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Association of polymorphisms in PRKCI gene and risk of prostate cancer in a sample of Iranian Population

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Abstract

The atypical protein kinase C iota (aPKCι) is an oncoprotein encoded by the *PRKCI* gene. It has been reported to play multifunctional roles in cellular maintenance, cell proliferation, survival, differentiation and apoptosis. In the present study we aimed to assess the impact of *PRKCI* rs546950 C>T and rs4955720 C>A polymorphisms on prostate cancer (PCa) risk in a sample of Iranian population. This case-control study was done on 169 patients with pathologically confirmed PCa and 182 benign prostatic hyperplasia (BPH). The PCR-RFLP method was used for detection rs546950 C>T and rs4955720 C>A polymorphisms. Our findings showed that rs546950 polymorphism of *PRKCI* decreased the risk of PCa in codominant (OR=0.35, 95%CI=0.19-0.64, P<0.001, CT vs CC) and dominant (OR=0.39, 95%CI=0.22-0.69, P=0.001, CT+TT vs CC) inheritance model tested. No significant association was found between rs4955720 C>A polymorphism and PCa. In the combined analysis of these two variants subjects carrying CT/CC, CT/CA, TT/AA and CT/AA significantly decreased the risk of PCa in comparison with rs546950 CC/rs4955720 CC genotype. Haplotype analysis indicated that rs546950T/rs4955720A decreased the risk of PCa compared to CC. In conclusion, the results revealed that *PRKCI* rs546950 variant decreased the risk of PCa in an Iranian population. Further studies with larger sample sizes and different ethnicities are required to confirm our findings.

Key words: Prostate cancer, PRKCI, polymorphism.

Introduction

Prostate cancer (PCa) is the most common cancer among men in the United States (1). It is increased rapidly in most of the low-risk populations. In Iran, the incidence rate of PCa is approximately 9.6 per 100,000 (varying from 3.2 to 16.0 per 100,000 according to different geographical setting) (2, 3). This is comparable to Asia-Pacific region (9.9 per 100,000) but significantly lower than the world (32.8 per 100,000) (4). The median age at diagnosis is approximately 66 years and the 5-year survival rate of PCa has been estimated to be 98.9% (5).

Accumulated evidence implies that genetic variations may contribute to the development and progression of PCa (6-8). Genome-wide association studies (GWASs) have shown an association between single nucleotide polymorphisms (SNPs) and risk of PCa (9-13). These findings propose that genetic susceptibility may play a potential role in the etiology of PCa.

Protein kinase C (PKC) includes a family of at least 12 distinctive serine/threonine kinase isoenzymes that have essential roles in transmembrane signal transduction pathways and have been described to regulate cell proliferation (14), differentiation (15), cell-to-cell interaction (16), secretion (17), cytoskeletal functions (18), gene transcription (19), apoptosis (20, 21), and drug resistance (22). Atypical protein kinase C lambda/iota (aPKCλ/ι), encoded by the *PRKCI* gene, has been reported to play multifunctional roles in cellular maintenance, cell proliferation, survival, differentiation and apoptosis (23, 24).

There is little and inconsistent data regarding the impact of *PRKCI* gene polymorphisms on risk/protection of PCa. Campa et al (25) have found a significant association between *PRKCI* rs546950 and rs4955720 polymorphisms and risk of prostate cancer. Recently, Li et al (26) investigated the impact of *PRKCI* rs546950 and rs4955720 polymorphisms on PCa. They found no significant association between the two variants and PCa risk in an Eastern Chinese population. Hence, the present study was aimed to find out the possible association between *PRKCI* rs546950 and rs4955720 polymorphisms and PCa in a sample of Iranian population.

Materials and methods

Patients

This case-control study was done on 169 unrelated men with histopathologically confirmed adenocarcinoma of prostate and 182 ages matched unrelated men with benign prostatic hyperplasia (BPH) with no history of any cancer. All the subjects were enrolled from Department of Urology, Shahid Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Ethical approvals for recruitment were obtained from local Ethics Committee of Zahedan University of Medical Sciences, and written informed consent was

obtained from all cases and controls. Blood samples were collected in EDTA-containing tubes and genomic DNA were extracted using salting out method as described previously (27).

Genotyping

Genotyping of rs546950 polymorphism of *PRKCI* was done by PCR-RFLP methods. The forward and reverse primers were 5`-ACTTAGATGCCTTTCTCATGGCCTGCATC- 3` and 5`- CAGATGTTGCCTTGTTAAAGTCTATCCC-3`, respectively. PCR was performed using commercially available Prime Taq premix (Genetbio, South Korea) according to the manufacturer's recommended protocol. In each 0.20 ml reaction, 1 µl of genomic DNA (~100 ng/ml), 1 µl of each primers and 10 µl of 2X Prime Taq Premix and 7 µl ddH2O were added. The PCR conditions were set as follows: 95°C for 5 min, 30 cycles of 95°C for 30s, 63° for 30s,and 72 °C for 30 s and a final extension step of 72 °C for 10 min. Ten microliter of PCR product digested by BseGI restriction enzyme (Fermentas). The C allele digested and produces 24- and 182-bp while the T allele undigested and produce 206-bp fragment (Figure 1).

Genotyping of rs4955720 polymorphism of *PRKCI* was done by PCR-RFLP methods. The forward and reverse primers were 5′-CTAACGTGGTTAAACCTCGTCTCTACA-3′ and 5′-ACATGACACAATTAGACTCTTGCTTGAT-3′, respectively. The PCR conditions were set as follows: 95°C for 5 min, 30 cycles of 95°C for 30s, 60°C for 30s, and 72 °C for 30s and a final extension step of 72°C for 10 min. Ten microliter of PCR product digested by MboII restriction enzyme (Fermentas). The C allele digested and produced 181 and 225 bp, while the A allele undigested (406 bp) (Figure 2).

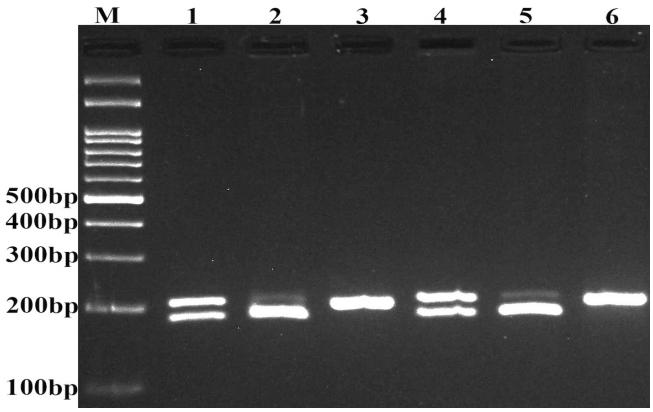

Figure 1. Electrophoresis pattern of the PCR-RFLP method for detection of *PRKCI* rs546950 C>T polymorphism. The C allele digested and produces 24 and 182 bp, while the T allele undigested and produce 206 bp fragment. M: DNA marker; Lanes 1, 4: CT; Lane 2, 5: CC; Lane 3, 6: TT.

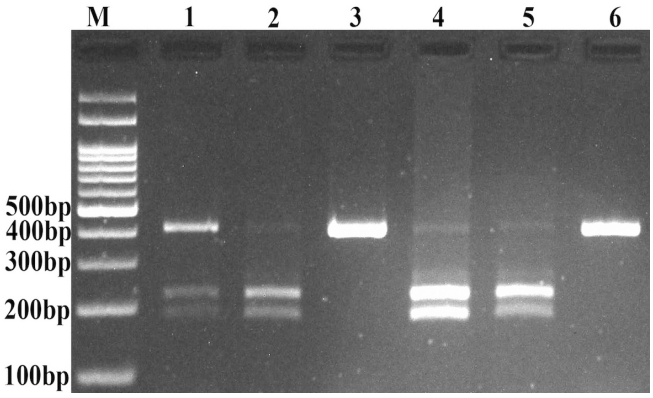

Figure 2. Electrophoresis pattern of the PCR-RFLP method for detection of *PRKCI* rs4955720 C>A polymorphism. The C allele digested and produced 181 and 225 bp, while the A allele undigested (406 bp). M: DNA marker; Lanes 1: CA; Lanes 2, 4, 5: CC; Lanes 3, 6: AA.

Statistical analysis

Statistical analysis was done using statistical package SPSS 18 software. Data were analyzed by independent sample t-test and $\chi 2$ test. Association between *PRKCI* polymorphisms and PCa were calculated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. Haplotype analysis was performed using SNPStats software (28). A p-value less than 0.05 were considered statistically significant.

Results

The study group consists of 169 Pca patients with an average age of 61.33 ±6.61 years and 182 benign prostatic hyperplasia (BPH) with a mean age of 62.51±7.67 years. No significant difference was found between the groups concerning age (p=0.123).

The genotypes and allele frequencies of *PRKCI* rs546950 *and* rs4955720 polymorphisms in PCa and control subjects are shown in table 2. A significant difference was found between cases and controls regarding rs546950 polymorphism ($\chi 2$=9.96; p=0.007). The findings showed that rs546950 polymorphism of *PRKCI* decreased the risk of PCa in codominant (OR=0.35, 95%CI=0.19-0.64, P<0.001, CT vs CC) and dominant (OR=0.39, 95%CI=0.22-0.69, P=0.001, CT+TT vs CC) inheritance model tested. While, the variant was not associated with the risk of PCa in recessive model (OR=1.18, 95%CI=0.69-1.99, P=0.593, TT vs CC+CT).

Table 1. Clinicopathological characteristics of prostate cancer Patients.

Characteristic	No. of patients (%)
Age (years), mean (range)	61.3 (42-79)
PSA at diagnosis mean ± SD (ng/ml)	14.9 ±14.3
Gealson Score	
≤6	57 (33.7)
7	73 (43.2)
>7	39 (23.1)
Stage	
pT1	8 (4.7)
pT2a	27 (16.0)
pT2b	11 (6.5)
pT2c	76 (45.0)
pT3a	13 (7.7)
pT3b	34 (20.1)
Perineural invasion	106 (62.7)
Impotency	26 (15.4)
Loss of Libido	24 (14.2)
Post-void residual, mean ± SD (ml)	27.2 ±25.2
Addiction	8 (4.7)
Hypertension	23 (13.6)
Diabetes mellitus	21 (12.4)
Any history of smoking	27 (16.0)
Alcohol drinking	7 (4.1)

Table 2. Genotypic and allelic frequencies of *PRKCI* polymorphisms in prostrate cancer (PCa) and control subjects.

PRKCI polymorphisms	Prostate Cancer n (%)	Control n (%)	OR (95%CI)	P-value
rs546950 C>T				
Codominant				
CC	41 (24.3)	20 (11.0)	1.00	-
CT	92 (54.4)	128 (70.3)	0.35 (0.19-0.64)	<0.001
TT	36 (21.3)	34 (18.7)	0.52 (0.25-1.05)	0.077
Dominant				
CC	41 (24.3)	20 (11.0)	1.00	-
CT+TT	128 (75.7)	162 (89.0)	0.39 (0.22-0.69)	0.001
Recessive				
CC+CT	133 (78.7)	148 (79.5)	1.00	-
TT	36 (21.3)	34 (18.7)	1.18 (0.69-1.99)	0.593
Allele				
C	174 (51.5)	168 (46.2)	1.00	-
T	164 (48.5)	196 (53.8)	0.81 (0.60-1.08)	0.174
rs4955720 C>A				
Codominant				
CC	86 (50.9)	80 (44.0)	1.00	-
CA	68 (40.2)	76 (41.7)	0.83 (0.53-1.30)	0.428
AA	15 (8.9)	26 (14.3)	0.54 (0.27-1.09)	0.085
Dominant				
CC	86 (50.9)	80 (44.0)	1.00	-
CA+AA	83 (49.1)	102 (56.)	0.76 (0.50-1.15)	0.201
Reccesive				
CC+CA	154 (90.1)	156 (85.7)	1.00	-
AA	15 (8.9)	26 (14.3)	0.54 (0.30-1.15)	0.135
Allelel				
C	240 (71.0)	236 (64.8)	1.00	-
A	98 (29.0)	128 (35.2)	0.75 (0.55-1.04)	0.090

Table 3. Interaction of rs546950 and rs4955720 polymorphisms of *PRKCI* gene on prostate cancer (PCa) risk.

rs546950 C>T	rs4955720 C>A	Prostate Cancer n (%)	Control n (%)	OR (95%CI)	P-value
CC	CC	30 (17.8)	15 (8.2)	1.00	-
CC	CA	11 (6.5)	4 (2.2)	1.38 (0.37-5.05)	0.754
CT	CC	46 (27.2)	58 (31.9)	0.41 (0.19-0.82)	0.013
TT	CC	10 (5.9)	7 (3.8)	0.72 (0.23-2.25)	0.714
CT	CA	41 (24.3)	60 (33.0)	0.34 (0.16-0.71)	0.004
TT	AA	10 (5.9)	15 (8.2)	0.33 (0.12-0.92)	0.044
TT	CA	16 (9.5)	12 (6.6)	0.67 (0.25-1.76)	0.461
CT	AA	5 (3.0)	10 (5.5)	0.25 (0.07-0.86)	0.034
CC	AA	0 (0.0)	1 (0.5)	-	-

The T allele was not associated with PCa (OR=0.81, 95%CI=0.60-1.08, P=0.174) compared with C allele.

As shown in table 2, The *PRKCI* rs4955720 variant was not associated with PCa in any inheritance models tested (co-dominant, dominant and recessive).

Additionally, in the combined analysis of these two variants subjects carrying CT/CC, CT/CA, TT/AA and CT/AA significantly decreased the risk of Pca in comparison with rs546950 CC/rs4955720 CC (table 3). Haplotype analysis is shown in table 4. The haplotype rs546950 T/rs4955720A marginally decreased the risk of PCa (OR=0.67, 95%CI=0.45-0.99, p=0.047) in comparison with rs546950 C/rs4955720 C.

As shown in table 5, the rs546950 C>T variant was marginally associated with clinicopathological characteristics such as age, stage, PSA (prostate specific antigen), While no significant association between rs4955720 polymorphism and clinicopathological characteristics were observed (table 5).

Discussion

In the present study we investigated the impact of *PRKCI* rs546950 and rs4955720 polymrphisms on PCa risk in a sample of Iranian population. Our findings showed a significant association between rs546950 polymorphism and PCa risk, so that CT as well as CT+TT genotypes decreased the risk of PCa in comparison with CC genotype. While no significant association was found between rs4955720 variant and PCa risk.

Furthermore, in the combined analysis of these two polymorphisms, subjects carrying CT/CC, CT/CA, TT/AA and CT/AA have significantly decreased risk of Pca in comparison with individuals carrying rs546950 CC/rs4955720 CC genotype (P<0.05). The Haplotype analysis indicated that rs546950T/rs4955720A decreased the risk of PCa compared to CC (p<0.05).

For the first time, Campa et al (25) investigated the impact of rs546950 and rs4955720 polymorphisms of *PRKCI* gene on risk of PCa in Caucasian. They found that both the variants contributed to a decreased PCa risk, with P-values of 0.0017 and 0.0004, respectively.

Li et al (26) investigated the impact of *PRKCI* rs546950 and rs4955720 polymorphism and PCa risk in an Eastern Chinese Han Population. In contrast to our findings, they found no significant association effects

Table 4. Haplotype association of PRKCI rs546950 and rs4955720 variants with prostate cancer (PCa) risk.

rs546950	rs4955720	Prostate Cancer (Frequency)	Control (Frequency)	OR (95%CI)	P-value
C	C	0.4503	0.3971	1.00	-
T	A	0.2254	0.2872	0.67 (0.45-0.99)	0.047
T	C	0.2598	0.2513	0.83 (0.53-1.31)	0.430
C	A	0.0645	0.0645	0.87 (0.40-1.90)	0.730

Table 5. Association of *PRKCI* polymorphisms with clinicopathologic parameters in prostate cancer (PCa) patients.

Factors	rs546950 C>T			P-value	rs4955720 C>A			P-value
	CC	CT	TT		CC	CA	AA	
Age at diagnosis Y, n				0.051				0.090
≤65	24	67	30		57	50	14	
>65	17	25	6		29	18	1	
Stage				0.050				0.773
pT1	6	1	1		4	4	0	
pT2a	8	15	4		13	13	1	
pT2b	0	8	3		4	6	1	
pT2c	16	41	19		37	29	10	
pT3a	3	9	1		8	4	1	
pT3b	8	18	8		20	12	4	
PSA at diagnosis (ng/ml), n				0.024				0.761
≤4	1	0	0		1	0	0	
4-10	26	37	21		40	37	7	
>10	14	55	15		45	31	8	
Gleason score, n				0.427				
≤6	18	27	12		28	35	23	0.748
7	14	45	14		24	32	12	
>7	9	20	10		5	6	4	
Perineural invasion, n				0.581				0.412
Positive	23	59	24		56	39	11	
Negative	18	33	22		30	29	4	
Surgical margin, n				0.291				0.596
Positive	12	40	15		31	30	6	
Negative	29	52	21		55	38	9	

for the two tested variants in the single locus analysis. While, individuals carrying homozygote wide-type form of these two polymorphisms had marginally reduced PCa risk (OR=0.63, 95% CI=0.40–0.99, P=0.045), compared with those carrying any of heterozygous or homozygous mutant genotypes.

The human *PRKCI* gene is mapped on 3q25-q27. It contains 18 exons and encodes a 587-amino acid protein with a molecular mass of approximately 67.3 KD. The rs546950 is located in first intron; lie in the 5' regulatory region of the gene, although the rs4955720 is situated in 3` untranslated region (3`UTR). The 3`UTR of mammalian mRNA is a repository of regulatory elements for mRNA stability, intracellular localization and translation.

Several studies have shown that PRKCI oncogene is amplified in various cancers such as lung squamous cell carcinoma (29), Alveolar rhabdomyosarcoma (30), breast ductal carcinoma (31), ovarian cancer (32) and PCa (33, 34).

The molecular mechanisms underlying carcinogenesis and progression of PCa have still not been entirely elucidated. The efforts to recognize molecular markers for early detection of PCa as well as personalize both patient prognosis and therapy are of critical clinical significance. The findings from several large case-control studies and cohort studies suggest that family history is a leading risk factor for PCa (35-39).

The limitations of the present study are the following: i) relatively small sample sizes, so replication with larger sample is needed. ii) We did not determine gene-environment interactions. It has been proposed that both genetic and environmental factors may contribute to prostate cancer susceptibility.

Taken together, the findings of the present study designated that rs546950 polymorphism of *PRKCI,* but not rs4955720 decreased the risk of PCa in a sample of Iranian population. Larger sample sizes with different ethnicities are necessary to verify our findings.

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References

1. Siegel, R., Ma J., Zou Z., Jemal A. Cancer statistics, 2014. *CA Cancer J Clin*. 2014, **64**:9-29. doi:10.3322/caac.21208

2. Farahmand, M., Khademolhosseini F., Mehrabani D. Trend of prostate cancer in Fars Province, Southern Iran, 2001-2007. *J Res Med Sci*. 2010, **15**:295-7.

3. Talaiezadeh, A., Tabesh H., Sattari A., Ebrahimi S. Cancer inci-

dence in southwest of iran: first report from khuzestan population-based cancer registry, 2002-2009. *Asian Pac J Cancer Prev*. 2013, **14**:7517-22.
4. Baade, P. D., Youlden D. R., Cramb S. M., Dunn J., Gardiner R. A. Epidemiology of prostate cancer in the Asia-Pacific region. *Prostate Int*. 2013, **1**:47-58. doi:10.12954/PI.12014
5. SEER stat fact sheets: prostate cancer. Surveillance, e., and end results program Web site. http://seer.cancer.gov/statfacts/ html/prost.html. Accessed September 2, 2014.
6. Pournourali, M., Tarang A. R., Yousefi M. The association between 1349T>G polymorphism of ApE1 gene and the risk of prostate cancer in northern Iran. *Cell Mol Biol (Noisy-le-grand)*. 2015, **61**:21-4.
7. Zhou, X., Wei L., Jiao G., Gao W., Ying M., Wang N., et al. The association between the APE1 Asp148Glu polymorphism and prostate cancer susceptibility: a meta-analysis based on case-control studies. *Mol Genet Genomics*. 2015, **290**:281-8. doi:10.1007/s00438-014-0916-3
8. Zhang, X., Weng W., Xu W., Wang Y., Yu W., Tang X., et al. The association between the migration inhibitory factor -173G/C polymorphism and cancer risk: a meta-analysis. *Onco Targets Ther*. 2015, **8**:601-13. doi:10.2147/OTT.S72795
9. Gudmundsson, J., Sulem P., Manolescu A., Amundadottir L. T., Gudbjartsson D., Helgason A., et al. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nat Genet*. 2007, **39**:631-7. doi:10.1038/ng1999
10. Thomas, G., Jacobs K. B., Yeager M., Kraft P., Wacholder S., Orr N., et al. Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet*. 2008, **40**:310-5. doi:10.1038/ng.91
11. Eeles, R. A., Kote-Jarai Z., Al Olama A. A., Giles G. G., Guy M., Severi G., et al. Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. *Nat Genet*. 2009, **41**:1116-21. doi:10.1038/ng.450
12. Al Olama, A. A., Kote-Jarai Z., Giles G. G., Guy M., Morrison J., Severi G., et al. Multiple loci on 8q24 associated with prostate cancer susceptibility. *Nat Genet*. 2009, **41**:1058-60. doi:10.1038/ng.452
13. Stadler, Z. K., Vijai J., Thom P., Kirchhoff T., Hansen N. A., Kauff N. D., et al. Genome-wide association studies of cancer predisposition. *Hematol Oncol Clin North Am*. 2010, **24**:973-96. doi:10.1016/j.hoc.2010.06.009
14. Lord, J. M., Pongracz J. Protein kinase C: a family of isoenzymes with distinct roles in pathogenesis. *Clin Mol Pathol*. 1995, **48**:M57-64.
15. Clemens, M. J., Trayner I., Menaya J. The role of protein kinase C isoenzymes in the regulation of cell proliferation and differentiation. *J Cell Sci*. 1992, **103 (Pt 4)**:881-7.
16. Freed, E., Gailit J., van der Geer P., Ruoslahti E., Hunter T. A novel integrin beta subunit is associated with the vitronectin receptor alpha subunit (alpha v) in a human osteosarcoma cell line and is a substrate for protein kinase C. *EMBO J*. 1989, **8**:2955-65.
17. Lord, J. M., Ashcroft S. J. Identification and characterization of Ca2+-phospholipid-dependent protein kinase in rat islets and hamster beta-cells. *Biochem J*. 1984, **219**:547-51.
18. Owen, P. J., Johnson G. D., Lord J. M. Protein kinase C-delta associates with vimentin intermediate filaments in differentiated HL60 cells. *Exp Cell Res*. 1996, **225**:366-73. doi:10.1006/excr.1996.0187
19. Berry, N., Nishizuka Y. Protein kinase C and T cell activation. *Eur J Biochem*. 1990, **189**:205-14.
20. Freemerman, A. J., Turner A. J., Birrer M. J., Szabo E., Valerie K., Grant S. Role of c-jun in human myeloid leukemia cell apoptosis induced by pharmacological inhibitors of protein kinase C. *Mol Pharmacol*. 1996, **49**:788-95.
21. Pongracz, J., Johnson G. D., Crocker J., Burnett D., Lord J. M. The role of protein kinase C in myeloid cell apoptosis. *Biochem Soc Trans*. 1994, **22**:593-7.
22. Dekker, L. V., Parker P. J. Protein kinase C--a question of specificity. *Trends Biochem Sci*. 1994, **19**:73-7. doi:10.1016/0968-0004(94)90038-8
23. Fields, A. P., Murray N. R. Protein kinase C isozymes as therapeutic targets for treatment of human cancers. *Adv Enzyme Regul*. 2008, **48**:166-78. doi:10.1016/j.advenzreg.2007.11.014
24. Murray, N. R., Kalari K. R., Fields A. P. Protein kinase Ciota expression and oncogenic signaling mechanisms in cancer. *J Cell Physiol*. 2011, **226**:879-87. doi:10.1002/jcp.22463
25. Campa, D., Husing A., Stein A., Dostal L., Boeing H., Pischon T., et al. Genetic variability of the mTOR pathway and prostate cancer risk in the European Prospective Investigation on Cancer (EPIC). *PLoS One*. 2011, **6**:e16914. doi:10.1371/journal.pone.0016914
26. Li, Q., Gu C., Zhu Y., Wang M., Yang Y., Wang J., et al. Two novel PRKCI polymorphisms and prostate cancer risk in an Eastern Chinese Han population. *Mol Carcinog*. 2014. doi:10.1002/mc.22130
27. Hashemi, M., Moazeni-Roodi A. K., Fazaeli A., Sandoughi M., Bardestani G. R., Kordi-Tamandani D. M., et al. Lack of association between paraoxonase-1 Q192R polymorphism and rheumatoid arthritis in southeast Iran. *Genet Mol Res*. 2010, **9**:333-9. doi:gmr728 [pii]10.4238/vol9-1gmr728
28. Sole, X., Guino E., Valls J., Iniesta R., Moreno V. SNPStats: a web tool for the analysis of association studies. *Bioinformatics*. 2006, **22**:1928-9. doi:10.1093/bioinformatics/btl268
29. Justilien, V., Walsh M. P., Ali S. A., Thompson E. A., Murray N. R., Fields A. P. The PRKCI and SOX2 oncogenes are coamplified and cooperate to activate Hedgehog signaling in lung squamous cell carcinoma. *Cancer Cell*. 2014, **25**:139-51. doi:10.1016/j.ccr.2014.01.008
30. Kikuchi, K., Soundararajan A., Zarzabal L. A., Weems C. R., Nelon L. D., Hampton S. T., et al. Protein kinase C iota as a therapeutic target in alveolar rhabdomyosarcoma. *Oncogene*. 2013, **32**:286-95. doi:10.1038/onc.2012.46
31. Turashvili, G., Bouchal J., Ehrmann J., Fridman E., Skarda J., Kolar Z. Novel immunohistochemical markers for the differentiation of lobular and ductal invasive breast carcinomas. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2007, **151**:59-64.
32. Haverty, P. M., Hon L. S., Kaminker J. S., Chant J., Zhang Z. High-resolution analysis of copy number alterations and associated expression changes in ovarian tumors. *BMC Med Genomics*. 2009, **2**:21. doi:10.1186/1755-8794-2-21
33. Cornford, P., Evans J., Dodson A., Parsons K., Woolfenden A., Neoptolemos J., et al. Protein kinase C isoenzyme patterns characteristically modulated in early prostate cancer. *Am J Pathol*. 1999, **154**:137-44. doi:10.1016/S0002-9440(10)65260-1
34. Murray, N. R., Jamieson L., Yu W., Zhang J., Gokmen-Polar Y., Sier D., et al. Protein kinase Ciota is required for Ras transformation and colon carcinogenesis in vivo. *J Cell Biol*. 2004, **164**:797-802. doi:10.1083/jcb.200311011
35. Carter, B. S., Beaty T. H., Steinberg G. D., Childs B., Walsh P. C. Mendelian inheritance of familial prostate cancer. *Proc Natl Acad Sci U S A*. 1992, **89**:3367-71.
36. Gronberg, H., Damber L., Damber J. E. Familial prostate cancer in Sweden. A nationwide register cohort study. *Cancer*. 1996, **77**:138-43. doi:10.1002/(SICI)1097-0142(19960101)77:1<138::AID-CNCR23>3.0.CO;2-5
37. Johnson, A. M., Zuhlke K. A., Plotts C., McDonnell S. K., Middha S., Riska S. M., et al. Mutational landscape of candidate genes in familial prostate cancer. *Prostate*. 2014, **74**:1371-8. doi:10.1002/pros.22849
38. Chen, L. S., Fann J. C., Chiu S. Y., Yen A. M., Wahlfors T., Tammela T. L., et al. Assessing interactions of two loci (rs4242382

and rs10486567) in familial prostate cancer: statistical evaluation of epistasis. *PLoS One*. 2014, **9**:e89508. doi:10.1371/journal.pone.0089508

39. Teerlink, C. C., Thibodeau S. N., McDonnell S. K., Schaid D. J., Rinckleb A., Maier C., et al. Association analysis of 9,560 prostate cancer cases from the International Consortium of Prostate Cancer Genetics confirms the role of reported prostate cancer associated SNPs for familial disease. *Hum Genet*. 2014, **133**:347-56. doi:10.1007/s00439-013-1384-2