	1.06	0.51	0.13	1.96		0.07
rs479744	>3991.7 ng/ml	127	70	7	122	52	9	1.32	0.74	2.35	0.76	0.23	2.51		0.72
	Δ4														
rs3800231	All subjects	260	234	42	253	210	52	1.16	0.83	1.61	0.86	0.49	1.53	0.05	0.8
rs3800231	<1.18144 ng/ml	102	77	14	72	74	19	0.75	0.38	1.45	0.60	0.19	1.96		0.28
rs3800231	>1.18144 ng/ml; <1.56389 ng/ml	75	83	14	92	71	16	2.11	1.10	4.07	1.96	0.64	5.96		0.05
rs3800231	>1.56389 ng/ml	83	74	14	89	65	17	0.92	0.52	1.65	0.61	0.23	1.59		0.39
rs9400239	All subjects	248	252	49	240	224	56	1.03	0.74	1.42	0.82	0.48	1.41	0.05	0.66
rs9400239	<1.18144 ng/ml	98	85	16	68	80	20	0.66	0.35	1.27	0.65	0.21	1.97		0.23
rs9400239	>1.18144 ng/ml; <1.56389 ng/ml	69	90	16	89	75	17	1.95	1.02	3.73	1.97	0.67	5.79		0.06
rs9400239	>1.56389 ng/ml	81	77	17	83	69	19	0.79	0.44	1.41	0.62	0.25	1.51		0.25
rs479744	All subjects	333	191	26	331	156	28	1.11	0.80	1.54	1.01	0.50	2.07	0.27	0.66
rs479744	<1.18144 ng/ml	127	64	10	94	59	11	0.58	0.30	1.14	1.37	0.31	6.16		0.41
rs479744	>1.18144 ng/ml; <1.56389 ng/ml	106	59	10	127	46	7	1.22	0.64	2.32	2.90	0.67	12.60		0.18
rs479744	>1.56389 ng/ml	100	68	6	110	51	10	1.21	0.67	2.19	0.47	0.14	1.50		0.65

Table II. Continued.

SNP	Stratum	Cases[a]			Controls[a]			OR Mm vs MM (95% CI)[c]			OR mm vs MM (95% CI)[c]			phtr[d]	ptrend
		MM[b]	Mm[b]	mm[b]	MM[b]	Mm[b]	mm[b]	OR	Low	Up	OR	Low	Up		
	ADIOL														
rs3800231	All subjects	264	235	42	256	211	52	1.14	0.82	1.58	0.85	0.48	1.52	0.97	0.95
rs3800231	<4.83769 ng/ml	93	83	21	76	75	14	0.79	0.44	1.45	1.47	0.48	4.50		0.98
rs3800231	>4.83769 ng/ml; <7.91243 ng/ml	84	79	12	85	73	20	1.43	0.77	2.67	0.65	0.23	1.81		0.99
rs3800231	>7.91243 ng/ml	87	73	9	95	63	18	1.41	0.73	2.70	0.47	0.15	1.55		0.77
rs9400239	All subjects	252	253	49	243	225	56	1.01	0.73	1.40	0.82	0.48	1.39	0.36	0.61
rs9400239	<4.83769 ng/ml	86	95	22	72	81	16	0.73	0.40	1.34	1.02	0.36	2.84		0.63
rs9400239	>4.83769 ng/ml; <7.91243 ng/ml	82	79	18	83	79	18	1.26	0.69	2.31	1.29	0.51	3.26		0.46
rs9400239	>7.91243 ng/ml	84	79	9	88	65	22	1.14	0.60	2.15	0.30	0.09	0.96		0.22
rs479744	All subjects	337	192	26	334	157	28	1.09	0.79	1.52	1.00	0.49	2.04	0.98	0.72
rs479744	<4.83769 ng/ml	118	71	12	102	59	5	0.69	0.39	1.25	5.96	0.66	53.81		0.98
rs479744	>4.83769 ng/ml; <7.91243 ng/ml	111	60	9	120	47	14	1.54	0.81	2.90	0.63	0.21	1.92		0.83
rs479744	>7.91243 ng/ml	108	61	5	112	51	9	1.07	0.56	2.05	0.71	0.15	3.25		0.92
	TESTO														
rs3800231	All subjects	239	215	38	238	191	49	1.17	0.83	1.67	0.77	0.41	1.42	0.48	0.93
rs3800231	<3.85474 ng/ml	80	72	12	80	66	19	1.77	0.81	3.86	0.57	0.15	2.17		0.85
rs3800231	>3.85474 ng/ml; <5.38003 ng/ml	75	62	9	75	68	12	0.84	0.40	1.78	0.37	0.09	1.54		0.23
rs3800231	>5.38003 ng/ml	84	81	17	83	57	18	1.05	0.52	2.10	1.21	0.43	3.42		0.74
rs9400239	All subjects	232	229	45	225	204	55	1.00	0.71	1.43	0.72	0.41	1.27	0.56	0.41
rs9400239	<3.85474 ng/ml	72	84	13	73	73	21	1.41	0.67	2.96	0.73	0.21	2.60		0.91
rs9400239	>3.85474 ng/ml; <5.38003 ng/ml	74	66	11	70	73	16	0.82	0.40	1.68	0.40	0.11	1.42		0.20
rs9400239	>5.38003 ng/ml	86	79	21	82	58	18	0.82	0.42	1.64	1.04	0.40	2.72		0.89
rs479744	All subjects	307	173	24	313	140	26	1.13	0.79	1.61	0.87	0.40	1.89	0.26	0.80
rs479744	<3.85474 ng/ml	105	57	6	98	54	12	1.21	0.57	2.57	0.46	0.10	2.19		0.75
rs479744	>3.85474 ng/ml; <5.38003 ng/ml	96	48	7	105	48	4	0.55	0.25	1.17	0.80	0.11	6.05		0.19
rs479744	>5.38003 ng/ml	106	68	11	110	38	10	1.46	0.73	2.93	1.33	0.38	4.67		0.34
	SHBG														
rs3800231	All subjects	249	220	40	241	202	51	1.11	0.78	1.57	0.77	0.42	1.39	0.42	0.76
rs3800231	<36.25549 nmol/l	85	78	13	86	66	19	0.91	0.47	1.75	0.46	0.15	1.38		0.25
rs3800231	>36.25549 nmol/l; <51.40571 nmol/l	84	72	20	72	81	13	1.10	0.55	2.20	1.63	0.46	5.76		0.50
rs3800231	>51.40571 nmol/l	80	70	7	83	55	19	1.32	0.65	2.69	0.39	0.11	1.39		0.52
rs9400239	All subjects	238	237	47	229	213	55	1.01	0.71	1.42	0.76	0.44	1.31	0.45	0.48
rs9400239	<36.25549 nmol/l	79	87	15	84	67	21	0.94	0.49	1.80	0.46	0.17	1.28		0.22
rs9400239	>36.25549 nmol/l; <51.40571 nmol/l	81	79	21	69	82	16	1.12	0.56	2.21	1.28	0.38	4.32		0.66
rs9400239	>51.40571 nmol/l	78	71	11	76	64	18	0.92	0.46	1.83	0.46	0.15	1.40		0.25
rs479744	All subjects	319	179	25	316	150	27	1.06	0.75	1.49	0.88	0.42	1.83	0.56	1.00
rs479744	<36.25549 nmol/l	112	64	8	108	52	9	0.87	0.46	1.66	0.45	0.10	1.95		0.34
rs479744	>36.25549 nmol/l; <51.40571 nmol/l	109	58	13	100	58	11	0.81	0.41	1.58	1.27	0.33	4.84		0.87
rs479744	>51.40571 nmol/l	98	57	4	108	40	7	1.72	0.82	3.61	0.46	0.07	3.18		0.54

Table II. Continued.

SNP	Stratum	Cases[a]			Controls[a]			OR Mm vs MM (95% CI)[c]			OR mm vs MM (95% CI)[c]				
		MM[b]	Mm[b]	mm[b]	MM[b]	Mm[b]	mm[b]	OR	Low	Up	OR	Low	Up	phtr[d]	ptrend
	BMI														
rs3800231	All subjects	757	615	135	919	780	176	0.93	0.79	1.09	0.89	0.68	1.17	0.97	0.28
rs3800231	Normal <25	242	199	47	261	254	62	0.89	0.67	1.19	0.87	0.54	1.40		0.42
rs3800231	Overweight ≥25 - <30	414	332	69	498	398	84	0.96	0.77	1.20	0.88	0.59	1.31		0.53
rs3800231	Obese ≥30	101	84	19	160	128	30	0.83	0.53	1.29	1.01	0.49	2.09		0.67
rs9400239	All subjects	736	649	139	917	796	182	0.97	0.83	1.14	0.91	0.69	1.19	0.85	0.51
rs9400239	Normal <25	241	207	46	267	251	65	0.95	0.71	1.27	0.84	0.52	1.34		0.47
rs9400239	Overweight ≥25 - <30	399	351	72	491	407	92	1.01	0.81	1.27	0.88	0.60	1.30		0.72
rs9400239	Obese ≥30	96	91	21	159	138	25	0.84	0.54	1.30	1.39	0.66	2.92		0.85
rs479744	All subjects	949	494	76	1209	578	93	1.04	0.88	1.23	1.00	0.70	1.43	0.39	0.73
rs479744	Normal <25	294	173	26	362	194	27	1.21	0.90	1.62	1.36	0.71	2.58		0.15
rs479744	Overweight ≥25 - <30	527	248	42	638	288	49	0.98	0.78	1.24	0.94	0.58	1.53		0.79
rs479744	Obese ≥30	128	73	8	209	96	17	1.00	0.64	1.55	0.84	0.30	2.31		0.83
	Disease aggressiveness														
rs3800231	All subjects	785	637	139	955	810	179	0.94	0.80	1.10	0.90	0.69	1.19	0.39	0.35
rs3800231	Aggressive	122	84	21	141	125	28	0.70	0.45	1.07	0.87	0.43	1.79		0.25
rs3800231	Non-aggr	663	553	118	814	685	151	0.98	0.83	1.17	0.91	0.67	1.22		0.58
rs479744	All subjects	971	510	78	1245	599	95	1.05	0.89	1.24	1.02	0.72	1.46	0.60	0.62
rs479744	Aggressive	148	66	15	188	94	16	0.77	0.50	1.20	1.36	0.57	3.25		0.77
rs479744	Non-aggr	823	444	63	1057	505	79	1.10	0.92	1.32	0.96	0.65	1.42		0.52
rs9400239	All subjects	762	665	142	939	824	187	0.95	0.81	1.12	0.89	0.68	1.16	0.47	0.36
rs9400239	Aggressive	113	93	21	140	120	31	0.89	0.58	1.35	0.70	0.35	1.39		0.30
rs9400239	Non-aggr	649	572	121	799	704	156	0.96	0.81	1.15	0.92	0.69	1.24		0.55

[a]Numbers may not add up to 100% of subjects due to genotyping failure. All samples that did not give a reliable result in the first round of genotyping were resubmitted to up to two additional rounds of genotyping. Data points that were still not filled after this procedure were left. [b]M/M, homozygotes for the common allele; M/m, heterozygotes; m/m, homozygotes for the rare allele. [c]Results of conditional logistic regression. OR Mm vs MM, odds ratios for the heterozygotes vs the homozygotes for the common allele; OR mm vs MM, odds ratios for the homozygotes for the rare allele vs. the homozygotes for the common allele; 95% CI, 95% confidence interval. [d]p of hetereogeneity.

Effects of genotyped SNPs in different population strata. None of the analysed SNPs were significantly associated with PC risk according to a priori subgroups of disease aggressiveness, circulating hormone levels, and BMI). The only possible exception was rs9400239 that was associated with a small increased PC risk in the intermediate tertile of the Δ4 hormone (P_{het} 0.045). Detailed results are presented in Table II.

Discussion

This is the first nested case-control study to report on the association between 3 SNPs in the FOXO3 genes that are associated with longevity (rs3800231, rs9400239 and rs479744), and subsequent PC risk.

Several genome-wide association studies to identify germline variants that are associated with prostate cancer risk have been conducted (23). However, only rs479744 is represented in the commercial SNPs arrays (Genome Browser) in the market while rs3800231 has not even been typed in the context of the Hapmap project making this SNP impossible to impute. In this study there was, for each of the polymorphism, a power greater than 80% to detect an association for a codominant model with OR=1.19 at α=0.016 (the experiment-wide significance threshold obtained by dividing 0.05 by three). Given the bad coverage in commercial arrays a the functional importance of the SNPs and the large number of subjects present in the study the EPIC cohort was the ideal setting to test the hypothesis that FOXO3 gene variants could affect PC risk.

Overall, none of these 3 SNPs were associated with PC risk and there the risk did not differ by disease aggressiveness, circulating levels of 6 hormones or their binding proteins, or BMI. Only the SNP rs9400239 showed an association with PC risk in men with low levels of circulating Δ4, although this association did not reach the experiment-wide significance threshold (p=0.016) and may be a chance finding.

Although over 97% of the EPIC subjects are estimated to be of Caucasian origin, differences in allelic frequencies across Europe could in theory cause confounding by population stratification. However, we did not observe major variations in allele frequencies across countries for the SNP studied here (data not shown). Moreover, cases and controls were systematically matched for EPIC recruitment center.

We conclude that polymorphisms in the *FOXO3* gene which are associated with longevity are not major risk factors for PC risk, in a population of Caucasian men.

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