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

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

3

4 **Short title:** GenoMELPREDICT performance in melanoma families

5

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105 **Abstract**

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

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

115 **Results** MELPREDICT performed well (AUC=0.752; 95% CI: 0.730, 0.775), and GenoMELPREDICT
116 performance was similar (AUC=0.748; 95% CI: 0.726, 0.771). Adding a reported history of pancreatic
117 cancer yielded discriminatory improvement ($p < 0.0001$) in GenoMELPREDICT (AUC=0.772; 95% CI:
118 0.750, 0.793; NRI=0.40). Including phenotypic risk factors did not improve performance.

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

123 **Capsule Summary**

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

129 **Introduction**

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

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

154 **Methods**

155 **Study population**

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

162 **Study sample**

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

169 ***CDKN2A* genotyping**

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

174 **Statistical analysis**

175 Using the MELPREDICT logistic regression model for which the probability of *CDKN2A*
176 mutation carriage is defined as $\frac{e^L}{1+e^L}$ with $L = 1.99 + [(0.92 \times \text{number of primary melanoma diagnoses}) +$
177 $(0.74 \times \text{number of additional family members diagnosed with melanoma}) - (2.11 \times \ln(\text{age at first}$
178 $\text{melanoma diagnosis}))]$, we estimated the predictive probability of *CDKN2A* mutation carriage among

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

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

202 **Results**

203 *CDKN2A* genotype was available for 711 (33.6%) mutation carriers and 1,405 (66.4%) non-
204 carriers belonging to 900 melanoma-prone families. *CDKN2A* mutations identified in GenoMEL families
205 have been previously published.[10, 17] Results of multivariable analyses for our 3-variable baseline and
206 4-variable GenoMELPREDICT model that included pancreatic cancer are presented in Table 2. Age at
207 first melanoma diagnosis, higher numbers of primary melanomas, higher numbers of family members
208 with a melanoma diagnosis, and a personal or family history of pancreatic cancer were independently
209 associated ($p < 0.0001$) with *CDKN2A* mutation carriage.

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

218 Adding phenotypic risk factors did not result in performance improvements of the 3-variable
219 baseline GenoMELPREDICT model (data not tabulated and available upon request). However, including
220 personal or family history of pancreatic cancer to the 3-variable baseline model significantly ($p < 0.0001$)
221 augmented its discriminatory performance, yielding an AUC=0.772 (95%CI: 0.750, 0.793) (Figure 1b).
222 The mean estimated probability of *CDKN2A* mutation carriage was 48.4% for mutation carriers and
223 26.2% for non-carriers. The NRI was 0.404, with noted improvement (79.6%) for reclassification of non-
224 carriers, but at the expense of reclassification of carriers (-39.2%). Adding phenotypic variables to the 4-
225 predictor model that included personal or family history of pancreatic cancer did not result in further
226 model improvement (data not tabulated and available upon request). Selecting a predicted probability
227 cutoff of 35% for this four variable model, which was similar to the theoretical best cutoff based on

228 Youden's index (34.4%), resulted in a sensitivity of 61%, specificity of 79%, positive predictive value of
229 60%, and a negative predictive value of 80%. A range of model metrics for the baseline and 4-predictor
230 GenoMELPREDICT models is available upon request. Consistent with results using observed phenotypic
231 data, adding imputed phenotypic variables did not result in performance improvement of either the
232 baseline or 4-predictor GenoMELPREDICT models (data not tabulated and available upon request).

233 In subgroup analyses, the AUCs for the 3- and 4-predictor GenoMELPREDICT models were
234 somewhat higher among Australian participants [0.809 (0.773, 0.844) for both], and similar or slightly
235 higher among participants living in Northern European countries [0.760 (0.718, 0.803) and 0.775 (0.734,
236 0.816), respectively]. Model performance was lower among participants from Southern European and
237 South American countries [0.625 (0.535, 0.714) and 0.635 (0.548, 0.722), respectively].

238 Models that excluded families with individuals who carried a mutation in other known melanoma
239 high penetrance genes, or excluded families without a verified report of personal or family history of
240 pancreatic cancer were consistent with our main results. In models excluding melanoma-prone families
241 from Sydney, which comprised one-third of all data used in our analysis, AUCs for the baseline (0.772;
242 95% CI: 0.747, 0.797) and 4-variable (0.784; 95% CI: 0.760, 0.808) GenoMELPREDICT models were
243 slightly higher compared to models using all available GenoMEL data. After excluding participants from
244 the Bethesda and Queensland centers, both of which contributed higher numbers of affected members
245 with *CDKN2A* genotype data per family (4.3 and 4.6 respectively), model AUCs were slightly lower than
246 those calculated from all available GenoMEL data (0.708; 95% CI: 0.681, 0.734 for baseline; and 0.740;
247 95% CI: 0.714, 0.765 for the 4-variable model).

248 **Discussion**

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

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

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

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

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

289 The 3- and 4-predictor GenoMELPREDICT models perform best among participants living in
290 Australia. This likely reflects the large influence of these individuals, who comprise nearly 40% of our
291 analytic sample, on overall model estimates. Conversely, 3- and 4-predictor GenoMELPREDICT models
292 perform poorest among participants living in Southern European and South American sites. This likely
293 reflects our working definition of a "melanoma-prone family," which minimally is two verified melanoma
294 cases in a first-degree blood relation. This definition may be too strict for populations that experience
295 lower incidence of melanoma for which a definition of two or more verified melanoma cases among
296 blood relatives may be better suited. Of the 900 families who had at least one member who contributed to
297 GenoMELPREDICT modeling, the Southern European and South American sites had, as expected, a
298 lower mean number of affected members per family (2.1) compared to that for the Northern European
299 (3.3) or Australian (3.6) sites.

300 We have reported on limitations of the GenoMEL study that include differences in amount of
301 data collected across centers, possible misclassification of *CDKN2A* mutations, lack of centralized
302 pathology review for reported cases of melanoma, and non-population-based ascertainment and sampling
303 of families at some centers based on known mutation status or number of familial melanoma cases.[10,
304 17] Although pathological verification of reported personal or familial cases of pancreatic cancer was low
305 (22%) in GenoMEL, the positive predictive value and sensitivity of self-report of family history for this
306 cancer are both reported to surpass 70%.[24]

307 GenoMELPREDICT is an effective predictor of *CDKN2A* mutational status, and statistical
308 performance improvement was made by adding any reported personal or family history of pancreatic
309 cancer. However only 5% to 10% of melanomas can be attributed to high penetrance germline genetics,
310 and thus only a small proportion of patients diagnosed with melanoma will benefit from genetic testing
311 for *CDKN2A*.[25] Despite controversy regarding the genetic testing of individuals in melanoma-prone
312 families,[26] there is burgeoning commercial availability of such tests. We have previously published in
313 this journal the challenges in developing a single encompassing worldwide recommendation to best guide
314 health professionals with respect to which patients should be considered for *CDKN2A* genetic testing.[8]
315 In Table 3, we republish our candidacy criteria for consideration of genetic testing.[8] Complementing
316 these criteria, GenoMELPREDICT may serve as a quick and robust tool, applicable worldwide, for
317 directing patients away from unnecessary genetic testing, especially in the event of a low carrier
318 probability estimate. Moreover, guidance considering the management of patients belonging to
319 melanoma-prone families in the context of genetic testing is available in a Continuing Medical Education
320 article published in this journal.[26] A user-friendly web-based interface to calculate the probability of
321 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.

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

Variable	No. (%) with mutation	GenoMELPREDICT			
		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%)	1.29 (1.20, 1.38)	<0.0001	1.26 (1.17, 1.32)	<0.0001
3	91/218 (28.6%)				
≥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
<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">• 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.

324 **Figure legend.**

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

326 Comparison of the ROC curves derived from the (Figure 1a) 3-variable baseline GenoMELPREDICT

327 model and MELPREDICT as reported by Niendorf *et al.*, 2006; and (Figure 1b) 3-variable baseline

328 GenoMELPREDICT model and the 4-variable GenoMELPREDICT model including any reported

329 personal or family history of pancreatic cancer. Legend results are cross-validated areas under the curve

330 (AUC) and 95% confidence intervals (CI) for GenoMELPREDICT models and AUC and 95% CI for

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