No Evidence for Linkage with Melanoma in Italian Melanoma-Prone Families

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Introduction

Melanoma-prone families are often defined as having two or more 1st-degree relatives with melanoma, with the number of cases per family varying according to the incidence rates in the respective populations and the presence of founder mutations (1). The germline mutations of two genes, the CDKN2A tumor suppressor gene on chromosome 9p21 (2) and the CDK4 oncogene on chromosome 12q14 (3), account for melanoma susceptibility in ~20% to 25% of families with multiple cases of cutaneous malignant melanoma worldwide (4), and mutation frequency increases with the number of melanoma cases in the family (1). The estimated cumulative risk of developing melanoma over a lifetime in the Italian population is ~0.5% (5), unlike in the United States, Australia, and New Zealand, where the risks are ~2.0 (6), ~3.3 (7), and ~5.7% (8), respectively. Thus, a familial aggregation in a Mediterranean country like Italy is suggestive of an inherited predisposition (9). Moreover, melanoma in a population with a wide range of pigmentary phenotypes, small sized nevi, and intense sun exposure may reveal susceptibility pathways specific to this population. Therefore, we did linkage analysis in CDKN2A-negative and CDK4-negative melanoma-prone families from Italy to take advantage of the increased statistical power from the genetically enriched clusters of individuals to identify novel genetic loci linked to melanoma susceptibility.

Materials and Methods

Our study population included 51 CDKN2A-negative and CDK4-negative melanoma-prone families with 2 to 5 melanoma patients (ages 18-87 y) and consisted of 280 relatives with 85 affected individuals (10). Ten additional families from central and northern Italy, including 2 to 4 affected individuals per family (ages 26-82 y), were added for the final fine-scale mapping.

We used the Applied Biosystems Inc. linkage mapping set with 414 microsatellite markers at an average density of 10 cM. PCR amplification of genomic DNA and marker interrogation was conducted as previously reported (10). Mendelian inconsistencies were identified with the use of PedCheck (version 1.1), and inconsistent genotypes were removed. Fine mapping was done at deCODE Genetics with an average marker density of one marker every 1 cM.

Power calculation for linkage analysis based on these families was done with the use of SLINK (11, 12). Parametric 2-point and multipoint linkage analyses, assuming an autosomal dominant inheritance model, were done with the use of the MLINK program of the FASTLINK software package (version 4.1P) and GENEHUNTER (2.1 release 6), respectively. The disease allele frequency was calculated as 0.0004 based on the prevalent sex averaged rate of melanoma in the Italian population of 60 per 100,000 (13). An affected-only model was used to reduce the effect of incomplete penetrance of the trait on the analysis by coding all unaffected individuals as “unknown.” Nonparametric multipoint linkage analysis was done with GENEHUNTER.

The predivided sample test (14) was used to test for heterogeneity between families based on age at diagnosis. Families were classified as “early” (age of onset below the study population mean of 50.8 y), “late” (age of onset above the mean), or “mixed” (affected individuals in family with both early and late ages of onset).

Parametric 2-point linkage analysis was done under a dominant model, with a redefined affection status (i.e.,
the presence of dysplastic nevi regardless of melanoma status); all other subjects were coded as unknown. This analysis included 125 subjects with dysplastic nevi.

**Results**

Based on our initial 51 families, we had 85% power to detect a logarithm of the odds (LOD) score > 3 under a dominant model with 50% penetrance and the assumption of no heterogeneity, with the use of a simulated marker with six equally frequent alleles.

The plots of the 2-point parametric maximum LOD scores are shown in Fig. 1. The highest LOD score observed was 1.83 at marker D11S908 on chromosome 11. No significant P values were observed with the nonparametric analyses (data not shown).

To minimize genetic heterogeneity, we did a predivided sample test based on the mean age at diagnosis for affected individuals, hypothesizing that families with a lower age of onset would more likely have a genetic basis for their disease. There were 8, 27, and 16 families in the early, mixed, and late onset classes, respectively. The predivided sample test revealed no significant genetic heterogeneity among the groups (data not shown). The highest LOD score observed was 3.27 on chromosome 8 at marker D8S277 (8.34 cM) in the late onset classification of families. However, the flanking markers 7 and 13 cM away were strongly negative. Moreover, there was no haplotype segregating with the disease, and the melanoma’s related alleles had similar frequencies in affected and unaffected individuals.

Linkage analyses with dysplastic nevi as the affection status showed no significant evidence for linkage (the maximum LOD score was 0.98 on chromosome 10). We selected 5 regions on 4 chromosomes with LOD scores above 1.5 with flanking positive LOD scores for

Figure 1. Parametric 2-point LOD scores for the genome-wide scan. All participating individuals were genotyped at 414 loci throughout the genome. Maximum 2-point LOD scores were calculated under dominant and recessive models in an affected-only analysis. Results are plotted as a function of order along the chromosomes. Black line, dominant model; gray line, recessive model.

Table 1. Fine-scale mapping: linkage analysis on a 1-cM map for particular regions of interest

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Number of markers</th>
<th>Parametric 2-point LOD scores</th>
<th>Nonparametric</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Maximum LOD</td>
<td>θ</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>0.57</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
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</tr>
<tr>
<td>11</td>
<td>39</td>
<td>1.06</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*cM location provided by deCODE Genetics.
fine-scale mapping. The corresponding LOD scores are shown in Table 1. In this follow-up analysis, no significant evidence of linkage was observed in the expanded population.

Discussion

A number of molecular studies on melanoma families with multiple affected individuals of Northern European descent have shown linkage to a couple of genetic loci on chromosome 1 (15-17). However, the gene(s) responsible for these associations are still unknown (18). We found no evidence for linkage to melanoma susceptibility genes in either chromosome 1 or other loci in 61 Italian melanoma-prone families with 2 to 5 cutaneous malignant melanoma that were mutation negative for both CDKN2A and CDK4.

Although possible, it is unlikely that familial aggregation is due to chance alone in this population because the incidence rates of melanoma in Italy are relatively low, with 8.5 cases per 100,000 for men and 10.2 cases per 100,000 for women (19). Aggregation might also be the result of intense sun exposure in susceptible individuals. 100,000 for women (19). Aggregation might also be the result of intense sun exposure in susceptible individuals. 100,000 for women (19). Aggregation might also be the result of intense sun exposure in susceptible individuals.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References


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