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MC1R variants increase melanoma risk in families with CDKN2A mutations: A meta-analysis

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ABSTRACT

Aim of the study: We performed a meta-analysis to assess whether *MC1R* variants increase the risk of melanoma in *CDKN2A* mutation carriers of melanoma-prone families.

Methods: Data from 96 *CDKN2A*-positive melanoma-prone families from seven independent populations of Europe, United States and Australia were included in the analysis. Summary risk estimates were calculated by random-effect models. We explored between-study heterogeneity and publication bias. Association between *MC1R* variants and age at diagnosis was assessed by the non-parametric Wilcoxon test.

Results: *CDKN2A* mutation carriers with $\geqslant 1$ *MC1R* variant showed a double melanoma risk as compared to *CDKN2A* mutation carriers without *MC1R* variants (Summary OR; 95%CI: 2.2; 1.1–4.5). *MC1R* heterozygous subjects had no significantly higher melanoma risk than wild-type subjects (1.6; 0.5–5.4) while carriers of multiple *MC1R* variants had a more than four-times higher melanoma risk (4.6; 1.3–16.4). Carriers of red hair colour (RHC) variants showed an increased melanoma risk with a Summary OR of 3.5 (95%CI: 1.3–9.9). *CDKN2A* mutation carriers with *MC1R* variants had a statistically significant lower median age at melanoma diagnosis than *CDKN2A* mutation carriers with no *MC1R* variants (37 years versus 47 years, p-value < 0.0001).

Conclusion: *MC1R* variants significantly increase penetrance of *CDKN2A* mutations in melanoma-prone families, especially with respect to multiple *MC1R* variants and to RHC variants. A significant anticipation of melanoma diagnosis is observed in *CDKN2A* mutation carriers with *MC1R* variants.

1. Introduction

Cutaneous melanoma is a complex and heterogeneous disease resulting from genetic, host/clinical and environmental risk factors. Genetic risk factors for melanoma include germline mutations in the cyclin-dependent kinase inhibitor 2A (*CDKN2A*, 9p21, MIM 600160) gene or in the cyclin-dependent kinase 4 (*CDK4*, 12q14, MIM 123829) gene, designated as 'high-risk' melanoma susceptibility genes, and allelic variants in the melanocortin-1 receptor (*MC1R*, 16q24, MIM 155555) gene, classified as a 'low-risk' melanoma susceptibility gene.[1]

The *CDKN2A* gene is the major known melanoma susceptibility gene with germline mutations of the *CDKN2A* being observed in ~40% of families with at least three affected members.[2] In contrast to its predisposing role in familial melanoma, the *CDKN2A* gene does not seem to be relevant in the

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genetic susceptibility of patients with sporadic melanoma.[3] Germline mutations in the *CDK4* gene are rare and have so far been identified in only few families worldwide.[2,4] Penetrance of *CDKN2A* mutations in the context of familial melanoma is incomplete and differs by age and geographical location.[5] Examination of the penetrance of *CDKN2A* mutations using data ascertained from 80 multiple-case melanoma families from Europe, Australia, and the United States has indicated geographic variation of this penetrance according to the population incidence rate of melanoma.[5] By age 80 years, *CDKN2A* mutation penetrance reached 58% in Europe, 76% in the United States and 91% in Australia. In addition, clinical features including age at melanoma diagnosis, presence and number of dysplastic nevi and occurrence of pancreatic cancer vary significantly among *CDKN2A* mutation carriers.

It has been hypothesised that the differences in *CDKN2A* penetrance could be related to modifier factors such as host/phenotypic characteristics, environmental sun exposure and/or different genetic background. Atypical/dysplastic nevi, high number of melanocytic nevi, poor tanning ability, or sunburns have been characterised as clinical and/or environmental risk factors in the development of melanoma in families with *CDKN2A* mutations.[6,7] The *MC1R* gene, which encodes for the melanocyte-stimulating hormone receptor and has a key role in the pigmentation process, has been found to increase the risk of sporadic melanoma.[8,9] Indeed several case–control studies worldwide have shown that melanoma risk is higher among *MC1R* variant carriers than among non-carriers, with the strongest effects observed for carriers of "red hair colour" or RHC variants (R151C, R160W, D294H)[8] and for carriers of multiple variants.[10–12] In melanoma-prone families, *MC1R* variants have been shown to modify the penetrance of *CDKN2A* mutations, and have been inconsistently associated with a reduction in age at melanoma diagnosis.[13–17]

We review herein all studies that investigated the relationship between *MC1R* variants and risk of melanoma in melanoma families with *CDKN2A* mutations, and performed a meta-analysis to assess whether *MC1R* variants increase the risk of melanoma in *CDKN2A* mutation carriers. A meta-analysis usually permits more conclusive results to be reached than single studies with moderate statistical power, and also sources of inconsistencies and variability in the estimates of the studied association can be better assessed.

2. Material and methods

2.1. Literature search and data extraction

We conducted a comprehensive, systematic bibliographic search of the literature to identify relevant studies on the association between *MC1R* variants and melanoma in *CDKN2A* mutation carriers from melanoma families. The literature search included papers published up to September 2009 cited in PubMed, ISI Web of Science (Science Citation Index Expanded) and Embase, using the keywords 'MC1R', 'melanocortin-1-receptor', 'CDKN2A' and 'Cyclin-Dependent Kinase inhibitor 2A' alone and in combination. We applied no search restriction. In addition, we reviewed the references from all retrieved articles to identify additional studies.

Studies suitable for this meta-analysis included familial melanoma cases and controls with available information on both *CDKN2A* mutations and *MC1R* variants. They had to report estimates of the association between *MC1R* variants and melanoma development in *CDKN2A* mutation carriers, with a corresponding measure of uncertainty (i.e. 95% confidence interval (CI), standard error, variance or *p*-value of the significance of the estimate). Alternatively, the frequency of *CDKN2A* mutation carriers with no or at least one *MC1R* variant had to be presented separately for cases and controls.

When available, we extracted fully adjusted risk estimates for melanoma development according to (1) the presence of at least one *MC1R* variant; (2) the presence of one and of two or more *MC1R* variants; and (3) the presence of at least one RHC variant (R151C, R160W, D294H) in *CDKN2A* mutation carriers. When adjusted estimates were not available, we calculated study-specific crude odds ratios (OR), with 95%CI for melanoma risk. For each study we recorded information on publication year, study location, *CDKN2A* mutations and *MC1R* variants identified and adjustment variables. All the included studies identified *MC1R* variants by direct sequencing analysis.

2.2. Statistical analysis

All the analyses were performed on individuals carrying a germline mutation in the *CDKN2A* gene.

We used random effects models with maximum likelihood estimate to evaluate summary estimation of the association between *MC1R* variants and melanoma development. In the analysis with separate pairwise comparisons for carriers of one and of two or more *MC1R* variants, the model took into account the correlation between the two risk estimates with a bivariate approach.[18] We performed a dose–response analysis with the number of *MC1R* variant alleles (one and two or more) using a linear model according to the method proposed by Greenland and Longnecker.[19]

Homogeneity of effects across studies was assessed using the χ^2 statistic, with significance level set at 0.10, and I^2, which represents the percentage of total variation across studies that is attributable to heterogeneity rather than to chance.[20] We explored between-study heterogeneity through sub-group analysis and meta-regression, and assessed the influence of single papers on the pooled risk estimate. Investigation of publication bias was carried out with funnel plots and Egger's test.[21]

We calculated gene–gene interaction for each of the three studies including the appropriate reference category (subjects with nor *CDKN2A* mutations nor *MC1R* variants). A product term was included in a logistic regression model and the *p*-value for interaction in each study was calculated by comparing, with the likelihood ratio test, the two models with and without the product term. A pooled estimate was not calculated due to the high variability between studies.

Association between *MC1R* variants and age at diagnosis was assessed by the non-parametric Wilcoxon two-sample test. We used the median or average age at diagnosis reported in each study for each case/control and *MC1R* variants

category, and weighted it for the number of subjects in each category.

P-value < 0.05 was considered statistically significant. The statistical analyses were performed using SAS, version 8.02.

3. Results

After initial screening of abstracts and references, 16 potentially relevant papers were identified and the full-text was retrieved for detailed evaluation. Out of these 16 papers, 10 were excluded for the following reasons: five studies[22–26] did not present association estimate for *MC1R* variants and melanoma nor cross data for *CDKN2A* mutations and *MC1R* variants, four studies[27–30] included only melanoma cases and no controls, and one investigated only patients with at least one *MC1R* variant.[31] One[17] of the remaining six papers[13–17,32] was a pooled-analysis of four independent studies: data from the United States and French populations have been more extensively published in previous papers already selected for the analysis,[15,16] therefore only data from the Italian and Spanish populations were retrieved from the pooled-analysis and used in our meta-analysis. Data from seven independent populations were eventually included in the meta-analysis.

The main characteristics of the seven study populations are presented in Table 1. Five studies were conducted in Europe, one in the United States and one in Australia. Overall, 96 melanoma families were investigated, including 504 subjects carrying at least one mutation in the *CKDN2A* gene and with information on *MC1R* variants.

Two out of the seven studies presented a significantly higher risk of melanoma for *CDKN2A* mutation carriers with one or more *MC1R* variants compared to *CDKN2A* mutation carriers without *MC1R* variant (Fig. 1); by pooling the seven studies we obtained a Summary OR (95%CI) of 2.2 (1.1–4.5), with no evidence of heterogeneity (χ^2 test *p*-value: 0.17; I^2: 33%) nor of publication bias (Egger's test *p*-value: 0.43). The Summary OR (95%CI) increased to 2.4 (1.2–5.1) after exclusion of the small study by Peric and colleagues,[32] which reported a negative association between *MC1R* variants and melanoma development.

When we analysed separately carriers of one and of two or more *MC1R* variants in the five populations reporting separate data or estimates (Fig. 2), we observed that *MC1R* heterozygous subjects had no significantly higher risk of melanoma compared to wild-type subjects (Summary OR; 95%CI: 1.6; 0.5–5.4), while carriers of two or more *MC1R* variants had a more than four-times higher risk of melanoma than wild-type subjects (Summary OR; 95%CI: 4.6; 1.3–16.4). The *p*-value for the linear trend considering none, one, and two or more *MC1R* variants was significant (*p*-value: 0.01), suggesting that melanoma risk increased with the increasing number of *MC1R* variant alleles in the genotype. No evidence of heterogeneity nor of publication bias was found in these further analyses (I^2, *p*-values for χ^2 and Egger's test were, respectively: 0%, 0.84 and 0.21 for heterozygous subjects; 29%, 0.23 and 0.95 for carriers of two or more *MC1R* variants).

Separate data for the three RHC variants R151C, R160W and D294H were reported in five studies (Fig. 3). One further study[14] presented separate data only on the R151C variant. When pooling data from these six studies, we found that Summary OR (95%CI) for carriers of RHC variants was 3.5 (1.3–9.9), with evidence of heterogeneity (χ^2 test *p*-value: 0.02; I^2: 62%), but no evidence of publication bias (Egger's test *p*-value: 0.87). By excluding the small study by Peric and colleagues[32] the Summary OR (95%CI) increased to 4.1 (1.4–11.9), with no more evidence of heterogeneity (χ^2 test *p*-value: 0.20; I^2: 33%).

When we assessed gene–gene interaction in each of the three studies[13,15,16] including the appropriate reference category – carriers of nor *CDKN2A* mutation nor *MC1R* variants – we found a non-significant positive interaction between the two genes, with ORs for the interaction term ranging from 1.2 to 10.3. A borderline *p*-value for interaction (0.08) was observed in the United States population.

Among the four studies with information on median or mean age at melanoma diagnosis, average age was 37 years for *CDKN2A* mutation carriers with one or more *MC1R* variants, that was significantly lower than the 47 years for *CDKN2A* mutation carriers with no *MC1R* variant (Table 2, *p*-value < 0.0001).

Table 1 – List of studies included in the meta-analysis and odds ratios (ORs) with 95% confidence intervals (CI) for the association between any *MC1R* variant and melanoma development among *CDKN2A* mutation carriers.

First author (publication year)	Population study country	No. families	Studied CDKN2A mutations	No. cases with any MC1R variant/No. of cases (%)	No. controls with any MC1R variant/No. of controls (%)	OR (95%CI)
Box et al. (2001)	Australia	15	Mixed	57/62 (92%)	11/16 (69%)	5.2 (1.3–21.0)
van der Velden et al. (2001)	The Netherlands	6	p-16 Leiden	35/38 (92%)	49/63 (78%)	3.3 (0.9–12.5)
Chaudru et al. (2005)	France	20	Mixed	37/42 (88%)	32/37 (86%)	1.2 (0.3–4.4)
Goldstein et al. (2005)	United States	16	Mixed	64/69 (93%)	49/72 (68%)	3.1 (0.8–11.5)[a]
Goldstein et al. (2007)	Spain	10	G101W	15/17 (88%)	13/23 (57%)	5.8 (1.1–31.3)
Goldstein et al. (2007)	Italy	22	G101W	24/34 (71%)	13/18 (72%)	0.9 (0.3–3.3)
Peric et al. (2008)	Slovenia	7	Mixed	5/9 (56%)	4/4 (100%)	0.1 (0.0–3.3)
All studies		96		237/271 (87%)	171/233 (73%)	2.2 (1.1–4.5)

[a] Adjusted by age, nevus and pigmentation factor. Nevus factors was created as combination of dysplastic nevi (absent, indeterminate, present) and total numbers of nevi. Pigmentation factor was created as combination of skin complexion (medium/dark, pale/fair) and extent of freckling (none/few, moderate, many).

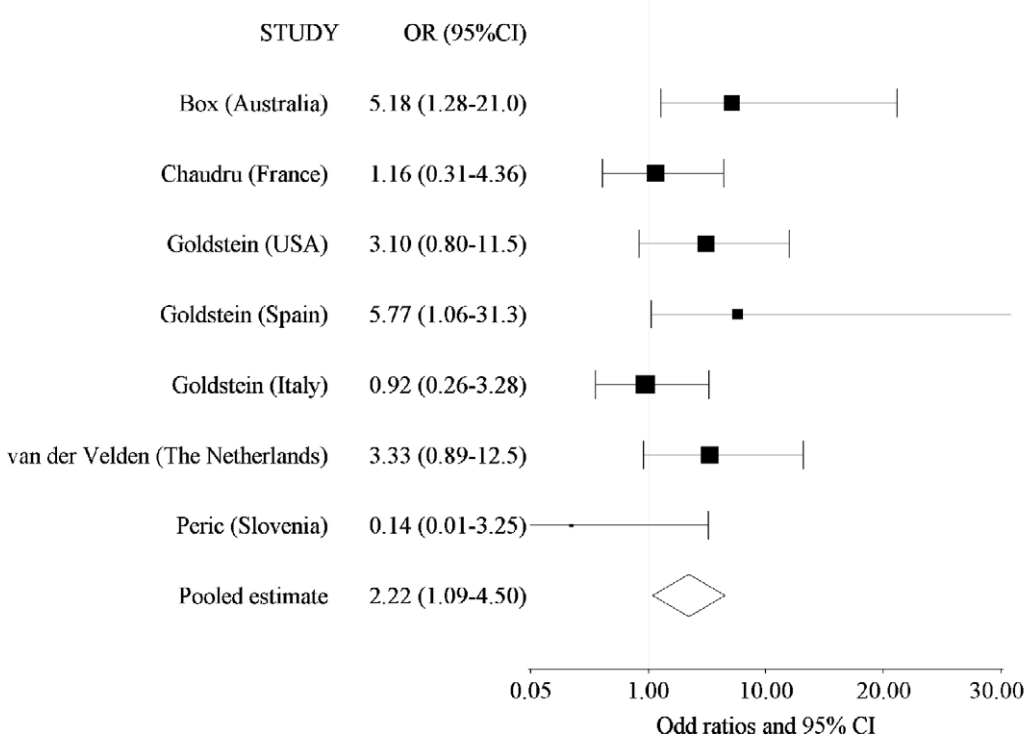

Fig. 1 – Study-specific and meta-odds ratio (OR) with 95% confidence intervals (CI) for the association between any *MC1R* variant and melanoma development among *CDKN2A* mutation carriers.

4. Discussion

Our meta-analysis evaluated the modifier effect of *MC1R* allelic variants on the penetrance of *CDKN2A* mutations in melanoma-prone families. Data from seven independent populations[13–17,32] including 96 melanoma families segregating *CDKN2A* mutations clearly showed that *MC1R* variants doubled melanoma risk in *CDKN2A* mutation carriers. Moreover, the risk is more then triple for carriers of RHC variants. Two studies, one on 15 Australian melanoma families carrying nine different *CDKN2A* mutations[13] and a second study of six Dutch families carrying the *p16-Leiden* deletion[13] showed that the presence of *MC1R* variants increased the risk of melanoma development among *CDKN2A* mutation carriers. Most of the effect of the *MC1R* genotype on melanoma risk was primarily related to the R151C variant in the Dutch families[14] and to the R151C, R160W, D294H RHC variants in the Australian families.[13] A significant association between multiple *MC1R* variants and melanoma risk was later reported in 16 melanoma-prone American families with *CDKN2A* mutations.[16] Interestingly, the presence of multiple *MC1R* variants was mainly associated in this study with the development of multiple primary melanomas. Consistent with findings in Australian, Dutch and American *CDKN2A* mutation-positive melanoma families, *MC1R* variants were also shown to significantly increase *CDKN2A* penetrance in 20 French melanoma-prone families.[15] Pooled RHC variants and the D294H and R163Q individual variants significantly increased cutaneous melanoma risk in this sample. A case–control study on melanoma patients and their unaffected relatives carrying the high-risk *CDKN2A* G101W founder mutation, from France, Italy, Spain, and the United States, provided additional evidence for a relationship between non-synonymous variants in *MC1R* and risk of melanoma in *CDKN2A* mutation carriers.[17] This study showed a significant association between increased number of *MC1R* variants and melanoma risk with substantial differences between the Italian sample and the other three groups.[17] No conclusive results could be drawn in a recent Slovenian study, due to the low number of *CDKN2A* mutation-positive affected family members carrying *MC1R* variants.[32] Removal of this small study from our evaluation increased the OR for the *MC1R*-melanoma association in *CDKN2A* mutation carriers to 2.4 (95%CI: 1.2–5.1) for any *MC1R* variant, and to 4.1 (95%CI: 1.4–11.9) for RHC variants. Apart from this latter study, ORs for the Italian and French samples were lower than those calculated for the other populations. The lower allele frequencies of the melanoma-associated R151C and R160W alleles in both Italian and French controls compared to other Caucasian samples[33] might explain this smaller association. When the analysis was restricted to RHC variants, a significantly higher risk of melanoma was observed in the French population, while a consistent low OR was found in the Italian population. This finding in the Italian sample might be related to the selected population, which included subjects from the Liguria region, characterised by a slightly lower occurrence and penetrance for melanoma as compared to both other European regions and adjacent Italian regions.[33] In addition, the frequency of red-haired individuals is also very low in this region and homozygosity or compound heterozygosity for high penetrance RHC variants are not necessarily associated with red hair.[34]

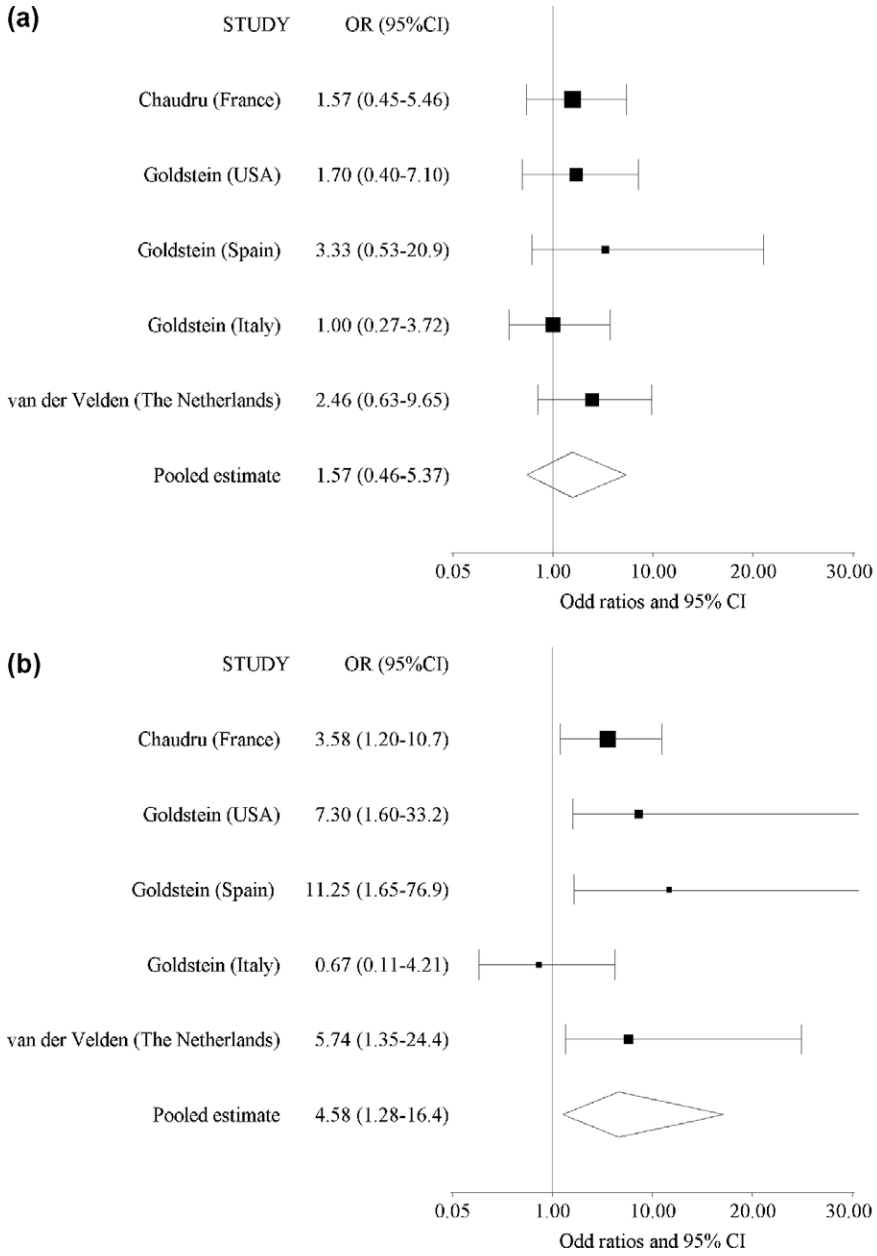

Fig. 2 – Study-specific and meta-odds ratio (OR) with 95% confidence intervals (CI) for the association between (a) one *MC1R* variant and melanoma development; (b) two or more *MC1R* variants and melanoma development. All subjects are *CDKN2A* mutation carriers. *Note*: *p*-value for linear trend: 0.01.

With regard to the number of *MC1R* variants in the genotype, our data showed a significant association between multiple *MC1R* variants and melanoma risk in *CDKN2A* mutation carriers. Carriers of multiple *MC1R* variants had an almost four-times higher risk of developing melanoma than wild-type *MC1R* subjects while there was no significant difference between *MC1R* heterozygous carriers and consensus carriers. Based on our results, the observed higher penetrance of *CDKN2A* mutations in Australia compared to the United States and Europe[5] may be explained by the higher frequency of multiple *MC1R* variants[13] and the higher frequency of the most common R151C and R160W RHC variants[33] in Australia than in the other countries. A higher melanoma risk was reported in carriers of two *MC1R* variants rather than just one variant in the Dutch, American and French *CDKN2A* mutation-positive families.[14–16] Conflicting data have been reported on the association of lower age and melanoma diagnosis in carriers of *MC1R* variants in addition to a *CDKN2A* mutation compared to carriers of a *CDKN2A* mutation alone in melanoma-prone families. Our pooled data showed that there was a statistically significant lower median age at melanoma diagnosis in *CDKN2A* mutation carriers with *MC1R* variants as compared to *CDKN2A* mutation carriers with no *MC1R* variant (37 years versus 47 years). A significant lower median age at melanoma diagnosis has been observed in the Australian[13] and American[16] study samples but not in the Dutch[14] and French[15] families and only marginally in the pooled G101W Spanish, French and Italian kindreds.[17] It

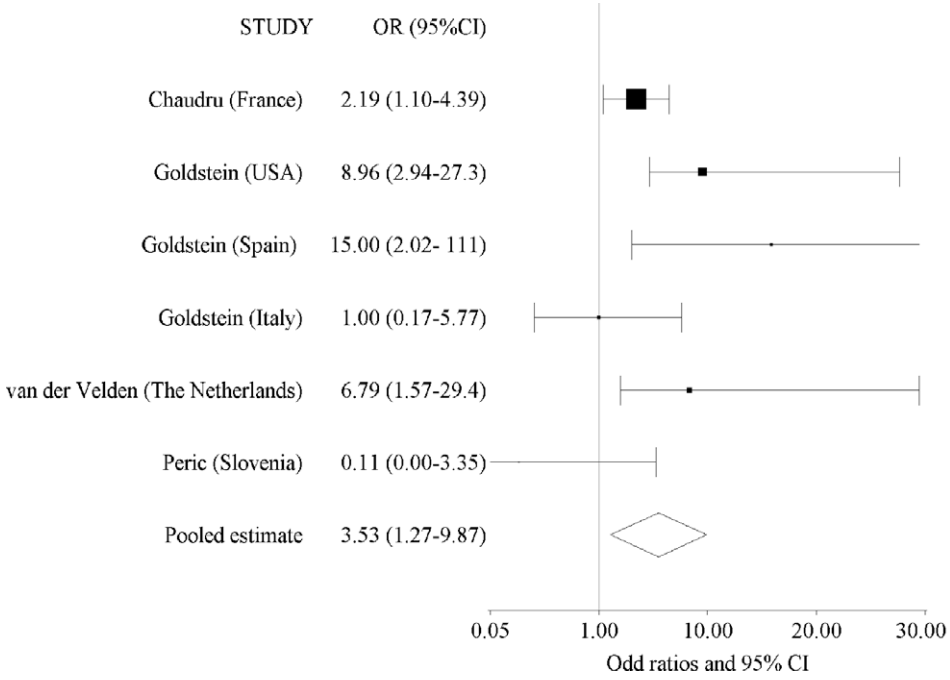

Figure 3 – Study-specific and meta-odds ratio (OR) with 95% confidence intervals (CI) for the association between RHC *MC1R* variants (R151C, R160W, D294H) and melanoma development among *CDKN2A* mutation carriers.

Table 2 – Median or average age at melanoma diagnosis according to the presence or not of any *MC1R* variant among *CDKN2A* mutation carriers.

First author (publication year)	Population study country	Median or average age at diagnosis (No. of cases)	
		No *MC1R* variant	At least one *MC1R* variant
Box et al. (2001)	Australia	58 (5)	38 (57)
Chaudru et al. (2005)	France	53 (5)	48 (37)
Goldstein et al. (2005)	USA	36 (5)	30 (64)
van der Velden et al. (2001)	The Netherlands	40 (3)	38 (35)
All studies		47 (18)*	37 (193)*

P-value for Wilcoxon two-sample test: <0·0001.

has been suggested that these controversial results might be explained by the number of multiple primary melanoma patients versus single primary melanoma patients included in the study samples, to differences in age distribution between samples, to differences in type or distribution of *CDKN2A* mutations across the different studies or to distribution of major melanoma risk factors including at risk phenotypic features and amount of sun exposure.[15–17]

The test for interaction between the *CDKN2A* and *MC1R* genes did not reach significance in the three studies analysed, although a borderline positive interaction was observed in the United States population.[13,15,16] However, it should be taken into account that detection of gene–gene interaction requires large sample sizes, especially when the main effects of the gene variants are small.

Besides the *MC1R* gene, additional genetic factors are likely to modulate melanoma risk in melanoma families. In a recent explanatory study, several common genetic polymorphisms in genes involved in the DNA repair, apoptosis and immune response pathways have been significantly associated with melanoma risk in US melanoma-prone families, with distinct effects in *CDKN2A*-positive and *CDKN2A*-negative families.[35] Analysis of the modifying effects of glutathione S-transferase (*GST*) genes on melanoma risk in French melanoma-prone families segregating *CDKN2A* mutations revealed a significant protective effect of null *GSTT1* allele on melanoma risk, while *GSTM1* null allele and *GSTP1* variants did not modify the risk significantly.[24]

This study aimed to review and summarise the main results from published papers investigating the association between *MC1R* variants and melanoma in *CDKN2A* mutation carriers. Due to the powerful approach of the meta-analysis we were able to highlight a significant role of *MC1R* variants on further increasing melanoma risk in high-risk subjects belonging to melanoma-prone families segregating a *CDKN2A* mutation. With regard to the individual studies, significant results have been observed in only two of the seven selected studies, probably because individual studies have generally not enough statistical power to find significant associations. Moreover, our meta-analysis allowed us to easily compare

differences in risk estimates identified in the different studies, probably due to the heterogeneity of the study populations, especially for what concerns skin pigmentation and sun exposure patterns.

The current study was limited by the small number of studies available in the literature examining the effect of *MC1R* variants on *CDKN2A* penetrance in melanoma kindreds carrying *CDKN2A* mutations. The small size precluded a deep investigation of the heterogeneity in the composition and characteristics of the study population or the methodological features of the performed studies, and to perform sub-group analyses. However, there was no evidence of a strong heterogeneity among risk estimates in the selected studies, therefore the pooled risk estimates could be considered representative of the study populations. Moreover, it was not possible to evaluate each *MC1R* variant individually, although separate analyses could be performed for the RHC variants. Finally, gene–gene interactions could be evaluated for only three of the selected studies where the reference category was available. In these three studies the precision of the ORs was small due to the very small number of subjects in the reference group (two cases and 62 controls).

In conclusion, our meta-analysis confirmed that *MC1R* variant alleles significantly increase the penetrance of *CDKN2A* mutations in melanoma-prone families, especially with respect to multiple *MC1R* variants and to RHC variants. We also found a statistically significant lower median age at melanoma diagnosis for *CDKN2A* mutation-positive patients who are carriers of *MC1R* variants as compared to non-carriers. These data on the *MC1R*-melanoma association in *CDKN2A* mutation carriers should strongly encourage further studies on the identification of other clinical, environmental and genetic factors possibly impacting on melanoma risk in order to improve the risk prediction in *CDKN2A* mutation-positive melanoma families and to provide appropriate counselling.

Conflict of interest statement

None declared.

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REFERENCES

1. Fargnoli MC, Argenziano G, Zalaudek I, Peris K. High- and low-penetrance cutaneous melanoma susceptibility genes. *Expert Rev Anticancer Ther* 2006;**6**:657–70.
2. Goldstein AM, Chan M, Harland M, et al. High-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. *Cancer Res* 2006;**66**:9818–28.
3. Begg CB, Orlow I, Hummer AJ, et al. Lifetime risk of melanoma in CDKN2A mutation carriers in a population-based sample. *J Natl Cancer Inst* 2005;**97**:1507–15.
4. Helsing P, Nymoen DA, Ariansen S, et al. Population-based prevalence of CDKN2A and CDK4 mutations in patients with multiple primary melanomas. *Genes Chromosomes Cancer* 2008;**47**:175–84.
5. Bishop DT, Demenais F, Goldstein AM, et al. Geographical variation in the penetrance of CDKN2A mutations for melanoma. *J Natl Cancer Inst* 2002;**94**:894–903.
6. Goldstein AM, Struewing JP, Chidambaram A, Fraser MC, Tucker MA. Genotype-phenotype relationships in U.S. melanoma-prone families with CDKN2A and CDK4 mutations. *J Natl Cancer Inst* 2000;**92**:1006–10.
7. Chaudru V, Chompret A, Bressac-de Paillerets B, et al. Influence of genes, nevi, and sun sensitivity on melanoma risk in a family sample unselected by family history and in melanoma-prone families. *J Natl Cancer Inst* 2004;**96**:785–95.
8. Raimondi S, Sera F, Gandini S, et al. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. *Int J Cancer* 2008;**122**:2753–60.
9. Sturm RA. Molecular genetics of human pigmentation diversity. *Hum Mol Genet* 2009;**18**:R9–R17.
10. Kennedy C, ter Huurne J, Berkhout M, et al. 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* 2001;**117**:294–300.
11. Landi MT, Kanetsky PA, Tsang S, et al. MC1R, ASIP, and DNA repair in sporadic and familial melanoma in a Mediterranean population. *J Natl Cancer Inst* 2005;**97**:998–1007.
12. Stratigos AJ, Dimisianos G, Nikolaou V, et al. Melanocortin receptor-1 gene polymorphisms and the risk of cutaneous melanoma in a low-risk southern European population. *J Invest Dermatol* 2006;**126**:1842–9.
13. Box NF, Duffy DL, Chen W, et al. MC1R genotype modifies risk of melanoma in families segregating CDKN2A mutations. *Am J Hum Genet* 2001;**69**:765–73.
14. van der Velden PA, Sandkuijl LA, Bergman W, et al. Melanocortin-1 receptor variant R151C modifies melanoma risk in Dutch families with melanoma. *Am J Hum Genet* 2001;**69**:774–9.
15. Chaudru V, Laud K, Avril MF, et al. Melanocortin-1 receptor (MC1R) gene variants and dysplastic nevi modify penetrance of CDKN2A mutations in French melanoma-prone pedigrees. *Cancer Epidemiol Biomarkers Prev* 2005;**14**:2384–90.
16. Goldstein AM, Landi MT, Tsang S, et al. Association of MC1R variants and risk of melanoma in melanoma-prone families with CDKN2A mutations. *Cancer Epidemiol Biomarkers Prev* 2005;**14**:2208–12.
17. Goldstein AM, Chaudru V, Ghiorzo P, et al. Cutaneous phenotype and MC1R variants as modifying factors for the development of melanoma in CDKN2A G101W mutation carriers from 4 countries. *Int J Cancer* 2007;**121**:825–31.
18. van Houwelingen HC, Arends LR, Stijnen T. Advanced methods in meta-analysis: multivariate approach and meta-regression. *Stat Med* 2002;**21**:589–624.
19. Greenland S, Longnecker M. Methods for trend estimation from summarized dose–response data, with applications to meta-analysis. *Am J Epidemiol* 1992;**135**:1301–9.
20. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;**21**:1539–58.
21. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;**315**:629–34.
22. Debniak T, Scott R, Masojc B, et al. MC1R common variants, CDKN2A and their association with melanoma and breast cancer risk. *Int J Cancer* 2006;**119**:2597–602.
23. Pastorino L, Bonelli L, Ghiorzo P, et al. CDKN2A mutations and MC1R variants in Italian patients with single or multiple primary melanoma. *Pigment Cell Melanoma Res* 2008;**21**:700–9.

24. Chaudru V, Lo MT, Lesueur F, et al. Protective effect of copy number polymorphism of glutathione S-transferase T1 gene on melanoma risk in presence of CDKN2A mutations, MC1R variants and host-related phenotypes. *Fam Cancer* 2009;**8**:371–7.
25. Council ML, Gardner JM, Helms C, et al. Contribution of genetic factors for melanoma susceptibility in sporadic US melanoma patients. *Exp Dermatol* 2009;**18**:485–7.
26. Höiom V, Tuominen R, Käller M, et al. MC1R variation and melanoma risk in the Swedish population in relation to clinical and pathological parameters. *Pigment Cell Melanoma Res* 2009;**22**:196–204.
27. Peris K, Fargnoli MC, Pacifico A, et al. CDKN2A and MC1R mutations in patients with sporadic multiple primary melanoma. *J Invest Dermatol* 2004;**122**:1327–30.
28. Cuéllar F, Puig S, Kolm I, et al. Dermoscopic features of melanomas associated with MC1R variants in Spanish CDKN2A mutation carriers. *Br J Dermatol* 2009;**160**:48–53.
29. Daniotti M, Ferrari A, Frigerio S, et al. Cutaneous melanoma in childhood and adolescence shows frequent loss of INK4A and gain of KIT. *J Invest Dermatol* 2009;**129**:1759–68.
30. Ghiorzo P, Pastorino L, Pizzichetta MA, et al. CDKN2A and MC1R analysis in amelanotic and pigmented melanoma. *Melanoma Res* 2009;**19**:142–5.
31. Borges AL, Cuéllar F, Puig-Butillé JA, et al. CDKN2A mutations in melanoma families from Uruguay. *Br J Dermatol* 2009;**161**:536–41.
32. Peric B, Cerkovnik P, Novakovic S, et al. Prevalence of variations in melanoma susceptibility genes among Slovenian melanoma families. *BMC Med Genet* 2008;**9**:86.
33. Gerstenblith MR, Goldstein AM, Fargnoli MC, Peris K, Landi MT. Comprehensive evaluation of allele frequency differences of MC1R variants across populations. *Hum Mutat* 2007;**28**:495–505.
34. Pastorino L, Cusano R, Bruno W, et al. Novel MC1R variants in Ligurian melanoma patients and controls. *Hum Mutat* 2004;**2**:103.
35. Yang XR, Pfeiffer RM, Wheeler W, et al. Identification of modifier genes for cutaneous malignant melanoma in melanoma-prone families with and without CDKN2A mutations. *Int J Cancer* 2009;**125**:2912–7.