Prostate Cancer Risk in Relation to a Single Nucleotide Polymorphism in the Insulin-like Growth Factor-binding Protein-3 (IGFBP3) Gene: a Meta-analysis

Ye-Qing Mao, Xin Xu, Yi-Wei Lin, Hong Chen, Zheng-Hui Hu, Xiang-Lai Xu, Yi Zhu, Jian Wu, Xiang-Yi Zheng, Jie Qin, Li-Ping Xie*

Abstract

Insulin-like growth factor-binding protein-3 (IGFBP3) has been identified as a putative tumor suppressor with multifunctional roles in the IGF axis. Recently, there have been a growing body of studies investigating the relation between the IGFBP3 A-202C polymorphism, circulating IGFBP3 and prostate cancer risk, but their outcomes varied leading to controversy. Hence, it is necessary to perform a meta-analysis covering all eligible studies to shed a light on the association of IGFBP3 A-202C and cancer risk. Finally, we included a total of 11 relevant articles between 2003 and 2010 covering 14 case-control studies including 9,238 cases and 8,741 controls for our analysis. Our results showed that A-202C was a marginal risk factor of prostate cancer (allele contrast: OR=1.08, 95% CI:1.01-1.16; dominant model: OR=1.11, 95% CI:1.01-1.22; heterozygote codominant model: OR=1.11, 95% CI:1.03-1.18; homozygote contrast: OR=1.19, 95% CI:1.03-1.37). Stratification analysis revealed that sample size and control source were two major heterogeneous meta-factors especially in the recessive model (source: Population-based control group: p=0.30,12=16.7%, Hospital-based control group: p=0.20,12=30.3%; sample size: Small: p=0.22,12=32.8%, Medium: p=0.09,12=48%, Large p=0.60,12=0.0%); However, contrary to previous findings, no significance was found in racial subgroups. No significant publication bias was found in our analysis. Considering the robustness of the results and the discrepancy among some studies, there might be some unsolved confounding factors, and further more critical large studies are needed for confirmation.

Keywords: IGFBP3 - prostate cancer - polymorphism - meta-analysis

Introduction

As one of the most prominent public health problems in the western, prostate cancer (PCa) was estimated to have claimed 33,720 deaths in the United States in 2011, ranking the second leading cause of cancer death (Brawley, 2012). In China, PCa is more and more concerned, for its growing incidence rate leaping from 1.6/105 PY (person per year) in 2002 to 4.3/105 PY in 2008 (Zhang et al., 2011). Although increasing risk factors such as age, family history of the disease, and race/ethnicity have been identified, the etiology of prostate cancer is still complex and elusive.

One of the factors involved is insulin-like growth factor binding protein-3 (IGFBP3), which regulates IGFs bioavailability to facilitate or inhibit IGF—IGF receptor interaction via binding to circulating IGFs (Collett-Solberg et al., 1996; Kelley et al., 1996). Some studies have demonstrated that decreased circulating IGFBP3 concentration portends higher cancer risk including breast, colorectal, lung and gastric cancer (Hankinson et al., 1998; Ma et al., 1999; Yu et al., 1999; Pham et al., 2007), and the individual variation of gene expression level may largely be attributed to genetic factors. IGFBP3 A-202C polymorphisms, an A-C transversion which is located 202 bp upstream of the transcription start site of IGFBP3, has been confirmed to be associated with basal promoter activity both in vitro and in vivo (Rohrbacher et al., 2005; Wagner et al., 2005), The [A] possessing stronger promoter activity yields higher IGFBP3 gene expression, while the [C] allele or A-202C leads to be a lower one (Deal et al., 2001; D’Aloisio et al., 2003; Costalonga et al., 2009).

Recently, more and more studies have focused on A-202C polymorphism and cancer susceptibility. As for PCa, the results are conflicting. The inconsistency might come from various study design, sample size, recruitment criteria or insignificant effect of polymorphisms. Therefore, it is necessary to perform a meta-analysis reviewing all the published case-control studies to reach a more reliable conclusion on the relation between IGFBP3 A-202C polymorphisms and PCa susceptibility.
Materials and Methods

Literature search

All the publications until Sep 20, 2012 in PubMed, Scopus, Web of Science and Chinese National Knowledge Infrastructure (CNKI) were identified with the search terms 'IGFBP3' or 'insulin-like growth factor-binding protein-3', 'polymorphism', 'variants', 'variation' and 'prostate' with restriction of 'Human'. The potentially associated articles as well as their bibliographies were read in full text or abstract to assess the appropriateness.

Inclusion criteria

The eligible studies should be case-control ones pertaining to IGFBP3 A-202C polymorphisms and PCAs, with sufficient data for odds ratio (OR) or relative risk (RR) and 95% confidence interval (CI) calculation. All the eligible studies with full text articles were retrieved.

Data extraction

The following data were carefully extracted from every identified article independently by two authors including: first author’s name, publication year, ethnicity, subject source, number of cases and controls, IGFBP3 A-202C genotypes distribution frequency. Necessary data for calculation in two articles were retrieved by email, if omitted by the authors. Ethnicity covered in this paper was classified as ‘Caucasian’, ‘Asian’ and ‘Mixed’ which could be further divided in subgroup analysis. Population-based and hospital-based studies were two kinds of subject source.

Statistical analysis

For the meta-analysis, association between IGFBP3 A-202C and PCa risk was demonstrated with pooled OR ±95% confidence intervals (CI) based upon A-202C genotype distribution and allele frequency in each case and control. The fixed-effects model (the Mantel-Haenszel method) or the random effects model (the DerSimonian and Laird method) was selected to calculate the pooled OR, according to Q-statistic and further I² metric in heterogeneity test (Lau et al., 1997; Higgins et al., 2002); if a significant heterogeneity between studies was found (P<0.10), random effects model was employed for the pooled OR calculation (Mantel et al., 1959; DerSimonian et al., 1986). The overall associations in every genetic model (dominant model, recessive model, heterozygote codominant model, allele contrast and homozygote contrast) were also examined by pooled odds ratio (ORs, 95% CI). Subgroup analysis was used to investigate the possible factor contributing to heterogeneity. Sensitivity analysis was performed by calculating the pooled ORs in the absence of every single study to indicate that study’s influence on overall results (Tobias, 1999). Publication bias was presented as funnel plots and assessed by Egger’s and Begg’s linear regression tests (Egger et al., 1997). Hardy-Weinberg equilibriums (HWE) of genotype distribution in all the control groups were performed by chi-square test. All the statistical analyses were performed through Stata software (Stata Corporation, College Station, TX).

Results

Summary statistics

Figure 1 presents the flowchart showing selection and identification process of eligible studies with specific reasons. A total of 11 case-control studies focusing on relation between IGFBP3 A-202C polymorphism and PCa susceptibility between 2003 and 2010, with 9,238 cases and 8,741 controls, were finally included. The sample sizes between studies varied widely ranging from 100 to 2633.

Table 1. Main Characteristics for All Eligible Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Ethnicity</th>
<th>Control source</th>
<th>Genotyping method</th>
<th>Sample size</th>
<th>Case no.</th>
<th>Control no.</th>
<th>P_HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nam</td>
<td>2003</td>
<td>Mixed</td>
<td>HBC</td>
<td>RFLP</td>
<td>Large</td>
<td>483</td>
<td>548</td>
<td>0.98</td>
</tr>
<tr>
<td>Wang</td>
<td>2003</td>
<td>Asian</td>
<td>HBC</td>
<td>RFLP</td>
<td>Medium</td>
<td>307</td>
<td>272</td>
<td>0.57</td>
</tr>
<tr>
<td>Li</td>
<td>2004</td>
<td>Mixed</td>
<td>PBC</td>
<td>RFLP</td>
<td>Medium</td>
<td>440</td>
<td>479</td>
<td>0.21</td>
</tr>
<tr>
<td>Schildkraut</td>
<td>2005</td>
<td>Mixed</td>
<td>HBC</td>
<td>RFLP</td>
<td>Small</td>
<td>100</td>
<td>92</td>
<td>0.3</td>
</tr>
<tr>
<td>Chen</td>
<td>2006</td>
<td>Mixed</td>
<td>HBC</td>
<td>RFLP</td>
<td>Small</td>
<td>213</td>
<td>213</td>
<td>0.69</td>
</tr>
<tr>
<td>Cheng</td>
<td>2006</td>
<td>Asian</td>
<td>PBC</td>
<td>RFLP</td>
<td>Medium</td>
<td>455</td>
<td>466</td>
<td>0.99</td>
</tr>
<tr>
<td>Cheng</td>
<td>2006</td>
<td>Caucasian</td>
<td>PBC</td>
<td>RFLP</td>
<td>Medium</td>
<td>451</td>
<td>444</td>
<td>0.16</td>
</tr>
<tr>
<td>Cheng</td>
<td>2006</td>
<td>Afriacan</td>
<td>PBC</td>
<td>RFLP</td>
<td>Large</td>
<td>666</td>
<td>642</td>
<td>0.24</td>
</tr>
<tr>
<td>Hernandez</td>
<td>2007</td>
<td>Caucasian</td>
<td>HBC</td>
<td>RFLP</td>
<td>Medium</td>
<td>401</td>
<td>366</td>
<td>0.79</td>
</tr>
<tr>
<td>Park</td>
<td>2009</td>
<td>Asian</td>
<td>HBC</td>
<td>RFLP</td>
<td>Small</td>
<td>225</td>
<td>225</td>
<td>0.74</td>
</tr>
<tr>
<td>Johansson</td>
<td>2009</td>
<td>Caucasian</td>
<td>PBC</td>
<td>QPCR</td>
<td>Large</td>
<td>2633</td>
<td>1715</td>
<td>0.35</td>
</tr>
<tr>
<td>Schumacher</td>
<td>2010</td>
<td>Caucasian</td>
<td>PBC</td>
<td>RFLP</td>
<td>Large</td>
<td>2626</td>
<td>2876</td>
<td>0.15</td>
</tr>
<tr>
<td>Safarinejad</td>
<td>2011</td>
<td>Caucasian</td>
<td>HBC</td>
<td>RFLP</td>
<td>Medium</td>
<td>168</td>
<td>336</td>
<td>0.59</td>
</tr>
</tbody>
</table>

HCC, hospital-based case-control; PCC, population-based case-control; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; q PCR, quantitative polymerase chain reaction; HWE, Hardy-Weinberg equilibrium
from approximately 100 to 5000, so we further classified them as follows: ‘Small’ denoted studies with numbers less than 500, ‘Medium’ for those between 500 and 1000 and ‘Large’ for those more than 1000. For ethnicity, there were 4 studies of Caucasian, 2 Asian and 5 mixed populations. Three articles with duplication on sample group and irrelevant IGFBP3 polymorphism sites were therefore excluded (Friedrichsen et al., 2005; Hoyo et al., 2007; Sarma et al., 2008). The main characteristics of selected articles were all listed in Table 1. Basically, all the controls (n = 8,741) were in consistent with HWE (p > 0.05), except for one study which contained a minority subgroup with disequilibrium (Cheng et al., 2003). Then, it was treated as four racial subdivisions independently but rather as a whole for analysis, due to the specialty of MEC (the Multiethnic Cohort study) (Kolonel et al., 2000).

**Main results**

Table 2 showed both case and controls’ genotypes distribution and allele frequency of every available study in the form of number and percentage. By intuitive judgment, we found a slightly favorable distribution of [A] allele and [AA] genotype for the controls, and a similar trend of [C]/[CC] for the cases. Table 3 indicated the main result of this meta-analysis. When all the 14 studies were pooled together, a moderate heterogeneity was revealed in the allele contrast and every genetic model (allele contrast: Q-statistic p = 0.03, I² = 47.0%; dominant model: p = 0.05, I² = 41.8%; recessive model: p = 0.12, I² = 31.5%; heterozygote codominant model: p = 0.13, I² = 30.3%; homozygote contrast: p = 0.02, I² = 47.8%). The pooled calculation (Figure 2) by random-effects model resulted in significant influence of A-202C polymorphism on cancer risk across all the genetic models except the recessive one (allele contrast: OR = 1.08, 95% CI: 1.01-1.16; dominant model: OR = 1.11, 95% CI: 1.01-1.22; heterozygote codominant model: OR = 1.11, 95% CI: 1.03-1.18; homozygote contrast: OR = 1.19, 95% CI: 1.03-1.37).

To explore the source of heterogeneity, we performed subgroup analyses stratified by control source, sample size and ethnicity respectively. The stratification analysis identified both ‘Control source’ and ‘Sample size’ as two major heterogeneous meta-factors especially in the recessive model (source: PBC: p = 0.30, I² = 16.7%; HBC: p = 0.20, I² = 30.3%; size: Small: p = 0.22, I² = 32.8%, Medium: p = 0.09, I² = 48%, Large p = 0.60, I² = 0.0%); the

Table 2. IGFBP3 A-202C Genotype Distribution and Allele Frequency in Cases and Controls

<table>
<thead>
<tr>
<th></th>
<th>Case Genotypes (N,%)</th>
<th>Control Genotypes (N,%)</th>
<th>Allele frequency (N,%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AC</td>
<td>CC</td>
</tr>
<tr>
<td>Nam 2003</td>
<td>135(28)</td>
<td>115(24)</td>
<td>233(48)</td>
</tr>
<tr>
<td>Wang 2003</td>
<td>189(62)</td>
<td>18(6)</td>
<td>100(33)</td>
</tr>
<tr>
<td>Li 2004</td>
<td>97(22)</td>
<td>126(29)</td>
<td>217(49)</td>
</tr>
<tr>
<td>Schildkraut</td>
<td>18(18)</td>
<td>27(27)</td>
<td>55(55)</td>
</tr>
<tr>
<td>Chen 2006</td>
<td>55(26)</td>
<td>67(31)</td>
<td>91(43)</td>
</tr>
<tr>
<td>Cheng 2006-Af</td>
<td>217(33)</td>
<td>308(46)</td>
<td>141(21)</td>
</tr>
<tr>
<td>Cheng 2006-C</td>
<td>103(23)</td>
<td>220(49)</td>
<td>128(28)</td>
</tr>
<tr>
<td>Cheng 2006-As</td>
<td>264(58)</td>
<td>161(35)</td>
<td>30(7)</td>
</tr>
<tr>
<td>Cheng 2006-H</td>
<td>22(31)</td>
<td>36(51)</td>
<td>12(17)</td>
</tr>
<tr>
<td>Hernandez 2007</td>
<td>112(28)</td>
<td>93(23)</td>
<td>196(49)</td>
</tr>
<tr>
<td>Park 2009</td>
<td>128(57)</td>
<td>21(9)</td>
<td>76(34)</td>
</tr>
<tr>
<td>Johansson 2009</td>
<td>891(34)</td>
<td>439(17)</td>
<td>130(49)</td>
</tr>
<tr>
<td>Schumacher 2010</td>
<td>724(28)</td>
<td>556(21)</td>
<td>136(51)</td>
</tr>
<tr>
<td>Safarinejad 2011</td>
<td>23(14)</td>
<td>60(36)</td>
<td>85(51)</td>
</tr>
</tbody>
</table>

Figure 2. Forest Plots of Cancer Risk Associated with IGFBP3 A-202C Polymorphism in Different Genetic Models (A. allele contrast, B. dominant model, C. codominant model, D. homozygote contrast). The squares and horizontal lines correspond to the study-specific odds ratio (OR) and 95% confidence interval (CI). The area of the squares reflects the study-specific weight. The diamond represents the pooled OR and 95% CI

Figure 3. Funnel Plot for IGFBP3 A-202C Polymorphism and Cancer Risk
Ye-Qing Mao et al

between different races especially in Africans
find any regular genotype distribution or association
overall calculation to some extent (Schumacher et al.,
studies as the largest weight in our analysis swayed the
controls screened from seven well-established cohort
population-based study including 2,626 cases and 2,876
biases producing significant heterogeneity. One available
SNP polymorphisms under investigation and potential
be attributed to suboptimal representativeness of hospital
former reports, but in discrepancy with Li's result derived
heterozygote codominant model (African : p=0.41, F=0.0%, Caucasian:
p=0.13, F=41.0%, Asian: p=0.27, F=23.0%) as well as in the recessive model. By stratification, significant
associations between A-202C and cancer risk were found
mainly within ‘population-based control’ group (allele
contrast: OR=1.06, 95% CI : 1.01-1.11; dominant model:
OR=1.13, 95% CI : 1.05-1.21; heterozygote codominant
model: OR=1.13, 95% CI : 1.05-1.22; homozygote
contrast: OR=1.11, 95% CI : 1.00-1.22), and ‘large’
sample group (allele contrast: OR=1.07, 95% CI : 1.02-
1.11; dominant model: OR=1.10, 95% CI : 1.02-1.19;
heterozygote codominant model: OR=1.11, 95% CI :
1.03-1.21). In the race subgroup, the association was
only found in Caucasians in the heterozygote codominant
model (OR=1.14, 95% CI : 1.05-1.24).

Other results
To examine the publication bias, Begg’s and Egger’s
tests for the alleles comparison were performed with
a Begg’s funnel plot (Figure 3) provided for visual
judgment. Both tests revealed no publication bias in this
analysis (Begg’s z=0.44 p=0.66, Egger’s t=0.84 p=0.42),
and no significant asymmetry was found in the funnel plot.
To explore whether the ORs were sufficiently robust under
various genetic model and contrasts, the sensitivity tests
were performed where the remaining studies were pooled
after every single one was deleted; The results showed that
none of the studies could considerably affect the overall
risk estimates in our meta-analysis (data were not shown).

Discussion
This meta-analysis including 9,238 cases and 8,741
controls represents the largest study to date investigating
the association between IGFBP3 A-202C polymorphisms
and PCa susceptibility as far as we know. Our results
revealed that C allele/[CC] genotype were slightly more
frequent than A allele/[AA] genotype at IGFBP3 A-202C
SNP site and A-202C is a potential risk factor for PCa,
which was especially more prominent within ‘population-
based control’ and ‘large’ subgroups with negligible
heterogeneity. This finding is generally in line with some
former reports, but in discrepancy with Li’s result derived
from a smaller sample size and a sole stratification (Li et
al., 2010). An increased cancer risk of C allele carriers
among the PBC groups but rather among HBC ones could
be attributed to suboptimal representativeness of hospital
controls with potential disease conditions involving the
SNP polymorphisms under investigation and potential
biases producing significant heterogeneity. One available
population-based study including 2,626 cases and 2,876
controls screened from seven well-established cohort
studies as the largest weight in our analysis swayed the
overall calculation to some extent (Schumacher et al.,
2011). Hence, a large population-based control is more
reliable in meta-analysis. For race stratification, we didn’t
find any regular genotype distribution or association
between different races especially in Africans
Despite a comprehensive study with substantial
data and insignificant publication bias, there were still
some limitations in our study: First, heterogeneity of
various levels existed among most subgroups and genetic
models, which meant some heterogeneity factors were
yet to be analyzed. One of the reasons might come from
inconformity of raw data that should be adjusted by age,
smoking status, drinking status, obesity, and environmental/
lifestyle factors. Second, unavailable details of race sub-
distribution in two studies prevented themselves from
inclusion for subgroup analysis, which lead to insufficient
samples in Africans and Asians subgroups compared with
Caucasians (Nam et al., 2003; Li et al., 2004). Besides,
it should be noteworthy that our conclusion actually
owed much to Safarinejad’s report (Safarinejad et al.,
2011) with the most prominently positive result of all.
While most other included studies yielded insignificant
results, which meant our conclusion was seemingly less
robust. Recently, it has been reported that IGFBP3 as a
multifunctional anti-proliferative protein gets involved in
benign prostatic hyperplasia (BPH) development in a
similar way with PCa (Neuhouser et al., 2008; Safarinejad
et al., 2011), suggesting that it was possible for some
BPH cases to be improperly grouped as controls, not
to mention the asymptomatic or underdiagnosed PCa
cases. On the other hand, the widely accepted hypothesis
that circulating concentration of IGFBP3 runs inversely
with PCa risk has been more and more challenged by
case-control studies (Severi et al., 1999; Li et al., 2004;
Hong et al., 2008). Recently, one study has focused on
intracellular level of instead of circulating level of IGFBP3
and identified a high expression of IGFBP3 in nucleus as
a poor prognostic biomarker (Seligson et al., 2012). If it is
further strengthened, the significance of IGFBP3 A-202C
should be re-defined.

In conclusion, our meta-analysis indicated that there
is a marginal association between IGFBP3 A-202C
polymorphisms and PCa risk. However, further studies
using well-defined large-scale controls, adjusted data
should be carried out critically with more detailed
stratifications. Only in this way, a more comprehensive
and insightful understanding of the IGFBP3 A-202C
polymorphism could be obtained.

Acknowledgements
This study was supported by grants from the National Natural Science Foundation of China (Grant
No. 30900552) and Zhejiang Provincial Natural Science
Foundation of China (Grant No. LY12H05006).

References
like growth factor (IGF)-1 and IGF-binding protein-3
concentrations and prostate cancer risk: results from the
European Prospective Investigation into Cancer and
Brawley OW (2012). Prostate cancer epidemiology in the United
Collett-Solberg PF, Cohen P (1996). The role of the insulin-like
growth factor binding proteins and the IGFBP proteases in
modulating IGF action. Endocrinol Metab Clin North Am,
25, 591-614.


