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Association between the GCG polymorphism of the selenium dependent *GPX1* gene and the risk of young onset prostate cancer

Z Kote-Jarai[1]*, F Durocher[2], SM Edwards[1], R Hamoudi[1], RA Jackson[1], A Ardern-Jones[1], A Murkin[1], DP Dearnaley[1], R Kirby[3], R Houlston[1], The CRC/BPG UK Familial Prostate Cancer Collaborators[1], DF Easton[2] & R Eeles[1]*

[1]*Section of Cancer Genetics, The Institute of Cancer Research and Royal Marsden NHS Trust, Sutton, Surrey, UK;* [2]*CRC Genetic Epidemiology Unit, Strangeways Research Laboratory, Cambridge, UK; and* [3]*Department of Urology, St Georges Hospital, Tooting, London, UK*

Epidemiological studies have suggested an association between low selenium levels and the development of prostate cancer. Human cellular glutathione peroxidase I (hGPX1) is a selenium-dependent enzyme that protects against oxidative damage and its peroxidase activity is a plausible mechanism for cancer prevention by selenium. The *GPX1* gene has a GCG repeat polymorphism in exon 1, coding for a polyalanine tract of five to seven alanine residues. To test if the *GPX1* GCG repeat polymorphism associates with the risk of young-onset prostate cancer we conducted a case–control study. The *GPX1 Ala* genotypes were determined for 267 prostate cancer cases and 260 control individuals using polymerase chain reaction (PCR) amplification with fluorescently labelled primers and an ABI 377 automated genotyper. Associations between specific genotypes and the risk of prostate cancer were examined by logistic regression. We found no significant association between the *GPX1* genotypes and prostate cancer. There was however an increased frequency of the *GPX1 Ala6/Ala6* genotype in the prostate cancer cases compared to controls (OR: 1.67; 95% CI: 0.97–2.87). The result of this study suggests that the *GPX1* genotype is unlikely to be associated with the risk of developing prostate cancer.
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Introduction

Prostate cancer is the second most common cancer in men in the UK. About 14 000 cases are diagnosed each year and it accounts for 9000 deaths annually.[1] Whilst most cases develop in elderly men, 13% of cases occur in men aged less than 65 y. Epidemiological studies have demonstrated familial aggregation of the disease that suggests genetic predisposition, particularly in younger men, to the disease.

Recent studies suggest an association between low levels of selenium and the risk of prostate cancer. Clark and colleagues[2] found striking evidence that selenium supplementation protected men against prostate cancer in a randomised trial in the USA (OR: 0.35; CI: 0.18–0.65). Another study has revealed an inverse association between advanced prostate cancer and toe-nail selenium levels, a surrogate marker of long-term selenium intake.[3] A large case–control study however failed to find a similar association between prostate cancer risk and toe-nail selenium, but the selenium level was inversely correlated with colon cancer risk.[4] Two recent reports also suggested the benefit of selenium in relation to incident

*Correspondence: Z Kote-Jarai or R Eeles, Cancer Genetics, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, UK.
E-mail: zsofia@icr.ac.uk, ros@icr.ac.uk
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prostate cancer,[5,6] however the association was mainly present in current or past cigarette smokers.[5] These findings provide some evidence for a possible protective role of selenium against the development of prostate cancer.

The human glutathione-peroxidase 1 (hGPX1) is a selenium-dependent enzyme which participates in the detoxification of activated oxygen species by catalysing reduction of these genotoxic compounds.[7] Therefore it is plausible that a genetic alteration or polymorphisms in the *GPX1* gene may also have an effect on prostate cancer risk. The presence of a GCG repeat polymorphism coding for alanine residues in a polyalanine tract of *GPX1* was described by Moscow *et al*.[8] They have also reported that other polymorphisms co-segregate with one of the alleles, a proline to leucine substitution at codon 198, a T to C substitution at +2 and a G for A substitution at −592. In a recent study it was shown that the Pro198Leu polymorphism was significantly associated with the risk of developing lung cancer in a case–control study confirming the role of *GPX1* as a potent risk factor in tumour development.[9]

Our aim in the present study was to investigate the relationship between the GCG repeat polymorphism in *GPX1* and prostate cancer risk using a large series of early onset prostate cancer cases and controls.

Materials and methods

Study population

Blood samples were obtained from 275 Caucasian patients living in the UK, who had histologically proven prostate cancer diagnosed before the age of 56 y. Ascertainment of cases was obtained through Consultants collaborating in the CRC/BPG UK Familial Prostate Cancer Study organised by The Institute of Cancer Research and The Royal Marsden NHS Trust. Patients were not selected on the basis of their family history and only a small proportion (12%) had family history of prostate cancer. Control blood samples were obtained from 270 geographically matched individuals who were spouses of patients in a population based study of colorectal cancer. No specific age matching was carried out but the mean age of the cases was similar to the control individuals (51 y). Controls with a previous diagnosis of any cancer were excluded.

Genotyping

Polymerase chain reaction (PCR) amplifications were performed using genomic DNA extracted from the blood samples of cases and controls by standard methods. For the genotyping of the *GPX1* GCG polymorphism, fluorescently labelled primers were used (sense: 5′–GAA ACT GCC TGT GCC ACG TGA CC–3′; antisense: 5′–CGA GAA GGC ATA CAC CGA CTG GGC–3′). Briefly, for all PCR reactions, 25 ng genomic DNA was used in a 15 µl reaction mixture. The PCR reactions contained 1.5 mM $MgCl_2$, 6 pM of each primer, 0.5 U of Amplitaq Gold polymerase (The Perkin Elmer Corp. Norwalk, CT, USA). The reactions were performed with an annealing temperature of 60°C for 35 cycles in a Hybaid Touchdown PCR machine. Following PCR amplification the repeat length of the samples was analysed on 6% polyacrylamide gels using an ABI 377 automated DNA Sequencer. The size of the amplified products was determined relative to size standards using Genescan and Genotyper Analysis software (Applied Biosystem). Allele calling was done without the knowledge of case or control status.

Statistical analysis

Association between the *GPX1* genotypes and the development of prostate cancer was presented in the usual way in terms of odds ratios (OR) based on logistic regression, but using 95% floating confidence limits (FCLs) rather than standard confidence intervals. In this case the analysis was performed under the restriction that the genotype frequencies in the controls and in the cases were in Hardy-Weinberg equilibrium and based significance levels on a likelihood ratio test. This approach has been shown to provide a more powerful test of a genotypic effect.[10] This sample size has approximately 80% power to detect an odds ratio of 2 for the putative high risk genotype significant at the 1% level.

Allele ID	Length: *Ala* s	bps	Frequency in controls
3	7	171	24%
2	6	168	29%
1	5	165	47%

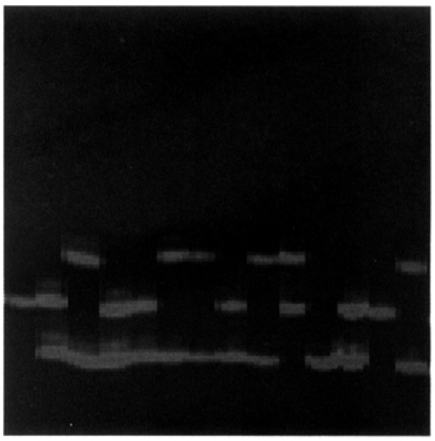

Figure 1 *(GPX1) GCG repeat polymorphism.*

Results

The *GPX1* trinucleotide polymorphism (GCG repeat) in exon 1 was genotyped successfully for 267 of the 275 prostate cancer cases and for 260 of the 270 control individuals. As shown in Figure 1, all three alleles coding for five, six or seven alanines in the polyalanine track (*Ala5*, *Ala6*, *Ala7*) occur with a frequency of 49, 29 and 24% respectively and about 70% of genotypes are heterozygous in the European CEPH sample set.[6]

Genotyping results are shown in Table 1. We found no significant association between the *GPX1* genotypes and the risk of young onset prostate cancer ($\chi^2_5 = 2.64$; $P = 0.12$). The *Ala6* homozygous genotype had slightly higher frequency in the prostate cancer group compared to the control population (12 *vs* 7%), corresponding to an odds ratio of 1.67 (CI:0.97 – 2.87) relative to the *Ala5* homozygotes. The *Ala6* allele was slightly more common overall in cases than in controls (33 *vs* 29%) but again the difference in allele frequencies between cases and controls was not significant ($\chi^2_2 = 1.88$, $P = 0.39$). Presently it is unknown whether this GCG repeat polymorphism has any effect on the properties of the *GPX1* enzyme. Besides the difference in the length of the polyalanine track the *Ala6* allele is in complete linkage disequilibrium with an amino acid substitution (Leu198Pro) in the coding region and two more base substitutions outside the coding regions.[8] Thus, this allele is the most likely to code for a functionally different protein.

Discussion

Amongst the many genetic and environmental factors that influence the risk for developing prostate cancer selenium intake is one possible candidate. So far, four studies have reported a significant reduction in risk for prostate cancer as the selenium intake increased. Two of them are based on the measurement of the long-term selenium intake using toe-nail clippings,[3,6] one is based on serum selenium measurements,[5] and one on selenium supplementation in a double-blinded study.[2] Three studies reported a reduction of risk for developing prostate cancer as the selenium level increased, suggesting that selenium can provide a two – three-fold protection against prostate cancer. One study showed an association only in smokers,[5] and one study failed to find any significant association between toe-nail selenium level and the risk of prostate cancer.[4]

Table 1 Association between the *GPX1* GCG polymorphism and early onset cancer of the prostate

Genotype	Prostate cancer <56 y ($n=267$)	Controls ($n=260$)	OR	95% FCI
Ala 5/5	48	55	1.00	0.68 – 1.47
Ala 5/6	75	75	1.15	0.84 – 1.58
Ala 5/7	62	60	1.18	0.83 – 1.68
Ala 6/6	32	22	1.67	0.97 – 2.87
Ala 6/7	38	34	1.28	0.81 – 2.03
Ala 7/7	12	14	0.98	0.45 – 2.12

How selenium might prevent prostate cancer development is a challenging question. As selenium is an important element in glutathione peroxidases (GPXs), a major antioxidant enzyme family, it is reasonable to suggest that it could have chemopreventative effect through the function of GPXs that provide protection against oxidative damage of DNA. As selenium intake seems to be an important environmental factor in the susceptibility for prostate cancer, *GPX* genes could be considered as candidate low penetrance prostate cancer genes.

Genetic predisposition is one important factor in the development of prostate cancer. We selected a cohort of young-onset prostate cancer cases (diagnosed at <56 y of age) to provide a suitable study population that may have developed prostate cancer as a result of a genetic predisposition. Human cellular glutathione peroxidase I (hGPX1) is a selenium dependent antioxidant enzyme and the gene, *GPX1* maps to 3p21.[6] In addition to an informative trinucleotide repeat polymorphism that has been genotyped in our study, three other common single nucleotide polymorphisms (SNPs) have been identified: a leucine to proline substitution at codon 198 (C > T at 593) and two further base changes in the non-coding region (C > T at +2 and G > A at −592). These three polymorphisms are in complete linkage disequilibrium with the *Ala6* repeat, therefore this allele is the most likely to be functionally distinct. It is interesting that we found some suggestions of an elevated risk in *Ala6* carriers and particularly *Ala6* homozygotes (RR = 1.67; 95% CI = 0.97 – 2.87). Furthermore, carriers of this allele were found to have a significantly elevated risk of developing lung cancer in other studies.[9]

The biochemical properties of hGPX1 have been extensively studied.[11] Glutathione peroxidase I is a selenoprotein, a primary antioxidant enzyme involved in the degradation of peroxides and hydroperoxides which damage cell membranes and DNA. The enzyme is a homotetramer, each subunit is 22 kD, and contains one atom of selenium. The selenium exists in the protein as a catalytically active selenocysteine residue at amino acid 47. The protein sequence is highly conserved, except for the last five amino acids, which are quite variable. If these residues are ignored, the homology of the human *GPX1* to the mouse is about 90%. The amino acids believed to be important in the function of the enzyme are all conserved in species where sequence data are available. Glu82 and Trp160 are in a hydrogen-bond to the selenocysteine selenolate; Arg52 and Arg179 are believed to form salt-bridges to the carboxylate groups of glutathione. Thus, the significance of the polymorphism of the human *GPX1* at the polyalanine track at codons 7 – 14 and the Pro to Leu substitution at codon 198 is presently unknown.

Since hGPX1 decomposes hydrogen peroxides and organic hydroperoxides produced during normal metabolism and after oxidative stress, it prevents peroxide-induced oxidative damage, lipid peroxidation and protein degradation. Recently it has been found that *GPX1* is inducible by etoposide, a topoisomerase II inhibitor, an apoptosis inducer and a TP53 activator.[12] DNA binding assays have proved that TP53 positively regulates an upstream promoter element of the *GPX* gene. This transactivation of *GPX1* by TP53 links the TP53 signalling pathway to the antioxidant pathway. As TP53 is activated by DNA damaging agents, this finding suggests an

important role of antioxidant enzymes in the cellular response not only to oxidative stress but also to DNA damage.

The exogenous selenium supply has been found to control the enzymatic activity of hGPX1. In a selenium deficient environment, cells have about 5% of normal hGPX1 activity.[13] However, the *GPX1* mRNA level is not affected by the selenium level which suggests that the human *GPX1* gene is regulated post-transcriptionally by selenium. It has also been demonstrated that selenium has a dose-dependent growth inhibition on prostate cancer cell lines[14] and this effect was not observed in normal cells. This indicates that selenium and perhaps *GPX1* activity as well could have a specific effect in prostate tumorigenesis. It would be interesting to know whether the *GPX1* polymorphism associates with any clinical parameters, such as survival or grade of the prostate cancer. We are currently collecting the data on the clinical and pathological characteristics of our cases, which could provide valuable information for stratification of our study population.

Based on previous studies, dietary selenium proved to be a strong candidate as an environmental factor, altering the risk for prostate cancer development. Whether the GCG polymorphism in the selenium dependent *GPX1* gene alters the *GPX1* antioxidant activity still remains to be seen. Our data do not show a significant association between this polymorphism and the development of young onset prostate cancer although we had a reasonable power to detect an odds ratio of 2. Based on this study it is unlikely that the *GPX1* polymorphisms have a significant role in prostate cancer development and the protective effect of selenium is more likely to be mediated by another mechanism.

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References

1 Eeles R, the UK Familial Prostate Study Co-ordinating Group and the CRC/BPG UK Familial Prostate Cancer Study Collaborators. Genetic predisposition to prostate cancer. *Prostate Cancer and Prostatic Disease* 1999; **2**: 9–15.
2 Clark LC *et al*. Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. *Br J Urol* 1998; **81**: 730.
3 Yoshizawa K *et al*. Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. *J Natl Cancer Inst* 1998; **90**: 1219–1224.
4 Ghadirian P *et al*. A case–control study of toe-nail selenium and cancer of the breast, colon and prostate. *Cancer Detec Prev* 2000; **24**: 305–313.
5 Nomura AM, Lee J, Stemmermann GN, Combs GF Jr. Serum selenium and subsequent risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 883–887.
6 Helzlsouer KJ *et al*. Association between alpha-tocopherol, gamma-tocopherol, selenium and susequent prostate cancer. *J Natl Cancer Inst* 2000; **20**: 2018–2023.
7 Flohe L. Glutathione peroxidase. *Basic Life Sci* 1988; **49**: 663–668.
8 Moscow JA *et al*. Loss of heterozygosity of the human cytosolic glutathione peroxidase I gene in lung cancer. *Carcinogenesis* 1994; **15**: 2769–2773.
9 Ratnasinghe D *et al*. Glutathione peroxidase codon 198 polymorphism variant increases lung cancer risk. *Cancer Res* 2000; **60**: 6381–6383.
10 Easton D, Peto J, Babiker AG. Floating absolute-risk: an alternative to relative risk in survival and case-control analysis avoiding an arbitary reference group. *Stat Med* 1991; **10**: 1025–1035.
11 Aumann KD, Bedorf N, Brigelius-Flohe R, Schomburg D, Flohe L. Glutathione peroxidase revisited-simulation of the catalytic cycle by computer-assisted molecular modeling. *Biomed Environ Sci* 1997; **10**: 136–155.
12 Tan M, Li S, Swaroop M, Guan K, Oberley LW, Sun Y. Transcriptional activation of the human glutathione peroxidase promoter by p53. *J Biol Chem* 1999; **274**: 12061–12066.
13 Chada S, Newburger P. Regulation of human glutathione peroxidase gene expression by selenium. *Glutathione Centennial*, Academic Press, 1989.
14 Menter DG, Sabichi AL, Lippman SM. Selenium effects on prostate cell growth. *Cancer Epidemol Biom Prev* 2000; **9**: 1171–1182.