MelaNostrum New Proposal Form ***(version May 2017)***

Investigators who would like to propose a new research project, please complete the form and send it back to Dr. Maria Teresa Landi (landim@mail.nih.gov)

I. Project Title

Evaluation of *CCDC83* as a candidate melanoma susceptibility gene

Date: May 25th 2017

II. Project Group Investigators

Project proposal: Miriam Potrony and Susana Puig
All Melanostrum members interested who have and wish to share *CCDC83* sequencing data
The proposal involves other groups within GenoMEL (for now David Adams has agreed to participate, other GenoMEL PI may participate in the near future)
Part of functional analysis will be performed at David Adams group. Other members from Barcelona may be collaborating in functional analysis (to be discussed later on depending on the results obtained from the sequencing data).

III. Background

We have performed a genome-wide linkage analysis + WGS in a melanoma-prone family with 6 *CDKN2A*-positive and two *CDKN2A*-negative cases under dermatological follow-up at the Melanoma Unit of Hospital Clinic of Barcelona (results presented at the GenoMEL meeting, Camogli May 2017). We have detected 6 candidate variants segregating with melanoma in the family (Table 1, Figure 1). This family also has multiple cases of cancer (3 breast, 1 cervix, 3 lung (one in a patient with melanoma), 1 vaginal in a patient with melanoma, 2 liver): 3 cancer cases among *CDKN2A*-negative individuals, 5 among *CDKN2A*-positive and 2 among cases without known *CDKN2A* status.

Table 1. Genetic candidate variants detected in family #1

chr	Genomic change	Aminoacid change	Gene	Variant type	ExAC	1000G	S	P	MT	GERP	CADD	reads IV-3	reads IV-5
1	g.197128680C>T	p.D847N	*ZBTB41*	Exonic non-synonymous	0.0007	0.001	D	B	D	5.63	23.1	18/34	24/44
1	g.203472742G>A	p.R298H	*OPTC*	Exonic non-synonymous	0.0001	-	T	D	D	3.94	33	21/31	16/38
6	g.14708411T>C	-	*LINC01108 JARID2*	intergenic	-	-	-	-	-	3.88	22	16/34	15/31
11	g.75637708C>A	-	*UVRAG*	intronic	-	0.001	-	-	-	3.43	21.7	19/44	17/39
11	g.85597369A>G	p.N157S	*CCDC83*	exonic non-synonymous	1.56E-05	-	T	D	D	5.09	23.3	19/34	20/36
11	g.85956484G>A	-	*EED*	intronic	-	-	-	-	-	4.33	20.5	17/37	15/25

S: SIFT functional prediction result (D=Deleterious, T=Tolerated)
P: Polyphen2 functional prediction result (D=Probably damaging, P=Possibly damaging, B=Bening)
MT: Mutation Taster functional prediction result (D=Disease Causing, N=Polymorphism)
ZBTB41:NM_194314:exon10:c.G2539A:p.D847N
OPTC:NM_014359:exon7:c.G893A:p.R298H
CCDC83:NM_001286159:exon5:c.A470G:p.N157S,CCDC83:NM_173556:exon5:c.A470G:p.N157S
GRCh37/hg19 genome version was used

Figure 1. Variant segregation confirmation by TaqMan assays

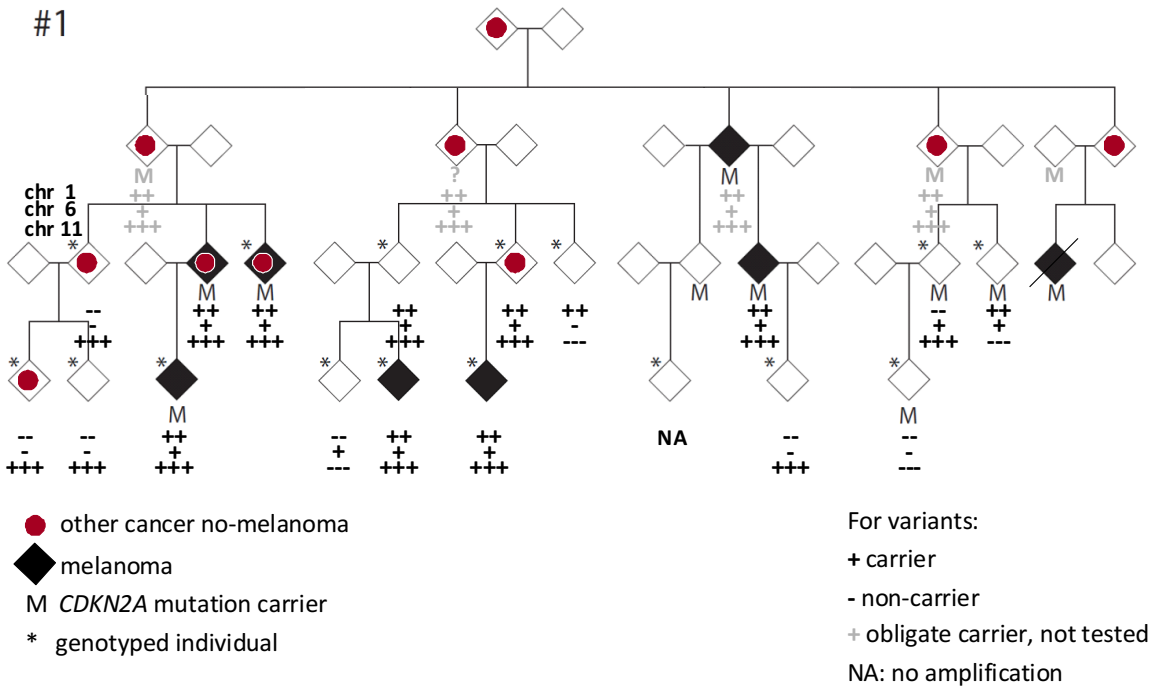

Table 2. Genetic candidate variants prevalence in controls and index cases

chr	Genomic change	Aminoacid change	Gene	Variant type	ExAC	1000G	Spanish controls	Index cases from melanoma families
1	g.197128680C>T	p.D847N	*ZBTB41*	exonic non-synonymous	0.0007	0.001	4/500 (0.008)	3/294 (0.010)
1	g.203472742G>A	p.R298H	*OPTC*	exonic non-synonymous	0.0001	-	1/500 (0.002)	3/299 (0.010)
6	g.14708411T>C	-	*LINC01108 JARID2*	intergenic	-	-	0/500 -	0/271 -
11	g.75637708C>A	-	*UVRAG*	intronic	-	0.001	0/500 -	0/294 -
11	g.85597369A>G	p.N157S	*CCDC83*	exonic non-synonymous	1.56E-05	-	0/500 -	0/293 -
11	g.85956484G>A	-	*EED*	intronic	-	-	3/500 (0.006)	3/289 (0.010)

We assessed the prevalence of the 6 variants in 500 cancer-free control individuals from Spain and a set of 300 index cases from *CDKN2A*-wildtype melanoma-prone families from Barcelona (Table 2).

Considering that the region in chromosome 11 is segregating not only with melanoma but other cancers in the family, that this region contains a non-synonymous mutation predicted to be pathogenic, we think that it is worth to assess the possible role of *CCDC83* in melanoma susceptibility.
This gene function is not well established. *CCDC83* has been reported as a possible cancer/testis antigen (Song *et al. Int J Oncol. 2012 Nov;41(5):1820-6*). Its expression in normal tissues is very low, except for testis, but it is expressed in multiple cancer cell lines including melanoma (*Cancer Cell Line Encyclopedia, https://portals.broadinstitute.org/ccle/home*). Furthermore *CCDC83* is mutated in melanoma and other tumors (*COSMIC, http://cancer.sanger.ac.uk/cosmic/*)

IV. Specific Aims

Evaluate whether germline *CCDC83* variants play a role in melanoma susceptibility.

V. Methods

Collect *CCDC83* data from melanoma-prone families and controls

Experimental plan:

1. To check the prevalence of loss of function CCDC83 and potentially interesting rare missense *CCDC83* variants (including the one detected in our study) in melanoma-prone families. If possible, complete cosegregation in families, assess LOH in tissue, recontact patients when necessary.
2. To collect information of other cancer information in the families with variants in CCDC83
3. To assess if we have a statistically significant enrichment of CCDC83 variants compared to controls.
4. To perform functional studies. Depending on the results of the previous steps. Time schedule to be defined.

VI. Materials or variables needed from the study PIs

CCDC83 germline variants data available from melanoma cases in families, unrelated cases and controls.

VII. Time line

Answer in principle on willingness to participate in the project- July 2017.
Verify cosegregation in families and LOH in tissue when available – November 2017.
End of data collection -November 2017
Prepare a report with the results to share with every group participating – December 2017
Planning functional studies – January 2018
Paper Drafting – during 2018 if functional analysis are available

VIII. Funding Sources and Declaration of Conflict of Interests. *To ensure full transparency and to protect collaborating study PIs, MelaNostrum requires the Project Leaders to disclose any circumstances that could give rise to a potential conflict of interests related to the proposed project activity in particular, or to melanoma in general, including but not limited to funding sources, employment and consulting, board membership and investment interests within the last 5 years.*

Each group's funding for its own sequencing
Teresa Landi's funding for Melanostrum sequencing
No conflict of interest

IX. Other remarks (e.g. dissemination plan, etc)

Publication time
To be discussed in the report prepared at the end of 2017. Maybe 2018?

Authorship
The study won't interfere with Melanostrum and each group publications plans.

Proposed authorship:
First author: Miriam Potrony
First co-authors: Main responsible for sequencing data analysis within Melanostrum and David Adams' group. Main responsible for functional analysis performance.
Co-authors: at least all PI accepting to share their data to perform this study. Order of appearance could correspond to the total number of families included, or families with variants detected. Other team members inclusion to be discussed based on the results and collaboration for additional work.

Last co-authors (order to be decided): Susana Puig, Teresa Landi, David Adams
Corresponding author: Susana Puig

To be discussed in detail with other participating groups from GenoMEL based on the number of families and relevant work.