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

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Citation

Potjer, T. P. (2019, May 29). *Familial Melanoma and Pancreatic Cancer: studies on genotype, phenotype and surveillance*. Retrieved from <https://hdl.handle.net/1887/73760>

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

Issue Date: 2019-05-29

General Introduction

CDKN2A, P16-LEIDEN AND FAMILIAL MELANOMA-PANCREATIC CANCER SYNDROME

Familial clustering of cutaneous melanoma has increasingly been documented since the 1970s, and one of the first studies that reported an excess of pancreatic ductal adenocarcinoma (henceforth referred to as pancreatic cancer; PC) in unbiased melanoma families was published in 1990 by Bergman and colleagues.¹ The families in this study originated from two genetically isolated towns in the vicinity of Leiden, the Netherlands. Shortly after the identification of the first melanoma predisposition gene *CDKN2A* (MIM #600160*) in 1994,^{2,3} a specific Dutch founder mutation† in the *CDKN2A* gene was described in these melanoma-pancreatic cancer prone families, a 19-base-pair deletion in exon 2 known as p16-*Leiden* (c.225_243del).^{4,5} An excess of PC in *CDKN2A*-mutated melanoma families was subsequently observed in other populations as well.^{6,7}

To date, the *CDKN2A* gene has remained the major high-risk predisposition gene for familial melanoma and germline mutations are identified in 10-40% of melanoma families.^{8,9} The *CDKN2A* gene encodes two distinct proteins by using different first exons (1α and 1β) that are translated in alternate reading frames (*figure 1*). The proteins, p16INK4a and p14ARF, are both tumour-suppressors that act in two different pathways. The p16-retinoblastoma(Rb)-pathway controls cell-cycle G1-phase exit, and the p14ARF-p53 pathway induces cell cycle arrest or apoptosis.¹⁰ Germline mutations associated with familial melanoma occur across the entire coding region of the *CDKN2A* gene, including both exon 1α and exon 1β. Heterozygous carriers of a germline mutation have a 70% lifetime risk for developing one or more cutaneous melanomas, and the first melanoma generally occurs at a young age (mean <45 years).¹¹⁻¹⁵ In a study that included 182 p16-*Leiden* mutation carriers, the mean age at melanoma diagnosis was 39 years and the risk of multiple primary melanomas was approximately 40%. Moreover, p16-*Leiden* mutation carriers that had a melanoma before age 40 had a twice as high risk to develop a second primary melanoma than carriers with a first melanoma after age 40.¹⁵

An increased risk for PC has been reported for various mutations in *CDKN2A* that affect the p16INK4a protein (exon 1α and exon 2, see *figure 1*).^{16,17} The PC risk is particularly high for p16-*Leiden* mutation carriers, approximately 15-20% with a mean age at diagnosis of 58 years.¹⁸⁻²⁰ In addition to melanoma and PC, several other cancers have been described in *CDKN2A* mutation carriers, including upper and lower respiratory tract cancers²¹⁻²⁴, digestive tract cancers^{21,25} and breast cancer^{26,27}. De Snoo *et al* specifically evaluated the non-melanoma cancer risks in a large cohort of 221 p16-*Leiden* mutation carriers and

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

† In this thesis, the word *mutation* is used as a synonym for *pathogenic variant*

668 first-degree relatives. They confirmed that these (proven or implied) carriers have a high risk for PC (RR 46.6) and additionally found an increased risk for particularly cancers of the lip, mouth and pharynx (RR 10.8), cancers of the respiratory system (RR 5.7, including laryngeal cancer), eye/brain tumours (RR 11.4) and non-melanoma skin cancers (RR 22.3).²¹ Germline mutations in the *CDKN2A* gene, including p16-*Leiden*, thus seem to cause a broad cancer predisposition syndrome.

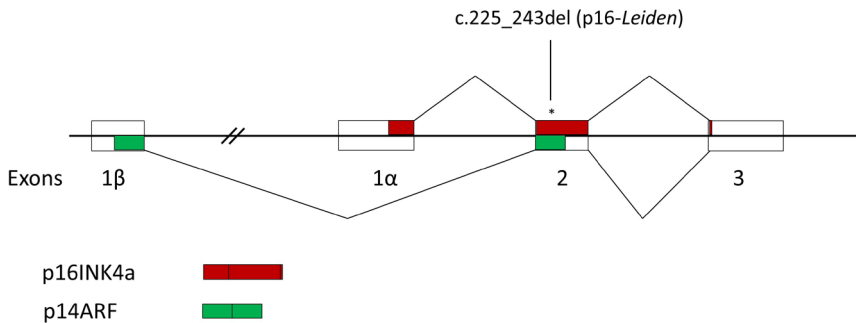


FIGURE 1. The *CDKN2A* gene and its two products, p16INK4a and p14ARF. The p16-*Leiden* mutation is located in exon 2 and affects both p16INK4a and p14ARF.

Adapted with permission from Pigment Cell Melanoma Research, 28, Aoude LG, Wadt KA, Pritchard AL, Hayward NK, Genetics of familial melanoma: 20 years after CDKN2A, 148-60 (2015)

In the first part of this thesis (chapters 2-6), we use the term Familial Atypical Multiple Mole Melanoma (FAMMM) syndrome when referring to familial melanoma with or without a known germline *CDKN2A* mutation. However, use of this term is avoided nowadays because the correlation between atypical multiple moles (nevi) and melanoma is more complex and the atypical nevi phenotype is often absent or shows incomplete co-segregation with the melanoma phenotype in many *CDKN2A*-mutated families.²⁸⁻³⁰ Therefore, in the second part of this thesis (chapters 7-9) we solely use the term familial melanoma, or hereditary melanoma when an underlying germline mutation has been identified.

CANCER SURVEILLANCE OF P16-LEIDEN MUTATION CARRIERS

MELANOMA SURVEILLANCE

Since the early 1980s, Dutch individuals from melanoma-prone families are offered yearly dermatologic surveillance at the specialized Pigmented Lesion Clinic of Leiden University

Medical Center (LUMC). A study from 1989 showed that melanomas that were detected during this surveillance (screen-detected) were at an earlier stage, i.e. lower Breslow thickness, and therefore had a more favorable prognosis than melanomas occurring in patients not participating in the surveillance program.³¹ Comparable studies in other high-risk cohorts confirmed this beneficial effect of regular surveillance on prognosis.^{32,33} When the p16-*Leiden* founder mutation was identified in the mid-1990s, many families participating in the Dutch surveillance program were found to carry this mutation. Van der Rhee *et al* subsequently studied the surveillance program in specifically p16-*Leiden* mutation carriers and again concluded that surveillance melanomas were significantly thinner than non-surveillance melanomas (Breslow thickness 0.50 mm and 0.98 mm, respectively).³⁴ The majority of melanomas in this study were detected within six months after the last surveillance and a considerable proportion were interval-melanomas (detected between regular screens; 20%). Carriers of the p16-*Leiden* mutation are therefore currently under more intensified, semi-annual, dermatologic surveillance.

PANCREATIC CANCER SURVEILLANCE – BACKGROUND

PC surveillance programs were first initiated in the United States two decades ago for families with a condition called Familial PC (FPC).^{35,36} Families with at least two first-degree relatives with a diagnosis of PC without an identifiable genetic cause are, by definition, referred to as FPC.³⁷ Although several cancer predisposition genes are currently known that confer an increased risk for PC, germline mutations are identified in only a small minority (<10%) of families predisposed to PC.³⁸⁻⁴¹ Therefore, most PC surveillance programs to date have focused on FPC families and generally have included only few individuals with a known underlying germline mutation.⁴²⁻⁴⁴

The 2013 guideline of the International Cancer of the Pancreas Screening (CAPS) Consortium defines the resection of potentially curable lesions, that is early-stage cancer or its high-grade precursor lesions, as a general goal of surveillance.⁴⁵ The dismal prognosis of PC (5-year survival rate <5%) is generally a consequence of late diagnosis, but when a tumour is resected at an early stage, the 5-year survival rate could improve drastically.^{46,47} Moreover, timely resection of high-grade precursor lesions of PC might prevent the development of PC at all. Intraductal papillary mucinous neoplasms (IPMN) and the more common pancreatic intraepithelial neoplasms (PanIN) are the most important precursor lesions that can be targeted by surveillance.⁴⁵ IPMNs are macroscopic cystic lesions, usually ≥ 5 mm, that have a high malignant potential when located in the main pancreatic duct (MD-IPMN) (*figure 2*).⁴⁸ A longitudinal study showed that approximately 60% of MD-IPMN displays high-grade dysplasia within 5 years, compared to 15% when the IPMN is located in one of the branch ducts (BD-IPMN).⁴⁹ PanINs are smaller, microscopic

lesions divided in grade 1 to 3 according to the degree of dysplasia and are located in the smaller pancreatic ducts (*figure 3*).⁵⁰ Low-grade PanINs (PanIN1-2) are found in a substantial proportion (28%) of non-PC specimens and can be indolent for many years or not progress to invasive cancer at all, whereas PanIN3 lesions are present in 58% of PC specimens and are considered carcinoma in situ.⁵¹ Precursor lesions, in particular IPMNs, can be detected with imaging of the pancreas because they manifest as small cystic lesions of the pancreatic ducts, i.e. ductectasias. Abdominal MRI combined with magnetic resonance cholangiopancreatography (MRCP) is considered the most sensitive imaging modality to detect these cystic lesions.⁵² Endoscopic ultrasonography (EUS) is better in detecting small solid pancreatic lesions, i.e. early-stage PC, compared to MRI/MRCP⁵² and it is able to detect secondary parenchymal changes caused by PanIN and IPMN lesions.⁵³ Current surveillance programs for PC generally use one of these modalities or a combination of both.⁴²⁻⁴⁵ PC surveillance programs have not (yet) implemented non-invasive (serum) biomarkers for PC in their protocols, since the only clinically approved biomarker carbohydrate antigen 19-9 (CA 19-9) has very limited diagnostic accuracy.⁵⁴ However, this is a subject of widespread investigation and various other biomarkers have shown promising results in detecting early-stage PC.^{55,56}

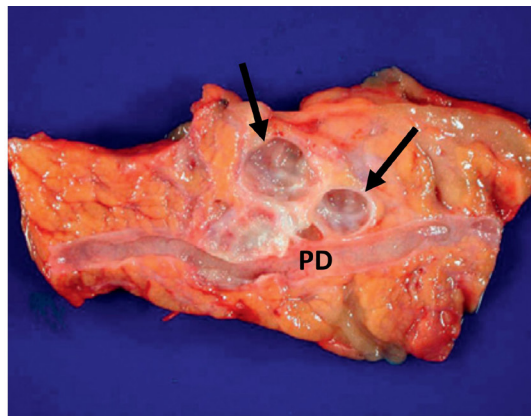


FIGURE 2. Surgical pathology specimen of resected pancreas that includes a branch-duct IPMN (arrows)
PD = main pancreatic duct

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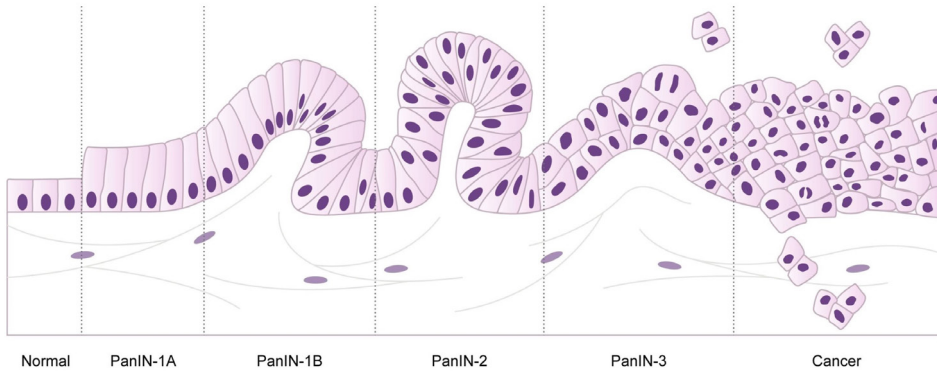


FIGURE 3. Progression model of pancreatic cancer from PanIN lesions. Normal ductal epithelial cells are short and cuboidal, while PanIN-1A lesions are flat and columnar. PanIN-1B lesions are identical to PanIN-1A, although papillary architecture can be observed in these lesions. PanIN-2 lesions can be flat or papillary and show moderate nuclear and architectural abnormalities. PanIN-3 lesions are papillary and show significant nuclear and cytological abnormalities, without the invasion of basement membrane. Pancreatic cancer (ductal adenocarcinoma) shows significant architecture and cytological abnormalities followed by basement membrane invasion.

Reprinted with permission from Susanto, J.M., 2017, Investigating the use of retinoids and epigenetic modification agents as new therapeutic strategies for the treatment of pancreatic cancer, PhD thesis, University of New South Wales, Sydney, available at <https://sites.google.com/site/josus123/pancreaticcancer> (accessed on December 2018).

Originally adapted from Modern Pathology, 16, Maitra A, Adsay NV, Argani P, Iacobuzio-Donahue C, De Marzo A, Cameron JL, Yeo CJ, Hruban RH, Multicomponent analysis of the pancreatic adenocarcinoma progression model using a pancreatic intraepithelial neoplasia tissue microarray, 902-12 (2003), with permission.

PANCREATIC CANCER SURVEILLANCE PROGRAM IN LEIDEN

At the LUMC, a PC surveillance program for high-risk individuals was started in the year 2000. The program is distinctive from other PC surveillance programs worldwide since it specifically focuses on the large and unique cohort of p16-*Leiden* mutation carriers that historically live in or originated from the vicinity of Leiden. All p16-*Leiden* mutation carriers, regardless of family history for PC, are eligible from age 45 and are offered annual surveillance by MRI/MRCP and, optionally, EUS. In the first evaluation by Vasen *et al* in 2011, PC was diagnosed in seven of 79 included individuals (9%) at a mean age of 59 years.⁵⁷ All patients had a resectable tumour with a size ranging 5-40 mm, although it was also shown that these tumours were aggressively growing since three of five tumours

increased in size by 10 mm or more in six months. Cystic duct lesions were detected in 11% of individuals, but ‘prophylactic’ surgery was performed in only one of these individuals, which revealed PanIN2 lesions on histologic examination. The authors concluded that small solid pancreatic tumours as well as small possible precursor lesions can be detected with MRI/MRCP-based surveillance of p16-*Leiden* mutation carriers, but the role of these precursor lesions in the development of PC and the timing and extent of (prophylactic) surgery remained to be determined.

GENETIC TESTING IN FAMILIAL MELANOMA

INDICATIONS FOR GERMLINE *CDKN2A* ANALYSIS

Criteria for performing germline *CDKN2A* mutation analysis in a melanoma family have been proposed in an international guideline published in 2009.⁵⁸ These criteria are based on the patient’s personal and family history for melanoma and PC and the geographic location of the family. In countries with a moderate to high incidence of melanoma such as the Netherlands and other Northern European countries, the guideline recommends *CDKN2A* mutation analysis to patients with melanoma if they have at least three primary melanomas, or when there are at least two additional diagnoses of melanoma and/or PC among close (first or second-degree) family members (“rule of threes”). For lower incidence countries such as those in Southern Europe, a comparable “rule of twos” was proposed. These patients/families have a presumed 10% or greater mutation probability. Current Dutch referral guidelines generally adhere to this international guideline, although patients with a juvenile melanoma (<18 years) and patients with both melanoma and PC are also eligible for *CDKN2A* diagnostics regardless of family history (*table 1*).

OTHER GENES ASSOCIATED WITH FAMILIAL MELANOMA

Several melanoma predisposition genes other than *CDKN2A* are currently known, but mutations in these genes are much rarer compared to mutations in *CDKN2A* (*table 2*).^{8,9} The *CDK4* gene, which functions in the same cell-cycle pathway as *CDKN2A*, i.e. the p16-retinoblastoma(Rb) pathway, was identified shortly after *CDKN2A* by using a candidate gene sequencing approach. *CDK4* mutations found in melanoma families are all located in codon 24 (p.R24H and p.R24C), leading to reduced p16INK4a inhibition of CDK4 and therefore an increase in CDK4 kinase activity and thus cell cycle progression. Melanoma families with a *CDK4* mutation are phenotypically comparable to *CDKN2A*-mutated families, although other cancers such as PC are not frequently seen in the very few families identified thus far.⁵⁹

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

Familial melanoma (diagnostic criteria)	<ul style="list-style-type: none"> ▪ 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	<ul style="list-style-type: none"> ▪ family with two first-degree relatives with melanoma ▪ family with two first- or second-degree relatives with melanoma and one first- or second-degree relative with pancreatic cancer ▪ person with three or more primary melanomas ▪ person with a juvenile melanoma (<18 years) ▪ person with both melanoma and pancreatic cancer

Reference: Vasen HFA, Hes FJ and de Jong MM. Erfelijke en familiale tumoren: Richtlijnen voor diagnostiek en preventie. Leiden: Stichting Opsporing Erfelijke Tumoren/Vereniging Klinische Genetica Nederland/Werkgroep Klinische Oncogenetica, 2017. Available from <https://www.stoet.nl/wp-content/uploads/2017/02/Richtlijnen-2017.jpg>

TABLE 2. Established melanoma predisposition genes other than *CDKN2A*

Gene	Pathway/Function	Non-melanoma cancers	Ref.
<i>CDK4</i>	Cell-cycle control	-	59
<i>TERT</i>	Telomere integrity	-	60
<i>POT1</i>	Telomere integrity	Glioma, leukaemia, possibly other cancers	61-64
<i>ACD</i>	Telomere integrity	Leukaemia	64,65
<i>TERF2IP</i>	Telomere integrity	Leukaemia	64,65
<i>BAP1</i>	DNA damage response	Uveal melanoma, malignant mesothelioma, renal cell carcinoma, basal cell carcinoma	66,67
<i>MITF</i>	Melanocyte homeostasis	Renal cell carcinoma, pancreatic cancer	68,69

The *CDKN2A* and *CDK4* genes were for many years the only known high-penetrance melanoma predisposition genes. The rise of new sequencing technologies in the last decade resulted however in the recent identification of several new predisposition genes and key pathways. One of these pathways controls telomere integrity and germline mutations have been reported in multiple genes involved in the regulation of telomere length (*TERT*) and telomere maintenance (*POT1*, *ACD*, *TERF2IP*) (figure 4). A specific mutation in the promotor region of *TERT* (c.-57T>G) causes an increased transcription of *TERT* and is found in only a few, although heavily affected, melanoma families.^{60,70} It is hypothesized that overexpression of *TERT* results in longer telomeres and therefore enhanced survival of cancerous cells, although this has not been proven for the c.-57T>G variant.⁷⁰ The shelterin complex protects the telomeres from DNA repair mechanisms and regulates *TERT* activity. Germline mutations have been identified in three of its six components, *POT1*, *ACD* and

TERF2IP, and it has been demonstrated that germline *POT1* mutations do indeed result in increased telomere length.^{61,62,65} Mutations in these genes are also found in families with a predisposition for glioma or leukaemia^{63,64} and these cancers are reported in some of the melanoma pedigrees as well. *POT1* germline mutations are also increasingly being reported in patients and families with a wide range of other cancers, including thyroid cancer⁷¹, colorectal cancer⁷², Hodgkin's lymphoma⁷³ and cancers in the Li-Fraumeni (*TP53*) spectrum, in particular (cardiac) angiosarcoma^{74,75}. The *POT1* gene might thus be associated with many different types of cancer other than melanoma. The *BAP1* (BRCA1-associated protein) gene is involved in several tumour suppressor pathways including the DNA damage response.

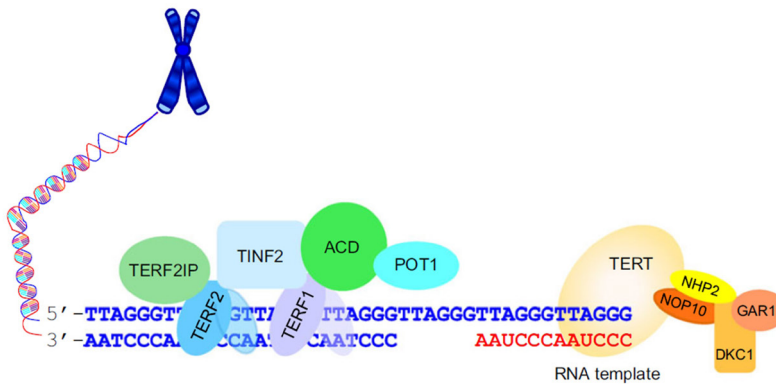


FIGURE 4 Schematic view of the telomere. The shelterin complex (TERF1, TERF2, TERF2IP, TINF2, ACD, POT1) is depicted on the left and the telomerase complex (TERT and other associated proteins) is depicted on the right. The telomerase complex adds telomere repeat sequences to the 3' end of the telomere. The shelterin complex is anchored to the double stranded TTAGGG region of the telomere by the subunits TERF1 and TERF2 and protects the telomeres from DNA repair mechanisms and regulates TERT activity.

Reprinted with permission from *Pigment Cell Melanoma Research*, 28, Aoude LG, Wadt KA, Pritchard AL, Hayward NK, *Genetics of familial melanoma: 20 years after CDKN2A*, 148-60 (2015)

Germline mutations in *BAP1* cause a specific cancer predisposition syndrome with a high penetrance for uveal melanoma (28%), malignant mesothelioma (22%), cutaneous melanoma (18%), renal cell carcinoma (9%) and basal cell carcinoma (6.5%). Also, specific benign skin lesions called atypical Spitz tumours (AST) or melanocytic *BAP1*-mutated atypical intradermal tumours (MBAIT) are typically found in *BAP1* mutation carriers.^{66,67} *MITF* is a lower (medium) penetrance melanoma predisposition gene and is involved in melanocyte homeostasis. Only one specific gain-of-function mutation in codon 318 (p.E318K), which causes an increase of *MITF* transcriptional activity, is associated with both

sporadic and familial melanoma.⁷⁶ *MITF* p.E318K carriers more frequently develop multiple primary melanomas and there is possibly an increased risk for renal cell carcinoma and pancreatic cancer as well.^{68,69} In *figure 5*, all these currently known melanoma predisposition genes are plotted relative to their frequency and effect size. More genes with a possible association with familial melanoma are presented in chapter 8.

In addition to these high- and medium-penetrance melanoma predisposition genes, several common risk variants (single nucleotide polymorphisms; SNPs) derived from large population-based genome wide association studies (GWAS) have been associated with (sporadic) melanoma (*figure 5*).⁷⁷⁻⁷⁹ These individual SNPs only marginally or moderately influence melanoma risk, but an aggregation of risk variants might substantially increase risk. One of the best established of these risk factors is the *MC1R* gene. The *MC1R* gene plays an important role in skin pigmentation and specific variants that are most strongly associated with a red hair colour phenotype (RHC variants) increase melanoma risk approximately twofold.⁸⁰ Other variants that are less strongly associated with red hair colour confer a much smaller melanoma risk and are called non-RHC variants. Studies have shown that both RHC and non-RHC variants also modify melanoma penetrance in *CDKN2A*-mutated families.^{81,82} Common susceptibility SNPs are typical candidates to be incorporated in a polygenic risk score (PRS) model, and such models have already shown to improve risk stratification in familial breast cancer.^{83,84}

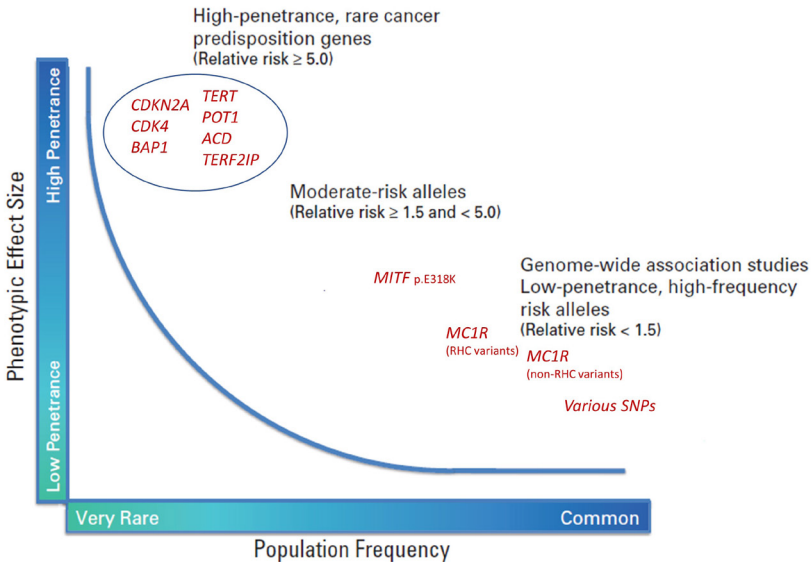


FIGURE 5. [Legend on the next page]

FIGURE 5. Graphic display of the phenotypic effect size of currently known genes involved in melanoma susceptibility, plotted against their frequency of occurrence. Note: the high-penetrance genes are randomly plotted within the blue circle. SNP = Single Nucleotide Polymorphism

Adapted with permission from Journal of Clinical Oncology, 28, Stadler ZK, Thom P, Robson ME, Weitzel JN, Kauff ND, Hurley KE, Devlin V, Gold B, Klein RJ, Offit K, Genome-wide association studies of cancer, 4255-67 (2010)

AIMS AND OUTLINE OF THIS THESIS

This thesis has three general aims.

- Our first aim is to investigate the full cancer phenotype of p16-*Leiden* mutation carriers and to study potential modifiers of cancer risk in these carriers (*PART I*).
- Our second aim is to evaluate and improve the p16-*Leiden* pancreatic cancer (PC) surveillance program.
- Our third and final aim is to evaluate and improve genetic testing for hereditary melanoma (*PART II*).

PART I Cancer phenotype and pancreatic cancer surveillance of p16-*Leiden* mutation carriers

In **chapter 2**, we prospectively evaluate a cohort of p16-*Leiden* mutation carriers for the occurrence of any cancer and we investigate the influence of tobacco use on cancer risk. In **chapter 3**, we genotype seven PC-associated SNPs in a nation-wide cohort of p16-*Leiden* mutation carriers and we investigate if these SNPs modify PC risk and could explain the interfamilial variability in the occurrence of PC among these families. In **chapter 4**, we compare the frequency, features and natural history of precursor lesions of PC and PC itself between two different high-risk groups (p16-*Leiden* vs. FPC surveillance cohorts). In **chapter 5**, we report two high-risk patients who developed a second primary PC after a limited resection of their first PC and we discuss the possible implications of these findings for the surgical management of patients with an early-stage screen-detected PC. In **chapter 6**, we investigate if a serum protein signature can differentiate between PC and non-PC in the p16-*Leiden* PC surveillance cohort and we discuss if this biomarker test has the potential to be implemented in the surveillance program.

PART II Genetic testing in familial melanoma; *CDKN2A* and beyond

In **chapter 7**, we study the association between germline *CDKN2A* mutations and several clinical features present in a melanoma family, and we develop a clinical scoring system (*CM-Score*) that can predict the presence of a germline *CDKN2A* mutation in melanoma

families. In **chapter 8**, we investigate the role of other (candidate) melanoma predisposition genes in a large cohort of Dutch non-*CDKN2A* melanoma families through comprehensive multi-gene panel testing.

In the final **chapter 9**, we discuss the main findings of these studies in the context of the most recent literature.

REFERENCES

1. Bergman W, Watson P, de Jong J, et al: Systemic cancer and the FAMMM syndrome. *Br J Cancer* 61:932-6, 1990
2. Hussussian CJ, Struewing JP, Goldstein AM, et al: Germline p16 mutations in familial melanoma. *Nat Genet* 8:15-21, 1994
3. Kamb A, Gruis NA, Weaver-Feldhaus J, et al: A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 264:436-40, 1994
4. Gruis NA, Sandkuijl LA, van der Velden PA, et al: CDKN2 explains part of the clinical phenotype in Dutch familial atypical multiple-mole melanoma (FAMMM) syndrome families. *Melanoma Res* 5:169-77, 1995
5. Gruis NA, van der Velden PA, Sandkuijl LA, et al: Homozygotes for CDKN2 (p16) germline mutation in Dutch familial melanoma kindreds. *Nat Genet* 10:351-3, 1995
6. Goldstein AM, Fraser MC, Struewing JP, et al: Increased risk of pancreatic cancer in melanoma-prone kindreds with p16INK4 mutations. *N Engl J Med* 333:970-4, 1995
7. Lynch HT, Brand RE, Hogg D, et al: Phenotypic variation in eight extended CDKN2A germline mutation familial atypical multiple mole melanoma-pancreatic carcinoma-prone families: the familial atypical mole melanoma-pancreatic carcinoma syndrome. *Cancer* 94:84-96, 2002
8. Aoude LG, Wadt KA, Pritchard AL, et al: Genetics of familial melanoma: 20 years after CDKN2A. *Pigment Cell Melanoma Res* 28:148-60, 2015
9. Read J, Wadt KA, Hayward NK: Melanoma genetics. *J Med Genet* 53:1-14, 2016
10. Sherr CJ: The INK4a/ARF network in tumour suppression. *Nat Rev Mol Cell Biol* 2:731-7, 2001
11. Bishop DT, Demenais F, Goldstein AM, et al: Geographical variation in the penetrance of CDKN2A mutations for melanoma. *J Natl Cancer Inst* 94:894-903, 2002
12. Goldstein AM, Struewing JP, Chidambaram A, et al: Genotype-phenotype relationships in U.S. melanoma-prone families with CDKN2A and CDK4 mutations. *J Natl Cancer Inst* 92:1006-10, 2000
13. Masback A, Olsson H, Westerdahl J, et al: Clinical and histopathological features of malignant melanoma in germline CDKN2A mutation families. *Melanoma Res* 12:549-57, 2002
14. Mantelli M, Pastorino L, Ghiorzo P, et al: Early onset may predict G101W CDKN2A founder mutation carrier status in Ligurian melanoma patients. *Melanoma Res* 14:443-8, 2004
15. van der Rhee JI, Krijnen P, Gruis NA, et al: Clinical and histologic characteristics of malignant melanoma in families with a germline mutation in CDKN2A. *J Am Acad Dermatol* 65:281-8, 2011
16. Goldstein AM: Familial melanoma, pancreatic cancer and germline CDKN2A mutations. *Hum Mutat* 23:630, 2004
17. Goldstein AM, Chan M, Harland M, et al: High-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. *Cancer Res* 66:9818-28, 2006
18. Vasen HF, Gruis NA, Frants RR, et al: Risk of developing pancreatic cancer in families with familial atypical multiple mole melanoma associated with a specific 19 deletion of p16 (p16-Leiden). *Int J Cancer*

- 87:809-11, 2000
19. de Vos tot Nederveen Cappel WH, Offerhaus GJ, van Puijenbroek M, et al: Pancreatic carcinoma in carriers of a specific 19 base pair deletion of CDKN2A/p16 (p16-leiden). *Clin Cancer Res* 9:3598-605, 2003
 20. Vasen H, Ibrahim I, Ponce CG, et al: Benefit of Surveillance for Pancreatic Cancer in High-Risk Individuals: Outcome of Long-Term Prospective Follow-Up Studies From Three European Expert Centers. *J Clin Oncol* 34:2010-9, 2016
 21. de Snoo FA, Bishop DT, Bergman W, et al: Increased risk of cancer other than melanoma in CDKN2A founder mutation (p16-Leiden)-positive melanoma families. *Clin Cancer Res* 14:7151-7, 2008
 22. Oldenburg RA, de Vos tot Nederveen Cappel WH, van Puijenbroek M, et al: Extending the p16-Leiden tumour spectrum by respiratory tract tumours. *J Med Genet* 41:e31, 2004
 23. Vinarsky V, Fine RL, Assaad A, et al: Head and neck squamous cell carcinoma in FAMMM syndrome. *Head Neck* 31:1524-7, 2009
 24. Cabanillas R, Astudillo A, Valle M, et al: Novel germline CDKN2A mutation associated with head and neck squamous cell carcinomas and melanomas. *Head Neck* 35:E80-4, 2013
 25. Mukherjee B, Delancey JO, Raskin L, et al: Risk of non-melanoma cancers in first-degree relatives of CDKN2A mutation carriers. *J Natl Cancer Inst* 104:953-6, 2012
 26. Ghiorzo P, Ciotti P, Mantelli M, et al: Characterization of ligurian melanoma families and risk of occurrence of other neoplasia. *Int J Cancer* 83:441-8, 1999
 27. Borg A, Sandberg T, Nilsson K, et al: High frequency of multiple melanomas and breast and pancreas carcinomas in CDKN2A mutation-positive melanoma families. *J Natl Cancer Inst* 92:1260-6, 2000
 28. Bishop JA, Wachsmuth RC, Harland M, et al: Genotype/phenotype and penetrance studies in melanoma families with germline CDKN2A mutations. *J Invest Dermatol* 114:28-33, 2000
 29. Nielsen K, Harbst K, Masback A, et al: Swedish CDKN2A mutation carriers do not present the atypical mole syndrome phenotype. *Melanoma Res* 20:266-72, 2010
 30. Ipenburg NA, Gruis NA, Bergman W, et al: The absence of multiple atypical nevi in germline CDKN2A mutations: Comment on "Hereditary melanoma: Update on syndromes and management: Genetics of familial atypical multiple mole melanoma syndrome". *J Am Acad Dermatol* 75:e157, 2016
 31. Vasen HF, Bergman W, van Haeringen A, et al: The familial dysplastic nevus syndrome. Natural history and the impact of screening on prognosis. A study of nine families in the Netherlands. *Eur J Cancer Clin Oncol* 25:337-41, 1989
 32. Masri GD, Clark WH, Jr., Guerry Dt, et al: Screening and surveillance of patients at high risk for malignant melanoma result in detection of earlier disease. *J Am Acad Dermatol* 22:1042-8, 1990
 33. Hansson J, Bergenmar M, Hofer PA, et al: Monitoring of kindreds with hereditary predisposition for cutaneous melanoma and dysplastic nevus syndrome: results of a Swedish preventive program. *J Clin Oncol* 25:2819-24, 2007

34. van der Rhee JI, de Snoo FA, Vasen HF, et al: Effectiveness and causes for failure of surveillance of CDKN2A-mutated melanoma families. *J Am Acad Dermatol* 65:289-96, 2011
35. Brentnall TA, Bronner MP, Byrd DR, et al: Early diagnosis and treatment of pancreatic dysplasia in patients with a family history of pancreatic cancer. *Ann Intern Med* 131:247-55, 1999
36. Kimmey MB, Bronner MP, Byrd DR, et al: Screening and surveillance for hereditary pancreatic cancer. *Gastrointest Endosc* 56:S82-6, 2002
37. Shi C, Hruban RH, Klein AP: Familial pancreatic cancer. *Arch Pathol Lab Med* 133:365-74, 2009
38. Zhen DB, Rabe KG, Gallinger S, et al: BRCA1, BRCA2, PALB2, and CDKN2A mutations in familial pancreatic cancer: a PACGENE study. *Genet Med* 17:569-77, 2015
39. Grant RC, Selander I, Connor AA, et al: Prevalence of germline mutations in cancer predisposition genes in patients with pancreatic cancer. *Gastroenterology* 148:556-64, 2015
40. Chaffee KG, Oberg AL, McWilliams RR, et al: Prevalence of germ-line mutations in cancer genes among pancreatic cancer patients with a positive family history. *Genet Med* 20:119-127, 2018
41. Hu C, Hart SN, Polley EC, et al: Association Between Inherited Germline Mutations in Cancer Predisposition Genes and Risk of Pancreatic Cancer. *Jama* 319:2401-2409, 2018
42. Overbeek KA, Cahen DL, Canto MI, et al: Surveillance for neoplasia in the pancreas. *Best Pract Res Clin Gastroenterol* 30:971-986, 2016
43. Corral JE, Mareth KF, Riegert-Johnson DL, et al: Diagnostic Yield From Screening Asymptomatic Individuals at High Risk for Pancreatic Cancer: a Meta-analysis of Cohort Studies. *Clin Gastroenterol Hepatol*, 2018
44. Signoretti M, Bruno MJ, Zerboni G, et al: Results of surveillance in individuals at high-risk of pancreatic cancer: A systematic review and meta-analysis. *United European Gastroenterol J* 6:489-499, 2018
45. Canto MI, Harinck F, Hruban RH, et al: International Cancer of the Pancreas Screening (CAPS) Consortium summit on the management of patients with increased risk for familial pancreatic cancer. *Gut* 62:339-47, 2013
46. Shimizu Y, Yasui K, Matsueda K, et al: Small carcinoma of the pancreas is curable: new computed tomography finding, pathological study and postoperative results from a single institute. *J Gastroenterol Hepatol* 20:1591-4, 2005
47. Takeda Y, Saiura A, Takahashi Y, et al: Asymptomatic Pancreatic Cancer: Does Incidental Detection Impact Long-Term Outcomes? *J Gastrointest Surg* 21:1287-1295, 2017
48. Tanaka M, Fernandez-del Castillo C, Adsay V, et al: International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. *Pancreatology* 12:183-97, 2012
49. Levy P, Jouannaud V, O'Toole D, et al: Natural history of intraductal papillary mucinous tumors of the pancreas: actuarial risk of malignancy. *Clin Gastroenterol Hepatol* 4:460-8, 2006
50. Hruban RH, Maitra A, Goggins M: Update on pancreatic intraepithelial neoplasia. *Int J Clin Exp Pathol* 1:306-16, 2008

51. Andea A, Sarkar F, Adsay VN: Clinicopathological correlates of pancreatic intraepithelial neoplasia: a comparative analysis of 82 cases with and 152 cases without pancreatic ductal adenocarcinoma. *Mod Pathol* 16:996-1006, 2003
52. Harinck F, Konings IC, Kluijft I, et al: A multicentre comparative prospective blinded analysis of EUS and MRI for screening of pancreatic cancer in high-risk individuals. *Gut* 65:1505-13, 2016
53. Brune K, Abe T, Canto M, et al: Multifocal neoplastic precursor lesions associated with lobular atrophy of the pancreas in patients having a strong family history of pancreatic cancer. *Am J Surg Pathol* 30:1067-76, 2006
54. Huang Z, Liu F: Diagnostic value of serum carbohydrate antigen 19-9 in pancreatic cancer: a meta-analysis. *Tumour Biol* 35:7459-65, 2014
55. Jimenez-Luna C, Torres C, Ortiz R, et al: Proteomic biomarkers in body fluids associated with pancreatic cancer. *Oncotarget* 9:16573-16587, 2018
56. Young MR, Wagner PD, Ghosh S, et al: Validation of Biomarkers for Early Detection of Pancreatic Cancer: Summary of The Alliance of Pancreatic Cancer Consortia for Biomarkers for Early Detection Workshop. *Pancreas* 47:135-141, 2018
57. Vasen HF, Wasser M, van Mil A, et al: Magnetic resonance imaging surveillance detects early-stage pancreatic cancer in carriers of a p16-Leiden mutation. *Gastroenterology* 140:850-6, 2011
58. Leachman SA, Carucci J, Kohlmann W, et al: Selection criteria for genetic assessment of patients with familial melanoma. *J Am Acad Dermatol* 61:677 e1-14, 2009
59. Puntervoll HE, Yang XR, Vetti HH, et al: Melanoma prone families with CDK4 germline mutation: phenotypic profile and associations with MC1R variants. *J Med Genet* 50:264-70, 2013
60. Horn S, Figl A, Rachakonda PS, et al: TERT promoter mutations in familial and sporadic melanoma. *Science* 339:959-61, 2013
61. Robles-Espinoza CD, Harland M, Ramsay AJ, et al: POT1 loss-of-function variants predispose to familial melanoma. *Nat Genet* 46:478-481, 2014
62. Shi J, Yang XR, Ballew B, et al: Rare missense variants in POT1 predispose to familial cutaneous malignant melanoma. *Nat Genet* 46:482-6, 2014
63. Bainbridge MN, Armstrong GN, Gramatges MM, et al: Germline mutations in shelterin complex genes are associated with familial glioma. *J Natl Cancer Inst* 107:384, 2015
64. Speedy HE, Kinnerley B, Chubb D, et al: Germ line mutations in shelterin complex genes are associated with familial chronic lymphocytic leukemia. *Blood* 128:2319-2326, 2016
65. Aoude LG, Pritchard AL, Robles-Espinoza CD, et al: Nonsense mutations in the shelterin complex genes ACD and TERF2IP in familial melanoma. *J Natl Cancer Inst* 107, 2015
66. Rai K, Pilarski R, Cebulla CM, et al: Comprehensive review of BAP1 tumor predisposition syndrome with report of two new cases. *Clin Genet* 89:285-94, 2016
67. Haugh AM, Njauw CN, Bublely JA, et al: Genotypic and Phenotypic Features of BAP1 Cancer Syndrome:

- A Report of 8 New Families and Review of Cases in the Literature. *JAMA Dermatol* 153:999-1006, 2017
68. Bertolotto C, Lesueur F, Giuliano S, et al: A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. *Nature* 480:94-8, 2011
 69. Ghiorzo P, Pastorino L, Queirolo P, et al: Prevalence of the E318K MITF germline mutation in Italian melanoma patients: associations with histological subtypes and family cancer history. *Pigment Cell Melanoma Res* 26:259-62, 2013
 70. Harland M, Petljak M, Robles-Espinoza CD, et al: Germline TERT promoter mutations are rare in familial melanoma. *Fam Cancer* 15:139-44, 2016
 71. Wilson TL, Hattangady N, Lerario AM, et al: A new POT1 germline mutation-expanding the spectrum of POT1-associated cancers. *Fam Cancer* 16:561-566, 2017
 72. Chubb D, Broderick P, Dobbins SE, et al: Rare disruptive mutations and their contribution to the heritable risk of colorectal cancer. *Nat Commun* 7:11883, 2016
 73. McMaster ML, Sun C, Landi MT, et al: Germline mutations in Protection of Telomeres 1 in two families with Hodgkin lymphoma. *Br J Haematol* 181:372-377, 2018
 74. Calvete O, Garcia-Pavia P, Dominguez F, et al: The wide spectrum of POT1 gene variants correlates with multiple cancer types. *Eur J Hum Genet* 25:1278-1281, 2017
 75. Calvete O, Martinez P, Garcia-Pavia P, et al: A mutation in the POT1 gene is responsible for cardiac angiosarcoma in TP53-negative Li-Fraumeni-like families. *Nat Commun* 6:8383, 2015
 76. Paillerets BB, Lesueur F, Bertolotto C: A germline oncogenic MITF mutation and tumor susceptibility. *Eur J Cell Biol* 93:71-5, 2014
 77. Barrett JH, Iles MM, Harland M, et al: Genome-wide association study identifies three new melanoma susceptibility loci. *Nat Genet* 43:1108-13, 2011
 78. Barrett JH, Taylor JC, Bright C, et al: Fine mapping of genetic susceptibility loci for melanoma reveals a mixture of single variant and multiple variant regions. *Int J Cancer* 136:1351-60, 2015
 79. Law MH, Bishop DT, Lee JE, et al: Genome-wide meta-analysis identifies five new susceptibility loci for cutaneous malignant melanoma. *Nat Genet* 47:987-995, 2015
 80. Raimondi S, Sera F, Gandini S, et al: MC1R variants, melanoma and red hair color phenotype: a meta-analysis. *Int J Cancer* 122:2753-60, 2008
 81. Demenais F, Mohamdi H, Chaudru V, et al: Association of MC1R variants and host phenotypes with melanoma risk in CDKN2A mutation carriers: a GenoMEL study. *J Natl Cancer Inst* 102:1568-83, 2010
 82. Fargnoli MC, Gandini S, Peris K, et al: MC1R variants increase melanoma risk in families with CDKN2A mutations: a meta-analysis. *Eur J Cancer* 46:1413-20, 2010
 83. Mavaddat N, Pharoah PD, Michailidou K, et al: Prediction of breast cancer risk based on profiling with common genetic variants. *J Natl Cancer Inst* 107, 2015
 84. Muranen TA, Mavaddat N, Khan S, et al: Polygenic risk score is associated with increased disease risk in 52 Finnish breast cancer families. *Breast Cancer Res Treat* 158:463-9, 2016

