Advanced prostate cancer risk, selenium, and oxidative stress: the role of genetic variation and environment

Summary / Samenvatting

Summary

Prostate cancer (PCa) is a common disease that is clinically heterogeneous. Only a subset of all PCa patients has advanced disease that is associated with a poor prognosis. Many other PCa patients have relatively slow-growing tumors that may never become clinically relevant when left untreated. The present thesis deals with PCa risk factors and specifically focuses on advanced PCa. Advanced PCa may have a different etiology compared to non-advanced PCa. Many previous studies on PCa risk factors only focused on overall PCa. In the present thesis, we investigated the role of selenium and the related oxidative stress pathway, which according to previous scientific research may play a role in (advanced) PCa development. Oxidative stress is a biological condition that results from an imbalance between reactive oxygen species and antioxidants in favor of the former. It has been hypothesized that oxidative stress is involved in aging and age-related diseases including PCa. Selenium is an essential micronutrient with antioxidant properties. The oxidative stress pathway includes many endogenous (genetic) and exogenous (environmental) pro- and antioxidants.

The various analyses in this thesis were conducted within the large prospective Netherlands Cohort Study, which included 58,279 men at baseline in September 1986. Netherlands Cohort Study participants completed a baseline questionnaire on many potential cancer risk factors including pro- and antioxidant intake. Study participants also provided toenail clippings, which were used to measure selenium concentrations and to isolate DNA for genotyping. We used a candidate gene association approach and studied single nucleotide polymorphisms (SNPs), which are the most common type of genetic variation in humans. We studied SNPs in genes encoding major selenoproteins (through which selenium exerts its biological activities) and oxidative stress-related proteins (e.g., pro- and antioxidant enzymes). Cancer cases were prospectively identified by annual record linkage to cancer registries. For the research described in this thesis, a follow-up of 17.3 years was used (1986–2003). Clinical and pathological TNM (Tumor, Node, Metastasis) stage was used to classify PCa. Two definitions of advanced PCa were used, i.e.: stage III/IV PCa (T3−4, N+, or M1) and stage IV PCa (T4, N+, or M1). The Netherlands Cohort Study employs a case–cohort design, which involves using a subcohort to estimate the person-time experience. The advantage of this design is that it considerably reduces data collection, which is important because costly and time-consuming methods for exposure monitoring and genotyping were used in the thesis.

In **Chapter 2** of the thesis we investigated the association between toenail selenium levels and advanced PCa risk. Toenail selenium is a selenium status biomarker that reflects long-term selenium intake (compared to blood selenium), and our study was the first large study of toenail selenium and advanced PCa risk. In our

study population of men from the Netherlands with low to moderate selenium status, we found that toenail selenium levels were inversely associated with advanced PCa risk. An inverse association between selenium status and advanced PCa risk has been shown previously in several other prospective studies that used blood selenium for exposure monitoring. Future research on selenium and PCa risk should focus on men who have low, suboptimal selenium levels (e.g., European men).

In **Chapter 3** we studied the associations between common genetic variants in the major selenoprotein genes *SEPP1* (5 tagging SNPs) and *GPX1* (3 tagging SNPs) and advanced PCa risk and examined potential gene–environment interactions with toenail selenium levels. The study showed that *SEPP1* and *GPX1* gene variants influenced advanced PCa risk (marginal associations) but the variants did not modify the association with toenail selenium (no gene–environment interactions). The positive findings for *SEPP1* and *GPX1* in our study suggest that selenoprotein genes may play a role in the etiology of advanced PCa.

In **Chapters 4 and 5** of the thesis we studied pro- and antioxidant intake in relation to overall and advanced PCa risk. The study showed that most antioxidant nutrients (e.g., vitamins, carotenoids) and pro-oxidant exposures (e.g., smoking, alcohol), either separately or combined in a score, were not associated. This is supported by previous research on this topic. However, our study also showed that intake of antioxidant flavonoids and black tea (a major source of flavonoids) was associated with a reduced risk of advanced but not total PCa. Few other studies examined flavonoids in relation to (advanced) PCa risk and these studies generally do not support an association. Our findings therefore require confirmation from other studies.

In **Chapter 6** we investigated associations between SNPs in oxidative stress-related genes and advanced PCa risk and examined potential interactions with pro- and antioxidant intake. The tested SNPs (n = 14) were candidate SNPs that have reported functionality or have been associated with PCa risk in previous studies. We found that a SNP in the antioxidant gene *CAT* (rs1001179) was associated with advanced PCa risk. The association has been reported in one previous study (for overall PCa) and is supported by functional data. Furthermore, we found seven novel gene–environment interactions that retained significance after adjusting for multiple testing. The statistically significant multiplicative gene–environment interactions in our study suggest that the oxidative stress pathway is involved in the etiology of advanced PCa. We therefore think that additional epidemiological research on this topic is needed.

In **Chapter 7**, the main study findings as well as the study limitations are discussed. Furthermore, recommendations for future studies are given.

Samenvatting

Prostaatkanker is een veelvoorkomende ziekte waarvan uiteindelijk slechts een subgroep advanced (d.i., gevorderd stadium) zal worden. Advanced prostaatkanker is een type prostaatkanker dat geassocieerd is met een slechte prognose. Deze thesis gaat over risicofactoren voor advanced prostaatkanker. Wij onderzochten de mogelijke rol van selenium en de gerelateerde oxidatieve stress pathway. Oxidatieve stress is een biologische toestand die het gevolg is van een verstoorde balans tussen schadelijke reactieve zuurstofvormen (meer) en antioxidanten (minder). Oxidatieve stress is mogelijk betrokken bij veroudering en ouderdomsgerelateerde aandoeningen zoals prostaatkanker. Selenium is een essentieel micronutriënt en antioxidant. De oxidatieve stress pathway omvat verschillende endogene (genetische) en exogene (omgevingsfactoren) pro- en antioxidanten.

De analyses beschreven in deze thesis zijn uitgevoerd in de prospectieve Nederlandse Cohortstudie (58279 mannen bij baseline in 1986). De studiedeelnemers hebben aan het begin van de studie een gedetailleerde vragenlijst (o.a. over dieet en andere leefstijlfactoren) ingevuld. Daarnaast hebben ongeveer 80% van de deelnemers een teennagelmonster aangeleverd. Dit biologisch materiaal werd in dit onderzoek gebruikt voor (1) het bestuderen van genetische variatie en (2) de bepaling van seleniumconcentraties. Wij onderzochten genetische variatie in belangrijke selenoproteïnegenen (de activiteit van selenium wordt uitgeoefend door deze genen) en genen van de oxidatieve stress pathway. Voor dit onderzoek werden de deelnemers van de studie 17.3 jaar gevolgd (1986–2003) voor het ontstaan van advanced (stadium III/IV of IV) prostaatkanker. De Nederlandse Cohortstudie maakt gebruik van het case–cohort design.

In **Hoofdstuk 2** van de thesis onderzochten wij de associatie tussen teennagelseleniumwaarden en het risico op advanced prostaatkanker. Wij vonden dat een lage seleniumwaarde geassocieerd was met een sterk verlaagd risico op advanced prostaatkanker.

In **Hoofdstuk 3** bestudeerden wij de associaties tussen (tagging) genetische varianten in twee selenoproteïnegenen, d.i., *SEPP1* en *GPX1*, en advanced prostaatkanker. Wij onderzochten tevens mogelijke gen–omgevingsinteracties met teennagelselenium. De studie toonde aan dat het risico op advanced prostaatkanker werd beïnvloed door de genetische varianten. De associatie tussen teennagelseleniumwaarden en advanced prostaatkanker werd echter niet gemodificeerd door de genetische varianten (d.i., geen gen–omgevingsinteractie).

In **Hoofdstuk 4 en 5** van de thesis bestudeerden wij de inname van pro- en antioxidanten in relatie tot totaal en advanced prostaatkanker. De meeste pro- en antioxidanten waren niet geassocieerd, d.i., als individuele factor of gecombineerd in

een score. Wij vonden wel dat een hogere inname van flavonoïden (een groep antioxidanten) geassocieerd was met een verlaagd risico op advanced prostaatkanker. Deze bevinding heeft nood aan bevestiging van andere studies.

In **Hoofdstuk 6** onderzochten wij de associaties tussen (kandidaat) genetische varianten in oxidatieve stress-gerelateerde genen en advanced prostaatkanker en bestudeerden potentiële interacties met inname van pro- en antioxidanten. Wij vonden dat een genetische variant in het catalase-gen (*CAT*) geassocieerd was met het risico op advanced prostaatkanker en identificeerden zeven statistisch significante gen–omgevingsinteracties.

Hoofdstuk 7 omvat een wetenschappelijke discussie, mogelijke beperkingen van het onderzoek, en aanbevelingen voor toekomstig onderzoek.