Isoform-Specific Loss of Function Variants in *CDKN2A* and Their Association with Cancer Phenotypes

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CDKN2A is a tumor suppressor gene that encodes two transcripts: p16 (p16INK) and p14 (p14ARF). These two transcripts have overlapping coding sequence but have distinct promoters and reading frames. Pathogenic alterations affecting p16 are associated with melanoma-pancreatic cancer predisposition, while the disease association for p14 is less well understood. In this study, we aimed to compare the melanoma prevalence observed in individuals with putative loss of function variants (pLoFV) in these two isoforms.

A retrospective review of individuals undergoing multigene panel testing for a variety of hereditary cancer indications from 2015-2023 at a single diagnostic laboratory was performed to identify cases with pLoFV in either the p16 or p14 transcripts. Nonsense, frameshift, initiation codon, and canonical splice variants, as well as gross deletions were defined as pLoF and included in this study. Variants in the overlapping sequence that cause pLoF in both isoforms, such as frameshifts, were categorized as p16 pLoFV. Otherwise, variants resulted in pLoF in only one of the two isoforms. Melanoma prevalence in the two groups was compared using the Chi Square Test.

A total of 258 individuals with 51 unique pLoFV were identified, including 192 individuals with variants impacting p16INK4a and 66 impacting p14ARF. Overall, personal history of melanoma was more common in individuals with pLoFV in p16 compared to p14 (30.7% vs 10.1%; p=0.001). In the p14 group, melanoma was rarely observed in individuals with nonsense/frameshift variants (2 of 48; 4.2%) or initiation codon variants (0 of 9; 0.0%) whereas 5 of 9 individuals (55.6%) with p14 splicing variants or gross deletions had melanoma.

In this study, we found that nonsense, frameshifting, and start loss in the p14 isoform were not associated with significant melanoma or pancreatic cancer phenotypes. In contrast, gross deletions involving the 5'UTR and variants impacting the donor site of p14 coding exon 1 were significantly associated with melanoma. Together, these results call into question whether the phenotypes observed in individuals with pLoFV in the p14ARF are due to a disruption of p16 rather than the degradation of the p14 protein. These data highlight the need for careful variant interpretation and clinical management guidelines for patients with variants impacting the p14 isoform, as there may be significant variant- and isoform-level stratification of risk.