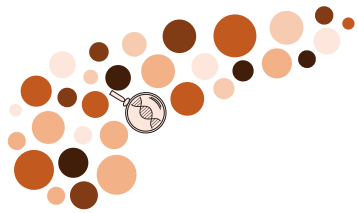
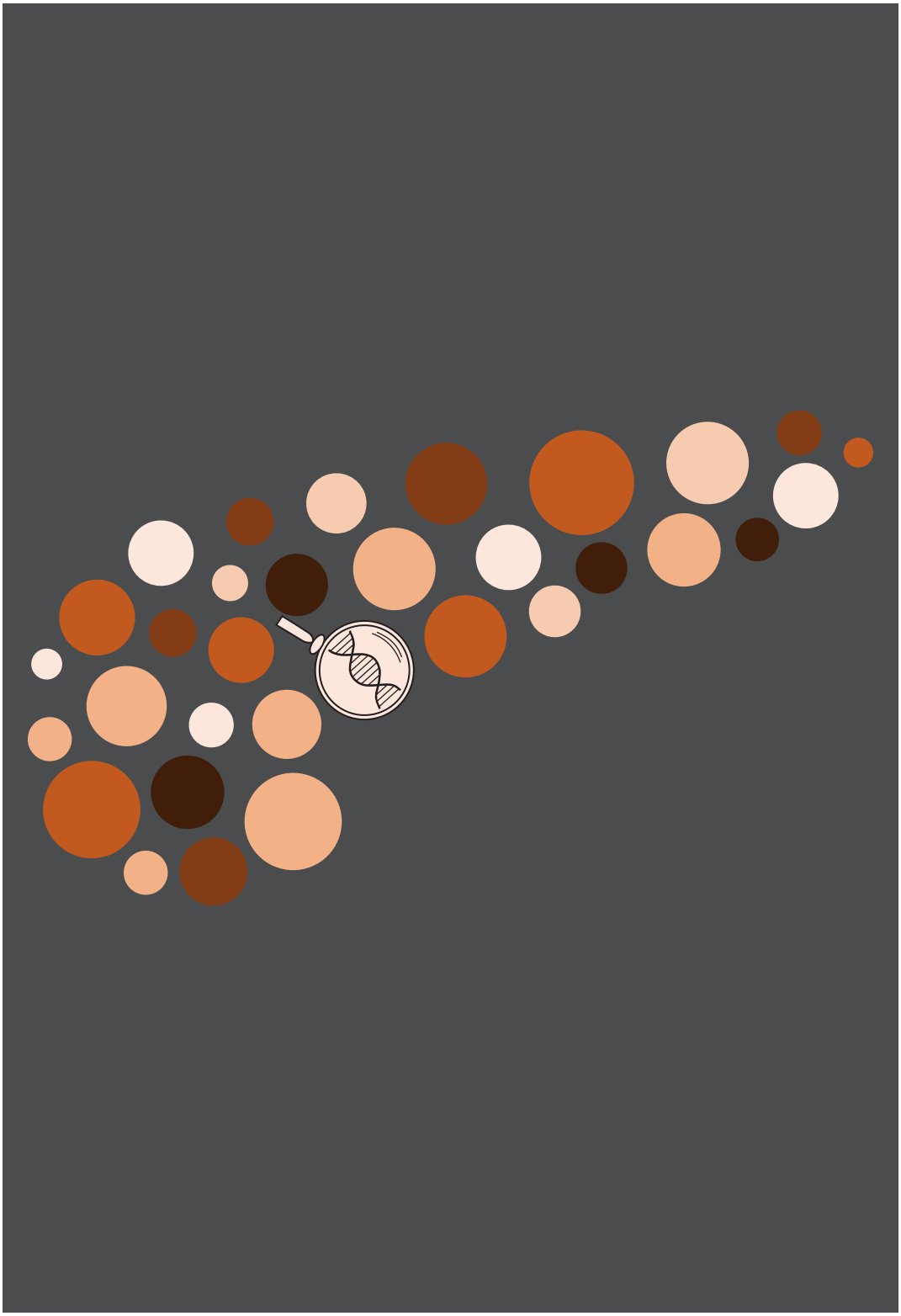
Familial Melanoma and Pancreatic Cancer: studies on genotype, phenotype and surveillance

Potjer, T.P.

PART I

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





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Prospective risk of cancer and the influence of tobacco use in carriers of the p16-*Leiden* germline variant

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Thomas P. Potjer, Heidi E. Kranenburg, Wilma Bergman, Wouter H. de Vos tot Nederveen Cappel, Hester S. van Monsjou, Daniela Q.C.M. Barge-Schaapveld, Hans F.A. Vasen

ABSTRACT

The p16-*Leiden* germline variant in the *CDKN2A* gene is associated with a high risk of melanoma and pancreatic cancer. The aims of this study were to assess the risk of developing other cancers and to determine whether tobacco use would alter cancer risk in carriers of such a variant. We therefore prospectively evaluated individuals with a p16-*Leiden* germline variant, participating in a pancreatic surveillance program, for the occurrence of cancer (n=150). Tobacco use was assessed at the start of the surveillance program. We found a significantly increased risk for melanoma (RR 41.3; 95% CI 22.9-74.6) and pancreatic cancer (RR 80.8; 95% CI 44.7-146). In addition, increased risks were found for cancers of the lip, mouth and pharynx (RR 18.8; 95% CI 6.05-58.2) and respiratory tumours (RR 4.56; 95% CI 1.71-12.1). Current smokers developed significantly more cancers of lip, mouth and pharynx, respiratory system and pancreas compared to former and never-smokers. In conclusion, this study shows that carriers of a p16-*Leiden* variant have an increased risk of developing various types of cancer and smoking significantly increases the risk of frequently occurring cancers. Smoking cessation should be an integral part of the management of p16-*Leiden* variant carriers.

INTRODUCTION

Familial atypical multiple mole melanoma (FAMMM) syndrome is an autosomal dominant tumour syndrome characterized by the development of melanoma and dysplastic naevi of the skin. Up to 40% of FAMMM families harbour a germline variant in the *CDKN2A* gene, making it the most frequently involved gene in FAMMM syndrome.[1] More than 65 different variants in the *CDKN2A* gene have been identified worldwide.[2] In the Netherlands, the p16-*Leiden* variant, a 19-base pair deletion (c.225_243del19; RefSeq NM_000077.4), is the most common *CDKN2A* germline variant.[3] In a previous study,[4] we demonstrated that carriers of such a variant have an increased risk of developing pancreatic cancer (15-20% lifetime risk). Since then a large cohort of patients is under pancreatic surveillance.[5]

Several studies reported an increased risk of tumours other than melanoma and pancreatic cancer for various *CDKN2A* germline variants.[6-10] However, these studies have used a variety of methodological approaches and some have been limited by inclusion of heterogeneous groups or by failure to determine individual mutation status. In addition, the influence of environmental factors (e.g. smoking) on the phenotypic variability in FAMMM syndrome is yet to be elucidated.

In the present study, we analysed the prospective risk of cancer in a unique cohort of individuals with the same p16 germline variant (p16-*Leiden).* Additionally, we examined the association between a personal history of smoking and the development of cancer.

PATIENTS AND METHODS

PATIENT COHORT

Individuals were included in this study on the basis of carrier status for the p16-*Leiden* germline variant and participation in a pancreatic surveillance program, which consisted of a yearly abdominal MRI combined with magnetic resonance cholangiopancreatography (MRCP) from age 45.[5]

A complete medical history was obtained at the start of the surveillance study. Following this first visit, patients revisited the gastroenterologist annually, at which point the occurrence of new cancers or other diseases was assessed. For the current study, all medical records (with pathological confirmation) were obtained for each individual from the electronic hospital information system. Only cancers that occurred after the first contact were included in the analysis. The study inclusion and follow-up period was from January

2000 to April 2013. The follow-up time for each individual started from inclusion until the last documented appointment with a medical specialist at the Leiden University Medical Center, or the date of death.

CANCER RISK ESTIMATES AND STATISTICAL ANALYSIS

The prospectively observed cancers were classified by International Classification of Diseases code 10 (ICD-10). To calculate the expected number of cancers, five-year cancer incidence rates of matching ICD codes, specific for sex and age, were obtained from the Netherlands Cancer Registry (NCR) for the province of South-Holland in the Netherlands.[11] To calculate the expected number of neuroendocrine tumours, national incidence rates were used for the period 2001-2010.[12] The relative risks were computed by dividing the observed cancer numbers in each group by the expected cancer numbers. Confidence intervals for the relative risks were calculated with the use of Poisson probabilities. To compute the impact of tobacco use on cancer development, individuals were classified as either ever-smokers (current or former) or never-smokers at inclusion in the study; χ^2 analysis was used for comparison. Acquired data was submitted to a public *CDKN2A* gene variant database (http://chromium.liacs.nl/LOVD2/home.php; submission ID #0014954)

RESULTS

PATIENT CHARACTERISTICS

A total of 150 proven or implied carriers of the p16-*Leiden* germline variant were included (64 males, median age at inclusion 51 years (range, 36-72 years)). One hundred and forty-four individuals had a proven p16-*Leiden* germline variant, including a homozygote for the p16-*Leiden* variant. The remaining 6 individuals had at least one melanoma in their medical history and a close relative with the p16-*Leiden* germline variant, which makes them highly likely of being a carrier (>97% according to Bayesian probabilities). The median time of follow-up was 43 months (range, 1-144 months; 1st-3rd quartile, 17-89 months). The total observation period was 682 person years.

PROSPECTIVE TUMOURS

A total of 47 prospective tumours were diagnosed in 36 (24%) of the 150 individuals. Due to the relatively small numbers of observed cancers, classification was based on organ system rather than individual site, with the exceptions of melanoma and pancreatic cancer. *Table 1* shows the relative risks for developing various types of cancer. Melanoma and pancreatic cancer were the most frequently occurring cancers (n=11 each, RR 41.3 (95% CI 22.9-74.6) and 80.8 (95% CI 44.7-146), respectively). When these tumours were excluded

from the analysis, the risk of developing any type of cancer remained significantly increased (RR 4.31; 95% CI 2.91-6.37). The highest risks were found for cancers of the lip, mouth and pharynx (RR 18.8; 95% CI 6.05-58.2), respiratory tumours (RR 4.56; 95% CI 1.71-12.1) and digestive tract tumours (RR 3.71; 95% CI 1.39-9.90). The relatively small numbers of observed cancers, however, resulted in broad confidence intervals, which is especially true for cancers of bone and soft tissue.

TABLE 1. Relative risk of developing cancer in a prospective series of p16-*Leiden* variant carriers (n=150)

Site/organ system	ICD-10 code	Observed (95% CI)	Expected	RR (95% CI)
Bone	c40-c41	1 (0.141-7.10)	0.0149	66.9 (9.43-475)*
Digestive	c15-c24, c26	4 (1.50-10.7)	1.08	3.71 (1.39-9.90)*
Female Breast	c50	3 (0.967-9.30)	1.15	2.61 (0.840-8.08)
Haematological	c81-c96	1 (0.141-7.10)	0.462	2.16 (0.305-15.3)
Lip, mouth, pharynx	c00-c14	3 (0.968-9.30)	0.160	18.8 (6.05-58.2)*
Male genital	c60-c63	1 (0.141-7.10)	0.689	1.45 (0.204-10.3)
Melanoma§	c43	11 (6.09-19.9)	0.266	41.3 (22.9-74.6)*
Nonmelanoma skin#	c44	4 (1.50-10.7)	0.327	12.3 (4.60-32.6)*
Pancreas	c25	11 (6.09-19.9)	0.136	80.8 (44.7-146)*
Respiratory	c32-c34	4 (1.50-10.7)	0.877	4.56 (1.71-12.1)*
Soft tissue	c38, c47-c49	2 (0.500-8.00)	0.0336	59.5 (14.9-238)*
Unknown primary site	c80	1 (0.141-7.10)	0.138	7.22 (1.02-51.3)
Urinary	c64-c68	1 (0.141-7.10)	0.333	3.00 (0.423-21.3)
All cancers		47 (35.3-62.6)	6.20	7.58 (5.69-10.1)*
All cancers except melanoma and pancreas		25 (16.9-37.0)	5.80	4.31 (2.91-6.37)*

** Significant*

§ First as well as subsequent melanomas are registered In the Netherlands Cancer Registry (NCR)

 # Basal cell carcinoma is not registered in the NCR and therefore not included in the calculation

Details of 21 prospective cancers (all cancers except those of skin and pancreas) are shown in *table 2*. Notably, the observed number of carcinoid tumours was higher than expected (0.0168; RR 119; 95% CI 29.7-475). When excluding carcinoid tumours from the risk calculation for digestive tract tumours, the increased risk for a digestive tract tumour no longer reached significance (RR 1.86; 95% CI 0.465-7.43).

Seven individuals developed a total of 11 melanomas during the follow-up period. However, a much larger number of individuals (91 out of 150) had a diagnosed melanoma prior to

starting surveillance for pancreatic cancer (median age at diagnosis of first melanoma 40 years). *Table 3* shows tumours diagnosed before inclusion, of which melanoma forms by far the major part. Only one individual developed a first melanoma during the follow-up period. Melanoma therefore remains the most frequently occurring cancer in this p16-*Leiden* study cohort and first melanomas mostly occur prior to the age of inclusion (45 years). A more exhaustive description of the melanoma phenotype in carriers of the p16-*Leiden* germline variant is given by van der Rhee *et al*.[13]

TABLE 2. Characteristics of prospective cancers (excluding skin cancer and pancreatic cancer)

Subject number	Sex	Tumour type/organ	Histopathology	Age at diagnosis
1	F	Caecum	Carcinoid	72
2	M	Appendix	Carcinoid	58
		Bone	Papillary squamous cell carcinoma of mandible	62
3	M	Stomach	Adenocarcinoma of cardia	64
4	M	Haematopoietic	Multiple myeloma	67
		Stomach	Adenocarcinoma	67
5	F	Breast	Ductal adenocarcinoma	48
6	F	Breast	Ductal adenocarcinoma	53
7	F	Breast	Ductal adenocarcinoma	49
8	M	Hypopharynx	Squamous cell carcinoma	51
		Lung	Squamous cell carcinoma	52
9*	M	Floor of mouth	Squamous cell carcinoma	58
		Larynx	Squamous cell carcinoma	58
10	F	Tongue	Carcinoma not specified	51
11	F	Lung	Non-small cell carcinoma	60
12	M	Larynx	Squamous cell carcinoma	55
13	F	Bladder	Small cell carcinoma	58
14	M	Prostate	Adenocarcinoma	69
15	F	Knee	Myxofibrosarcoma	48
16	M	Neck	Leiomyosarcoma	66
17	F	Unknown	Metastatic adenocarcinoma	67

**This patient had two primary tumours detected concurrently.*

TOBACCO USE

With regard to a personal history of smoking, information was complete for 147 (98%) out of 150 individuals. At inclusion, 92 individuals were ever-smokers (of which 26 were current smokers). Eleven of 92 ever-smokers (12%) and four of 55 never-smokers (7%) developed

pancreatic cancer, respiratory cancer or cancer of the lip, mouth and pharynx (p=0.364). Four of 11 patients with pancreatic cancer were never-smokers. When only current smokers were considered, seven of 26 (27%) developed above mentioned cancers, versus only eight of 121 (7%) of the former and never-smokers. Therefore, current smokers in our cohort have a fourfold increased risk of developing these types of cancer when compared to former and never-smokers (p=0.002).

CHRONIC DISEASES

We also evaluated the occurrence of other (chronic) diseases. We found that six out of 150 individuals (4%) had a medical history of sarcoidosis, which is much higher than expected (estimated prevalence in Europe approximately 15-20 per 100,000 individuals).[14] There was no kinship between these individuals.

CAUSES OF DEATH

Eighteen of the 150 individuals died during follow-up (median age of death 62 years (range, 49-78 years)). Seventeen individuals died from cancer; seven from pancreatic cancer (median age 59 years) and four from melanoma (median age 61 years).

TABLE 3. Tumours diagnosed before inclusion in the surveillance program

Site/organ system	Observed cancer	Individual(s)
Digestive	1	1
Female Breast	4	4
Female Genital	1	1
Lip, mouth, pharynx	3	2
Melanoma	194	91
Nonmelanoma skin	2	2
Respiratory	4	4
Urinary	1	1
All cancers	210	98

DISCUSSION

This prospective study analysed the risk of cancers in a cohort of homogeneous *CDKN2A* variant carriers (p16-*Leiden*). A significantly increased risk of both melanoma and pancreatic cancer was found. However, when excluding these cancers from the risk calculation, a marked increased risk for developing any cancer (RR 4.31; 95% CI 2.91-6.37) remained. Most notable were the increased risk of respiratory and lip, mouth and pharynx cancer,

and the relatively frequent occurrence of carcinoid tumours. Being a current smoker at the start of surveillance was significantly associated with the development of tumours of the pancreas, respiratory tract and head and neck region. In addition, we found an association between the p16-*Leiden* variant and sarcoidosis.

Without considering melanoma and pancreatic cancer, tumours of the respiratory tract (including laryngeal tumours) and of the lip, mouth and pharynx were the most frequently occurring tumours in our cohort. A previous retrospective study by de Snoo *et al* also found significantly increased risks for these tumours in a cohort of p16-*Leiden* variant carriers.[9] Oldenburg *et al* described a p16-*Leiden* variant positive family in which many relatives had developed lung cancer and head and neck tumours.[15] Several other case reports have also described the occurrence of head and neck tumours in *CDKN2A* variant positive families.[16,17] In sum, it seems that tumours of the head and neck and respiratory tract are part of the spectrum of cancers occurring in *CDKN2A* variant-positive FAMMM families.

Two interesting observations were the relatively frequent occurrence of carcinoid tumours and sarcoidosis in unrelated variant carriers in our cohort. Both have not been previously reported in carriers of a *CDKN2A* variant. Although only two individuals developed a carcinoid tumour during follow up, another individual had a medical history of carcinoid. It has been shown that p16 inactivation plays a role in the pathogenesis of sporadic neuro-endocrine tumours, as a substantial amount of these tumours show loss of p16 expression,[18] and also promoter methylation of the p16 gene is frequently found [19]. Further studies are needed to confirm the possible association between a *CDKN2A* germline variant and carcinoid tumours or sarcoidosis.

Our current study has several strengths. Due to its prospective design, patient participation was not influenced by the occurrence of tumours. In addition, due to the yearly follow-up at the outpatient Department of Gastroenterology, it is unlikely that cancers and other important medical information were missed. Another strength is the homogeneity of the cohort; all individuals have the same *CDKN2A* germline variant. An important limitation was, however, the relatively high age of inclusion of individuals (median age 51 years), which was due to the threshold of 45 years of age for inclusion in the pancreatic surveillance program. Tumours generally occurring before this age were therefore not included in the calculations, which is reflected by the observation of a high incidence of melanoma prior to start of the surveillance program. Because the number of participants and observed cancers was relatively small, risk factor analysis for each cancer separately could not be carried out.

Pancreatic cancer is the leading cause of death in our cohort. Pancreatic cancer surveillance may improve survival, as most tumours are detected in a resectable stage.[5] In view of the increased risk of head and neck tumours (including tumours of the larynx), patients should be advised to contact their doctor if they have complaints of hoarseness, dysphagia or ulcers in mouth or throat. A low threshold for reference to an otolaryngologist should be advocated. A surveillance program for tumours of the head and neck region should possibly be considered in the future, which could simply consist of yearly inspection of the mouth and throat. The clear relation of many of the frequently occurring cancers in our cohort to smoking indicates that active intervention to quit smoking is of the utmost importance in this group.

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