# Modelling MC1R rare variants: A structural evaluation of variants detected in a Mediterranean case-control study.

Maider Ibarrola-Villava[1], Maria Peña-Chilet[1], Marta J. Llorca-Cardeñosa[1], Sara Oltra[1], Conrado-Martinez-Cadenas[2], Jeronimo Bravo[3,*], Gloria Ribas[1,*].

[1]Department of Haematology and Medical Oncology, INCLIVA Biomedical Research Institute, 46010, Valencia, Spain;

[2]Department of Medicine, University of Castellon Jaume I, 12071, Castellon, Spain.

[3]Department of genomics and proteomics. Instituto de Biomedicina de Valencia - CSIC, 46010, Valencia, Spain.

To whom correspondence should be addressed:

*Dr. Gloria Ribas.

Department of Haematology and Medical Oncology, INCLIVA Biomedical Research Institute, Av. Blasco Ibañez 17, 46010, Valencia, Spain.

Telephone number: +34963862894; FAX: +34963987860

Email: gribasdespuig@gmail.com

*Dr. Jeronimo Bravo.

Department of genomics and proteomics. Instituto de Biomedicina de Valencia - CSIC. Calle Jaume Roig 11, 46010, Valencia, Spain.

Telephone number: +34963391760; FAX: +34963690800.

Email: jbravo@ibv.csic.es

**TO THE EDITOR**

Many diseases are caused by single amino acid substitutions on key genes due to loss of stability, protein misfolding and changes in the interaction properties (Goldberg, 2003; Honig and Nicholls, 1995). Protein structures can be used to evaluate the effect of missense mutations on disease associated proteins, especially on highly polymorphic genes, where functional studies are likely to be incomplete.

Considering the involvement of the highly polymorphic melanocortin-1 receptor gene (*MC1R*) in melanoma predisposition, we investigated the role of the rare variants (minor allele frequency <1%) found in this gene in the Spanish population. A structural analysis was performed by evaluating the amino acid substitutions in the context of a three-dimensional model and comparing their effects with available functional data.

The *MC1R* gene, which is located on chromosome 16q24.3 and codes for a melanocyte-stimulating hormone receptor with seven transmembrane helices, is to date, the main genetic contributor to sporadic melanoma predisposition (Kennedy *et al.*, 2001). It has already been associated to melanoma in different Caucasian populations (Fernandez *et al.*, 2007; Ibarrola-Villava *et al.*, 2010; Ibarrola-Villava *et al.*, 2012). It is highly polymorphic, with more than 100 non-conservative reported variants (Gerstenblith *et al.*, 2007). Most of these changes are relatively rare, but their frequencies vary among populations, and it appears that they are more commonly found in South European populations than in those of North European descent (Gerstenblith *et al.*, 2007; Williams *et al.*, 2011).

Of the 1170 individuals sequenced (710 non-related melanoma cases and 460 cancer-free controls), 697 (59.6%) carried at least one *MC1R* variant, with 78 (11.2%) of them actually carrying a rare variant. Overall, 6.7% (78 of 1170 individuals) of the Spanish population carry an *MC1R* rare variant. We detected a total of 41 different *MC1R* variants. Among these, 31 variants appear rarely in Spain and variant Y298H appeared for the first time in our population. We have obtained statistically significant individual associations with melanoma for six

variants: V60L, S83P, R151C, I155T, R160W and D294H (Supplementary Table S1). The highest OR was estimated for I155T and R160W (OR: 3.81; p-*value*=0.003 and OR: 3.83; p-*value*=0.001 respectively). The estimated OR associated with carrying at least one non-synonymous variant was 1.79 (p-*value* = $4.93x10^{-11}$). However, the OR for carrying only one rare variant was 0.97 (p-*value* = 0.93). There are many common non-synonymous *MC1R* variants associated with a red hair colour (RHC) phenotype: D84E, R142H, R151C, I155T, R160W and D294H. The association of other common variants such as V60L, V92M and R163Q with the RHC phenotype has not been clearly established, and therefore these variants have been considered non-red hair colour (NRHC) (Wong and Rees, 2005). Almost all carriers of rare changes in the Spanish population tend to have fair skin colour. However, only 40% of them also harboured an RHC or NRHC variant. Thus, rare variants might slightly modulate skin pigmentation.

Different studies have demonstrated the functional consequences of some of these *MC1R* changes. Thus, mutations in positions 84, 142, 151, 160 and 294 have been previously reported to alter protein function (Dessinioti *et al.*, 2011; Garcia-Borron *et al.*, 2005; Newton *et al.*, 2007; Ringholm *et al.*, 2004; Sanchez-Laorden *et al.*, 2009). In addition, previous functional studies have validated some of the 3D model prediction results. Position M128 appears to have no effect according to *in silico* predictions; however, the 3D model suggests it could be a putative folding disrupter, later confirmed in a functional study (Perez Oliva *et al.*, 2009).

Due to the importance of *MC1R* variants in pigmentation and melanoma predisposition we have undertaken a comprehensive three-dimensional structure modelling of 29 non-synonymous changes detected in our population in order to further explain putative implications of rare variants (Table 1 and Figure 1). After evaluating all changes according to MC1R structure, variant location and nature of the amino acid changes, 25 variants seemed to have structural implications and cluster in three different regions that may explain their differences in functionality: the GTPase/PKC signalling region, an alleged α-MSH-binding extracellular region, and a novel central core protein region. The first and better-known region harbours some

of the common RHC (R142H, R151C, I155T and R160W) and NRHC (R163Q) variants. Although these variants do not seem to bring about structural changes under the 3D-model, they are located in an intracellular region thought to be a GTPase and PKC interaction area that initiates the receptor downstream signalling pathway and internalization of the receptor which confirms previously reported functionality (Dessinioti *et al.*, 2011; Garcia-Borron *et al.*, 2005; Newton *et al.*, 2007). Interestingly, side chains of residues I155, T157, R160 and R163 define a common solvent accessible area, a PKC binding domain comprising $^{157}$TLPR$^{160}$ and $^{160}$ARR$^{163}$ (Sanchez-Laorden *et al.*, 2009), and therefore, variants in these positions could explain impairment of PKC activity. Rare variants R142C, R213W and T308M are located near this region.

The second important region includes the rest of the RHC (D84E and D294H) and NRHC (V60L and V92M) variants, plus other rare variants (S83P, G89R, M128T and Y298H). Variants located in this central region may be involved in protein folding processes, and therefore may be important in the maintenance of the receptor's integrity.

Finally, we propose a third new functional region which includes S41F, P268R, T272M, K278E, N281S and A285V rare variants. All except S41F are located in the extracellular end helical tips and though there is no evident structural implication, we believe this domain may be involved in α-MSH union (Perez Oliva *et al.*, 2009).

Rare variants located in positions 41, 128, 89, 213 and 308 are described to alter protein function (Ozola *et al.*, 2013; Perez Oliva *et al.*, 2009; Sanchez-Laorden *et al.*, 2007). M128T and R213W showed markedly loss of function and displayed reduced cell surface expression and agonist binding affinity while variants S41F and G89R showed almost complete loss of function with no detectable cell surface expression and complete inability to activate the cAMP pathway. Finally, residue T308 is described to be implicated in protein phosphorilation. Therefore, the ensuing outcomes of this *MC1R* variant modelling are highly consistent with data from previously published functional *MC1R* studies.

To sum up, changes S41F, S83P, G89R, R142C, M128T, R213W, P268R, Y298H and T308M (Figure 1b, highlighted in deep blue) are predicted to have structural consequences, while variants T272M, K278E, N279K, N281S and A285V (Figure 1b highlighted in cyan) may have mild or no structural implications, but may be potentially important for protein recognition due to their near extracellular location. SNP3d *in silico* predictions represent a useful tool to evaluate amino acid change effects in a protein structure. However, they can only discriminate between damaging or benign effect. Our model combines folding effects, nature of the amino acid substitution and location of both individual variants and clusters.. The use of 3D models may constitute a robust and exploratory prediction method, as it combines folding and visualization of the amino acid changes, and therefore could be a promising tool in order to assess further functional *MC1R* variant studies. To our knowledge, this is the first time that a large number of rare *MC1R* variants are evaluated by taking into account the structural effects of the resulting residue changes.

## CONFLICT OF INTEREST

The authors state no conflict of interest.

## ACKNOWLEDGEMENTS

This study was supported by a Grant from the Ministerio de Salud Carlos III (ISCIII) (FI10-00405) and Ministerio de Economia y Competitividad (SAF2012-31405). MP-C is funded by the VALi+d from the Generalitat Valenciana (ACIF/2011/207). MJLC is funded by the Generalitat Valenciana under a Geronimo Forteza contrat (FPA/2013/A/037). GR is funded by the Ministerio de Salud Carlos III under a ‘Miquel Servet’ contract (CP08-00069). We thank the Madrid College of Lawyers and all the participants from all contributing Hospitals.

## REFERENCES

Dessinioti C, Antoniou C, Katsambas A*, et al.* (2011) Melanocortin 1 receptor variants: functional role and pigmentary associations. *Photochem Photobiol* 87:978-987.

Fernandez L, Milne R, Bravo J*, et al.* (2007) MC1R: three novel variants identified in a malignant melanoma association study in the Spanish population. *Carcinogenesis* 28:1659-1664.

Garcia-Borron JC, Sanchez-Laorden BL, Jimenez-Cervantes C (2005) Melanocortin-1 receptor structure and functional regulation. *Pigment Cell Res* 18:393-410.

Gerstenblith MR, Goldstein AM, Fargnoli MC*, et al.* (2007) Comprehensive evaluation of allele frequency differences of MC1R variants across populations. *Hum Mutat* 28:495-505.

Goldberg AL (2003) Protein degradation and protection against misfolded or damaged proteins. *Nature* 426:895-899.

Honig B, Nicholls A (1995) Classical electrostatics in biology and chemistry. *Science* 268:1144-1149.

Ibarrola-Villava M, Fernandez LP, Pita G*, et al.* (2010) Genetic analysis of three important genes in pigmentation and melanoma susceptibility: CDKN2A, MC1R and HERC2/OCA2. *Exp Dermatol* 19:836-844.

Ibarrola-Villava M, Hu HH, Guedj M*, et al.* (2012) MC1R, SLC45A2 and TYR genetic variants involved in melanoma susceptibility in southern European populations: results from a meta-analysis. *Eur J Cancer* 48:2183-2191.

Kennedy C, ter Huurne J, Berkhout M*, et al.* (2001) Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color. *J Invest Dermatol* 117:294-300.

Newton RA, Roberts DW, Leonard JH*, et al.* (2007) Human melanocytes expressing MC1R variant alleles show impaired activation of multiple signaling pathways. *Peptides* 28:2387-2396.

Ozola A, Azarjana K, Donina S*, et al.* (2013) Melanoma risk associated with MC1R gene variants in Latvia and the functional analysis of rare variants. *Cancer Genet* 206:81-91.

Perez Oliva AB, Fernendez LP, Detorre C*, et al.* (2009) Identification and functional analysis of novel variants of the human melanocortin 1 receptor found in melanoma patients. *Hum Mutat* 30:811-822.

Ringholm A, Klovins J, Rudzish R*, et al.* (2004) Pharmacological characterization of loss of function mutations of the human melanocortin 1 receptor that are associated with red hair. *J Invest Dermatol* 123:917-923.

Sanchez-Laorden BL, Herraiz C, Valencia JC*, et al.* (2009) Aberrant trafficking of human melanocortin 1 receptor variants associated with red hair and skin cancer: Steady-state retention of mutant forms in the proximal golgi. *J Cell Physiol*.

Sanchez-Laorden BL, Jimenez-Cervantes C, Garcia-Borron JC (2007) Regulation of human melanocortin 1 receptor signaling and trafficking by Thr-308 and Ser-316 and its alteration in variant alleles associated with red hair and skin cancer. *J Biol Chem* 282:3241-3251.

Williams PF, Olsen CM, Hayward NK*, et al.* (2011) Melanocortin- 1-receptor and risk of cutaneous melanoma: A meta-analysis and estimates of population burden. *Int J Cancer*.

Wong TH, Rees JL (2005) The relation between melanocortin 1 receptor (MC1R) variation and the generation of phenotypic diversity in the cutaneous response to ultraviolet radiation. *Peptides* 26:1965-1971.

# TABLES

Table 1. *In silico* analysis of *MC1R* variants

| Mutation | SNPs3d | Polyphen | Pub Funct data | 3D-Model | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Possible effects of mutation | Folding | Surface exposed | Region[a] | Predict |
| V60L (r) | Benign | ● | F | Conserved change | No effect | Yes | Core | ▨ |
| D84E (R) | Affect | ● | F | Conserved charge larger residue | Affect | No | Core | ▨ |
| V92M (r) | Benign | ○ | NF | Conserved change | No effect | No | - | ▨ |
| R142H (R) | Affect | ● | F | Cytosolic conserved | No effect | No | PKC/GTPase sig. | ▨ |
| R151C (R) | Affect | ● | F | Cytosolic non conserved | No effect | Yes | PKC/GTPase sig. | ▨ |
| I155T (R) | Affect | ● | F | Cytosolic non conserved | No effect | Yes | PKC/GTPase sig. | ▨ |
| R160W (R) | Affect | ◍ | F | Cytosolic conserved | No effect | Yes | PKC/GTPase sig. | ▨ |
| R163Q (r) | Benign | ○ | F | Cytosolic conserved | No effect | Yes | PKC/GTPase sig. | ▨ |
| D294H (R) | Affect | ● | F | Charge change | Affect | No | Core | ▨ |
| C35Y | Affect | ● | F | Non conserved | No effect | Yes | - | ▨ |
| S41C | Affect | ○ | - | Conserved change | No effect | No | MSH binding | ▨ |
| S41F | Affect | ○ | F | Bulkier hydrophobic change | Affect | No | MSH binding | ▨ |
| F45L | Affect | ● | - | Conserved change | No effect | No | - | ▨ |
| S83P | Affect | ● | - | Induces distortion of helix 2 | Affect | No | Core | ▨ |
| G89R | Affect | ◍ | F | Charged bulkier residue | Affect | Yes | Core | ▨ |
| V92L | Benign | ○ | - | Conserved change | No effect | No | Core | ▨ |
| T95M | Affect | ◍ | - | Conserved change | No effect | No | - | ▨ |
| V122M | Benign | ○ | - | Conserved change | No effect | Yes | - | ▨ |
| M128T | Benign | ○ | F | Hydrophobic to small polar | Affect | No | Core | ▨ |
| R142C | Affect | ● | - | Non conserved | Affect | No | PKC/GTPase sig. | ▨ |
| R213W | Affect | ○ | F | Bulky hydrophobic replacement | Affect | Yes | PKC/GTPase sig. | ▨ |
| P268R | Affect | ● | - | Charged bulkier residue | Affect | Yes | MSH binding | ▨ |
| T272M | Affect | ● | F | Change to large apolar | No effect | Yes | MSH binding | ▨ |
| K278E | Benign | ◍ | - | Charge change | No effect | Yes | MSH binding | ▨ |
| N279K | Affect | ● | - | Non conserved change | No effect | Yes | MSH binding | ▨ |
| N281S | Affect | ○ | NF | Change to smaller residue | No effect | Yes | MSH binding | ▨ |
| A285V | Benign | ○ | - | Bulkier residue | No effect | Yes | MSH binding | ▨ |
| Y298H | Affect | ● | - | Charge change | Affect | No | Core | ▨ |
| T308M | Affect | ● | - | Change to large apolar | Affect | Yes | PKC/GTPase sig. | ▨ |

SNPs3d (http://www.snps3d.org/) and Polyphen (http://genetics.bwh.harvard.edu/pph2/) online tools
Pub Funct data: Previous published functional data on different *MC1R* variants
3D-Model: three dimensional model variant evaluation according to MC1R structure and variant location
Predict: Global evaluation of prediction model
(R), Red hair colour variants; (r), Non red hair colour variants
F, functional data available; NF, non-funtional data available, -, non described
● Probably damaging; ◍ Possibly damaging; ○ Benign
▨ Predictive effect; ▨ Predictive No effect
[a]Location in the three putative regions described by our group, PKC/GTPase sig., GTPase signalling; (-) estimated neutral variants that do not correspond to any sub-region of the protein

**FIGURE LEGENDS**

Figure 1: MC1R three-dimensional model. Representation of 25 non-synonymous variants described in the Spanish population. a) Location of Red Hair Color (RHC) and Non Red Hair Color (NRHC) variants. Positions RHC are colored in red whereas NRHC are colored in orange. b) Location of potentially-functional rare variants according to their structure. Positions colored in deep blue and cyan. c) Magnification of receptor's core central region. d) Detail of important residues in PKC MC1R binding domain. Residues are colored according to atom nature. Oxygen in red and nitrogen in purple.