GENETIC RISK AND MELANOMA

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Abstract

This paper reviews current understanding of the role of genetic factors in the causation of melanoma. Three genes have a proven role in influencing melanoma susceptibility: CDKN2A (p16INK4A) and its alternative product p14ARF, CDK4 and MC1R. The former two genes are frequently mutated in the context of familial melanoma, though rarely otherwise, and can raise risk to extreme levels. MC1R regulates melanocyte pigment production and its variants contribute strongly to risk in European populations because they are very prevalent, modulating individual risk by two to three fold. There are many unanswered questions about the genetic epidemiology of melanoma and the place of genetic testing in melanoma risk assessment; these are the subject of intense international collaborative research.

In one sense all melanoma is genetic. The main causes of melanoma are mutations, ie. permanent DNA sequence changes, affecting key genes in melanocytes. Melanocytic naevi are also now believed to result from short-lived clonal proliferation of melanocytes after similar mutagenic events. These may be induced somatically by solar ultraviolet radiation, other mutagens or DNA replicative errors, or may be inherited in the germline. Over the last 10 years a gradual revolution has been under way in our understanding of how they contribute to risk of melanoma.

An average Australian's risk of melanoma is currently estimated as 3.5% for women and 4.5% for men, though it is twice as high in the tropics and lower by half in the far south. It is best to regard an individual's risk as lying on a continuum, with many separate risk factors cooperating to raise or lower the probability that melanoma will develop at some time during their life. Some of these risk factors are genetic in the sense that they have been inherited and are therefore likely to be shared with parents and other close relatives. For example, it has long been understood that skin colour is a heritable trait and that fair-red, easy burning skin is associated with an above average risk of all forms of skin cancer. The other major phenotypes strongly associated with melanoma are the number, size and density of normal and atypical melanocytic naevi; these are quantitative traits with complex genetic origins. Sun exposure itself can also be strongly shared among close relatives, especially early in life; this is because families share a common environment, such as geographic location, and often share patterns and habits of sun exposure and protection.

The upshot of these considerations is that many cases of melanoma will involve related individuals. In other words melanoma will show familial aggregation. In some cases this will be due to one or a few strong genetic factors, or the additive effects of several genes, each of modest effect. In others it will be largely due to a shared high sun-exposure environment. Finally, in any large population it is inevitable that some clusters will have occurred purely by chance.

There is a separate sense in which a person's susceptibility to melanoma may be hard-wired genetically, but not inherited: critical mutations or epimutations, ie. fixed gene expression abnormalities without DNA sequence change, may have occurred in a melanocyte progenitor during embryonic-fetal development. This is an important area for future research to define but will not be discussed further here.

Historically, attention was first drawn to familial melanoma by observations of familial clusters of melanoma-susceptible individuals. These were characterised by early age of onset, multiple primary melanomas and frequent presence of atypical melanocytic naevi. Because the cases described were on the "same side" of the family, ie. shared common ancestors, it was postulated that a single gene, autosomal dominant Mendelian trait causing both melanoma and the naevi was responsible. Further research has shown that this was an oversimplification. There is no evidence yet that a syndrome of multiple banal or atypical naevi is caused by a single gene, even though familial melanoma can be.

What do we know of the genes that influence melanoma risk and their effects? Can this knowledge be utilised clinically? What more do we need to know and how is local and international collaborative research meeting this challenge? Readers have been directed in the references to comprehensive recent reviews,[1,2] to original reports with essential reference data, especially if post-dating those reviews, and to policy statements and unpublished studies of the Melanoma Genetics Consortium.

Genes that influence melanoma risk – CDKN2A

Major genetic effects are most easily discovered in families with multiple cases of melanoma. A combination of genetic linkage analysis of such families in and fine mapping of DNA deletions in the region of peak linkage to familial melanoma eventually led to the identification of the CDKN2A locus ("p16") in 1994, which was soon found to carry germline mutations in many melanoma kindreds. CDKN2A was ultimately found to produce two unrelated proteins by alternative splicing, a situation unique in the genome. The product discovered first, p16INK4A, was a known cyclin-dependent kinase (CDK) inhibitor that regulated the cell proliferation cycle at the G1-S checkpoint. Subsequently, the p14ARF product was identified and its complex functions include regulation of p53 levels and therefore pathways mediating responses to DNA damage.

CDKN2A mutations have been found in hundreds of familial melanoma kindreds throughout the world. The proportion of these kindreds with mutations varies from close to 100% in the very largest kindreds in low-incidence countries such as the UK to less than 5% in clusters of only two related individuals in Australia. Worldwide, 40% of dense kindreds (three or more cases) carry CDKN2A mutations, whereas the rate in Australian kindreds of the same density is 20-25%. These proportions increase somewhat if

any cases have had multiple primary melanoma.[3]

Other putatively predisposed individuals can carry CDKN2A mutations. In the limited number of studies of multiple primary melanoma so far, frequencies from 2-15% have been observed. A recent population-based analysis of cases of melanoma under 40 years in Australia yielded a frequency of 2%; most of these carriers did not have a strong family history of melanoma.[3] Based on these and other estimates, it is unlikely that more than 1/200 melanoma cases in Australia carry a CDKN2A mutation. These mutations are observed throughout the p16INK4A exons of the gene and most of them encode proteins with altered function: altered binding to CDKs, failure to inhibit CDK activity, abnormal trafficking in the cell, or evidence of protein instability. Some mutations can affect both p16INK4A and p14ARF proteins while others affect p16INK4A, or rarely p14ARF, alone.

Phenotypes caused by CDKN2A mutation

Mouse knockouts specific for p16INK4A yield melanomas at a low rate, but this is greatly enhanced by mutagens if they are bred on to a p14ARF heterozygous background. The combined p16INK4A/p14ARF knockout is melanoma-prone, especially in the presence of activating oncogenic mutations. Taken together, there is strong genetic evidence that inherited, inactivating mutations in both products of this dual-function locus contribute to risk of melanoma and other tumours. Certainly the locus is a major target of deletion events in melanoma formation and these usually inactivate both genes, causing deregulation of a key cell-cycle checkpoint and the loss of an activator of p53-mediated apoptosis.

Carriers of CDKN2A mutations within mutation-positive families cannot be recognised clinically, although there is an association with increased number of naevi and atypical naevi. The risk of melanoma to carriers of these mutations has so far only been estimated in the context of familial melanoma, but confidence limits are still very broad and the estimates vary across geographic regions. Australian carriers of CDKN2A mutations had the highest lifetime risks, averaging 90%, in contrast to carriers in Europe in which they were less than half as high, especially in middle age. These effects are presumably due to differing regional levels of sun exposure.[4]

CDKN2A mutations, as a class, cause a significant increase in the risk of pancreatic cancer, estimated at 17% lifetime risk in one study of a common Dutch founder mutation.[5] Interestingly, recent analysis of data from around the world has shown that familial melanoma kindreds in Australia do not exhibit this association, except perhaps for carriers of the same Dutch mutation.[3] In five melanoma families worldwide, a germline CDK4 mutation prevents p16INK4A binding. The phenotype of these families is so far indistinguishable from that of CDKN2A families, but the data are simply too few to be sure of this.[3]

Other genes influencing melanoma risk – MC1R

Progress has also been made in identifying so-called low-penetrance genes, notably the melanocortin-1 receptor (MC1R) which is highly polymorphic, especially in fair-skinned populations. Many of the variant forms of this protein favour production of red-yellow (phaeo) melanins over brown-black eumelanin and are therefore associated with red hair, freckling and fair, sun-susceptible skin types. These variants are also convincingly associated with risk of both melanoma and non-melanoma skin cancer in population-based studies. The extra risk produced is modest, approximately two-fold per variant allele carried, and is independent of skin colour; individuals with

only one variant allele will exhibit a darker, eumelanin-based skin type, but still have increased risk of melanoma. Due to the high frequency of MC1R variants in the population, their overall contribution to disease prevalence (attributable risk) will be much larger than for rare, high-penetrance alleles of CDKN2A. It is anticipated that genes influencing naevus number, once they are discovered, will prove to be equally important.

The exciting discovery in 2002 that the BRAF gene is mutated at high frequency in melanoma has not had an impact on our understanding of melanoma risk. As with the second most common activating oncogenic mutation target in melanoma, NRAS, these mutations occur almost exclusively during life and are not inherited.

Genetic assessment of melanoma risk

Individual risk assessment must take a comprehensive view of personal and family history of melanoma, other risk factors such as the number and type of melanocytic naevi, skin pigmentation and evidence of sun sensitivity and past and present sun exposure. Is there a role for CDKN2A mutation testing, ie. should the gene be screened, and if a family mutation has been discovered, should an individual be tested for it? The cited reviews and position papers canvas the issues more thoroughly than space permits here.[6, 7, 8]

The probability of detecting a CDKN2A mutation is only substantial (>10%) in the context of a strong family history of melanoma, ie. three or more relatives affected by melanoma on the same side of the family. Importantly, a family history of melanoma cannot be taken at face value but must be confirmed from medical or cancer registry records. Previous Australian studies have shown that up to 40% of reports of melanoma in close relatives cannot be substantiated and analyses of different cohorts 15 years later show little has changed.[3]

Within the restricted context of proven high-density melanoma kindreds, in Australia, it is clear that CDKN2A mutation carriers have a substantially increased risk of melanoma. However these estimates are very imprecise (more so than for *BRCA1* carriers in familial breast cancer, for example) and are probably strongly modulated by both sun exposure and pigmentation. We also know little of the risk to non-carriers in such families, however there are grounds to believe that it would be elevated, albeit to a much lesser extent than for the carriers.

Crucially, the outcome of genetic testing is unlikely to alter the risk management of the patient. All members of familial melanoma kindreds must be regarded as at increased risk of melanoma, irrespective of mutation status, and ought to be enrolled in programs of heightened surveillance. This would suggest that genetic testing has little positive to offer. There is also potential for negative consequences such as abandonment of preventive and screening behaviours in the event of a negative test. However the decision to test for carrier status of a family mutation is one for patients to make after weighing up their options and preferences. This is best done in the context of a family cancer genetics clinic.

The current research agenda

The Melanoma Genetics Consortium (now known as GenoMel) has been supported by the US National Institutes of Health (2001-6), and has recently attracted a European Union network of excellence grant (2005-9), to study the genetic epidemiology of melanoma. Partners in the consortium come from 18 centres in 11 countries and include all Australian groups working in melanoma genetics. Recruitment of people with a strong family history of melanoma remains active in Australia.

Are there more melanoma susceptibility genes to be found? Genome-wide linkage searches of the majority of familial melanoma kindreds without CKDN2A or CDK4 mutations have established that a new high-penetrance locus exists on chromosome 1p and possibly more.[9] Efforts to map and identify these genes continue. Pigmentation, naevus, sun-sensitivity and DNA repair phenotypes remain largely undefined genetically and some of the genes regulating them will undoubtedly influence melanoma risk via medium/low-penetrance alleles. In addition to direct genetic analysis of those phenotypes, there is a need for well controlled genome-wide association studies of melanoma to map and identify the relevant genes directly.

How common are these genetic variants, how strong are their effects and how do they interact with sun exposure to cause melanoma? These are questions that ideally require two types of resources: large cohorts of carriers of high-penetrance mutations, largely recruited from familial melanoma kindreds, and population-based cohorts of cases of melanoma and their relatives. Provided the appropriate risk factors have been measured, modelling of risk to the cases and their relatives will enable the strongest independent predictors of risk to be identified. Several large studies of this kind have been mounted in Australia and are currently completing data collection and analysis.

What are the issues and best practice approaches in management of people at high risk of melanoma? Now that a group at extremely high lifetime risk can be identified, at least in the context of familial melanoma, it is essential that they and their relatives at putatively lower risk be followed prospectively to resolve the uncertainties over the relationship of carrier status to risk. Psychosocial research is also required to establish the issues and consequences of genetic risk assessment in melanoma, which may differ from those in other familial cancers. Most importantly, longitudinal studies are needed to determine which clinical measures are most effective and efficient in preventing, detecting and treating future melanomas in high-risk patients.

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