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Patterns of familial aggregation of three melanoma risk factors: great number of naevi, light phototype and high degree of sun exposure

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Background Besides melanoma susceptibility genes and shared environmental exposures, part of the familial clustering of cutaneous malignant melanoma (CMM) might be due to familial aggregation of melanoma-associated phenotypes. Our goal was to assess the patterns of familial aggregation of three melanoma risk factors: great number of naevi (GNN), light phototype (LP) and high degree of sun exposure (HDSE).

Methods Familial aggregation of GNN, LP and HDSE was investigated in 66 French families with at least two CMM cases and was measured by the asssociation of the relatives' traits with the probands' traits, using the generalized estimating equations approach. The probands were the melanoma cases leading to ascertainment of the families, subdivided into cases (with the trait studied) and controls (without the trait).

Results We found significant evidence for familial aggregation of GNN only among sibs (OR = 3.7, 95% CI : 1.4–10.5, $P = 0.01$), of LP among blood relatives (OR = 3.8, 95% CI : 1.8–8.0, $P = 0.004$) and of HDSE among blood relatives (OR = 4.5, 95% CI : 2.1–9.9, $P < 0.001$) and spouses (OR = 44.3, 95% CI : 5.1–382.2, $P < 10^{-3}$). These results suggest that genetic factors might account for the clustering of GNN and LP and shared environment for the aggregation of HDSE. The GNN clustering was lower in families with increasing numbers of CMM ($\geqslant$3 cases) or presence of p16 mutations, the opposite being observed for LP and HDSE. Moreover, the familial aggregation of LP was significantly lower in families with highly sun-exposed members.

Conclusion Melanoma might not only result from specific genetic and environmental factors but also from those underlying melanoma-associated traits involving complex gene-gene and gene-environment interactions.

Keywords Melanoma, familial aggregation, naevus, phototype, sun exposure, generalized estimating equations

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The incidence of cutaneous malignant melanoma (CMM) has been increasing markedly over the past 40 years in white-skinned populations from industrialized countries.[1] In France, age-standardized incidence rates were estimated to be 7.7 per 100 000 in females and 6.3 per 100 000 in males in 1995, showing an increase of 103% in females and 152% in males when compared to the 1975 rates.[2] These rates are similar to those observed in other European countries (UK, Southern Europe) and are lower than those reported in the US and Australia.[1] It is now well known that genetic and environmental factors both play a role in the development of CMM. Sun exposure, pigmentary traits (including atypical naevi, a high number of naevi, poor ability to tan, propensity to sunburn, red

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hair, fair skin) and a family history of melanoma have been reported to be risk factors for CMM by numerous epidemiological studies.[3–6] Approximately 8–12% of CMM cases occur in a familial setting.[7] Recent progress in the genetics of CMM has led to the identification of two melanoma susceptibility genes: the tumour suppressor gene CDKN2A encoding the $p16^{ink4A}$ protein on chromosome 9p21[8,9] and the CDK4 gene, on chromosome 12q13, that encodes a protein normally inhibited by the p16 protein.[10] CDKN2A mutations have been detected in at most 50% of melanoma-prone families that have been examined in Europe, North America, and Australia[11] and CDK4 mutations have been described in only three families.[10,11] Therefore, other genes remain to be identified. These highly penetrant genes can explain the high incidence of CMM in rare families[12,13] while the familial clustering of few CMM cases might result from low penetrant susceptibility genes and/or shared environmental exposures.[14] Moreover, part of the clustering of CMM might be due to familial aggregation of melanoma-associated phenotypes including pigmentary traits (e.g. fair skin that fails to tan, red hair and freckling), and increased number of common and atypical melanocytic naevi, their expression being modulated by sun exposure.

To better understand the effects and interactions of genetic and environmental factors in CMM aetiology, a family study was conducted at Institut Gustave Roussy (IGR), Villejuif, France, that led to the collection of 295 families ascertained by 295 CMM probands during the period 1986–1989. A family history of CMM was reported in 22 cases and was found to be associated significantly with red hair and presence of atypical moles, and to a lesser extent with a great number of naevi, a young age at diagnosis and multiple primary melanomas.[15] Segregation analysis of CMM in this sample showed evidence for the transmission of a rare dominant gene interacting with age and propensity to sunburn,[16] while the transmission of a great number of naevi appeared to involve more complex genetic mechanisms.[17] To investigate the part of familial clustering of CMM that might be due to familial aggregation of melanoma risk factors, our first series of 22 familial CMM probands was extended to a total of 100 probands with at least one affected relative with CMM. Clinical and epidemiological data in probands and relatives were obtained in a total of 66 families. The method of generalized estimating equations (GEE) was used to assess the patterns of family resemblance of three melanoma risk factors: great number of naevi (GNN), light phototype (LP) and high degree of sun exposure (HDSE).

Subjects and Methods

Recruitment of families and data collection

One hundred Caucasian CMM probands with at least one affected relative were recruited: 89 from the Department of Dermatology of Institut Gustave Roussy (IGR), France and 11 from other French hospitals during the period 1986–1996. Inclusion criteria included a histologically confirmed diagnosis of CMM in a Caucasian subject who had been living in France for more than 10 years and reporting a familial history of CMM at first interview. Of all patients contacted, 99% agreed to participate and gave their informed consent. Among the 100 probands ascertained, 86 were newly diagnosed cases while the other ones were followed-up cases. Family data collected at time of the probands' interview included names and addresses of their first-, second- and third-degree relatives together with their sex, date and place of birth, status with respect to CMM and other cancers, age at diagnosis of the cancer, age and cause of death if dead. Once histological confirmation of at least one CMM case among these relatives was obtained, the family entered the study and was investigated further. Detailed information on clinical and epidemiological variables was gathered in all first-degree relatives of histologically confirmed CMM cases as well as in intervening family members between two cases. Clinical and histological data of CMM cases were obtained from medical records. Pathology slides were reviewed by a pathologist. A physical examination was conducted by a trained dermatologist in all probands and in relatives coming to the hospital to determine their total naevus count. This naevus count was classified into three categories (<10, 10–49, $\geqslant 50$) and the presence of atypical moles (diameter >10 mm with irregular margins and variegated colour) was also recorded. Subjects, who were not seen in the hospital (about 75% of the probands' relatives), self-reported their naevus phenotype, as belonging to either one of the three categories (<10, 10–49, $\geqslant 50$ naevi) on a questionnaire. In a subset of 50 relatives who both underwent a physical examination and reported their naevus phenotype category on the questionnaire, the self-reported naevus phenotype agreed with the one assigned by the physician in 45 of them (90%). The questionnaire was completed by the probands and relatives seen at the hospital at time of examination and distributed by the probands to all other relatives. Besides the usual demographic characteristics and the naevus count, epidemiological data included skin colour (pale or dark), eye colour (pale or dark), hair colour (red, blonde, light brown, dark brown, or dark), presence (or not) of freckles and café au lait spots, degree of exposure to sunlight (low, medium, or high) during their holidays, leisure time and work time, artificial UV exposure (yes or no), long stay (>1 year) in a sunny country, skin reactions to sunlight evaluated by the ability to tan (low, medium, or high), and the propensity to sunburn (low, medium or high). Epidemiological information in dead relatives was obtained through the proband or a contact person within the family and could be completed in most cases for the skin, eye, and hair colour and the degree of sun exposure, and coded as unknown for the other risk factors in most instances. Among 100 families ascertained through 100 probands, at most 66 families were suitable for the analysis of familial aggregation of melanoma risk factors, since they included the proband and at least one relative with known information for at least one of the three traits of interest: GNN, LP, HDSE. These 66 families included 66 probands and their 316 relatives (292 blood relatives and 24 spouses) (Table 1). The 292 blood relatives included 55 parents, 72 children, 93 sibs, 72 second- and third-degree relatives. The distribution of the 66 families according to the number of CMM cases was 51 families with 2 cases, 11 with 3 cases, 3 with 4 cases and 1 with 5 cases. Regarding each trait separately, the number of families used was 56 for GNN, 60 for the phototype, and 58 for sun exposure, that included the corresponding number of probands (one per family) and 227, 248 and 233 probands' relatives, respectively. There were 47 families, including 196 probands' relatives, which were used for all three traits. Blood samples were obtained in a total of 48 families that were tested for CDKN2A and CDK4 mutations.[11]

Table 1 Distribution of probands and probands' relatives according to sex, age, cutaneous malignant melanoma (CMM) status, and their known status for each of the three traits studied: great number of naevi (GNN), light phototype (LP) and high degree of sun exposure (HDSE)

	No.	Sex ratio (M/F)	Mean age (years)	No. of CMM cases	No. of subjects with known GNN	No. of subjects with known LP	No.of subjects with known HDSE
Probands	66	0.64	48.2	66	56	60	58
Probands' parents	55	0.58	68.1	12	36	43	40
Probands' children	72	0.62	25.7	9	59	64	50
Probands' sibs	93	0.58	42.8	11	71	72	82
Probands' 2nd and 3rd degree relatives	72	0.58	43.9	12	61	69	61
Total probands' blood relatives	292	0.59	35.6	44	227	248	233
Probands' spouses	24	0.37	53.7	0	20	21	19

Traits analysed

From the information on naevus phenotype, (recorded as one of three categories: <10, 10–49, ⩾50 naevi), GNN was defined as having ⩾50 versus <50 naevi at the time of study. The choice of 50 naevi to dichotomize the trait was based on published studies in Europe.[3] Different cutoff points according to age could not be used since a polychotomous variable (three classes) instead of a full naevus count was obtained in most relatives. Using a simplification of Fitzpatrick's scale,[18] LP was defined as red hair and/or low ability to tan versus medium or high ability to tan. High degree of sun exposure was classified according to indicators of sun exposure over the whole life: long stay in a sunny country or high sun exposure in at least two of the three types of exposure (during holidays, leisure time, work time) versus other degrees of sun exposure.

Statistical methods

The familial case-control approach[19] was used to assess the familial aggregation. The probands were the melanoma cases leading to ascertainment of the families and subdivided into cases if they had the studied trait (GNN, LP or HDSE) and controls if they did not have it. Familial aggregation was measured by the association of the relatives' trait with the probands' trait. In family k, the association of the ith relative's trait, y_{ik}, with the proband's trait, c_k ($c_k = 1$ if k is a case, $c_k = 0$ if k is a control), was modelled by a logistic function, where the logit, θ_{ik}, is:

$$\theta_{ik} = \alpha c_k + \beta'_1 x_{ik} + \beta'_2 z_k, \qquad (1)$$

the regression coefficient α being the log odds ratio (OR) that quantifies the familial aggregation, and β'_1 and β'_2 being the vectors of regression coefficients for covariates specific to the proband's relative (x_{ik}) and to the proband (z_k). The primes denote transpose.

Models

From the general model given in (1), different models were considered to test various patterns of familial aggregation.

Model 1

This model was used to measure the association of the probands' traits with those observed in two types of relatives respectively: blood relatives (first, second and third-degree relatives) and spouses, in order to distinguish genetic from environmental sources of familial aggregation.

$$\theta_{ik} = \alpha_1 c_k \text{blood relative}_{ik} + \alpha_2 c_k \text{spouse}_{ik} + \beta'_1 x_{ik} + \beta'_2 z_k$$

where $\text{blood relative}_{ik} = 1$ if the *ik*th relative was a blood relative and $\text{blood relative}_{ik} = 0$ otherwise; and similarly for the spouse_{ik} variable.

Model 2

Model 2 was similar to model 1, but the relationships to probands were specified as parents, children, sibs, a pooled category of second- and third-degree relatives, and spouses:

$$\theta_{ik} = \alpha_1 c_k \text{parent}_{ik} + \alpha_2 c_k \text{childg}_{ik} + \alpha_3 c_k \text{sib}_{ik} + \alpha_4 c_k \text{relative2–3}_{ik} + \alpha_5 c_k \text{spouse}_{ik} + \beta'_1 x_{ik} + \beta'_2 z_k$$

Model 3

Model 3 derived the familial aggregation dependent on the number of CMM cases in the family, mel3_k, where mel3_k was equal to 1 if the family included three or more melanoma cases and was equal to 0 if it had only two cases.

$$\theta_{ik} = \alpha_1 c_k \text{blood relative}_{ik} + \alpha_2 c_k \text{mel3}_k \text{blood relative}_{ik} + \beta'_1 x_{ik} + \beta'_2 z_k + \beta_3 \text{mel3}_k.$$

The familial aggregation among blood relatives was measured by $\exp(\alpha_1)$ if the family included two CMM cases and by $\exp(\alpha_1 + \alpha_2)$ if the family included three or more CMM cases.

Model 4

Model 4 derived the familial aggregation dependent on the presence of p16 mutations in the family, p16_k, where p16_k was equal to 1 if a p16 mutation was detected in the family and was equal to 0 otherwise (the p16 symbol was used instead of the usual CDKN2A symbol for sake of simplicity).

$$\theta_{ik} = \alpha_1 c_k \text{blood relative}_{ik} + \alpha_2 c_k \, \text{p16}_k \text{blood relative}_{ik} + \beta'_1 x_{ik} + \beta'_2 z_k + \beta_3 \text{p16}_k$$

Model 5

Model 5 derived the familial aggregation varying according to the relatives' sun exposure (expo_{ik}) and was used when the traits studied were GNN and LP.

$$\theta_{ik} = \alpha_1 c_k \text{blood relative}_{ik} + \alpha_2 c_k \, \text{expo}_{ik} \text{blood relative}_{ik} + \beta'_1 x_{ik} + \beta'_2 z_k + \beta_3 \text{expo}_{ik}$$

The familial aggregation among blood relatives was measured by $\exp(\alpha_1)$ if the *ik*th relative was not highly sun exposed and by $\exp(\alpha_1 + \alpha_2)$ if the *ik*th relative was highly sun exposed.

Model 6

The familial aggregation of either one of the three traits (trait1) was measured while adjusting for the possible confounding effects of the two other traits in probands ($trait2_k$, $trait3_k$) and in probands' relatives ($trait2_{ik}$, $trait3_{ik}$), that were included as covariates in the model:

$$\theta_{ik} = \alpha_1 c_k \text{blood relative}_{ik} + \beta'_1 x_{ik} + \beta'_2 z_k + \beta_3 \text{trait2}_k + \beta_4 \text{trait3}_k + \beta_5 \text{trait2}_{ik} + \beta_6 \text{trait3}_{ik}$$

In all models, the adjustment variables specific to probands (z_k) were age and sex and those specific to probands' relatives (x_{ik}) were age, sex and melanoma status. CMM status was taken into account since it might be a potential confounder when assessing familial aggregation of melanoma-associated traits. Analyses were also repeated without adjusting for CMM status. Spouses were not included in models 3 to 6 due to their small number.

Parameter estimation

Since the traits studied were correlated within families, we used the GEE approach to estimate the regression coefficients in the logistic model to obtain valid standard errors.[20,21] The GEE method does not need the specification of the joint distribution of each trait in a family, but only requires specifying the traits' means, variances and correlations among family members. The mean μ_{ik} for relative i in family k with trait y_{ik} is $\mu_{ik} = \exp(\theta_{ik})/[1 + \exp(\theta_{ik})]$ which depends on the parameters measuring familial aggregation, $\alpha' = (\alpha_1, \ldots, \alpha_n)$, and the β coefficients for covariates, the expected variance is $\sigma_{ik} = \mu_{ik}(1-\mu_{ik})$ and the correlation between each pair of relative (ij) is assumed to be function of a constant term, δ, with $\sigma_{ijk} = \sigma_{ik}\sigma_{jk}\delta$.

Estimates of the parameters (α, β, δ) were obtained by solving a system of p equations (p being the number of parameters estimated):

$$\sum_{i=1}^{K} D_i^T V_i^{-1} f_i = 0,$$

where K is the number of families, D_i is the matrix of the derivatives of each moment with respect to the parameters, V_i is a weight matrix and f_i is the matrix of the differences between the observed trait values, observed variances and covariances and respectively the expected means, variances and covariances computed from the above expressions.

The parameter estimates obtained from the GEE approach have an asymptotic normal distribution. For example, under model 1, the null hypothesis of interest is H_0: $\exp(\alpha_1) = 1$, i.e. there is no familial aggregation among blood relatives. The test statistic $T_1 = \hat{\alpha}_1/\hat{\sigma}_1$ has an asymptotically standardized normal distribution under the null hypothesis. So, an absolute value of $T_1 > 1.96$ at the 5% level indicates a significant familial aggregation among blood relatives. Another null hypothesis of interest, considered in model 2, is whether the familial aggregation among blood relatives is significantly different from the one among spouses, i.e. H_0: $\alpha_1 = \alpha_2$. The test statistic is $T_2 = (\alpha_1 - \alpha_2)/\hat{\varsigma}_{12}$ with $\hat{\varsigma}_{12}{}^2 = \hat{\sigma}_1^2 + \hat{\sigma}_2^2 - 2\hat{\sigma}_{12}$, the estimated variance of $(\alpha_1 - \alpha_2)$.

The computer program QGE ('EE: Estimating Equations', Fred Hutchinson Cancer Research Center, Quantitative Genetic Epidemiology, Technical Report 126) was used to perform all computations. This program incorporates the Newton-Raphson algorithm to estimate the parameters.

Results

The OR measuring the familial aggregation of the three traits while adjusting for sex, age and melanoma status are presented in Table 2 and Table 3 under the six models considered. When pooling all blood relatives together (model 1), these OR were significant for LP (OR = 3.8, 95% CI : 1.8–8.0, $P = 0.004$) and HDSE (OR = 4.5, 95% CI : 2.1–9.9, $P < 0.001$) but they were not significant for GNN (OR = 2.3, 95% CI : 0.9–5.9, $P = 0.09$). The phenotypic resemblance between spouses was significant for HDSE (OR = 44.3, 95% CI : 5.1–382.2, $P < 0.001$) and the OR was greater than one for LP but did not reach significance. The familial resemblance of HDSE in spouses was significantly higher than that observed among blood relatives ($P < 0.03$). When considering different types of relationships to probands (model 2), the familial aggregation of all three traits among sibs was significant: the OR being 3.7 (95% CI : 1.4–10.5, $P = 0.01$) for GNN, 5.1 (95% CI : 1.6–15.4, $P = 0.005$) for LP and 4.7 (95% CI : 1.4–15.7, $P = 0.01$) for HDSE. The familial aggregation of LP and HDSE was also significant among children ($P = 0.002$ and $P < 0.001$, respectively). However, there was no significant evidence for familial resemblance in parents and pooled second- and third-degree relatives of probands for any of the three traits.

The familial aggregation of the three traits among all blood relatives did not vary significantly according to the type of family defined by the number of CMM cases (⩾3 versus <3 CMM cases) (model 3) and presence of p16 mutations (model 4). However, as seen in Table 3, the familial aggregation of GNN was lower in families including ⩾3 CMM cases and in families with p16 mutations, the opposite being observed for LP and HDSE. Moreover, the familial aggregation of LP was significantly lower among highly sun-exposed than among poorly sun-exposed blood relatives (OR = 3.1 versus OR = 4.4, $P = 0.02$) (model 5), the same trend being observed for GNN (OR = 1.5 versus OR = 1.9, not significant). In model 6, when each trait was adjusted for the effects of the two other traits of interest (e.g. GNN adjusted for LP and HDSE), the familial aggregation of LP (OR = 7.0, 95% CI : 3.4–14.1, $P < 10^{-6}$) and HDSE (OR = 9.3, 95% CI : 3.8–22.9, $P < 10^{-5}$) became more significant than without adjustment while the OR measuring the familial clustering of GNN decreased (OR = 1.8, 95% CI : 0.6–5.5). When all analyses were repeated without including the relatives' melanoma status as a covariate, the OR estimates, measuring the different patterns of familial aggregation, were similar.

Discussion

Our analyses, conducted in a sample of 66 French families with at least two melanoma cases, provided evidence for familial aggregation of three melanoma-associated traits: GNN, LP and HDSE, with different patterns of family resemblance. We found a significant familial aggregation of GNN only among sibs, of LP among blood relatives (all pooled as well as in sibs and children separately) and of HDSE among blood relatives and spouses. The OR measuring the association of the relatives' traits with the probands' traits decreased according to the degree of relationship

Table 2 Estimated odds ratios (OR) measuring the familial aggregation of great number of naevi (GNN), light phototype (LP) and high degree of sun exposure (HDSE) in models 1 and 2 (defined in the text) adjusted for sex, age and cutaneous malignant melanoma status

	GNN			LP			HDSE		
Models	% GNN[a]	OR[b] (95% CI)	*P*-value[c]	% LP[a]	OR[b] (95% CI)	*P*-value[c]	% HDSE[a]	OR[b] (95% CI)	*P*-value[c]
Model 1									
Controls' blood relatives	24.7			20.1			12.2		
Cases' blood relatives	33.3	2.3 (0.9–5.9)	0.09	30.4	3.8 (1.8–8.0)	0.004	46.6	4.5 (2.1–9.9)	<0.001
Controls' spouses	14.3			7.1			9.1		
Cases' spouses	0.0	–	–	14.3	1.8 (0.2–14.4)	ns	87.5	44.3 (5.1–382.2)	<0.001
Model 2									
Controls' parents	0.0			17.2			11.1		
Cases' parents	25.0	1.9 (0.6–6.1)	ns[d]	28.6	2.9 (0.8–10.3)	0.1	23.1	1.7 (0.3–10.2)	ns
Controls' children	35.1			7.7			6.5		
Cases' children	40.9	1.9 (0.4–7.6)	ns	32.0	5.8 (1.9–8.1)	0.002	57.9	11.6 (3.1–43.1)	<0.001
Controls' sibs	30.0			9.3			20.7		
Cases' sibs	20.9	3.7 (1.4 –10.5)	0.01	33.3	5.1 (1.6–15.4)	0.005	41.7	4.7 (1.4–15.7)	0.01
Controls' 2nd and 3rd degree relatives	20.5			12.8			3.4		
Cases' 2nd and 3rd degree relatives	22.7	1.5 (0.5–4.6)	ns	22.7	2.5 (0.8–7.5)	ns	31.2	3.3 (0.9–11.8)	0.06

[a] % of individuals with GNN, LP or HDSE.

[b] Odds ratios adjusted for sex, age and melanoma status.

[c] Probability that the estimated OR differs significantly from 1.

[d] ns = not significant ($P > 0.10$).

Table 3 Odds ratios (OR) measuring the familial aggregation of great number of naevi (GNN), light phototype (LP) and high degree of sun exposure (HDSE) in models 3 to 6 (defined in the text)

	GNN			LP			HDSE		
Models	OR[a]	95% CI	*P*-value[b]	OR[a]	95% CI	*P*-value[b]	OR[a]	95% CI	*P*-value[b]
Model 3									
Blood relatives: ⩾3 CMM[c]	0.9	(0.1–10.0)		8.1	(1.6–39.8)		35.1	(3.5–355.3)	
Blood relatives: <3 CMM	3.3	(1.0–10.4)	ns[g]	3.1	(1.4–6.9)	ns	2.8	(1.1–7.4)	0.09
Model 4									
Blood relatives: p16 +ive[d]	0.6	(0.1–4.2)		1.6	(0.5–4.9)		3.2	(0.6–16.7)	
Blood relatives: p16 –ive	1.3	(0.4–4.6)	ns	1.1	(0.3–5.1)	ns	2.7	(1.1–6.7)	ns
Model 5									
Blood relatives: sun exposure +ive[e]	1.5	(0.3–8.4)		3.1	(1.5–6.4)		–	–	–
Blood relatives: sun exposure –ive	1.9	(0.7–5.1)	ns	4.4	(2.1–9.2)	0.02	–	–	–
Model 6									
Blood relatives[f]	1.8	(0.6–5.5)	ns	7.0	(3.4–14.1)	$<10^{-6}$	9.3	(3.8–22.9)	$<10^{-5}$

[a] All odds ratios are adjusted for sex, age and melanoma status.

[b] Probability that the regression coefficient α_2 differs from 0 in models 3, 4 and 5 and α_1 differs from 0 in model 6.

[c] Belonging to families with ⩾3 cutaneous malignant melanoma (CMM) cases.

[d] Belonging to families with p16 mutations.

[e] Odds ratios adjusted for the relatives' HDSE status.

[f] Odds ratios adjusted for sex, age, melanoma status and the two other melanoma-associated traits (e.g. for GNN : LP and HDSE).

[g] ns = not significant ($P > 0.10$).

with probands for GNN and LP, while, for HDSE, they were significantly higher in spouses than in blood relatives. These patterns of familial aggregation suggest the presence of genetic factors accounting for the clustering of GNN and LP and shared environment for HDSE. However, this interpretation should be noted with caution, given the small number of spouses. Moreover, significance of the tests should be interpreted with respect to the number of tests conducted. All these results applied to families with at least two CMM cases and familial aggregation of these melanoma-associated traits might depend on the strength of association of CMM with each of them. However, similar results were obtained whether the relatives' melanoma status was included or ignored in the analyses, indicating that familial clustering of GNN, LP or HDSE is rather a cause than a consequence of CMM familial aggregation. Interestingly, the familial clustering of GNN decreased in families with an increased number of CMM cases and with co-segregating p16 mutations, the opposite being observed for LP and HDSE. Although not significant, these observations suggest that these risk factors might also partly explain the aetiological heterogeneity of melanoma.

Familial aggregation of increased numbers of naevi and atypical naevi was first described in melanoma-prone families, and recognized as the familial atypical multiple mole melanoma (FAMMM) syndrome or dysplastic naevus syndrome (DNS).[22–24] One study suggested that the co-segregation of melanoma and DNS might result from the pleiotropic effect of the same gene.[25] However, the difficulty in reaching a clear consensus on the DNS definition hampered further studies to clarify its genetic determinism. Twin studies and segregation analyses applied to more objective naevus phenotypes, naevus count and/or naevus density, suggested that these phenotypes were under genetic control,[26–29] but their familial transmission appeared more complex than the Mendelian transmission of a single major gene.[17,28,30] A recent twin study found a substantial contribution of genetic influences to the colour and size of naevi and also a significant environmental contribution to colour.[31] In our present series of 66 French families, familial aggregation of GNN was only significant among siblings. Although this might be due to the influence of recessive-like genetic factors, as also suggested by segregation analysis of GNN in our first series of 295 melanoma families,[17] the lack of significant results in other relatives might be due to the use of a binary phenotype instead of a more informative quantitative measure which could not be obtained in relatives not seen at the hospital. The familial aggregation of GNN, lower in families with at least three CMM cases and in those with p16 mutations, suggests that distinct genetic factors might be involved in GNN and melanoma. This result is in agreement with a recent study of 20 American melanoma-prone families[32] where the presence of dysplastic naevi (DN) was found to interact significantly with the p16 mutation-carrier status in melanoma risk, with DN being a stronger risk factor for CMM subjects without p16 mutations versus those with mutations. However, a combined linkage and association analysis in Australian twins has recently shown that a CDKN2A-linked gene influences the number of flat moles but has no effect on raised moles or atypical moles.[33] No distinction between the different types of moles, flat or raised, was made in our study.

Familial aggregation of LP has been little studied and was suggested by a twin study of skin reflectance.[34] Our results indicate the possible influence of genetic determinants. This is supported by the recent finding of a significant association between variants of the melanocortin 1 receptor (MCR1) gene with red hair and propensity to sunburn,[35] these variants being also associated with melanoma independent of skin type.[36] We found that the familial aggregation of LP was higher in families with three or more melanoma cases and in those with p16

mutations, suggesting possible common genetic determinants underlying LP and melanoma and/or interactions between their respective determinants. A significant interaction between a putative melanoma gene and propensity to sunburn was found in our first series of 295 French melanoma families ascertained through one melanoma proband.[16] Moreover, in Dutch melanoma families sharing the same p16 mutation,[37] polymorphisms of the MCR1 gene associated with LP were suggested to be modifiers of the risk of melanoma.

Familial clustering of melanoma might also be due to shared environmental factors. The pattern of familial aggregation of HDSE in our families strongly suggests a common behaviour with respect to sun exposure that appears to be shared by spouses more strongly than among blood relatives. The higher association of children's HDSE (OR = 11.6, $P < 0.001$) than sibs' HDSE (OR = 4.7, $P = 0.01$) with the probands' HDSE supports this view since the probands, their spouses and children, living in the same household, are more likely to share the same type of sun exposure. The clustering of HDSE increasing with the number of CMM cases or presence of p16 mutation suggests that sun exposure may enhance the expression of melanoma genes. Putative p16 gene carriers that develop melanoma[38] or subjects belonging to melanoma high risk families[14] were also reported to have been more exposed to sun.

Interactions between the traits studied were also observed. The familial aggregation of LP was significantly lower in families with highly sun-exposed members, the same trend being observed for GNN although it was not significant. This might be explained by protective behaviours of individuals with a fair skin at higher risk for melanoma who cluster more in families with limited sun exposure. Moreover, while adjusting each of these traits for the effect of the other two, the familial aggregation of LP and HDSE was twice as high than without adjustment, the opposite trend being observed for GNN. These results underline the complex confounding relationships among LP, HDSE and GNN. As shown before, there is a negative confounding relationship between LP and HDSE, which may explain the higher familial aggregation of each of these traits when adjusting for the other. On the other hand, the positive association of a high number of naevi with a light phototype and high sun exposure[4] suggests that clustering of GNN might be partly accounted for by clustering of LP and HDSE. However, as mentioned earlier, there might be different genetic factors with complex interactive effects underlying these traits. Further combined segregation-linkage analysis, considering simultaneously the transmission of these phenotypes, may help in disentangling the mechanisms that are common or specific to these traits.

In conclusion, this study underlines the importance of taking into account melanoma risk factors to dissect the complex mechanisms causing the development of CMM. Melanoma may not only result from specific genetic and environmental determinants but also from those underlying melanoma-associated phenotypes with complex gene-gene and gene-environment interactions. Further genetic and epidemiological studies directed towards these melanoma-associated phenotypes, especially the phototype and number of naevi, may help in unravelling the multiple factors causing this cancer.

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