Insights into genetic susceptibility to melanoma by gene panel testing: potential pathogenic variants in *ACD, ATM, BAP1,* and *POT1*

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Objective

- Until six years ago, *CDKN2A/ARF* and *CDK4* were the only known melanoma-predisposition genes tested in clinical practice (20-45% pos familial CM cases [1] and 11%-19% of multiple primary melanomas (MPM) [2]), but, recently, novel rare high-risk variants have been identified in BAP1, POT1, ACD,TERF2IP) and TERT promoter.
- We performed germline sequencing of CM patients through a multigene panel containing all established and two selected candidate* CM susceptibility genes, with the following aims:
  - to validate this comprehensive gene panel in high-risk melanoma cases
  - to evaluate the potential impact of this panel in the clinical practice in terms of increased diagnostic yield and of interpretational challenges of novel variants.

**ATM and PALB2. They were included under the hypothesis the aggregation of pancreatic cancer (PC) in our CM families could be partly ascribed to those two genes*

Methods and Materials

Study cohort

273 consecutive CDKN2A/CDK4-negative index CM cases selected for genetic testing using criteria proposed to assess CM susceptibility (3), of which:

- 167 familial melanoma cases
- 84 MPM cases
- 167 familial melanoma cases
- 22 cases with familiarity for PC, BAP1-TPDS, or with atypical Spitz nevus

80 cancer-free controls

DNA sequencing

- Panel size: 198 amplicons, 55.5 Kb.
- Custom targeted sequencing: coding exons and splice junctions of CDKN2A/ARF, CDK4 exon2, ACD, BAP1, MITF exon 10, POT1, TERF2IP, ATM and PALB2
- TERT promoter was analyzed by Sanger sequencing

Results

Out of 273 probands who underwent gene panel testing, we identified:

- 16 (5.9%) pathogenetic (P) or likely pathogenic (LP) variants in the established CM susceptibility genes BAP1 (2.2%; n=6), POT1 (0.7%; n=2), ACD (0.37%; n=1) and MITF (2.6%; n=7). A novel POT1 splice variant found in this cohort is described in Figure 1
- 8 variants of uncertain significance (VUS): 1 in BAP1, 6 in POT1, and 1 in TERF2IP
- 4 deleterious variants and 5 potentially deleterious variants (3.3%) as well as 6 rare VUS in ATM, whereas no rare variants were found in PALB2. (Fig2)

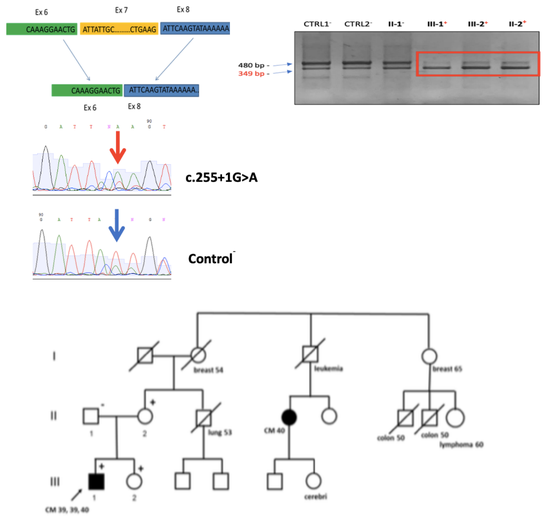

Fig.1 (a) Schematic representation of exon 7 splicing (113bp) resulting from the c.255 + 1G>A variant.
(b) Electrophoresis of cDNA from 4 family members, 3 (affected proband, sister and mother) carrying the c.255 + 1G>A variant, the non-carrier father from the unaffected branch of the family, and two healthy controls (CTRL-). The shortest of the two transcripts, resulting from the skipping of exon 7, is overrepresented in carriers compared to noncarriers. (c) cDNA sequencing confirmed that the mutant allele produced the shorter isoform, with skipping of exon 7, in a higher proportion of the transcript in carriers vs non carriers. The blu arrow indicates the lower relative abundance of the spliced isoform (ex 6-8) in noncarriers vs carriers (red arrow). (d) Pedigree diagram of the family carrying the c.255 +1G>A variant. Dark symbo=CM. Cancer type and age at diagnosis are indicated under each symbol. Arrow= proband. +=carrier, -=non-carrier.

Conclusion

To our knowledge, this is the first study to report a high percentage of deleterious ATM variants in melanoma families (3.3%, plus 2.2% rare VUS), and has led to an ongoing multicenter international collaboration to define the role of ATM in CM susceptibility.

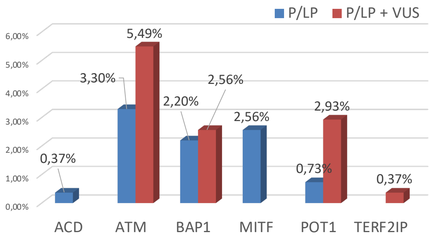

Fig.2 Graph showing the percentage of pathogenic and likely pathogenic (P/LP) and VUS variants in each gene

References

1. Aoude, L.G.et al.Genetics of familial melanoma: 20 years after CDKN2A. Pigment Cell Melanoma Res. 2015, 28, 148–160.
2. Bruno, W.et al.,Multiple primary melanomas (MPMs) and criteria for genetic assessment: MultiMEL, a multicenter study of the Italian Melanoma Intergroup. J. Am. Acad. Dermatol. 2016, 74, 325–332.
3. Leachman, S.A.; Lucero, O.M.; Sampson, J.E.; Cassidy, P.; Bruno, W.; Queirolo, P.; Ghiorzo, P. Identification, genetic testing, and management of hereditary melanoma. 2017, 1–14.