Genetic epidemiology of malignant melanoma susceptibility

Dimitrios Papakostas^{*1}, Irene Stefanaki¹ & Alexander Stratigos¹

Practice points

- Fair skin and hair type, dysplastic nevi, multiple common melanocytic nevi, excessive intermittent sun exposure predispose to melanoma.
- *CDKN2A* mutations already associated with familial melanoma susceptibility.
- GWAS and DNA sequencing techniques elucidate the genetic basis of melanoma susceptibility.
- Available data accessible via online databases like MelGene.
- Molecular pathways involved in melanoma susceptibility.
- MAPK/ERK kinase transduction pathway involved in pathogenesis of melanoma.
- *BRAF* overexpressed in melanocytic nevi contributing to their expansion.
- Melaninogenesis (*MC1R*, *MITF*, *TYR*, *OCA2*) and nevus count (*CDKN2A*, *MTAP*) pathways involved in melanoma susceptibility in conformity with epidemiological observations.
- Emerging role of DNA repair pathways (*PARP1*, *APEX1*, *XP*, *ATM*, *TERT*, *p53*).
- High-penetrance mutations are associated with familial melanoma.
- Germline *CDKN2A* mutations present in 30–40% of familial melanoma cases
- *CDKN2A* mutations are associated with lifetime melanoma risk up to 90%. Sporadic familial melanoma cases are attributed to *CDKN2A* downstream mutations affecting *CDK4* and *Rb1*. The role in familial melanoma susceptibility of additional pathways involving the *MITF*, *TERT*, *POT1* and *BAP1* genes has been currently set on research focus.
- *MC1R* is a moderate risk gene for melanoma susceptibility
- Common low-penetrance polymorphisms of genes involved in the melaninogenesis, melanocytic proliferation, cell cycle regulation and DNA repair pathways are associated with melanoma susceptibility.
- Twenty single nucleotide polymorphisms in ten genetic loci reached genome-wide significance in a recent meta-analysis.

SUMMARY Germline *CDKN2A* mutations were the first to be associated with familial melanoma. *MC1R* polymorphisms are associated, in conformity with epidemiological observations, with fair skin phenotype and a moderately increased risk for melanoma. The wider implementation of genome-wide association studies along with improved whole exome sequencing techniques made possible the identification of novel high-penetrant mutations (*TERT*, *MITF*, *POT1*, *BAP1*) beyond the established pathways of pigmentation and nevus count suggesting an additional role for pathways involved in cell cycle control and DNA repair. A multitude of common polymorphisms in the general population have been associated through candidate gene studies with a low risk for melanoma, supporting the hypothesis of a complex disease.

¹Department of Dermatology, Dermatoooncology Unit, A. Syggros Hospital, University of Athens, Greece

^{*}Author for correspondence: Tel.: +30 21 07231731; Fax: +30 21 07231731; dimitrios.papakostas@gmail.com

KEYWORDS

- *CDKN2A* • GWAS • MC1R
- melanoma • MITF
- polymorphism • POT1
- susceptibility

Cutaneous melanoma accounts for 5% of all skin malignancies, but is responsible for more than 80% of all skin cancer associated deaths. There has been a steady increase in the incidence of the disease among fair skinned individuals during the past decades, with the highest incidence rates being reported in Australia and New Zealand [1]. Phenotypical characteristics such as fair skin type, dysplastic nevi and multiple common nevi along with environmental factors such as excessive intermittent sun exposure mainly in childhood have been already identified as risk factors for melanoma pathogenesis. Interestingly, the clustering of melanoma cases within families accounting for 5–10% of all melanoma cases enabled a first insight into the genetic basis of melanoma susceptibility. *CDKN2A* mutations, very rare in the general population, were the first to be associated with increased melanoma risk in the 1990s [2,3]. In the past decade, the emergence of new diagnostic modalities in human genetics such as genome-wide association studies (GWAS) and improved DNA sequencing techniques helped to elucidate the genetic basis of melanoma susceptibility with the identification of a multitude of new genetic loci associated with melanoma [4]. In contrast to the rare high-penetrant mutations with familial aggregation, these polymorphisms are rather common in the general population conferring a relatively low risk for melanoma. Not surprisingly, they are associated with known melanoma endophenotypes, phenotypical traits associated with melanoma susceptibility, such as pigmentation or nevi count. In the past years the role of novel low-penetrance mutations in pathways of cell cycle control and DNA repair has been set on research focus, promising to utterly revolutionize our understanding of melanoma susceptibility and how low to moderate risk genes interact with environmental factors predisposing patients to melanoma [5,6]. This will be of utmost importance not only in order to identify, properly educate and clinically follow persons at risk but also for the development of novel targeted treatments.

The initial melanoma susceptibility research was based on linkage analysis and candidate gene association studies. In the past 6 years, the broader implementation of GWAS resulted in a load of new data and information on new susceptibility genes. GWAS are based on the examination of a great number of genetic variations known as single nucleotide polymorphisms

(SNPs) in order to detect associations between common SNPs and certain diseases. Systematic meta-analyses have been performed to help interpret the increasing available data that are currently online accessible via web-based databases like MelGene [4,7,8]. Novel whole exome sequencing techniques are especially effective in the identification of rare genetic variants in the entire genome of an individual and will be increasingly used in the years to come to identify new target genes comparing the entire genome of patients with similar clinical features [9].

In the past years melanoma research has focused on the elucidation of molecular pathways involved in melanoma pathogenesis and susceptibility. Novel treatments with elaborate monoclonal antibodies targeting key molecular mutations of the MAPK/ERK kinase transduction pathway such as BRAF and MEK dramatically improved the therapeutic approach of melanoma, the first major breakthrough in more than 30 years of therapeutic stagnation. *BRAF* is the most commonly mutated oncogene in melanoma. 40–60% of melanomas harbor indeed an activating *BRAF* mutation. The V600E mutation is a gain of function mutation that accounts for more than 90% of the *BRAF* mutations. Activation of the MAPK/ERK signaling cascade results in increased proliferation and survival of melanoma cells [10].

To broaden our understanding of melanoma pathogenesis and improve our prevention strategies decisive is the identification of genes involved in melanoma susceptibility. As expected from current epidemiological data, molecular pathways playing a role in melaninogenesis and overall nevus count are the most appropriate candidates for genetic linkage and candidate genes association studies (Table 1) [3].

Variants of MC1R favoring the production of pheomelanin instead of eumelanin are associated with fair hair and light skin phenotype and a moderately increased risk for melanoma [11]. Binding of the melanocyte-stimulating hormone to MC1R activates a G protein signaling pathway inducing the expression of MITF, a regulator of the expression of multiple genes associated with melaninogenesis, such as *TYR*, *TYRP-1*, *OCA2* and *SLC45A2* [12].

Nevus count is the second important and well-documented melanoma endophenotype. The oncogene BRAF is overexpressed in melanocytic nevi contributing to their expansion and increased formation. The role of genes

involved in cell growth and apoptosis regulation of melanocytes such as *CDKN2A* and *MTAP* has been highlighted by recent GWAS results. Currently there is increased evidence that a multitude of low-risk genes may contribute to nevi formation and thus be associated with melanoma risk [13,14].

Beyond these two well-documented endophenotypes associated with melanoma risk the overwhelming new evidence from recent and ongoing GWAS pinpoints the role of molecular pathways involved in DNA repair. Impaired DNA repair in the setting of increased ultraviolet radiation (UVR) exposure should be logically associated with increased melanoma risk. UVR can damage the DNA directly or indirectly through reactive oxygen species production. *PARP1*, *APEX1* and the xeroderma pigmentosum compound genes are integral parts of the base excision repair system, whereas the nucleotide repair system constitutes of DNA damage detection by the ATM enzyme and subsequent p53 activation. TERT protects the telomeres from UVR-induced erosion. A disequilibrium in these interconnected pathways as a result of common gene polymorphisms may be associated with increased melanoma susceptibility as proposed by the new hits in recent GWAS [15].

The understanding of the composite interactions between these molecular pathways and their role in melanoma susceptibility promise to change the genetic map of melanoma. The multitude of new GWAS hits can be stratified according to their association with melanoma to high and low to moderate risk genes. This could prove of great assistance for genetic counseling and risk prediction. Eventually, it could help revolutionize our preventive and therapeutic approach to this complex disease.

High-penetrance genes associated with familial melanoma

Germline *CDKN2A* mutations in melanoma patients with positive familial history were the first to be associated with melanoma susceptibility in the 1990s. The mutation is present in 30–40% of familial melanoma cases. Only 1–2% of sporadic melanoma cases and 3% of patients with multiple primary melanomas are *CDKN2A* positive [16]. The penetrance of *CDKN2A* mutation depends on geographic location, which may reflect the geographical variations of UVR and the interaction with other composite genetic factors such as *MC1R* polymorphisms [17]. *CDKN2A* mutations are associated with a lifetime risk in familial melanoma kindreds up to 90% in Australia by the age of 80 years compared with a more moderate 28% for non-familial carriers of the mutation [3,4]. *CDKN2A* gene encodes two alternate proteins, p14ARF and p16INK4a. p16 functions as a CDK4 inhibitor and p14 regulates cell cycle through interaction with p53. CDK4 is a downstream regulator in the MAPK/ERK kinase transduction pathway, acting as phosphorylator of the Rb1. Though 20–40% of familial melanomas are caused by *CDKN2A* mutations, sporadic familial cases are attributed to downstream mutations affecting *CDK4* and *Rb1*. *Rb1* positive patients that survive retinoblastoma have an 80% risk for melanoma [18].

Beyond the CDK4/CCND1 pathway ongoing research suggests a role for additional pathways involving the *MITE*, *TERT*, *POT1* and *BAP1* genes in familial melanoma susceptibility. MITE, as previously described, is a downstream regulator in the MC1R pathway. MC1R activation via melanocyte stimulating hormone after cutaneous UVR-induced DNA damage induces *MITE* expression which in turn increases

Table 1. Molecular pathways and related susceptibility genes.

Molecular pathways	High risk	Moderate risk	Low risk
Melaninogenesis	<i>MITE</i>	<i>MC1R</i>	<i>SLC45A2</i> <i>TYR</i> <i>ASIP</i> <i>CDK10</i>
Nevus count	<i>CDKN2A</i> <i>CDK4</i> <i>Rb1</i>		<i>MTAP</i> <i>CASP8</i>
DNA repair	<i>TERT</i> <i>POT1</i> <i>BAP1</i>		<i>ATM</i> <i>FTO</i> <i>AFG3L1</i>

expression of the two alternate *CDKN2A* products, thus blocking cell cycle progression till DNA damage is repaired [6]. Germline *MITF* mutation E318K is associated with both familial and sporadic melanoma, multiple primary melanomas and renal carcinoma [19]. Functional analysis of the polymorphism revealed a sumoylation defect impairing MITF-regulated target transcription. In sporadic melanoma cases a 2.4 odds ratio suggests E318K as a moderate risk polymorphism [20].

TERT protects the telomeres from erosion and *POT1* is a member of the shelterin complex which regulates telomere length and protects chromosome ends. Rare germline *TERT* promoter mutations have been recently identified in both familial and sporadic melanomas [21], whereas UVR-induced somatic mutations appear to be very frequent in both primary and metastatic melanomas and associated with poor prognosis. Rare loss-of-function polymorphisms of *POT1* predispose to melanoma via a direct effect on telomeres suggesting *POT1* as a high-risk gene for familial melanoma [22,23].

BAP1 is a tumor suppressor gene that plays a role in cell cycle regulation, cellular differentiation, apoptosis and response to DNA damage. Recent research evidence involves germline *BAP1* mutations in the pathogenesis of the COMMON syndrome predisposing to multiple atypical melanocytic tumors, uveal and cutaneous familial melanoma, mesothelioma and other malignancies [24]. Additionally, germline *BAP1* mutations in patients with uveal melanoma are associated with a more aggressive phenotype and increased risk for metastasis [25].

Low-to-moderate-risk genes

The *MC1R* gene is the best documented moderate-risk gene for melanoma susceptibility. Polymorphisms favoring the production of pheomelanin instead of eumelanin are associated as expected with fair skin type and hair as well as with a 40% increased risk for melanoma, in conformity with the epidemiologically well-established role of fair skin phenotype in melanoma susceptibility [6,11]. However, the *MC1R* gene is a highly polymorphic gene, a multitude of polymorphisms have been associated with skin phenotype and/or moderately increased melanoma risk, suggesting alternate pathways different than the melaninogenesis pathway with a possible contribution to melanoma risk.

The identification of a multitude of new genetic loci after the first GWAS wave and the evaluation of their role in melanoma susceptibility suggest a multifactorial background with a multitude of low-risk genes interacting with environmental factors to influence melanoma susceptibility. GWAS in recent years have been a major breakthrough in melanoma research, since they made possible the identification of novel genetic loci and involved pathways beyond the well-established candidates. In total, 20 SNPs in ten genetic loci reached genome-wide significance in a recent meta-analysis by Antonopoulou *et al.* These are common low penetrance polymorphisms of genes involved in the melaninogenesis (*SLC45A2*, *TYR*, *ASIP*, *CDK10*), melanocytic proliferation (*MTAP*, *CASP8*, *TERT*, *MITF*), cell cycle regulation and DNA repair pathways (*ATM*, *FTO*, *AFG3L1*). Their association with melanoma susceptibility confirms the polygenetic nature of melanoma and provides some helpful insight into the genetics of this complex tumor [4,7].

Conclusion

Recent advances in genetic testing including GWAS and whole exam sequencing techniques provide us with a broader insight into the pathogenesis of cutaneous melanoma, a genetically heterogeneous disease. High-penetrance genes like *CDKN2A* play a decisive role in familial melanoma, which accounts for 10% of all melanoma cases. Recent evidence from GWAS pinpoints the role of very rare novel high-penetrance mutations with familial aggregation such as *POT1*, *TERT*, *MITF* and *BAP1*. In contrast to these high-penetrance mutations associated with an increased risk in the familial setting a multitude of low-penetrance polymorphisms rather common in general population seem to be associated with a low risk for melanoma. This new evidence on the genetic basis of sporadic melanoma supports the idea of a complex interaction between a multitude of low to moderate risk genes and environmental factors.

Future perspective

In the coming years the elucidation of new pathways and candidate genes beyond the established and well-documented pathways of pigmentation and nevus count will improve our understanding of melanoma pathogenesis and susceptibility. Final goal remains the identification of new targets for the development of innovative high-impact treatments which may further improve

the treatment of systemic disease or help us even prevent melanoma in familiarly predisposed patients.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a

financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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