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A comparison of patients with familial (n = 51) and sporadic (n = 163) cutaneous melanoma who attended the Yale Pigmented Lesion Clinic between January 31, 1995 and January 31, 1996 was performed. The two groups did not differ in their phenotypic characteristics or in the histologic subtypes, Breslow depth, or location of their melanomas. Familial melanoma patients were found to have a greater number of clinically atypical nevi ($p = 0.02$), to develop melanoma at a younger age ($p = 0.05$), to have had more skin biopsies of benign lesions performed prior to their diagnosis of melanoma ($p = 0.02$), and to have had at least 3 histologically confirmed atypical nevi removed in the first five years after their diagnosis ($p = 0.007$). As a reflection of medical surveillance, the number of skin biopsies performed in both groups of patients and their histologic diagnoses were reviewed. Age had a positive correlation [0.20] with the total number of biopsies prior to the diagnosis of melanoma ($p = 0.004$). Independent of family history, the number of skin biopsies performed in the five years after diagnosis of the initial melanoma correlated positively with the physician's estimate of the number of clinically atypical nevi ($p = 0.0001$). However, having had skin biopsies performed in the five years, two years or one year prior to the diagnosis of melanoma did not predict a thinner Breslow depth. Our data demonstrate that dermatologists routinely detect thinner melanomas than non-dermatologists, i.e., while ≤ 0.40 mm ($p = 0.05$). Familial patients

had more clinically atypical nevi and developed melanoma at a younger age. Therefore, one explanation for why medical surveillance did not predispose to thinner Breslow depths is the lack of total body skin examinations.

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Introduction

Melanoma represents a malignant proliferation of melanocytes and its incidence has been increasing worldwide (Cook, 1985). During the period 1980 to 1990, the number of melanomas diagnosed annually in the United States doubled while the population increased by only 11%. (Grin-Jorgensen, 1992) More specifically, the incidence has been increasing by approximately 5% per year. (Armstrong, 1992) The increasing incidence has been accompanied by a smaller increase in mortality. For example, while the incidence of melanoma increased approximately six-fold since 1950, the mortality rate from melanoma doubled. (Grin-Jorgensen, 1992) This increase in mortality argues in favor of a true increase in the incidence of the disease and not just of an increase in diagnosis.

Because mortality rates have been shown to be directly proportional to the depth of invasion of the tumor, researchers have attempted to define risk factors that would allow identification of individuals who would benefit from medical screening and education concerning skin self-awareness. Risk factors predisposing to cutaneous melanoma include a personal history of melanoma, greater than 50 melanocytic nevi, greater than 5 atypical nevi, and lastly, a family history of melanoma.

Few investigators (Barnhill, 1992; Grange, 1995; Kopf, 1986) have directly compared familial and sporadic cutaneous melanoma patients. Moreover, the proportion of familial melanoma patients in these studies was less than the proposed national incidence of 10%. This thesis will compare familial and sporadic cutaneous melanoma in a patient population with a higher proportion of familial melanoma. In addition, it will

analyze whether familial melanoma patients are aware of their increased risk of developing melanoma, and whether they therefore seek medical screening (e.g., full body skin examinations).

Familial Melanoma - Historical Perspective

Although the article by Cawley (1951) is cited as the first to describe familial melanoma (Moschella, 1961), Norris (1820) actually described this entity 150 years earlier. In his article entitled "Case of Fungoid Disease", Norris reports a male patient who developed a tumor on his lower abdomen at the site where a mole had been previously. The tumor was "nearly of half the size of a hen's egg, of a deep brown colour, of a firm and fleshy feel, ulcerated on the surface". The patient developed satellitosis and regional lymphnode metastases and died soon thereafter. At autopsy, there was evidence of metastases to multiple organs including the lung, liver and brain. Norris stressed the fact that the patient's father had died of a similar disease approximately thirty years earlier, and that not only the father, but also the patient's brother and children, had many moles. He concluded that the case "would incline [him] to believe that the disease is hereditary".

The next mention of familial melanoma in the literature is actually one of familial ocular melanoma. While ocular melanoma is not the focus of this review, this early recognition of a familial aspect deserves mentioning. Davenport (1926) described three generations, spanning from 1871 to 1926, in which two siblings of each generation had an ocular melanoma. He noted that the average age of the family members at the time of diagnosis was only 28.6 years.

In 1951, Cawley published a study of three individuals in one family who had melanoma. He discussed a 60-year-old man and his son and daughter, each of whom had multiple nevi, a fair complexion, light hair and blue eyes. The father and son developed metastases, and the son died as a result of his disease. Given the knowledge of hereditary melanoma in other animals and hereditary ocular melanoma, Cawley surmised that these cases of cutaneous melanoma had a hereditary component. He reasoned that if one of every eight to ten people living past the age of forty years died of cancer, the chance of three of five family members having cutaneous melanoma was 4.4 in a billion.

After Cawley's article, several reports of hereditary melanoma appeared in the literature (Table 1). Part of the apparent increase may have resulted from a new awareness of the entity familial melanoma, but a portion also may have resulted because of the increasing incidence of melanoma which was already occurring at that time. After these case reports, the number of affected patients became great enough to initiate studies consisting of only these patients.

Further insight into familial melanoma came in 1978, when Lynch et al. published their article entitled "Familial Atypical Multiple Mole-Melanoma (FAMMM) Syndrome". Lynch et al. (1978) based their autosomal dominant syndrome on a family in which three successive generations had either cutaneous melanoma or an atypical mole phenotype. These authors defined the atypical mole phenotype as multiple large moles with irregular borders and colors ranging from reddish brown to pink with signs of pigmentary leakage. They credit Clark (1976) as first suggesting that this phenotype might be associated with cutaneous melanoma. In support of Clark's theory, the article also mentions a young

female in whom a clinically suspicious mole, originally biopsied and diagnosed as an intradermal nevus with junctional activity, underwent malignant transformation within two years to a melanoma.

The familial melanoma/precursor nevi syndrome acquired a second name in 1978, the B-K mole syndrome. (Clark, 1978) B-K moles were named after two patients with melanoma who originally presented with multiple nevi with distinctive clinical and histologic findings. The clinical definition of a B-K mole is included. The patients could have from less than 10 to greater than 100 nevi with predominance on the trunk, as well as nevi with diameter of approximately 10 mm, an irregular outline and a mixture of colors -- tan, brown, black and pink. Clark et al. (1978) point out that the distribution of B-K moles was similar among family members. In addition, patients with the syndrome often had more nevi on non--sun-exposed surfaces than the general population. The authors felt that once patients were clinically identified, histologic confirmation was necessary to assign the patients this syndrome (Table 2). In addition to a compound nevus, one would see atypical melanocytic hyperplasia, mesenchymal changes within the papillary dermis and a lymphocytic infiltrate.

Further support for an association between B-K moles and melanoma came from two patients with the syndrome and a prior diagnosis of melanoma who had a second primary melanoma diagnosed after changes were noted in a B-K mole through serial photography. (Clark, 1978) The authors reviewed prior reports of familial melanoma in the literature, such as Cawley's family, and proposed that, in fact, these families would most likely qualify as the B-K syndrome. (Clark, 1978)

Familial Melanoma - Genetic Aspects

As the first case reports of familial melanoma in humans were being published, investigators were already examining the genetic aspects of melanoma in other species. In 1926, Caylor et al. reported that successive generations of swine developed metastatic melanoma. In addition, it was noticed in the 18th century that gray horses developed melanoma, and in the early 20th century, Van Dorssen (cited by Cawley, 1951) suggested that if gray horses lived long enough, all of them would eventually develop melanoma. Work in *Drosophila* also revealed the approximate location of a gene which might be responsible for generating melanoma. (Salamon, 1963) As early as 1915, it was known that if a gene in the *Drosophila* was homozygous, a melanotic tumor would develop, killing the larvae. Studies on salamanders revealed that melanoma has a faster growth rate in offspring than in the parents. (Cawley, 1951)

After reviewing pedigrees of familial melanoma, most investigators believed that the genotype was inherited in an autosomal dominant pattern with incomplete penetrance. (Greene, 1983; Lynch, 1983) Using a pedigree analysis of four kindreds, Lynch et al. (1983) found evidence to support an autosomal dominant inheritance pattern among melanoma patients with a penetrance rate of 93%. However, when all of the family members with dysplastic nevi were included in the analysis, neither an autosomal dominant inheritance nor a purely environmental cause accounted for the distribution. The authors argued that because dysplastic nevi occur sporadically in a significant proportion of the population, the analysis would be invalid until sporadic and familial dysplastic nevi could be distinguished. (Lynch, 1983)

Anderson (1967) argued that certain cases of familial melanoma could result from recessive inheritance, as in the report by Moschella (1961) of two sisters. However, he believed that the pattern actually resulted from a lack of knowledge concerning the full family pedigree. (Anderson, 1967) Other clinicians believed that melanoma was a polygenic trait. (Anderson, 1967; Duggleby, 1981; Wallace, 1971) One group argued that polygenic inheritance was likely because the risk of melanoma in family members grew as the number of affected relatives increased. (Wallace, 1971) However, the authors note that polygenic inheritance cannot be differentiated completely from dominant inheritance with incomplete penetrance. (Wallace, 1971)

Schoch (1963) began the search for a genetic explanation of human melanoma in 1963. His family consisted of a mother and two sons with melanoma. Karyotypes of chromosomes from their peripheral blood leukocytes and dermal fibroblasts were normal. He concluded that a hereditary factor must exist to explain melanoma occurring in three family members, but the factor must exist at a sub-chromosomal level, e.g., at the level of genes.

With new technology allowing DNA analysis, the search for both an abnormal allele in sporadic melanoma and a common allele in familial melanoma patients began. Genetic linkage analysis has proved to be a powerful method for mapping the location of genes, especially when they have classic Mendelian patterns of dominant, recessive or X-linked inheritance. (Piepkorn, 1994) Linkage analysis tests the hypothesis that a DNA polymorphism is co-inherited with a susceptibility to the disease in question, in this case melanoma.

Chromosomes 1 and 6

The first results of linkage studies showed promising results for chromosomes 1 and 6. In 1982, Pellegris et al. studied six families with familial melanoma and found that HLA-B12 was present in 5 of 6 families. They hypothesized that two complementary factors (one being the HLA complex on chromosome 6p) predisposed to melanoma and that the predisposition occurred in a dominant pattern with incomplete penetrance. (Pellegris, 1982) Greene et al. (1983) performed linkage analysis on 14 kindreds and found that a gene predisposing to dysplastic nevi *and* melanoma could be located on chromosome 1p near the *Rh* locus. Studies using two markers on chromosome 9 and 11 showed no linkage. (Greene, 1983)

The theory that the familial melanoma (MLM) locus resided on chromosome 1 was discredited in 1987 by Gerhard et al., who found no association in either sporadic or familial patients with the Ha-*ras*-1 allele. Four other groups also discredited chromosome 1 as the site for the MLM locus. (van Haeringen, 1989; Cannon-Albright, 1990; Kefford, 1991; Nancarrow, 1992a) The original studies that supported chromosome 1 as the MLM locus included patients with histologically confirmed atypical nevi but without a history of melanoma. The studies that discredited chromosome 1 included only those patients with melanoma. (Gerhard, 1987)

Further proof that the MLM locus did not reside on chromosome 1 was provided by Nancarrow et al. (1992b). Using 172 microsatellite markers, they scanned all autosomes in 3 familial kindreds. They were able to exclude most of chromosome 1p.

Unable to exclude regions of 6, 9cen and 10qter, they suggested further study of these regions. (Nancarrow, 1992b)

In 1994, chromosome 6 was studied by Walker et al. Using linkage analysis, they found that a region between D6S105 and HLAF segregated with melanoma in 5 of 16 familial kindreds. They found this surprising as these same families had shown previously linkage to chromosome 9, but the authors concluded that the families might be susceptible to melanoma because of a gene within the HLA region on chromosome 6. (Walker, 1994)

Chromosome 9

The idea of a tumor suppressor gene being responsible for melanoma formation was first suggested by studies of mouse melanoma cells and diploid fibroblasts. Jonasson et al. (1977) showed that a regular elimination of murine chromosome 4, which is homologous to human chromosome 9p, occurred. This supported the theory of a tumor suppressor gene.[1]

Further localization of MLM came in 1992, when a woman without a family history of melanoma presented with eight primary cutaneous melanomas. Cytogenetic studies of her lymphocytes revealed a reciprocal translocation involving chromosomes 5p and 9p with the breakpoint near 9p21. (Petty, 1993a) In situ hybridization and gene dosage studies demonstrated a deletion of at least 6 Mb of chromosome 9p21. (Petty, 1993b) This finding was consistent with the notion that her melanoma susceptibility arose via the loss of a tumor suppressor gene.

Cannon-Albright et al. (1992) focused on the 9p21 region as it had been implicated by the presence of homozygous deletions in melanoma tumors (Fountain, 1992)

and the presence of a germline deletion in the above-mentioned woman. Using the D9S126 and interferon-α (IFNA) gene markers to perform linkage analysis on eleven large familial melanoma kindreds, they localized the MLM locus to the chromosomal region 9p13-p22. (Cannon-Albright, 1992) Within two years, Cannon-Albright had confirmed her findings in more familial kindreds and had provided further localization. Using newly found markers, she found that the MLM locus resided in a 2-cM region proximal to D9S736 and distal to D9S171 (Table 3). (Cannon-Albright, 1994)

Holland et al. (1994) did further deletion mapping on chromosome 9p using sporadic and familial metastatic melanoma specimens. Using markers spanning 19cM across 9p21-22, they found that 57% of the samples displayed an area of deletion. Two areas of common loss resided on either side of the IFNA marker. This was the first time a region distal to IFNA had been described, and the authors suggested that a second tumor suppressor might be involved.

Further work supporting the theory that a MLM locus resided on chromosome 9p was done by Goldstein et al. (1994), who performed linkage analysis using markers IFNA and D9S126 on families previously examined in the chromosome 1p linkage studies. They found evidence for linkage of the IFNA locus in 50% of patients with invasive melanoma, melanoma in situ and dysplastic nevi. Three pedigrees previously shown to have no linkage to 1p now showed linkage to 9p, while 3 pedigrees which had shown linkage to 1p were also linked to 9p; 2 pedigrees displayed linkage to neither chromosome. This reconfirmed the concept of heterogeneity among melanoma patients. The authors recognize that Cannon-Albright et al.'s (1992) research did not offer evidence of

heterogeneity but stress that both Cannon-Albright et al. (1992) and Nancarrow et al. (1992b) restricted their studies to melanoma, excluding dysplastic nevi. (Goldstein, 1994) Further studies by Goldstein (1996) simultaneously examined the two loci, 1p and 9p, in these same families. For melanoma, the linkage to 9p was twice as strong. When dysplastic nevi were studied as well as melanoma, there was a comparable linkage with both 1p and 9p.

Isshiki et al. (1994) directly analyzed twenty microsatellite loci on chromosome 9 from 25 uncultured, freshly excised melanoma tumors (22 sporadic and 3 familial). They found a loss of heterozygosity (LOH) affecting 9p in 72% of the melanomas. They found the same LOH in metastatic lesions in one patient and in a vertical growth phase tumor in another patient, supporting the theory that the mutation occurred early in tumorigenesis. In eleven cases, chromosome 9 was completely deleted, while in six cases, all or part of chromosome 9p was lost. The finding of a somatic interstitial deletion of 9p between D9S162 and D9S169 in one patient corresponded with prior localization of the MLM locus. (Isshiki, 1994) Similarly, Puig et al. (1995) utilized 12 microsatellite markers to analyze 54 paired melanoma and normal tissues. They reported a 54% LOH at 9p with one tumor having a homozygous deletion.

MTS1: The MLM Locus

Genes that directly regulate the cell cycle are likely candidates for tumorigenesis. Kamb et al. (1994) recognized that within the 2-cM region on chromosome 9 proximal to D9S736 and distal to D9S171 lay the Multiple Tumor Suppressor 1 (MTS1)[2] gene that encodes $p16^{INK4}$, the inhibitor of cyclin-dependent kinase 4 (CDK4). In the cell cycle,

$p16^{INK4}$ forms a complex with the D-type cyclins to prevent passage through the G_1 phase of the cell cycle. Normally, the binding of cyclins to cyclin-dependent protein kinases (CDK) allows the phosphorylation of CDK, and therefore activation. The active unit is then inhibited by phosphorylation of another amino acid pair or by binding to CDK inhibitory subunits (CKI). $p16^{INK4}$, along with $p15^{INK4B}$, is structurally related to CKIs specific for CDK4- and CDK6-cyclin complexes. $p16^{INK4}$ is a protein, consisting of four tandemly repeated ankyrin domains, which binds to and inhibits the catalytic activity of the CDK-cyclin complex, thereby preventing the phosphorylation of the retinoblastoma gene product. (Morgan, 1995; Parry, 1996) Therefore, the inactivation of the $p16^{INK4}$ inhibitor would promote tumorigenesis. UVC irradiation has been shown to cause a large increase in the $p16^{INK4}$ protein, delaying cells in the G_1 phase of the cell cycle, which resides as the levels of $p16^{INK4}$ decrease. (Wang, 1996)

In this original study, Kamb et al. (1994) found that MTS1 was deleted homozygously in many tumor cell lines, including astrocytomas, glioma, breast, osteosarcoma and kidney. Furthermore, in the melanoma cell lines that carried at least one copy of the gene, there were often nonsense, missense or frameshift mutations in the gene. They found eighteen mutations in 14 of 34 melanoma cell lines. Additional studies of human non-small cell lung carcinomas showed that MTS1/CDK4I mutations, either deletions or missense, were present in 19 of 64 tumors, suggesting that inactivation of this gene was important during carcinogenesis. (Hayashi, 1994) Okamoto et al. (1994) found that neither $p16^{INK4}$ nor the RB protein was present in 28 of 29 tumor cell lines from human lung, esophagus, liver, colon and pancreas. In addition, they found that

homozygous deletions of MTS1 were present in many of the cell lines and that transfection of the MTS1 cDNA expression vector into carcinoma cells inhibited the colony forming efficiency. These transfected cells also were selected against with continued passage *in vitro*.

Nobori et al. (1994) also reported the presence of MTS1 mutations within multiple tumor cell lines. Using positional cloning on 46 human malignant cell lines, they found that the most frequently deleted region resided within 9p21. Polymerase chain reaction (PCR) analysis revealed homozygous deletions of the MTS1 fragment in 61% of melanomas, 87% of gliomas, 36% of non-small-cell lung cancers and 64% of leukemia cell lines. While the MTS1 gene transcripts were not present in tumor cell lines, reverse transcriptase PCR showed that they were present in normal cells. Notably, the authors also reported a germ-line nonsense mutation within this gene in a patient with the dysplastic nevus syndrome. This provided further support that MTS1 was the MLM locus.

To prove that mutations in MTS1 occurred *in vivo* and not just as artifacts of *in vitro* cell culture formation, analysis of actual tumors had to be performed. Ohta et al. (1994) tested for somatic mutations in this gene using DNA from 30 surgically resected metastatic melanoma tumors, both cutaneous and uveal in origin, from sporadic melanoma patients. No mutations were found in the coding region of MTS1 in the uncultured tumors, but mutations or deletions were detected in 60% of the cultured melanoma cell lines DNAs. This agreed with prior sequencing of uncultured tumors of various other organs. From their results, the authors concluded that the MTS1 was a likely candidate

for the MLM gene, but that another gene on 9p21 might be the target of the frequent LOH in melanomas. In addition, if the MTS1 were the MLM gene, it might be inactivated by homozygous deletion or by another mechanism which would not alter the coding sequence of the gene. Later studies by Ohta et al. (1996) re-examined whether the allelic loss of 9p21 observed in sporadic cutaneous melanoma included the MTS1 locus. Using similar methods to above but with new microsatellite markers, they found that somatic mutations of the locus are rare (3.1%) in sporadic melanomas with allelic loss being more common (63%). This again suggested that genes other than MTS1 in the 9p21-23 region might be involved in the development of melanoma.

Similarly, Gruis et al. (1995c) studied primary bladder carcinomas along with primary and metastatic melanomas and reported mutations of MTS1 in 3 of 33 bladder carcinomas and 5 of 34 melanomas. Although other authors found that homozygous deletions were two to three times more common than mutations (Kamb, 1994a), Gruis et al. (1995c) did not look for chromosomal deletions within these samples, because the presence of some normal cells within their tissue would bias the results.

As Kamb et al. (1994a) had already identified homozygous deletions in 75% of melanoma cell lines, suggesting that the MTS1 locus acted as a tumor suppressor gene, Hussussian et al. (1994) studied whether the mutations or deletions of MTS1 cosegregated with the 9p21 linked familial melanoma patients. They found that within their 18 families, while a majority of the melanomas occurred in patients with dysplastic nevi, only 30% of dysplastic nevi cases had detectable MTS1 mutations while 92% of the melanoma cases had a mutation. This suggested that while the MTS1 mutations may be a

cause of melanoma, they were not a cause of dysplastic nevi. They also found that in their familial patients who linked to 9p21, 4 of 6 families had MTS1 mutations. Hussussian et al. (1994) proposed that in the two families without mutations, there might be a silent mutation or a normal MTS1 gene which is tightly linked to a different hypervariable locus whose mutations led to melanoma development. They concluded that while their data was consistent with the identification of MTS1 as the MLM gene, mutations or deletions within the gene were not necessary for melanoma development within familial patients.

Kamb et al. (1994b) then did further studies to determine whether their prior observation of point mutations in MTS1 in melanoma cell lines segregated with melanoma susceptibility in 9p-linked pedigrees. Although they found two potential mutations of the gene in cell lines, they found no disruptive MTS1 mutations in the pedigree patients. The authors suggest that the low incidence of observed mutations might signal that MTS1 and the MLM gene are separate and distinct genes or that the majority of predisposing mutations occur outside the p16 coding region. Given all of the previous data suggesting that this gene was in fact the MLM gene, the authors suggest that because mutations in MTS1 had been reported in malignant tumors, other than melanoma, a mutation in this gene might just predispose to any cancer within linked families.

Further support for those who believed in the heterogeneity of malignant melanoma was provided by MacGeoch et al. (1994), who studied six melanoma families in England using linkage analysis. They reported that only 3 of 6 families were linked to 9p12-23. A study of Australian pedigrees by Holland et al. (1995) also suggested the heterogeneity of MLM. Mutation analysis of MTS1 in 17 pedigrees revealed only one

family with a germline mutation in exon 1 along with two previously reported polymorphisms of an untranslated region in exon 3. Walker et al. (1995) performed a similar study in 18 melanoma families and discovered that 7 of 18 pedigrees segregated with MTS1 mutations. Within the 7 pedigrees, 46 of 51 family members with melanoma segregated with the mutations. The remaining cases were assumed to be sporadic.[3] Another study analyzed 14 melanoma cell lines with varying metastatic potential in nude mice, and discovered mutations of MTS1 in 3 of 14 lines and homozygous deletions in 6 of 14 lines. (Luca, 1995) Gruis et al. (1995b) analyzed 15 Dutch familial pedigrees and found that 13 of 15 contained a 19 basepair germline deletion causing a reading frame shift.[4] Borg et al. (1996) reported that 2 of 10 Swedish kindreds had a novel germline mutation in exon 2. Analysis of 33 consecutive familial melanoma patients revealed mutations in 5 of 28 unrelated patients. Fitzgerald et al. (1996) speculate that the number of mutations is lower than in the studies using large kindreds because other effects, such as excessive sunlight exposure, would be more likely to affect two to three family members in this study compared with the larger numbers in the kindred studies.

Further proof that MTS1 was the MLM gene was provided by a kindred with multiple family members who had melanoma and/or pancreatic cancer. (Whelan, 1995) All of the affected family members shared a mutated allele (Gly93Trp) of MTS1 along with a wild-type allele, while the unaffected members were homozygous for the wild-type gene.

Maestro et al. (1995) took a different view in arguing that mutations within MTS1 predisposed to melanoma. Knowing that p53 mutations occurred in sun-related

tumors such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), they analyzed melanoma cell lines. Their results displayed an excess of C:G to T:A transitions at adjacent pyrimidine sites which is identical to those induced by UV radiation in BCC and SCC. They interpreted the information as strong support for UV radiation causing melanoma. Kamb (1995) responded to this new data and suggested that while Maestro et al. (1995) were correct in their interpretation, the data also supported the idea that the point mutations they had found occurred *in vivo* and were not an artifact of cell culture. Pollock et al. (1995) performed a similar experiment on 30 melanoma cell lines. They discovered that 19 of 30 lines carried partial or complete homozygous deletions. In addition, of the remaining cell lines, 8 were shown by direct sequencing by PCR to have a total of 9 different mutations within exon 1 and exon 2. The highest mutation rate was found to be C:G to T:A transitions, which agrees with Maestro et al.'s (1995) results. Similarly, the authors concluded that UV radiation plays a role in melanoma formation and that the target is MTS1.[5,6] (Pollock, 1995)

Because of its similarity in structure to MTS1 and its close location on chromosome 9p (<35kb), the p15 gene was studied as a possible MLM. The p15 gene, also known as MTS2, is induced by TGF-β and is thought to cause G_1 arrest either by growth inhibition or cell contact inhibition. It is known that approximately 80% of homozygous deletions that inactivate MTS1 also inactivate the p15 gene. The authors searched for p15 gene mutations in tumor cell lines and for linkage with melanoma kindreds, but were unable to find any mutations. They concluded that although p15 plays a role in growth regulation, it was an unlikely candidate for the MLM. (Stone, 1995)

Glendening et al. (1995) found homozygous deletion of the p15 gene in six metastatic melanoma cell lines in addition to hemizygous loss of MTS1. The p16 protein was present within all of the lines. They concluded that the loss of one allele was enough to signal melanoma formation. As Gruis (1995b) had reported a living patient with a homozygous deletion, Glendening et al. (1995) argued that this is biologically equivalent to a 50% loss in function and that a redundancy in the genome, possibly provided by the p15 protein, compensated for the loss.

Further answers to the mechanism of MLM came from a study of cell lines from head and neck squamous cell carcinomas and from a variety of non-melanoma primary tumors. Having already discovered a *de novo* methylation of a 5' CpG island that caused a transcriptional block of full-length $p16^{INK4}$, Mao et al. (1995) identified alternative MTS1 transcripts of a sequence in exon 1ß in both unmethylated and methylated cell lines. The authors suggest that this different sequence might alter cell cycle regulation.

To strengthen the argument that MTS1 corresponded to the MLM gene, the biological importance of the protein needed to be proven. Liu et al. (1995) proved that a mutated form of the gene encoded a dysfunctional protein. They described a family in which a mutation in exon 2 segregated with the disease. In fact, 3 of 3 family members with melanoma displayed the mutation in addition to two elderly family members without melanoma. As had been cited previously, the authors argued that obligate carriers do not necessarily display the disease and that because of the current increase in UV radiation exposure, the younger generations are more likely to develop melanoma. (Cannon-Albright, 1994; Battistutta, 1994) They reported that the protein did not bind CDK4 *in*

vitro, thereby losing its inhibitory function, and that *in vivo*, the mutated gene failed to inhibit formation of G418-resistant colonies in a transfection assay while the wild types succeeded. They concluded that MTS1 was indeed the MLM gene that predisposed to melanoma but that other genetic or epigenetic factors participated in tumorigenesis. (Liu, 1995)

Reymond et al. (1995) also studied the function of the mutant $p16^{INK4}$ protein. They found that most variants were deficient in interacting with CDK4 and CDK6. These results were consistent with the theory that a decrease in CDK interaction predisposes individuals to an increased risk of cancer. Wick et al. (1995) studied both $p16^{INK4}$-CDK4 binding and CDK4/D1 kinase activity and found that the melanoma-associated mutants were defective. Koh et al. (1995) demonstrated that several tumor-derived alleles of MTS1 of osteosarcoma origin encoded dysfunctional proteins *in vitro*, causing a lack of inhibition in progression through the G_1 phase of the cell cycle. *In vivo*, they proved that a functional retinoblastoma protein is necessary but not sufficient for full sensitivity to $p16^{INK4}$-mediated inhibition. Parry et al. (1996) described a variety of $p16^{INK4}$ mutations, some of which caused altered function while others did not. Some were found to be temperature-sensitive for $p16^{INK4}$ protein binding to CDK4 and CDK6 *in vitro*, for inhibiting cyclin activity, and for increasing the proportion of G_1-phase cells.

The biological significance of a dysfunctional $p16^{INK4}$ complex was further elucidated when it was found that the mutated complex acted as a tumor-specific antigen recognized by autologous cytolytic T lymphocytes. (Wölfel, 1995) The mutated CDK4 was present in autologous cultured primary melanoma and metastatic tissues but not

within the patient's lymphocytes. The point mutation, an arginine-to-cysteine exchange, was present in 2 of the 28 melanomas analyzed. The mutation prevented binding of the $p16^{INK4}$ protein but not to other CDK4 inhibitors. This same mutation was reported in a kindred in which 11 of the 11 melanoma patients had it along with 2 of 17 unaffected. (Zuo, 1996) The mutation was found to alter the $p16^{INK4}$ protein binding site but did not affect the ability of CDK4 to bind to cyclin D and form a functional kinase. This mutation appeared to act as a dominant oncogene.

Another tactic taken to prove that MTS1 corresponded to the MLM was to demonstrate that mutations within the gene correlated with metastatic potential. Varying results have been reported. Studying human melanoma cell lines with varying metastatic potential in nude mice, Luca et al. (1995) found no correlation between the presence of a mutation within or deletion of MTS1 and metastatic potential. In contrast, Puig et al. (1995) studied 54 paired melanoma tissues and normal tissues and found that 87.5% of the genes with large deletions were associated with a high risk of metastases compared with those without deletions or with a loss of fewer than 8 microsatellite markers. Using immunohistochemistry to evaluate the expression of the $p16^{INK4}$ protein, it was found that while loss of expression was not necessary for melanoma formation (Reed, 1995; Glendening, 1995), its loss was more likely to be related to the tumor's invasiveness or ability to metastasize. The protein was partially or completely absent in 53% of the primary tumors and 72% of the metastatic tumors. (Reed, 1995)

In summary, linkage analysis, LOH studies and studies of a woman with a reciprocal translocation involving chromosomes 5p and 9p all support chromosome 9p as

containing the MLM locus. Further investigations have found that MTS1, a tumor suppressor gene involved in regulation of the cell cycle, is most likely the MLM locus.

Materials and Methods

Patients were eligible for the study if they had at least one invasive cutaneous melanoma. The diagnosis of an *in-situ* melanoma was not sufficient for entry into the study, but some of the patients with multiple primary melanomas had *in-situ* lesions. Of note, in the patients with multiple primaries, the invasive melanoma was not always their initial cutaneous melanoma. Melanoma stage in the multiple primary patients was based upon the most advanced lesion. Two hundred and eighteen patients meeting these criteria visited the Yale Pigmented Lesion Clinic between January 31, 1995 and January 31, 1996. Four patients were excluded from the statistical analysis because their family history of melanoma was unknown.

Patient information was obtained through chart review, questionnaires, patient visits, and telephone interviews. Upon entering the clinic, patients completed an epidemiologic questionnaire that addressed family history of melanoma, nevi and skin cancer, the patient's personal assessment of number of nevi and their characteristics, personal and family history of non-cutaneous malignancies, occupation, UV light exposure, and phenotypic characteristics.

A full-body skin examination was completed by a physician at the time of each visit to the clinic. The total nevus count was ascertained from the patient's first visit. The total number of clinically atypical nevi and actinic keratoses included those recorded from the patient's first clinic visit through January 31, 1996. At the patient's first clinic visit,

the nurse recorded all previous skin biopsies and their approximate dates as well as the name of the physicians who had performed any skin biopsies or surgical excisions. With patient consent, the pathology reports were obtained, as were the medical records from previous physicians. The latter allowed a further review of the number of biopsies. Multiple attempts were often necessary in order to obtain both the reports and records. Only histologically confirmed biopsies were included in the analysis. Previous and subsequent skin biopsies were recorded chronologically in relationship to the patient's melanoma(s) and according to type. The four subgroups of biopsy specimens were: (1) atypical nevi based upon architectural disorder and cellular atypia (National, 1992), (2) benign nevi, (3) non-melanoma skin cancers (BCC, SCC, keratoacanthoma), and (4) benign (non-nevi) lesions. One analysis included only the number of skin biopsies performed in the five years prior to and in the five years after the diagnosis of melanoma. If five years had not yet passed since the diagnosis of melanoma, blanks were inserted in calculating the number of biopsies so as not to assume that the patient did not have biopsies in those given years.

The histologic slides of all the melanomas diagnosed at outside institutions were re-read by one of the dermatopathologists in our department utilizing standard criteria. (Lever, 1990) In addition, slides that originally had been read at Yale but did not have complete descriptions were re-reviewed. Those melanomas that could not be clearly classified histologically were labeled 'unclassified'. Medical documentation, via pathologic reports, of family members with melanoma was attempted for each patient with a positive family history. Relatives' information obtained by death certificate or medical

records was considered clinically confirmed but not histologically confirmed. Family members' melanomas were considered confirmed for the purpose of statistical analysis only if histologically documented by a pathology report.

Data collection was done by the author with review by a physician. Statistical analysis was performed using SAS programming. Data were entered by a neutral party using KP-5, a data entry program written by Peter Charpentier at Yale University, and were then verified. Chi-square tests, t-tests, correlations and unconditional logistic regression were used to analyze the data. In instances where more than 20% of the cells in the chi-square test had expected values of less than five, the Fisher's exact (two-tailed) test was used to assess significance ($p \leq 0.05$). Data was broken into subgroups based on accepted standards or on the nature of the data ascertained by univariate analysis. All tests that examined the potential effect of family history were run in triplicate, i.e., patients with no family history ("sporadic patients") were compared to: (1) all familial patients [group 1], (2) patients with a positive history in a first-degree relative [group 2], and (3) patients with a confirmed melanoma history in a first-degree relative [group 3].

Results

Patient Characteristics

All 218 melanoma patients who attended the Yale Pigmented Lesion Clinic from January 31, 1995 to January 31, 1996 were Caucasian. As seen in Table 4, there was no significant difference in the sex of the patients in the familial melanoma group (n = 51) versus the sporadic melanoma group (n = 163) (all patients: F 61.2%; M 38.8%). Two

patients were immunosuppressed, both due to infection with the Human Immunodeficiency Virus.

Of the 214 patients included in statistical analysis, 51 (23.6%) had a positive history of familial melanoma ("familial patients"). Of this group, 6 patients had two affected relatives, one patient had three affected relatives, and one patient had eight affected relatives. Of the familial patients, 31 (60.8%) had at least one first-degree relative with the diagnosis of melanoma while 20 (39.2%) patients had only second-degree relative(s) affected. Pathology reports were obtained for 85.3% of the first-degree relatives (Table 5) and 70.6% of the second-degree relatives. Approximately the same proportion of females and males reported a family history (25.3% versus 21.7%, $p = 0.62$). Two of the familial patients had relatives diagnosed with melanoma after their own diagnosis. These two patients were excluded from those statistical analyses that examined screening prior to the diagnosis of melanoma.

As mentioned previously, all tests that examined the potential effect of family history were run in triplicate, i.e., patients with no family history ("sporadic patients") were compared to: (1) all familial patients ($n = 51$, group 1), (2) patients with a positive history in a first-degree relative ($n = 31$, group 2), and (3) patients with a confirmed melanoma history in a first-degree relative ($n = 26$, group 3). Unless otherwise specified, the results are from the comparison of sporadic patients to group 1. The comparison of sporadic patients to groups 2 and 3 did not produce statistically significant results but contained a similar trend to the results found when using group 1.

The age of familial patients at the first diagnosis of melanoma was significantly different from that of sporadic patients ($p = 0.05$). Familial patients were more likely to be diagnosed with melanoma at a younger age. As seen in Table 4, 64.7% of the familial patients were diagnosed between the ages of 25 and 45 years while only 44.5% of the sporadic patients were. In addition, 48.5% of the sporadic patients were diagnosed over the age 45 years as compared to 31.3% of the familial patients. When age was examined as a continuous variable, however, the t-test results displayed no significant difference ($F = 41.8 \pm 12.3$ yrs; $S = 45.4 \pm 14.6$ yrs, $p = 0.09$).

As seen in Table 4, there was no significant difference in the hair color or eye color of the two groups. In addition, the patients' estimates of their own skin's susceptibility to sunburn and ability to tan were similar. As everyone in the study had the diagnosis of invasive melanoma, one would expect a majority of the patients to have lighter hair and eye color along with a susceptibility to burning and an inability to tan. (Armstrong, 1992) Questions concerning sun exposure both in terms of time outdoors and percentage of time wearing different strengths of sunscreen cannot be considered valid, because some patients answered for current post-melanoma practices while others answered based on prior practices. Females were much more likely to have visited a suntanning parlor at least once ($F = 88.8\%$; $M = 11.5\%$, $p = 0.001$). Patients who had visited a salon had a younger average age than those who reported no visits ($Y = 38.2 \pm 10.4$ yrs; $N = 46.0 \pm 14.5$ yrs, $p = 0.0004$). No significant difference existed between sporadic and familial patients in their likelihood of having visited a suntanning salon ($p =$

0.82). A majority of subjects (60%) had visited a salon less than ten times. The greatest number of visits was 60.

The remaining phenotypic characteristics studied were the patients' and the physician's estimate of the total number of nevi and atypical nevi. No significant difference existed between familial and sporadic patients concerning the physician's estimate of total number of nevi ($p = 0.61$). Approximately 50% of the patients in each group fell into the subgroup of 0-32, while approximately 25% of patients in each group fell into the subgroups of 33-66 or > 66. The physician's estimate of the number of atypical nevi was significantly higher among familial patients ($p = 0.02$). Equal proportions of patients had 2-5 clinically atypical nevi (F = 37.2%; S = 37.6%), but a greater proportion of sporadic patients had 0-1 clinically atypical nevi (F = 17.7%; S = 32.7%) while a smaller proportion had either 6-10 or greater than 10 clinically atypical nevi (F = 45.1%; S = 29.7%, $p = 0.02$). Of interest, the estimated number of total body nevi did correlate positively with the number of recorded clinically atypical nevi ($p = 0.0001$).

Most patients in the study (86%) had at least one clinically atypical nevus, and approximately 70% of patients in both the familial and sporadic groups had histologically confirmed atypical nevi ($p = 0.10$). (National, 1992) The distribution of atypia of the nevi among the two groups was roughly the same ($p = 0.20$), with most nevi having mild atypia. It bears noting that while most of the nevi were diagnosed at this institution, there is a significant discrepancy among institutions in the classification of atypia as mild, moderate, or severe.

The majority of the patients in both the sporadic and the familial melanoma groups did not have a history of a non-melanoma skin cancer. No significant difference existed between the two groups ($p = 0.24$). In addition, no difference existed between the familial and sporadic groups in the type of non-cutaneous malignancies ($p = 0.70$).

In multivariable analysis (Table 6), a family history of non-melanoma skin cancer (OR = 2.08; 95% CI = 1.07 to 4.05), a surrogate report of atypical nevi in family members (OR = 2.27; 95% CI = 1.16 to 4.43), and the presence of a secondary primary melanoma (OR = 2.40; 95% CI = 1.12 to 5.15) predicted a positive family history of melanoma.

Melanoma Characteristics

The distribution of the sites of initial primary melanomas was similar to previously reported series. (Armstrong, 1992) As seen in Table 7, melanomas were more likely to occur in females on upper or lower extremities, while men had significantly more melanomas at all other sites ($p = 0.001$). This significant difference between females and males existed among both familial patients ($p = 0.008$) and sporadic patients ($p = 0.001$). When patients of both sexes were grouped together, there was no difference between familial and sporadic patients in the distribution of melanoma sites ($p = 0.30$). Notable characteristics of familial patients, compared to sporadic patients, were a larger proportion of males having melanomas of the anterior trunk while an equal proportion of the sexes had melanomas of the posterior trunk ($p = 0.008$). The anatomical distribution of melanomas among sporadic patients was similar to the expected distribution described above, with females having the greatest proportion of tumors on the legs and males having the greatest proportion on the posterior trunk ($p = 0.001$). (Armstrong, 1992)

With respect to histologic diagnoses and Breslow depths, no significant differences existed between familial and sporadic patients, as seen in Table 7. Nodular melanomas were deeper than superficial spreading or lentigo maligna melanomas at the time of diagnosis. The majority of nodular melanomas had Breslow depths > 0.75 mm at the time of diagnosis.

As expected from the similar Breslow depths, the distribution of melanoma stages and the outcomes were similar in familial and sporadic patients ($p = 0.18$; 1.0, respectively). By the nature of the study, all patients were alive upon entry, and only two patients died while three currently have evidence of metastatic disease. Only six patients ever had evidence of metastatic disease. This is not surprising as the patient population consisted of many patients who had a dermatologist, and not an oncologist, as their primary caregiver for their melanoma.

No difference existed between the treatment of melanoma in the familial and sporadic patients. All patients had excisions, and both groups contained patients with lymphadenectomies. The margins of excision in the familial and sporadic groups were considered equal ($F = 1.7 \pm 1.4$ cm; $S = 1.7 \pm 1.1$ cm, $p = 0.05$). Few patients had received additional treatment such as vaccination, chemotherapy or radiation.

Multiple Primaries

Eighty-three percent of the patients ($n = 177$) had only one cutaneous melanoma. Twenty-five patients had two melanomas, 10 patients had three melanomas and 6 patients had four or more. In contrast to the initial cutaneous melanomas, where patients were most likely to have noticed a suspicious lesion, dermatologists discovered a majority

(80%) of the second primary tumors (F 87.5%; S 75%, p = 0.25). The majority of second primaries (72.5%) were diagnosed within five years of the diagnosis of the initial melanoma, with approximately half of the diagnoses made within the first year. No differences existed between the familial and sporadic groups with regard to multiple primaries or in the intervals during which they developed (p = 1.00; 0.83, respectively). On average, in the first five years after the initial melanoma diagnosis, one of every 142.9 biopsies of atypical nevi [group A] and benign nevi [group B] was a second primary melanoma. When the denominator includes non-nevi benign [group C] lesions (i.e., A+B+C), a second primary melanoma was diagnosed for every 200 skin biopsies performed.

The Breslow depths of subsequent melanomas were thinner than the originals. When looked at as a continuous variable, initial melanomas were found to have thicker Breslow depths than subsequent melanomas (p = 0.01). Only 32% of initial melanomas were less than or equal to 0.40 mm, while 56% of subsequent lesions fell into this group (p = 0.35). The Breslow depths of second primaries were similar for familial and sporadic patients. Among those patients who had discovered their initial melanoma, 10.5% again noticed their subsequent lesions, while 84.2% of such lesions were noted as suspicious by a dermatologist. Most (99%) of the second primary melanomas were diagnosed histologically either as superficial spreading melanomas or *in situ* lesions.

Predictors of Breslow Depth

As discussed above, a personal history of melanoma was predictive of a thinner Breslow depth (p = 0.01), while a family history of melanoma was not (p = 0.56).

Whether or not the patient had skin biopsies performed within the five years prior to the first melanoma diagnosis -- including atypical nevi [A] ($p = 0.39$), benign nevi [B] ($p = 0.30$), non-nevi benign lesions [C] ($p = 0.20$), non-melanoma skin cancer [group D] ($p = 0.15$), or a combination of A+B+C+D ($p = 0.53$) -- did not influence the Breslow depth. Likewise, having had a skin biopsy performed in the two years or one year preceding the diagnosis of melanoma did not predict a thinner Breslow depth ($p = 0.50$ and 0.40, respectively) (Table 9). Age was not predictive of Breslow depth among males, females or both when grouped ($p = 0.15$-0.52); however, a positive correlation between age and Breslow depth did exist ($p = 0.03$). Use of artificial UV light for tanning purposes did not predispose to thicker lesions ($p = 0.18$). The patients who frequented the salon greater than ten times were not more likely to have a thicker lesion at diagnosis ($p = 0.11$).

For all patients, the anatomical site of the melanoma was predictive of Breslow depth. Melanomas of the head and neck or lower extremities were more likely to be > 1.5 mm in depth ($p = 0.04$), although a majority (77.4%) were still ≤ 1.5 mm. In females, site did not correlate with depth ($p = 0.69$). Among males, however, lesions of the head and neck were equally likely to be > 1.5 mm as ≤ 1.5 mm ($p = 0.03$), while lesions of the trunk or extremities were more likely to be ≤ 1.5 mm ($p = 0.03$).

An analysis of who noticed the first melanoma as a predictor of Breslow depth yielded interesting results. As seen in Table 9, when the patient noticed the suspicious lesion as opposed to a friend, family member or physician, the Breslow depth was more likely to be 0.41-4.00 mm as opposed to ≤ 0.40 mm ($p = 0.005$). The same was true if a patient noticed the lesion as opposed to a physician ($p = 0.001$). Dermatologists

discovered more of the melanomas that were diagnosed while still ≤ 0.75 mm in depth than did other physicians ($p = 0.03$). In fact, a majority of lesions diagnosed by dermatologists were ≤ 0.4 mm in depth ($p = 0.05$). Dermatologists, compared to non-dermatologists, did not discover more melanomas in those patients with more clinically atypical nevi or with histologically confirmed atypical nevi ($p = 0.18$; 0.09, respectively).

Biopsies Pre- and Post- First Melanoma

The average number of skin biopsies performed in the five years prior to the diagnosis of melanoma was 0.7, while the average total number at any time prior to the diagnosis was 1.1. Age had a positive correlation [0.20] with the total number of biopsies prior to melanoma ($p = 0.004$). When comparisons of all types of biopsy combinations were made (e.g., A,B,C,D or various combinations of the types), familial patients were more likely than sporadic patients to have had benign nevi biopsied at any time prior to their melanoma ($p = 0.03$); this was not true when looking at any of the biopsy combinations in the five years prior to the diagnosis of melanoma (Table 8).

The average number of skin biopsies performed was 5.6 in the first five years after the melanoma was diagnosed and 8.1 from the time of diagnosis through January, 1996. The average length of follow-up was 77.5 months after the diagnosis of the first melanoma. Independent of family history, the number of skin biopsies performed in the first five years after the diagnosis of the first melanoma correlated positively with the number of clinically atypical nevi ($p = 0.0001$). Familial patients were more likely to have had more than two histologically confirmed atypical nevi removed in the first five years after the diagnosis of their initial melanoma, while sporadic patients were more likely to

have had 0 or 1-2 biopsies of atypical nevi ($p = 0.007$). Patients with a second primary melanoma were more likely to have had > 5 biopsies in the first five years after the first melanoma, while patients with only one primary tended to have 1-5 biopsies ($p = 0.001$).

Patient Perceptions

In general, patients were able to assess the condition of their own skin, as reflected in a comparison of the physician analysis with patient answers to the questionnaire. When compared to the physician estimate of total body nevi, patients correctly estimated their number of nevi ($p = 0.001$) and whether they had more nevi ($p = 0.001$) or larger nevi ($p = 0.02$) than the general population. Familial patients with a history of histologically confirmed atypical nevi thought that they had more nevi than average ($p = 0.02$) and that they had irregular nevi ($p = 0.005$). Importantly, a majority of patients who had atypical nevi biopsied prior to their melanoma diagnosis were aware that they had more than an average number of nevi ($p = 0.001$) and irregular nevi ($p = 0.02$). In fact, patients with greater than 5 histologically proven atypical nevi removed prior to the diagnosis of their melanoma were more likely to think that their nevi were irregular than were patients with no prior histologically confirmed atypical nevi or 1-5 histologically confirmed atypical nevi ($p = 0.02$). When patient perceptions were compared to the physician estimates of the number of clinically atypical nevi, patients with greater than 5 clinically atypical nevi felt that they had more nevi than the general population ($p = 0.001$) and that these nevi were irregular in shape ($p = 0.04$). Patients whose melanoma was discovered by a dermatologist, as opposed to a non-dermatologist, were more likely to think that they had more nevi than average ($p = 0.04$).

The patients who had noticed a suspicious lesion, which was in fact a melanoma, were analyzed to assess any differences that might predict more self-awareness. Although not significant, a trend existed indicating that females were more likely to have noticed their lesion ($p = 0.78$). As mentioned previously, familial patients were not more likely to have discovered their own melanoma. Patients who had detected their own melanoma were more likely to have tumors located on the head and neck, anterior trunk, or extremities as opposed to tumors of the posterior trunk ($p = 0.001$). Patients noticing their own melanoma were no more likely to have had non-melanoma skin cancers prior to or subsequent to the diagnosis of their melanoma ($p = 0.39$), have had biopsies within the five years prior to melanoma ($p = 0.23$), differ in age ($p = 0.94$), or think they had more nevi, irregular nevi or larger nevi than average ($p = 0.21$; 0.55; 0.60, respectively). Interestingly, a majority of patients who had detected their melanoma thought they had 0-32 total body nevi ($p = 0.02$), as compared to estimates of greater numbers of nevi among other patients including the group whose melanoma was discovered by a physician ($p = 0.005$). In addition, those patients who had noticed their own lesion were more likely to underestimate their total body nevi count, while the patients whose melanoma was discovered by someone besides themselves tended to overestimate their nevi count when compared to the physician's estimate ($p = 0.001$; 0.001, respectively).

Familial melanoma patients were more likely than sporadic patients to report a positive familial history of atypical nevi ($p = 0.02$). We were not able to document many of these reports either clinically or histologically.

Discussion

Family History of Melanoma

It has been estimated that approximately 10% of melanomas are familial in origin. In our study, 51 (23.6%) of the patients had a positive family history of melanoma when including first- and second-degree relatives, and 31 (14.5%) were familial melanoma patients when only including first-degree relatives. To date, only a few studies have compared sporadic and familial melanoma directly. One previous study consisted of 7 (4.6%) familial melanoma patients of a total of 151 patients with melanoma; the family members' melanomas were undocumented. (Barnhill, 1992) Six patients had at least one first-degree relative affected while the seventh reported two second-degree relatives. Another study completed in France compared 22 (8%) familial patients to 273 sporadic patients. (Grange, 1995) Fourteen patients had at least one histologically confirmed first-degree relative with the diagnosis of melanoma while the remaining eight had histologically confirmed second- or third-degree relatives with melanoma. Kopf et al. (1986) studied a cohort consisting of 69 (5.9%) familial patients and 1100 sporadic patients. No histologic or clinical documentation of first- or second-degree relatives is mentioned.

Of the 31 patients with at least one first-degree relative with melanoma, 85.3% of the family members' melanomas were histologically confirmed, and of the 20 patients with a second-degree relative with melanoma, 75% of the relatives' melanomas were confirmed. The importance of confirmation of patients' reports concerning family members lies in the fact that non-melanoma skin cancers can be confused with melanoma.

For example, Aitken et al. (1994) found that 44.5% of positive family histories were false positives. In this study, we did not observe such a high false positive rate. Medical records of only two family members failed to confirm melanoma (one family member had an atypical nevus while a second had a basal cell carcinoma). Even if those first- and second-degree relatives who lacked confirmation are excluded from our study, 41 patients (19.2%) would still be considered familial. However, as the Yale Pigmented Lesion Clinic serves as a regional tertiary care site, the number of difficult cases, e.g., patients with multiple primaries, positive family histories, and numerous atypical moles, would presumably be represented disproportionately. However, having at least the estimated proportion of familial patients in a study increases the likelihood of observing any true differences.

Most studies, including ours, found no phenotypic differences between sporadic and familial patients. (Barnhill, 1992) Grange et al. (1995), however, found a significant association with red hair and an inability to tan in familial patients. In addition, the majority of studies have a greater proportion of females (Barnhill, 1992; Grange, 1995), and in our study as well, the proportion of females was greater in both the familial and sporadic groups (all patients: F 61.2%; M 38.8%). Of note, approximately the same proportion of males and females reported a positive family history, which indicates the absence of bias in the sexes' knowledge of family medical history.

Surprisingly, among Barnhill's (1992) patient cohort, familial patients were on average older at the time of diagnosis (52.3 years vs. 46.7 years). Although our results were not significant, we have found, as have others (Grange, 1995), that familial patients

tend to be younger at diagnosis (this study: 41.8 years vs. 45.4 years) (Table 4). It is thought that familial patients are younger at the time of diagnosis because of earlier detection based upon increased medical surveillance and because of development of melanoma at an earlier age.

While melanoma clusters in some families because of a mutated gene causing melanoma susceptibility or increased numbers of dysplastic nevi which predispose to melanoma, all familial patients do not segregate into these categories. Melanoma may cluster in some families because of increased UV light exposure or increased susceptibility to UV light. Neither Barnhill et al. (1992) nor Kopf et al. (1986) reported a difference in the number of total body nevi or clinically atypical nevi, but Grange et al.'s (1995) and Aitken et al.'s (1994) familial patients tended to have a higher total body nevus count. In our patients, a trend of more clinically atypical nevi existed (> 6 AN: F 45.1%; S 29.7%, p = 0.02). At this time, it is difficult to clearly define differences in the skin of familial and sporadic melanoma patients.

In our study, the Breslow depths of the melanomas did not differ between the two groups, suggesting that lesions in familial patients were not removed earlier in the disease. While Grange et al.'s (1995) and Kopf et al.'s (1986) results agreed with ours, Barnhill et al. (1992) have reported thinner lesions in familial patients (i.e., F 1.54 mm, S 2.11 mm). Greene (1985b) reported a thinner Breslow depth among familial patients compared to that of an unselected population, but the results may be biased, as only familial patients were being studied directly. All concur that the histologic grouping of melanomas does not differ among the two populations.

The results of our multivariate analysis of familial patients versus sporadic patients differed somewhat from those of prior studies. Aitken et al. (1994) report an association of familial patients with poorer tanning ability, lighter skin color and a greater number of total body nevi while Grange et al. (1995) found a higher incidence of red hair and clinically atypical nevi. We found an association with a familial history of atypical nevi and non-melanoma skin cancer (Table 6). Familial melanoma patients may be able to describe family members' nevi more accurately than sporadic patients. A family history of non-melanoma skin cancer may be a reflection of tanning ability, skin color or cumulative sun exposure. An association between the development of a second primary melanoma and a family history of melanoma was found in multivariate but not univariate analysis. This finding has been reported previously in a prospective study of familial patients. (Greene, 1985) One group's analysis is not more true than another, because results will vary depending on the variables entered and the different means by which they were measured.

Prior Dermatologic Surveillance

Given that early detection of melanoma can improve survival, skin cancer screening has received much attention. (Tucker, 1988; Geller, 1992a & 1992b; Roush, 1992) Visual screening by qualified health care providers should increase the proportion of melanomas diagnosed at an early stage. (Koh, 1991) Geller et al. (1992a) studied the use of health services prior to the diagnosis of cutaneous melanoma in a group of patients diagnosed with melanoma between January 1 and December 31, 1987. The study did not include a control population. Through questioning of approximately half of the patients,

the group found that, compared to published figures of national population utilization, a slightly higher proportion of these patients had regular physicians and had seen the physician more often in the past year (87% versus 82% and 67% versus 63%, respectively). Only 20% of the patients had their skin examined in the year prior to diagnosis, while only 24% had examined their own skin. Eleven percent of this patient population was labeled as having a family history of melanoma, although it is not mentioned how this group was designated nor whether the history was substantiated. Of the familial melanoma patients, only 24% responded that they saw a dermatologist on a regular basis, compared to 20% of all of the study patients.

By examining the number of skin biopsies in the years prior to the diagnosis of melanoma as a measure of surveillance by medical personnel, we also studied whether familial melanoma patients were being screened. We found that while patients with a positive family history of melanoma did have more benign skin biopsies performed at any time prior to their melanoma diagnosis ($p = 0.02$), patients with a family history of melanoma did not have more skin biopsies performed in the one year, two years or five years prior to the diagnosis of melanoma than did sporadic melanoma patients (Table 8). In addition, having had a skin biopsy performed prior to the diagnosis of melanoma and therefore having been under medical surveillance did not predict a melanoma of thinner depth among either familial or sporadic melanoma patients (Table 7). Since melanoma, with the exception of nodular melanoma, would be apparent on the skin as a suspicious lesion for at least a year prior to its diagnosis, one possible explanation is that these patients did not undergo full-body skin examination. The results also suggest that among

medical personnel, dermatologists do diagnose lesions at an earlier stage than do other physicians.

Why are familial melanoma patients not receiving greater skin surveillance? Part of the onus lies with medical personnel who must stress the importance of screening for skin cancer. A 1989 survey of New England dermatologists found that 70% regularly recommended family screening to melanoma patients. (Geller, 1992b) In the Pigmented Lesion Clinic, patients are regularly reminded to have at least first-degree relatives undergo a full-body skin exam. In reviewing medical records, however, it appears that not all patients take this suggestion seriously. The charts reveal that when patients are asked about the results of family screening, they respond that members have not yet been screened. Attempts to offer free public screening to relatives of patients with melanoma have met with somewhat limited success. (unpublished observation) Possibly the relatives fear the results of a screening exam or may not consider themselves at risk.

Post-Melanoma Dermatologic Surveillance

Dermatologic surveillance after the diagnosis of cutaneous melanoma is important not only for early detection of recurrence or progression of disease, but also for screening for the development of another primary melanoma. It is estimated that approximately 6% of patients develop multiple primary melanomas. Our data, as did that of others (Carey, 1994), demonstrate that subsequent melanomas are thinner (1st: 1.1 ± 1.2 mm; 2nd: 0.5 ± 0.8 mm).

Following diagnosis of melanoma, multiple biopsies are performed. The majority are of nevi and benign growths. When familial and sporadic patients were compared, the

familial group was found to have more atypical nevi removed in the first five years after melanoma ($p = 0.007$). This does not suggest that sporadic patients should not have suspicious nevi removed as 0.005 secondary melanomas were diagnosed based on each nevi biopsied.

Patient Perception

Our data demonstrates that melanoma patients can accurately estimate their number of moles ($p = 0.001$) and determine an increased number of nevi ($p = 0.001$) or larger nevi ($p = 0.02$) compared to the general population. This suggests that these patients are aware that something is different about their skin. Our results do indicate that patients were more likely to detect their melanoma if it was not located on their posterior trunk. However, patients who were the first to notice their suspicious pigmented lesion and bring it to medical attention did not have thinner lesions. In fact, their melanomas were deeper (Table 7). This emphasizes the need for patient education.

Conclusion

In summary, this study has directly compared familial ($n = 51$) and sporadic ($n = 163$) patients seen in the Yale Pigmented Lesion Clinic between January 31, 1995 and January 31, 1996. Familial melanoma patients were found to develop melanoma at a younger age ($p = 0.05$) and to have had more benign biopsies performed prior to their diagnosis of melanoma ($p = 0.02$). The two patient populations did not differ in their sex distribution, hair and eye color, or the ability to tan/burn. In addition, no difference between the two populations was seen when comparing the histology, Breslow depth, or site of their respective melanomas. Multivariable analysis showed that predictors of a

positive family history of melanoma included a personal history of melanoma, a family history of non-melanoma skin cancer, and a surrogate report of familial atypical nevi. Excluding the greater number of benign skin biopsies performed on familial melanoma patients prior to their diagnosis of melanoma, the number of skin biopsies performed within the five years prior or at any time prior to the diagnosis was the same between the familial and sporadic melanoma patients.

Patients who had skin biopsies performed in the years prior to a melanoma diagnosis did not have thinner melanomas. One explanation is that patients might not be receiving full-body skin examinations in the year prior to their melanoma diagnosis. The ability of dermatologists to detect early melanomas was demonstrated. Dermatologists were much more likely than other physicians or the patients themselves to diagnose lesions at an earlier point in development. Screening by dermatologists would greatly reduce the number of patients who do develop advanced melanoma. The benefit of skin examinations is also evident after seeing that second primary lesions are diagnosed at a thinner Breslow depth.

Table 1: Summary of Familial Melanoma Case Reports		
Authors	**Year of Publication**	**Proband and Relative(s)**
Cawley	1951	male, son and daughter
Greifelt	1952	male and father
Moschella	1961	female and sister
Miller et al.	1962	male and daughter
		female and mother
		female and mother
		male and maternal grandfather
		male and mother
		female and maternal grandfather
		female and sister
		female and sister
Schoch	1963	male, mother and brother
Salamon et al.	1963	female and father
		male and mother
		female and maternal grandfather
		female and paternal aunt
Turkington	1965	female and maternal aunt
		male and mother
		male and maternal grandfather
		female and father
		female and father
		male and maternal and paternal grandfathers
		female, sister and father
		male, sister and maternal and paternal uncles
		female and sister
		female and maternal cousin
		female and father
		male and paternal grandfather
		female and mother
		male, father and paternal cousin
		male and father
Katzenellenbogen et al.	1966	fraternal twins(male and female)
Smith et al.	1966	female and son
Anderson et al.	1967	female, mother, maternal aunt and uncle, maternal cousin, 10 distant maternal relatives (question if 7 were documented)

Table 1: Summary of Familial Melanoma Case Reports		
Authors	**Year of Publication**	**Proband and Relative(s)**
Andrews	1968	male and sister
St-Arneault et al.	1969	male identical twins
Lynch et al.	1978	male, sister, mother, maternal aunt, maternal grandfather and his sister, and maternal grandmother and her sister

Table 2: Histologic Criteria for Atypical or B-K Moles	
FAMMM Syndrome	**B-K Mole Syndrome**
Compound nevus	Compound nevus
Melanocytic dysplasia (mild to severe)	Atypical melanocytic hyperplasia
Fibroplasia of the papillary dermis (variable)	Fibroplasia of the papillary dermis
Lymphocytic infiltrate of the papillary dermis (variable--may or may not be present)	Lymphocytic infiltrate of the papillary dermis
Histology not always similar to a regressing malignant melanoma or halo nevus	Histology similar to a regressing malignant melanoma or halo nevus

Table 3: Marker Location From 9pter to Proximal 9p
D9S156-D9S157-D9S162-IFNA-D9S736-D9S171-D9S126-D9S169-D9S104

TABLE 4: Clinical Characteristics of Patients			
	+ Family History (n=51) n (%)	**- Family History (n=163) n (%)**	**P value**
Sex			0.62
Female	33 (64.7%)	98 (60.1%)	
Male	18 (35.3)	65 (39.9)	
Age at 1st Melanoma			0.05
0 < years ≤ 25	2 (3.9)	11 (6.8)	
25 < years ≤ 45	33 (64.7)	73 (44.8)	
45 < years ≤ 65	15 (29.4)	60 (36.8)	
years > 65	1 (2.0)	19 (11.6)	
Eye Color			0.46
Blue	22 (43.1)	66 (40.0)	
Green	3 (5.9)	22 (13.3)	
Hazel	6 (11.8)	32 (19.4)	
Grey	1 (2.0)	4 (2.4)	
Light Brown	7 (13.7)	13 (7.9)	
Dark Brown	11 (21.6)	26 (15.8)	
Hair Color			0.72
Blond	9 (17.7)	30 (18.2)	
Red	2 (3.9)	12 (7.3)	
Light Brown	12 (23.5)	52 (31.5)	
Dark Brown	26 (51.0)	66 (40.0)	
Black	1 (2.0)	4 (2.4)	
Skin Burnability			0.39
Severe Sunburn (Blistering)	4 (7.8)	11 (6.7)	
Painful Sunburn (Peeling)	23 (45.1)	78 (47.3)	
Mildly Burned (with Tanning)	22 (43.1)	60 (36.4)	
Brown (no Sunburn)	2 (3.9)	6 (3.6)	
Skin Tannability			0.69
Deeply Tanned	7 (13.7)	22 (13.3)	
Moderately Tanned	31 (60.8)	93 (56.4)	
Mildly Tanned (Hx Peeling)	9 (17.7)	34 (20.6)	
No Suntan (Freckling)	2 (3.9)	12 (7.3)	

Table 5: Family Members with Melanoma - Not Histologically Confirmed			
	Age at Diagnosis (yrs)	**Site**	**Outcome**
Patient 1 (Sister)	29	posterior trunk	alive and well
Patient 2 (Sister)	unknown	unknown	alive and well
Patient 3 (Sister)	45	arm	alive and well
Patient 4 (Sister)	24	right arm	alive and well
Patient 5 (Father)	57	head and neck	deceased

Table 6: Multivariable Analysis - Predicting a Positive Familial Melanoma History		
	Odds Ratio	**95% Confidence Interval**
+ Family History of Non-Melanoma Skin Cancer	2.08	1.07 - 4.05
Surrogate Report of Atypical Nevi in Family Members	2.27	1.16 - 4.43
Second Primary Melanoma	2.40	1.12 - 5.15

Table 7:
Melanoma Characteristics

	+ FH (n=51) n (%)	- FH (n=163) n (%)	P value	Female (n=131) n (%)	Male (n=83) n (%)	P value	Mean Breslow Depth (in mm)
Histology			0.73			0.17	
Superficial Spreading	37 (72.6)	120 (76.0)		97 (75.2)	60 (75.0)		0.86
Nodular	6 (11.8)	19 (12.0)		11 (8.5)	14 (17.5)		2.40
Lentigo Maligna	1 (2.0)	0 (0)		1 (0.8)	0 (0)		0.30
Acrolentiginous	1 (2.0)	1 (0.6)		2 (1.5)	0 (0)		0.45
Desmoplastic	1 (2.0)	2 (1.3)		3 (2.3)	0 (0)		5.5
Unclassified	5 (9.8)	16 (10.1)		15 (11.6)	6 (7.5)		0.84
Site			0.30			0.001	
Head & Neck	7 (13.7)	16 (9.8)		11 (8.4)	12 (14.5)		
Posterior Trunk	8 (15.7)	52 (31.9)		26 (19.9)	34 (41.0)		
Anterior Trunk	10 (19.6)	21 (12.9)		11 (8.4)	20 (24.1)		
Upper Extremity	8 (15.7)	34 (20.9)		36 (27.5)	6 (7.2)		
Lower Extremity	18 (35.3)	39 (23.9)		46 (35.1)	11 (13.3)		
Other	0 (0)	1 (0.6)		1 (0.8)	0 (0)		
Breslow Depth (in mm)			0.56			0.24	
BD $\leq$ 0.75	29 (59.2)	80 (52.6)		73 (59.3)	36 (46.2)		
0.75 < BD $\leq$ 1.5	11 (22.5)	41 (27.0)		29 (23.6)	23 (29.5)		
1.5 < BD $\leq$ 4.0	8 (16.3)	29 (19.1)		20 (16.3)	17 (21.8)		
BD > 4.0	1 (2.0)	2 (1.3)		1 (0.8)	2 (2.6)		

Table 8: Biopsies Prior to the Diagnosis of Melanoma				
		+ FH (n=51) n (%)	- FH (n=163) n (%)	P value
Total # in 5 Years Prior				
Atypical Nevi	0	47 (92.2)	151 (92.6)	1.00
	≥1	4 (7.8)	12 (7.4)	
Benign Nevi	0	42 (82.4)	149 (91.4)	0.07
	≥1	9 (17.7)	14 (8.6)	
Non-Nevi Benign	0	45 (88.2)	143 (87.7)	0.92
	≥1	6 (11.8)	20 (12.3)	
Non-Melanoma	0	50 (98.0)	152 (93.2)	0.30
Skin Cancer	≥1	1 (2.0)	11 (6.8)	
All Lesions	0	36 (70.6)	128 (78.5)	0.25
	≥1	15 (29.4)	35 (21.5)	
Total # At Any Time Prior				
Atypical Nevi	0	47 (92.2)	150 (92.2)	1.00
	≥1	4 (7.8)	13 (8.0)	
Benign Nevi	0	36 (70.6)	138 (84.7)	0.03
	≥1	15 (29.4)	25 (15.3)	
Non-Nevi Benign	0	43 (84.3)	134 (82.2)	0.73
	≥1	8 (15.7)	29 (17.8)	
Non-Melanoma	0	50 (98.0)	151 (92.6)	0.31
Skin Cancer	≥1	1 (2.0)	12 (7.4)	

Table 9: Predictors of Breslow Depth (in mm) (All Patients)					
n = 201	**BD ≤ 0.4 mm n (%)**	**0.4 mm < BD ≤ 0.75 mm n (%)**	**0.75 mm < BD ≤ 1.5 mm n (%)**	**BD > 1.5 mm n (%)**	**P value**
Biopsy Hx					
Skin Bx in 1 yr Prior 0	43 (25.2))	47 (27.5)	48 (28.1)	33 (19.3)	0.40
≥ 1	11 (36.7)	8 (14.6)	4 (13.3)	7 (23.3)	
Skin Bx in 2 yrs Prior 0	43 (25.6)	46 (27.4)	46 (27.4)	33 (19.6)	0.50
≥ 1	11 (33.3)	9 (27.3)	6 (18.2)	7 (21.2)	
Skin Bx in 5 yrs Prior 0	38 (24.7)	44 (28.6)	42 (27.3)	30 (19.5)	0.51
≥ 1	16 (34.0)	11 (23.4)	10 (21.3)	10 (21.3)	
Prior Non-Melanoma Skin Cancer					0.09
Yes	13 (22.9)	13 (22.9)	15 (26.3)	16 (28.1)	
No	41 (28.5)	42 (29.2)	37 (25.7)	24 (16.7)	
Noticed Melanoma					
Self	18 (18.6)	25 (25.8)	31 (32.0)	23 (23.7)	0.005
Physician, Friend, Family	36 (34.6)	30 (28.9)	21 (20.2)	17 (16.4)	
Self	18 (18.6)	25 (25.8)	31 (32.0)	23 (23.7)	0.001
Physician	22 (45.8)	14 (29.2)	8 (16.7)	4 (8.3)	
Physician: Non-Dermatologist	8 (38.1)	4 (19.1)	5 (23.8)	4 (19.1)	0.05
Dermatologist	14 (51.9)	10 (37.0)	3 (11.1)	0 (0)	

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[1] Two copies of each tumor suppressor gene exist in each cell. Familial predisposition to cancer in the tumor suppressor model originated from studies of retinoblastoma and the 'two-hit' formulation. One defective allele (whose protein product is defective in suppressing tumor growth) is inherited. The protein product of the second allele suppresses tumorigenesis, but mutation or deletion of this allele unleashes the suppressor effect.

[2] MTS1 is also known as CDK41, $p16^{INK4}$ gene and CDKN2.

[3] In addition, Walker et al. (1995) reported a 55-100% penetrance of the different mutations in the kindreds.

[4] Within one kindred, the authors reported that two family members were homozygous for the deletion, demonstrating that homozygotes are viable despite the loss of a cell cycle regulator. One member had not had a melanoma at the time of the first evaluation at age 54 but died a year later of an adenocarcinoma. Two of her three children did have melanoma. The authors point out that although it would be rare to find two homozygotes in the given number of patients, they were from an endogamous region.

[5] Luca et al. (1995) reported similar results.

[6] A recent study, which proved that mutations within p53 do not play a large role in melanoma tumorigenesis, did find C:G to T:A transitions in p53 in 20% of the metastatic melanomas consistent with UV radiation damage. (Hartmann, 1996)

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