

Increased Risk of Cancer Other Than Melanoma in CDKN2A Founder Mutation (p16-Leiden)-Positive Melanoma Families

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Abstract **Purpose:** We report the largest study to date analyzing the risk of cancers other than melanoma in melanoma families positive for the same *CDKN2A* mutation.
Experimental Design: We studied family members of 22 families positive for the p16-Leiden founder mutation who had attended a surveillance clinic or were their close relatives. Within this cohort, observed and expected rates of cancer were computed by mutation status consisting of 221 (proven plus obligate) carriers, 639 (proven plus obligate) noncarriers, and 668 first-degree relatives whose carrier risk was estimated from the relationship to known carriers and the age and melanoma status of that person and their relatives.
Results: Our analysis shows a relative risk (RR) of cancer other than melanoma and non-melanoma skin cancer of 4.4 [95% confidence interval (95% CI), 3.3-5.6], predominantly attributable to the increased risk for pancreatic cancer (RR, 46.6; 95% CI, 24.7-76.4), but also for other cancers. We provide substantial proof for pancreatic cancer being a key component of the p16-Leiden phenotype. Inclusion of this cancer in a penetrance analysis leads to an estimated RR of pancreatic cancer for mutation carriers of 47.8 (95% CI, 28.4-74.7).
Conclusions: This study shows clear evidence of increased risk of cancers other than melanoma in *CDKN2A* families carrying the p16-Leiden mutation. Further research is necessary to determine if similar risks apply to families with *CDKN2A* mutations other than p16-Leiden.

Hereditary predisposition to melanoma is known to be caused by at least three genes (two of which have been cloned): *CDKN2A*, which encodes p16INK4A (commonly referred to as p16) and p14ARF (alternative reading frame), and *CDK4* (1) and a melanoma susceptibility gene shown to be located on chromosome 1p22 (2). The majority of mutations involve *CDKN2A*, found in up to 40% of 3 case families (3). The risk of melanoma has been addressed in such families (4), but the risk of cancer other than melanoma has not been studied in detail.

Before the identification of *CDKN2A* an increased risk of cancer other than melanoma was identified in several mela-

noma families (5, 6) and the potential for selection bias debated (7, 8). When restricting the analysis to melanoma and/or dysplastic nevus patients only, the relative risk (RR) for digestive cancers in one study was 5.6, although overall no significantly increased risk of cancer was found. (9).

After the identification of *CDKN2A* as the melanoma susceptibility gene, it became apparent that an increased risk of other cancers existed in mutation carriers. Goldstein et al. found an increased risk of digestive cancer in 10 mutation-positive families with a standardized incidence ratio of 13.1 for pancreatic cancer (10). Ghiorzo et al. showed a RR of 14.8 for pancreatic cancer in 9 families positive for the Ligurian *CDKN2A* founder mutation, Gly¹⁰¹Trp, opposed to no cases in 12 mutation-negative melanoma families (11). Eighty females from 9 Swedish founder mutation (113insArg)-positive families were analyzed and a standardized mortality ratio of 39 for pancreatic cancer, as well as an increased standardized mortality ratio of 3.8 for breast cancer, was noted (12).

None of these studies were able to analyze the risks for the mutation carriers only but rather for the mutation-positive families as a whole by including nongenotyped family members. Recently, the first analysis on genotyped family members only has been done. A prospective study of 15 mutation-positive families consisting of 117 mutation-positive and 136 mutation-negative family members showed a RR of 2.3 for cancers other than melanoma mainly caused by the risk of pancreatic cancer (RR, 52; ref. 13).

Although these studies have been limited in size and have included several different *CDKN2A* mutations, the overall

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Translational Relevance

Genetic counseling in familial melanoma is exceptionally troublesome mainly due to the unclear associated risks of pancreatic cancer and probably other tumors with CDKN2A mutations. Insight into these associated risks will help focus clinical monitoring and also facilitate the investigation of preventative measures, of particular relevance to both pancreatic cancer and melanoma. We feel this study is a clear example of how basic research can help clinicians with better care for patients. Our research pinpoints to what field clinical research should be aimed at in familial melanoma and hopefully initiates similar initiatives in the melanoma clinics.

conclusion is that there is an excess risk of cancers other than melanoma in families with CDKN2A mutations. This is justified by the consistency of the findings to date, but there still remains a lack of precision to these estimates and hence larger more detailed studies are required. There also remains the issue of allelic effects so that differing mutations may have different effects on risk.

A frequent mutation in CDKN2A in Dutch melanoma families *r.225-243del* is usually referred to as p16-Leiden (14). The families originate from two towns, located in the vicinity of Leiden and known for being isolated both geographically and genetically and for their tight family connections. The finding of a shared haplotype across p16-Leiden families, together with the isolated character of the population, strongly suggests a common founder to these families, which was genealogically confirmed with the most recent common ancestor dating back to 1707 (15). Most melanoma families originating from the founder population have been identified in the early 1980s and followed up clinically ever since. This has led to a unique collection of extended pedigrees, all with the same CDKN2A mutation. The careful monitoring of family members, the geographical proximity of the families, and, for many, their attendance at a single medical facility (Leiden University Medical Center) have produced a highly documented set of families, making the examination of the risk of cancer among these persons possible.

A previous retrospective analysis in 19 of the p16-Leiden families composed of melanoma patients, tested carriers, and their first-degree relatives revealed an increased cumulative risk of 17% for putative mutation carriers for developing pancreatic cancer (16). With many more family members genotyped and a recent family history questionnaire, we have analyzed the risk of cancers other than malignant melanoma within these families to obtain an improved estimate of the magnitude of this risk.

Materials and Methods

Study inclusion. The majority of families were ascertained through the Pigmented Lesions Clinic of the Department of Dermatology of the Leiden University Medical Center from 1980. Family members were invited to attend the clinic annually independently of their mutation status, which was unknown to both the individual and the medical

personnel. At these clinics, skin examinations were done and a family tree constructed for each kindred; this pedigree information was updated on a regular basis including the vital status and medical history of relatives. Before and following the identification of the p16-Leiden mutation in 1995, many family members voluntarily gave a blood sample for research purposes after signing an informed consent. The participants were informed that carrier information was not to be transmitted back to them.

For this analysis of cancer risk, families in which two first-degree (21 families) or two second-degree (1 family) relatives have been diagnosed with invasive melanoma and have been found positive for the p16-Leiden mutation were considered eligible. Much effort has been put in including as many of the family members as possible, usually leading to the inclusion of all descendants from the founding parents in each kindred.

Cancer verification. Cancer information for all family members was collected during the annual visits to the Pigmented Lesions Clinic, which started in 1980. Also, on several occasions, most recently in 2001, all consented family members (510) were invited to the clinic for research purposes (blood withdrawal and questionnaires on medical history). This research was approved by the local ethical committee. The attendance rate for the 2001 invitation was 96%. We consider that the information is complete on relatives from 1970 onwards. Confirmation of cancers was gathered through pathology reports and medical records. This confirmation was facilitated by the attendance of many family members at the same local medical facility (Leiden University Medical Center).

Family member selection and carrier risk estimates. The analysis is based on family members within these mutation-positive pedigrees. Carrier status has been determined in those family members who attended the clinic and voluntarily (with informed consent) gave a blood sample but whose carrier status is unknown to both the family members and the clinicians and research nurses. To avoid selection bias, we have included all first-degree relatives of genotyped family members in our analysis, weighted by their risk of being a mutation carrier. To calculate the risk of being a mutation carrier, we assumed that the p16-Leiden mutation had the melanoma penetrance calculated by Bishop et al. (4) for mutation carriers worldwide, which is consistent with these data (data not shown). The risk of being a mutation carrier is estimated based on carrier status information of the participants' family members as well as information on medical history of melanoma of the participant and the participants' family members. The carrier frequency for p16-Leiden in the general population was taken to be 1 in 500 (although results only varied marginally with the precise value). The risks were calculated using suitably adapted MENDEL software (17).

The study start date for those attending clinic was taken to be the date of first attendance so that cancer diagnosed before that date were excluded; this assumption attempts to remove the potential bias of affected persons being more likely to attend clinic. The study start date for all other family members was taken to be 1970 or their date of birth if later than 1970; thus, all cancers diagnosed before 1970 were ignored as this information was considered likely to be incomplete as well as the inherent difficulty of confirming family reports of diagnosis before that date. Each person's end date for the study was taken to be their date of death or their last date of clinic attendance.

We assumed in the analysis that attendance at the clinic did not depend on mutation status (unknown to family members and medical staff). The cohort of persons included in the analysis consists of

1. Persons attending clinic for screening at least once (in the period from 1986 to 2004), who gave a blood sample.
2. First-degree relatives of persons in the above category.

Cancer risk estimates. For computing the expected number of cancers (except melanoma), we used the cancer incidences for the southwest Netherlands from 1988 to 1992. Cancer risk analysis was divided into two periods (from 1970 to start of clinic and from start of

clinic to current time) to reflect differing ascertainment. The RRs were also reviewed by gender. RRs by cancer site were computed by dividing the observed number of cancers in each group (weighted by the probability of being a gene carrier) by the expected number computed from the population incidence rates. 95% Confidence intervals (95% CI) were estimated for these RRs using the approach described elsewhere (18) and taking the confidence interval to be union of the confidence intervals for the integers immediately below and above the observed numbers of cancers. This approach assumes that probability of carrier status is known precisely for each person in the pedigree and not only the genetically tested persons.

The statistical methods combine the information on mutation status and medical history, because the determination of mutation status required the analysis of a blood sample taken subsequent to first attending clinic there is the potential for differential mortality between mutation carriers and noncarriers to translate into biased estimates of RR. We therefore report results for persons proven to be mutation carriers but also weighted across the probability that each person is a mutation carrier, which should not be biased by differential mortality.

Segregation analysis. The risk model for estimating a family member's probability of being a mutation carrier is based on the melanoma penetrance model described by Bishop et al. (4). As pancreatic cancer has also been suggested to be a component of the phenotype associated with being a mutation carrier, a joint phenotype of melanoma status and pancreatic cancer status was investigated to estimate the risk of being diagnosed with either cancer. In this model, carriers have a risk of being diagnosed with melanoma, which is an age-dependent hazard function of the population incidence of melanoma. Also, risk of pancreatic cancer among carriers was taken to be related similarly to the population risks (except with different age-specific hazards to those for melanoma). Noncarriers of the p16-Leiden mutation are assumed to have the population age-specific incidence rates for both melanoma and pancreatic cancer.

Results

A total of 22 families were ascertained for this study. In these families, there were 209 tested carriers and 597 tested noncarriers. Three of the carriers are homozygote for the p16-Leiden mutation; of which two are described by Gruis et al. (14), the third has not been reported yet. In total, the families consisted of 2,604 persons of whom 1,528 (58.7%) contributed to the life-table analysis by being alive after 1970 and either attending or having a first-degree relative attending the Pigmented Lesions Clinic. In total, 639 persons were either tested noncarriers or noncarriers based on the reported family structure, whereas 221 were either tested carriers or obligate carriers based on the reported family history. Of the remaining 668 persons, the average carrier risk was estimated to be 0.21 with a range from 0.0001 to 0.9999. Three hundred ninety (58%) had a carrier risk <0.1, 72 (11%) had a risk between 0.1 and 0.4, and the remainder (31%) had a risk of at least 0.4 (see Fig. 1 for a pedigree example).

Overall, 279 cancers were reported in this cohort of participants from the beginning of 1970, of which 122 (44%) were melanoma; these diagnoses of melanoma are not considered further in this analysis. Of the remaining 157 reports, 14 (9%) could not be confirmed through hospital records or pathology records and so were excluded. Twenty-one of the diagnoses were made before first attending clinic and so are excluded. The analysis is based on the 122 confirmed diagnoses that were distributed across 28 anatomical sites, with the most common diagnoses being nonmelanoma skin cancer

($n = 25$; 21%), pancreatic cancer ($n = 22$; 15%), lung cancer ($n = 16$; 11%), and breast cancer ($n = 12$; 9%). No other diagnoses were made more than 10 times. Only 3 (2%) were of unknown primary site.

Seventeen persons had more than one diagnosis in the follow-up period of whom 5 had <50% chance of being a carrier. For 8 of these 17 persons, one diagnosis was of non-melanoma skin cancer.

For each person's follow-up period, the risk of cancer at each anatomical site was calculated and then the observed and expected numbers of cancers were compared. These values were weighted by the carrier risk. There were no notable differences by gender so results were pooled across males and females. These comparisons were made for each of the two periods (from 1970 to 1985 and from 1986 to current time). However, there were no substantive differences between risks calculated for the two periods separately (data not shown) so the two periods were combined (Table 1). Overall, 26,246 person-years were included in the follow-up period from 1970 (or first attending clinic or birth, if later) apportioned as an estimated 4,555.7 person-years among the carriers.

Table 1 shows the observed numbers of cancers among proven carriers, among proven and implied carriers, and among proven and implied noncarriers. Persons untested for p16-Leiden are divided between implied carriers and implied noncarriers in proportion to their probability of carrying p16-Leiden. Also included in the table are expected numbers of cancer by *International Classification of Diseases* code and the calculated RR and 95% CI.

For all cancers, except skin cancer in the cohort, there were 97 diagnoses and 69.1 cancers expected (a marginal increase, $P < 0.01$); of these 97 diagnoses, 35 were in mutation carriers, 13 in mutation-negative persons, and the remainder in untested persons (Table 1). However, the risk was most notably increased in proven and implied mutation carriers for which there were 75.3 cancers observed and only 15.0 expected (RR, 5.0; 95% CI, 3.9-6.3). Because the increased risk of pancreatic cancer is of particular interest, we regarded this cancer by itself and did not include it in the digestive cancer group. A significantly increased risk was found for pancreatic cancer (observed, 15.9; RR, 46.6; 95% CI, (24.7-76.4), Table 1 assuming melanoma only penetrance) consistent with previous literature. Numerically, this increase explained the greatest proportion of the excess risk, but equivalent differences were found for cancer of the respiratory system (observed, 13.9; RR, 5.7; 95% CI, 2.8-9.6). Other significant increases in risk were found for cancers of the lip, oral cavity and pharynx, nonmelanoma skin tumors, and tumors of the eye/brain (one uveal melanoma diagnosis, others are brain tumors).

Among nonmutation carriers for all cancers, except skin cancer, there were marginally less observed (46.7) than expected (56.9; RR, 0.8; 95% CI, 0.6-1.1). The only cancer site with an increase in risk was pancreatic cancer (observed, 6.1; RR, 4.9; 95% CI, 1.8-11.6). Otherwise cancer rates were consistent with the general population.

For nonmelanoma skin cancer, there were excess risks for both mutation carriers and noncarriers with an estimated 22.3-fold increased risk for the former and a 5.7-fold increased risk among nonmutation carriers. These increases are consistent with an intense dermatologic service but also show a significant increase in mutation carriers over noncarriers ($P = 0.001$).

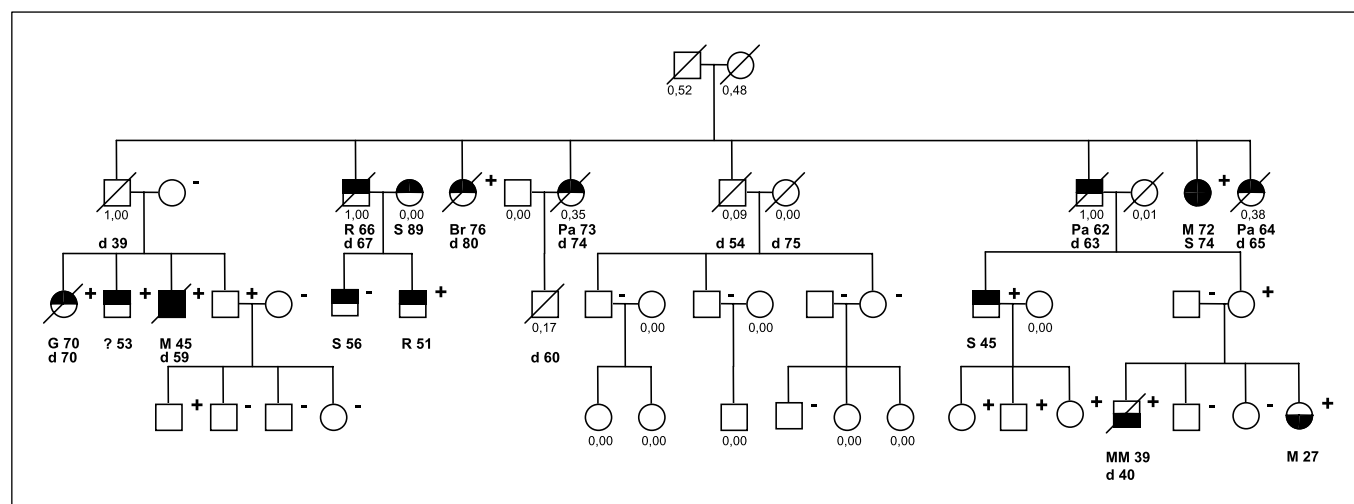


Fig. 1. Pedigree of one of the p16-Leiden families. The number with two decimals depicted in the first line underneath many individuals is the estimated risk of being a carrier. The second and third lines indicate type and age of diagnosed tumors. *G*, genital cancer; *?*, cancer of unknown site; *M*, melanoma; *MM*, multiple melanoma; *S*, nonmelanoma skin cancer; *R*, respiratory cancer; *Br*, brain tumor; *Pa*, pancreatic cancer. *d*, age of death. Symbols in which the lower half is shaded, individuals with melanoma; symbols in which the upper half is shaded, individuals with other cancer. +, tested carriers; -, tested noncarriers. The pedigree was slightly modified to preserve confidentiality.

Examination of the risks across the cohort showed that non-carriers who had never attended clinic had a nonsignificantly increased risk of being diagnosed with skin cancer (RR, 2.6; 95% CI, 0.6-6.2), whereas noncarriers attending the clinic had a significantly increased risk (RR, 38.0; 95% CI, 16.4-81.4) and mutation carriers attending clinic had apparently even greater risk (RR, 60.2; 95% CI, 19.6-157.3).

The carrier probability for the p16-Leiden mutation forms the basis for dividing members of the cohort between mutation carriers and noncarriers. The penetrance for melanoma was taken from the analysis of Bishop et al. (4), which combines mutation carriers across Europe. We reestimated this penetrance for these families (Fig. 2) and obtained numerical results marginally lower than in the previous analysis although not significantly so (data not shown). However, given the strength of the observation that pancreatic cancer is also part of the phenotype of this germ-line mutation, we reestimated the penetrance assuming that mutation carriers have both an increased risk of melanoma and also of pancreatic cancer. We estimated the penetrance based on time to first melanoma or pancreatic cancer using methodology as described by Bishop et al. (4), estimating separately the increased risk of melanoma and also for pancreatic cancer under a competing hazards model. This estimated penetrance was then used to reassess the carrier probability for each person in the cohort. The only notable change in this analysis was the removal of the increased risk of pancreatic cancer in nonmutation carriers, essentially as persons who were untyped for the mutation but developed pancreatic cancer were far more likely to be mutation carriers in this analysis. Under this revised analysis, represented as the melanoma and pancreatic cancer penetrance in Table 1, an estimated 1.1 pancreatic cancers would be expected to be diagnosed in noncarriers (compared with 0 observed) and an estimated 0.5 among mutation carriers; the RR for mutation carriers is therefore estimated to be 47.8 (95% CI, 28.4-74.7), comparable with the estimate from the proven mutation carriers (Table 1).

Finally, as these data provide convincing evidence of pancreatic cancer being a major component of the phenotype, we have reestimated the penetrance under two assumptions. We have assumed that (a) the occurrence of pancreatic cancer in a close relative did not influence the likelihood that family members attended the screening clinic and that (b) pancreatic cancer did have such an influence. For the former, we did the likelihood analysis assuming that only melanoma contributed to family ascertainment ["Pancreas (M)"] in Fig. 2], whereas for the latter we assumed that the combination of phenotypes was involved in family ascertainment ["Pancreas (M + P)"] in Fig. 2]. The model assumes that, for each cancer, the age-specific rate for each site is taken to be a multiple of the corresponding rate in the general population. These multiples are themselves age-dependent by being considered in three age ranges (0-45, 46-65, and ≥ 66 years). Figure 2 shows the estimated penetrance for melanoma and pancreatic cancer under the best-fitting model.

Discussion

We report the largest study to date analyzing the risk of cancers other than melanoma in melanoma families positive for one and the same CDKN2A mutation. We studied 22 families positive for the p16-Leiden founder mutation and were able to include 1,528 persons in our analysis. These consisted of 221 (proven plus obligate) carriers, 639 (proven plus obligate) noncarriers, and 668 first-degree relatives, weighted by their probability of being a carrier. Our analysis estimated a RR of 4.4 for cancers other than melanoma and nonmelanoma skin cancer, predominantly caused by the increased risk of pancreatic cancer, cancer of the lip, mouth and pharynx, respiratory tumors, nonmelanoma skin tumors, soft-tissue tumors, and tumors of the eye/brain.

The association between CDKN2A mutation status and risk of cancer other than melanoma is comparable with (and numerically marginally higher than) the risk of cancer other

than breast cancer among women carrying a BRCA1 (19) or a BRCA2 (20) mutation. These sets of studies indicate that although there is a predominant anatomic site (or number of sites) at notably increased risk among mutation carriers of such germ-line predispositions, there also appears to be an increased risk of cancer more systemically. Such an effect would be consistent with the basic biological processes in-

fluenced by the predisposing gene (e.g., cell cycle regulation for CDKN2A), which would be expected to have an effect in many cell types.

There are several strengths to our study, most notably that it was based around a long-term clinic in a population whose health care is provided by a single institution. We were able to collect a reliable family history by the frequent visits of these

Table 1. RRs of nonmelanoma cancer for proven and implied carriers and noncarriers

Site	International Classification of Diseases code	Proven carriers			Proven and implied carriers		
		Observed	Expected	RR (95% CI)	Observed	Expected	RR (95% CI)
Bone	170	0	0.02	0 (0-184)	0	0.04	0 (0-92.2)
Female breast	174	3	2.01	1.5 (0.3-4.4)	6.3	2.95	2.1 (0.7-4.9)
Digestive	150-156, 158-159	6	1.62	3.7 (1.4-8.1)	8.1	2.97	2.7 (1.2-5.8)
Eye/brain*	190-191	2	0.18	11.4 (1.3-40.1)	3.4	0.3	11.4 (2.1-34.1)
Female genital	180-184	4	0.77	5.2 (1.4-13.3)	4	1.15	3.5 (0.9-10.1)
Hematologic	201, 203-206	0	0.62	0 (0-5.9)	0	1.1	0 (0-3.4)
Lip, mouth, pharynx	140-149	3	0.19	15.5 (3.3-46.1)	3.7	0.35	10.8 (1.8-29.3)
Male genital	185-187	1	0.39	2.6 (0.1-14.3)	1.3	0.97	1.4 (0-7.4)
Pancreas (melanoma penetrance only)	157	9	0.19	47.9 (21.7-90.0)	15.9	0.34	46.6 (24.7-76.4)
Pancreas (melanoma and pancreatic penetrance)	157	9	0.19	47.9 (21.7-90.0)	21.9	0.46	47.8 (28.4-74.7)
Respiratory	160-163	6	1.23	4.9 (1.8-10.6)	13.9	2.44	5.7 (2.8-9.6)
Nonmelanoma skin	173	10	0.27	37 (17.8-68.1)	12.1	0.54	22.3 (11.5-41.2)
Soft tissue	171	0	0.02	0 (0-184)	1	0.03	31 (0.8-240.8)
Thyroid	193-194	0	0.07	0 (0-52.7)	0	0.11	0 (0-33.5)
Unknown	199	1	0.41	2.5 (0.1-13.6)	2	0.74	2.7 (0.3-11.8)
Urinary	188-189	0	0.46	0 (0-8.0)	1.4	0.89	1.6 (0-8.1)
All cancers		45	8.59	5.2 (3.8-7.0)	75.3	15.04	5 (3.9-6.3)
All except nonmelanoma skin cancer		35	8.32	4.2 (2.9-5.9)	63.2	14.5	4.4 (3.3-5.6)
Site	International Classification of Diseases code	Proven noncarriers			Proven and implied noncarriers		
		Observed	Expected	RR (95% CI)	Observed	Expected	RR (95% CI)
Bone	170	0	0.11	0.0 (0.0-27.0)	0	0.18	0 (0-20.5)
Female breast	174	3	6.85	0.4 (0.1-1.5)	4.7	8.78	0.5 (0.1-1.3)
Digestive	150-156, 158-159	4	8.11	0.5 (0.1-1.4)	6.9	10.97	0.6 (0.2-1.3)
Eye/brain†	190-191	1	0.82	1.2 (0-8.8)	0.6	1.25	0.5 (0-5.8)
Female genital	180-184	0	2.62	0.0 (0-2.1)	0	3.4	0 (0-1.1)
Hematologic	201, 203-206	0	3.04	0.0 (0-1.1)	0	4.36	0 (0-0.8)
Lip, mouth, pharynx	140-149	0	0.99	0.0 (0-3.4)	1.3	1.35	1 (0-5.4)
Male genital	185-187	1	3.67	0.3 (0-2.1)	2.7	5.12	0.5 (0-1.7)
Pancreas (melanoma penetrance only)	157	0	0.92	0.0 (0-3.2)	6.1	1.24	4.9 (1.8-11.6)
Pancreas (melanoma and pancreatic penetrance)	157	0	0.92	0.0 (0-3.2)	0.1	1.12	0.1 (0-4.6)
Respiratory	160-163	1	7.84	0.1 (0-0.9)	7.1	10.67	0.7 (0.3-1.5)
Nonmelanoma skin	173	11	1.64	6.7 (3.3-12.8)	12.9	2.25	5.7 (2.8-9.9)
Soft tissue	171	0	0.80	0.0 (0-3.8)	0	0.12	0 (0-30.7)
Thyroid	193-194	2	0.31	6.5 (0.8-28.3)	2	0.45	4.5 (0.5-19.5)
Unknown	199	0	2.06	0.0 (0-1.5)	0	2.81	0 (0-1.3)
Urinary	188-189	1	2.66	0.4 (0-2.7)	1.6	3.64	0.4 (0-2)
All cancers		24	42.0	0.6 (0.4-0.9)	46.7	56.86	0.8 (0.6-1.1)
All except nonmelanoma skin cancer		13	40.36	0.3 (0.2-0.6)	33.8	54.61	0.6 (0.4-0.9)
All except nonmelanoma skin cancer and pancreatic cancer		13	39.38	0.3 (0.2-0.6)	27.7	53.37	0.5 (0.3-0.8)

NOTE: Proven carriers are persons shown to carry the p16-Leiden mutation; proven noncarriers have been shown not to carry the mutation; all other individuals are assessed for their carrier status by segregation analysis of the p16-Leiden mutation.

*Brain tumors and one uveal melanoma diagnosis.

†Brain tumor.

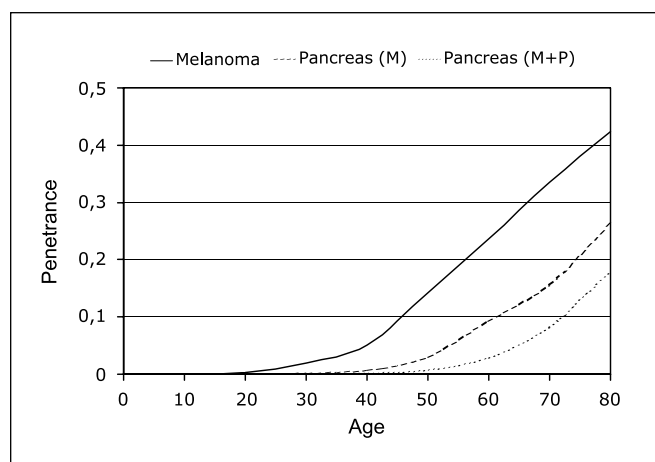


Fig. 2. Estimated penetrance for melanoma and pancreatic cancer under the best-fitting model. *Pancreas (M)*, only melanoma contributed to family ascertainment; *Pancreas (M + P)*, the combination of phenotypes (melanoma and pancreatic cancer) was involved in family ascertainment.

family members to the Pigmented Lesions Clinic in Leiden. Mutation carrier status was not known until recently and has not been transmitted to the families. In addition to the intense skin-screening follow-up, several research studies have been conducted throughout the follow-up time. The most recent study in 2001 was extensive and more than 500 family members attended. Because most family members go to this same hospital, only 14 of 157 (9%) reports of cancers other than melanoma could not be confirmed (and were disregarded in our analysis).

Several measures were taken to overcome the potential pitfalls of biases in such studies. The bias of family selection when regarding retrospective data was overcome by adding up the retrospective and prospective periods only after confirmation of no significant differences between these two (data not shown). We also calculated that the increased risk among clinic attendees was comparable with that among their first-degree relatives (data not shown). Testing bias was overcome by including first-degree relatives of genotyped family members and by weighting the contribution of untested persons by their estimated mutation carrier status. There is, however, still the potential for more subtle forms of bias. Because attendance at the surveillance clinic was voluntary, we do not know the factors that promoted attendance. For instance, if the risk of all cancers among mutation carriers varied systematically across different parts of the family (because of other genetic factors or nongenetic exposures), then the branches with higher cancer rates might be more likely to attend such clinics leading to an inflated risk that would actually reflect the risk in those branches attending the clinic.

We showed also an increased risk for skin cancers other than melanoma (basal and squamous cell carcinomas) in both mutation carriers and noncarriers, although mutation carriers developed significantly more tumors than noncarriers. This risk increase in nonmutation carriers can probably be explained by the intense skin screening: the risk was nonsignificantly increased in the noncarriers who had never attended clinic, whereas the risk was significantly increased in the clinic-attending noncarriers.

The allelic effects of particular CDKN2A mutations remain unclear. For the p16-Leiden mutation and also for the G101W mutation prevalent in Italy, the greatly increased risk of pancreatic cancer is clear. The mutation commonly found in Sweden (113insArg) has also been associated with an increased risk of breast cancer (12), but this is not confirmed by either our study or the prospective study of Goldstein et al. (13). However, neither this study nor the Goldstein et al. analysis could rule out such an increase in risk.

In our initial analysis, we found an estimated increased risk for pancreatic cancer in noncarriers of 4.9. Our model used for the risk estimation of carrier probability for first-degree relatives of genotyped family members most likely causes this increase. This model is based on the penetrance for melanoma only. When we reestimated the penetrance assuming that mutations carriers have both an increased risk of melanoma and also of pancreatic cancer, the increased risk for pancreatic cancer in noncarriers disappeared. Under this revised analysis, the estimated RR for mutation carriers for pancreatic cancer was as high as 47.8 (95% CI, 28.4-74.7). The age-specific penetrance of pancreatic cancer cannot be estimated precisely in such analyses because of the limited knowledge of ascertainment and the limited number of cases. Figure 2 gives the best estimates under two different scenarios with the expectation that the precise estimate from this study would lie between these two estimates.

The evidence for an associated increased risk of tumors other than melanoma and specifically pancreatic carcinoma in CDKN2A-positive families has increased analogous to the availability of mutation detection and follow-up time (10-13, 21). Although an associated increased risk of other cancers is undisputed, precise risk estimates are not available for most CDKN2A mutations. Collaborative efforts through GenoMEL⁶ have recently shown that indeed there is a strong association between pancreatic cancer and CDKN2A mutations, but this relationship differs by mutation. The geographical variation in the type and frequency of CDKN2A mutations and the variation in cancer rates complicate the estimation of the effects of CDKN2A (22).

Our study provides yet more compelling proof of a strong increased risk for a variety of other tumors in CDKN2A mutation-positive family members, particularly pancreatic carcinoma, stressing the need for adequate preventive (research) measures. We have addressed this need for the p16-Leiden families by offering a research screening program. Family members ages ≥ 45 years who test positive for the mutation at the department of Clinical Genetics are offered to attend this program, which consists of an annual magnetic resonance imaging of the abdomen. Results on the first 5 years of this research screening program will be published in due course.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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⁶ www.genomel.org

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