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Evidence against the Reported Linkage of the Cutaneous Melanoma–Dysplastic Nevus Syndrome Locus to Chromosome 1p36

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Summary

The reported linkage between cutaneous melanoma and the dysplastic nevus syndrome (CM/DNS) to markers located on the distal portion of the short arm of chromosome 1 was examined in three Utah kindreds ascertained for multiple cases of melanoma. Family members in these kindreds were genotyped for the two markers reported to be most closely linked in the Bale study, PND and D1S47. Both melanoma alone and a combined melanoma/DNS phenotype were analyzed; no evidence for linkage was found. By multipoint linkage analysis the CM/DNS locus was excluded from an area of 55 cM containing the PND–D1S47 region. Diagnostic or genetic heterogeneity are alternate explanations for the discrepancy between our observations and those of Bale et al.

Introduction

Families with a high incidence of cutaneous melanoma (CM) have been described in which melanoma cosegregates with multiple atypical moles, known as dysplastic nevi (Clark et al. 1978; Lynch et al. 1978). Linkage studies of families displaying both melanoma and the dysplastic nevus syndrome (DNS) have yielded conflicting results. Greene et al. (1983) initially presented evidence that a gene predisposing to CM and DNS (CM/DNS) was linked to Rh on the short arm of chromosome 1. Bale et al. (1989) presented evidence that this gene maps to chromosome band 1p36 between the PND locus and an anonymous DNA segment, D1S47. van Haeringen et al. (1989) almost simultaneously reported exclusion of the DNS locus from the short arm of chromosome 1 by using a different set of markers and families. In the present paper we examine the reported linkage of CM/DNS to the PND and D1S47 loci in a set of Utah melanoma families which are part of an ongoing study of melanoma and the dysplastic nevus.

Methods

Three pedigrees were ascertained from local physicians for the presence of two or more closely related individuals with melanoma. These pedigrees contained 14, 4, and 2 cases of CM, respectively. The most informative branches were selected for linkage studies (see fig. 1). All individuals shown except those marked as deceased were examined. The age of the individual at the time of examination, the clinical phenotype, and the two marker genotypes are shown. The pedigree drawings also note those individuals with at least one biopsied nevus classified as histologically dysplastic by the NIH (1984) consensus criteria. Informed consent was obtained from each participant, and the study was approved by the Institutional Review Board of the University of Utah Medical Center.

All available relatives who had achieved puberty and available spouses in these pedigrees attended a screen-

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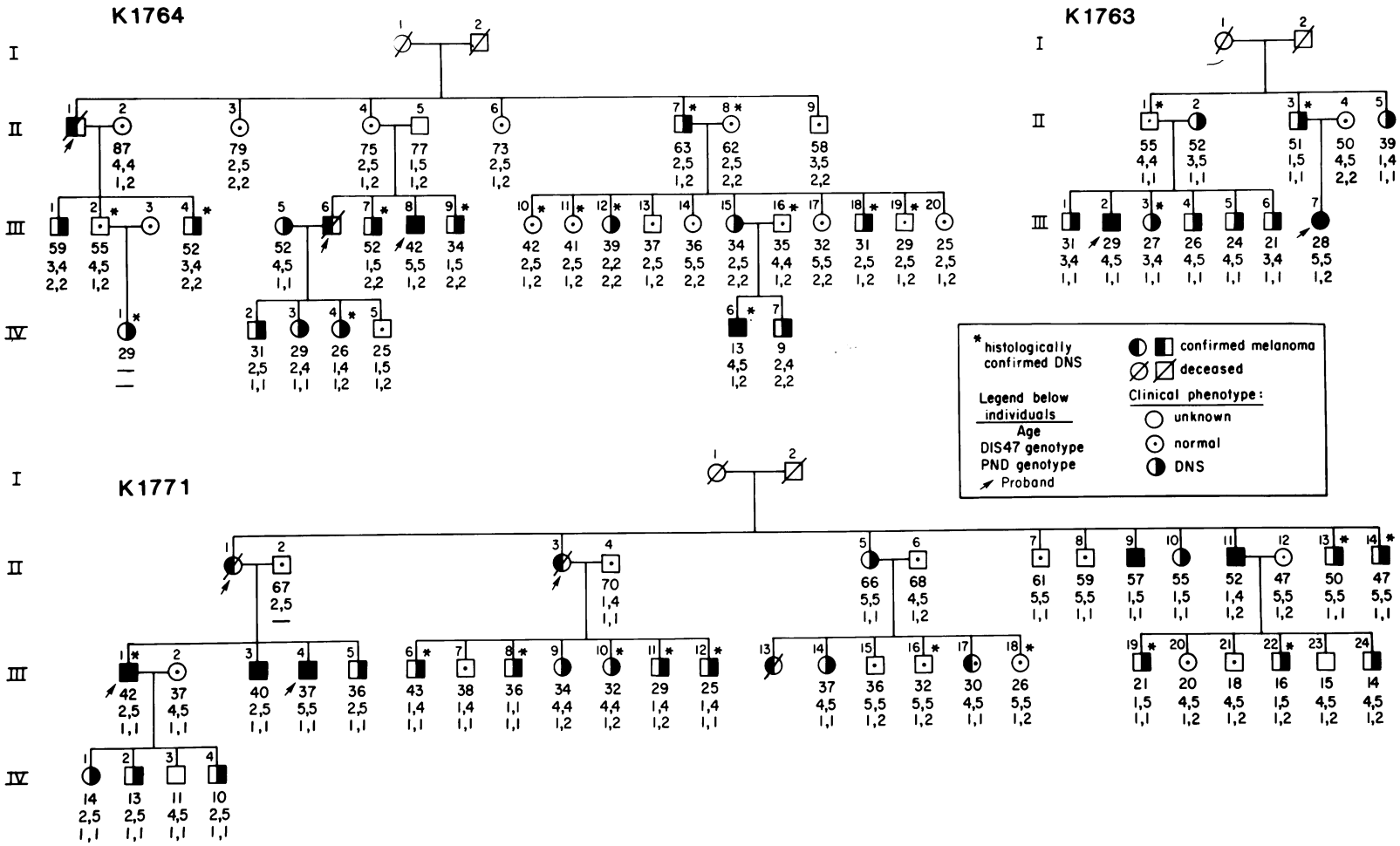

Figure 1 Three Utah pedigrees ascertained for melanoma cases and screened for DNS. Clinical phenotype, histology, age, and marker genotypes for two chromosome 1 markers are shown.

ing clinic for a complete skin examination. All nevi present and their size and appearance were recorded on a body chart, and up to two nevi per person, usually clinically atypical, were removed for pathological interpretation.

In early studies, the presence of a histologically dysplastic melanocytic nevus on an individual was sufficient for the diagnosis of DNS (Elder et al. 1980, 1981, 1982; Clark et al. 1984). More recently, there has been greater reliance on the clinical phenotype, including the diagnosis of clinically dysplastic nevi without the use of nevus biopsy, i.e., solely on the basis of clinical appearance (Cooke et al. 1989). Bale et al. (1989) state that for their analysis "cases of dysplastic nevi [were diagnosed] according to histologic or clinical criteria." Our study and the study of Bale et al. (1989) relied on Greene et al. (1985) for the clinical criteria for atypical nevi and for the clinical diagnosis of DNS in an individual. Greene et al. (1985) state that "dysplastic nevi often have irregular borders, a haphazard mixture of tan, brown, dark brown, and pink colors, and a margin that tends to be indistinct, fading into the adjacent normal skin. . . . They virtually always retain a macular component." Greene et al. (1985) also state that "the patient with familial dysplastic nevi typically has an increased number of larger-than-normal, irregularly shaped, indistinctly bordered, variably pigmented nevi that retain a macular or pebbly plaque component." Since Greene et al. (1985) note that affected individuals may have "more than 100 nevi . . . [or] . . . only one or a few," we have attempted to formalize their criteria with a more rigorous definition. In our study the DNS phenotype of each individual was established utilizing the following clinical guidelines: (1) many nevi, usually more than 50 clinically detectable nevi, (2) five or more clinically atypical nevi, and/or (3) five or more nevi ≥ 5 mm in diameter.

All diagnoses were made by one physician (L.J.M.), who was blinded to the family/spouse status of the individual. Individuals with melanoma were considered affected in all analyses. Because of the difficulty of diagnosis in young people, individuals under age 18 years who appeared unaffected were classified as unknown.

Four analyses were performed, one for the CM phenotype alone and three (analyses I–III) for the combined CM/DNS phenotype. In the analysis of CM alone, all individuals without confirmed melanoma were considered unknown, and the CM trait was analyzed as fully penetrant. Subsequent analyses included DNS in the definition of the abnormal phenotype, and we attempted to follow the definition used by Bale et al. (1989). Since the definition of DNS through clinical *or* histologic criteria can be interpreted in various ways, we present three analyses which vary this definition. In analysis I affected individuals were required to have melanoma or clinically diagnosed DNS with histologic confirmation by NIH consensus criteria. The four individuals (ages 14, 21, 30, and 14 years), with clinical DNS who were not biopsied were considered unknown, and all biopsied individuals with nevi which did not meet the NIH consensus criteria for dysplasia were considered unaffected. In analysis II the CM/DNS phenotype is almost the same, except that the biopsied individuals without histological confirmation were considered unknown rather than unaffected. In analysis III of the CM/DNS phenotype all individuals with clinically diagnosed DNS were considered affected regardless of whether histologic confirmation was obtained. In all three analyses individuals with neither melanoma nor clinically diagnosed DNS were considered unaffected.

Genomic DNA samples were digested to completion with a fivefold excess of enzyme according to the specifications of the supplier, except that 1 mM spermidine was added to the restriction digest. The digested samples were electrophoresed in 0.9% agarose gels and transferred to a nylon membrane filter (Zetabind; CUNO, Inc.) according to the method of Barker et al. (1984).

The filters were prehybridized for a minimum of 1 h at 42°C in hybridization solution (50% formamide, 10% dextran sulfate, 0.1 mg denatured sonicated salmon-sperm DNA/ml, 5 × SSC, 1 × Denhardt's solution, 0.02 M sodium phosphate pH 6.7). Probe inserts were gel purified and radioactively labeled by nick translation (Rigby et al. 1977) with [^{32}P]-alpha-dCTP (3,000 Ci/mmol; New England Nuclear) to specific activities of 1–3 × 10^8 cpm/μg. The radioactive probes were hybridized to the genomic blots at 42°C for 18 h in hybridization solution. Hybridization competition with total human DNA was used with probe CRI-LA336 (D1S47) to reduce background hybridization due to repetitive elements present in the probe (Ardeshir et al. 1983; Litt and White 1985). The filters were washed once at room temperature in 2 × SSC, 0.1% SDS and then twice in 0.1 × SSC, 0.1% SDS at 65°C for 30 min. The filters were exposed to Kodak XAR-5 film backed by an intensifying screen (Dupont Lightning Plus) at −70°C for 1–7 d.

The PND locus was typed by hybridizing clone pJA110 (Nemer et al. 1984, 1986; Yang-Fen et al. 1985) to *Xho*I blots. pJA110 was acquired from the American Type Culture Collection. The D1S47 locus was typed by

Table 1

LOD Scores between CM/DNS and D1S47 for Four Analyses

Model and Family	Recombination Fraction					
	.00	.05	.10	.20	.30	.40
Melanoma affecteds only:						
1771	−1.51	−1.11	−.73	−.32	−.12	−.03
1763	−1.80	−.69	−.43	−.19	−.08	−.02
1764	−1.99	−.65	−.42	−.21	−.09	−.02
Total[a]	−5.31	−2.46	−1.58	−.72	−.29	−.07
CM/DNS analysis I:						
1771	−11.77	−3.48	−2.07	−.80	−.28	−.08
1763	−2.40	−.66	−.40	−.17	−.06	−.02
1764	−15.03	−3.81	−2.36	−1.08	−.55	−.10
Total	−29.20	−7.96	−4.83	−2.04	−.78	−.20
CM/DNS analysis II:						
1771	−10.66	−2.60	−1.16	−.04	+.27	+.20
1763	−2.40	−.72	−.44	−.19	−.08	−.02
1764	−16.63	−3.94	−2.41	−1.11	−.54	−.20
Total	−29.70	−7.26	−4.02	−1.34	−.35	−.02
CM/DNS analysis III:						
1771	−12.81	−6.59	−4.43	−2.16	−1.01	−.36
1763	−3.41	−1.58	−1.15	−.53	−.20	−.04
1764	−12.15	−3.79	−2.41	−.93	−.27	−.02
Total	−28.37	−11.96	−7.98	−3.62	−1.48	−.42

[a] Because of rounding error, total LOD scores may not equal the sum of the individual family LODs.

hybridizing probe CRI-LA336 (Donis-Keller et al. 1987) to *Hin*dIII blots. CRI-LA336 was obtained from David Barker (University of Utah).

For comparative purposes linkage analysis of the three combined CM/DNS phenotypes was performed assuming the segregation model described by Bale et al. (1989), i.e., a dominant gene with a penetrance of .99, no allowance for sporadic cases, and a gene frequency of .001. The program LINKAGE (Lathrop et al. 1985) was used for both pairwise and multipoint linkage analyses assuming equal rates of recombination in males and females. All analyses were performed on an Apollo 4500 workstation.

Results

Table 1 presents the LOD scores for linkage between D1S47 and the CM/DNS locus for the four analyses described. All four exhibited strong evidence against tight linkage. Linkage could be rejected (LOD < -2.00) at recombination fractions $<.07$ for melanoma and at .16 for the least informative CM/DNS analysis (II). Table 2 presents the corresponding results for the PND locus for all four analyses. There was essentially no evidence with regard to linkage when melanoma alone was considered, and there was consistent evidence against linkage for the CM/DNS analyses. No evidence for heterogeneity for either locus was present among the three pedigrees studied.

Multipoint linkage analysis was performed for the CM/DNS phenotype (analysis II) and the two marker loci PND and D1S47. Analogous to the analysis reported by Bale et al. (1989), the likelihood for various genetic map positions of the hypothesized CM/DNS locus was computed relative to a fixed map of the two marker loci. These likelihoods were then transformed to location scores by subtracting the $\log_{10}$ likelihood of the data when the CM/DNS gene was assumed to be at a map position X on the fixed D1S47-PND map from the $\log_{10}$ likelihood assuming that the CM/DNS gene was unlinked to chromosome 1p. This analysis (see fig. 2) excluded linkage from the region between PND and D1S47 with odds of $>10^9$:1. For this analysis, we assumed the distance between PND and D1S47 to be 14.6 cM, as reported by Dracopoli et al. (1988). When a threshold of a location score <-2.0, corresponding

Table 2

LOD Scores between CM/DNS and PND for Four Analyses

Model and Family	Recombination Fraction					
	.00	.05	.10	.20	.30	.40
Melanoma affected only:						
1771	−.12	−.14	−.13	−.09	−.05	−.01
1763	+.12	+.10	+.08	+.05	+.02	+.01
1764	+.02	+.01	.00	.00	.00	.00
Total[a]	+.02	−.02	−.04	−.05	−.03	−.01
CM/DNS analysis I:						
1771	−5.29	−1.98	−1.23	−.58	−.26	−.07
1763	+.09	+.07	+.06	+.03	+.02	.00
1764	−8.42	−1.32	−.47	+.16	+.26	+.13
Total	−13.62	−3.24	−1.65	−.39	+.02	+.06
CM/DNS analysis II:						
1771	−5.52	−1.85	−1.09	−.45	−.19	−.06
1763	−.17	−.13	−.10	−.05	−.02	.00
1764	−8.46	−.94	−.13	+.39	+.38	+.16
Total	−14.15	−2.92	−1.32	−.11	+.17	+.09
CM/DNS analysis III:						
1771	−5.02	−3.04	−1.81	−.86	−.36	−.08
1763	−.10	−.08	−.06	−.04	−.02	−.01
1764	−8.30	−1.28	−.42	+.20	+.30	+.14
Total	−13.42	−4.12	−2.29	−.69	−.07	+.06

[a] Because of rounding error, total lod scores may not equal the sum of the individual family lods.

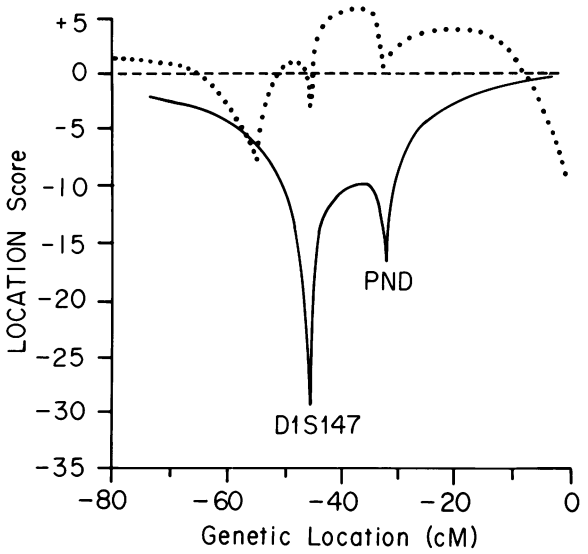

Figure 2 Location-score graph for CM/DNS (analysis II) between PND and D1S47. The solid line is the location score for the Utah pedigrees. The dotted line is the location-score graph presented by Bale et al. (1989), which was created using a larger set of markers than shown.

to odds of 100:1 is used, a region spanning ~55 cM can be excluded from containing the CM/DNS gene segregating in these three families. For comparison, figure 2 also presents a similar location-score map for the data presented by Bale et al. (1989) (fig. 2, dotted line). A comparison of the two maps indicates that across the region between PND and D1S47 the combined odds against linkage are at least 10,000:1.

Several pedigree observations directly illustrate the above results. The lack of linkage to melanoma alone is observed in kindred 1764, where individuals III-6 and III-8 are siblings concordant for melanoma who share no alleles identical by descent at the D1S47 locus. Similarly, in kindred 1763, III-2 and III-7 are affected cousins who share no alleles identical by descent at D1S47. The exclusion of the CM/DNS gene from between D1S47 and PND is shown in kindred 1771. Individual III-8, a 36-year-old male, had a total of 85 nevi, all <5 mm in diameter, five of which were clinically atypical. Two nevus biopsies were taken, both of which showed histopathology consistent with NIH consensus criteria for histologically dysplastic nevi. In-

dividual III-9, a 34-year-old female, had 90 total nevi including five atypical and four large (diameter >4 mm) nevi. No dysplasia was apparent in the two biopsied nevi. The mother of these individuals had a pathologically confirmed melanoma but was not examined in clinic. Their father, II-3, had 13 clinically normal small nevi. The two affected siblings inherited from their affected mother different alleles at both D1S47 and PND, indicating a double recombinant in this region, if, as reported, the CM/DNS locus were located between these two markers.

Discussion

The data from the Utah families presented here, in conjunction with the data presented by van Haeringen et al. (1989), present strong evidence for the exclusion of the CM/DNS locus from the distal short arm of chromosome 1. Diagnostic or genetic heterogeneity are alternate explanations for the discrepancy between these studies and the study of Bale et al. (1989).

Diagnostic heterogeneity is a potential problem for linkage studies of DNS. A small number of individuals have a very abnormal phenotype and are easy to classify; however, the DNS syndrome as described remains highly variable: "it is not unusual to observe more than 100 dysplastic nevi in an affected family member. More commonly, such persons will have 25 to 75 abnormal nevi, although some patients may have only one or a few" (Greene et al. 1985). Since the phenotype itself is highly variable and is influenced by sun exposure and skin type, it is prone to an uncertain diagnosis.

Because of diagnostic uncertainty in the DNS phenotype, the evidence of linkage for CM alone should be considered to be of prime importance in any attempt to map the CM/DNS locus. Our evidence against linkage between CM alone and the chromosome 1p36 markers (tables 1 and 2) is consistent with the evidence against linkage for the combined CM/DNS phenotype. Results for CM alone were not presented by Bale et al. (1989). Since all recombination events found by Bale et al. (1989) occurred in individuals with melanoma, rather than in individuals with the more subjective diagnosis of DNS, it is likely that support for linkage to melanoma alone was weak.

The use of histopathological analysis in the diagnosis of DNS remains a controversial topic. In one part of our study which has been reported elsewhere (Meyer et al. 1988; Piepkorn et al. 1989), all individuals were routinely biopsied twice, and over half of the spouse controls had at least one nevus which met the NIH (1984) consensus criteria for histologic dysplasia. Thus use of histology to confirm the DNS phenotype is nonspecific and could be misleading. This can be seen in our pedigrees presented in figure 1, in which individuals with a normal clinical diagnosis were found to have histologically dysplastic nevi. These patients would presumably not have been biopsied under the protocol of Bale et al. (1989) because they were clinically normal. When the CM/DNS phenotype is expanded to include either clinical DNS *or* histological abnormalities in clinically normal individuals, linkage between DNS and the PND-D1S47 region is excluded with similar odds (data not shown).

There are several notable examples of diseases with ambiguous phenotypic descriptions which have been reported as linked to a chromosome in one study and not linked to that chromosome in other studies. Affective disorder was mapped to chromosome 11 (Egeland et al. 1987) in an Amish kindred, and schizophrenia mapped to chromosome 5 (Sherrington et al. 1988); both results have been refuted in subsequent studies (Detera-Wadleigh et al. 1987; Kennedy et al. 1988; Kelsoe et al. 1989; St. Clair et al. 1989). One reasonable explanation for the discrepancies in linkage analyses of affective disorder, schizophrenia, and DNS is the difficulty of establishing an unambiguous diagnosis. Another explanation often invoked is genetic heterogeneity, although it is difficult to understand why there would be homogeneity within a study and heterogeneity across studies, unless the underlying populations were quite different. These discrepancies emphasize (1) the need for independent verification of a reported linkage before accepting the results and (2) the need for use of a broad phenotype (such as DNS) to suggest linkage but requiring confirmation with a narrow definition (melanoma).

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