# Association of *FGFR4* Genetic Polymorphisms with Prostate Cancer Risk and Prognosis

Liesel M. FitzGerald, PhD[1*], Eric Karlins, M.S.[2*], Danielle M. Karyadi, PhD[2], Erika M. Kwon, M.S.[2], Joseph S. Koopmeiners M.S.[1], Janet L. Stanford, PhD[1,3] , and Elaine A. Ostrander, PhD[2,4]

[1]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, 1124 Fairview Ave N., Seattle, WA 20854

[2]National Human Genome Research Institute, Cancer Genetics Branch, National Institutes of Health, Building 50, 50 South Drive, Bethesda, MD 20892

[3]Department of Epidemiology, School of Public Health and Community Medicine, University of Washington, Seattle, Washington, USA

[*]These authors contributed equally to this work. [4]To whom correspondence may be addressed to: Elaine A Ostrander, Ph.D., Building 50, Room 5351, 50 South Drive, Bethesda MD 20892; Email: eostrand@mail.nih.gov, Phone: 301-594-5284; FAX 301-480-0472.

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**Running Title:** *FGFR4* polymorphisms and prostate cancer risk.

## Abstract

The *fibroblast growth factor receptor 4* (*FGFR4*) is thought to be involved in many critical cellular processes and has been associated with prostate cancer risk. Four single nucleotide polymorphisms within or near *FGFR4* were analysed in a population-based study of 1458 prostate cancer patients and 1352 age-matched controls. We found no evidence to suggest that any of the *FGFR4* SNP genotypes were associated with prostate cancer risk or with disease aggressiveness, Gleason score or stage. A weak association was seen between rs351855 and prostate cancer-specific mortality. Subset analysis of cases that had undergone radical prostatectomy revealed an association between rs351855 and prostate cancer risk. While our results confirm an association between *FGFR4* and prostate cancer risk in radical prostatectomy cases, they suggest that the role of *FGFR4* in disease risk and outcomes at a population-based level appears to be minor.

**Keywords:** prostate cancer; population based association analysis; *FGFR4*; radical prostatectomy.

## Introduction

*FGFR4* belongs to a family of four transmembrane tyrosine kinase receptors (*FGFR1-4*) and is activated by several members of the fibroblast growth factor (FGF) family coupled with an accessory molecule of heparin sulfate proteoglycan (1,2). *FGFR*s are thought to play a role in critical cellular processes including cell cycle regulation, migration, metabolism, survival, and cellular proliferation and differentiation (2). Activation of the extracellular domain of *FGFR*s leads to intracellular signaling of multiple signal transduction pathways including the *Erk*, *MAPK*, *PI3K-Akt* pathways and *WNT* pathways, which are thought to be involved in cancer onset and progression (3,4).

*FGF*s and *FGFR*s have been associated with the occurrence and prognoses of many types of cancer including that of the prostate, breast and lung (3-16). Findings suggest that rs351855, a missense change at codon 388 (Gly388Arg) in the transmembrane domain of the *FGFR4* gene, could play a role in tumorigenesis and disease progression of these cancers (15-21). In the case of prostate cancer, *FGFR4* was found to be associated with disease occurrence, tumor proliferation, and aggression (13,15,16). Wang and colleagues (2004) genotyped rs351855 in 329 cases who underwent radical prostatectomy and 191 controls and found a significant association between the Arg allele and prostate cancer occurrence, pelvic lymph node metastasis, and prostate-specific antigen (PSA) recurrence in Caucasians (16). Additional evidence to suggest a role in prostate cancer has come from protein expression and cell culture studies. *FGFR4* expression is elevated in tumor epithelial cells as compared to normal epithelium (13,16) and cells expressing the Gly allele of rs351855 grow in tighter colonies, show a slower closure rate in wound assays, and are less invasive in Matrigel than Arg expressing cells (16).

In spite of the numerous genetic studies analyzing associations between variants in *FGFR4* and cancer, only one non-synonymous SNP (rs351855) has been genotyped in these studies. In addition, many of these studies were performed on a small number of samples and in selected populations. To address these issues we analyzed four tag SNPs, including rs351855 and two other non-synonymous SNPs, in and near the *FGFR4* gene in 1458 men with prostate cancer and 1352 age-matched controls from a population-based case-control study.

## Materials and methods

The study population consists of participants from two population-based case-control studies of prostate cancer in Caucasian and African American residents of King County, Washington (Study I and Study II), whose collection methodologies have been previously described (22,23). Incident cases with histologically confirmed prostate cancer were ascertained from the Seattle-Puget Sound SEER cancer registry. In Study I, cases were diagnosed between January 1, 1993, and December 31, 1996 and were 40-64 years of age at diagnosis. In Study II, cases were diagnosed between January 1, 2002, and December 31, 2005 and were 35-74 years of age at diagnosis. Overall, 2,244 eligible prostate cancer patients were identified and 1,754 (78.2%) were interviewed. The main reasons for non-response were patient refusal (13.9%), physician refusal to allow patient contact (2.1%), patients were too ill to participate (0.9%), or died before interview (1.4%). Blood samples yielding sufficient DNA for genotyping were drawn from 1,457 (83.1%) cases who completed the study interview.

A comparison group of controls without a self-reported history of prostate cancer, residing in King County, Washington, was identified using random digit dialing (RDD). Controls were frequency matched to cases by five-year age groups and recruited evenly

throughout each ascertainment period for cases. During the first step of RDD, complete household census information was obtained for 94% and 81% of the residential telephone numbers contacted for Study I and Study II, respectively. A total of 2,448 men were identified who met the eligibility criteria and 1,645 (71.7%) completed a study interview. The main reasons for non-participation included refusal (29.1%) or too ill to participate (1.4%). Blood samples were drawn and DNA prepared from 1,352 (82.2%) interviewed controls using standard protocols.

Subjects in both studies completed in-person interviews conducted by trained male interviewers using standardized questionnaires. The questions pertained to the time period up to the reference date, i.e., the date of prostate cancer diagnosis for cases and a randomly pre-assigned date for controls that approximated the distribution of cases' diagnosis dates. Information was collected on family structure and cancer history, medical history, and social and demographic factors. Clinical information on cases, including Gleason score, tumor stage, serum PSA level at diagnosis and primary treatment, was obtained from the SEER cancer registry. All study procedures were approved by the Fred Hutchinson Cancer Research Center institutional review board and the National Human Genome Research Institute. Written informed consent was obtained from all study participants prior to participation.

The Genome Variation Server (http://gvs.gs.washinton.edu/GVS/) was utilized to select *FGFR4* tag SNPs. Only data from the HapMap CEU population were screened with analysis parameters set as a minor allele frequency of 5% or higher and an $r^2$ threshold of 0.80. A total of four SNPs were selected for analysis: rs1966265 (Ile10Val), rs376618 (Leu136Pro), rs351855 (Gly388Arg) and rs7708357. The former three are coding non-synonymous SNPs within *FGFR4*, while rs7708357 is situated 3' of the gene and was selected to distinguish the six

haplotypes that occur in the LD block spanning *FGFR4*.

The Applied Biosystems (ABI) SNPlex™ Genotyping System was used to genotype the SNPs and proprietary GeneMapper® software was used for allele calling (www.appliedbiosystems.com). Discrimination of the specific SNP allele was carried out on the ABI 3730*xl* DNA Analyzer and is based on the presence of a unique sequence assigned to the original allele-specific oligonucleotide. Quality control included genotyping of 140 blind duplicate samples distributed across all genotyping batches. There was 100% agreement between the blinded samples for each of the four SNPs. Each 384-well batch of DNA aliquots genotyped incorporated similar numbers of case and control samples, and all laboratory personnel were blinded to the case-control status of samples.

Departure from Hardy-Weinberg equilibrium was assessed for each SNP separately in controls using the allele procedure in SAS version 9.1.3. Pairwise linkage disequilibrium (LD) was estimated between SNPs based on the $r^2$ statistic calculated in controls, using Haploview software version 4.0 (http://www.broad.mit.edu/mpg/haploview/).

Unconditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) to measure the association between individual SNP genotypes and prostate cancer risk (24), as implemented in SAS version 9.1.3. Potential confounding factors, including age at reference date, PC screening history and first-degree family history of prostate cancer, were examined to see if such factors changed the risk estimates by ≥ 10%. After these tests, only age at reference date was included in the final models. Polytomous regression models were used to generate ORs and 95% CIs for the association between SNP genotypes and cases with prostate cancer stratified by disease aggressiveness (less versus more), Gleason score {≤7 (3+4) versus ≥ 7 (4+3)}, and tumor stage (local versus regional/distant) compared to controls.

More aggressive cases were those with either a Gleason score of ≥ 7 (4+3), regional or distant stage disease, or a PSA level ≥ 20 ng/mL at diagnosis. Both codominant (additive) and dominant genetic models were considered for each variant allele, depending on the distribution of genotypes.

A permutation procedure was used to account for the effect of multiple testing. Pairs of case-control labels and ages were permuted in order to approximate the distribution of the age-adjusted p-values under the null hypothesis. Ages and case-control labels were permuted together to preserve any relationship that may exist between age and case-control status and allow age-adjusted p-values to be calculated for each permutation that are consistent with the original analysis. For each permutation, codominant and dominant models were fit for all SNPs and the minimum of the p-values kept for each SNP. The p-values were ordered to approximate the null distribution of the order statistics for the p-values, i.e., minimum p-value, second smallest p-value, etc. The original p-values were also ordered and permutation p-values were calculated by comparing the ordered p-values to the null distribution for the appropriate order statistic. Permutation p-values can be interpreted as the probability of observing a p-value less than or equal to what was observed for the given order statistic under the null hypothesis of no association with disease for any of the 4 SNPs. For example, the minimum p-value was compared to the null distribution for the minimum p-value and the corresponding permuted p-value can be interpreted as the probability of the minimum p-value being less than or equal to the observed minimum p-value under the null hypothesis. The same is true for the second smallest p-value, the third smallest p-value, etc. The permutation approach to approximating the null distribution of the order statistics will be valid regardless of any correlation between the SNPs. A SNP was considered to be significantly associated with prostate cancer risk if the nominal p-

value and the permuted p-value were both less than 0.05. In the results section, we report unadjusted p-values.

Haplotype analyses were performed using HPlus (http://qge.fhcrc.org/hplus/). The primary endpoint for the survival analyses was time to death from prostate cancer. Survival time, i.e., time elapsed from diagnosis until death, was the time dependent variable used. In each case, a death certificate was obtained to confirm the event. Living cases were censored as of November 15th, 2007. The association between survival and *FGFR4* genotype was evaluated using Kaplan-Meier estimator functions and Cox's proportional hazard models (25) to estimate hazard ratios (HR) and 95% confidence intervals (CI). Final survival analysis models were adjusted for age at diagnosis, Gleason score and diagnostic PSA value.

## Results

The characteristics of the 1458 prostate cancer cases and 1352 controls enrolled in this study are portrayed in Table 1. By design, cases and controls did not differ significantly by race or age. Due to the low number of African Americans recruited to this study, analyses of these men were restricted to logistic regression only. The genotypic frequencies of the four *FGFR4* SNPs did not deviate significantly from Hardy-Weinberg equilibrium in the control samples nor was there apparent linkage disequilibrium between the four SNPs.

There was no significant evidence of an association between any of the *FGFR4* SNPs whether investigated individually (Table 2) or in haplotypes (data not shown) and the risk of prostate cancer in Caucasian or African American men. Similarly, evaluation of prostate cancer risk in Caucasians within categories defined by aggressiveness, Gleason score, or stage of disease in polytomous models did not yield significant results (data not shown).

Subset analyses looking at only those cases that had undergone radical prostatectomy (57% of all cases) compared to controls showed associations between prostate cancer risk and SNPs rs351855, rs1966265 and rs7708357 under a dominant model (Table 3). While the effects of the minor alleles of rs1966265 and rs7708357 were only of borderline significance in terms of disease risk (OR=0.82; 95% CI 0.7-0.99; OR=1.21; 95% CI 1.0-1.47 respectively), carriers of the minor T allele of rs351855 had a significant increase in prostate cancer risk (OR=1.34; 95% CI 1.11-1.62; p=0.002). To further investigate the effect of rs351855, we performed polytomous analyses to examine whether it is associated with aggressive disease, Gleason score and/or tumor stage. As presented in Table 4, the risk for prostate cancer did not differ significantly by disease aggressiveness, Gleason score or stage for carriers of the minor rs351855 T allele.

Survival analyses were performed in the overall case dataset to evaluate prostate cancer-specific survival in the presence of each of the *FGFR4* SNP genotypes. There was no decrease in prostate cancer-specific survival associated with SNPs rs1966265, rs376618 and rs7708357 (data not shown). However, cases with the C allele of rs351855 experienced a significantly worse cause-specific survival (Figure 1) relative to men with only T alleles, with a hazard ratio of 1.7 (95% CI, 1.01-2.92; p=0.04).

## Discussion

Our study found no significant associations between any of the four *FGFR4* tag SNPs and prostate cancer risk overall or when considering disease aggressiveness, Gleason score or tumor stage. Cases with one or two copies of the Gly allele of rs351855 did, however, have somewhat lower prostate-specific survival. In order to replicate the study design by Wang et al. (2004), we also analyzed only those patients who had undergone a radical prostatectomy compared to

controls. While risk did not differ significantly in terms of disease aggressiveness, Gleason score or stage, cases that had undergone radical prostatectomy as primary therapy and carried one or two copies of the rs351855 Arg allele had a greater risk of prostate cancer overall. The significance of this observation in only the subset of cases treated with radical prostatectomy is not clear.

*FGF*s and *FGFR*s have been associated with occurrence and prognoses in lung, bladder, cervical, breast, colorectal, and prostate cancers. In lung adenocarcinoma, the presence of either one or two copies of the Arg allele at rs351855 was significantly associated with an earlier age of disease onset (median 60.2 years vs. 64.6 years for Gly/Gly genotype; p=0.009) (19). The Arg allele was also associated with advanced clinical stage and more frequent lymph node metastases than the Gly/Gly genotype in lung cancer patients (20).

There is also evidence that the interaction between *FGF19* and *FGFR4* contributes to progression in liver, lung, and colon tumors (26). Specifically, blocking the interaction of *FGF19* and *FGFR4* inhibited tumor growth in colon xenografts and prevented hepatocellular carcinomas in *FGF19* transgenic mice. In addition, the *FGFR4* Arg allele of rs351855 has been associated with early lymph node metastasis and advanced lymph node metastasis in colon cancer patients (17). It is also implicated in reducing disease-free survival time and overall survival as well as attenuating the effects of adjuvant systemic therapy in colon cancer (17,21). In contrast to the effects of the Arg allele, in breast cancer the Gly allele of rs351855 appears to function as a tumor suppressor in tissue culture assays, suppressing the cell motility of invasive breast cancer cells (20). However as compelling as these biological results are, subsequent case only and case-control studies have shown no epidemiological evidence for an association between the *FGFR4* Gly388Arg mutation and the above mentioned cancers (18,27,28).

However, in relation to prostate cancer, Wang *et al.* (2004) reported a significant association between the Arg allele and disease occurrence where 15% of radical prostatectomy cases carried the Arg/Arg genotype at codon 388 compared to only 4% of controls, (p=0.005) (16). The Arg allele was also overrepresented in radical prostatectomy cases with lymph node metastases (p=0.04) and PSA recurrence (p=0.02). To examine the biological effects of the two rs351855 alleles, Wang and colleagues (2004) established cell lines expressing either the Gly or Arg alleles using the prostate epithelial cell line PNT1A (17). Cells expressing Gly grew in tightly connected colonies while Arg expressing cells grew in a more scattered, irregular morphology. To investigate differences in cell motility, which when increased can contribute to metastatic disease, a wound assay was also performed (17). At both 24 and 48 hours the cells expressing Gly alleles showed a slower closure rate than those expressing Arg alleles. Additionally, a Matrigel assay was used to confirm that the Arg expressing cells displayed higher invasiveness than their Gly expressing counterparts (17). The increased motility and invasiveness seen in cells expressing Arg may be attributed to the associated increase in uPAR, part of the urokinase activator system suggested to be involved in invasion and metastasis of prostate (29) and other cancers (30). Whilst we also observe a significant association between disease risk and the rs351855 Arg allele in radical prostatectomy cases, this association is not observed at the population-based level or when considering disease aggressiveness, Gleason score or tumor stage.

There are a number of strengths and limitations that should be considered when interpreting our results. Our study has a much larger sample size than previous studies of *FGFR4* SNP genotypes and prostate cancer risk (13,15,16). Follow-up studies in other types of cancer have also experienced difficulty in replicating results derived from small datasets when

larger sample sizes are considered (18,27,28). In addition, the data presented here are population-based and thus reflect the disease as it presents in the general population. However a limitation of this study is the small numbers of African American men present in the population of Western Washington. Also, the limited number of prostate cancer-specific deaths in this population reduces power for the survival analyses. Finally, although the *FGFR4* tag SNPs distinguish all six haplotypes that occur in the Caucasian HapMap LD block, we cannot rule out possible associations that may exist between other SNPs in or near the *FGFR4* gene and prostate cancer.

The findings of this study do not support a major role of *FGFR4* in relation to prostate cancer risk overall or among patients with more aggressive disease, higher Gleason score or advanced tumor stage. There was a weak association between the Gly388Arg SNP and worse prostate cancer-specific survival as well as prostate cancer risk among men who underwent a radical prostatectomy; however, the effect of the former is small and the significance of the latter is unclear.

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**Table 1 Demographic and clinicopathologic characteristics of the population-based study participants, King County, Washington**

| Characteristic | Cases N=1,458 (%) | Controls N=1,352 (%) |
|---|---|---|
| Race | | |
| African American | 149 (10.2) | 85 (6.3) |
| Caucasian | 1309 (89.8) | 1267 (93.7) |
| Age | | |
| 35 - 49 | 118 (8.1) | 127 (9.4) |
| 50 - 54 | 214 (14.7) | 209 (15.5) |
| 55 - 59 | 357 (24.5) | 358 (26.5) |
| 60 - 64 | 433 (29.7) | 348 (25.7) |
| 65 - 69 | 177 (12.2) | 164 (12.1) |
| 70 - 74 | 158 (10.8) | 146 (10.8) |
| First-degree Family History of PC | | |
| No | 1144 (78.5) | 1200 (88.8) |
| Yes | 313 (21.5) | 152 (11.2) |
| PC Screening History | | |
| Never | 157 (10.8) | 183 (13.5) |
| DRE only | 257 (17.6) | 519 (38.4) |
| PSA +/- DRE | 1043 (71.6) | 650 (48.1) |
| Aggressiveness of Disease | | |
| Less Aggressive | 975 (66.9) | - |
| More Aggressive | 482 (33.1) | - |
| Gleason Grade | | |
| ≤7 (3+4) | 1221 (84.2) | - |
| ≥7 (4+3) | 230 (15.8) | - |
| Tumor Stage | | |
| Local | 1132 (78.2) | - |
| Regional/Distant | 315 (21.3) | - |
| Radical Prostatectomy as Primary Treatment | | |
| No | 626 (43) | - |
| Yes | 831 (57) | - |

Abbreviations: PSA, prostate-specific antigen; DRE, digital rectal examination.

**Table 2 Association between *FGFR4* genotypes and prostate cancer risk by race**

| SNP, Genotype | Cases (%) | Controls (%) | OR (95% CI) | P-value[1] |
|---|---|---|---|---|
| Caucasians | | | | |
| rs1966265 | | | | |
| GG | 782 (62.1) | 742 (59.2) | Reference | 0.18 |
| AG | 405 (32.2) | 447 (35.6) | 0.86 (0.73 - 1.02) | |
| AA | 72 (5.7) | 65 (5.2) | 1.05 (0.74 - 1.49) | |
| rs376618 | | | | |
| AA | 703 (56.8) | 712 (57.2) | Reference | 0.73 |
| AG | 448 (36.2) | 437 (35.1) | 1.04 (0.88 - 1.23) | |
| GG | 87 (7) | 96 (7.7) | 0.92 (0.67 - 1.25) | |
| rs351855 | | | | |
| CC | 587 (46.8) | 631 (50.4) | Reference | 0.15 |
| CT | 544 (43.4) | 496 (39.1) | 1.18 (0.99 - 1.39) | |
| TT | 123 (9.8) | 124 (9.9) | 1.06 (0.80 - 1.39 | |
| rs7708357 | | | | |
| GG | 459 (36.5) | 507 (40.4) | Reference | 0.05 |
| AG | 632 (50.2) | 569 (45.4) | 1.23 (1.04 - 1.46) | |
| AA | 167 (13.3) | 178 (14.2) | 1.03 (0.81 - 1.32) | |
| African Americans | | | | |
| rs1966265 | | | | |
| GG | 132 (89.8) | 70 (87.5) | Reference | 0.57 |
| AG | 15 (10.2) | 10 (12.5) | 0.77 (0.31 - 1.93) | |
| rs376618 | | | | |
| AA | 65 (44.8) | 38 (47.5) | Reference | 0.25 |
| AG | 59 (40.7) | 38 (47.5) | 0.96 (0.52 - 1.75) | |
| GG | 21 (14.5) | 4 (5) | 2.46 (0.81 - 9.28) | |
| rs351855 | | | | |
| CC | 104 (71.2) | 60 (75) | Reference | 0.40 |
| CT | 39 (26.7) | 18 (22.5) | 1.29 (0.66 - 2.59) | |
| TT | 3 (2.1) | 2 (2.5) | 0.34 (0.05 - 2.86) | |
| rs7708357 | | | | |
| AA | 23 (15.8) | 9 (11.5) | Reference | 0.38 |
| AG | 74 (50.7) | 35 (44.9) | 0.89 (0.34 - 2.18) | |
| GG | 49 (33.6) | 34 (43.6) | 0.60 (0.23 - 1.50) | |

Abbreviations: CI, confidence interval; FGFR4, fibroblast growth factor receptor 4; OR, odds ratio; SNP, single nucleotide polymorphism.

[a]Indicates P-values that are significant at the 0.05 level after adjusting for multiple comparisons.

**Table 3 Association between *FGFR4* genotypes and prostate cancer risk in Caucasian cases who had radical prostatectomy as primary treatment compared to controls**

| SNP, Genotype | Cases (%) | Controls (%) | OR (95% CI) | P-value[1] |
|---|---|---|---|---|
| rs1966265 | | | | |
| GG | 470 (63.7) | 742 (59.2) | Reference | 0.04 |
| AG±AA | 268 (36.3) | 512 (40.8) | 0.82 (0.68 - 0.99) | |
| rs376618 | | | | |
| AA | 404 (56) | 712 (57.2) | Reference | 0.72 |
| AG±GG | 317 (44) | 533 (42.8) | 1.04 (0.86 - 1.23) | |
| rs351855 | | | | |
| CC | 321 (43.8) | 631 (50.44) | Reference | 0.002[a] |
| CT±TT | 411 (56.2) | 620 (49.6) | 1.34 (1.11 - 1.62) | |
| rs7708357 | | | | |
| GG | 263 (35.7) | 507 (40.4) | Reference | 0.05 |
| AG±AA | 474 (64.3) | 747 (59.6) | 1.21 (1.00 - 1.47) | |

Abbreviations: CI, confidence interval; FGFR4, fibroblast growth factor receptor 4; OR, odds ratio; SNP, single nucleotide polymorphism.

[a]Indicates P-values that are significant at the 0.05 level after adjusting for multiple comparisons.

**Table 4 Association between *FGFR4* rs351855 genotype and risk of prostate cancer in Caucasian cases who had radical prostatectomy as primary therapy, stratified by clinical features, compared to controls**

| rs351855 | Controls | Cases, Less Aggressive | | | Cases, More Aggressive | | | p-value[a] |
|---|---|---|---|---|---|---|---|---|
| Genotype | N (%) | N (%) | OR | 95% C I | N (%) | OR | 95% CI | |
| CC | 631 (50.4) | 199 (43.5) | 1 | Reference | 122 (44.4) | 1 | Reference | |
| CT±TT | 620 (49.6) | 258 (56.5) | 1.36 | 1.10 – 1.70 | 153 (55.6) | 1.31 | 1.00 – 1.70 | 0.78 |
| | | Cases, Gleason Score ≤ 7 (3+4) | | | Cases, Gleason Score ≥ 7 (4+3) | | | |
| | | N (%) | OR | 95% C I | N (%) | OR | 95% CI | |
| CC | 631 (50.4) | 281 (44.4) | 1 | Reference | 40 (40.4) | 1 | Reference | |
| CT±TT | 620 (49.6) | 352 (55.6) | 1.32 | 1.08 – 1.60 | 59 (59.6) | 1.50 | 0.99 – 2.28 | 0.55 |
| | | Cases, Local Stage | | | Cases, Regional/Distant Stage | | | |
| | | N (%) | OR | 95% C I | N (%) | OR | 95% CI | |
| CC | 631 (50.4) | 228 (43.8) | 1 | Reference | 93 (44.1) | 1 | Reference | |
| CT±TT | 620 (49.6) | 293 (56.42 | 1.35 | 1.09 – 1.66 | 118 (55.9) | 1.33 | 0.99 – 1.78 | 0.92 |

Abbreviations: CI, confidence intervals; FGFR4, fibroblast growth factor receptor 4; OR, odds ratio; SNP, single nucleotide polymorphism.
[a]Test for homogeneity of ORs across clinical features.

**Titles and Legends to Figures**

Figure 1 Kaplan-Meier survival estimates for Caucasian prostate cancer patients according to rs351855 genotype.