Supplemental data for

# Germline variation at *CDKN2A* and associations with nevus phenotypes among members of melanoma families

**Short title:** *CDKN2A* mutations among melanoma family members

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**Supplemental Tables:**



**Supplementary Figures:**

**Supplemental Table 1. Characterization of *CDKN2A* variants in GenoMEL**

| DNA sequence | p16 protein change | p14ARF protein change | Location | Classification by GenoMEL |
|---|---|---|---|---|
| c.-19443G>A (c.62G>A in p14ARF) | N/A | p.R21K | Exon 1β | Pathogenic |
| c.-19312G>C (c.193G>C in p14 ARF) | N/A | splice variant | Exon 1β | Pathogenic |
| c.-19311G>C (c.193+1G>C in p14ARF) | N/A | splice variant | Intron 1 (p14ARF) | Pathogenic |
| c.-19311G>A (c.193+1G>A in p14ARF) | N/A | splice variant | Intron 1 (p14ARF) | Pathogenic |
| c.-19310T>C (c.193+2T>C in p14ARF) | N/A | splice variant | Intron 1 (p14ARF) | Pathogenic |
| c.-19309A>G (c.193+3A>G in p14ARF) | N/A | possible splice mutation | Intron 1 (p14ARF) | Pathogenic |
| c.-19307G>A (c.193+5G>A in p14ARF) | N/A | splice variant | Intron 1 (p14ARF) | Pathogenic |
| c.-5057Ex1b_-2740Ex1adel | | p14ARF Exon 1b deletion | Exon 1β | Pathogenic |
| c.-196p14Ex1b_-11233p16Ex1adel | | | Exon 1β, Exon 1α | Pathogenic |
| | Macro deletion of p16INK4a Exons 1a, 2 and 3 | | Exon 1α, 2 and 3 | Pathogenic |
| c.-735G>A | | N/A | Promoter | Benign |
| c.-493A>T | | N/A | Promoter | Benign |
| c.-292A>G | | N/A | Promoter | Benign |
| c.-252A>T | | N/A | Promoter | Benign |
| c.-191A>G | | N/A | Promoter | Benign |
| c.-95G>C | | N/A | Promoter | Benign |
| c.-56G>C | | N/A | Promoter | Benign |
| c.-34G>T | c.-34_-32>p.M1 | N/A | Promoter | Pathogenic |
| c.-34G>A | | N/A | Promoter | Pathogenic |
| c.-33G>C | | N/A | Promoter | Benign |
| c.-25C>T | | N/A | Promoter | Benign |
| c.-21C>T | | N/A | Promoter | Benign |
| c.-2G>A | | N/A | Promoter | Benign |
| c.9_32del24 | p.A4_P11delAAGSSMEP | N/A | Exon 1α | Pathogenic |
| c.9_32dup24 | p.A4_P11dup | N/A | Exon 1α | Pathogenic |

| DNA sequence | p16 protein change | p14ARF protein change | Location | Classification by GenoMEL |
|---|---|---|---|---|
| c.10G>A | p.A4T | N/A | Exon 1α | Pathogenic |
| c.13G>A | p.A5T | N/A | Exon 1α | Pathogenic |
| c.35C>A | p.S12X | N/A | Exon 1α | Pathogenic |
| c.44G>A | p.W15* | N/A | Exon 1α | Pathogenic |
| c.45G>A | p.W15* | N/A | Exon 1α | Pathogenic |
| c.46delC | p.L16fs* | N/A | Exon 1α | Pathogenic |
| c.47T>C | p.L16P | N/A | Exon 1α | Pathogenic |
| c.47T>G | p.L16R | N/A | Exon 1α | Pathogenic |
| c.52_57dup | p.T18_A19dup | N/A | Exon 1α | Pathogenic |
| c.67G>C | p.G23R | N/A | Exon 1α | Pathogenic |
| c.67G>A | p.G23S | N/A | Exon 1α | Pathogenic |
| c.68G>A | p.G23D | N/A | Exon 1α | Pathogenic |
| c.71G>C | p.R24P | N/A | Exon 1α | Pathogenic |
| c.73delG | p.V25* | N/A | Exon 1α | Pathogenic |
| c.79G>T | p.E27* | N/A | Exon 1α | Pathogenic |
| c.88delG | p.A30fs* | N/A | Exon 1α | Pathogenic |
| c.94_99dup | p.L32_E33dup | N/A | Exon 1α | Pathogenic |
| c.95T>C | p.L32P | N/A | Exon 1α | Pathogenic |
| c.95T>G | p.L32R | N/A | Exon 1α | Pathogenic |
| c.103G>A | p.G35R | N/A | Exon 1α | Pathogenic |
| c.104G>C | p.G35A | N/A | Exon 1α | Pathogenic |
| c.104G>A | p.G35E | N/A | Exon 1α | Pathogenic |
| c.104G>T | p.G35V | N/A | Exon 1α | Pathogenic |
| c.106delG | p.A36fs* | N/A | Exon 1α | Pathogenic |
| c.106G>C | p.A36P | N/A | Exon 1α | Pathogenic |
| c.123C>G | p.P38R | N/A | Exon 1α | Pathogenic |
| c.142C>A | p.P48T | N/A | Exon 1α | Pathogenic |

| DNA sequence | p16 protein change | p14ARF protein change | Location | Classification by GenoMEL |
|---|---|---|---|---|
| c.143C>T | p.P48L | N/A | Exon 1α | Pathogenic |
| c.143C>A | p.P48Q | N/A | Exon 1α | Pathogenic |
| c.146T>C | p.I49T | N/A | Exon 1α | Pathogenic |
| c.146T>G | p.I49S | N/A | Exon 1α | Pathogenic |
| c.149A>G | p.Q50R | N/A | Exon 1α | Pathogenic |
| c.150+2T>C | putatative aberrant splicing | | Intron 1 | Pathogenic |
| c.150+37G>C | | | Intron 1 | Benign |
| c.150+459T>C | | | Intron 1 | Benign |
| c.150+833A>T | | | Intron 1 | Benign |
| c.150+838_839insT | | | Intron 1 | Benign |
| c.150+926G>T | | | Intron 1 | Benign |
| c.150+1104C>A | aberrant splicing | aberrant splicing | Intron 1 | Pathogenic |
| c.150+1255C>A | | | Intron 1 | Benign |
| c.150+1277G>A | | | Intron 1 | Benign |
| c.151-1G>A | putatative aberrant splicing | | Intron 1 | Pathogenic |
| c.151-1G>T | putatative aberrant splicing | | Intron 1 | Pathogenic |
| c.151-2A>G | putatative aberrant splicing | | Intron 1 | Pathogenic |
| c.151-10C>G | | | Intron 1 | Benign |
| c.151-75G>A | | | Intron 1 | Benign |
| c.151-122G>C | | | Intron 1 | Benign |
| | p.(M52_D156delinsRTDDLRICTLR ASKAQSIHFPAQKVQPGRPVSGLAS AHAPIGGTA) | | Intron 1 | Pathogenic |
| c.151-820A>G | | | Intron 1 | Benign |
| c.151-1104C>G | | | Intron 1 | Pathogenic |
| c.151-1456T>C | | | Intron 1 | Benign |
| c.151G>T | p.V51F | p.G65V | Exon 2 | Pathogenic |

| DNA sequence | p16 protein change | p14ARF protein change | Location | Classification by GenoMEL |
|---|---|---|---|---|
| c.158T>C | p.M53T | p.D67D | Exon 2 | Pathogenic |
| c.159G>C | p.M53I | p.D68H | Exon 2 | Pathogenic |
| c.167_197del31 | p.56fs* | p.Q70fs* | Exon 2 | Pathogenic |
| c.167G>T | p.S56I | p.Q70H | Exon 2 | Pathogenic |
| c.170C>T | p.A57V | p.R71R | Exon 2 | Pathogenic |
| c.172C>T | p.R58* | p.P72L | Exon 2 | Pathogenic |
| c.176T>G | p.V59G | p.S73R | Exon 2 | Pathogenic |
| c.178G>C | p.A60P | p.G74A | Exon 2 | Pathogenic |
| c.179C>G | p.A60G | p.G74G | Exon 2 | Pathogenic |
| c.185T>C | p.L62P | p.A76A | Exon 2 | Pathogenic |
| c.188T>C | p.L63P | p.A77A | Exon 2 | Pathogenic |
| c.192G>A | p.L64L | p.A79T | Exon 2 | Benign |
| c.194T>C | p.L65P | p.A79A | Exon 2 | Pathogenic |
| c.194insGCT | p.L194_H195insL | p.A79insA | Exon 2 | Pathogenic |
| c.198C>T | p.H66H | p.R81W | Exon 2 | Benign |
| c.199G>A | p.G67S | p.R81Q | Exon 2 | Pathogenic |
| c.199del15 | p.G67delGAEPN | p.R81delRRGAQ | Exon 2 | Pathogenic |
| c.202_203GC>TT | p.A68L | pR82L | Exon 2 | Pathogenic |
| c.203C>G | p.A68G | p.R82R | Exon 2 | Pathogenic |
| c.206A>G | p.E69G | p.G83G | Exon 2 | Pathogenic |
| c.212A>G | p.N71S | p.Q85Q | Exon 2 | Pathogenic |
| c.213C>A | p.N71K | p.L86M | Exon 2 | Pathogenic |
| c.220G>T | p.D74Y | p.R88L | Exon 2 | Pathogenic |
| c.225_243del19 | p.P75fs* | p.R90Vfs* | Exon 2 | Pathogenic |
| c.240_253del14 | p.R80fs* | p.A95fs* | Exon 2 | Pathogenic |
| c.241C>T | p.P81S | p.T95I | Exon 2 | Pathogenic |
| c.250G>A | p.D84N | p.R98Q | Exon 2 | Pathogenic |

| DNA sequence | p16 protein change | p14ARF protein change | Location | Classification by GenoMEL |
|---|---|---|---|---|
| c.250G>C | p.D84H | p.R98P | Exon 2 | Pathogenic |
| c.251A>C | p.D84A | p.R98R | Exon 2 | Pathogenic |
| c.259C>T | p.R87W | p.P101L | Exon 2 | Pathogenic |
| c.260G>C | p.R87P | p.P101P | Exon 2 | Pathogenic |
| c.262G>T | p.E88X | p.G102V | Exon 2 | Pathogenic |
| c.281T>C | p.L94P | p.A108A | Exon 2 | Pathogenic |
| c.281T>A | p.L94Q | p.A108A | Exon 2 | Pathogenic |
| c.296G>C | p.R99P | p.P113P | Exon 2 | Pathogenic |
| c.301G>T | p.G101W | p.R115L | Exon 2 | Pathogenic |
| c.304_305insC | p.A102fs* | | Exon 2 | Pathogenic |
| c.305delCGCG | p.R103fs* | | Exon 2 | Pathogenic |
| c.318G>A | p.V106V | p.A121T | Exon 2 | Benign |
| c.322G>A | p.D108N | p.R122Q | Exon 2 | Pathogenic |
| c.334C>G | p.R112G | p.P126R | Exon 2 | Pathogenic |
| c.337_338insGTC | p.R112_L113insR | p.S127_A128insS | Exon 2 | Pathogenic |
| c.339G>C | p.L113L | p.A128S | Exon 2 | Benign |
| c.340C>T | p.P114S | p.A128V | Exon 2 | Pathogenic |
| c.352G>A | p.A118T | p.G132D | Exon 2 | Pathogenic |
| c.353C>T | p.A118V | p.G132G | Exon 2 | Pathogenic |
| c.358delG | p.E120fs* | N/A | Exon 2 | Pathogenic |
| c.365G>T | p.G122V | N/A | Exon 2 | Pathogenic |
| c.373G>C | p.D125H | N/A | Exon 2 | Pathogenic |
| c.377T>A | p.V126D | N/A | Exon 2 | Pathogenic |
| c.379G>C | p.A127P | N/A | Exon 2 | Pathogenic |
| c.379G>T | p.A127S | N/A | Exon 2 | Pathogenic |
| c.380_381insGTC | p.A140_R141insS | N/A | Exon 2 | Pathogenic |
| c.384_457del74 | p.R128fs* | N/A | Exon 2 | Pathogenic |

| DNA sequence | p16 protein change | p14ARF protein change | Location | Classification by GenoMEL |
|---|---|---|---|---|
| c.385_386delAT | p.Y129fs* | N/A | Exon 2 | Pathogenic |
| c.413G>C | p.R138T | N/A | Exon 2 | Pathogenic |
| c.430C>T | p.R144C | N/A | Exon 2 | Pathogenic |
| c.442G>A | p.A148T | N/A | Exon 2 | Benign |
| c.457G>T | p.D153spl | N/A | Exon 2 | Pathogenic |
| c.457+1G>T | p.Ex1a-Ex3delEx2 | p.R128fs* | Intron 2 | Pathogenic |
| c.457+474T>C | | | Intron 2 | Benign |
| c.457+678T>C | | | Intron 2 | Benign |
| c.458-2A>C | putatative aberrant splicing | | Intron 2 | Pathogenic |
| c.458-89C>T | | | Intron 2 | Benign |
| c.458-105A>G | putatative aberrant splicing | | Intron 2 | Pathogenic |
| c.458-279C>T | | | Intron 2 | Benign |
| c.500C>G | | | Exon 3 (inc 3'UTR) | Benign |
| c.540C>T | | | Exon 3 (inc 3'UTR) | Benign |

*Genome build Hg19(GRCh37) and reference sequences (NM_000077.4 for p16) and (NM_058195.3 for p14ARF) were used for the described variants. Classifications are based on known functional impact on CDKN2A and evidence of cosegregation wihin melanoma families or bioinformatically inferred impact on CDKN2A function.

**Supplemental Table 2. Associations between *CDKN2A* mutational status and nevus phenotype among members of melanoma families by study center according to latitude**

| Nevus phenotype | Individual *CDKN2A* mutational status | By latitude (closer to the equator → farther from the equator)[1] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Porto Alegre (BR), São Paulo (BR), Queensland (AU), Tel Aviv (IL), Sydney (AU), Montevideo (UY), Santiago (CL) (n=179) | | Philadelphia (US), Bethesda (US), Salt Lake City (US), Barcelona (ES), Valencia (ES) (n=605) | | Cesena (IT), Genoa (IT), Ljubljana (SI), Paris (FR) (n=219) | | Leiden (NL), Leeds (UK), Lund (SE), Riga (LV), Stockholm (SE) (n=648) | | |
| | | OR (95% CI) | p-value | OR (95% CI) | p-value | OR (95% CI) | p-value | OR (95% CI) | p-value | p-interaction[2] |
| 2 mm nevi | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 1.33 (0.98, 1.81) | 0.19 | 1.04 (0.87, 1.26) | 0.65 | 0.91 (0.52, 1.61) | 0.75 | 0.93 (0.78, 1.11) | 0.42 | 0.25 |
| 5 mm nevi | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 1.28 (0.87, 1.87) | 0.30 | 1.50 (0.81, 2.77) | 0.28 | 1.60 (0.88, 2.91) | 0.21 | 0.95 (0.71, 1.26) | 0.71 | 0.26 |
| Atypical nevi | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 1.88 (1.32, 2.68) | 0.05 | 3.21 (1.02, 10.13) | 0.17 | 1.21 (0.68, 2.19) | 0.54 | 1.27 (0.75, 2.14) | 0.39 | 0.13 |
| Mole gestalt (3 vs. 0)[3] | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 2.40 (0.80, 7.15) | 0.20 | 1.61 (0.89, 2.93) | 0.13 | 4.47 (0.72, 27.65) | 0.17 | 1.43 (0.83, 2.45) | 0.21 | 0.17 |
| Mole gestalt (2 vs. 0)[4] | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 0.92 (0.28, 3.07) | 0.89 | 1.17 (0.62, 2.22) | 0.63 | 21.55 (2.97, 156.43) | 0.004 | 1.36 (0.87, 2.12) | 0.19 | 0.82 |
| Mole gestalt (1 vs. 0)[5] | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 3.48 (1.31, 9.25) | 0.02 | 1.34 (0.73, 2.48) | 0.36 | 7.38 (1.24, 43.96) | 0.006 | 0.85 (0.51, 1.44) | 0.55 | 0.08 |

[1]Adjusted for age at phenotyping, gender, age at phenotyping*gender, melanoma affected status, center, and familial clustering within study center. Married-in relatives not belonging to a melanoma family lineage are excluded. P-values correspond to overall score tests.

[2] P-value for the association between the interaction of *CDKN2A* mutation carriage with GenoMEL center grouping and nevus phenotype.

[3] Mole gestalt is modeled in a GEE model excluding individuals with values of "1" and "2" for mole gestalt to achieve the contrast estimates.

[4] Mole gestalt is modeled in a GEE model excluding individuals with values of "1" and "3" for mole gestalt to achieve the contrast estimates.

[5] Mole gestalt is modeled in a GEE model excluding individuals with values of "2" and "3" for mole gestalt to achieve the contrast estimates.

**Supplemental Table 3. Associations between *CDKN2A* mutational status and nevus phenotype among members of melanoma families according to relative UV exposure categorization of anatomic site of first verified melanoma**

| Nevus phenotype | Individual *CDKN2A* mutational status | By exposure site of first verified melanoma (n=749)[1] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Usually exposed (n=106) | | Intermittently exposed (n=414) | | Usually unexposed (n=142) | | |
| | | OR (95% CI) | p-value | OR (95% CI) | p-value | OR (95% CI) | p-value | p-interaction[2] |
| 2 mm nevi | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 1.28 (0.91, 1.81) | 0.17 | 1.11 (0.88, 1.40) | 0.39 | 1.35 (0.92, 1.96) | 0.15 | 0.71 |
| 5 mm nevi | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 0.70 (0.36, 1.36) | 0.34 | 1.24 (0.79, 1.93) | 0.39 | 1.17 (0.55, 2.48) | 0.71 | 0.40 |
| Atypical nevi | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 1.10 (0.44, 2.72) | 0.87 | 1.38 (0.71, 2.67) | 0.40 | no estimate | n/a | 0.13 |
| Mole gestalt (3 vs. 0)[3] | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 0.23 (0.04, 1.52) | 0.10 | 1.00 (0.51, 1.98) | 0.99 | no estimate | n/a | 0.28 |
| Mole gestalt (2 vs. 0)[4] | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 0.37 (0.08, 1.69) | 0.20 | 0.78 (0.40, 1.52) | 0.47 | 1.26 (0.35, 4.54) | 0.73 | 0.28 |
| Mole gestalt (1 vs. 0)[5] | No known pathogenic | 1.00 | | 1.00 | | 1.00 | | |
| | Pathogenic | 0.22 (0.05, 0.97) | 0.04 | 1.02 (0.53, 1.94) | 0.96 | 1.30 (0.39, 4.22) | 0.67 | 0.09 |

[1]Adjusted for age at phenotyping, gender, age at phenotyping*gender, study center and familial clustering within study center. P-values correspond to overall score tests. Case participants missing anatomic site data (n=87) are excluded.

[2] P-value for the association between the interaction of *CDKN2A* mutation carriage with relative UV exposure categorization of anatomic site of first verified melanoma and nevus

[3] Mole gestalt is modeled in a GEE model excluding individuals with values of "1" and "2" for mole gestalt to achieve the contrast estimates.

[4] Mole gestalt is modeled in a GEE model excluding individuals with values of "1" and "3" for mole gestalt to achieve the contrast estimates.

[5] Mole gestalt is modeled in a GEE model excluding individuals with values of "2" and "3" for mole gestalt to achieve the contrast estimates.

**Supplemental Figure 1.**

2 mm, 5 mm, and atypical nevus count distributions among GenoMEL melanoma family members across all ascertainment centers according to *CDKN2A* mutational status and case status. Crude nevus counts are plotted and are not representative of center-specific measures adopted for statistical modeling. Heavy horizonal lines indicate 50$^{th}$ percentile counts, boxes indicate 25$^{th}$ and 75$^{th}$ percentile counts, whiskers indicate 5$^{th}$ and 95$^{th}$ percentile counts, and circles represent values in the top or bottom 5% of counts.