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

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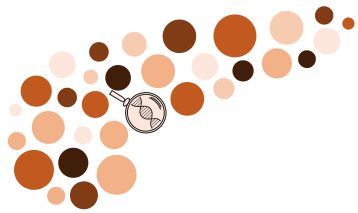
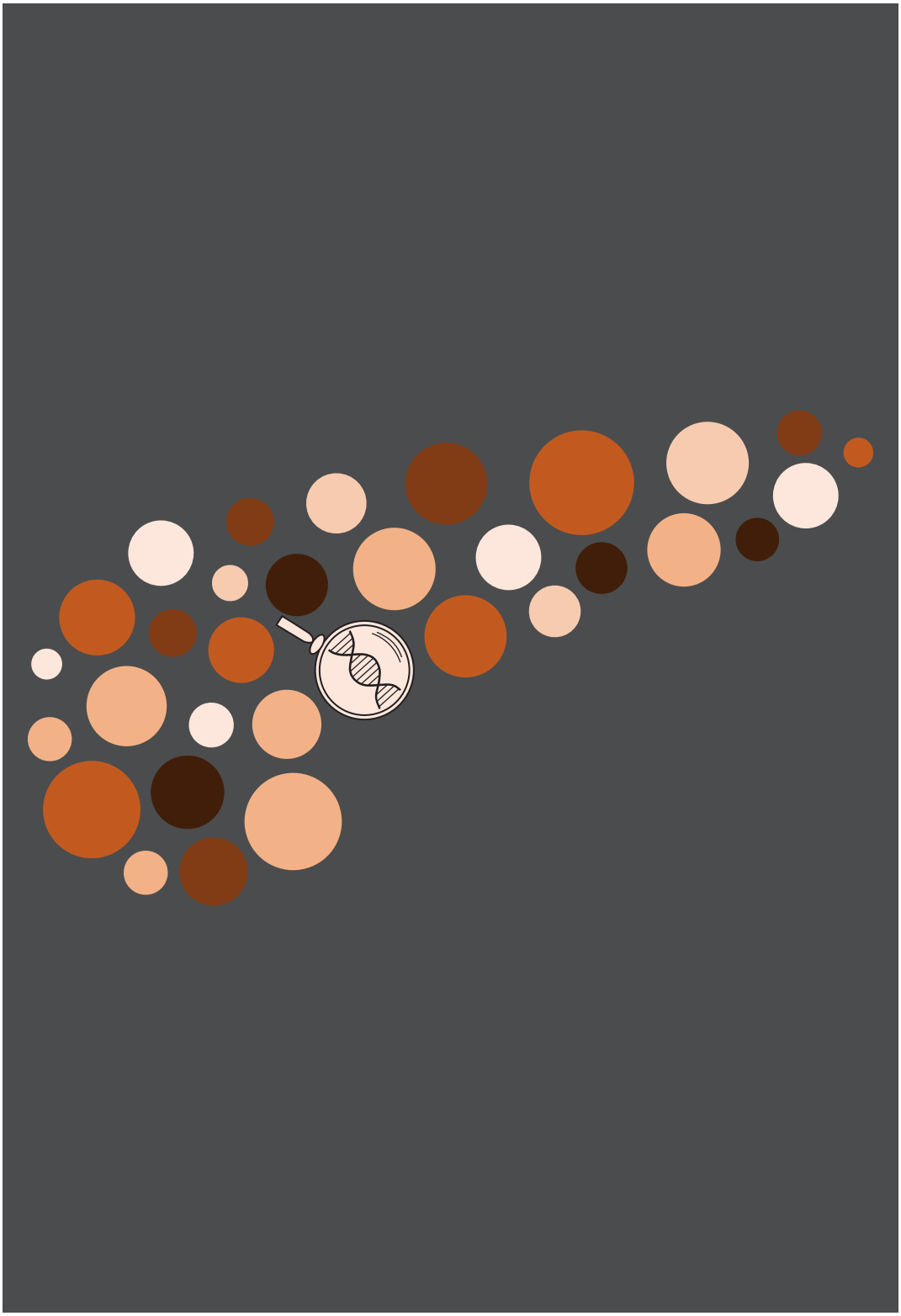
PART III





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General Discussion

BRIEF SYNOPSIS

In this thesis, we performed several genotype and phenotype studies in hereditary and familial melanoma patient cohorts. In the first part, our studies focused on patients with the p16-*Leiden* founder mutation in the *CDKN2A* gene. This founder mutation is the major cause of the familial melanoma-pancreatic cancer syndrome in the Dutch population. Since many p16-*Leiden* mutation carriers live in the vicinity of Leiden and are under frequent dermatologic and/or pancreatic cancer (PC) surveillance at Leiden University Medical Center (LUMC), the relatively large and homogeneous p16-*Leiden* cohort of the LUMC is unique in its kind. We studied the cancer phenotype (**chapter 2**) and modifiers of cancer risk (**chapters 2 and 3**) in these p16-*Leiden* mutation carriers. We also evaluated the p16-*Leiden* PC surveillance program by studying the role of precursor lesions in the development and early detection of PC (**chapter 4**), by reflecting on the surgical management of early-stage screen-detected PC (**chapter 5**) and by studying potential biomarkers for the early detection of PC (**chapter 6**). The LUMC has also been the primary sequencing facility for *CDKN2A* in the Netherlands since 1998 and therefore, a large collection of DNA samples and clinical information of melanoma families from across the Netherlands has been acquired in the last two decades. In the second part of this thesis, our studies focused on this familial melanoma cohort, of which ~85% do not harbour a germline *CDKN2A* mutation. We studied which clinical features are associated with the presence of a *CDKN2A* mutation in a melanoma family and created a scoring system to predict *CDKN2A* mutation probability (**chapter 7**). Furthermore, we genetically characterized melanoma families without a *CDKN2A* mutation for variants in other (candidate) melanoma predisposition genes (**chapter 8**). In this final chapter, we discuss the main findings of these studies in the context of the most recent literature.

THE EXTENDED CANCER PHENOTYPE OF *CDKN2A*

The *CDKN2A* gene is primarily a melanoma predisposition gene, and up to 40% of melanoma-prone families harbour a germline mutation in *CDKN2A*.[1,2] In many *CDKN2A*-mutated melanoma families, a clear excess of PC has also been reported,[3,4] and the lifetime risk for PC is estimated to be approximately 15-20% for carriers of the p16-*Leiden* founder mutation.[5,6] Because of the frequent co-occurrence of these two cancers in *CDKN2A*-mutated families, the condition is sometimes referred to as familial melanoma-pancreatic cancer syndrome (MIM #606719^). There is now increasing evidence that *CDKN2A* mutation carriers are also at risk for cancers at sites other than skin and pancreas, in particular cancer of the upper respiratory tract, i.e. head and neck region (larynx, pharynx and oral region), and possibly also cancer of the lower respiratory tract (lung). Head and neck cancers and lung cancer were initially reported in several case studies of *CDKN2A*-mutated families with various mutations.[7-9] In a large study by de Snoo *et al* in 2008, a cohort of 221 p16-*Leiden* mutation carriers and 668 first-degree relatives was retrospectively evaluated for the occurrence of any cancer and significant risks were found for cancers of the lip, mouth and pharynx (RR 10.8) and cancers of the respiratory system including laryngeal cancer (RR 5.7).[6] In **chapter 2**, we aimed to confirm the findings of the de Snoo *et al* study by prospectively evaluating a cohort of 150 p16-*Leiden* mutation carriers that were participating in the PC surveillance program and we again found a particular excess of cancers of the lip, mouth and pharynx (n=3, RR 18.8) and cancers of the respiratory system (n=4, RR 4.6). Since two of the four respiratory system cancers in our study were laryngeal cancers, a total of five cancers were located in the head and neck cancer region, making it the third most frequent cancer site in these p16-*Leiden* mutation carriers (following skin and pancreas). This number is striking considering the relatively low incidence of head and neck cancers in the general population (approximately 3000 new cases each year in the Netherlands; age standardized rate 9 per 100.000).[10] A study with very similar results was concomitantly published by a Swedish group that assessed cancer risks in a cohort of 120 individuals with a specific Swedish *CDKN2A* founder mutation (p.Arg112dup).[11] In this study, significantly increased risks other than melanoma and PC were again observed for cancers of the upper digestive tract (including oral region and pharynx; RR 17.1) and respiratory tract (including larynx; RR 15.6), and the majority of these cancers were indeed located in the head and neck region. In a subsequent collaboration study with the Swedish group (**chapter 7**), we further investigated the occurrence of cancers in the head and neck region* in *CDKN2A* mutation carriers by incorporating this

^ *Mendelian Inheritance in Man*; Catalog of Human Genes and Genetic Disorders (http://www.omim.org)

* In chapter 7, the term upper airway cancer (UAC) is used as a synonym for cancers of the head and neck region (larynx, pharynx, oral region).

clinical feature and four additional features in a *CDKN2A* mutation probability scoring system (*CM-Score*). We showed that the presence of these cancers in Dutch melanoma families was significantly associated with the presence of a germline *CDKN2A* mutation (OR 6.0). Since the scoring system could accurately predict *CDKN2A* mutation status in both the Dutch training cohort and the Swedish validation cohort, we demonstrated that cancers in the head and neck region are indeed an important component of the *CDKN2A* cancer phenotype in different populations with different *CDKN2A* mutations. Therefore, it might be more appropriate to use the term '*CDKN2A*-associated cancer predisposition syndrome' when referring to the familial melanoma-pancreatic cancer syndrome, because this term better reflects the broader tumour spectrum to which *CDKN2A* mutation carriers are predisposed to. It should however be noted that ~80% of both Dutch and Swedish *CDKN2A*-mutated families in chapter 7 had a *CDKN2A* founder mutation located in exon 2 (p16-*Leiden* and p.Arg112dup, respectively), which might indicate a possible genotype-phenotype correlation between specific *CDKN2A* (exon 2) mutations and head and neck cancers. Yet, case studies reporting a high incidence of head and neck cancers in *CDKN2A*-mutated families have not been confined to families with mutations in exon 2; Cabanillas *et al* reported a melanoma family with multiple cases of head and neck cancer and a truncating *CDKN2A* mutation in exon 1α.[8] Replication studies in large cohorts with different *CDKN2A* mutations are needed to further explore possible genotype-phenotype correlations. Only one such study has recently been published, in which 29 American *CDKN2A*-mutated melanoma families were evaluated over a relatively long period of four decades. No increased risk for cancers other than melanoma or PC were found in this study. Unfortunately, the specific *CDKN2A* mutations found in these families were not reported.[12]

MODIFIERS OF CANCER RISK IN *CDKN2A* MUTATION CARRIERS

NON-GENETIC MODIFIERS

Several phenotypic characteristics known to be associated with increased melanoma risk in the general population, such as the number of dysplastic nevi, poor tanning ability and sunburns in childhood, have also been shown to modify melanoma risk in carriers of a germline *CDKN2A* mutation.[13] Therefore, early patient education on sun protection is essential for carriers and their first-degree relatives. Interestingly, the most important non-melanoma cancers associated with *CDKN2A* mutations, i.e. PC, head and neck cancer and lung cancer, are strongly related to tobacco use in the general population.[14,15] Previously, it was already shown that tobacco use is an independent risk factor for

developing PC in the setting of familial PC (FPC).[16] We investigated in **chapter 2** if tobacco use is also associated with a higher risk of PC and other tobacco-related cancers in p16-*Leiden* mutation carriers. In total, 27% of current smokers in the cohort developed a tobacco-related cancer versus 7% of the former- and never-smokers. Current smokers therefore had a fourfold increased risk of developing these types of cancers (P=0.002). These findings were confirmed in the Swedish *CDKN2A* cancer risk study, where ever-smokers had an odds ratio of 9.3 for developing tobacco-related cancers compared to never-smokers.[11] Thus, tobacco use seems a significant modifier of cancer risk in *CDKN2A* mutation carriers and carriers should be strongly discouraged to smoke.

GENETIC MODIFIERS

A well-established modifier gene of melanoma risk in germline *CDKN2A* mutation carriers is *MC1R*, a gene involved in skin pigmentation.[17,18] In contrast, little is known about genetic modifiers that might influence the risk of other cancers such as PC in *CDKN2A* mutation carriers. Since there is notable interfamilial variability in the occurrence of PC among p16-*Leiden* families that might not be fully explained by differences in tobacco use or other non-genetic risk factors among mutation carriers, we investigated in **chapter 3** whether common Single Nucleotide Polymorphisms (SNPs) that are associated with PC in the general population might influence PC risk in p16-*Leiden* families. We genotyped seven PC-associated SNPs in a cohort of 185 p16-*Leiden* mutation carriers of whom 50 were diagnosed with PC, but found no significant association with PC for any of these SNPs. The study might have been underpowered for a detectable effect but it might also be possible that other PC-associated SNPs that were not genotyped in this study modify PC risk in p16-*Leiden* families. No additional studies have yet been performed that investigate SNPs as potential genetic modifiers of PC in *CDKN2A*-mutated families. However, Yang *et al* investigated if patients with PC and a germline *CDKN2A* mutation might also have one or more rare variants in 24 other (putative) PC-related genes.[19] In this study, nominally significant associations between PC and rare variants in the mismatch repair (*MMR*) genes were found, and some patients had more than one rare variant in PC-related genes. However, no loss-of-function variants were detected in these patients and only a subset of variants was classified as potentially deleterious. Therefore, the relevance of these findings remains uncertain and additional studies are needed.

PANCREATIC CANCER SURVEILLANCE AND -MANAGEMENT OF P16-*LEIDEN* MUTATION CARRIERS

P16-*Leiden* mutation carriers are offered yearly PC surveillance at the LUMC from the age of 45. Promising results of this program and several other PC surveillance programs worldwide have been published in the last two decades, however, the diagnostic yield of these programs varies greatly.[20-22] One explanation might be that there are differences in the definition of diagnostic yield among these programs. For instance, some programs included histologically confirmed lower-grade precursor lesions (PanIN1-2) in their diagnostic yield and it can be questioned if the resection of these lesions is relevant in these patients. Moreover, cystic duct lesions detected with pancreatic imaging might very well be benign or lower-grade lesions if there is no histological confirmation, and including such lesions in the diagnostic yield of a surveillance program can cause significant bias. There are also differences in which high-risk individuals are included in the surveillance programs. Most programs largely focus on individuals from FPC families, but some also include a significant number of individuals with a germline mutation in a known cancer predisposition gene such as *CDKN2A* or *BRCA1/2*. Since the PC surveillance program of the LUMC is almost entirely focused on *CDKN2A* mutation carriers, the majority of which have the p16-*Leiden* founder mutation, it is distinctive from other PC surveillance programs worldwide. To further explore possible phenotypic differences between high-risk groups that might influence diagnostic yield, we studied in **chapter 4** the frequency and behaviour of precursor lesions and PC in two different surveillance cohorts, that is, the LUMC p16-*Leiden* cohort (n=116) and a German FPC cohort (n=125). We reported substantially more diagnoses of PC in the p16-*Leiden* cohort (7% versus 0.8% in the FPC cohort), but cystic duct lesions were much more common in the FPC cohort (42% versus 16% in the p16-*Leiden* cohort). Histological examination of cystic lesions in the FPC cohort often revealed IPMN or PanIN2-3 lesions, whereas in the p16-*Leiden* cohort few such lesions were seen. Yet, cystic lesions in the p16-*Leiden* cohort frequently showed growth or malignant transformation compared to cystic lesions in the FPC cohort. Recently, our findings were confirmed in a similar study by Konings *et al* that compared the prevalence of cystic lesions and their natural behaviour between 88 FPC individuals and 98 carriers of a germline mutation that predisposes to PC (of which 64 were *CDKN2A* mutation carriers (65%)).[23] In this study, 5% of mutation carriers had a pancreatic cyst >10 mm compared to 16% in FPC individuals, but cysts in mutation carriers were more likely to grow or develop radiological features that suggest high-grade dysplasia (16% vs 2% in FPC individuals). Interestingly, the only two patients that developed PC in this study were a *CDKN2A* mutation carrier (exact mutation not reported) and a patient with the rare but highly penetrant Peutz-Jeghers syndrome. These studies show that *CDKN2A*/p16-*Leiden* mutation carriers have a much higher risk

for PC than FPC individuals. Furthermore, these studies suggest that precursor lesions in these carriers might have a higher malignant potential compared to precursor lesions in FPC individuals. However, the role of precursor lesions in the development of PC in p16-*Leiden* mutation carriers has been questioned in a recent study by Ibrahim *et al* that found a similar malignancy rate in p16-*Leiden* mutation carriers with a cystic lesion compared to those without cystic lesions.[24] Still, 50% of larger cysts (>10 mm) progressed to PC in this study. The authors also reported a particular high growth rate of PC of approximately 15 mm/year and thus confirmed previous observations of aggressively growing tumours in p16-*Leiden* mutation carriers.[20] Taken together, p16-*Leiden* mutation carriers have a particularly high risk for aggressively growing PCs, and cystic lesions, especially when >10 mm, are often instable at follow-up and might precede the development of PC in some carriers. Therefore, a more intensive PC surveillance program, for instance semi-annual surveillance with alternating MRI/MRCP and EUS, might be considered for this group if future studies show this program to be cost-effective.

The ultimate goal of PC surveillance is to improve survival of high-risk individuals, through timely resection of early-stage PC or its high-grade precursor lesions. In 2016, a large multicentre study by Vasen *et al* was the first to show a beneficial effect of regular surveillance on survival.[25] In the subset of *CDKN2A*-p16-*Leiden* mutation carriers (n=178), the resection rate of PC was high (75% compared to 15% in sporadic PC) and patients with screen-detected PC had a substantial improved 5-year survival rate (24% compared to only 4-7% in symptomatic sporadic PC). The survival benefit in the FPC subset (n=233) was less clear, since only one PC and a few high-grade precursor lesions were detected in this subset. However, Canto *et al* recently demonstrated a positive effect of surveillance on survival in FPC individuals as well.[26] In this large (n=354) and long-term follow-up (16 years) surveillance study, which mainly included FPC individuals, nine of ten PCs detected during surveillance were resectable and the 3-year survival rate of these patients was greatly improved to 85%. An additional ten individuals had a resection of one or more high-grade precursor lesions and none of these patients died during follow-up (median 7.9 years). In general, the survival rate after surgical resection of PC is largely determined by the chance of local disease recurrence or distant metastases. In the Vasen *et al* surveillance study, the resection margin was free of cancer in 78% of p16-*Leiden* mutation carriers that underwent surgery, and 56% had cancer-free lymph nodes.[25] However, in patients with a hereditary predisposition for PC such as p16-*Leiden*, the occurrence of other primary cancers (melanoma in particular) might also influence survival. For instance, one p16-*Leiden* mutation carrier with PC reported by the Vasen *et al* study died 10 months after diagnosis from melanoma metastases. Also, second primary melanomas are frequently seen in p16-*Leiden* mutation carriers, and an increased risk for developing second primary cancers in

the same tissue is characteristic for many hereditary forms of cancer. Therefore, it is very well conceivable that p16-*Leiden* mutation carriers who underwent a partial resection for PC are still at risk for a second primary PC in the remnant pancreas, and developing such a second primary PC will also influence survival. In **chapter 5**, we report the first p16-*Leiden* mutation carrier with a second primary PC after a partial resection. This patient had a small (5 mm) early-stage PC that was detected during surveillance and developed a second primary PC 4.5 years later that was also detected by continuing surveillance. Subsequently, several additional p16-*Leiden* mutation carriers with a second primary PC (synchronous and metachronous) have been reported by our group,[27,28] and more patients will probably be diagnosed in the future because of improved survival of p16-*Leiden* mutation carriers with a screen-detected PC, as reported by Vasen *et al*.[25] The development of a second primary PC in these patients can only be completely prevented when a total pancreatectomy of the first cancer is performed. Prevention of a second primary PC is especially relevant for patients that are diagnosed with an early-stage and prognostically favourable PC, and we therefore recommend to consider a total pancreatectomy as one of the surgical options in these patients. Although a total pancreatectomy is a major operation, the post-surgical outcomes and quality of life are, present-day, comparable to those who underwent a partial pancreatectomy.[29,30] The disadvantages of a total pancreatectomy, such as the development of (brittle) diabetes, should however be carefully discussed with these patients and shared-decision making is essential. Future studies that assess the actual risk for developing a second primary PC are needed and results from these studies might aid in the difficult decision of what type of surgery should be performed in p16-*Leiden* mutation carriers with an early-stage PC.

To further improve PC surveillance programs, some important limitations of the current surveillance programs need to be considered. The most challenging limitations are the suboptimal diagnostic performance of imaging modalities that are currently being used (MRI/MRCP and EUS) and the fact that extensive surgery (partial or total pancreatectomy) is the only way to remove a suspicious lesion. Pancreatic lesions might not always be clearly visible or properly interpreted by the radiologist, and patients that had surgical resection for a non-relevant lesion on histologic examination (false-positives) have repeatedly been reported.[31,32] The Vasen *et al* surveillance study showed that at least 5 out of 13 FPC individuals that had a surgical resection of a suspicious lesion had a non-relevant (precursor) lesion on histological examination.[25] In the p16-*Leiden* subset, only two patients without PC had surgery for suspicious lesions. One of these patients had multifocal PanIN1-2 and branch duct (BD-)IPMNs combined with severe multifocal fibrosis (we also reported this patient in chapter 4), but the second patient only had one low-grade IPMN. Since pancreatic resection is associated with significant morbidity and mortality,

it is very important to minimize the amount of false-positive findings and unnecessary resections in otherwise healthy individuals participating in a PC surveillance program.[32] When these individuals do develop PC, a successful surveillance program should be able to consistently detect it in the earliest possible stage (high sensitivity; few false-negatives). One way to improve the diagnostic performance of surveillance programs is to add (serum) biomarkers to the program that can differentiate between PC, relevant precursor lesions and non-relevant or no lesions. Among several types of biomarkers that are currently being investigated in this context, microRNA panels and global protein profiling (proteomics) are relatively new and results are encouraging.[33-35] In the LUMC, a specific proteomic biomarker signature for PC was previously identified in cohorts of patients with sporadic PC.[36-38] In **chapter 6**, we investigated this biomarker signature in a cohort of 66 p16-*Leiden* mutation carriers, of which 5 had developed PC. We could accurately distinguish patients with PC from patients without PC using this biomarker signature. Many included p16-*Leiden* mutation carriers had a (recent or non-recent) medical history of one or more melanomas (62%), but this did not influence the biomarker signature. The patient with histologically confirmed multifocal PanIN1-2 and BD-IPMNs previously reported in chapter 4 could also be distinguished from controls. The results of this preliminary study are very promising, but additional studies that include more patients with PC and with (relevant and non-relevant) precursor lesions will be needed before this biomarker test can be implemented in the p16-*Leiden* PC surveillance program.

The effectiveness of a cancer surveillance program does not only depend on its technical performance, but also on the motivation of participants and their adherence to the surveillance protocol. Adherence might be jeopardized when participants experience significant psychological distress, which is imaginable when there is a continuing risk for a highly lethal form of cancer such as PC that might already have occurred in (close) family members. Factors that determine or influence psychological distress should therefore be identified and recognized in an early stage.[39] Several recent studies that have investigated the psychological feasibility of PC surveillance in high-risk groups have shown that most participants have a positive attitude towards the program and find that the advantages of PC surveillance outweigh the limitations.[40-42] Approximately one third of participants worry significantly about the possibility of getting cancer, but this does not affect their mood or interfere with their daily activities.[40,41] Cancer worries decrease each following year and getting a positive surveillance result does not influence the level of cancer worries.[41,42] The only reported predictor of cancer worries is having a first degree relative with PC under the age of 50 years.[42] Importantly, anxiety and depression levels of participants are comparable with the general population and stable during follow-up.[40,41] Based on these studies, surveillance for PC seems well feasible from a psychological point of view.

GENETIC TESTING AND COUNSELLING OF MELANOMA FAMILIES: *CDKN2A* AND BEYOND

Identifying a causative germline mutation such as p16-*Leiden* in a melanoma family is relevant, since carriers can be enrolled in targeted cancer surveillance programs and their family members have the possibility to undergo presymptomatic genetic testing. Moreover, gene-specific lifestyle advices and patient education on early cancer symptoms can be given to confirmed carriers. For instance, *CDKN2A* mutation carriers are strongly advised to refrain from smoking and to seek medical advice in an early stage when there are possible signs of head and neck cancer (hoarseness, dysphagia, ulcers in mouth or throat), as we discussed in chapter 2. Knowledge about individual mutation status might also influence reproductive choices, since most cancer predisposition syndromes (*CDKN2A* included) have an autosomal dominant inheritance pattern with a 50% risk for (future) offspring to inherit the predisposition. Pre-implementation genetic diagnosis (PGD) is an assisted reproductive technique that can prevent a future child from inheriting this predisposition, but is, by definition, only available for patients with a known underlying germline mutation and has indeed been performed for *CDKN2A* mutation carriers in the Netherlands.[43] Although some patients might experience significant psychological distress from *CDKN2A* testing, several studies have shown that, in general, *CDKN2A* testing in melanoma families does not result in increased psychological distress or cancer worries among carriers and may even enhance compliance with lifestyle advices such as sun protection behaviour.[44-48]

Genetic testing for hereditary melanoma can thus be beneficial for patients and their family members and should therefore be routinely offered to melanoma families. However, the chance of identifying a causative germline mutation strongly depends on the cancer burden in a family and therefore, selection criteria for performing germline *CDKN2A* analysis were proposed in 2009.[49] These criteria are based on the total number of melanomas and PCs in a family but do not include age at melanoma diagnosis or the presence of other cancers in a family such as head and neck cancers. Recently, a French melanoma research group suggested to add age at melanoma diagnosis (<40 years) to the 2009 criteria in order to improve the detection rate of *CDKN2A* mutations.[50] In **chapter 7**, we included all the above mentioned features in a multivariate logistic regression model and found significant associations with the presence of a germline *CDKN2A* mutation for every feature in a cohort of 1227 melanoma families. For further practical purposes we developed *CM-Score*, a non-computerized scoring system that can accurately predict *CDKN2A* mutation status based on the five clinical features from the logistic regression model. Clinical geneticists, but also dermatologists and oncologists, can use this tool in

daily clinical practice to address questions on heritability of melanoma patients before genetic testing is performed. In our model, median age at melanoma diagnosis <50 years and the presence of head and neck cancer[†] in a family were both strong predictors for a germline *CDKN2A* mutation (OR 8.5 and 6.0, respectively). We therefore propose to add these features to the current Dutch referral criteria for germline *CDKN2A* diagnostics as shown in *table 1*. By adding these features, the overall mutation detection rate will likely improve and genetic testing of families with a very low probability for a *CDKN2A* mutation (<5%) will be more avoided. Of note, these criteria for *CDKN2A* diagnostics and our *CM-Score* system are only applicable to melanoma index patients and families and are not designed for families with familial pancreatic cancer (FPC). Yet, germline *CDKN2A* mutations are also found in FPC families without any occurrence of melanoma, and genetic testing for *CDKN2A* mutations is therefore recommended to these families as well.[51,52]

TABLE 1. Proposed referral criteria for germline *CDKN2A* diagnostics

Familial melanoma (diagnostic criteria)	▪ family with three relatives with melanoma, of which two are first-degree relatives (all first- and second-degree relatives) ▪ family with two first-degree relatives with melanoma, of which one has multiple primary melanomas
Other families	▪ family with two first-degree relatives with melanoma *with a mean age at diagnosis <50 years* ▪ family with two first- or second-degree relatives with melanoma and one first- or second-degree relative with pancreatic cancer *or head and neck cancer (larynx, pharynx, oral region)* ▪ person with three or more primary melanomas ▪ person with a juvenile melanoma (<18 years) ▪ person with both melanoma and pancreatic cancer *or head and neck cancer (larynx, pharynx, oral region)*

The parts in *italic* are the proposed additions to the current referral criteria.

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* (see *table 2* in chapter 1).[1,2] Pathogenic germline mutations in these genes are however much more rare and the yield when tested separately is very low (approximately 0-1%; *MITF* 0-3%).[53-57] But when these genes are incorporated in

[†] In chapter 7, the term upper airway cancer (UAC) is used as a synonym for cancers of the head and neck region (larynx, pharynx, oral region).

a multi-gene panel test for hereditary melanoma, the diagnostic yield of genetic testing can increase significantly. We performed such a multi-gene panel test in **chapter 8**, and report an additional 4% diagnostic yield in established melanoma predisposition genes in a cohort of 451 non-*CDKN2A/CDK4* melanoma families. The most important genes in our panel were *MITF* (p.E318K variant) and *BAP1*, in which we found pathogenic mutations in 15 (3.4%) and 3 (0.7%) families, respectively. Conversely, in the genes involved in telomere integrity, we only detected two variants of uncertain significance (VUS) in this large cohort, suggesting a minor role for these genes in the Dutch population. The additional yield of 4%, a number comparable to that reported by similar studies in non-*BRCA1/2* breast cancer families,[58,59] sufficiently justifies multi-gene panel testing in familial melanoma, but the increased chance of finding a VUS in one of the genes should be carefully discussed with the patient before such a test is requested.[60] When a patient is hesitant about multi-gene panel testing, or when there is a particularly high probability for a germline *CDKN2A* mutation based on the presence of PCs and/or head and neck cancers in a family (and consequently a high *CM-Score*, as discussed in chapter 7), then targeted *CDKN2A* diagnostics might be more appropriate as an initial test. The referral criteria for *CDKN2A* diagnostics proposed in *table 1* can also be applicable when a multi-gene panel test is being considered in a melanoma patient or family, because most patients with a germline mutation in one of these genes are expected to have a (familial) melanoma phenotype. One clear exception is *BAP1*, which we also recommend to test if there are occurrences of uveal melanoma, malignant mesothelioma, renal cell carcinoma or multiple basal cell carcinomas in a melanoma patient or his/her family members.[61,62] In our multi-gene panel test, we additionally included several candidate melanoma predisposition genes, of which the most interesting variants were found in the genes *OCA2*, *BRIP1* and *POLE*. Because of the exponential increase of WES/WGS technologies and possibilities in recent years, new candidate genes are continually being discovered by research groups worldwide.[63-66] These candidate genes have the potential to be added to a diagnostic gene panel test for hereditary melanoma in the future, which will likely result in a further increase in diagnostic yield of the panel. Moreover, adding a melanoma-specific polygenic risk score (PRS) to the panel will enable us to even better differentiate between patients with a higher and lower genetic risk for melanoma. This individual risk stratification is an integral part of personalized medicine and will further enhance the selection of patients that should or should not be offered dermatologic surveillance. The development of a PRS model based on common susceptibility SNPs derived from large Genome Wide Association Studies (GWAS) is part of a current research project of our group and recently, promising results of melanoma PRS models have been published by others.[67-69] Altogether, there is great potential for the individual patient to undergo multi-gene panel testing and future research will undoubtedly give more insight in the genetic background of melanoma families with a yet unknown hereditary predisposition.

SUMMARY AND CONCLUDING REMARKS

The studies in this thesis were aimed at I) better understanding the clinical phenotype of *CDKN2A*-p16-*Leiden* mutation carriers and improving the PC surveillance program for these carriers, and II) improving genetic testing for hereditary melanoma. In **part I**, we have shown that *CDKN2A*-p16-*Leiden* mutation carriers have an increased risk for several types of cancer including head and neck cancers, and therefore we have introduced the term '*CDKN2A*-associated cancer predisposition syndrome'. We have demonstrated that smoking is an important modifier of cancer risk in p16-*Leiden* mutation carriers and we therefore argued that smoking should be actively discouraged. We have also shown that, when compared to individuals from FPC families, p16-*Leiden* mutation carriers have a higher risk for (aggressively growing) PC and a lower frequency of precursor lesions of PC, but precursor lesions in these carriers might have a particular high malignant potential. Future studies should assess the feasibility of a more intensive PC surveillance program for this group. Since it is very likely that p16-*Leiden* mutation carriers that have been curatively treated for PC have a substantial risk for developing a second primary PC in the longer term, we have recommended to consider a total pancreatectomy when an early-stage and prognostically favourable PC is diagnosed in these carriers. Furthermore, we have demonstrated that a specific proteomic biomarker test for the early detection of PC is a very promising candidate for implementation in the PC surveillance program. In **part II**, we created a scoring system (*CM-Score*) to predict *CDKN2A* mutation probability and, based on several strong predictive features in this model, we have proposed to update the current Dutch referral criteria for *CDKN2A* diagnostics. Lastly, we have demonstrated that multi-gene panel testing in non-*CDKN2A* melanoma families results in an additional 4% diagnostic yield and that *MITF* and *BAP1* are important genes to include in such a panel. We have hypothesized that future incorporation of melanoma candidate genes in the panel, supplemented with an individual PRS calculation, will most likely increase the diagnostic yield of the panel (although more VUSs will be found as well) and will improve individual cancer risk assessment and individual recommendations for cancer surveillance. 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.

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