

Where are all the other melanoma predisposition genes?

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Introduction

Since the identification of germline mutations in the genes encoding p16 and CDK4 in some melanoma families over 10 years ago (1-4) it might appear that little progress has been made in establishing which other genes contribute to melanoma susceptibility. However, I would argue that considerable progress has been, and is being made towards this goal. Although, with a couple of notable exceptions, much of this success has not led to the actual identification of novel low and high penetrance melanoma predisposition genes, significant advances have been made in determining the appropriate approaches to employ in this endeavour, as well as in ascertainment of the relevant samples – both family-based and case-control – in which to search for these genes.

High penetrance genes

The CDKN2A locus is unusual in that it encodes two completely unrelated products – p16INK4A and p14ARF – that are derived by alternative splicing and translation in different reading frames. While the p16 component is the principal target of mutation in melanoma-prone families (5) germline deletions or splice mutations specifically affecting p14 have been found in some kindreds (6-10). Thus p14ARF is the third high penetrance melanoma predisposition gene to be identified, although like CDK4, it only contributes to a small fraction of the overall burden of familial melanoma.

The obvious question remains: how many more high penetrance genes are there and how do we find them? The first part of this question is difficult to answer, although as indicated below, an estimate of its lower limits might be extrapolated from current data. The answer to the second half of the question is more straightforward as it still relies very much on the standard family-based approaches of linkage mapping and candidate gene analysis. So what are the lower estimates of yet to be discovered high penetrance melanoma predisposition genes? Linkage mapping has recently identified a locus on 1p22 associated with early-onset melanoma families (11). This locus appears to be responsible for ~20% of the families that do not possess p16/p14/CDK4 mutations or show linkage to the CDKN2A region on 9p21. Interestingly, the families utilized in this genome scan show two clear age of onset peaks – the first around 33 years and the second around 56 years. The latter group of families does not show linkage to the 1p22 locus. Two obvious explanations could account for this: either there is another gene responsible for late age of onset familial melanoma, or, the majority of families that show later onset are clusters of cases that do not share a Mendelian gene defect predisposing them to melanoma. If the experiences of the Breast Cancer Linkage Consortium (BCLC) are anything to go by the second scenario may be the most likely. Despite exhaustive linkage analyses the BCLC have failed to find convincing evidence for BRCA1 or BRCA2 mutations (12, 13). It thus seems reasonable to consider melanoma predisposition in most late onset melanoma families to be due to oligogenic inheritance, i.e. a small number of low penetrance genes. Such families may thus prove useful in genome-wide association studies (see below).

Worldwide a number of families with cases of cutaneous and ocular melanoma have been identified. With a single exception (14), these families do not carry mutations in the three known melanoma predisposition loci, thus it appears likely that novel loci exist that predispose to both of

these forms of melanoma. Indeed, recent linkage analysis in two such Danish families mapped a susceptibility gene to 9q21 (15). These families were heavily weighted for predisposition to ocular melanoma, rather than cutaneous melanoma. This contrasts with the bulk of the other families showing ‘mixed’ melanoma predisposition, in which the majority of cases have cutaneous tumors and only one or two cases have ocular melanoma. It is tempting to speculate therefore that a novel, yet to be mapped locus, exists for the latter families (the tally is now up to 3 unknown high penetrance melanoma genes). It is also possible that there are other genes responsible for melanoma in an exceedingly small proportion of families. But because of their small overall effect, and the known locus heterogeneity for melanoma susceptibility, it would be extremely difficult to identify such families through conventional linkage analysis. Instead, we must revert to candidate gene approaches. Notably, this tack has already proved successful for melanoma as it was this approach that identified germline mutations in the CDK4 gene (4). Good candidate genes for sequencing in familial melanoma cases are those encoding other members of the INK4 family of CDK-inhibitors, other CDKs, as well as products that impact on the function of the pRb and p53 pathways (since these are regulated by the three known melanoma predisposition genes). Although some of these genes have already been assessed in a small number of melanoma families (16-20), sequencing of these and other candidates in a much larger cohort of families is warranted.

Low penetrance genes

In addition to family-based studies, interest is increasing rapidly to identify the low penetrance genes that are assumed to contribute to the risk of melanoma in the 90-95% of sporadic melanoma patients. While there have been numerous candidate gene studies assessing the potential of various loci and variants within them as low penetrance melanoma risk alleles (reviewed in 21) there have been no systematic genome-wide single nucleotide polymorphism (SNP) association studies conducted to date to search for such genes. The bulk of the candidate gene/SNP studies in melanoma have been disappointing. Most have been grossly underpowered or have used poorly matched controls. Some of the candidate genes analysed so far encode DNA repair enzymes, xenobiotic metabolizing enzymes, cytokines and other immune regulators, and growth factors and their receptors. To my knowledge, for the handful of putative positive associations initially reported for some of these genes, none have been replicated. What is the problem here – is the choice of candidate genes bad, or are the studies inadequate? The vexing issue here is of type 1 and type 2 errors. There is a bias towards publishing positive findings, often generated from analysis of very small case-control comparisons, leading to many type 1 errors. This is compounded by potentially abundant type 2 errors, i.e. possible associations being missed because the sample size is too small or because the gene haplotypes (central to the tenet of finding a SNP in linkage disequilibrium with the causal variant) are not covered fully. But the tide appears to be turning. Some more recent melanoma association studies have attempted to address these issues by assessing many SNPs in the same gene in relatively large case-control samples. This has led to the suggestion that haplotypes of the BRAF locus might confer a modest risk for melanoma (22). This association has been replicated in one study (23) but not in another (24). So while the jury might still be out on the potential involvement of BRAF as a low penetrance melanoma susceptibility gene, the current study designs have improved markedly, and offer hope that the candidate gene approach will have some success in the near future.

There is already one shining example of how successful such candidate gene studies can be – the association between functional variants of the melanocortin 1 receptor (MC1R) and melanoma. This is arguably one of the most replicated of case-control associations in all of biology. Some MC1R variants (termed red hair color variants because of their association with red hair) confer an approximately two-fold risk of melanoma for carriers of a single variant allele, and a four-fold risk if both alleles are variant (reviewed in 21). Since MC1R is a pigmentation gene, it is not unreasonable to presume that other pigmentation genes might also be low penetrance melanoma genes. Consequently, there are a number of studies currently underway assessing this possibility.

What are other rational candidate low penetrance melanoma genes? As mentioned above, gene products involved in DNA repair, carcinogen metabolism, maintaining redox balance, and immune response, all appear biologically plausible. But are candidate gene approaches the right way to go? I would advocate they are, since they are within the technical and budgetary reach of most groups working in this field. And if conducted well, i.e. using a combination of functional and haplotype-tagging SNPs in very large case-control samples, they have the potential to comprehensively rule in, or rule out, a particular gene.

But how do we more systematically identify low penetrance genes and their associated variants? The standard approach is to carry out a genome-wide association study (GWAS) using whole-genome SNP chips containing 100,000 – 500,000 SNPs. The drawback to this kind of study to date has been primarily budgetary – it requires millions of dollars to conduct on a scale large enough to be statistically robust. Additionally, this scale usually means that no single group has enough samples to run on their own – a consortium is needed. In the melanoma field one such consortium has been established (GenoMEL, <http://www.genomel.org/>) and has recently secured European Union funding to carry out a melanoma GWAS using thousands of DNA samples contributed by 10 or more member groups. Although at least a year away, the outcome of this study is eagerly awaited, and hopefully will provide a number of important leads to follow up.

In conclusion, we are currently enjoying the fruits of the genomic era, and with the aid of new high-throughput genotyping technologies combined with large sample collections, we are firmly poised to identify many new genes that predispose to melanoma.

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