Familial Melanoma and Pancreatic Cancer: studies on genotype, phenotype and surveillance

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SUMMARY

Approximately 5-10% of melanoma patients have a familial predisposition for melanoma, and up to 40% of familial patients harbor a germline mutation in the major high-risk melanoma susceptibility gene *CDKN2A*. Carriers of a germline *CDKN2A* mutation have a 70% lifetime risk for developing (multiple) melanoma, which generally occurs at a young age (<40 years). As *CDKN2A*-mutated melanoma families show pancreatic cancer (PC) as a second major type of cancer, the condition is sometimes referred to as familial melanoma-pancreatic cancer syndrome. In the Netherlands, most of these *CDKN2A* families have a specific Dutch founder mutation known as p16-*Leiden*. In the first part of this thesis, our studies focused on patients with this p16-*Leiden* founder mutation. Our first aim was to investigate the full cancer phenotype associated with the p16-*Leiden* mutation and to study potential genetic and non-genetic modifiers of cancer risk. In addition to regular dermatologic surveillance, p16-*Leiden* mutation carriers are offered yearly MRI-based PC surveillance at Leiden University Medical Center. The second aim of this thesis was to evaluate and improve the p16-*Leiden* PC surveillance program by focusing on a) the role of precursor lesions (PanIN, IPMN) in the development and early detection of PC, b) the surgical management of early-stage screen-detected PC, and c) potential biomarkers for possible implementation in the PC surveillance program. The third and final aim of this thesis was to to evaluate and improve genetic testing for hereditary melanoma in general, a subject that will be addressed in the second part of this thesis.

In **chapter 1**, we provide an introduction to familial and hereditary melanoma, the p16-*Leiden CDKN2A* founder mutation, and the PC surveillance program in Leiden.

In **chapter 2**, we studied the prospective risk of cancer in a cohort of 150 p16-*Leiden* mutation carriers participating in the PC surveillance program. As expected, melanoma and PC were the most frequently observed cancers in these individuals, but we also found an overall increased risk for other cancers, most notably cancers in the head and neck region (lip, mouth, pharynx, and larynx). Moreover, a higher than expected number of (unrelated) individuals had a carcinoid tumor or a medical history of sarcoidosis. The risk for developing a tobacco-related cancer such as PC or head and neck cancer was increased fourfold for current smokers compared to former- and never-smokers. We concluded that active intervention to quit or refrain from smoking is very important to the prevention of these frequently occurring cancers, and that p16-*Leiden* mutation carriers should be advised to contact their doctor if they have complaints of hoarseness, dysphagia or ulcers in mouth or throat. An annual inspection of the mouth and throat might potentially contribute to the early detection of head and neck cancers in these individuals.

Among p16-*Leiden* families, there can be striking variability in the number of family members diagnosed with PC. Although tobacco use might explain some of this variability, it is also possible that there are additional genetic risk factors (*modifiers*) in these carriers that influence PC risk. In **chapter 3**, we performed a case-control study of 185 p16-*Leiden* mutation carriers, of whom 50 were diagnosed with PC, and investigated whether a diagnosis of PC was associated with any of seven selected Single Nucleotide Polymorphisms (SNPs) that were derived from large genome wide association studies (GWAS) of sporadic PC. In our analyses we found no significant associations with any of these SNPs. We then hypothesized that either the cohort was too small to detect an effect, that some of the controls might develop PC in the future and therefore could have biased the results, or that modification of PC risk in p16-*Leiden* mutation carriers might actually be due to other SNPs that were not selected in this study.

The studies presented in chapters 4 to 6 focused on the p16-*Leiden* PC surveillance program. In **chapter 4**, we compared the frequency and behavior of precursor lesions and PC in the p16-*Leiden* PC surveillance cohort (n=116) to a German Familial Pancreatic Cancer (FPC) surveillance cohort (n=125). We showed that the frequency of PC was ten times higher in the p16-*Leiden* cohort, but MRI-detected cystic lesions of the pancreatic ducts were much more frequent in the FPC cohort. Resected specimens in the FPC cohort also frequently revealed IPMN and PanIN2-3 precursor lesions, which were only rarely seen in the p16-*Leiden* cohort. However, as precursor lesions in p16-*Leiden* mutation carriers more often progressed to PC, these lesions appear to have a higher malignant potential in p16-*Leiden* mutation carriers. These findings suggest that a more intensive PC surveillance program might be considered for this high-risk group.

In **chapter 5**, we described two high-risk patients (one p16-*Leiden* mutation carrier and one *BRCA2* mutation carrier) who developed a second primary PC two to four years after a partial resection of an early-stage (T1-2N0M0) PC. These cases point to a probable increased risk for developing a second primary PC in high-risk patients who survive long enough after the initial diagnosis. We therefore discussed the advantages and disadvantages of total pancreatectomy (TP) and concluded that TP should be considered in high-risk patients (p16-*Leiden* mutation carriers in particular) diagnosed with an early-stage and prognostically favorable PC.

An important limitation of current PC surveillance programs is the suboptimal diagnostic performance of imaging modalities. Deciding whether a patient actually needs pancreatic surgery can therefore be challenging. In **chapter 6**, we evaluated a previously identified proteomic biomarker signature for PC for its potential inclusion in the Leiden PC surveillance

program as an additional, non-invasive, screening modality. Using this biomarker signature, we could accurately distinguish cases with PC (n=5) from controls without PC (n=61). We could also distinguish the only patient with histologically confirmed precursor lesions (multifocal PanIN1-2 and IPMN) from other controls. Importantly, the biomarker signature was not disturbed by a (recent or non-recent) medical history of melanoma. Since this was a preliminary study with limited sample size, additional studies will be needed before this biomarker test can be implemented in the current PC surveillance program.

Chapters 7 and 8 comprise the second part of this thesis, in which we focused on genetic testing for hereditary melanoma. In **chapter 7**, we studied the association between germline *CDKN2A* mutations and the presence of five clinical features in a melanoma family. One of these features, the presence of head and neck cancer(s) in a family, had not been previously studied in relation to the probability of a *CDKN2A* mutation. Using multivariate logistic regression analysis, significant associations were found for every feature in a large cohort of 1227 Dutch melanoma families (13.7% with a *CDKN2A* mutation). For practical purposes we further developed *CM-Score*, a non-computerized scoring system derived from the logistic regression model. In a predominantly Swedish familial melanoma validation cohort (n=421; 9.0% with a *CDKN2A* mutation), *CM-Score* showed a good ability to discriminate between families with and without a *CDKN2A* mutation (Area under the Curve 0.94). The commonly used threshold of 10% mutation probability was approximated to a *CM-Score* of 16 out of 49 points; the mutation probability below this score was very low (≤4%). We therefore concluded that *CDKN2A* diagnostics should at least be recommended to families with a *CM-Score* ≥16 points.

Although the *CDKN2A* gene is the most important melanoma predisposition gene that should be part of any genetic test for hereditary melanoma, several other high- and medium-penetrance melanoma predisposition genes are currently known and could potentially be tested in addition to *CDKN2A*. In **chapter 8**, we performed multigene panel testing of 30 established and candidate melanoma predisposition genes in a cohort of 451 Dutch melanoma-prone families without a known *CDKN2A* (or *CDK4)* mutation. We found pathogenic mutations in *BAP1* (n=3 families) and *MITF* (p.E318K variant) (n=15 families), which together resulted in a diagnostic yield of 4.0%. In the *BAP1* families, there were no reported diagnoses of *uveal* melanoma or malignant mesothelioma, both of which are major *BAP1*-associated cancers. Based on these findings, we concluded that both *BAP1* and *MITF* genes should always be included in a multigene panel test for *cutaneous* melanoma. In the known melanoma predisposition genes involved in telomere integrity (*POT1*, *ACD*, *TERF2IP*, *TERT*), we only identified two variants of uncertain significance, in *ACD* and *TERF2IP*, suggesting only a minor role for these genes in the Dutch melanoma population.

Additionally, we found several variants of interest in candidate melanoma predisposition genes, in particular in *OCA2*, *BRIP1* and *POLE*, but more research is warranted before these or other candidate genes can be included in regular diagnostic testing for hereditary melanoma.

In the final chapter, **chapter 9**, we discuss the main findings of the studies presented in this thesis in the context of the most recent literature. In this chapter, we introduce the term "*CDKN2A*-associated cancer predisposition syndrome" and we propose a revision of the current referral criteria for *CDKN2A* diagnostics in familial melanoma. We emphasize that *CDKN2A* mutation carriers are a distinctive group within PC surveillance programs and we discuss the possible implications that this may have for clinical practice. In the future, improved referral criteria combined with increasing possibilities for elaborate multigene panel testing will enable us to better identify individuals with a high genetic risk for melanoma and other cancers. Together with further improvement of complex cancer surveillance programs such as that for PC, we are confident that these developments will eventually lead to better survival and quality of life for these individuals.