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Cutaneous Malignant Melanoma and Familial Dysplastic Nevi: Evidence for Autosomal Dominance and Pleiotropy

SHERRI J. BALE,[1] ARAVINDA CHAKRAVARTI,[2] AND MARK H. GREENE[1]

SUMMARY

Segregation of familial cutaneous melanoma has been shown to be compatible with autosomal dominant transmission with incomplete penetrance. However, the combined phenotype of melanoma and a known melanoma-precursor lesion, the dysplastic nevus (DN), has not previously been found to fit a Mendelian model of inheritance using complex segregation analysis. Employing a life-table and disease-free survival analysis approach, we estimated the lifetime incidence of melanoma in the sibs and offspring of DN-affected individuals to be 46%, consistent with a highly penetrant, autosomal dominant mode of inheritance. To further elucidate the relationship between the two traits, we conducted a linkage analysis between the melanoma locus and a hypothetical DN locus, and obtained a maximum lod score of 3.857 at $\theta = .08$. Furthermore, all families giving evidence for linkage were in the coupling phase and the maximum likelihood estimate of θ was not significantly different from 0 ($P = .1$). This provides evidence that the DN and melanoma traits may represent pleiotropic effects of a single, highly penetrant gene behaving in an autosomal dominant manner.

INTRODUCTION

Among the neoplasias that affect adults, familial clusters of cutaneous malignant melanoma (CMM) occur with a high frequency. It has been estimated that

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[1] Environmental Epidemiology Branch, National Cancer Institute, Bethesda, MD 20205.

[2] Human Genetics Program, Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA 15261.

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8%–12% of all CMM occur in persons with a positive family history of melanoma [1], the first report of a high-risk family dating back to 1820 [2]. The mode of inheritance in such high-risk families has been variously reported as autosomal dominant [3, 4] and polygenic [5, 6]. The two studies giving evidence for a polygenic model arrived at quite different estimates of the heritability of liability to the phenotype. Wallace et al. estimated 11% heritability [5], while Duggleby et al. found that nearly half the liability could be attributed to the additive effect of many genes [6]. It was noted, however, that it can be quite difficult to distinguish between an autosomal dominant model with incomplete penetrance and a polygenic model [5]. Certainly, there are also environmental factors that might exacerbate the melanoma risk in members of such families. Cells derived from patients with familial melanoma have been found to demonstrate in vitro sensitivity to the cytotoxic and mutagenic effects of ultraviolet radiation [7–10]. Case-control studies have demonstrated an increased risk of melanoma in persons with a sun-sensitive cutaneous phenotype [11] and in persons with excessive cumulative sun exposure, especially if sustained at a young age [12].

To help clarify the genetic contribution to melanoma susceptibility, Greene et al. employed the Elston-Stewart maximum likelihood method of segregation analysis to 14 CMM-prone kindreds and suggested that autosomal dominant inheritance explained the pattern of disease in these families [13]. An additional dimension of this study was its inclusion of the dysplastic nevus (DN) syndrome. DN are clinically [14–16] and histologically [17, 18] distinctive melanocytic lesions that have been implicated as the substrate from which most cases of familial CMM arise [19–25]. Further segregation analysis, considering as affected those family members who had either CMM or DN, showed that the data were not consistent with a Mendelian pattern of inheritance [13], since the probability that the affected heterozygote transmits the disease (τ_2) gene was estimated to be 1.0 rather than .5 in these families. However, such a finding might result from sporadic cases of DN in the families. That 2% of controls in a case-control study of CMM were found to have DN lends support to this explanation [26]. More recently, 4.8% of 881 consecutive patients in a private dermatology practice were shown to have biopsy-proven DN [27], although even a sporadic rate as high as this would not completely account for the inflated estimate of τ_2 as found in the original study. It is more likely that the ascertainment correction used in the original model was not sufficient to account for the fact that these were "loaded" families, that is, families in which there was an excess of CMM, and, hence, DN, than if the probands were randomly ascertained from a group of truly autosomal dominant cases of CMM. This would surely inflate the heterozygote transmission probability.

Although no major gene was found to be consistent with the observed data by segregation analysis, linkage analysis for the DN/CMM phenotype was still performed, with DN/CMM vs. the Rh locus on chromosome 1 giving a lod score of 1.56 at a recombination value of 30% [13], under the assumption of autosomal dominant inheritance using penetrance estimates from the melanoma-alone analysis. Although it is not the usual practice to attempt link-

age analysis when a major locus is undetectable by segregation analysis, in view of the uncorrected ascertainment bias and recent simulation studies which showed that in those cases where the major locus could not be detected by segregation analysis [28] linkage to a marker locus could still be detected under certain conditions, this linkage result may be valid. Lynch et al. also observed an apparent dominant inheritance for the combined DN/CMM phenotype in four families [29].

Here, we consider further the data collected by Greene et al. using the method of Chase et al. [30] that allows testing genetic hypotheses for a disease with a late, variable age of onset. Chase's life-table approach allows the use of information on persons of all ages to produce an estimate of the probability of disease onset by a given age. This is crucial for the following reason. If it were possible to follow all the first-degree relatives of a patient with an adult-onset, autosomal dominant disease to the end of their lives, and if they all lived long enough to become affected, one-half would be expected to develop the disease, under the assumption that the disease gene is completely penetrant. In reality, such an ideal follow-up situation rarely occurs. Family studies nearly always involve the analysis of cross-sectional data. To complicate matters further, subjects die from competing causes prior to disease onset. Therefore, a simple count of the number of affected persons among all individuals at risk will underestimate the segregation ratio for the disease.

To help clarify the relationship between DN and melanoma in these families, we also performed a standard linkage analysis between these traits. A high lod score with no recombination would provide evidence that the same gene predisposes to both the precursor lesion and melanoma, although incomplete penetrance can simulate recombination.

MATERIALS

The method of ascertainment for the 14 families studied by Greene et al. was described in detail in [13–19]. As these same families were used for the current analysis, we will only briefly discuss the collection of data. The Environmental Epidemiology Branch (EEB) of the National Cancer Institute maintains a nonpopulation-based registry of high-risk cancer families. These kindreds are referred to the Family Studies Section of the EEB by National Institutes of Health physicians, independent practitioners in the community, and by family members themselves. This database was searched to identify all kindreds in which there were at least two living members with pathologically-confirmed CMM. Twenty-five kindreds fit these criteria, of which 14 participated in the study. Pathologic diagnoses were required to classify any individual as affected with melanoma. Both clinical and histological criteria defined each person's status with respect to DN. Four hundred one members of these 14 high-risk families were classified regarding affection by DN and/or CMM.

METHODS

Life-Table Analysis

Chase's method involves calculating the cumulative probability of escaping disease onset for a series of first-degree relatives of affected individuals [30]. This value, when subtracted from unity, yields the lifetime incidence, which is an estimate of the segrega-

tion ratio for a highly penetrant disease. If the 95% confidence interval containing this estimate includes 0.5 (the segregation ratio under the hypothesis of Mendelian inheritance), there is evidence that the disease results from a highly penetrant, autosomal dominant gene.

This method was employed to evaluate the lifetime risk of melanoma in first-degree relatives of individuals who had DN. Since DN usually appear by adolescence [31] and malignant melanoma shows a late, variable age of onset [32], application of the life-table technique should be a powerful method for estimating the segregation ratio, from which we may then infer the mode of transmission. Furthermore, since the DN status of study participants was unknown when these families were ascertained, using DN to identify probands avoids the referral bias inherent in using melanoma status for this purpose. Also, since this method reduces the analytic unit to nuclear families, it circumvents the problem of having originally enrolled multiplex pedigrees.

The 14 pedigrees were coded so that the investigator was blind to the melanoma status of all family members. Probands were defined according to the following criteria: (1) affected with DN; (2) age 19 or older; and (3) having at least one sibling or offspring. The last criterion was necessary because the probands were not included in the analysis, so that a proband with no siblings or offspring was uninformative. In this way, 46 probands were identified from the 14 pedigrees. No sibship was identified by more than one proband (i.e., there were no multiple ascertainments).

After breaking the melanoma status codes, we evaluated the melanoma experience of the relatives using Chase's method [30]. Similarly, a cohort of relatives of melanoma patients at risk of DN was identified and a segregation ratio computed.

Linkage Analysis

We next performed a standard linkage analysis between DN and melanoma on the assumption of autosomal dominance as suggested by our data. Using the average annual incidence of melanoma as reported for the years 1968–1972 [33], the average cumulative incidence of melanoma to age 90 was found to be .00599. To obtain an upper bound on the gene frequency, all cases were assumed to result from the action of a completely penetrant autosomal dominant gene. The gene frequency for melanoma, p_M, was then estimated to be .00599/2 = .003. For DN, again assuming autosomal dominant inheritance and a population prevalence of 8% [25], the estimate of the gene frequency becomes p_{DN} = .04. Allowing a normally distributed age of onset for familial CMM (mean = 30 years, standard deviation = 12 years as computed from our data), we performed standard linkage analysis using LIPED [34]. We also performed tests of linkage heterogeneity on the sample of families using Morton's [35] and Smith's [36, 37] methods.

RESULTS

The 46 DN probands used to identify the sample were excluded from the life-table analysis of melanoma. The 95 sibs and 65 offspring of these probands ranged in age from 5.5 to 76.7 years. Thirty-three cases of melanoma occurred among these first-degree relatives of the probands (see table 1). The Kaplan-Meier estimate of the probability of escaping melanoma for the first-degree relatives of DN probands was 0.5428 (SE = 0.0792). This value was achieved by age 58, as there were no incident cases of melanoma in the 10 individuals between ages 59 and 76. This approach yields an estimate of the segregation ratio for melanoma of 0.46, a value not significantly different from the predicted value of 0.50 (P = .58).

Similarly, 35 individuals with melanoma identified 116 first-degree relatives at risk of DN. Since DN are evident by adolescence, an age correction for this

TABLE 1

MELANOMA EXPERIENCE OF 160 SIBS AND OFFSPRING OF 46 PROBANDS WITH DN

Age range	No. alive at start of interval	No. incident melanoma cases	No. withdrawals or deaths	No. surviving
0–9	160	0	2	158
10–19	158	6	22	130
20–29	130	14	35	81
30–39	81	7	37	37
40–49	37	3	10	24
50–59	24	3	14	7
60–69	7	0	6	1
70–79	1	0	1	0

trait in our data is unnecessary. Under the hypothesis of autosomal dominance, 50% of those at risk would be expected to have DN. In our data, 54.31% (63 individuals) were affected. The standard error of this estimate is 4.63% so that the observed number of DN cases does not differ significantly from that expected under the autosomal dominant hypothesis.

Linkage analysis between the melanoma and DN loci gave an interpolated maximum lod score of 3.857 at $\hat{\theta} = .08$ for the 14 families combined. There was no evidence of heterogeneity using Morton's test [35] ($\chi^2 = 22.3$; d.f. = 13; $.7 > P > .6$) or Smith's test [36, 37] ($\alpha = 1.0$; $\theta = .10$; $Z = 3.84$). (Values of θ and Z were not interpolated when using Smith's procedure.) The results of the lod score analysis for each family are shown in table 2.

TABLE 2

LOD SCORES FOR MELANOMA VS. DN

		THETA					
FAMILY	(SIZE)	0	.05	.10	.20	.30	.40
255	(34)	−.2061	−.1417	−.0794	−.0021	.0179	.0080
342	(44)	.7754	.7207	.6502	.4655	.2451	.0598
372	(21)	.6715	.5873	.5008	.3263	.1644	.0444
373	(29)	.4905	.4442	.3924	.2757	.1521	.0477
377	(39)	1.5909	1.5648	1.4758	1.1598	.7136	.2399
479	(17)	−.6152	−.5175	−.4307	−.2838	−.1659	−.0720
480	(9)	.2751	.2347	.1951	.1205	.0580	.0154
481	(9)	−.1606	−.1250	−.0955	−.0510	−.0219	−.0054
567	(35)	−.2129	−.0922	−.0212	.0436	.0554	.0389
623	(51)	−.7990	−.5448	−.3697	−.1543	−.0485	−.0073
873	(80)	1.2278	1.0613	.8895	.5496	.2518	.0564
909	(9)	.2240	.1662	.1159	.0414	.0020	−.0084
928	(27)	−.2953	−.0688	.0267	.0876	.0796	.0450
1016	(28)	.3044	.5201	.5875	.5336	.3601	.1618
Total	(432)	3.2705	3.8093	3.8374	3.1124	1.8637	.6242

DISCUSSION

Our analyses provide further support for an autosomal dominant mode of inheritance for familial cutaneous melanoma. We also provide the first concrete evidence using statistical methods in high-risk families that the combined traits of CMM/DN follow an autosomal dominant pattern of inheritance with a high rate of penetrance. We used individuals with DN to identify nuclear families in which the gene for CMM could be segregating. By selecting as probands only those individuals who had already passed through adolescence, and evaluating only melanoma status in their first-degree relatives, we avoid the problem that each trait has a different age-of-onset pattern. This is an improvement over the previous segregation analysis of melanoma alone or of both DN and CMM together using the Elston-Stewart method [13]. Maximum likelihood segregation analysis of CMM alone implied the presence of a dominant gene with 64% penetrance because individuals with DN were not recognized as expressing the gene. Segregation analysis of the combined DN/CMM phenotype forced a single age-of-onset distribution to be fitted to an outcome that is actually the result of two very different underlying age patterns. It is likely that this approach caused inaccurate assignments of the probability of being a gene carrier to those individuals who were unaffected at the time of observation. Our use of the life-table approach avoids these pitfalls.

The second problem encountered in the published segregation analysis of DN/CMM was that the heterozygote transmission probability approached 1.0, causing rejection of any Mendelian model [13]. The life-table technique, in which only the proband's DN status is considered, does not address this question. However, if an individual with nonfamilial DN were chosen as a proband (in which case none of his first-degree relatives would be expected to have CMM), the life-table analyses would underestimate the segregation ratio. This is a likely explanation for the fact that the observed segregation ratio of CMM in these families is less than 0.5.

The difference between our estimate of the segregation ratio ($1 - .5428 = .4572$) and 0.5, although not significant, could also be explained by incomplete penetrance of the melanoma-DN gene. (Although an estimate of the penetrance could be found by computing the ratio of the estimated segregation ratio with that expected [i.e., .4572/.5000 = .9144], it must be noted that the penetrance is *not* estimated independently in this manner and is totally confounded with the segregation ratio.) Another possibility is that this small deviation from the theoretical value is due to there being a limited number of relatives of advanced age being available for analysis.

Linkage analysis between the loci for the two traits showed that the maximum likelihood estimate of θ was 0.08. This value is not significantly different from zero, ($\chi^2 = 2.7042$, $P = .1$), supporting our hypothesis that pleiotropy, a single gene producing multiple effects, could account for the development of both melanoma and DN. Importantly, there is clinical (histologic) evidence that DN is a precursor lesion in the development of melanoma [14, 16, 19, 26, 27]. The apparent recombination of 8% between the two loci could possibly be due to as yet undetected linkage heterogeneity.

We observed that six families showed zero recombination between DN and melanoma ($\hat{\theta} = 0$) while three families showed free recombination ($\hat{\theta} = .5$). Inspection of the pedigrees revealed that the apparent disruption in cosegregation of the DN and CMM loci occurred only in the youngest generations of these three families, that is, there were many people at risk of melanoma who had DN but had not developed cancer. One explanation for this finding is that there is differential follow-up from family to family. The majority of regular follow-ups of study participants is provided by their local dermatologists, each exercising his or her own judgment in the management of these patients. Undoubtedly, there is considerable variation in the aggressiveness with which individual study participants are managed. Thus, the observed disruption of cosegregation of the loci in the younger individuals may be due to alert physicians who biopsy any slightly changing mole in high-risk individuals. As a consequence of aggressive medical care, those people may never develop melanoma; seven subjects underwent removal of changing nevi that proved to be histologically borderline lesions, that is, severely dysplastic nevi that might have become malignant had they not been removed [19]. In fact, this is the most probable explanation of the aberrant melanoma pattern in these three families, since their physicians have performed 32 biopsies on seven people (family 479), 54 biopsies on four people (family 481), and 55 biopsies on five people (family 623) with DN! These between-family differences in management can also simulate recombination events.

Alternatively, there may be another separate genetic locus for DN. As mentioned above, there is evidence that DN is not an uncommon finding in otherwise healthy people [26, 27]. Four of 90 spouses in our families had DN. Such nonfamilial cases within the families would also simulate recombination. Lastly, there may be cases of truly sporadic or nonfamilial DN within these families. At present, it is not possible to distinguish, either clinically or histologically, the familial melanoma-associated DN, other familial DN, or the sporadic DN.

The only way to prove pleiotropy is to isolate the gene and understand its function at the molecular level. Such studies are now underway, using restriction fragment length polymorphisms to clarify our earlier standard linkage results; if these studies confirm that the CMM/DN gene is on the short arm of chromosome 1, attempts to identify and isolate the gene can proceed. Until that time, we have provided strong support for the plausible, biologic hypothesis that DN and CMM result from the pleiotropic actions of a single, autosomal dominant gene.

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REFERENCES

1. GREENE MH, FRAUMENI JF: The hereditary variant of malignant melanoma, in *Human Malignant Melanoma,* edited by CLARK WH, GOLDMAN L, MASTRANGELO M, New York, Grune and Stratton, 1979, pp 139–166

2. Norris W: A case of fungoid disease. *Edinb Med Surg J* 16:562–565, 1820
3. Anderson DE, Smith JL, McBride CM: Hereditary aspects of malignant melanoma. *J Am Med Assoc* 200:741–746, 1967
4. Lynch HT, Krush AJ: Hereditary and malignant melanoma—implications for early cancer detection. *Can Med Assoc J* 99:17–21, 1968
5. Wallace DC, Exton LE, McLeod GRC: Genetic factors in melanoma. *Cancer* 27:1262–1266, 1971
6. Duggleby WF, Stoll H, Priore HL, Greenwald P, Graham S: A genetic analysis of melanoma—polygenic inheritance as a threshold trait. *Am J Epidemiol* 114:63–72, 1981
7. Smith PJ, Greene MH, Devlin DA, McKeen EA, Paterson MC: Abnormal sensitivity to UV radiation in cultured skin fibroblasts from patients with hereditary cutaneous malignant melanoma and dysplastic nevus syndrome. *Int J Cancer* 30:39–45, 1982
8. Howell JN, Greene MH, Corner RC, Maher VM, McCormick JJ: Fibroblasts from patients with hereditary cutaneous malignant melanoma are abnormally sensitive to the mutagenic effect of simulated sunlight and 4-NQO. *Proc Natl Acad Sci USA* 81:1179–1183, 1984
9. Perera MIR, Greene MH, Kraemer KH: Dysplastic nevus syndrome—increased ultraviolet mutability in association with increased melanoma susceptibility. *Clin Res* 31:595A, 1983
10. Ramsay RG, Chen P, Imray FP, Kidson C, Lavin MF, Hockey A: Familial melanoma associated with dominant ultraviolet radiation. *Cancer Res* 42:2909–2912, 1982
11. Holman CD, Armstrong BK: Pigmentary traits, ethnic origin, benign nevi, and family history as risk factors for cutaneous malignant melanoma. *J Natl Cancer Inst* 72:257–266, 1984
12. Holman CDJ, Armstrong BK: Cutaneous malignant melanoma and indicators of total accumulated exposure to the sun—an analysis separating histogenetic types. *J Natl Cancer Inst* 73:75–82, 1984
13. Greene MH, Goldin LR, Clark WH, et al.: Familial cutaneous malignant melanoma: autosomal dominant trait possibly linked to the Rh locus. *Proc Natl Acad Sci USA* 80:6071–6075, 1983
14. Reimer RR, Clark WH, Greene MH, Ainsworth A, Fraumeni JR Jr: Precursor lesions in familial melanoma—a new genetic pre-neoplastic syndrome. *J Am Med Assoc* 239:744–746, 1978
15. Greene MH, Clark WH, Tucker MA, et al.: Precursor nevi in cutaneous malignant melanoma: a proposed nomenclature. *Lancet* 2:1024, 1980
16. Greene MH, Clark WH, Tucker MA, et al.: Acquired precursors of cutaneous malignant melanoma—the familial dysplastic nevus syndrome. *N Engl J Med* 312:91–97, 1985
17. Clark WH, Reimer RR, Greene MH, Ainsworth A, Mastrangelo M: Origin of familial malignant melanoma from heritable melanocytic lesions. *Arch Dermatol* 114:732–738, 1978
18. Clark WH, Elder DE, Guerry D, Epstein MH, Greene MH, van Horn M: A study of tumor progression—the precursor lesions of superficial spreading and nodular melanoma. *Hum Pathol* 15:1147–1165, 1984
19. Greene MH, Clark WH, Tucker MA, Kraemer KH, Elder DE, Fraser MC: The high risk of malignant melanoma in melanoma-prone families with dysplastic nevi. *Ann Intern Med* 102:458–465, 1985
20. Lynch HT, Frichot BC III, Lynch JF: Familial atypical multiple mole melanoma syndrome. *J Med Genet* 15:352–356, 1978
21. Braun-Falco O, Landthaler M, Rychkmanns F: BK-mole syndrome. *Fortschr Med* 97:1494–1498, 1979

22. HAUESISEN S, SARBIN H: Auftreten des "B-K naevus syndrome" bei einer patientin mit melanoma und deren vater. *Aktuel Dermatol* 5:61–65, 1979
23. BOVET R, BACHMANN B, DELACRETAZ J: Is the B-K mole syndrome always an autosomal dominant trait? *Ann Dermatol Venereol* 107:1173–1178, 1980
24. HUNDEIKER M, RUPPEL R, SCHMITT H: Familiare maligne melanome und atypische disseminierte pigmentale (B-K-mole-syndrom). *Der Hautarzt* 31:42–46, 1980
25. NATIONAL INSTITUTES OF HEALTH CONSENSUS DEVELOPMENT CONFERENCE: Precursors to malignant melanoma. *J Am Med Assoc* 251:1864–1866, 1984
26. RHODES AR, SOBER AJ, MIHM MC, FITZPATRICK TB: Possible risk factors for primary cutaneous malignant melanoma (abstr.) *Clin Res* 28:252, 1980
27. CRUTCHER WA, SAGEBIEL RW: Prevalence of dysplastic naevi in a community practice. *Lancet* 1:729, 1984
28. GOLDIN LR, COX NJ, PAULS DL, GERSHON ES, KIDD KK: The detection of major loci by segregation and linkage analysis: a simulation study. *Genet Epidemiol* 1:285–296, 1984
29. LYNCH HT, FUSARO RM, KIMBERLING WJ, LYNCH JF, SHANNON DANES B: Familial atypical multiple mole melanoma (FAMMM) syndrome—segregation analysis. *J Med Genet* 20:342–344, 1983
30. CHASE GA, FOLSTEIN MF, BREITNER JC, BEATY TH, SELF SG: The use of life tables and survival analysis in testing genetic hypotheses, with an application to Alzheimer's disease. *Am J Epidemiol* 117:590–597, 1983
31. TUCKER MA, GREENE MH, CLARK WH, KRAEMER KH, FRASER MC, ELDER DE: Dysplastic nevi on the scalp of prepubertal children from melanoma-prone families. *J Pediatr* 103:65–69, 1983
32. STEVENS RG, MOOLGAVKAR SH: Malignant melanoma: dependence of site-specific risk on age. *Am J Epidemiol* 119:890–895, 1984
33. DEPT. HEALTH, EDUCATION AND WELFARE: *The Third National Cancer Survey Advanced Three Year Report,* DHEW publication no. (NIH) 75-637, Biometry Branch, NCI, NIH, Bethesda, Md., February 1974
34. OTT J: A computer program for linkage analysis of general pedigree data. *Am J Hum Genet* 28:528–529, 1976
35. MORTON NE: Sequential tests for the detection of linkage. *Am J Hum Genet* 7:277–318, 1956
36. SMITH CAB: Testing for heterogeneity of recombination values in human genetics. *Ann Hum Genet* 27:175–182, 1963
37. HODGE SE, ANDERSON CE, NEISWANGER K, SPARKES RS, RIMOIN DL: The search for heterogeneity in insulin-dependent diabetes mellitus (IDDM): linkage studies, two-locus models, and genetic heterogeneity. *Am J Hum Genet* 35:1139–1155, 1983