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Genetic epidemiology of melanoma

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Abstract

Melanoma incidence has risen in many Caucasians populations over the last 20 years and research on the potential environmental and genetic risk factors has led to some interesting new findings but also to many more questions. The relationship between melanoma and ultraviolet radiation is complex and this area of research is controversial especially regarding the use of sunbeds and sunscreens. In terms of genetic factors, the discovery of two genes CDKN2A and CDK4 has been a great advance with more understanding of melanocyte biology in relation to defects in senescence. For phenotypic risk factors such as fair skin and high numbers of naevi, the role of genetic factors is clearly evident but these traits are complex and the discovery of genes involved in skin pigmentation and naevi formation is not an easy task. Research on the MC1R gene has not only shown the importance of this gene in hair and skin pigmentation but also in senescence and immunity. Functional studies involving CDKN2A and MC1R are leading to important new findings. There is also some hope regarding the use of micro-arrays in helping to dissect many genetic events in melanoma. The collection of large datasets including family, twin and case-control studies as well as tumour banks with collaborations between countries will hopefully lead to more discoveries. For the primary and secondary prevention of this tumour, efforts need to be sustained in public health campaigns on sun exposure and the recognition of individuals at high risk.

1. Changes in incidence

Melanoma although a relatively uncommon cancer has become an important public health issue because of rising incidence in Caucasian populations. It is estimated that over the last 50 years, the incidence has risen steadily by around 6% every year leading to a 10-fold increase in incidence since the late 1950s [1]. However, most recent figures have shown that in parts of the United States of America (USA), Canada, Australia and Europe, the incidence rates have reached a plateau or decreased [2]. The increase in melanoma incidence up until the mid-1990s is thought to be mostly attributed to increases in sun exposure over the last 30 years. It is therefore tempting to think that the recent plateau or downturn in incidence is caused by the success of public health campaigns. However, this is not proven especially as this downturn is also observed in countries with no or little public health campaigns and the lag period between possible changes in behaviour and reduction in incidence has been quite short. Furthermore, the plateau and downturn in melanoma incidence occurred at the same time in many Caucasian populations across the world. These countries have very different levels of ultraviolet (UV) exposure so the increased awareness of melanoma and the possible changes in behaviour may not be the only explanation for this recent trend.

2. Environmental factors

2.1. Sun exposure

The association between melanoma and sun exposure although supported by latitude studies, migration studies and case-control studies is very complex. One of the obvious confounding factors in all studies looking at the effect of sun exposure is skin type. Although melanoma mainly affects fair-skinned individuals, it is still commonly found in subjects with skin types 3 and 4, who may tan very easily. The obvious difference in melanoma incidence between Australia (melanoma incidence in Queensland is 50/100 000) and Europe (melanoma incidence in UK is 10/100 000) has clearly been a robust proof that sun must play a role in its causation. Melanoma is the 4th most common tumour in Australia when including non-melanoma skin cancers compared with the UK where it is the 18th most common tumour [3]. Case-control studies from all over the world looking at sun exposure in terms of sunbathing and sunburns have yielded very small odds ratios. So sun exposure *per se* is not a very good predictor

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of melanoma risk. Furthermore, cumulative occupational exposure to the sun as well as exposure in good tanners appears to be protective so the causal effects of sunshine are difficult to dissect [4]. Exposure in childhood seems to be the most detrimental which suggests that ultraviolet radiation may programme cells at an early stage [5]. Melanoma is also more common on intermittently sun exposed areas especially in young age groups which is also not in support of a dose-response effect [6]. In Australia, melanoma mainly affects fair-skinned individuals of Irish and Scottish descent [7]. The genetic pool in terms of migration patterns to Australia for example is relevant when examining the association with sun exposure and future studies looking at mutations and polymorphisms in relevant genes will shed light on possible founder effects in melanoma causation. The case-control study model may not be sensitive enough to detect the deleterious effects of sun exposure which may only be relevant in genetically susceptible individuals. Furthermore, it is subject to poor recall and recall bias. Gene environment interactions are difficult to study as the genetics of melanoma are presently poorly understood and the collection of sun exposure data can be inaccurate, but collaborations between countries collecting standardised genetic and epidemiological data in melanoma families and large melanoma cohorts should help to dissect the role of genes and environment in melanoma [8].

3. Sunscreens and sunbeds

Sunscreens have long been promoted for skin cancer prevention, but their efficacy in reducing melanoma is unclear [9]. Moreover, recent studies have shown that the use of sunscreens can be associated with an increased risk of melanoma and this risk is likely to be explained by the suppression of sunburns which lead to longer exposure to UVR mainly within the UVA range [10]. Higher sun protection factor (SPF) sunscreens also increase mean cumulative sun exposure of young subjects in Europe and also seem to affect naevus counts [11,12]. For sunbeds, the association with melanoma is more controversial as several studies have shown no association between sunbeds and melanoma, whilst others have shown a weak, but elevated, risk, especially in young women [13–15]. The design of these studies is critical as exposure to natural sunlight acts as a major confounder.

It is important to maintain the public health messages to raise melanoma awareness for secondary prevention. In terms of primary prevention, reduction in sun exposure is still recommended as apart from the complex association with melanoma, excessive sun exposure leads to premature ageing and non-melanoma skin cancers which are very prevalent. The controversy lies in what level of sun exposure is considered excessive and whether sun avoidance may have deleterious health effects. Individuals with fair skin should be particularly targeted for reducing sun exposure whilst health campaigns target the whole population. Publications have also pointed towards the potential protective role of ultraviolet radiation for osteoporosis and solid cancers [16,17]. The incidence of ovarian and colon cancer is also influenced by a latitude gradient with an inverse relationship as observed in melanoma and this decrease in solid tumours with decreasing latitude may be explained by the anti-cancer effects of vitamin D following UVR exposure [18,19]. A recent study showed a negative association between mortality caused by breast, colon, ovarian and prostate cancer and residential exposure to sunlight [20]. Vitamin D receptor polymorphisms have also been shown to be protective in melanoma and more work is needed to investigate the effects of sun avoidance, especially in European countries where UV exposure is only relevant for a few months of the year [21].

4. Genetic factors

4.1. Germline mutations

Melanoma like many other cancers has a genetic basis. It has long been observed that melanoma can cluster in some families. However, this is rare and the genes implicated in large melanoma families are likely to play a minor role in population-based melanomas. In melanoma genetics, the first germline (transmitted from one generation to the next) mutations in *CDKN2A* or *p16* on chromosome 9p21 were discovered in large melanoma pedigrees in 1994 by the Utah group [22]. Mutations in *CDK4*, another gene involved in melanoma families and closely linked to *CDKN2A* or *p16*, was identified by Zuo and colleagues in 1996 [23]. *CDKN2A* or *p16* acts as a true tumour suppressor gene and has a crucial role in cell cycle regulation and senescence. It is an unusual gene with two alternative reading frames producing two distinct proteins: p16 and p14 ARF (Alternative Reading Frame). Both of these proteins have a crucial role in cell death and apoptosis via the Rb pathway for p16 and p53 pathway for p14 ARF [24]. The p16 protein is a cyclin-dependent kinase inhibitor (by binding to CDK4) and mutations in *p16* may account for up to 25% of familial melanomas worldwide, whilst *CDK4* mutations on chromosome 12 have only been found in a few rare families [8,23,25]. The *p16* gene appears to be a rare, highly penetrant gene which is transmitted in most families in an autosomal dominant fashion. The estimated frequency of the mutated *p16* gene in the general population is 0.01% [8]. The Melanoma Genetics Consortium has recently studied melanoma families with confirmed *p16* mutations pooled from all

over the world and found an overall penetrance of the gene of 30% by the age of 50 years (95% Confidence Interval (CI) 12 to 62%). The authors also reported a higher penetrance in countries with a high melanoma incidence such as the USA and Australia compared with Europe that has a lower incidence of the disease [8]. This may suggest that the environmental exposure may affect *p16* penetrance, although other genetic causes may explain the differences in *p16* penetrance worldwide. For example, the types of mutations in respective countries may affect penetrance estimates. Screening for *p16* mutations in a typical mole syndrome (AMS)/melanoma families is currently not recommended in UK clinical practice (outside of research settings) because of its poor predictive value, in terms of lifetime risk of melanoma, the possible involvement of yet undiscovered melanoma genes and the fact that mutation status would not affect the follow-up strategies [26]. The frequency of follow-up visits is on average 6 monthly, but this varies greatly depending on the severity of the AMS phenotype, the family history, the level of anxiety about being "at risk" and also whether patients are happy to keep an eye on their lesions and request more interval follow-up visits when needed.

p16 Mutations have also been linked to multiple melanoma primaries, with or without a family history of the disease. However, the prevalence of *p16* mutations, even in these highly susceptible individuals, was low [27]. A French study of the prevalence of *p16* mutations in multiple primary melanoma cases has shown that 9% of the cases had *p16* mutations, but that these mutations were similar to the founder mutations observed in the previously reported melanoma families and were not *de novo* germline mutations [28]. This shows that the absence of a family history of melanoma in patients with multiple primary tumours does not rule out the involvement of p16 and these patients and their families may need screening. It is likely that a number of low penetrance genes play a role in melanoma and, like many other cancers, it is suspected that sporadic melanomas have a complex mode of inheritance [29].

Melanoma susceptibility has also been described in the context of various family cancer syndromes with an excess of breast, throat, gastrointestinal cancers, but especially, pancreatic cancers [30–32]. Melanoma can also occur in rarer family cancer syndromes (such as retinoblastoma, Li Fraumeni and Neurofibromatosis type I) as well families prone to ocular melanoma, Non Hodgkin's lymphoma and nervous system tumours [33–35]. A study in families with melanoma and a susceptibility to other solid cancers has shown that p16 can be involved in some multi-site cancer families, although this was a rare occurrence [36]. In Holland, the association with pancreas cancer is common in most Leiden melanoma families, but the founder mutation responsible for familial melanomas in that region may explain why pancreas is also affected by this specific *p16* mutation. The overall susceptibility to cancer in the families of melanoma patients is important to detect by taking a thorough family history and offering genetic counselling and appropriate cancer screening. However, care should be taken not to raise anxiety and genetic counselling in susceptible families is helpful. However, more studies are needed to determine if the risk of solid tumours, other than pancreatic cancer, is significantly increased in these susceptible families. The potential link between melanoma and other cancers is also important to investigate as these studies may unravel genetic pathways which melanoma may share with other common cancers.

Other genes such as *PTEN*, *MC1R* (Melanocortin 1 Receptor) and DNA repair genes have also been implicated in melanoma. However, they do not appear to play a major role [37,38]. There is no evidence that melanoma patients, including patients with a genetic susceptibility to the disease, have significant defects in DNA repair following UVR exposure, but this has only been assessed in lymphocytes and only in a few subjects *in vivo* in epidermal cells [39,40]. New techniques using high liquid chromatography can now detect more accurately UV-induced DNA lesions and more work is needed to investigate DNA damage and repair in the epidermis in melanoma cases and controls.

4.2. Somatic changes

Genetic changes which may occur in the precursor lesions (naevi) or at every stage of melanoma progression are also important to study. Melanoma may occur because of step-wise random or non-random mutations in genes which are important in melanocyte differentiaton. However, the study of somatic changes in tumours has also been successful in locating important germline mutations in tumour suppressor genes. Somatic changes have long been investigated in melanoma and have helped, in part, in locating the *p16* gene on chromosome 9p21 in 1994 [41]. Loss of heterozygosity studies (LOH) in sporadic tumours confirm the importance of the 9p21 region in the pathogenesis of melanoma. Additional chromosome arms shown to be lost include 1p, 9q, 17p, 3p, 6q, 10q, 11q and 18q.[42,43] Cytogenetic studies using comparative genomic hybridisation (CGH) and fluorescent *in situ* hybridisation (FISH) investigating sporadic melanoma have shown losses from chromosomes 1, 6, 9, 10, 17 and 7 and gains for chromosomes 1, 6, 7, 9, 10 and 17 [44,45].

More recent work on the 9p21 region has shown that mutation, inactivation and loss of the *CDKN2* gene at 9p21 does not entirely explain all the LOH observed on the 9p21 locus and this suggests the presence of other tumour suppressor genes on 9p21 [46,47]. Linkage analyses have also pointed to another gene on 9p as some 9p21-linked melanoma families have no *CDKN2A* exon mutations [48,49]. The investigation of the non-coding

Table 1
Atypical mole syndrome

- The atypical mole syndrome (AMS) is a powerful predictor of risk in melanoma and may indicate a genetic susceptibility to melanoma.
- Although the AMS is common in *p16*- or *CDKN2A*-linked families, it is not a reliable predictor of carrier status, as gene carriers may not express multiple atypical naevi.
- Atypical naevi are more a marker of risk than true precursor lesions in susceptible individuals and the random removal of a large number of atypical naevi is not recommended.
- The removal of lesions in susceptible individuals should still be guided by clinical signs indicating a transformation into early melanoma.

region of the *p16* gene suggests that mutations in the promoter region are unlikely to play a significant role in the linked 9p21 families without *p16* exonic mutations [50].

More recently, somatic changes have been described in the *BRAF* gene affecting up to 60% of melanoma tumours [51]. This gene is one of three serine/threonine kinases that are regulated by binding to RAS. This is a well known oncogene affecting up to 15% of all cancers. When mutated, the protein has increased kinase activity and the role of this oncogenic pathway in melanoma may open up new avenues for therapeutic agents in melanoma.

4.3. Genetics of the atypical mole syndrome or the "at risk phenotype"

Over the last 20 years, epidemiological evidence has shown that host factors (high naevus counts and a fair skin phenotype) are the most powerful predictors of risk for melanoma and discovering which genes are involved in skin pigmentation and naevus expression may help in understanding melanoma susceptibility [52,53]. In UK white people, more than 100 common naevi and the presence of 2 to 3 atypical naevi give odds ratios between 5 and 10, whilst fair skin (skin types 1 and 2) is associated with a 3-fold increase in melanoma risk [53]. In the UK, the presence of 100 or more naevi accounts for 22% of melanomas below the age of 40 years. As the naevi number decreases significantly with age, only 12% of melanomas are attributable to high numbers of naevi above the age of 40 years. For all ages, skin type 1 accounts for 9% of all melanomas in the UK [54]. In Australia, where sun exposure is thought to be the most important factor in explaining the rising incidence, naevi number remains the most powerful predictor of risk [7]. This is also the case for adolescents with melanoma in Australia, in whom, 100 or more naevi are associated with an odds ratio for melanoma of 46.5 and no differences were observed between cases and controls regarding sun exposure habits [55]. This highlights the crucial role of host factors in melanoma susceptibility which should lead to increased research in genes influencing the "at risk phenotype" i.e. the number of naevi or susceptibility to sunburn.

The link between melanoma and naevi was first reported in melanoma families. These families often have an abnormal naevus phenotype which has been labelled the BK mole syndrome (BK for the initials of the family described), the FAMMM (familial atypical multiple mole melanoma) and the AMS (Atypical Mole Syndrome) phenotype. This phenotype, characterised by a large number of common and atypical naevi, as well as naevi on unusual sites such as the buttocks, breast and scalp, is more a marker of risk rather than a true precursor phenotype [56]. The AMS phenotype can also be found in 2% of the normal population in the UK and is associated with relative risks for melanoma of 10 to 20 and screening of all AMS cases is therefore not feasible [53]. The AMS phenotype can also be found in cancer-susceptible families, with or without melanoma, and pancreas cancer has clearly been linked to both melanoma and AMS [30,32]. The rate of malignant transformation of atypical naevi in these high-risk patients is thought to be very low. It is recommended that atypical naevi be removed only if the clinical features suggest an early melanoma, whilst in the past it was not uncommon to remove many atypical naevi in high-risk patients. This is not helpful in decreasing the melanoma risk as melanoma may appear *de novo* and the selection of lesions which may or may not progress is almost impossible. The diagnosis of the AMS is clinical and the confirmation of histological dysplasia in a few lesions does not add anything in terms of management (Table 1).

Germline mutations in *p16* which appears to be quite specific to melanoma families (as well as pancreatic

Table 2
p16 and Melanoma

- Germline mutations in *p16* or *CDKN2A* are found in up to 25% of melanoma families worldwide.
- The penetrance of *p16* or *CDKN2A* is estimated to be around 60% by the age of 80 years.
- *p16* or *CDKN2A* is likely to be involved in the expression of naevi, but other naevogenic genes remain to be discovered.
- Detection of the *p16* mutation status is not yet recommended in clinical practice because clinical care and follow-up strategies are not affected by this mutation status.
- Other genes, such as *MC1R*, are likely to play a role in susceptibility to melanoma.

families) are likely to be implicated in the expression of naevi as well. (p16 has also been implicated in other cancers, but not affecting the germline). In UK melanoma families, *p16* mutation carrier status correlates with high mean naevus counts [57]. However, the relationship between *p16* status and naevi number is not straightforward, as *p16* status is not always correlated to high naevus counts in all melanoma families [58,59]. Naevi are very common and therefore likely to be influenced by common low penetrance genes. Using the twin model, it is estimated that up to 60% of the naevus phenotype may be attributed to additive genetic factors and family studies also suggest that the mode of inheritance of naevi is complex [60,61]. The Australian study by Zhu and colleagues has also shown that the 9p21 locus containing the *p16* gene may influence naevus numbers [61] (Table 2).

In most melanoma families, the expression of the AMS phenotype may help in the recognition of potentially affected individuals, but this phenotype is not specific enough to be relied upon in terms of deciding who may need long-term follow-up within a family [58,59]. The presence of *p16* mutations does not appear to give rise to different types of atypical naevi or melanomas in terms of biological behaviour, including prognosis, so follow-up strategies do not differ according to the presence or absence of *p16* mutations [25,59]. Family and personal history therefore remains the most important criteria for screening high-risk groups.

Individuals with the AMS phenotype not only develop multiple atypical lesions, but also do not show an involution of naevi from middle age as is seen in individuals with fewer naevi. Indeed, it is often observed clinically that new naevi still appear well after 30 years of age in many susceptible families. This clinical observation fits well with the recently discovered role of *p16* as a senescence gene. Senescence is believed to be a mechanism which protects against cancer by eliminating cells with acquired genetic changes. It is now established that genetic changes in *p16* cause defects in melanocyte senescence and differentiation which could lead to tumorigenesis [62]. Further studies looking at *p16* function and *p16*-knockout cell lines will help to unravel genetic pathways which may lead to therapeutic targets for melanoma and other cancers.

Another important risk factor for melanoma is fair skin/hair type with a propensity of sunburn. Not surprisingly, these phenotypic traits are genetically determined. Using the twin model, freckle count has been found to be a highly heritable trait, with 90% of the variation in freckle counts explained by additive genetic factors [60]. Polymorphisms in the *MC1R* gene have been linked to a fair hair/skin phenotype, with or without freckles. There is a large number of *MC1R* variants and the relationship between *MCIR* variants and hair/skin colour, as well as the relationship between *MC1R* variants and melanoma, are complex. New *MC1R* variants have now been strongly associated with melanoma, irrespective of skin type [63,64]. Furthermore, studies from the Leiden group, have shown that the penetrance of the *p16* deletion observed in almost all cases of familial melanoma in that area can be increased by the presence of *MC1R* polymorphisms [65]. This remained after adjusting for skin type showing that the role of the *MC1R* genotype in increasing melanoma risk in these high-risk families is not only linked to the expression of a fair and sun-sensitive skin type. In Australia, a similar increase in *p16* penetrance has been observed in melanoma families which have one of three *MC1R* polymorphims associated with red hair, fair skin and a poor ability to tan [66].

5. Conclusions

Melanoma is an important public health issue and research strategies are attempting to elucidate the relative contribution of genes and environmental factors in its causation. Major progresses have been made, but new discoveries lead to many more questions than answers. The role of UVR in melanoma has long been known, but this association is very complex to dissect. Host responses to UVR, as well as important genes involved in the cell cycle and senescence, such as *p16* or *CDKN2A*, are leading the way in genetic epidemiology research of melanoma. Studies that are currently taking place include a genome-wide search using family, case-control and twin data, in the hope of discovering new melanoma genes or naevogenic genes. DNA microarrays using melanoma tumours are also likely to be helpful in understanding which genes are involved in tumour progression. Recent functional studies of *p16* or *CDKN2A* are also important to explore how this gene affects melanocyte behaviour. Melanoma is not a common tumour, and families are even rarer, so future progress will need worldwide collaborations. The data created by the new hardware and software in biotechnology will also require the combined knowledge of clinicians, cell biologists, epidemiologists, geneticists and biostatisticians. When new melanoma/naevus genes are discovered, epistatic (gene-gene) and gene-environmental interactions will be investigated and the impact of sun exposure on the expression of naevi and the progression to melanoma will then be analysed more closely. Public health campaigns and screening would then be targeted towards subjects who are genetically most at risk.

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