The role of MC1R gene variants and phenotypical features in predicting high nevus count

Poel, L.A.J. van der; Bergman, W.; Gruis, N.A.; Kukutsch, N.A.

The role of *MC1R* gene variants and phenotypical features in predicting high nevus count

Louise A.J. van der Poel, Wilma Bergman, Nelleke A. Gruis* and Nicole A. Kukutsch*

Variants in the Melanocortin 1 Receptor (*MC1R*) gene have been associated with an increased risk of melanoma, but the role in nevus count is unclear. We investigated if specific *MC1R* gene variants or the number of *MC1R* gene variants and phenotypical features were associated with nevus count. A total of 494 participants of the 'Leiden skin cancer study' were included and the MC1R gene coding sequence was analysed by single-strand conformation polymorphism analysis followed by sequencing of unknown variants. The association between *MC1R* gene variants and nevus count and the association between age, gender and phenotypical features and nevus count were studied using the Chi-square test. Study of nine frequently occurring *MC1R* gene variants in participants without skin cancer ($n=203$) showed that the 'r' Val60Leu variant was significantly associated with high nevus count (>50 nevi) ($P=0.017$). This association was very strong among women ($P<0.001$), but not present among men. Having one or two *MC1R* variants in general did not show a significant difference in the nevus count. Hair colour, skin type, eye colour and age were not significantly associated with nevus count, whereas gender showed a significant association ($P=0.008$), with the highest nevus counts in female. The Val60Leu variant of the MC1R gene could be a promising candidate as an independent predictor of high nevus count, particularly in women. This information about the genetic makeup could promote personalized follow-up strategies and might help to prevent skin cancer in the future. *Melanoma Res* 30: 511–514 Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

Melanoma Research 2020, **30**:511–514

Keywords: MC1R gene, nevus count, phenotype, predictor, Val60Leu

Department of Dermatology, Leiden University Medical Centre, Leiden, The Netherlands

Correspondence to Louise A.J. van der Poel, Department of Dermatology, B1-Q, Leiden University Medical Centre, Postbus 9600, 2300 RC Leiden, The Netherlands
Tel: +31 71 5262630; fax: +31 71 5248106;
e-mail: l.a.j.van_der_poel@lumc.nl

*Nelleke A. Gruis and Nicole A. Kukutsch contributed equally to the writing of this article.

Received 27 February 2020 **Accepted** 26 June 2020

High nevus count is a known risk factor for melanoma and is determined by biological, (epi-)genetic and external factors [1–3]. A recent study investigated the association between phenotypic characteristics and melanoma in a large prospective cohort study. Individuals with moles compared to individual with no moles had a significantly higher melanoma incidence [4].

The association between nevus count and phenotypic characteristics was inconsistent in previous studies [5,6]. The Melanocortin 1 Receptor (*MC1R*) gene is associated with phenotypical characteristics such as skin, eye and hair colour and variants in *MC1R* have been associated with an increased skin cancer risk, including melanoma. However, the role of *MC1R* gene variants in the nevus count is still unclear [1,3]. Until now, only a few other genes were found to be associated with nevus count [2,7–9].

We investigated if specific *MC1R* gene variants or the number of *MC1R* gene variants were associated with the nevus count and if the nevus count was associated with phenotypical features.

For the purpose of the study, 203 (131 female) participants from the 'Leiden skin cancer study', aged 30–60 years without skin cancer and with skin types I through IV as defined by Fitzpatrick, were included for the first analysis [10]. With the aid of standardized interviews and physical examination performed by a dermatologist, the phenotypical characteristics, number and localization of common and atypical melanocytic nevi were noticed. Nevi were classified as atypical if they showed a minimum of any three out of the following criteria: diameter of 5 mm or larger, asymmetrical shape, ill-defined border, irregular brown pigmentation and erythema [11].

MC1R gene variants were determined as described earlier [10]. In short, the *MC1R* gene coding sequence was analysed by single-strand conformation polymorphism followed by sequencing of unknown variants. Comparisons of *MC1R* gene variants, gender, age and phenotypic variables such as skin type, hair colour and eye colour between nevus count groups [low (0–10), intermediate (11–50), high (>50)] were performed using the Chi-square test (SPSS21.0). Validation of the findings for *MC1R* variants was performed in a second analysis in participants of the 'Leiden skin cancer study' ($n=291$) aged 30–60 with a history of at least one of the following skin cancers: squamous cell carcinoma, basal cell carcinoma and melanoma [10].

DOI: 10.1097/CMR.0000000000000687

Twenty-seven different *MC1R* genetic variants were found in the study cohort. The most frequently occurring *MC1R* variants were Arg160Trp, Arg151Cys, Arg163Gln, Val60Leu, Val92Met, Asp84Glu, Asp294His, His260Pro and Arg142His. About 38% of the participants had no *MC1R* variant, 45% had one *MC1R* variant and 16.7% had a combination of two variants. Patients with one or two variants did not show a significant difference in low (0–50) versus high (>50) nevus count ($P=0.954$, respectively, $P=0.754$).

Basic characteristics and phenotypical data in combination with low (0–10), intermediate (11–50) and high (>50) nevus counts are shown in Table 1. Subjects with skin types II and III showed a high nevus count (40.9 and 47.3%) compared to skin types I and IV (8.4 and 3.4%). Overall skin type was not significantly associated with nevus count ($P=0.735$). Dark blond and/or blond hair presented with a high nevus count (65.7%) compared to black/brown (28.4%), but overall hair colour was not found to be significantly associated with nevus count (*P* value 0.316). Twelve individuals had red hair; they all had a nevus count fewer than 50. Overall age and eye colour were not significantly associated with the nevus count ($P=0.516$, respectively, $P=0.082$). Gender was significantly associated with the nevus count ($P=0.008$), with the highest nevus counts in female.

Comparing the groups of nevus count and the most common *MC1R* variants, only the Val60Leu variant was significantly associated with a high nevus count ($P=0.017$) (Table 2). Analyses stratified for women and men showed a strong association among women ($P<0.001$), but not among men (Table 2). In contrast to these results, we did not find a significant association between Val60Leu and the nevus count among women and men in the patients in the second analysis with a history of skin cancer (data not shown).

Based on our findings, the Val60Leu variant of the *MC1R* gene and female gender could be a promising candidate as an independent predictor of the high nevus count. Duffy *et al.* [12] investigated the relationship between *MC1R* gene variants and degree of freckling and nevus count in 2331 adolescent twins, their sibs and parents in 645 twin families. *MC1R* variants were combined as strong 'R' (Asp84Glu, Arg151Cys, Arg160Trp and Asp294His) and weak 'r' (Val60Leu, Val92Met and Arg163Gln) red hair alleles [12]. They found a positive correlation between nevus count and freckling score. The 'r' allele made a small contribution to this positive correlation. In this study, the R/R genotype displayed the lowest number of nevi and the highest number of freckles, but for the whole group, they found an overall positive correlation between freckling and nevus count which in their eyes might be related to sun exposure [12]. A recent study of Duffy *et al.* [3] determined if high nevus count and red hair or MC1R alleles, act synergistically in melanoma risk. In contrast to our study, they included participants living in Queensland, Australia, a high ultraviolet radiation area. They showed that the combination of a total nevus count of more than 20 nevi (>5 mm diameter) with the R/R genotype resulted in a strong risk profile for melanoma for patients living in a high sun exposed area [3]. The R/r genotype was significantly associated with the total nevus count in both the control as the case group. Compared to our study, they did not distinguish between the different MC1R alleles but grouped them as wild-type, r and R.

Baron *et al.* [13] investigated different kinds of sun exposure and interaction with genotype combination (MC1Rand HERC2/OCA2 rs12913832) in children aged 6 through 10 years ($n=477$). They concluded that waterside vacation increased the number of nevi in children with rs12913832 blue eye colour alleles and sunburn

Table 1 Study population characteristics and association with nevus count (*N*=203)

Nevus count[a]		Low, *N* (%)	Intermediate, *N* (%)	High, *N* (%)	Total, *N* (%)	*P* value[b]
Gender	Women	59 *(74.7)*	59 *(62.8)*	13 *(43.3)*	131 *(64.5)*	0.008
	Men	20 *(25.3)*	35 *(37.2)*	17 *(56.7)*	72 *(35.5)*	
Age (years)	Mean	51.8 (*N*=79)	49.1 (*N*=94)	46 0.1 (*N*=30)	49.7 (*N*=203)	0.516
	Range	(35.9–60.4)	(34.8–60.4)	(34.6–59.6)	(34.6–60.4)	
Skin type	Fitzpatrick I	6 *(7.6)*	9 *(9.6)*	2 *(6.7)*	17 *(8.4)*	0.735
	Fitzpatrick II	35 *(44.3)*	37 *(39.4)*	11 *(36.7)*	83 *(40.9)*	
	Fitzpatrick III	36 *(45.6)*	43 *(45.7)*	17 *(56.7)*	96 *(47.3)*	
	Fitzpatrick IV	2 *(2.5)*	5 *(5.3)*	0	7 *(3.4)*	
Hair colour (*n*=201)	Red	7 *(9.0)*	5*(5.4)*	0	12 *(6.0)*	0.316
	Dark blond-Blond	46 *(59)*	65 *(69.9)*	21 *(70)*	132 *(65.7)*	
	Black-brown	25 *(32.1)*	23 *(24.7)*	9 *(30.0)*	57 *(28.4)*	
Eye colour	Brown/ green	28 *(35.4)*	29 *(30.9)*	16 *(53.3)*	73 *(36)*	0.082
	Blue/grey	51 *(64.6)*	65 *(69.1)*	14 *(46.7)*	130 *(64)*	

[a]Divided in groups: low (0–10), intermediate (11–50) and high (>50).
[b]*P* value calculated using Pearson's Chi-square test asymp sig (two sided).

Table 2 MC1R genetic variants and nevus count (*N*=203)

Variation	Nevus count[a]	Absent, *N* (%)	Present, *N* (%)	*P* value[b]
Arg160Trp	0–10	63 (38.4)	16 (41.0)	0.413
	11–50	79 (48.2)	15 (38.5)	
	>50	22 (13.4)	8 (20.5)	
Arg151Cys	0–10	69 (37.9)	10 (47.6)	0.358
	11–50	84 (46.2)	10 (47.6)	
	>50	29 (15.9)	1 (4.8)	
Arg163Gln	0–10	70 (37.4)	9 (56.3)	0.140
	11–50	87 (46.5)	7 (43.8)	
	>50	30 (16)	0	
Val60Leu	0–10	69 (40.4)	10 (31.3)	0.017
	11–50	82 (48)	12 (37.5)	
	>50	20 (11.7)	10 (31.3)	
Val92Met	0–10	67 (38.5)	12 (41.4)	0.767
	11–50	80 (46)	14 (48.3)	
	>50	27 (15.5)	3 (10.3)	
Asp84Glu	0–10	79 (39.1)	0	0.558
	11–50	93 (46)	1 (100)	
	>50	30 (14.9)	0	
Asp294His	0–10	79 (39.5)	0	0.338
	11–50	92 (46)	2 (66.7)	
	>50	29 (14.5)	1 (33.3)	
His260Pro	0–10	79 (39.3)	0	0.310
	11–50	92 (45.8)	2 (100)	
	>50	30 (14.9)	0	
Arg142His	0–10	79 (39.3)	0	0.310
	11–50	92 (45.8)	2 (100)	
	>50	30 (14.9)	0	
Val60Leu Women	0–10	52 (47.3)	7 (33.3)	
	11–50	52 (47.3)	7 (33.3)	< 0.001
	>50	6 (5.5)	7 (33.3)	
Val60Leu Men	0–10	17 (27.9)	3 (27.3)	
	11–50	30 (49.2)	5 (45.5)	0.950
	>50	14 (23.0)	3 (27.3)	

[a]Divided in groups: low (0–10), intermediate (11–50) and high (>50).
[b]*P* value calculated using Pearson's Chi-square test asymp sig (two sided).

increase the nevus count in children with MC1R r/r, R/+ in combination with OCA2$^{Brown/Brown}$ [13].

MC1R Val60Leu, which showed a positive association with high nevus count in our study, is considered an 'r' variant with a weaker association with the red hair colour phenotype [14,15]. Also Tagliablue *et al.* [16] who analysed seven *MC1R* variants (Val60Leu, Asp84Glu, Arg142His, Arg151Cys, Ile155Thr, Arg160Trp and DAsp294His) found the lowest association for red hair, skin type I/II or freckles with the Val60Leu variant. Orlow *et al.* [17] investigated the *MC1R* gene and 85 SNPs in genes associated with pigmentary, DNA repair, telomere/senescence and other pathways and did not find a significant association between *MC1R* variants and nevus count. Another group investigated whether pigmentation genes involved in the melanogenesis contributed to melanoma predisposition and total nevus count, they sequenced the *MC1R* gene and 32 pigmentary SNP markers and did not find a significant association for *MC1R* and the amount of nevi [8]. Hu *et al.* looked into the role of several *MC1R* variants in melanoma risk and nevus count. In total, 79 different *MC1R* variants in 1131 melanoma patients and 869 healthy controls were found, consisting of 69 rare 'r' alleles, five frequent 'r' alleles (Val60Leu, Val92Met, Ile155Thr, Arg163Gln and Thr314Thr) and five 'R' alleles (Asp84Glu, Arg142His, Arg151Cys, Arg160Trp and Asp294His). In their study, the 'r' alleles were significantly associated with nevus count (<10, 10–50, 51–100 and >100) (*P* value 0.002). However, they did not discriminate for different variants within the group of 'r' alleles [18].

Although our results did not show a significant association between nevus count and phenotypical features, which might be due to low numbers of participants per subgroup, they do support the finding that individuals with a more sun-sensitive phenotype (apart from skin type I) are prone to have a high nevus count. An interaction between gender and Val60Leu as risk factors for nevus count has not been reported earlier, but an interaction between gender and nevi has recently been published in association with thick melanomas: among women thick melanomas occurred more frequently in patients with <10 nevi; no such association was found among men [19].

This study showed that the Val60Leu variant of the *MC1R* gene could be a promising candidate as an independent predictor of high nevus count, especially for women without skin cancer. Further studies should investigate its functional role and the possible relation with other genes that promote high nevus count. Information about the genetic makeup could promote personalized follow-up strategies and might help to prevent skin cancer in the future.

Conflicts of interest

There are no conflicts of interest.

Acknowledgements

We thank J.N. Bouwes Bavinck for his valuable assistance with the statistical analyses.

References

1 Newton-Bishop JA, Chang YM, Iles MM, Taylor JC, Bakker B, Chan M, *et al*. Melanocytic nevi, nevus genes, and melanoma risk in a large case-control study in the United Kingdom. *Cancer Epidemiol Biomarkers Prev* 2010; **19**:2043–2054.

2 Roos L, Sandling JK, Bell CG, Glass D, Mangino M, Spector TD, *et al*. Higher nevus count exhibits a distinct DNA methylation signature in healthy human skin: implications for melanoma. *J Invest Dermatol* 2017; **137**:910–920.

3 Duffy DL, Lee KJ, Jagirdar K, Pflugfelder A, Stark MS, McMeniman EK, *et al*. High naevus count and MC1R red hair alleles contribute synergistically to increased melanoma risk. *Br J Dermatol* 2019; **181**:1009–1016.

4 Olsen CM, Pandeya N, Thompson BS, Dusingize JC, Green AC, Neale RE, Whiteman DC; QSkin Study. Association between phenotypic characteristics and melanoma in a large prospective cohort study. *J Invest Dermatol* 2019; **139**:665–672.

5 Oliveria SA, Satagopan JM, Geller AC, Dusza SW, Weinstock MA, Berwick M, *et al*. Study of Nevi in Children (SONIC): baseline findings and predictors of nevus count. *Am J Epidemiol* 2009; **169**:41–53.

6 Taylor NJ, Thomas NE, Anton-Culver H, Armstrong BK, Begg CB, Busam KJ, *et al*.; GEM Study Group. Nevus count associations with pigmentary phenotype, histopathological melanoma characteristics and survival from melanoma. *Int J Cancer* 2016; **139**:1217–1222.

7 Falchi M, Bataille V, Hayward NK, Duffy DL, Bishop JA, Pastinen T, *et al*. Genome-wide association study identifies variants at 9p21 and 22q13 associated with development of cutaneous nevi. *Nat Genet* 2009; **41**:915–919.

8 Johansen P, Andersen JD, Madsen LN, Ullum H, Glud M, Børsting C, *et al*. Pigmentary markers in danes–associations with quantitative skin colour, nevi count, familial atypical multiple-mole, and melanoma syndrome. *PLoS One* 2016; **11**:e0150381.

9 Duffy DL, Iles MM, Glass D, Zhu G, Barrett JH, Höiom V, *et al*.; GenoMEL. IRF4 variants have age-specific effects on nevus count and predispose to melanoma. *Am J Hum Genet* 2010; **87**:6–16.

10 Bastiaens M, ter Huurne J, Gruis N, Bergman W, Westendorp R, Vermeer BJ, Bouwes Bavinck JN. The melanocortin-1-receptor gene is the major freckle gene. *Hum Mol Genet* 2001; **10**:1701–1708.

11 Kennedy C, Bajdik CD, Willemze R, De Gruijl FR, Bouwes Bavinck JN; Leiden Skin Cancer Study. The influence of painful sunburns and lifetime sun exposure on the risk of actinic keratoses, seborrheic warts, melanocytic nevi, atypical nevi, and skin cancer. *J Invest Dermatol* 2003; **120**:1087–1093.

12 Duffy DL, Box NF, Chen W, Palmer JS, Montgomery GW, James MR, *et al*. Interactive effects of MC1R and OCA2 on melanoma risk phenotypes. *Hum Mol Genet* 2004; **13**:447–461.

13 Barón AE, Asdigian NL, Gonzalez V, Aalborg J, Terzian T, Stiegmann RA, *et al*. Interactions between ultraviolet light and MC1R and OCA2 variants are determinants of childhood nevus and freckle phenotypes. *Cancer Epidemiol Biomarkers Prev* 2014; **23**:2829–2839.

14 Raimondi S, Sera F, Gandini S, Iodice S, Caini S, Maisonneuve P, Fargnoli MC. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. *Int J Cancer* 2008; **122**:2753–2760.

15 García-Borrón JC, Abdel-Malek Z, Jiménez-Cervantes C. MC1R, the cAMP pathway, and the response to solar UV: extending the horizon beyond pigmentation. *Pigment Cell Melanoma Res* 2014; **27**:699–720.

16 Tagliabue E, Gandini S, García-Borrón JC, Maisonneuve P, Newton-Bishop J, Polsky D, *et al*.; M-SKIP Study group. Association of melanocortin-1 receptor variants with pigmentary traits in humans: a pooled analysis from the M-skip project. *J Invest Dermatol* 2016; **136**:1914–1917.

17 Orlow I, Satagopan JM, Berwick M, Enriquez HL, White KA, Cheung K, *et al*. Genetic factors associated with naevus count and dermoscopic patterns: preliminary results from the Study of Nevi in Children (SONIC). *Br J Dermatol* 2015; **172**:1081–1089.

18 Hu HH, Benfodda M, Dumaz N, Gazal S, Descamps V, Bourillon A, *et al*. A large French case-control study emphasizes the role of rare Mc1R variants in melanoma risk. *Biomed Res Int* 2014; **2014**:925716.

19 De Giorgi V, Scarfì F, Gori A, Maida P, Trane L, Silvestri F, *et al*. Nevi and Breslow thickness in melanoma: sex differences? *Melanoma Res* 2020; **30**:179–184.