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Genetic variability of the fatty acid synthase pathway is not associated with prostate cancer risk in the European Prospective Investigation on Cancer (EPIC)

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ABSTRACT

A western lifestyle, characterised by low rates of energy expenditure and a high-energy diet rich in animal protein, saturated fats and refined carbohydrates, is associated with high incidence of prostate cancer in men. A high-energy nutritional status results in insulin/IGF signalling in cells, which in turn stimulates synthesis of fatty acids. We investigated whether the genetic variability of the genes belonging to the fatty acid synthesis pathway is related to prostate cancer risk in 815 prostate cancer cases and 1266 controls from the European Prospective Investigation on Cancer (EPIC). Using a tagging approach and selecting 252 SNPs in 22 genes, we covered all the common genetic variation of this pathway. None of the SNPs reached statistical significance after adjusting for multiple comparisons. Common SNPs in the fatty acid synthase pathway are not major contributors to prostate cancer risk.

1. Introduction

A western lifestyle, characterised by low rates of energy expenditure and a high-energy diet rich in animal protein, saturated fats and refined carbohydrates, is associated with high incidence of prostate cancer (PC).[1–3] This is also supported by geographic epidemiology evidence, showing that PC incidence is consistently correlated with western lifestyle.[4] Even within Europe, a North-South and West-East gradient of PC incidence supports this hypothesis.[5]

Nutritional status is an important determinant of blood and tissue concentrations of insulin-like growth factor-I, which has a key role in regulating anabolic (growth) processes.[6,7] Experimental and epidemiologic evidence indicates that excess stimulation of cells by IGF-I can promote tumour development by inhibiting apoptosis and stimulating cell proliferation.[8] Excess energy intake and obesity also cause insulin resistance, which is associated with elevated blood levels of glucose and insulin.[2,9–16]

One of the several outcomes of insulin/IGF-I signalling is the synthesis of fatty acids.[17] Fatty acid synthesis is important for cellular proliferation by allowing the cell to store energy in adipose tissue,[18] and also by providing precursors of critical signal molecules for proliferation and differentiation.[19]

Evidence suggests that high-fat diets and obesity are risk factors for PC.[1,20,21] Indeed, a lipogenic phenotype is a distinctive feature of many cancer cell types, including PC cells.[22]

Additional lines of evidence in favour of involvement of this pathway in prostate carcinogenesis include the fact that expression of acetyl-CoA carboxylase alpha (ACC-alpha) and fatty acid synthase (FAS), the two most important components of the pathway, results in proliferation of PC cells.[23–25]

ACC-alpha is the rate-limiting enzyme for long-chain fatty acid synthesis that catalyses the carboxylation of acetyl-CoA to malonyl-CoA. FAS catalyses the NADPH-dependent condensation of acetyl-CoA and malonyl-CoA to produce predominantly the 16-carbon saturated free palmitate. A high level of palmitic acid in plasma phospholipids has been associated with prostate cancer risk in a nested case–control study within EPIC.[26] Transcription of the FAS gene is controlled synergistically by the transcription factors ChREBP (carbohydrate response element-binding protein) and SREBP-1 (sterol response element-binding protein-1). ChREBP is induced by glucose signalling.[27] Glucose is transported into cells by a number of specific carriers, which are members of the solute carrier families 2 and 5. SREBP-1 on the other hand is stimulated by insulin signalling[18,28] and its function requires a co-factor CBF/NF-Y.[29] Intake of dietary fatty acids, specifically poly-unsaturated fatty acids (PUFAs), results in suppressed lipogenesis in liver and other tissues,[30] through direct action on SREBP-1 and NF-Y.[31] Fatty acids are transported into cells by members of the solute carrier family 27.[32] Key components of the pathway and their relations are represented in Supplementary Fig. 1.

Since molecules in the FAS pathway and upstream of it are centrally implicated in prostate carcinogenesis, we hypothesised that polymorphic alleles of their encoding genes could modify their expression or activity, thus conferring altered PC susceptibility. In this report we investigated the genetic variability of 22 key genes in the above mentioned pathway. We tested the association of 252 tagging SNPs with PC risk in a study of 815 invasive PC cases and 1266 controls nested within the European Prospective Investigation into Cancer and Nutrition (EPIC). To our knowledge this is the first report on polymorphisms of these genes and PC risk.

2. Materials and methods

2.1. *The EPIC cohort*

A fully detailed description of the EPIC cohort has been published elsewhere.[33]

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, standardised 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.

2.2. Selection of case and control subjects

Case subjects were selected among men who developed PC after blood collection. Control subjects (1–2 controls per case) were selected randomly by incidence density sampling, matching the cases for centre of recruitment, age at blood donation and duration of follow-up. A total of 815 invasive PC cases and 1266 controls were included in the present study. The controls include 10 subjects who were selected twice due to the incidence density matching procedure. Each control should have been free of cancer up to the duration of follow-up of the index case. The study was approved by the ethical 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 centres.

2.3. SNP selection

For each of the 22 candidate genes we selected a genomic region between 5 kb 5′ of the beginning of the first known exon and 5 kb 3′ of the end of the last known exon. A list of SNPs in all 22 gene regions was compiled using data from HapMap (release 22, based on dbSNP version 126 and NCBI genome build 36), and tagging SNPs were selected by the use of the Tagger algorithm,[34] as implemented in the Haploview software. Parameters used for Tagger selection were minor allele frequency (MAF) $\geqslant$ 5% in Caucasians, minimum $r^2 = 0.8$ between each pair of tagged and tagging SNPs, pairwise tagging. This selection resulted in a list of 275 tagging SNPs (Table 1). SNPs that were predicted to perform poorly with Illumina GoldenGate genotyping technology were either replaced by SNPs in high linkage disequilibrium ($r^2 \geqslant 0.8$, as calculated from HapMap data), or dropped from the list if no proxy was available. This gave us a list of 260 SNPs, for which genotyping was attempted on cases and controls. Finally, for quality control purpose, we added 30 SNPs that had been genotyped on the same samples in an unrelated project.

2.4. Sample preparation and genotyping

DNA was extracted from blood samples on an Autopure instrument (Qiagen, Hilden, Germany) with Puregene chemistry (Qiagen, Hilden, Germany). The order of DNAs from cases and controls was randomised on PCR plates in order to ensure that an equal number of cases and controls could be analysed simultaneously.

Genotyping was carried out using the Illumina GoldenGate technology (San Diego, CA, USA), according to the protocol specified by the manufacturer.

Table 1 – Candidate genes and their SNPs.

Gene	Trivial name(s)	# SNPs MAF $\geqslant$ 0.05[a]	# tagSNPs $r^2 > 0.8$[b]	# SNPs genotyped[c]
ACACA	ACC alpha	137	22	21
ACACB	ACC beta	115	41	38
FASN	FAS	13	7	6
MLXIPL	MIO; CHREBP; MONDOB; WBSCR14; WS-bHLH	11	5	3
NFYA	CBF-A nuclear transcription factor Y, alpha	22	5	5
NFYB	CCAAT-binding factor/nuclear factor_Y nuclear transcription factor Y, beta	17	8	7
NFYC	CBF-G nuclear transcription factor Y, gamma	77	9	9
SLC27A1	FATP1	25	12	11
SLC27A2	FATP2	55	19	15
SLC27A3	FATP3	12	10	9
SLC27A4	FATP4	7	7	5
SLC27A6	FATP6	111	21	19
SLC2A1	GLUT, GLUT1	47	16	15
SLC2A12	GLUT12, GLUT8	58	22	21
SLC2A4	GLUT4	11	8	8
SLC2A5	GLUT5	24	10	10
SLC2A8	GLUT8, GLUTX1	17	7	6
SLC5A1	SGLT1	47	11	10
SLC5A2	SGLT2	6	5	4
SLC5A8	SMCT	55	15	15
SREBF1	SREBP1	11	1	1
SREBF2	SREBP2	43	14	14
Total		921	275	252

[a] SNPs found in HapMap (release 22) with MAF $\geqslant$ 0.05 in CEPH Caucasians (CEU), considering a region between 5 kb 5′ of the beginning of the first known exon and 5 kb 3′ of the end of the last known exon for each gene.

[b] Tagging SNPs selected from each gene region with the use of the Tagger algorithm, before replacements/exclusions due to low Illumina design scores.

[c] SNPs successfully genotyped and used for statistical analysis (after exclusions due to low call rate and strong deviation from HWE).

2.5. Hormone level measurement

Hormone measurements on serum insulin-like growth factor 1 (IGF-I), IGF-binding protein-3 (IGFBP-3) androstenedione (Δ4), androstanediol glucuronide (ADIOL), testosterone (TESTO) and sex-hormone-binding-globuline (SHBG) were available on 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.[35,36]

Androstenedione and androstanediol glucuronide 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' immunoradiometric assay (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, analysed 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.

2.6. Data filtering and statistical analysis

Any sample where more than 25% of attempted SNPs failed was excluded from analysis. SNPs that failed for 25% of samples or more were excluded, as well as SNPs that showed statistically significant ($p < 10^{-5}$) deviations from Hardy–Weinberg equilibrium (HWE) among controls.

We analysed the association between PC risk and genotypes for each SNP 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). We also performed analyses stratifying by disease aggressiveness (defined as extraprostatic extension (stage C/D) or high histologic grade (Gleason score $\geqslant 8$)).

Finally, we performed analyses stratifying by circulating levels of IGF-I, IGFBP-3 and sex steroid hormones (ADIOL, Δ4, SHBG and TESTO). The different strata were defined as

Table 2 – Characteristics of the study population.

		Controls	Cases
		1,266	815
Age at diagnosis (Median, Mean, Std)		60.5 (61.3,6.1)	60.4 (60.7,5.8)
Severity of disease[a]			
	Non-aggressive	–	657
	Aggressive	–	158
Subjects with measurements of circulating hormones			
IGF-I	Missing	654	228
	<133.94 ng/ml	204	156
	>133.94 ng/ml; <194.15 ng/ml	204	234
	>194.15 ng/ml	204	197
IGFBP-3	Missing	653	228
	<3368.5 ng/ml	205	174
	>3368.5 ng/ml; <3991.7 ng/ml	204	190
	>3991.7 ng/ml	204	223
ADIOL	Missing	652	226
	<4.84 ng/ml	205	215
	>4.84 ng/ml; <7.91 ng/ml	205	187
	>7.91 ng/ml	204	187
Δ4	Missing	656	231
	<1.18 ng/ml	204	205
	>1.18 ng/ml; <1.56 ng/ml	203	197
	>1.56 ng/ml	203	182
SHBG	Missing	684	261
	<36.26 nmol/l	194	196
	>36.26 nmol/l; <51.41 nmol/l	194	191
	>51.41 nmol/l	194	167
TESTO	Missing	698	279
	<3.85 ng/ml	190	176
	>3.85 ng/ml; <5.38 ng/ml	189	160
	>5.38 ng/ml	189	200

[a] Disease aggressiveness was defined as extraprostatic extension (stage C/D) or high histologic grade (Gleason score $\geqslant 8$).

Table 3 – Associations between SNPs in candidate genes and prostate cancer risk. All associations supported by $p < 0.05$ in at least one of the two tests are reported. For genes where no significant association were found, the SNPs with the lowest p-value in either test is reported.

Gene	rs number	Chr	Position[a]	OR het (95% CI)[b]	OR hom (95% CI)[b]	p_{2df}	p_{trend}
ACACA	rs878520	17	32,836,220	0.81 (0.68–0.98)	0.99 (0.75–1.3)	0.07	0.32
ACACA	rs8073074	17	32,577,062	0.90 (0.74–1.09)	0.69 (0.4–1.17)	0.24	0.11
ACACB	rs3742023	12	108,178,365	0.92 (0.76–1.10)	1.30 (0.99–1.71)	0.04	0.28
ACACB	rs2268387	12	108,128078	0.91 (0.75–1.11)	0.78 (0.60–1.02)	0.19	0.07
FASN	rs4485435	17	77,638,375	0.98 (0.81–1.20)	0.73 (0.45–1.19)	0.45	0.39
MLXIPL	rs11760752	7	72,660,998	1.01 (0.84–1.23)	1.64 (1.05–2.55)	0.09	0.17
NFYA	rs9296352	6	41,160,852	1.22 (0.99–1.50)	1.63 (0.86–3.07)	0.07	0.02
NFYB	rs2466551	12	103,037,048	0.83 (0.67–1.02)	1.19 (0.67–2.12)	0.16	0.27
NFYB	rs17806516	12	103,053,896	1.21 (0.94–1.54)	1.68 (0.52–5.43)	0.24	0.10
NFYC	rs17357062	1	40,994,802	0.81 (0.59–1.12)	9.69 (1.04–90.05)	0.06	0.60
NFYC	rs3767953	1	41,002,374	0.91 (0.73–1.15)	0.93 (0.40–2.17)	0.73	0.45
SLC27A1	rs12985511	19	17,455,473	1.14 (0.91–1.41)	1.96 (0.95–4.04)	0.11	0.07
SLC27A2	rs933857	15	48,277,334	1.19 (0.99–1.44)	0.78 (0.47–1.27)	0.08	0.42
SLC27A2	rs11854881	15	48,260,667	0.92 (0.73–1.14)	0.52 (0.22–1.21)	0.24	0.15
SLC27A3	rs6682411	1	152,014,240	0.76 (0.57–1.00)	1.09 (0.44–2.71)	0.13	0.10
SLC27A4	rs10987969	9	130,163,826	0.94 (0.79–1.13)	0.76 (0.54–1.06)	0.26	0.14
SLC27A6	rs257897	5	128,341,991	0.86 (0.71–1.03)	1.23 (0.91–1.67)	0.05	0.91
SLC27A6	rs17767297	5	128,363,032	0.81 (0.61–1.06)	0.36 (0.04–3.01)	0.21	0.08
SLC2A1	rs3820546	1	43,170,672	0.85 (0.69–1.05)	1.17 (0.92–1.50)	0.02	0.32
SLC2A1	rs4658	1	43,164,837	1.14 (0.94–1.37)	1.18 (0.77–1.82)	0.36	0.16
SLC2A12	rs2811675	6	134,371,690	1.13 (0.92–1.38)	0.91 (0.70–1.18)	0.18	0.71
SLC2A12	rs9493790	6	134,356,656	0.83 (0.64–1.07)	0.96 (0.31–2.95)	0.36	0.18
SLC2A4	rs222847	17	7,126,141	0.78 (0.58–1.06)	0.97 (0.29–3.23)	0.29	0.15
SLC2A5	rs12736085	1	9,053,237	0.94 (0.78–1.13)	0.68 (0.47–0.98)	0.12	0.07
SLC2A8	rs3824414	9	129,206,678	1.10 (0.90–1.34)	1.12 (0.86–1.45)	0.58	0.33
SLC2A8	rs10987637	9	129,208,147	1.01 (0.83–1.22)	0.87 (0.66–1.15)	0.55	0.45
SLC5A1	rs9606899	22	30,777,811	0.78 (0.63–0.97)	1.24 (0.62–2.49)	0.06	0.10
SLC5A1	rs9609421	22	30,791,497	0.75 (0.53–1.06)	ND	0.26	0.06
SLC5A2	rs3116150	16	31,405,522	1.16 (0.96–1.40)	1.27 (0.88–1.84)	0.18	0.07
SLC5A8	rs7962305	12	100,126,478	1.06 (0.88–1.28)	0.75 (0.49–1.14)	0.28	0.68
SLC5A8	rs2671434	12	100,099,783	1.21 (0.93–1.56)	1.20 (0.42–3.43)	0.35	0.16
SREBF1	rs4925114	17	17,651,995	0.96 (0.80–1.16)	0.89 (0.66–1.19)	0.71	0.42
SREBF2	rs133290	22	40,597,551	1.32 (1.07–1.64)	1.01 (0.79–1.30)	0.01	0.79
SREBF2	rs11702960	22	40,599,478	0.93 (0.75–1.16)	0.47 (0.21–1.06)	0.17	0.16

[a] NCBI genome build 36.
[b] OR het: odds ratios of heterozygotes; OR hom: odds ratios of homozygotes for the less common allele; 95%CI: 95% confidence interval; homozygotes for the common allele were the reference group for all tests.

tertiles of raw measurement values of all control subjects. 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, 3369 and 3992 ng/ml for IGFBP-3, 36.3 and 51.4 nmol/l for SHBG and 3.9 and 5.4 ng/ml for TESTO. Within each of these strata we estimated the effect of genotype variation on breast cancer risk and testing for heterogeneity of these effects across strata.

In order to take into account the large number of tests performed in this project, we calculated for each gene the number of effective independent variables, M_{eff}, using the SNP Spectral Decomposition approach.[37] We obtained a gene-wide M_{eff} value for each gene and also a study-wide M_{eff} value, by adding up the gene M_{eff}'s.

All statistical analyses were performed with SAS version 9.2.

3. Results

In this study we analysed 252 SNPs of 22 genes involved in the fatty acid synthase pathway in 815 PC cases and 1266

matched controls. Summary characteristics of the study population are shown in Table 2.

3.1. Genotyping success rates and quality control

We attempted genotyping of 290 SNPs, of which 30 analysed in previous studies were included for quality control. Seven SNPs were excluded from association analysis because of low call rates (<75%), and one SNP (rs7800944 *MLXIPL* gene) was excluded because of departure from HWE ($p < 10^{-5}$ in controls). The genotype concordance for the 30 quality control SNPs with previous studies was 100%. Random duplicate samples ($n = 54$) were also included and concordance of their genotypes was 99.99%. The number of SNPs included in the association analysis was 252 (97% of attempted SNPs) and the average call rate for these SNPs was 99.9%.

We initially included 2110 samples, and after removing subjects samples with a call rate lower than 75% ($n = 39$), we had a dataset including 815 PC cases and 1266 matched controls.

3.2. Main effects of genotyped SNPs

We calculated M_{eff} values for each candidate gene separately and for the whole study (by adding the individual gene M_{eff} values). The pathway-wide M_{eff} was 207 (the M_{eff} values for each gene are reported in Supplementary Table 1). We therefore considered a study-wide significance p-value threshold of 0.05/207 = 0.00024 statistically significant.

In overall PC risk analysis no associations emerged at the study-wide significance threshold between any of the polymorphisms genotyped (p_{trend} 0.0227–1.0; p_{2df} 0.0122–0.9965). Table 3 shows overall results of the SNP from each investigated gene with the lowest p-value, and for all SNPs with p-values below 0.05, and Supplementary Table 2 shows the corresponding results for all analysed SNPs.

3.3. Effects of genotyped SNPs in different population strata

Using the above mentioned tertile thresholds we found no statistically significant association between analysed SNPs and PC risk in the different strata considered (disease aggressiveness, levels of hormones in blood). Detailed results are presented in Supplementary Tables 3–9.

When stratifying for tertiles of Δ4 levels, the SNP rs4925114 of the *SREBF1* gene had a heterogeneity test P value which was close to multiple-testing-corrected significance ($P_{heterogeneity}$ = 0.0003). The association with PC risk was limited to the intermediate tertile of Δ4 levels (P_{value} = 0.00118) (Supplementary Table 4).

4. Discussion

Up-regulation of genes involved in lipogenesis is a common feature of many cancers, as documented by the fact that many human tumour cells synthesise fatty acids instead of using fatty acids from the diet.[38]

In this study we investigated if common genetic variation in genes belonging to the FAS pathway plays an important role in the development of prostate cancer based on their key function in lipogenesis. Overall no clear association between any of the 252 SNPs from 22 candidate genes was detected.

A strong evidence in favour of involvement of fatty acid synthesis pathway in prostate carcinogenesis include the fact that expression of ACC-alpha and FAS, the key limiting enzymes of fatty acid synthesis, results in proliferation of PC cells.[23,25,39–42]

In this report we sought to thoroughly examine the common genetic variability of 22 key genes in the pathway in relation to possible association with PC risk. For doing so we selected 252 tagging SNPs which tagged 921 alleles in a pairwise manner, covering in this way at least 88.9% of the genetic variation in each gene. We exhaustively analysed the possible associations between the SNPs and PC risk. By way of estimating codominant and trend models we had a fair chance to also detect recessive or dominant effects if there were any. Moreover, subgroup analyses were performed based on disease aggressiveness. Finally we performed stratified analysis taking into account the circulating levels of 6 hormones or their binding proteins, namely IGF-I, IGFBP-3, ADIOL, Δ4, SHBG and testosterone, looking for heterogeneity of SNP effects as a sign of interaction.

Using a stringent threshold for statistical significance, which takes into account the large number of tests performed, we have found no statistical association between the polymorphisms and PC risk regardless of the model of inheritance (dominant, codominant and recessive) or the stratum (hormone level or disease aggressiveness) considered. It is interesting to report that SNP rs4925114 of the *SREBF1* gene showed an association with PC risk in subjects with an intermediate range of values of circulating Δ4. However, since the significance level did not reach the experiment-wide significance threshold, and considering the fact that it was significant only in the intermediate stratum, this result could be explained as a false positive finding.

Within the EPIC study population, which for men covers eight western European countries, there is some variation in prostate cancer incidence rates, as well as in stage and grade, which could be at least partly due do different extent of PSA screening across the different EPIC countries. However, it should be stressed that our analysis does not focus on these geographic differences but on possible genetic determinants within the full EPIC study population.

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).

By estimating the co-dominant model we had a possibility to detect both recessive and dominant effects in addition to trend effects (power = 0.80 for co-dominant model to detect OR = 1.36 at alpha = 0.00024 for a SNP with a MAF of 0.30). None of the SNPs we tested reached this level of significance. We conclude that polymorphisms in 22 candidate genes sensible to energy intake belonging to the FAS pathway are not major risk factors for PC risk, in a population of Caucasian men.

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The only role of the funding sources was to provide funding. No other conditions were attached.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.09.029.

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