Genetic susceptibility of cervical cancer

Zoodsma, Margreet

Summary

Cervical cancer is the third most common cancer in women worldwide. Lifetime risks for cervical cancer range from 0.4% in Israel to 5.3% in Colombia, where cervical cancer is the most common malignancy in women. In general, the incidence of cervical cancer is higher in underdeveloped countries and less frequent in Western and industrialized countries, as a result of effective screening programs.
Infection with oncogenic types of human papillomavirus (HPV) is the main causal factor of cervical cancer and its precursor lesions called cervical intraepithelial neoplasia (CIN). During their lifetime many women will become infected with HPV, but only a minority will develop CIN or cervical cancer. Consequently, there have to be other factors, e.g. genetic factors that play a role in the development of CIN or cervical cancer. Candidate genes are genes involved in immunomodulatory and metabolic pathways. These genes probably confer only a small to moderate increase in the lifetime risk for CIN or cervical cancer.Variants in these so-called low penetrance genes are expected to be present in a large number of people and therefore the population attributable risk may be high. In subjects carrying low penetrance gene variants, environmental factors, possibly interacting with genetic factors are expected to affect the risk of developing cancer. One subject may be 10-200 times more sensitive than another and may therefore develop cancer at the same level of exposure.
This thesis addresses the role that a set of selected candidate genes play in the susceptibility to CIN or cervical cancer.

After an introduction to the thesis in **Chapter 1** common and genetic risk factors that previously have been considered to be involved in the development of CIN and cervical cancer are discussed in **Chapter 2**. The goal of this review was the evaluation of polymorphisms that are put forward as candidates for either association with CIN and/or cervical cancer. We pooled results reported for DQA1, DQB1 and DRB1 alleles and 10 other genes that have been evaluated in more than one study. An association of polymorphisms with CIN and cervical cancer at or below 5% significance was found for 15 human leukocyte antigen (HLA) II alleles. Four

polymorphisms (the tumor suppressor gene Tp53, IL-10, CYP2D6 and Methylenetetrahydrofolate reductase (MTHFR)) were associated with an increased CIN and cervical cancer risk. However, only the pooled analyses of the DQB1 alleles, the HLA-DR variants and Tp53 genes had sufficiently large sample sizes to confirm or exclude the proposed association. The sample size for DQA1 and several other genes, despite the pooling of data, was still insufficient to have an adequate power for conclusions about the presence or absence of meaningful associations with CIN and cervical cancer. Our data indicate that further analysis in larger sample sizes, especially for genes other than the HLA genes, is necessary to describe the exact relations between these genes and susceptibility for CIN and cervical cancer with an adequate power.

In **Chapter 3** three Dutch families with familial clustering of (pre) neoplastic cervical disease are described and a review of the literature is given on familial risks of CIN and cervical cancer. In the literature familial clustering of cervical cancer is associated with an elevated relative risk of 1.5-2.3 for female family members, but genes have not been identified yet. Future studies should include not only carcinoma in situ, but also CIN grade II-III and the paternal relatives. Practical guidelines for women from families with familial clustering of cervical cancer are discussed. When in the future genetic risk for cervical cancer can be identified at the molecular level, families with clustering of cervical cancer should be offered genetic testing to find out whether they carry a genetic predisposition for cervical cancer. The identification of such a predisposition could stimulate compliance to screening programs, or, in regions without population screening, it might make cervical cancer screening available to the women involved. Moreover, when genetic susceptibility indeed turns out to act through a decreased host response to HPV infection, women with this particular susceptibility are good candidates for prophylactic HPV vaccination.

The variability in host immunogenetic background, such as the HLA class I or II type antigens is an important parameter in determining the overall cellular immune response to HPV infections. In **Chapter 4** the involvement of the HLA region in CIN and cervical cancer susceptibility was analyzed. The aim of the study was to explore

whether the HLA-DQ and/or HLA-DR genes are responsible for the earlier reported association with the disease or whether, due to strong linkage disequilibrium in the HLA-region, other genes in the vicinity of these genes are responsible. Markers covering the entire HLA-region were genotyped in a large sample of CIN and cervical cancer patients, as well as in controls. A total of 311 CIN patients, 695 cervical cancer patients as well as 115 family-based and 586 unrelated controls were included. Two markers showed an association with susceptibility to cervical neoplasia, G511525 and MICA, respectively. Marker G511525, close to the region containing the HLA-DQ and -DR genes, was most strongly associated, showing a decrease in frequency of allele 221 from 6.7% to 3.3% in squamous cell cancer patients. Furthermore, an association was found for the MICA marker (allele 184) with squamous cell cancer patients (allele 184: OR=1.31 (CI 1.13-1.53); homozygotes, OR=1.48 (CI 1.06-2.06)). No associations were observed with either adenocarcinoma or CIN. In conclusion, our study firmly establishes the association of the region containing the HLA-DQ and -DR genes with the risk of developing squamous cell carcinoma. Furthermore, an increased risk was observed for carriers of allele 184 at the MICA locus and in particular for homozygotes, suggesting a recessive effect.

Cellular immunity may be critical in the elimination of HPV harboring cells. IL-10, a T-helper 2-type cytokine, has a suppressive effect on cell-mediated immunity. Resistance to apoptosis through the apoptotic death receptor signaling Fas pathway might enable many cancers to escape the immune system. Therefore we examined, as described in **Chapter 5,** whether three polymorphisms in the Il-10 gene and a polymorphism at position –670 of the Fas promotor affects susceptibility for CIN. In addition it was studied whether these polymorphisms were causal and not merely associated by typing microsatellite markers in the region surrounding both genes. A total of 311 CIN, 695 cervical cancer patients and 115 family-based as well as 586 unrelated controls were analyzed. Association analysis revealed an increased CIN (CIN II-III (OR 1.44 (1.06-1.97)) and squamous cell carcinoma of the cervix (OR 1.35 (1.04-1.75)) for individuals heterozygous for the A-allele of the IL-10 –592 polymorphism. In contrast to previous findings, no association was found for the IL-

10 –1082 polymorphism, while an increased risk for AC in heterozygotes for the Fas –670 polymorphism (OR 1.59 (1.02-2.48)) was observed.
Conclusion: our study shows a possible role for the IL-10 gene in CIN and squamous cell cervical cancer susceptibility in the Caucasian population, simultaneously there might be a role for the Fas gene in the development of adenocarcinoma of the cervix. Further investigations with a higher density of markers and functional studies are necessary to identify causal mutation(s).

The tumor suppressor gene p53 has a major role in maintaining the integrity of the cellular DNA. Upregulation of p53 causes G1 arrest, resulting in a delayed gene amplification allowing genetic damage to be repaired. HPV 16 and 18 encode two major oncoproteins, E6 and E7. The E6 protein binds to and induces the degradation of p53 protein. A sequence polymorphism at position 72 results in the presence of either a proline or an arginine at this position. The arginine form of p53 is more susceptible for E6-degradation than the proline form.
The p21 gene, also known as WAF1 or CIP1, located on chromosome 6p21.2, has been cloned and identified as a p53 mediator and an inhibitor of G1 cyclin-dependent kinases. Alterations in this gene may adversely affect the regulation of cellular proliferation and increase cancer susceptibility. In **Chapter 6** we aimed to explore whether the codon 72 polymorphism at the p53 locus affects susceptibility for cervical neoplasia. As the role of the p21 polymorphism is unknown with regard to cervical neoplasia in the Caucasian population, we also investigated the effect of this polymorphism on cervical neoplasia susceptibility and its interaction with the p53 codon 72 polymorphism. A total of 266 CIN grade II/III patients, 695 invasive cervical cancer patients as well as 115 family-based and 586 unrelated controls were included. Association analysis revealed an increase of the Arg allele/Arg homozygotes frequency of the p53 codon 72 among cervical cancer cases (OR Arg allele=1.25, 95% CI 1.05-1.49; OR Arg homozygotes homozygotes =1.57, 95% CI 1.03-2.41). The p21 codon 31 polymorphism also revealed a significant difference of the Ser allele/Ser homozygotes frequency between cervical cancer cases and controls (OR Ser allele=1.36, 95% CI 1.00-1.83; OR Ser homozygotes =1.38, 95% CI 1.01-1.90). Cervical cancer cases were significantly more often doubly homozygous than controls

(OR 1.79, 95% CI 1.10-2.90). The risks for single homozygotes were also increased, although not significant. These findings could however be explained by independent effects of the two polymorphisms, implying that the two genes likely do not interact with each other. No associations were found for both polymorphisms with CIN patients. In conclusion, in a large sample of Caucasian cases and controls, an association with the codon 72 polymorphism of the p53 gene and with the codon 31 polymorphism of the p21 gene with susceptibility to cervical cancer but not to CIN was observed. Analysis of the combined genotypes of the two polymorphisms suggests that there is no interaction between the two genes.

MTHFR is a critical enzyme regulating the metabolism of folate and methionine. The potential influence of MTHFR activity on DNA methylation and on the availability of uridylates and thymidylates for DNA synthesis and repair presents MTHFR as a candidate for a cancer predisposing gene. **Chapter 7** describes our study, which tested in a large study population whether the C677T polymorphism at the MTHFR locus affects susceptibility for CIN. In addition it was studied whether this polymorphism is causal and not merely associated by typing microsatellite markers in the region surrounding the MTHFR gene. A total of 311 CIN, 695 cervical cancer patients as well as 115 family-based and 586 unrelated controls were analyzed. Association analysis showed a decreased cervical cancer risk for individuals heterozygous or homozygous for the T-allele, both for squamous cell carcinoma (heterozygous OR 0.66 (0.51-0.86); homozygous OR 0.76 (0.49-1.16)) and adenocarcinoma (heterozygous OR 0.71 (0.49-1.03); homozygous OR 0.34 (0.14-0.81)). No difference was found for high grade CIN (heterozygous OR 1.03 (0.76-1.40); homozygous OR 0.91 (0.54-1.55). A microsatellite haplotype containing the C allele was associated with an increased risk for cervical cancer and CIN (both among squamous cell carcinomas, adenocarcinomas and CIN II-III) (OR=2.61 (1.59-4.27). In conclusion, our study lends further support to the hypothesis that the MTHFR C677T polymorphism is involved in susceptibility cervical cancer, but also illustrates that despite the large sample size analysed still larger studies are needed to fully establish the nature of this association.

Transplant patients might comprise a special group with respect to HPV infection and cervical cancer risk. Transplant recipients, who all receive immunosuppressants, have an increased incidence of malignancies compared to the normal population, while immunosuppressive therapy in itself has also been shown to be an independent risk factor for developing de novo malignancies. Immunosuppressants in general diminish the immunological defence against spontaneous mutations, continuous exposure to antigens and oncogenic viruses. Therefore in **Chapter 8** the incidence of cervical neoplasia and non-melanoma skin cancer in female organ transplant recipients were determined and to identify possible HLA class I or class II loci related to cervical neoplasia and non-melanoma skin cancer occurrence, these were also explored. Information regarding HLA typing, medication, immunosuppressive therapy prior to and after transplantation, underlying diseases, gynecological history and cancer was retrieved from the transplantation databases (444 renal transplant recipients and 170 orthotopic liver transplant recipients). Data on cervical neoplasia and its risk factors were retrieved by a questionnaire. For cervical cancer, its precursor CIN grade III and squamous cell cancer and basal cell cancer of the skin, standardized incidence ratios (SIRs) were calculated. Multivariable analysis for risk factors was performed by logistic regression analysis. SIRs for CIN III and squamous cell cancer and basal cell cancer of the skin were highly increased: 9.8 (95% CI: 4.3-24.5), 76.4 (95% CI: 56.3-101.3) and 15.9 (95% CI: 1.8-20.9), respectively. Multivariable analysis showed an increased hazard ratio (HR) for CIN in patients with a history of sexually transmitted diseases (13.4 (95% CI: 3.5-52, p=0.000)), in patients with multiple sexual partners (7.0 (95% CI: 2.1-33.3, p=0.002)), in orthotopic liver transplant recipients (5.3 (95% CI: 1.7-16.7)) and in HLA-A28 patients (3.8 (95% CI: 1.1-13.3, p=0.03)), while a decreased HR for CIN was observed in HLA B7 patients (0.11 (95% CI: 0.01-0.90, p=0.04)). An increased HR was also detected for squamous cell cancer of the skin in HLA-B18 (2.52 (95% CI: 1.06-5.99, p= 0.033)), but no increased HR for basal cell cancer of the skin and HLA. In conclusion, in our transplant population overall SIRs for HPV related CIN III and non-melanoma skin cancer are highly increased and for CIN III and squamous cell cancer of the skin associated with different HLA types. HRs due to classic risk factors for cervical neoplasia far outweigh increased HRs due to specific HLA types.

Results are shown from study in a large series of female orthotopic liver or renal transplant recipients. In these patients the incidence of non-melanoma skin cancer and cervical neoplasia was studied as well as a possible HLA class I or class II loci related to the development of non-melanoma skin cancer and cervical neoplasia.

Future perspectives

Genetic studies into effects of frequent genetic variations suggests that substantial genetic effects require quite large studies, especially in relation to common study size, before they can be detected by association studies. If significant associations are detected, functional interpretation and even larger studies are required to know which variation is causal and why. There is little known about the relation of common variants and gene function and our current understanding of genome sequence variation needs to be improved because pure statistical identification of causal variants is likely almost impossible. Currently genome wide association studies are technically possible and are expected to result in systematic identification of genomic regions, where variants in genes contribute to disease susceptibility.
The complexity of gene-gene and gene-environment interaction is such that only studies at an international scale will be able to bring the resources required to have statistical power to detect even the most important ones.