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Estimating *CDKN2A* mutation carrier probability among global familial melanoma cases using GenoMELPREDICT

Short title: GenoMELPREDICT performance in melanoma families

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Abstract

Background Although rare in the general population, highly penetrant germline mutations in *CDKN2A* are responsible for 5-40% of melanoma cases reported in melanoma-prone families. We sought to determine whether MELPREDICT was generalizable to a global series of melanoma families and whether performance improvements can be achieved.

Methods 2,116 familial melanoma cases were ascertained by the international GenoMEL Consortium. We recapitulated the MELPREDICT model within our data (GenoMELPREDICT) to assess performance improvements by adding phenotypic risk factors and history of pancreatic cancer. We report areas under the curve (AUC) with 95% confidence intervals (CI) along with net reclassification indices (NRI) as performance metrics.

Results MELPREDICT performed well (AUC=0.752; 95%CI: 0.730, 0.775), and GenoMELPREDICT performance was similar (AUC=0.748; 95% CI: 0.726, 0.771). Adding a reported history of pancreatic cancer yielded discriminatory improvement ($p<0.0001$) in GenoMELPREDICT (AUC=0.772; 95%CI: 0.750, 0.793; NRI=0.40). Including phenotypic risk factors did not improve performance.

Conclusion The MELPREDICT model functioned well in a global dataset of familial melanoma cases. Adding pancreatic cancer history improved model prediction. GenoMELPREDICT is a simple tool for predicting *CDKN2A* mutational status among melanoma patients from melanoma-prone families and can aid in counselling these patients towards genetic testing or cancer risk counselling.

Capsule Summary

- Available prediction tools for *CDKN2A* status were developed among small, homogeneous populations and lack generalizability. GenoMELPREDICT is a globally generalizable and simple clinical tool for predicting *CDKN2A* mutational status among familial melanoma patients.
- GenoMELPREDICT can aid in appropriate patient management, whether that is genetic testing or cancer risk counselling.

Introduction

Inherited mutations in the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene are major risk factors for familial melanoma.[1-3] The frequency of *CDKN2A* mutations in melanoma-prone families varies widely (<5% to 40%) with the number of family members diagnosed with melanoma and the number of primary melanomas diagnosed within an individual.[1, 4-6] The penetrance of *CDKN2A* mutations in melanoma-prone families is a function of population incidence rates of melanoma and is modified by environmental factors, melanoma-associated phenotypes, and *MC1R* variants.[3, 7] In light of geographic variability in mutation penetrance, a standard guideline for recommending *CDKN2A* genetic testing has not been suitable for heterogeneous populations.[8] GenoMEL, the International Melanoma Genetics Consortium, supports a qualitative framework to identify candidate individuals for *CDKN2A* mutation testing based on population-based melanoma incidence rates, diagnosis of multiple primary melanomas, and a verified family history of melanoma and/or pancreatic cancer.[8] Rapid identification of familial melanoma patients with low probability of a germline mutation in *CDKN2A* could aid to direct patients toward risk counseling and away from inappropriate genetic testing, especially since a negative test result is unlikely to influence their risk management, and/or in fostering potential conversation about genetic testing for mutations in other known, but much rarer, high-penetrance melanoma genes.

MELPREDICT is a published logistic regression model to predict *CDKN2A* mutation carrier status.[9] MELPREDICT performed well (area under the curve (AUC)=0.881) among melanoma patients (n=116) belonging to melanoma-prone families in Boston, Massachusetts, USA, and similarly (AUC=0.803) among those from melanoma-prone families in Toronto, Ontario, Canada (n=143).[9] We sought to determine whether MELPREDICT was generalizable to a large series of melanoma families from 20 countries participating in GenoMEL. Further, we evaluated whether improvements in model performance can be achieved by adding personal or family history of pancreatic cancer and/or phenotypic risk factors for melanoma.

Methods

Study population

The GenoMEL consortium comprises 29 study centers from Australia, Europe, the Middle East, and North and South America. GenoMEL used a common protocol to obtain research data as previously described.[10] Written informed consent was obtained from each participant, and individual GenoMEL centers received study approval from their respective institutional review boards. Consenting participants completed a self-administered questionnaire that solicited information on phenotypic characteristics, and personal and family history of melanoma and other cancers.[10, 11]

Study sample

Our study sample reflects 2,116 melanoma patients with *CDKN2A* genotype. These participants were from 900 melanoma-prone families defined by the presence of three or more verified melanoma cases among blood relatives (individuals who share a common ancestor and are not related by marriage) or two verified melanoma cases in first-degree blood relatives recruited at 20 GenoMEL centers (Table 1). There were 359 reports in 122 families of a personal or family history of pancreatic cancer, and pathologic verification was available for 79 (22%) of these reports; the remainder were self-reported.

CDKN2A genotyping

Germline DNA was screened for mutations in *CDKN2A* (including exons 1α, 1β, 2 and 3), and mutations were classified as pathogenic (i.e. positive) or non-pathogenic (i.e. negative) as previously described.[10, 11] Eleven families had at least one member who was known to carry a mutation in another melanoma high-penetrance gene; these families were included in our analyses.

Statistical analysis

Using the MELPREDICT logistic regression model for which the probability of *CDKN2A* mutation carriage is defined as $\frac{e^L}{1+e^L}$ with L = 1.99 + [(0.92 × number of primary melanoma diagnoses) + (0.74 × number of additional family members diagnosed with melanoma) – (2.11 × ln(age at first melanoma diagnosis))], we estimated the predictive probability of *CDKN2A* mutation carriage among

study participants, and the AUC was derived from the set of predictive probabilities.[9, 12] Using data from GenoMEL, we modeled the probability of *CDKN2A* mutation carriage as a function of these three variables and considered this our baseline model (GenoMELPREDICT). We used a generalized estimating equation with a logit link function and independence covariance structure with robust standard errors to account for familial clustering. We evaluated changes in baseline model performance associated with the addition of reported personal or family history of pancreatic cancer (yes, no), facial freckling (none, very few, few, some many, very many), proclivity to burn (tan with no burning, mild sun burning, sun burning with peeling, severe sun burning with blistering), proclivity to tan (very tanned, moderate tanning, mild tanning, no tanning), eye color (brown or black, blue, other), hair color (black, brown, blonde or fair, red), and skin type (very fair, fair, olive or brown or black), including all pairwise and triplet combinations of these phenotypic variables.

We used the empirical method of DeLong[13] to estimate and compare (via a Wald test) paired AUCs of receiver operating characteristic (ROC) curves. For each model, AUCs and 95% confidence intervals (CI) were calculated by ten-fold cross validation to evaluate discrimination between *CDKN2A* mutation carriers and non-carriers, and we used one-stage cluster sampling to randomly assign all members of a family to the same fold. Optimal discrimination was determined by maximizing sensitivity and specificity. Improvement in model performance was assessed by measuring the difference between paired model AUCs and by event and non-event net classification indices (NRI).[13-15] Models incorporating phenotypic factors were performed on sample sizes that varied according to factor missingness; for each augmented model, we reran our baseline model on the corresponding reduced sample size. Multiple imputation by the fully conditional specification method was used to restore missing values.[16] All analyses were performed using SAS v.9.4 (SAS Institute, Cary, NC) and R (R Core Team; http://www.R-project.org/).

Results

CDKN2A genotype was available for 711 (33.6%) mutation carriers and 1,405 (66.4%) non-carriers belonging to 900 melanoma-prone families. *CDKN2A* mutations identified in GenoMEL families have been previously published.[10, 17] Results of multivariable analyses for our 3-variable baseline and 4-variable GenoMELPREDICT model that included pancreatic cancer are presented in Table 2. Age at first melanoma diagnosis, higher numbers of primary melanomas, higher numbers of family members with a melanoma diagnosis, and a personal or family history of pancreatic cancer were independently associated ($p<0.0001$) with *CDKN2A* mutation carriage.

Using the published MELPREDICT model parameter coefficients to predict *CDKN2A* mutation carriage in the GenoMEL sample set resulted in an AUC = 0.752 (95% CI: 0.730, 0.775); the mean estimated probability of *CDKN2A* mutation carriage was 42.7% for mutation carriers, and 13.0% for non-carriers. *De novo* modeling, *i.e.* GenoMELPREDICT, of age at first melanoma diagnosis, number of primary melanoma diagnoses, and number of additional family members diagnosed with melanoma resulted in an AUC = 0.748 (95% CI: 0.726, 0.771). For this model, the mean estimated probability of *CDKN2A* mutation carriage was 46.4% for mutation carriers, and 27.2% for non-carriers. The difference in AUC values between models was not statistically significant ($p = 0.21$) (Figure 1a).

Adding phenotypic risk factors did not result in performance improvements of the 3-variable baseline GenoMELPREDICT model (data not tabulated and available upon request). However, including personal or family history of pancreatic cancer to the 3-variable baseline model significantly ($p < 0.0001$) augmented its discriminatory performance, yielding an AUC=0.772 (95%CI: 0.750, 0.793) (Figure 1b). The mean estimated probability of *CDKN2A* mutation carriage was 48.4% for mutation carriers and 26.2% for non-carriers. The NRI was 0.404, with noted improvement (79.6%) for reclassification of non-carriers, but at the expense of reclassification of carriers (-39.2%). Adding phenotypic variables to the 4-predictor model that included personal or family history of pancreatic cancer did not result in further model improvement (data not tabulated and available upon request). Selecting a predicted probability cutoff of 35% for this four variable model, which was similar to the theoretical best cutoff based on

Youden's index (34.4%), resulted in a sensitivity of 61%, specificity of 79%, positive predictive value of 60%, and a negative predictive value of 80%. A range of model metrics for the baseline and 4-predictor GenoMELPREDICT models is available upon request. Consistent with results using observed phenotypic data, adding imputed phenotypic variables did not result in performance improvement of either the baseline or 4-predictor GenoMELPREDICT models (data not tabulated and available upon request).

In subgroup analyses, the AUCs for the 3- and 4-predictor GenoMELPREDICT models were somewhat higher among Australian participants [0.809 (0.773, 0.844) for both], and similar or slightly higher among participants living in Northern European countries [0.760 (0.718, 0.803) and 0.775 (0.734, 0.816), respectively]. Model performance was lower among participants from Southern European and South American countries [0.625 (0.535, 0.714) and 0.635 (0.548, 0.722), respectively].

Models that excluded families with individuals who carried a mutation in other known melanoma high penetrance genes, or excluded families without a verified report of personal or family history of pancreatic cancer were consistent with our main results. In models excluding melanoma-prone families from Sydney, which comprised one-third of all data used in our analysis, AUCs for the baseline (0.772; 95% CI: 0.747, 0.797) and 4-variable (0.784; 95% CI: 0.760, 0.808) GenoMELPREDICT models were slightly higher compared to models using all available GenoMEL data. After excluding participants from the Bethesda and Queensland centers, both of which contributed higher numbers of affected members with *CDKN2A* genotype data per family (4.3 and 4.6 respectively), model AUCs were slightly lower than those calculated from all available GenoMEL data (0.708; 95% CI: 0.681, 0.734 for baseline; and 0.740; 95% CI: 0.714, 0.765 for the 4-variable model).

Discussion

We show that the published MELPREDICT model used to predict *CDKN2A* mutational status is generalizable to the global community of melanoma-prone families represented in GenoMEL. We also provide evidence that adding personal and family history of pancreatic cancer to the model, a variable that can be collected with very little additional associated cost, leads to some improvement in the ability to predict *CDKN2A* mutational status, and we call this augmented model GenoMELPREDICT. Predictive performance of GenoMELPREDICT is comparable to other clinical tools used to predict *BRCA1* and *BRCA2* mutational status among breast cancer patients.[18-20]

The diverse global sample of familial melanoma cases recruited by GenoMEL allowed us to detect a broader spectrum of *CDKN2A* mutations compared to the limited number (18 variants) reported by the original MELPREDICT developers.[9] A total of 85 unique, putatively pathogenic mutations were identified among GenoMEL cases, allowing for a more representative appraisal of GenoMELPREDICT's performance.

MelaPRO[21] and CM-Score[22] are two other published algorithms for *CDKN2A* mutation prediction among melanoma prone families. MelaPRO incorporates melanoma risk among unaffected family members, uses a Bayesian approach to predict carrier status, and incorporated penetrance estimates for areas of high and low baseline incidence, and one derived from the population-based Genes, Environment, and Melanoma Study.[23] MelaPRO was tested on a patient sample drawn from the same ascertainment center used by Niendorf *et al.* to test the MELPREDICT algorithm, and it outperformed (n=195; AUC=0.86) MELPREDICT on prediction of carrier status among the same homogeneous familial cohort. The CM-Score algorithm is a multivariate logistic regression model developed among a training cohort of 1,227 Dutch melanoma-prone families and incorporates five clinical features (number of family members with melanoma and with multiple primary melanomas, median age at diagnosis, and presence of pancreatic cancer or upper airway cancer in a family member) to predict germline *CDKN2A* mutational status. CM-Score was validated in a combined Swedish and Dutch cohort of 421 melanoma-prone families. CM-Score demonstrated excellent performance characteristics among a homogeneous

group of Northern Europeans (AUC=0.94; 95%CI: 0.90, 0.98), possibly due to the high incidence of specific founder mutations in this population.[22]

We opted to assess MELPREDICT rather than MelaPRO or CM-Score. CM-Score was developed among a cohort of Swedish and Dutch melanoma-prone families with a high incidence of specific founder mutations, reducing generalizability. Due to the increased incidence of upper airway cancers observed among carriers of these Swedish and Dutch founder mutations, the CM-Score algorithm incorporates any history of such cancers and may be inappropriate for a heterogeneous population of familial melanoma kindreds.[22] In our dataset, there were 295 reports of a personal or family history of laryngeal, pharyngeal, and oral cavity cancers within 97 families; pathologic verification was available for 30 (10%) of these reports. MelaPRO requires users to specify *CDKN2A* penetrance associated with the population under study, which involves more complex assessments of the source populations from which individual cases arise; this aspect may potentially limit MelaPRO's utility in clinical practice. Because the GenoMEL consortium includes melanoma-prone families from around the world and simultaneous modeling of multiple *CDKN2A* penetrances was not feasible, our preference was to evaluate generalizability and enhancement of MELPREDICT.

The 3- and 4-predictor GenoMELPREDICT models perform best among participants living in Australia. This likely reflects the large influence of these individuals, who comprise nearly 40% of our analytic sample, on overall model estimates. Conversely, 3- and 4-predictor GenoMELPREDICT models perform poorest among participants living in Southern European and South American sites. This likely reflects our working definition of a "melanoma-prone family," which minimally is two verified melanoma cases in a first-degree blood relation. This definition may be too strict for populations that experience lower incidence of melanoma for which a definition of two or more verified melanoma cases among blood relatives may be better suited. Of the 900 families who had at least one member who contributed to GenoMELPREDICT modeling, the Southern European and South American sites had, as expected, a lower mean number of affected members per family (2.1) compared to that for the Northern European (3.3) or Australian (3.6) sites.

We have reported on limitations of the GenoMEL study that include differences in amount of data collected across centers, possible misclassification of *CDKN2A* mutations, lack of centralized pathology review for reported cases of melanoma, and non-population-based ascertainment and sampling of families at some centers based on known mutation status or number of familial melanoma cases.[10, 17] Although pathological verification of reported personal or familial cases of pancreatic cancer was low (22%) in GenoMEL, the positive predictive value and sensitivity of self-report of family history for this cancer are both reported to surpass 70%.[24]

GenoMELPREDICT is an effective predictor of *CDKN2A* mutational status, and statistical performance improvement was made by adding any reported personal or family history of pancreatic cancer. However only 5% to 10% of melanomas can be attributed to high penetrance germline genetics, and thus only a small proportion of patients diagnosed with melanoma will benefit from genetic testing for *CDKN2A*.[25] Despite controversy regarding the genetic testing of individuals in melanoma-prone families,[26] there is burgeoning commercial availability of such tests. We have previously published in this journal the challenges in developing a single encompassing worldwide recommendation to best guide health professionals with respect to which patients should be considered for *CDKN2A* genetic testing.[8] In Table 3, we republish our candidacy criteria for consideration of genetic testing.[8] Complementing these criteria, GenoMELPREDICT may serve as a quick and robust tool, applicable worldwide, for directing patients away from unnecessary genetic testing, especially in the event of a low carrier probability estimate. Moreover, guidance considering the management of patients belonging to melanoma-prone families in the context of genetic testing is available in a Continuing Medical Education article published in this journal.[26] A user-friendly web-based interface to calculate the probability of carriage of a *CDKN2A* mutation is available at www.genomel.org.

Table 1: Number of participants and families by ascertainment center

GenoMEL Center	Participants*	Families†	Average number of participants per family‡	Average number of affected members per family¶
Barcelona, ES	44	25	1.8	2.1
Bethesda, US	199	46	4.3	4.8
Cesena, IT	50	24	2.1	2.1
Copenhagen, DK	47	34	1.4	2.5
Genoa, IT	34	16	2.1	2.3
Leeds, GB	158	77	2.1	2.8
Leiden, NL	210	60	3.5	4.6
Ljubljana, SI	9	4	2.3	2.3
Lund, SE	20	7	2.9	4.4
Montevideo, UY	8	4	2.0	2.0
Paris, FR	341	176	1.9	2.5
Philadelphia, US	78	36	2.2	2.4
Porto Allegre, BR	9	5	1.8	2.2
Queensland, AU	96	21	4.6	6.2
Riga, LV	5	5	1.0	2.6
Santiago, CL	3	2	1.5	2.0
São Paulo, BR	13	8	1.6	2.1
Stockholm, SE	39	21	1.9	2.8
Sydney, AU	722	305	2.4	3.4
Tel Aviv, IL	21	18	1.2	2.0
Valencia, ES	10	6	1.7	2.2
Total	2116	900	2.2	3.1

* Verification of melanoma was available for >99% of participants by: pathology report (74%), physician letter or clinical document verifying melanoma diagnosis (23%), cancer registry data (2%), or death certificate (<1%). Excludes affected individuals with a diagnosis of non-cutaneous melanoma or who are members of melanoma families by marriage and not ancestry.

† Family members with a melanoma of the uveal tract or conjunctiva did not contribute to defining a melanoma family.

‡ Includes only participants who contribute to prediction modeling.

¶ Includes family members who may not contribute to prediction modeling because of missing data.

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Table 2. Distribution of pathogenic *CDKN2A* mutations among GenoMEL cases and model estimates for the baseline and 4-predictor GenoMELPREDICT models.

		GenoMELPREDICT			
Variable	**No. (%) with mutation**	**OR (95% CI)***	**P**	**OR (95% CI)***	**P**
Ln(age at diagnosis)		0.29 (0.22, 0.39)	<0.0001	0.28 (0.22, 0.37)	<0.0001
Number of primary melanomas					
1	378/1426 (26.5%)				
2	153/380 (40.3%)	1.20 (1.10, 1.31)	<0.0001	1.20 (1.10, 1.32)	<0.0001
≥3	180/310 (58.1%)				
Number of other family members with melanoma					
1	132/669 (19.7%)				
2	146/560 (26.0%)				
3	91/218 (28.6%)	1.29 (1.20, 1.38)	<0.0001	1.26 (1.17, 1.32)	<0.0001
≥4	342/569 (60.1%)				
Personal or family history of pancreatic cancer					
No	495/1757 (28.2%)				
Yes	216/359 (60.2%)			3.05 (1.97, 4.74)	<0.0001

* Odds ratios and 95% confidence intervals were estimated from a generalized estimating equation (GEE) model using a logit link function and with adjustment for familial clustering. For reference, age at first cutaneous melanoma diagnosis is modeled as ln(age at first diagnosis) with range 2.30 (10 years old) to 4.55 (95 years old). A ln(age) of 3.0 corresponds to a 20 year old, a ln(age) of 3.5 to a 33 year old, and a ln(age) of 4.0 to a 55 year old.

Table 3. Candidacy for consideration of genetic testing

Low melanoma incidence area/population	Moderate to high melanoma incidence area/population
• Two (synchronous or metachronous) primary melanomas in an individual and/or • Families with at least one invasive melanoma and one or more other diagnoses of melanoma and/or pancreatic cancers among first- or second-degree relatives on the same side of the family	• Three (synchronous or metachronous) primary melanomas in an individual and/or • Families with at least one invasive melanoma and two or more other diagnoses of invasive melanoma and/or pancreatic cancer among first- or second-degree relatives on the same side of the family

This table refers to pathologically confirmed invasive melanoma. Table reprinted from Leachman et al., *J Am Acad Dermatol* 2009.

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Figure legend.

Figure 1. Receiver operator characteristic (ROC) curves for GenoMELPREDICT models.

Comparison of the ROC curves derived from the (Figure 1a) 3-variable baseline GenoMELPREDICT model and MELPREDICT as reported by Niendorf *et al.*, 2006; and (Figure 1b) 3-variable baseline GenoMELPREDICT model and the 4-variable GenoMELPREDICT model including any reported personal or family history of pancreatic cancer. Legend results are cross-validated areas under the curve (AUC) and 95% confidence intervals (CI) for GenoMELPREDICT models and AUC and 95% CI for MELPREDICT.

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