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Genetic Predisposition to Melanoma

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INTRODUCTION

CUTANEOUS MALIGNANT melanoma (CMM) is one of the more common sites of cancer in populations worldwide. In the United States alone, there are approximately 32 000 new cases of CMM diagnosed annually and 7800 melanoma-related deaths. Furthermore, U.S. incidence rates for melanoma have been increasing more rapidly than for any other cancer except lung cancer [1]. In areas where fair-skinned individuals have high sun exposure, the lifetime risk is 1 in 60 [2].

Familial occurrence of melanoma has been recognised for over 40 years. Approximately 10% of melanoma cases arise in a familial setting [3]; these cases are hypothesised to carry an inherited susceptibility to melanoma. An increased risk of 2.0 for first degree relatives of melanoma cases [4] and 3.0 for relatives of one or more cases have been reported [5]. Analysis of the Utah Population Database [6] indicates a risk of 6.5 [7] for relatives of melanoma cases diagnosed before age 50 years, and 13.9 for relatives of two melanoma cases in the same family diagnosed at any age [8].

In addition to recognising the importance of family history in determining melanoma risk, epidemiological studies have indicated the importance of sun exposure [9], the number of naevi borne by an individual [10], skin type, hair and eye colour, and the distribution of naevi (commonly called moles) on the skin [11].

Prior to linkage analyses of melanoma susceptibility, the mode of inheritance of familial melanoma had not been estabished, and some investigators even debated the existence of a major gene [12–14]. The relationship between familial melanoma and an associated trait, the dysplastic nevus syndrome (DNS), has also been the subject of considerable debate.

Recently, melanoma susceptibility loci were assigned to chromosomes 1 [15] and 9 [16] although the chromosome 1 assignment remains controversial. A cell cycle regulatory gene, *CDKN2* (also known as *p16* and *MTS1*) [17], was found at the chromosome 9 location within the shortest region of overlap of a series of nested deletions in melanoma and other tumour cell lines [18–20]. However, conclusive demonstration of the role of this gene in melanoma susceptibility has been elusive [21].

The unfolding story of melanoma susceptibility demonstrates the many difficulties and pitfalls of the study of a complex phenotype that does not appear to follow simple Mendelian inheritance. We review this work from the initial description of familial melanoma and associated nevus phenotypes to the discovery of a tumour suppressor, which may be the chromosome 9-linked melanoma susceptibility gene.

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DYSPLASTIC NEVUS SYNDROME (DNS)

Histologically, the dysplastic nevus is an apparent precursor lesion to malignant melanoma. As such, it is an obvious extension of the melanoma phenotype, and its analysis could clarify the genetics of susceptibility to melanoma. However, there is considerable diagnostic heterogeneity when dysplastic naevi are used as a trait definition since both the clinical phenotype of DNS and the histological phenotype of dysplastic naevi are ambiguously described. For example, Greene and associates [22] states that "it is not unusual to observe more than 100 dysplastic naevi in an affected family member. More commonly such persons have 25 to 75 abnormal naevi, although some patients may have one or a few". In addition to the wide range in number and description of naevi which can be diagnostic for DNS, there is considerable debate about appropriate criteria for histological diagnosis of dysplastic naevi, which leads to disagreement on the diagnoses of specific lesions by different observers. Although DNS is thought to be rare (less than 1%) estimates of population prevalence of individuals with clinically or histologically defined dysplastic naevi of 4.9% [23], 20% [24] and 53% [25] have been reported. Six pathologists reviewed a set of random nevus biopsy specimens from Caucasian population controls. Their estimate of the prevalence of dysplasia in a single nevus varied from 7% to 32% [26]. In fact, the NIH consensus development conference on melanoma [27] suggested that: "The term 'dysplastic nevus' has been used by various investigators in significantly different ways, thereby generating a great deal of controversy, and should be avoided."

Melanoma susceptibility has been associated with DNS since 1978, when it was recognised that multiple large naevi of variable clinical appearance appeared to be associated with familial risk for melanoma [28, 29]. Clinically atypical moles from the relatives of cases showed evidence of dysplasia upon pathological evaluation of biopsies. The syndrome was thus defined as DNS [30].

Greene and associates [31] reported results from a segregation analysis of the combined trait of melanoma and DNS on a number of families ascertained for multiple melanoma cases. Members of the families were defined to have melanoma, DNS or neither. Although segregation analysis did not reject a dominant genetic model for melanoma alone, the analysis of melanoma and DNS together did not conform to the expectations for a dominant gene. This analysis provided evidence of the imprecision of the genetic analysis when a nevus trait was included. Bale and colleagues [32] subsequently used a different method of analysis, and argued that the distribution of melanoma and DNS in families was as expected for a dominant, disease-causing gene.

LINKAGE OF MELANOMA SUSCEPTIBILITY AND DNS TO CHROMOSOME 1

Just as the genetic analyses of melanoma and the DNS have yielded conflicting results, so have linkage studies analysing the combined trait. Evidence was presented that a gene predisposing to melanoma and DNS was linked to Rh on the short arm of chromosome 1 [31], a region demonstrating loss of heterozygosity (LOH) in melanomas [33]. Bale and associates [15] refined the linkage analysis, showing linkage of the combined trait to two genetic markers using a set of families collected at the NIH. Linkage to the pronatiodilatin (*PND*) locus was reported with a maximum LOD score (the base 10 logarithm of the odds for linkage) of 3.09 at a recombination fraction of 0.08, and to an anonymous DNA segment, D1S47, with a LOD score of 3.63 at a recombination fraction of 0.11.

Goldstein and colleagues [34] reanalysed updated data on the combined trait in the NIH families with the Bale model [15] and found similar LOD scores for D1S47 and somewhat reduced LOD scores for *PND*. However, when they analysed the families for melanoma alone, the maximum LOD score for linkage to D1S47 was reduced to 1.38 at a recombination fraction of 0.02, and the maximum LOD score for linkage to *PND* was 0.09 at a recombination fraction of 0.3. Seven additional unpublished NIH families were studied for chromosome 1p linkage [34] and gave a LOD score for linkage to D1S47 of 1.99 at a recombination fraction of 0.05 for melanoma alone, and a LOD score of linkage to *PND* of 0.06 at a recombination fraction of 0.4. When the Bale model [15] and a combined trait of melanoma and the DNS were used, the LOD scores for D1S47 and *PND* were negative at all recombination values. At a recombination fraction of 0.10, the LOD score for D1S47 was -4.32 and the LOD score for *PND* was -1.46. Thus, the updated analysis of the 13 NIH families for linkage to the combined trait of melanoma and DNS indicated that there was no linkage to chromosome 1.

In addition, data from each of five other analyses of linkage of melanoma to this region have refuted the 1p linkage findings [35–39]. One possibility for this discrepancy is genetic heterogeneity; however, this seems to be an unlikely explanation for the 1p linkage findings since all of the families in all of the studies were ascertained by the same criterion, namely at least two cases of melanoma. Given the prevalence of dysplastic naevi in the population, it is likely that the original study misclassified individuals as susceptible based on their nevus histology.

GENETIC ANALYSIS OF MELANOMA SUSCEPTIBILITY AND NEVUS COUNTS

Because of the controversies surrounding both the trait defintion and the report of linkage to chromosome 1p, the Genetic Analysis Workshop Study Group decided to focus a comparative genetic analysis on datasets relevant to the analysis of melanoma, naevi, and linkage of these traits to chromosome 1p. Appropriate datasets were requested from researchers and distributed by the workshop organisers to all interested parties for examination with a large variety of methods selected without constraint by each workshop participant. The five datasets included a twin study [40], three linkage studies [41–43] and a linkage study which included quantitative assessment of number and size of naevi [44]. The analyses were published in the *Genetic Analysis Workshop* 7 proceedings [45]. Numerous issues were addressed regarding melanoma, DNS, nevus phenotype, environmental exposures, and the presence of a combined melanoma and DNS susceptibility locus on chromosome 1.

The workshop identified the definition of affected individuals as perhaps the most difficult issue in analysis of this complex disease. Since clinical or histopathological definitions of naevi are not uniform, total number of naevi was examined at the workshop and in other genetic analyses of susceptibility as a melanoma-associated phenotype [45, 46]. These studies suggest that a quantitative trait based on number and size of naevi is inherited as a common codominant major locus in some kindreds [45, 46]. The summary of all analyses presented at the workshop noted that "among the great diversity of results and conclusions, the one finding that was consistent across all analyses was that dominant inheritance of (nevus phenotypes) was strongly rejected". This conclusion is of particular interest, because this is precisely the model that had been postulated for CMM and dysplastic nevus [47]. Other segregation analyses of dysplastic naevi have also contradicted a dominant mode of inheritance [48–50].

LINKAGE OF MELANOMA SUSCEPTIBILITY TO CHROMOSOME 9p21 (*MLM*)

Concurrent with the analysis of the linkage of melanoma susceptibility to chromosome 1, several different studies pinpointed a region on the short arm of chromosome 9 as one involved in the early stage development of melanoma tumours. Cytogenetically detectable loss or rearrangement of chromosome 9p was demonstrated in approximately 46% of all melanomas, and 9p rearrangements were identified in both dysplastic naevi and primary lesions, implying a 9p locus with an early role in melanoma development [51]. Loss of heterozygosity (LOH) studies also indicated the existence of a tumour suppressor locus mutated in most, if not all, melanomas [52, 53]. Fountain and colleagues [54] showed that 86% of melanoma tumours and cell lines harboured hemi- or homozygous deletions of DNA markers in this region, and defined a region of 2–3 Mb on 9p21 where a putative tumour suppressor gene must lie.

Further support for a melanoma susceptibility gene on chromosome 9p was indicated by the identification of a 34-year-old Caucasian woman, with multiple atypical moles and eight primary cutaneous melanomas, who has a *de novo* constitutional cytogenetic rearrangement involving chromosomes 5p and 9p [55]. Molecular analysis of this germline cytogenetic abnormality showed loss of material from the 9p21 region. D9S126 and *IFNA*, two genetic markers which map to the critical region, were used in comparison studies between the patient's genotypes and those of her unaffected parents. Homozygosity was found at both loci by gene dosage studies, suggesting that the germline loss of this gene predisposed to melanoma [55].

Cannon-Albright and associates [16] examined genetic markers on chromosome arm 9p for genetic linkage to melanoma using 11 large kindreds with multiple cases of invasive melanoma. Genetic markers from this region were analysed in these kindreds with a partially penetrant dominant genetic model for melanoma susceptibility. The susceptibility, called the *MLM* locus, was localised to 9p21 with a LOD score of 12.71. The original localisation [16] and subsequent confirmations [56–58] placed *MLM* near alpha-interferon (IFNA) on chromosome 9p. Further analysis by Cannon-Albright and associates [59] placed *MLM* in a 2 centiMorgan region, which has D9S736 as a distal boundary and D9S171 as a proximal boundary.

As the chromosome 9p linkage was being reported, Nancarrow and colleagues [60] were publishing the results of a linkage study of Australian pedigrees with familial melanoma using 172 microsatellite markers, distributed across all autosomes. LOD scores greater than 1.0 in a single family were noted for 13

different markers, including D9S104 on 9p as well as markers on chromosomes 3,4,5,6,10,12,14,18 and 21. However, no significant evidence for linkage was found.

Chromosome 9p linkage has since been investigated and confirmed in the set of 13 NIH families in which the 1p36 linkage was originally established [58]. Heterogeneity analysis estimated the proportion of families linked to chromosome 9p to be 50% and the proportion linked to chromosome 1p also to be 50%. Two of the 11 families for which data are presented show conditional probabilities greater than 90% of being linked to chromosome 1p. Interestingly, three of the original families provided the majority of the evidence of 1p linkage, and they are now seen to show conditional probabilities greater than 90% for 9p linkage in this latest study [58].

Bale and Dracopoli [61] had previously analysed the linkage between a melanoma and DNS susceptibility locus and the marker D9S3, a marker proximal to *IFNA*, in 4 of the NIH hereditary melanoma families and excluded close linkage. Two of these 4 families which they analysed now show conditional probabilities of 9p linkage to melanoma alone of 0.99 [58].

Neither the original linkage study [16] nor two of the linkage studies which confirmed the chromosome 9 linkage provided evidence for existence of genetic heterogeneity [16, 56, 57]. The only published evidence for genetic heterogeneity comes from the report of linkage of melanoma and dysplastic nevus syndrome to chromosome arms 1p and 9p [58]. Therefore, although it is difficult to rule out linkage to chromosome 1 in some families, it is still not well supported. The question of genetic heterogeneity for melanoma susceptibility will be best answered when the 9p susceptibility locus has been cloned and tested in a series of melanoma cases and their relatives.

PENETRANCE AND EXPRESSIVITY OF *MLM*

Kindreds showing evidence of chromosome 9p linkage have been analysed to estimate the penetrance of the 9p melanoma susceptibility locus, and to define the expression of the gene with respect to both melanoma and naevi [62]. In three kindreds in which the melanoma susceptibility gene is known to segregate, 124 gene carriers were identified by a haplotype consisting of four markers flanking the susceptibility locus. The probability of being affected with melanoma was estimated to be 53% by the age of 80 in gene carriers.

Nevus counts, skin type and sun exposure histories were gathered in a clinical examination of 119 individuals in two kindreds. Gene carriers were found to have higher nevus counts and higher nevus densities than non-gene carriers. Among gene carriers, individuals with melanoma were found to have more sun exposure within each skin type than gene carriers without melanoma. These analyses suggest that the 9p melanoma susceptibility is related to total number of naevi, and that it interacts with other genetic and environmental factors to induce melanoma.

Independent studies are also needed to examine the relationship of the *MLM* locus to precursor nevus phenotypes, and to assess the interaction of the *MLM* locus with known environmental risk factors. The ultimate identification of the germline mutations will allow estimation of the proportion of melanoma kindreds that segregate the 9p susceptibility, the frequency of the disease allele, and age- and sex-specific penetrances.

MLM AND THE *p16/MTS1/CDKN2* GENE

Molecular studies of the 9p21 region provided firm evidence for the order of markers used in previous genetic studies. Weaver-Feldhaus and associates [19] constructed a detailed physical map of the *MLM* region based on 54 STSs they generated between *IFNA* and D9S171. Analysis of YAC clones, P1 clones, and deletions in tumour lines yielded the order: *IFNA* - D9S736 -D9S171 -D9S126 -D9S161 [19]. D9S736 was isolated from P1 452 (obtained from Genome Systems, St Louis, Missouri, U.S.A.) and is located on CEPH YACs 761 A5 and 802 B11, placing it between *IFNA* and D9S171. These results agree with the relative localisation of Genethon markers [63] provided by Kwiatkowski and colleagues [64], reporting the order *IFNA* - D9S171 - D9S161, as well as with the order inferred from the Utah genetic data.

As described above, 9p21 is the site of frequent chromosomal aberration in tumours and cell lines. The aberrations are not confined to melanomas. 9p21 rearrangements have been reported in gliomas, leukaemias, and lymphomas, and other analyses have shown homozygous deletions in bladder cancer [65], and in a series of 10 tumour cell line types ([18] and references therein). Because of the high frequency of homozygous deletion in this region, classical deletion mapping was used to determine the shortest region of overlap, using a large set of melanoma cell lines and a similarly large set of cell lines representing 11 other tumour types.

The deletion analysis on chromosome 9 pinpointed a gene, *CDKN2* (also known as *MTS1*) that encodes a putative cell cycle regulator named p16 by its discoverers [17]. *CDKN2* was identified by its ability to bind and inhibit cyclin-dependent kinases (CDKs) *in vitro* [17]. CDKs, along with their associated positive regulatory factors, the cyclins, are principal determinants of the initiation of DNA replication and mitosis (for review, see Sherr [66]). As *p16/MTS1/CDKN2* encodes a putative inhibitor of cell division, *MTS1* has the appearance of a tumour suppressor gene. The *p16/MTS1/CDKN2* gene consists of 3 coding exons: E1 (131 bp), E2 (307 bp) and E3(11 bp). A percentage of melanoma lines as well as primary and metastic melanomas [67] that do not contain homozygous deletions contain smaller hemizygous genetic lesions in either E1 or E2 of *p16/MTS1/CDKN2*, such as frameshift, nonsense and missense mutations [18]. The high percentage of homozygous deletions and mutations in melanoma cell lines as well as the deletion of point mutations in primary melanomas suggest that *p16/MTS1/CDKN2* is involved in formation of melanomas and, as such *p16/MTS1/CDKN2* is an appealing candidate for MLM.

To test whether this gene is *MLM*, *p16/MTS1/CDKN2* coding sequences were analysed in 13 pedigrees that segregate 9p melanoma susceptibility, as well as in individuals from 38 other melanoma-prone families in whch linkage has not been determined. In only 2 cases were potential predisposing mutations identified [21]. Both were linked to the carrier chromosome, while neither was detected in the normal population. Neither involved a conservative amino acid substitution. These mutations were not seen in over 100 population controls or in over 50 other cases of familial and sporadic melanomas, suggesting that they are both rare. Further, Southern blot analysis of RFLPs gave no evidence for heterozygous deletions of *p16/MTS1/CDKN2* in the germline of melanoma-prone individuals [21].

In the absence of biochemical or structural information, the possibility that the two rare missense mutations are neutral cannot be excluded. Regardless of whether or not these changes are neutral, the frequency of *p16* mutations observed in melanoma-prone kindreds was much less than would be expected if

p16 were *MLM*: 2/13 in 9p21-linked families and 0/38 in familial melanoma cases.

There are two possible explanations for the low frequency of *p16* mutations observed: (1) *p16* and *MLM* are distinct genes or (2) *p16* is *MLM*, but the majority of predisposing mutations occur outside the *p16* coding region and adjoining splice junction sequences. To prove or disprove the hypothesis that *p16* is *MLM* will require an extensive search for predisposing mutations that lie in noncoding regions of the *p16* gene or in neighbouring genes.

Note added in proof—A second independent report of six *CDKN2* mutations in 9/18 NIH melanoma kindreds provides evidence that *CDKN2* is *MLM* [68]. Mutations have now been found in both the Utah and the NIH kindreds. Interestingly, those kindreds from both studies with the strongest statistical evidence for 9p linkage did not have *CDKN2* coding mutations. This supports the hypothesis that regulatory, non-coding mutations are responsible for those 9p-linked kindreds in which no *CDKN2* mutation has yet been found. There is some evidence that the kindreds in which *CDKN2* mutations have been identified may be more penetrant than those kindreds without known mutations. The two Utah kindreds found to have mutations include the most penetrant Utah kindreds. The nine NIH high risk melanoma kindreds found to have disease-related *CDKN2* mutations are smaller, more dense clusters of melanoma than the Utah kindreds, with an overall estimated penetrance of 69%. The analysis of *CDKN2* mutations in the NIH kindreds suggests that 10 of the 18 kindreds have a conditional probability of 9p linkage greater than 0.70. *CDKN2* mutations were found in three of the eight remaining kindreds, suggesting that the 9p susceptability locus is responsible for most familial melanoma.

1. National Cancer Institute. 1987 Annual Cancer Statistics Review NIH publication No. 88-2789, 1988 C. Frey and A. Hartman. *J Natl Cancer Inst* 1991, **83**, 170.
2. Sober AJ, Lew RA, Koh HK, Barnhill RL. Epidemiology of cutaneous melanoma. *Dermatol Clin* 1991, **9**, 617–629.
3. Greene MH, Fraumeni JF Jr. The hereditary variant of malignant melanoma. In Clark WH Jr, Goldman LI, Mastrangelo MJ, eds. *Human Malignant Melanoma*. New York, Grune and Stratton, 1979.
4. Wallace DC, Exton LA, McLeod GR. Genetic factor in malignant melanoma. *Cancer* 1971, **27**, 1262–1266.
5. Holman CD, Armstrong BK. Pigmentary traits, ethnic origin, benign nevi, and family history as risk factors for cutaneous malignant melanoma. *JNCI* 1984, **72**, 257–266.
6. Skolnick MH. The Utah genealogical data base: a resource for genetic epidemiology. In Cairns J, Lyon JL, Skolnick M, eds. *Banbury Report No 4: Cancer Incidence in Defined Populations*. New York, Cold Spring Harbor Laboratory, 1980, 285–297.
7. Goldgar DE, Easton DF, Cannon-Albright LA, Skolnick MH. A systematic population-based assessment of cancer risk in first degree relatives of cancer probands. *JNCI* 1994, **86**, 1600–1608.
8. Goldgar D, personal communication.
9. Green A, Swerdlow AJ. Epidemiology of melanocytic nevi. *Epidemiol Rev* 1989, **11**, 204–221.
10. Swerdlow AJ, English J, Mackie RM, O'Doherty CJ, Hunter JAA, Clark J. Benign naevi associated with high risk of melanoma. *Lancet* 1984, **2**, 168.
11. Swerdlow AJ, Green A. Melanocytic naevi and melanoma: an epidemiological perspective. *Br J Dermatol* 1987, **117**, 137–146.
12. Anderson DE. Clinical characteristics of the genetic variety of cutaneous melanoma in man. *Cancer* 1971, **28**, 721–725.
13. Wallace DB, Beardmore GL, Exton LA. Familial malignant melanoma. *Ann Surg* 1973, **177**, 15–20.
14. Duggleby WF, Stoll H, Priore RI, Greenwald P, Graham S. A genetic analysis of melanoma polygenic inheritance as a threshold trait. *Am J Epidemiol* 1981, **114**, 61–72.
15. Bale SJ, Dracopoli NC, Tucker MA, *et al.* Mapping the gene for hereditary cutaneous malignant melanoma-dysplastic naevus to chromosome 1p. *N Engl J Med* 1989, **320**, 1367–1372.
16. Cannon-Albright LA, Goldgar DE, Meyer LJ, *et al.* Assignment of a locus for familial melanoma MLM, to chromosome 9p13-22. *Science* 1992, **258**, 1148–1152.
17. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK3. *Nature* 1993, **366**, 704.
18. Kamb A, Gruis NA, Weaver-Feldhaus J, *et al.* A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 1994, **264**, 436.
19. Weaver-Feldhaus J, Gruis NA, Neuhausen S, *et al.* Localization of a putative tumor suppressor gene using homozygous deletions in melanomas. *Proc Natl Acad Sci USA* 1994, **91**, 7563–7567.
20. Nobori T, Miura K, Wu DJ, Lois A, Takabayashi K, Carson DA. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature* 1994, **368**, 753–756.
21. Kamb A, Shattuck-Eidens D, Eeles R, *et al.* Analysis of MTS1 as a candidate for the chromosome 9p melanoma susceptibility locus (MLM). *Nature Genet* 1994, **8**, 22–26.
22. Greene MH, Clark WH Jr, Tucker MA, Kraemer KH, Elder DE, Fraser MC. High risk of malignant melanoma in melanoma-prone families with dysplastic nevi. *Ann Intern Med* 1985, **102**, 458–465.
23. Crutcher WA, Sagebiel RW. Prevalence of dysplastic naevi in a community practice. *Lancet* 1984, **1**, 729.
24. Holly EA, Kelly JW, Shpall SN, Chiu S-H. Number of melanocytic nevi as a major risk factor for malignant melanoma. *J Am Acad Dermatol* 1987, **17**, 459–468.
25. Piepkorn M, Meyer LJ, Goldgar D, Seuchter S, Cannon-Albright LA, Skolnick MH. The dysplastic melanocytic nevus: a prevalent lesion that correlates poorly with clinical phenotype. *J Am Acad Derm* 1989, **29**, 407–415.
26. Piepkorn MW, Barnhill RL, Cannon-Albright LA, *et al.* A multiobserver, population-based analysis of histologic dysplasia in melanocytic nevi. *J Am Acad Derm* 1994, **30**, 707–714.
27. NIH Consensus Panel on Early Melanoma. Diagnosis and treatment of early melanoma. *JAMA* 1992, **268**, 1314–1319.
28. Clark WH, Reimer RR, Greene M, Ainsworth AM, Mastrangelo M. Origin of familial malignant melanomas from heritable melanocyte lesions: the B-K mole syndrome. *Arch Dermatol* 1978, **114**, 732.
29. Lynch HT, Frichot BC, Lynch JF. Familial atypical multiple mole melanoma syndrome. *J Med Genet* 1978, **15**, 352–356.
30. Elder DG, Kraemer KH, Greene KH, Clark WH Jr, Guerry D. The dysplastic nevus syndrome: our definition. *Am J Dermatopathol* 1982, **4**, 455–459.
31. Greene MH, Goldin LR, Clark WH, *et al.* Familial malignant melanoma: autosomal dominant trait possibly linked to the Rhesus locus. *Proc Natl Acad Sci USA* 1983, **80**, 6071–6075.
32. Bale SJ, Dracopoli NC, Tucker MA, *et al.* Cutaneous malignant melanoma and familial dysplastic nevi—evidence for autosomal dominant inheritance and pleiotropy. *Am J Human Genet* 1986, **38**, 188–196.
33. Dracopoli N, Alhadeff B, Houghton AN, Old LJ. Loss of heterozygosity at autosomal and X-linked loci during tumor progression in a patient with melanoma. *Cancer Res* 1987, **47**, 3995–4000.
34. Goldstein AM, Dracopoli NC, Ho EC, *et al.* Further evidence of a locus for cutaneous malignant melanoma-dysplastic nevus (CMM/DN) on chromosome 1p, and evidence for genetic heterogeneity. *Am J Human Genet* 1993, **52**, 537–550.
35. Cannon-Albright LA, Goldgar DE, Wright EC, *et al.* Evidence against the reported linkage to the cutaneous melanoma-dysplastic naevus syndrome locus to chromosome 1p36. *Am J Human Genet* 1990, **46**, 912–918.
36. Gruis NA, Bergman W, Frants RR. Locus for susceptibility to melanoma on chromosome 1p. *N Engl J Med* 1990, **322**, 853–854.
37. Nancarrow DJ, Palmer JM, Walters NK *et al.* Exclusion of the familial melanoma locus (MLM) from the PND/D1S47 and LMYC regions of chromosome arm 1p in 7 Australian pedigrees. *Genomics* 1992, **12**, 18–25.
38. Kefford RF, Salmon J, Shaw HM, Donald JA, McCarthy WH. Hereditary melanoma in Australia: variable association with dysplastic naevi and absence of genetic linkage to chromosome 1p. *Cancer Genet Cytogenet* 1991, **51**, 45–55.
39. van Haeringen A, Bergman W, Nelen MR, *et al.* Exclusion of the dysplastic nevus syndrome (DNS) locus from the short arm of chromosome 1 by linkage studies in Dutch families. *Genomics* 1989, **5**, 61–64.
40. Easton DF, Cox GM, MacDonald AM, Ponder BAJ. The study of nevi in British twins: study design and description of the data set. *Cytogenet Cell Genet* 1992, **59**, 165–166.
41. Bale SJ, Goldstein AM, Tucker MA. Description of the National Cancer Institute melanoma families. *Cytogenet Cell Genet* 1992, **59**, 159–160.

42. Bergman W, Gruis NA, Frants RR. The Dutch FAMMM family material: clinical and genetic data. *Cytogenet Cell Genet* 1992, **59**, 161–164.
43. Salmon JA, Rivers JK, Donald JA, Shaw HM, McCarthy WH, Kefford RF. Clinical aspects of hereditary melanoma in Australia. *Cytogenet Cell Genet* 1992, **59**, 170–172.
44. Meyer LJ, Goldgar DE, Cannon-Albright LA, *et al.* Number, size, and histopathology of nevi in Utah kindreds. *Cytogenet Cell Genet* 1992, **59**, 167–169.
45. Genetic Analysis Workshop 7. Issues in gene mapping and detection of major genes. *Cytogenet Cell Genet* 1992, **59**.
46. Goldgar DE, Cannon-Albright LA, Meyer LJ, Piepkorn MW, Zone JJ, Skolnick MH. Inheritance of nevus number and size in melanoma and dysplastic nevus syndrome kindreds. *J Natl Cancer Inst* 1991, **83**, 1726–1733.
47. Risch N, Sherman S. Genetic analysis workshop 7: summary of the melanoma workshop. *Cytogenet Cell Genet* 1992, **59**, 148–158.
48. Pascoe L. The inheritance of cutaneous malignant melanoma (CMM) and dysplastic nevus syndrome (DNS). *Am J Human Genet* 1987, **40**, 464.
49. Traupe H, Macher E, Hamm H, Happle R. Mutation rate estimates are not compatible with autosomal dominant inheritance of the dysplastic nevus "syndrome". *Am J Med Genet* 1989, **32**, 155–157.
50. Happle, R, Traupe H, Vakilzadeh F, Macher E J. Arguments in favor of a polygenic inheritance of precursor nevi. *J Am Acad Derm* 1981, **6**, 540–543.
51. Cowan JM, Halaban R, Franke U. Cytogenetic analysis of melanocytes from premalignant nevi and melanomas. *J Natl Cancer Inst* 1988, **80**, 1159–1164.
52. Fountain JW, Karayiorgou M, Graw SL, *et al.* Chromosome 9p involvement in melanoma. *Am J Human Genet* 1991, **49**, (suppl.), A223.
53. Olopade OI, Jenkins R, Linnenbach AJ, *et al.* Molecular analysis of chromosome 9p deletion in human solid tumors. *Proc Am Assoc Cancer Res* 1990, **31**, 318.
54. Fountain JW, Karayiorgou M, Ernstoff MS, *et al.* Homozygous deletions within human chromosome band 9p21 in melanoma. *Proc Natl Acad Sci* 1992, **89**, 10557–10561.
55. Petty EM, Gibson LH, Fountain JW, *et al.* Molecular definition of a chromosome 9p21 germ-line deletion in a woman with multiple melanomas and a plexiform neurofibroma implications for 9p tumor-suppressor gene(s). *Am J Human Genet* 1993, **53**, 96–104.
56. Gruis NA, Sandkuijl LA, Weber JL, *et al.* Linkage analysis in Dutch familial atypical multiple mole-melanoma (FAMMM) syndrome families. Effect of naevus count. *Melanoma Res* 1993, **3**, 271–277.
57. Nancarrow DJ, Mann GJ, Holland EA *et al.* Confirmation of chromosome 9p linkage in familial melanoma. *Am J Human Genet* 1993, **53**, 936–942.
58. Goldstein AM, Dracopoli NC, Engelstein M, Fraser MC, Clark WH, Tucker MA. Linkage of cutaneous malignant melanoma/dysplastic nevi to chromosome 9p, and evidence for genetic heterogeneity. *Am J Human Genet* 1994, **54**, 489–496.
59. Cannon-Albright LA, Goldgar DE, Neuhausen S, *et al.* Localization of the 9p melanoma susceptibility locus to a 2cM region between D9S736 and D9S171. *Genomics* 1994, **23**, 265–268.
60. Nancarrow DJ, Walker GJ, Weber JL, Walters MK, Palmer JM, Hayward NK. Linkage mapping of melanoma (MLM) using 172 microsatellite markers. *Genomics* 1992, **14**, 939–947.
61. Bale SJ, Dracopoli NC. Chromosome 9p and hereditary cutaneous malignant melanoma. *J Natl Cancer Inst* 1989, **81**, 70.
62. Cannon-Albright L, Meyer LJ, Goldgar DE, *et al.* Penetrance and expressivity of the chromosome 9p melanoma susceptibility locus. *Cancer Res* 1994.
63. Weissenbach J, Gyapay G, Dib C, *et al.* A second-generation linkage map of the human genome. *Nature* 1992, **359**, 794–801.
64. Kwiatkowski DJ, Dib C, Slaugenhaupt SA, Povey S, Gusella JF, Haines JL. An index marker map of chromosome 9 provides strong evidence for positive interference. *Am J Human Genet* 1993, **53**, 1279–1288.
65. Ruppert JM, Tokino K, Sidransky D. Evidence for two bladder cancer suppressor loci on human chromosome 9. *Cancer Res* 1993, **53**, 5093–5094.
66. Sherr CJ. Mammalian G1 cyclins. *Cell* 1993, **73**, 1059–1065.
67. Gruis NA, Weaver-Feldhaus J, Liu Q, *et al.* The MTS1 and TP53 genes may involve separate pathways of tumorigenesis. Submitted, 1994.
68. Hussussian CJ, Struewing JP, Goldstein AM *et al.* Germline p16 mutations in familial melanoma. *Nature Genet* 1994, **8**, 15–21.

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Recent Advances in the Genetics of Metastasis

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INTRODUCTION

METASTATIC SPREAD of tumour cells appears, at least formally, to be a complex multi-step process. Cells need to detach from their tissue of origin and from neighbouring primary tumour cells, migrate through basement membrane (in the case of epithelial cancer cells) and interstitial matrix, and invade the lymph and blood transport system. In most instances, metastases occur first in lymph nodes and, at some later stage, leave the lymphoid tissue to enter the blood stream. Adhesion to vascular endothelium is thought to lead to extravasation and nesting in new tissue, such as the lung. The complexity of this process suggests the participation of a variety of different proteins, the loss or gain of each possibly accounting for the individual specific step proposed. It is plausible that tumour cells acquire properties by mutation and selection, and that the rare cell that has assembled all the necessary properties will metastasise. However, it is also possible that a genetic programme is elicited by the coregulation of various genes, which may reduce the complexity considerably.

To accomplish metastatic behaviour, matrix degrading pro-

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