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CDKN2A Mutations and Melanoma Risk in the Icelandic Population

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Abstract

Background—Germline *CDKN2A* mutations have been observed in 20-40% of high-risk melanoma-prone families, however little is known about their prevalence in population-based series of melanoma cases and controls.

Methods—We resequenced the *CDKN2A* gene, including the p14ARF variant and promoter regions, in approximately 703 registry-ascertained melanoma cases and 691 population-based controls from Iceland, a country in which the incidence of melanoma has increased rapidly.

Results—We identified a novel germline variant, G89D that was strongly associated with increased melanoma risk and appeared to be an Icelandic founder mutation. The G89D variant was present in about 2% of Icelandic invasive cutaneous malignant melanoma cases. Relatives of affected G89D carriers were at significantly increased risk of melanoma, head & neck cancers, and pancreatic carcinoma compared to relatives of other melanoma patients. Nineteen other germline variants were identified, but none conferred an unequivocal risk of melanoma.

Conclusions—This population-based study of Icelandic melanoma cases and controls showed a frequency of disease-related *CDKN2A* mutant alleles ranging from 0.7% to 1.0%, thus expanding our knowledge about the frequency of *CDKN2A* mutations in different populations. In contrast to North America and Australia where a broad spectrum of mutations was observed at a similar frequency, in Iceland, functional *CDKN2A* mutations consists of only one or two different variants. Additional genetic and/or environmental factors are likely critical for explaining the high incidence rates for melanoma in Iceland. This study adds to the geographic regions for which population-based estimates of *CDKN2A* mutation frequencies are available.

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CDKN2A Isoform4 (p14ARF): NM_058195

Conflict of Interest None declared.

Keywords

melanoma; *CDKN2A*; G89D; pancreatic cancer; population-based

Introduction

The *CDKN2A* (MIM# 600160) gene is the major gene conferring high-risk of cutaneous malignant melanoma. *CDKN2A* encodes several cell cycle inhibitors translated from alternatively spliced transcripts. The Isoform1 (α) transcript comprising exons 1α, 2 and 3 encodes the cyclin dependent kinase inhibitor p16 (INK4a).[1] Isoform4 (β), comprises an alternative first exon, 1β, spliced to exons 2 and 3 in common with Isoform1 leading to a shifted reading frame relative to Isoform1; the alternative reading frame protein is designated p14ARF.[2] p14ARF acts via the p53 pathway to induce cell cycle arrest or apoptosis.[3, 4] Isoform3, whose expression has only been seen in the pancreas, utilizes an alternative splice donor at the end of exon 1 resulting in a 12 kiloDalton protein that exhibits Retinoblastoma-independent cell growth inhibition.[5]

The frequency of mutations in *CDKN2A* has been investigated extensively using family-based approaches but rarely using population-based approaches. Germline *CDKN2A* mutations have been observed in 20-40% of melanoma-prone families. The mutation frequency varies by geographic region with higher frequencies in regions with lower incidence.[6] Outside of high-risk families, the prevalence of mutations is much lower. The two major population-based studies assessing *CDKN2A* mutation frequencies were based primarily on melanoma patients from Australia and North America and showed prevalences of functional *CDKN2A* mutations ranging from 0.2-2%.[7, 8, 9]

Melanoma incidence has increased rapidly in Iceland in the last decades. During 2000-2004, the age-standardized incidence was 10.9/100,000 for males and 19/100,000 for females and had tripled from the 1980 incidence.[10, 11, 12] No population-based estimates of disease-related *CDKN2A* mutations (outside of families) are yet available from Northern Europe. The goal of this study was to estimate the frequency of *CDKN2A* mutations in population-based Icelandic melanoma cases and controls, assess the risk of melanoma in identified variants, and examine evidence for associations with other cancers (pancreatic cancer (PC)), breast cancer (BC), neural system tumors (NST) and head & neck cancers) previously associated with *CDKN2A* mutations.[13]

Methods

Patients and design

Study approval was granted by the Icelandic National Bioethics Committee, the Icelandic Data Protection Authority, and the National Cancer Institute Institutional Review Board. Records of melanoma diagnoses, all histologically confirmed, from 1955-2005 were obtained from the Icelandic Cancer Registry (ICR). Invasive cutaneous malignant melanoma (CMM) was identified through ICD10 code C43. Diagnoses of melanoma-in-situ (MIS) from 1980-2003, were identified by ICD10 code D03. Ocular melanoma (OM) was identified by code C69 in combination with a SNOMED morphology code indicating melanoma. Melanomas arising at mucosal sites (primarily oral, nasal, and genital) were identified by listing non-C43 codes that were associated with a SNOMED morphology code indicating melanoma, then manually inspected to remove misclassification errors; verified entries associated with a mucosal site ICD10 code were designated mucosal melanoma. Metastatic melanoma was identified by a SNOMED morphology code indicating melanoma with a /6 suffix, regardless of the ICD10 code. Breast cancers were identified by ICD10

code C50. Head & neck tumours were identified by codes C00-C09, C11, C13, C14, C30-C33. NSTs were identified by codes C70-C72 or C47. Pancreatic tumours were identified by code C25.

All melanoma patients identified through the ICR were invited to a recruitment center where they signed an informed consent form, provided a blood sample and answered a questionnaire with the aid of a study nurse. Study consent was obtained from 776 melanoma patients comprising 527 invasive CMM, 217 MIS, 17 OM, 13 mucosal melanoma and 2 metastatic melanoma cases. The overall recruitment success rate was approximately 80%.

The population-based control group comprised 827 individuals, unrelated to each other at 3 meioses, selected randomly from the Icelandic Genealogical Database with all individuals aged 18-70 years. Medical records of the controls were not investigated and melanoma patients were not excluded if they occurred amongst the controls. Patients who were selected both as melanoma cases and controls (4 individuals) were treated statistically as two separate individuals. Further details of the selection and recruitment of the control group have been described previously.[14]

Genotyping

CDKN2A exons 1α, 1β, 2, and 3 and about 1 kilobase of the promoter regions upstream of exons 1α and 1β from approximately 703 melanoma patients and 691 controls were successfully sequenced. This corresponds to sequencing yields of 90.5% and 83.6% for cases and controls, respectively. Procedures for sequencing are described in [14]. The common IVS2-105A>G mutation [15] was sequenced; no mutations were detected. All identified *CDKN2A* variants were confirmed by manual inspection of primary signal traces. Twenty-one variants were detected. The call rate for the IVS2-278C>T variant was <66% in cases and controls, so this variant was excluded from further evaluation. For the remaining variants, the call rate was 94% for cases and 98% for controls.

Statistical Analysis

Differences in *CDKN2A* variant frequencies between cases and controls were evaluated by Fisher's exact or Wilcoxon Mann Whitney (WMW) test using StatXact (version 4.0). Point estimates and 95% confidence intervals (CI) of unadjusted odds ratios (OR) were calculated and used as the measure of association between melanoma risk and specific individual *CDKN2A* variants.

High-penetrance mutations in *CDKN2A* would be expected to result in a higher risk of melanoma in relatives of affected mutation carriers. Therefore, we determined a familial relative risk ($fRR_{1°\text{-}3°}$) parameter which compares, for each *CDKN2A* variant, the risk of melanoma in first through third degree (1°-3°) relatives of melanoma-affected variant carriers with the risk of melanoma in 1°-3° relatives of the overall group of (genotyped) melanoma patients. First through third degree relatives of each melanoma patient were identified through the Icelandic Genealogical Database.[16] Recorded diagnoses of melanomas and other cancers within this circle of relatives were obtained from the ICR. The familial relative risk (*fRR*) method has been described previously [17]. Briefly, for each *CDKN2A* variant, the *fRR* was determined as:

$$fRR = \frac{a/r}{x/n}$$

where *r* is the number of relatives of melanoma-affected carriers of the *CDKN2A* variant (counting individuals multiple times who are related to more than one *CDKN2A* variant

carrier[18]) and *a* is the number of relatives of the *CDKN2A* variant carriers who are themselves affected with the disease being queried (*i.e.*, melanoma, BC, head & neck cancer, NSTs, or PC), again counting individuals multiple times who are related to more than one carrier. In the denominator, *n* is the total number of relatives of the genotyped melanoma patients and *x*, the number of those relatives who are affected with the disease being queried. *fRR* was calculated for relative circles at each degree of relatedness from 1°-3°. A combined *fRR* for 1°-3° relatives ($fRR_{1°\text{-}3°}$) was then derived using a weighting scheme as described previously.[17] Also, as in the previous study, the *fRR* for each degree of relation is a weighted average over subpopulations, where the population is stratified according to sex and in 5-year birth periods.

We assessed the significance of $fRR_{1°\text{-}3°}$ by simulation. The observed $fRR_{1°\text{-}3°}$ value was compared to a set of empirical $fRR_{1°\text{-}3°}$ values derived from 1,000 groups of matched controls, each group being the same size as the number of carriers for each *CDKN2A* variant, drawn at random from amongst the genotyped melanoma patients. The p-value is the proportion of the 1,000 control groups that gave a $fRR_{1°\text{-}3°}$ as large as or larger than the $fRR_{1°\text{-}3°}$ for the variant carriers. This *fRR* approach compares the risk to relatives of variant-carrying melanoma patients to the risk to relatives of the overall group of melanoma patients studied but not to the general population risk. Icelandic population-based *fRR* assessments for melanoma have been published previously.[17]

The predicted biochemical severity of the coding variants was evaluated using the BLOSUM62 matrix.[19] Using BLOSUM62, missense mutations were categorized as non-conservative or conservative.

Results

Twenty variants were reliably identified, 16 in *CDKN2A* and 4 in p14ARF. Table 1 presents the variants and their frequencies in all melanoma cases combined, invasive CMM, MIS, mucosal, metastatic and OM patients.

The major known variants, including the -981G>T, -735G>A, and -493A>T promoter variants, c.-191G>A 5′ UTR variant, and Nt500C>G and Nt540C>T 3′ UTR variants, were all detected. Coding *CDKN2A* variants were A57V, G89D, and A148T. A57V and G89D occur in the overlapping coding sequence of p14ARF, however, both result in synonymous p14ARF variants, designated R112R and G143G, respectively.

Because some observed variants were rare, we assessed their potential for affecting disease risk using three complementary methods. First, we looked for association with disease incidence. Second, we examined whether relatives of patients who carried mutations were at increased risk of melanoma or several other cancers. Third, we examined whether the non-synonymous coding variants made amino acid changes that were predicted to be non-conservative.

The only variant that showed a significant association with melanoma was the missense variant G89D (p=0.011) (Table 1). The G89D association strengthened when the analysis was restricted to invasive CMM patients (p=0.0015). G89D was observed in nine invasive CMM patients and one control (OR=13.8, 95% CI, 1.9-606). Two rare non-coding *CDKN2A* variants (IVS1-122G>C and IVS1+37G>C) were observed in one and two melanoma cases, respectively, but not in any controls. The previously described IVS1+37G>C variant, believed to be a neutral polymorphism [9, 20], generates a non-synonymous G63R mutation in exon 1 of the *CDKN2A* Isoform3 (p12) protein. None of the known neutral polymorphic or novel rare non-coding variants were significantly associated with melanoma (Table 1).

Further, no disease-related non-synonymous variants were detected in any ocular or mucosal melanoma patients.

We used the familial relative risk ($rRR_{1°\text{-}3°}$) approach to examine whether relatives of the patients who carried the four detected missense variants (A57V, G89D, A148T, and IVS1+37G>C (Iso3 G63R) were at increased risk of melanoma. Patients who carried G89D had a significantly increased risk of melanoma amongst their 1°-3° relatives compared to the overall melanoma group (table 2). There was no detectable increase in melanoma risk among 1°-3° relatives of melanoma patients who carried A148T or IVS1+37G>C (Iso3 G63R). For A57V, the point estimate of *fRR* was over 3-fold, however, this estimate was not significant (p=0.066).

The *fRR* method was also used to compare the risks of PC, head & neck cancers, NSTs and BC among relatives of variant-carrying melanoma patients compared to relatives of the overall group of melanoma patients. Relatives of melanoma patients who carried G89D had significantly greater risks of head & neck cancers and pancreatic carcinomas than relatives of the general group of melanoma patients (table 2). A148T exhibited a non-significant *fRR* of 1.83 for head & neck cancers (p=0.059). There was no evidence that any of the *CDKN2A* variants conferred high-penetrance cross-risk for either BC or NSTs.

In addition, because of the purported relationship between p14ARF and NSTs, we evaluated *fRR* for the four exon 1β-specific variants TS-229A>T, TS-102C>T, TS-89C>T and c. 316-121T>C. No increased familial risk for melanoma, NSTs, head & neck cancers, pancreatic carcinoma or BC was detected (data not shown).

We used the BLOSUM62 matrix to evaluate the biochemical severity of the three *CDKN2A* missense variants. According to BLOSUM62, G89D was classified as a non-conservative amino acid change. In contrast, A57V and A148T were classified as conservative amino acid replacements using the BLOSUM62 matrix. In summary, the observations are fully consistent with the notion that G89D is a highly penetrant melanoma risk allele.

The germline G89D variant has not been reported outside Iceland and may therefore be a unique Icelandic founder mutation. All carriers of G89D shared a SNP haplotype background for the *CDKN2A* region (data not shown). Reference to the Icelandic Genealogical Database identified a most recent common ancestor linking all G89D carriers (case and control) at 10 generations. The common ancestor was a female who lived from approximately 1605-1665 in Hunavatnssysla county in northern Iceland.

Table 3 shows the frequency of potentially disease-related *CDKN2A* variants in Icelandic melanoma patients and population-based controls. The allelic frequency of *CDKN2A* disease-related variants in melanoma patients is 0.7% (95% CI, 0.3-1.3%) for G89D alone and 1.0% (95% CI, 0.55-1.7%) for G89D and A57V together. The population-based allelic frequency for these variants in controls is 0.08% (95% CI, 0.002 -0.43%) for G89D alone and 0.38% (95% CI, 0.13-0.90%) for G89D and A57V combined. These estimates are consistent with those reported by the population-based Genes and Environment in Melanoma (GEM) study[8] whether G89D is considered alone (p= 0.62) or both G89D and A57V are classified as disease-related variants (p= 0.75).

Discussion

This population-based study of Icelandic melanoma cases and controls showed a frequency of disease-related *CDKN2A* mutant alleles ranging from 0.7%-1.0% (combined 95% CI: 0.3-1.7%) in melanoma cases and 0.08%-0.38% (combined 95% CI: 0.002-0.9%) in population-based controls, thus expanding our knowledge about the frequency of *CDKN2A*

mutations in different populations. Since these are allelic frequencies, the carrier frequencies will be approximately double these values. The frequency of *CDKN2A* mutations in melanoma patients observed in this study is thus consistent with previous population-based estimates from predominantly North American and Australian populations.[7, 8, 9] However, in North America and Australia, a broad spectrum of mutations is observed whereas in Iceland a similar frequency of functional mutations consists of only one or two different variants. This pattern is similar to what is seen in BRCA2 in Iceland, where the frequency of mutation carriers is as high or higher than in other countries yet mutation carriers all harbor a single founder mutation, BRCA2 999del5.[21, 22] The predominance of a single *CDKN2A* founder mutation is similar to what has been observed in Sweden and the Netherlands.[6, 23, 24, 25, 26]

This study identified G89D as the major high penetrance *CDKN2A* mutation in Iceland. G89D had the strongest, most consistent association with melanoma and showed a 9-fold increase in risk of any melanoma and a 13-fold increase for invasive CMM. Further, based on the *fRR* evaluation, G89D conferred a high-penetrance cross-risk for head & neck cancers and pancreatic carcinoma. In addition, similar to most *CDKN2A* mutations, G89D may be a founder mutation, with all carriers stemming from a common ancestor who resided in northern Iceland in the 1600's. Finally, G89D was estimated to be carried by 1.4% of Icelandic melanoma patients and over 2% of invasive CMM patients.

The evidence for A57V being a disease-related mutation is less clear. A57V was not significantly associated with melanoma, being present in 4 cases and 4 controls. Further investigation revealed that one of the controls had a family history of melanoma (with a parent and an aunt with CMM), while the other three controls did not. In addition, the patients who carried A57V showed an increased non-significant *fRR* estimate for melanoma compared to other patients. Moreover, evaluation of the biochemical severity showed that the amino acid change was conservative. Also, Alanine 57 is not conserved across 14 animal species previously examined. Further, Valine is at position 57 in the rat, mouse, and opossum species considered further suggesting that A57V does not result in a functional defect in the p16 protein. [6, 27] This variant has, however, been observed in a pancreatic tumour [28], in a melanoma-prone family[29], and two multiple primary melanoma patients from GEM.[8] Thus, A57V may moderately increase melanoma risk; however, additional studies will be required to completely determine its relationship with melanoma.

The A148T variant is a frequently reported polymorphism. In the current study, its allelic frequency was 2.5% in cases and 2.7% in controls; there was no evidence for A148T conferring increased melanoma risk. While most reports have classified it as a neutral polymorphism, Debniak et al[30] reported that the variant conferred a 2.5-fold increased CMM risk in Polish subjects. The variant also appeared to confer cross-risk of breast and other cancers in the Polish study.[30] No such cross-risk of BC, PC, or NSTs was observed in the Icelandic sample. Debniak et al[30] speculated that the risk might be due to A148T being in linkage disequilibrium with a promoter polymorphism -493A>T. This promoter variant was observed in 32 cases and 36 controls in the current study, was in strong linkage disequilibrium with A148T and showed no association with melanoma risk (OR=0.88, 95% CI: 0.54-1.44). Taken together, our association data support the view that both A148T and -493A>T are neutral polymorphisms in the Icelandic population.

The *CDKN2A* IVS1+37G>C variant generates a G63R coding mutation in the Isoform3 p12 protein.[5] It was observed in 2 melanoma cases and 0 controls. Neither case exhibited an increased family history of melanoma, pancreatic or other cancers. Although this variant would be classified as a non-conservative amino acid using the BLOSUM62 matrix, there was no evidence for an association to melanoma in this study. In addition, this variant was

originally observed in a non-carrier melanoma case from an Italian multiplex melanoma family harboring a R24P *CDKN2A* mutation; the variant was predicted to have no effect on mRNA processing using the Splice View program.[20] The variant was also classified by the GEM study as a non-functional variant and observed in 0.58% of single-primary-melanoma and 0.52% of multiple-primary-melanoma patients.[9] Thus, this variant does not appear to be disease-related.

A number of cancers including BC, NSTs, and PC have previously been associated with *CDKN2A* mutations.[13] The strongest and most consistent association has been observed between specific *CDKN2A* variants and PC.[6, 13, 31] NSTs have also been reported to be associated with large deletions and/or mutations that alter p14ARF but this relationship has not been fully resolved.[6, 13] In addition, a significantly increased risk of BC was reported in *CDKN2A* melanoma-prone families from Sweden mainly with the predominant Swedish founder mutation (R112_L113insR)[24] but this cross-risk has not been observed for other *CDKN2A* mutations. There are also reports of a relationship between head & neck cancers and germline *CDKN2A* mutations.[13, 32, 33] We used the *fRR* approach to examine these non-melanoma cancers and specific *CDKN2A* variants. The major Icelandic melanoma-related variant G89D showed significant *fRR* for both pancreatic and head & neck cancers, suggesting a high-penetrance cross-risk for these tumours. Indeed, carriers of G89D had more frequent familial connections to head & neck cancer cases than to melanoma cases (frequencies 0.0122 and 0.0100 respectively), suggesting that the absolute risks for both cancers might be similar in these families. None of the exon 1β-specific variants or missense variants showed an increased familial risk for NSTs. Similarly, no cross-risk was detected for BC suggesting that this risk may be limited to the R112_L113insR mutation.

In conclusion, this study of *CDKN2A* mutations from Iceland expands the geographic regions for which there are population-based estimates of prevalences of *CDKN2A* variants in melanoma patients and controls. This study also identified a unique Icelandic founder mutation G89D that confers increased risk of melanoma and familial risks of pancreatic carcinoma and head & neck cancers. Further, G89D is the key high penetrance *CDKN2A* variant in the Icelandic population. Thus, although specific *CDKN2A* mutations substantially increase risk for melanoma, based on the mutation frequency estimated in this study, the overall impact of *CDKN2A* mutations on Icelandic melanoma patients and the general Icelandic population is expected to be low. Additional genetic and/or environmental factors are likely critical for explaining the high incidence rates for melanoma in Iceland.

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TABLE 1

ALLELE FREQUENCIES FOR *CDKN2A* VARIANTS IDENTIFIED IN POPULATION-BASED ICELANDIC MELANOMA CASES AND CONTROLS

		Allele Frequency									
Variant	**Class**	**All Melanoma (n~703)**	**Invasive CMM (n~470)[1]**	**Melanoma In Situ (n~209)**	**Mucosal & Metastatic Melanoma (n~11)**	**Ocular Melanoma (n~14)**	**Controls (n~691)**	**WMW[2] P-value All Melanoma**	**OR for All Melanoma (95% CI)**	**WMW[2] P-value invasive CMM**	**OR for invasive CMM (95% CI)**
CDKN2A p16 Variants											
-981G>T	Promoter	0.03	0.028	0.035	0	0.042	0.034	0.59	0.88 (0.57-1.36)	0.46	0.83 (0.50-1.36)
-735G>A	Promoter	0.018	0.019	0.018	0	0	0.027	0.15	0.67 (0.40-1.12)	0.26	0.71 (0.40-1.27)
-493A>T	Promoter	0.024	0.026	0.02	0.045	0	0.027	0.71	0.91 (0.56-1.48)	1	0.98 (0.58-1.66)
c.-191G>A	5′ UTR	0.41	0.4	0.41	0.45	0.43	0.38	0.15	1.12 (0.96-1.31)	0.24	1.11 (0.94-1.32)
c.-33G>C	5′ UTR	0.005	0.005	0.005	0	0	0.006	0.8	0.86 (0.27-2.74)	1	0.93 (0.30-2.84)
c.-14C>T	5′ UTR	0.0014	0.0011	0.0024	0	0	0.0007	1	--	1	--
IVS1+27A/C	Intron 1	0.0022	0.0032	0	0	0	0.0007	0.62	2.9 (0.2-154)	0.31	4.4 (0.4-232)
IVS1+37G/C	Intron 1	0.0014	0.0011	0.0024	0	0	0	0.25	--	0.4	--
IVS1-122G/C	Intron 1	0.0008	0	0.0025	0	0	0	0.5	--	--	--
A57V	Exon 2	0.003	0.0035	0.0026	0	0	0.003	1	1.01 (0.19-5.44)	1	1.14 (0.17-6.75)
G89D	Exon 2	0.007	0.011	0	0	0	0.0008	**0.011**	**9.2 (1.3 - 404)**	**0.0015**	**13.8 (1.9-606)**
A148T	Exon 2	0.025	0.025	0.025	0.05	0	0.027	0.72	0.91 (0.56-1.46)	0.79	0.91 (0.53-1.55)
IVS2+83C/T	Intron 2	0.0015	0.0023	0	0	0	0.0015	1	1.02 (0.07-14.13)	1	1.54 (0.11-21.22)
IVS2+227A/G	Intron 2	0.014	0.012	0.012	0.05	0.045	0.008	0.19	0.6 (0.25-1.34)	0.38	1.53 (0.60-3.91)
Nt500C>G	3′ UTR	0.17	0.17	0.16	0.27	0.17	0.16	0.49	1.08 (0.88-1.32)	0.56	1.07 (0.85-1.35)
Nt540C>T	3′ UTR	0.12	0.11	0.14	0.09	0.04	0.11	0.33	1.13 (0.89-1.43)	0.78	1.04 (0.80-1.37)
p14/ARF Variants											
TS -229A>T[3]	Promoter	0.0015	0.0023	0	0	0	0.0029	0.69	0.53 (0.05-3.68)	1	0.79 (0.07-5.50)
TS -102C>T[3]	Promoter	0.018	0.022	0.011	0	0	0.022	0.48	0.80 (0.46-1.39)	1	0.98 (0.54-1.76)
TS -89C>T[3]	Promoter	0.0025	0.0024	0.0028	0	0	0.003	1	0.83 (0.12-4.90)	1	0.82 (0.07-5.75)
c.316+121T>C	Intron	0.1	0.11	0.09	0	0.12	0.1	0.8	0.96 (0.74-1.24)	0.89	1.02 (0.77-1.35)

[1] One patient had both invasive CMM and Ocular Melanoma. This case was included in the calculations for both melanomas.

[2] Wilcoxon Mann-Whitney test

[3]Locations relative to transcription start site, defined as first base in the sequence NM_058195

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TABLE 2

FAMILIAL RELATIVE RISK (*FRR*) FOR MELANOMA AND OTHER CANCERS IN 1°-3° RELATIVES OF MELANOMA-AFFECTED CARRIERS OF SELECTED *CDKN2A* VARIANTS.

Variant	Affected Relative Phenotype	$fRR_{1°\text{-}3°}$	P-value
G89D	Melanoma	2.89	0.020
	Breast Cancer	0.47	0.958
	Head and Neck	4.84	0.016
	Neural System Tumours	1.12	0.749
	Pancreas	4.17	0.006
A148T	Melanoma	1.13	0.617
	Breast Cancer	0.75	0.729
	Head and Neck	1.83	0.059
	Neural System Tumours	1.22	0.120
	Pancreas	1.15	0.413
A57V	Melanoma	3.05	0.066
	Breast Cancer	0.51	0.807
	Head and Neck	0.44	0.751
	Neural System Tumours	1.02	0.212
	Pancreas	1.34	0.452
IVS1+37G>C (Iso3 G63R)	Melanoma	0.00	0.494
	Breast Cancer	0.52	0.798
	Head and Neck	0.00	0.493
	Neural System Tumours	0.00	0.133
	Pancreas	0.00	0.394

TABLE 3

FREQUENCY OF G89D AND A57V VARIANTS IN THE ICELANDIC POPULATION

CDKN2A variant	Melanoma Patients		Population-based controls	
	Allele freq (%)	95% CI	Allele freq (%)	95% CI
A57V	0.3	0.08 – 0.79	0.3	0.08 – 0.78
G89D	0.7	0.3 – 1.3	0.08	0.002 – 0.43
A57V + G89D	1.0	0.55 – 1.7	0.38	0.13 – 0.90