ASSOCIATION OF MELANOCORTIN-1 RECEPTOR VARIANTS WITH PIGMENTARY TRAITS IN HUMANS: A POOLED-ANALYSIS FROM THE M-SKIP PROJECT

Elena Tagliabue (1), Sara Gandini (1), José C García-Borrón (2), Patrick Maisonneuve (1), Julia Newton-Bishop (3), David Polsky (4), DeAnn Lazovich (5), Rajiv Kumar (6), Paola Ghiorzo (7)(8), Leah Ferrucci (9), Nelleke A Gruis (10), Susana Puig (11), Peter A Kanetsky (12), Tomonori Motokawa (13), Gloria Ribas (14), Maria Teresa Landi (15), Maria Concetta Fargnoli (16), Terence H Wong (17), Alexander Stratigos (18), Per Helsing (19), Gabriella Guida (20), Philippe Autier (21), Jiali Han (22), Julian Little (23), Francesco Sera (24) and Sara Raimondi (1) for the M-SKIP Study group

(1) Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy; (2) Department of Biochemistry, Molecular Biology and Immunology, University of Murcia and IMIB-Arrixaca, Murcia, Spain; (3) Section of Epidemiology and Biostatistics, Institute of Cancer and Pathology, University of Leeds, Leeds, UK; (4) The Ronald O. Perelman Department of Dermatology, New York University School of Medicine, NYU Langone Medical Center, New York, NY, USA; (5) Division of Epidemiology and Community Health, University of Minnesota, MN, USA; (6) Division of Molecular Genetic Epidemiology, German Cancer Research Center, Heidelberg, Germany; (7) Department of Internal Medicine and Medical Specialties, University of Genoa, Italy; (8) IRCCS AOU San Martino-IST, Genoa, Italy (9) Department of Chronic Disease Epidemiology, Yale School of Public Health, Yale Cancer Center, New Haven, CT, USA; (10) Department of Dermatology, Leiden University Medical Center, Leiden, The Netherlands; (11) Melanoma Unit, Dermatology Department, Hospital

Clinic Barcelona; University of Barcelona; CIBER de Enfermedades Raras, Spain; (12) Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute2, Tampa, FL, USA; (13) Skin Research Department, POLA Chemical Industries, Yokohama, Japan; (14) Department of medical oncology and hematology, Fundación Investigación Clínico de Valencia Instituto de Investigación Sanitaria- INCLIVA, Valencia, Spain; (15) Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, MD, USA; (16) Department of Dermatology, University of L'Aquila, L'Aquila, Italy; (17) NHS Forth Valley, UK; (18) First Department of Dermatology, Andreas Sygros Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece; (19) Department of Pathology, Oslo University Hospital, Oslo, Norway; (20) Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari, Bari, Italy; (21) International Prevention Research Institute, Lyon, France; (22) Department of Epidemiology, Richard M. Fairbanks School of Public Health, Melvin & Bren Simon Cancer Center, Indiana University, Indianapolis, USA;(23) School of Epidemiology, Public Health and Preventive Medicine, University of Ottawa, Ottawa, Canada; (24) Department of Social and Environmental Health Research, London School of Hygiene & Tropical Medicine, London, UK

Short title: MC1R and pigmentary traits: a pooled-analysis

Abbreviation used: MC1R, melanocortin-1 receptor; RHC, red hair color; UVR, ultra violet radiation; α-MSH, α-melanocyte stimulating hormone; cAMP, cyclic adenosine monophosphate; NMSC, Non Melanoma Skin Cancer; WT, wild-type; HW, Hardy-Weinberg; SORs, Summary Odds Ratios; CI, Confidence Interval; MCA, multiple correspondence analysis

* **Correspondence to:**

Sara Raimondi, PhD

Division of Epidemiology and Biostatistics

European Institute of Oncology

Via Ripamonti 435, 20141 Milan, Italy

Tel: +39 02 94372711

Fax: +39 02 57489922

Email: sara.raimondi@ieo.it.

To the Editor

Skin pigmentation is due to the accumulation of eumelanin, which is brown-black pigment and photoprotective, and pheomelanin, which is yellow-red pigment and may promote carcinogenesis (Valverde et al., 1995). The melanocortin-1 receptor (MC1R) gene regulates the amount and type of pigment production and is a major determinant of skin phototype (Garcia-Borron et al., 2005; Valverde et al., 1995). Binding of α-melanocyte stimulating hormone (α-MSH) to MC1R stimulates the enzymatic activity of adenylate cyclase enzyme, thereby elevating intracellular cyclic adenosine monophosphate (cAMP) levels. MC1R is a highly polymorphic, especially in Caucasian: more than 200 coding region variants have been described to date (Garcia-Borron et al., 2014; Gerstenblith et al., 2007; Perez Oliva et al., 2009). Six variants - D84E, R142H, R151C, I155T, R160W and D294H - have been designated as 'R' alleles due to their strong association with the 'red hair color' (RHC) phenotype characterized by red hair, fair skin, freckles and sun sensitivity. The V60L, V92M and R163Q variants are found to have a weaker association with the RHC phenotype and have been designated as 'r' alleles (Garcia-Borron et al., 2014; Raimondi et al., 2008).

Previous studies demonstrated that several alleles are associated with phenotypic characteristics and that MC1R variants are associated both with melanoma and non-melanoma skin cancer (NMSC) (Han et al., 2006; Pasquali et al., 2015; Scherer et al., 2008; Tagliabue et al., 2015) with a stronger role for darker-pigmented populations, suggesting that non-pigmentary pathways link MC1R with skin cancer development. Since the role and strength of each MC1R variant in determining specific phenotypic characteristics and the RHC phenotype remains unclear, we performed a pooled-analysis of individual-level data from the M-SKIP project, described in full elsewhere (Raimondi et al., 2012). We selected from the M-SKIP database all 5,366 cancer-free

controls with MC1R gene sequenced and information on at least one of the following phenotypic characteristics: hair color, eye color, skin type and freckles, thus including 16 independent studies from 18 publications (Table S1).

We found greater Summary Odds Ratios (SORs) for carriers of two MC1R variants compared with carriers of only one variant allele (Table 1). Furthermore carriage of any MC1R variant, one variant and two or more variants, compared with not having such variants (i.e. wild-type (WT) subjects), were significantly associated with fair hair color, skin type I/II and presence of freckles. Red hair color was significantly associated with carrying any MC1R variant (SOR; 95%CI: 3.54; 1.91-6.55) and with carrying two or more variants (SOR; 95%CI: 10.17; 5.28-19.58), but not with carrying one MC1R variant (SOR; 95%CI: 1.18; 0.57-2.44). No significant association was observed for light eye color and MC1R. Sensitivity analyses indicated that the observed between-study heterogeneity may be attributable to single studies: when we excluded the studies that were outliers, we obtained similar pooled-ORs as the original ones, but no longer with evidence of heterogeneity (results not shown). No evidence of publication bias was found by Egger's test. All the investigated MC1R variants compared with WT subjects were positively associated with skin type I/II and freckles (Table S2). The three variants that seemed to play the most important role in skin type determination and presence of freckles were D84E, R151C and D294H. Red hair color was significantly associated with all MC1R variants except for V92M and R163Q.

We visualized the associations between hair color, eye color, skin type, freckles and the three main studied geographical areas by Multiple Correspondence Analysis (MCA) (Figure S1a/b). A two-dimension MCA solution, with Dimension 1 (Dim1) on the horizontal axis and Dimension 2 (Dim2) on the vertical axis, was considered the most adequate because the first and second

dimension presented Benzecri-adjusted inertias of 85.31% and 11.31% respectively (Table S3), accounting for 96.62% of the total association. The extreme RHC phenotype (red-hair, skin type I and freckles) was associated either with carrying at least 2 MC1R variants (Figure S1a) or with the presence of major penetrant ('R') alleles (Figure S1b). We suggest that Dim1 can be interpreted as a "pigmentation score" because it differentiates well between dark and fair phenotypic characteristics. The median pigmentation score increased with increasing number of MC1R variants, and for single MC1R variants it was higher ($p<0.0001$) compared with WT subjects (Figure S2).

Seven of the nine MC1R variants analyzed in this study, V60L, D84E, R142H, R151C, I155T, R160W and D294H, are clearly hypomorphic with significant reduction in cAMP signaling potential (Beaumont et al., 2007; Herraiz et al., 2012; Kadekaro et al., 2010; Scott et al., 2002). Within this group of variants, the lowest SOR for red hair, skin type I/II or freckles corresponds to V60L. Interestingly, this variant was also the one with the smallest functional impairment in terms of coupling to the cAMP pathway, when the seven variants analyzed here were compared under identical experimental conditions (Herraiz et al., 2012).

Results also showed that V92M and R163Q behave as 'r' alleles, with a weak albeit significant association with cutaneous phenotypic traits. In heterologous systems, V92M has been reported to display either a slight functional impairment (Herraiz et al., 2012) or normal coupling to the cAMP pathway (Beaumont et al., 2007), whereas R163Q apparently signals as efficiently as WT. Therefore, it appears that the ability of V92M or R163Q to activate the cAMP pathway is similar, if not identical to WT. This suggests that other mechanisms account for their association with cutaneous phenotypic characteristics, for example, V92M or R163Q might impair functional coupling to signaling module(s) different from the cAMP cascade. MC1R

promiscuously binds to a variety of intracellular partners with signaling potential and this ability might depend on WT conformation. However, little is known as to the effects of other variants on MC1R binding to its various protein partners, and the phenotypic consequences of such molecular interactions also remain largely unknown. Further research is needed to understand the scaffolding properties of MC1R, the functional consequences of the formation of signaling complexes orchestrated by the receptor, and the effects on these processes of the myriad of natural variants in the MC1R gene.

Conflict of Interest Disclosures: None reported

Acknowledgments:

This work was supported by the Italian Association for Cancer Research (grant number: MFAG 11831). The Melanoma Susceptibility Study (PAK) was supported by the National Cancer Institute [CA75434, CA80700, CA092428]. The Nurses' Health Study and the Health Professionals Follow-Up Study (JH) were supported by NIH R01 CA49449, P01 CA87969, UM1 CA186107, and UM1 CA167552. We would like to thank the participants and staff of the Nurses' Health Study, the Health Professionals Follow-Up Study for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. Genoa study was supported by AIRC IG 15460 to PG.

The M-SKIP study group consists of the following members: Principal Investigator: Sara Raimondi (European Institute of Oncology, Milan, Italy); Advisory Committee members: Philippe Autier (International Prevention Research Institute, Lyon, France), Maria Concetta Fargnoli (University of L'Aquila, Italy), José C. García-Borrón (University of Murcia, Spain), Jiali Han (Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA), Peter A. Kanetsky (Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA), Maria Teresa Landi (National Cancer Institute, NIH, Bethesda, MD, USA), Julian Little (University of Ottawa, Canada), Julia Newton-Bishop (University of Leeds, UK), Francesco Sera (UCL Institute of Child Health, London, UK); Consultants: Saverio Caini (ISPO, Florence, Italy), Sara Gandini and Patrick Maisonneuve (European Institute of Oncology, Milan, Italy); Participant Investigators: Albert Hofman,

Manfred Kayser, Fan Liu, Tamar Nijsten and Andre G. Uitterlinden (Erasmus MC University Medical Center, Rotterdam, The Netherlands), Rajiv Kumar and Dominique Scherer (German Cancer Research Center, Heidelberg, Germany), Tim Bishop, Julia Newton-Bishop and Faye Elliott (University of Leeds, UK), Eduardo Nagore (Instituto Valenciano de Oncologia, Valencia, Spain), DeAnn Lazovich (Division of Epidemiology and Community Health, University of Minnesota, MN, USA), David Polsky (New York University School of Medicine, New York, NY, USA), Johan Hansson and Veronica Hoiom (Karolinska Institutet, Stockholm, Sweden), Paola Ghiorzo and Lorenza Pastorino (University of Genoa, Italy), Nelleke A. Gruis and Jan Nico Bouwes Bavinck (Leiden University Medical Center, The Netherlands), Paula Aguilera, Celia Badenas, Cristina Carrera, Pol Gimenez-Xavier, Josep Malvehy, Miriam Potrony, Susana Puig, Joan Anton Puig-Butille, Gemma Tell-Marti (Hospital Clinic, IDIBAPS and CIBERER, Barcelona, Spain), Terence Dwyer (Murdoch Childrens Research Institute, Victoria, Australia), Leigh Blizzard and Jennifer Cochrane (Menzies Institute for Medical Research, Hobart, Australia), Ricardo Fernandez-de-Misa (Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain), Wojciech Branicki (Institute of Forensic Research, Krakow, Poland), Tadeusz Debniak (Pomeranian Medical University, Polabska, Poland), Niels Morling and Peter Johansen (University of Copenhagen, Denmark), Susan Mayne, Allen Bale, Brenda Cartmel and Leah Ferrucci (Yale School of Public Health and Medicine, New Haven, CT, USA), Ruth Pfeiffer (National Cancer Institute, NIH, Bethesda, MD, USA), Giuseppe Palmieri (Istituto di Chimica Biomolecolare, CNR, Sassari, Italy), Gloria Ribas (Fundación Investigación Clínico de Valencia Instituto de Investigación Sanitaria- INCLIVA, Spain), Chiara Menin (Veneto Institute of Oncology, IOV-IRCCS, Padua, Italy), Alexander Stratigos and Katerina Kypreou (University of Athens, Andreas Sygros Hospital, Athens,

Greece), Anne Bowcock, Lynn Cornelius and M. Laurin Council (Washington University School of Medicine, St. Louis, MO, USA), Tomonori Motokawa (POLA Chemical Industries, Yokohama, Japan), Sumiko Anno (Shibaura Institute of Technology, Tokyo, Japan), Per Helsing and Per Arne Andresen (Oslo University Hospital, Norway), Gabriella Guida and Stefania Guida (University of Bari, Bari, Italy), Terence H. Wong (University of Edinburgh, UK), and the GEM Study Group.

Participants in the GEM Study Group are as follows: Coordinating Center, Memorial Sloan-Kettering Cancer Center, New York, NY, USA: Marianne Berwick (PI, currently at the University of New Mexico), Colin Begg (Co-PI), Irene Orlow (Co-Investigator), Urvi Mujumdar (Project Coordinator), Amanda Hummer (Biostatistician), Klaus Busam (Dermatopathologist), Pampa Roy (Laboratory Technician), Rebecca Canchola (Laboratory Technician), Brian Clas (Laboratory Technician), Javiar Cotignola (Laboratory Technician), Yvette Monroe (Interviewer). Study Centers: The University of Sydney and The Cancer Council New South Wales, Sydney (Australia): Bruce Armstrong (PI), Anne Kricker (co-PI), Melisa Litchfield (Study Coordinator). Menzies Institute for Medical Research, University of Tasmania, Hobart (Australia): Terence Dwyer (PI), Paul Tucker (Dermatopathologist), Nicola Stephens (Study Coordinator). British Columbia Cancer Agency, Vancouver (Canada): Richard Gallagher (PI), Teresa Switzer (Coordinator). Cancer Care Ontario, Toronto (Canada): Loraine Marrett (PI), Beth Theis (Co-Investigator), Lynn From (Dermatopathologist), Noori Chowdhury (Coordinator), Louise Vanasse (Coordinator), Mark Purdue (Research Officer). David Northrup (Manager for CATI). Centro per la Prevenzione Oncologia Torino, Piemonte (Italy): Roberto Zanetti (PI), Stefano Rosso (Data Manager), Carlotta Sacerdote (Coordinator). University of California, Irvine (USA): Hoda Anton-Culver (PI), Nancy Leighton (Coordinator), Maureen

Gildea (Data Manager). University of Michigan, Ann Arbor (USA): Stephen Gruber (PI), Joe Bonner (Data Manager), Joanne Jeter (Coordinator). New Jersey Department of Health and Senior Services, Trenton (USA): Judith Klotz (PI), Homer Wilcox (Co-PI), Helen Weiss (Coordinator). University of North Carolina, Chapel Hill (USA): Robert Millikan (PI), Nancy Thomas (Co-Investigator), Dianne Mattingly (Coordinator), Jon Player (Laboratory Technician), Chiu-Kit Tse (Data Analyst). University of Pennsylvania, Philadelphia, PA (USA): Timothy Rebbeck (PI), Peter Kanetsky (Co-Investigator), Amy Walker (Laboratory Technician), Saarene Panossian (Laboratory Technician). Consultants: Harvey Mohrenweiser, University of California, Irvine, Irvine, CA (USA); Richard Setlow, Brookhaven National Laboratory, Upton, NY (USA).

References

Beaumont KA, Shekar SN, Newton RA, James MR, Stow JL, Duffy DL, et al. Receptor function, dominant negative activity and phenotype correlations for MC1R variant alleles. Hum Mol Genet 2007 Sep 15;16(18):2249-2260.

Garcia-Borron JC, Sanchez-Laorden BL, Jimenez-Cervantes C. Melanocortin-1 receptor structure and functional regulation. Pigment Cell Res 2005 Dec;18(6):393-410.

Garcia-Borron JC, Abdel-Malek Z, Jimenez-Cervantes C. MC1R, the cAMP pathway, and the response to solar UV: extending the horizon beyond pigmentation. Pigment Cell Melanoma Res 2014 Sep;27(5):699-720.

Gerstenblith MR, Goldstein AM, Fargnoli MC, Peris K, Landi MT. Comprehensive evaluation of allele frequency differences of MC1R variants across populations. Hum Mutat 2007 May;28(5):495-505.

Han J, Kraft P, Colditz GA, Wong J, Hunter DJ. Melanocortin 1 receptor variants and skin cancer risk. Int J Cancer 2006 Oct 15;119(8):1976-1984.

Herraiz C, Journe F, Ghanem G, Jimenez-Cervantes C, Garcia-Borron JC. Functional status and relationships of melanocortin 1 receptor signaling to the cAMP and extracellular signal-regulated protein kinases 1 and 2 pathways in human melanoma cells. Int J Biochem Cell Biol 2012 Dec;44(12):2244-2252.

Kadekaro AL, Leachman S, Kavanagh RJ, Swope V, Cassidy P, Supp D, et al. Melanocortin 1 receptor genotype: an important determinant of the damage response of melanocytes to ultraviolet radiation. FASEB J 2010 Oct;24(10):3850-3860.

Pasquali E, Garcia-Borron JC, Fargnoli MC, Gandini S, Maisonneuve P, Bagnardi V, et al. MC1R variants increased the risk of sporadic cutaneous melanoma in darker-pigmented Caucasians: a pooled-analysis from the M-SKIP project. Int J Cancer 2015 Feb 1;136(3):618-631.

Perez Oliva AB, Fernendez LP, Detorre C, Herraiz C, Martinez-Escribano JA, Benitez J, et al. Identification and functional analysis of novel variants of the human melanocortin 1 receptor found in melanoma patients. Hum Mutat 2009 May;30(5):811-822.

Raimondi S, Sera F, Gandini S, Iodice S, Caini S, Maisonneuve P, et al. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. Int J Cancer 2008 Jun 15;122(12):2753-2760.

Raimondi S, Gandini S, Fargnoli MC, Bagnardi V, Maisonneuve P, Specchia C, et al. Melanocortin-1 receptor, skin cancer and phenotypic characteristics (M-SKIP) project: study

design and methods for pooling results of genetic epidemiological studies. BMC Med Res Methodol 2012 Aug 3;12:116-2288-12-116.

Scherer D, Bermejo JL, Rudnai P, Gurzau E, Koppova K, Hemminki K, et al. MC1R variants associated susceptibility to basal cell carcinoma of skin: interaction with host factors and XRCC3 polymorphism. Int J Cancer 2008 Apr 15;122(8):1787-1793.

Scott MC, Wakamatsu K, Ito S, Kadekaro AL, Kobayashi N, Groden J, et al. Human melanocortin 1 receptor variants, receptor function and melanocyte response to UV radiation. J Cell Sci 2002 Jun 1;115(Pt 11):2349-2355.

Tagliabue E, Fargnoli MC, Gandini S, Maisonneuve P, Liu F, Kayser M, et al. MC1R gene variants and non-melanoma skin cancer: a pooled-analysis from the M-SKIP project. Br J Cancer 2015 Jul 14;113(2):354-363.

Valverde P, Healy E, Jackson I, Rees JL, Thody AJ. Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. Nat Genet 1995 Nov;11(3):328-330.

Table 1. Summary Odds Ratios for the association between combined MC1R variants and phenotypic characteristics

Phenotypic characteristic	MC1R	studies/controls	SOR (95% CI)	I^2 (%)	p-value[3]
Hair color - fair vs. dark[1]	Wild-type	13/1371	1.00 (reference)		
	Any variant	13/2758	**1.91 (1.38-2.65)**	**59**	**<0.01**
	1 variant	13/1991	**1.55 (1.12-2.15)**	39	0.07
	2+ variants	13/767	**3.32 (2.34-4.72)**	**62**	**<0.01**
Hair color - red vs. others	Wild-type	7/705	1.00 (reference)		
	Any variant	7/1474	**3.54 (1.91-6.55)**	0	0.80
	1 variant	7/1016	1.18 (0.57-2.44)	0	0.83
	2+ variants	7/458	**10.17(5.28-19.58)**	0	0.77
Eye color - fair vs. dark[2]	Wild-type	14/1530	1.00 (reference)		
	Any variant	14/2832	1.12 (0.96-1.30)	12	0.33
	1 variant	14/2079	1.11 (0.94-1.32)	10	0.35
	2+ variants	14/753	1.16 (0.93-1.45)	0	0.80
Skin type - I, II vs. III, IV	Wild-type	14/1540	1.00 (reference)		
	Any variant	14/3046	**2.26 (1.81-2.83)**	**49**	**0.02**
	1 variant	14/2211	**1.95 (1.51-2.53)**	41	0.06
	2+ variants	14/835	**3.58 (2.68-4.78)**	**42**	**0.05**
Freckles - yes vs. no	Wild-type	9/1067	1.00 (reference)		
	Any variant	9/2257	**2.52 (1.99-3.20)**	33	0.16
	1 variant	9/1528	**2.00 (1.52-2.64)**	36	0.13
	2+ variants	9/729	**4.47 (3.25-6.15)**	38	0.12

SOR=Summary Odds Ratio, CI=Confidence Intervals. Note: significant ORs and p-values are in bold

[1]Fair hair color were: red, blond, dark blonde, light brown. Dark hair color were: brown, black, dark brown. [2]Fair eye color were: blue, green, grey, hazel. Dark eye color were: brown, black. [3]Q test p-value