Molecular Epidemiology of Melanoma

Anne E. Cust, Hensin Tsao, Marianne Berwick, Graham J. Mann, and Mark M. Iles

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A. E. Cust (✉)
Cancer Epidemiology and Prevention Research, Sydney School of Public Health, The University of Sydney, Sydney, NSW, Australia

Melanoma Institute Australia, The University of Sydney, Sydney, NSW, Australia
e-mail: anne.cust@sydney.edu.au

H. Tsao
Skin Cancer Genetics Laboratory/Wellman Center for Photomedicine, Melanoma and Pigmented Lesion Center/Department of Dermatology/Melanoma Genetics Program, Massachusetts General Hospital, Cancer Center, Harvard Medical School, Boston, MA, USA
e-mail: htsao@mgh.harvard.edu

M. Berwick
Department of Internal Medicine, University of New Mexico Cancer Center, University of New Mexico, Albuquerque, NM, USA
e-mail: mberwick@salud.unm.edu

G. J. Mann
Melanoma Institute Australia, The University of Sydney, Sydney, NSW, Australia

Centre for Cancer Research, Westmead Institute for Medical Research, The University of Sydney, Sydney, NSW, Australia
e-mail: graham.mann@sydney.edu.au

M. M. Iles
Leeds Institute for Data Analytics, University of Leeds, Leeds, UK
e-mail: M.M.Iles@leeds.ac.uk

C. M. Balch et al. (eds.), *Cutaneous Melanoma*,
https://doi.org/10.1007/978-3-030-05070-2_48

Abstract

There have been major advances in our understanding of the molecular epidemiology of melanoma over the past decade. Comprehensive cataloguing of a landscape of driver mutations has enabled identification of the key pathways that underscore the biological processes and therapeutic opportunities in melanoma. This new knowledge has been complemented by an improved understanding of the genetic susceptibility of melanoma, in pigmentation, nevus, telomere, and other biological pathways, identified mainly by genome-wide association studies. This chapter describes the genetic basis for melanoma, including cutaneous melanoma of non-desmoplastic and desmoplastic types, acral, mucosal, and uveal melanomas. It describes analyses of gene-environment, gene-gene (epistasis), and gene-phenotype interactions that have led to an improved understanding of the biological processes involved in melanoma development and more accurate prediction of an individual's melanoma risk. The research presented highlights the exciting developments that have come from combining different types of data, including somatic, germline, clinical, pathologic, phenotypic, and environmental risk factors.

Molecular Characterization of Melanoma

Overview of Genomic Features

Cancer classification, such as that for melanoma, can be accomplished at clinical, genomic, and molecular levels. While a "grand unified" theory of melanoma remains elusive, large-scale sequencing efforts, such as whole exome sequencing (WES) data from The Cancer Genome Atlas Skin Cutaneous Melanoma project (TCGA-SKCM) and whole genome sequencing (WGS) data from the Australian Melanoma Genome Project, have provided a comprehensive view of melanoma. Study of somatic DNA variation offers insights into overall mutational burden, underlying environmental etiologies (such as UV signature changes) and critical dependencies that mediate the proliferation, survival, and metastasis of melanomas. For this section, we will focus largely on key genetic elements that feed into crucial pathophysiologic pathways. We will take a top-down approach to examine some recent insights into the genomic classification of melanoma.

Skin cancers, including cutaneous melanoma, Merkel cell carcinoma, cutaneous squamous cell carcinoma, and basal cell carcinoma, harbor the highest density of mutations among all known cancers (Fig. 1a). The vast majority of these mutations are UV signature changes (i.e., C➔T at dipyrimidine sites, shown as pink bars in Fig. 1a).

The most comprehensive data on melanomas are from the TCGA-SKCM (Cancer Genome Atlas 2015) and Australian Melanoma Genome Project (Hayward et al. 2017). As shown in Fig. 1b, the overall substitution/indel rate varies by subtype of melanoma: acral and mucosal melanomas average 2.64 mutations/Mb, while cutaneous melanomas average 49.17 mutations/Mb ($p < 1.0E-7$). However, acral and mucosal melanomas appear to be more vulnerable to larger

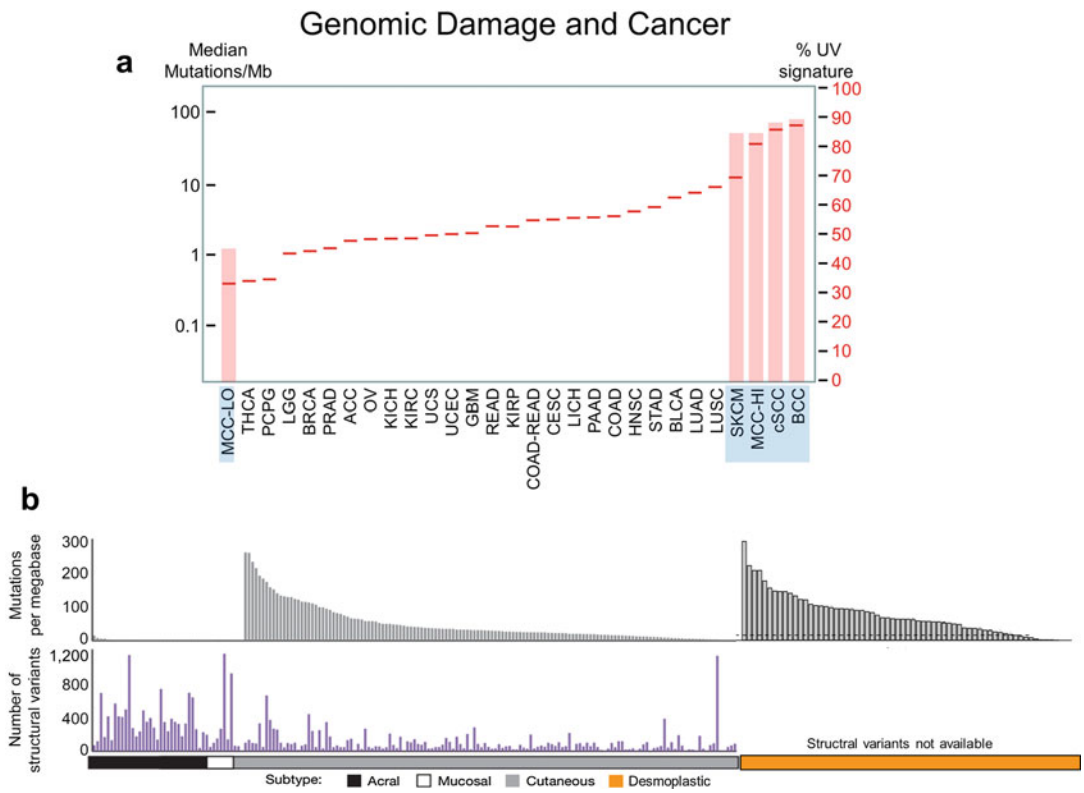

Fig. 1 Overview of genomic damage and cancer. (a) The mutation burden (red line; median no of mutations/Mb) ranked from lowest to highest among the TCGA tumors. The percentage of missense mutations which reflect UV signature changes (pink bars). Skin tumors are highlighted in blue and harbor the greatest number of mutations, especially UV signature changes, among all cancers. The lowest mutation burden is Merkel cell carcinoma due to the MCC virus (MCC-Lo). Abbreviations for all TCGA cancers are listed on the website (https://gdc.cancer.gov/resources-tcga-users/tcga-code-tables/tcga-study-abbreviations). Additional abbreviations include SKCM (skin cutaneous melanoma), cSCC (cutaneous squamous cell carcinomas), BCC (basal cell carcinoma), MCC-Hi (Merkel cell carcinoma-high mutation burden), and MCC-Lo (Merkel cell carcinoma-low mutation burden). (b). Mutation burden, by melanoma subtype, in a collection of whole genome-sequenced tumors from the Australian Melanoma Genome Project. (Modified from Fig. 1a in Hayward et al. (2017)); density of desmoplastic melanoma is modified from Fig. 1 from Shain et al. (2015)

structural variation compared to cutaneous melanoma (mean 342.4 vs. 101, $P < 1.0E\text{-}6$). In addition, Shain et al. performed whole exome sequencing on desmoplastic melanoma – a rarer subtype of melanoma – and found a relatively high density of mutations, especially UV signature ones (Fig. 1b; mean 62 mutations/Mb) (Shain et al. 2015). A general synthesis of mucocutaneous melanomas indicates two classes of genomic injury. Point and indel mutagenesis, especially those generated by UV radiation, appears to promote sun-exposed melanoma subtypes (cutaneous, desmoplastic), while structural variations are more commonly associated with sun-hidden subtypes (acral, mucosal). In nearly all cutaneous melanomas, even those occurring in young adults (Wilmott et al. 2018), UV-associated signatures are dominant; sub-variations (signatures 7a–c) correspond to the various mechanisms of DNA damage and occur in different proportions in different individuals (Hayward et al. 2017). In contrast, the mutation signatures responsible for the bulk of the mutation load in acral and mucosal melanomas cannot be attributed to known carcinogens. Six signatures not previously reported in melanoma were seen in these melanomas: signatures 2 and 13, resulting from endogenous deamination and DNA editing; signature 8 and 18 also

seen in breast cancer, medulloblastoma, and neuroblastoma; and signatures 1 and 5 which are seen ubiquitously, all having unknown mechanisms. Further clinical and epidemiologic studies are needed to understand the causes of acral and mucosal melanoma.

It is notable to mention that high-output variant discovery creates a signal-to-noise problem. Among all mutations detected, it is often difficult, if not impossible, to ascertain only the functionally important lesions. Stratton introduced the idea of "driver" and "passenger" mutations to better associate individual mutations with functional correlates (Stratton et al. 2009). *Driver* mutations are those with evidence of selection during tumor formation (e.g., through the loss-of-function of a tumor suppressor or the gain-of-function of an oncogene), whereas passenger mutations lack evidence for such selection (Stratton et al. 2009). The impact of a passenger mutation on biology of a given tumor would be incidental, for example, by contributing to the overall load of tumor neoantigens. Comprehensive cataloguing of a landscape of driver mutations has enabled identification of the key pathways that underscore the biological processes and therapeutic opportunities in melanoma. Cutaneous melanoma is dominated by largely complementary mutations in the MAP kinase pathway, which define four genetic subtypes.

Cutaneous Melanoma (Non-desmoplastic and Non-acral Types)

RAS pathway. Both the TCGA (Cancer Genome Atlas 2015) and Australian Melanoma Genome Project (Hayward et al. 2017) identified four distinct classes of cutaneous melanoma based on the predominant mutated driver gene: *BRAF*, *RAS*, *NF1*, and *triple wild-type* subtypes (Fig. 2). While the prevalence rates for each subclass are different, the *BRAF* subtype is the most common and is present in approximately 40–50% of tumors. Members of this class harbor *BRAF* (v-raf murine sarcoma viral oncogene homolog B1) oncogenic mutations primarily in the V600 and K601 amino acid residues. About 30% of cutaneous melanomas exhibit mutations in *N- K-* and *H-RAS*. There is strict exclusivity between *BRAF*(V600E) and *RAS* (G12/Q61) oncogenic lesions suggesting that co-activation of the MAPK pathway is either redundant or possibly toxic. In addition to a reciprocal relationship observed at the cellular level, *BRAF*- and *NRAS*-mutated melanomas are clinically distinct. Compared to *BRAF*-mutated tumors, *NRAS*-mutant tumors more commonly occur in older patients (Heppt et al. 2017; Sakaizawa et al. 2015; Thomas et al. 2015), in congenital nevi (Bauer et al. 2007) than acquired benign nevi (Johnson and Puzanov 2015) and in thicker primary tumors (Ellerhorst et al. 2011; Johnson and Puzanov 2015). *NRAS* mutations occur on sun- and non-

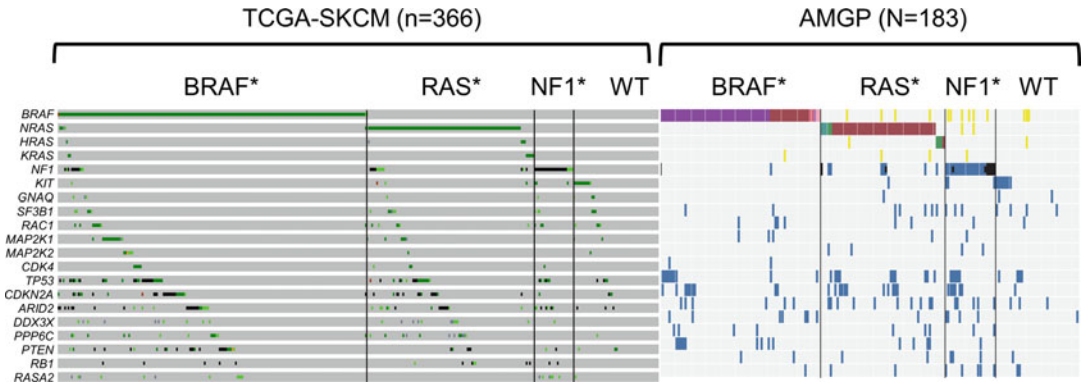

Fig. 2 Comparison of "Rasopathic" Groups between The Cancer Genome Atlas (TCGA) (Cancer Genome Atlas 2015) and the Australian Melanoma Genome Project (AMGP) (Hayward et al. 2017)

sun-exposed skin, mucosal, and acral sites, whereas *BRAF* mutations predominate more on intermittently sun-exposed sites and in acquired benign nevi (Johnson and Puzanov 2015).

NF1 mutations occur in about 12–14% of cutaneous melanomas (Cancer Genome Atlas 2015; Hayward et al. 2017). The gene product, neurofibromin, is a GTPase-activating protein (GAP) and serves to downregulate RAS signaling by accelerating the hydrolysis of RAS-GTP to RAS-GDP (Kiuru and Busam 2017). Compared to *BRAF*- and *NRAS*-mutant tumors, *NF1*-mutants appear more clinically aggressive. They are identified in older patients and are more prevalent in males. Tumors with *NF1* mutations are associated with poorer disease-free and overall survival (Cirenajwis et al. 2017). *NF1* mutation status is also an important predictive factor for therapy response, with *NF1* mutants showing decreased sensitivity to *BRAF* inhibitors in vitro and in vivo (Maertens et al. 2013; Whittaker et al. 2013). *NF1* mutations inversely correlate with *BRAF* hotspot mutations, but not with hotspot mutations in *NRAS* (Cancer Genome Atlas 2015).

Triple wild-type (i.e., no mutations in *BRAF*, *RAS,* or *NF1*) tumors are uncommon (Fig. 2) and are composed of a smaller (~5%) subset of tumors with *KIT* alterations among other genes. Clinically, this rare subtype does not significantly differ from the common *BRAF*- and *NRAS*-mutant subtypes but shows a slight male predisposition and an average age at diagnosis of approximately 60–70 years (Cirenajwis et al. 2017).

Apart from the MAPK pathway, the PI3K pathway is also stimulated with *RAS* oncogenesis. A recurrent *PIK3CA* mutation (E545K) occurs in less than 5% of melanomas (Cancer Genome Atlas 2015). Activation of the PI3K pathway can also be achieved through inactivation of *PTEN*, which occurs in about 10% of cases. Co-occurrence of *BRAF*(V600E) and *PTEN* loss has long been demonstrated (Tsao et al. 2004).

Rb/p53 pathway. Within the Rb pathway, the most commonly targeted locus is *CDKN2A*. Silencing of this locus by deletion, mutation, and methylation occurs in about 60% of cases. Recurrent activating mutations in the p16 cognate partner, *CDK4*, occur in <5% of cases. With loss of p14ARF, which results from inactivation of *CDKN2A*, *MDM2* is unconstrained and thus accelerates the proteosomal destruction of p53 (Pomerantz et al. 1998); *TP53* itself is mutated in about 15% of tumor specimens.

Alterations in epigenetic control. Mutations and deletions of *ARID* genes (*ARID2*, 15–20%; *ARID1A/1B*, 20–30%) are among a new wave of mutated epigenetic regulators which have been uncovered in cutaneous melanoma. These proteins function as part of the SWI/SNF chromatin remodeling complex and serves to globally regulate gene expression. A spectrum of mutations in other epigenetic regulators has also been catalogued though the precise functional consequences remain to be fully elucidated. These include *IDH1*, *EZH2*, *SETD2*, and *HDAC9*.

Telomerase promoter mutations. Perhaps one of the most mutationally targeted regions in the melanoma genome is the *TERT* promoter. Around 70% of melanoma specimens harbor point mutations within this region, often of putative UV derivation (Horn et al. 2013; Huang et al. 2013). Mechanistically, *TERT* promoter mutations (positions −134, −138, −146) create novel Ets/TCF binding sites and are associated with shorter telomeres, fewer structural rearrangements but more mutations per Mb (Hayward et al. 2017). Since the promoter mutations are often UV-induced, these variants may be a marker of UV damage which could explain the correlation with fewer structural changes and a higher mutation density.

Cutaneous Melanoma (Desmoplastic Type)

Whole exome sequencing of 20 desmoplastic melanomas reveals a dramatically different landscape compared to the more common types of cutaneous melanoma (Fig. 3). Clinically, desmoplastic melanomas occur more frequently on the sun-exposed sites of elderly patients (Tsao et al. 1997). Unlike other types of melanoma, the most prevalent alterations are found in the *CDKN2A* (47%), *TP53* (48%), and *NF1* (55%) tumor suppressor genes (Shain et al. 2015). *TERT* promoter mutations occur in 90% of the

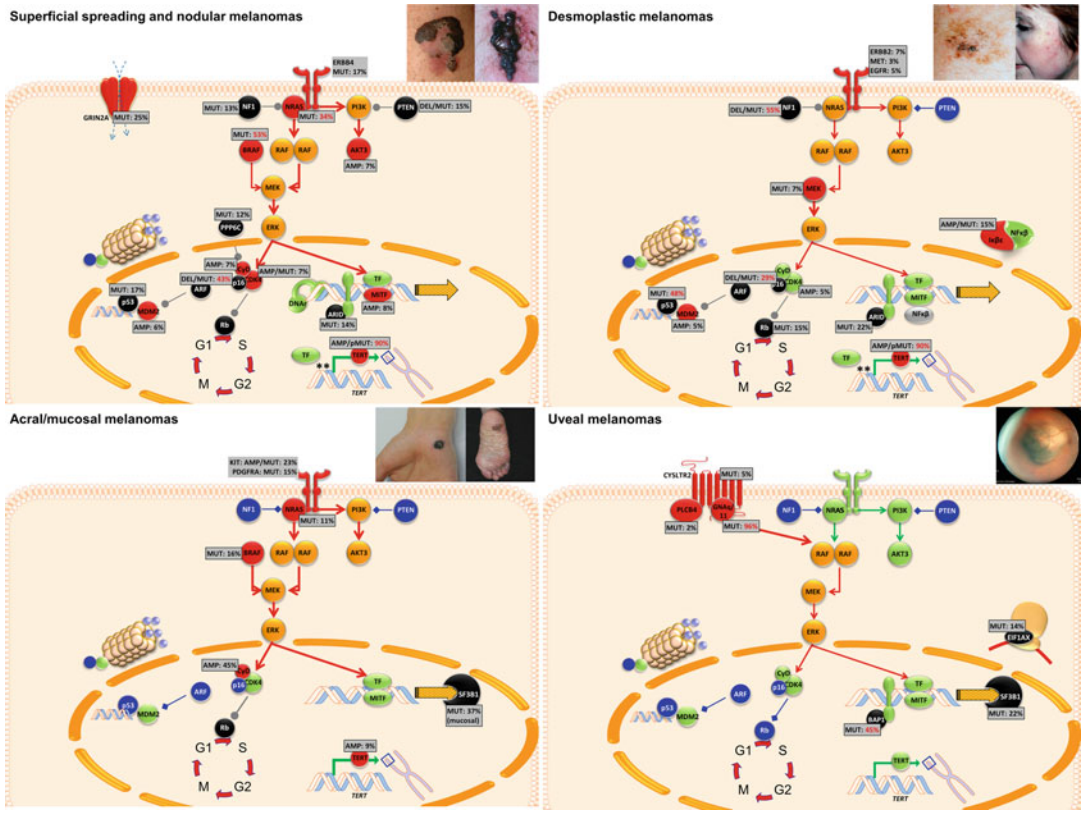

Fig. 3 Integrated map of mutations in melanoma. Various subtypes of melanoma showing the clinical picture and physiological sites of mutations. Figure legend: red circles indicate oncogenic activation; black circles indicate inactivation of tumor suppressors; blue and orange circles indicate normal physiological interaction; blue diamond arrowheads indicate inhibition; red arrowheads indicate stimulation. Mutation rates are shown in gray boxes. MUT, mutation; AMP, amplification; DEL deletion; red numbers indicate a high prevalence mutation rate

samples but may once again reflect the enrichment for UV exposure (88% of changes are UV signature mutations). Unlike traditional cutaneous melanoma, no single oncogene predominate, though errant activation of various receptor tyrosine kinases (RTKs) such as *ERBB2*, *EGFR,* and *MET*, and rare activating lesions in *NRAS*, *PIK3CA*, *MAP 3 K1*, *MAP 2 K1,* and *RAC1* have also been observed. The other distinguishing feature is the inactivation of NF-κB nuclear translocation and signaling via gain-of-function mutagenesis of *NFKBIE* (15%). In synthesis, desmoplastic melanoma appears to be a "suppressor-deficient" tumor at the genomic level due to random inactivating events which result from UV radiation and which impinge upon the usual pathways.

Acral and Mucosal Melanomas

As alluded to above, acral and mucosal melanomas share certain similarities including a dearth of point mutations, a wealth of structural variants and a higher frequency of *KIT* involvement (Fig. 3) (Hayward et al. 2017). Amplified drivers include proto-oncogenes such as *PDGFRA*, *KIT*, *CCND1*, and *MDM2*. In a recent targeted sequencing analysis of 2793 acral melanomas specifically, the prevalence of mutations in *BRAF*, *NRAS*, *KIT*, *pTERT(C228T)*, *pTERT (C250T)*, and *PDGFRA*, was 23.7% (641/2706), 10.4% (242/2325), 8.0% (223/2793), 5.9% (32/545), 5.5% (30/545), and 1.4% (40/2325), respectively (Bai et al. 2017). The dominant variants in *BRAF*, *KIT*, and *NRAS* were p.V600 (96%),

p.L576 (22%), and p.Q61 (70%). Interestingly, there were 13 AM cases which exhibited concurrent *BRAF*(V600E) and *NRAS*(Q61R/K or G12D) alterations suggesting that these lesions may not be functionally epistatic in acral melanoma. Moreover, with nearly a quarter of acral melanomas harboring *BRAF*(V600) alterations, it is important to note that acral tumors may in fact be susceptible to current anti-MAPK therapies.

In a single institution case series of 19 mucosal melanomas (5 anorectal, 9 vulvovaginal, and 5 nasopharyngeal), whole exome sequencing found that *KM2TC* was the most frequently mutated gene (52%), followed by *KIT*, *DIAPH1*, and *LAMA3* (50%) and *NF1* (37%); interestingly, 6 of the 7 *NF1* mutant cases had concurrent *KIT* alterations (Hintzsche et al. 2017). Unlike cutaneous melanoma, recurrent *SF3B1* R625H/S/C mutations were identified in 7 of 19 (37%) mucosal melanomas, which was a similar finding observed in an Australian cohort (Hayward et al. 2017).

Uveal Melanoma

Uveal melanomas arise from the uveal tract and are distinguished clinically, histologically, and genetically from other ocular melanomas such as conjunctival melanoma. As shown in Fig. 3, there are largely two clusters of mutations – those that actively engage G-protein signaling and those that involve the "BSE" mutations (*BAP1*, *SF3B1*, and *EIF1AX*).

Gq signaling pathway. Mutations in *GNA11* and *GNAQ* were among the first oncogenic mutations to be described in uveal melanomas and are the most common oncogenic drivers (Onken et al. 2008; Van Raamsdonk et al. 2009, 2010). A recent meta-analysis of whole exome sequencing data from 139 uveal melanomas showed that Gq pathway genes were mutated in 99% of the samples (47% *GNAQ*, 46% *GNA11*, 5% *CYSLTR2*, and 2% *PLCB4*). *CYSLTR2* is the cysteinyl leukotriene receptor 2 and is a G-protein coupled receptor (Park et al. 2018). *PLCB4* encodes phospholipase C beta 4, is downstream of Gq signaling, and is the enzyme that catalyzes the formation of inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate (Park et al. 2018). Like the MAPK pathway, the Gq signaling cassette appears to be mutually, and universally, activated through these four genes.

"***BSE*" *genes***. A second cluster of mutations occur in three distinct genes – *BAP1*, *SF3B1*, and *EIF1AX* ("BSE"). While these are somewhat exclusive, the relationship is not as tightly epistatic as *GNAQ* and *GNA11*. *BAP1* (BRCA1-associated protein 1), a deubiquinating hydrolase, is inactivated genetically in about half of all uveal melanomas, especially those with metastatic potential. The protein encoded by *BAP1* normally regulates proteins via removal of ubiquitin molecules. One of the key downstream targets of *BAP1*-mediated de-ubiquitination is histone H2A. Thus, *BAP1* function is critical for the proper regulation of gene expression in multiple genomic regions (Scheuermann et al. 2010). While the function of *BAP1* is not fully understood, loss of *BAP1* may revert uveal melanoma cells to a more "stem cell-like" and phenotypically aggressive state (Landreville et al. 2012). *BAP1* resides on chromosome 3p21.1. Clinically, monosomy 3 correlates with a poorer prognosis, although the specific target(s) on chromosome 3 that drives this aggression has not been fully elucidated (Robertson et al. 2017).

EIF1AX and *SF3B1* mutations are less common than *BAP1* and *GNAQ*/*GNA11*, each found in at most one-fifth of samples. The proteins encoded by these genes regulate nuclear processes of translation initiation and pre-mRNA splicing, respectively. Mutations in *EIF1AX* and *SF3B1* are nearly mutually exclusive with each other and with *BAP1*. *EIF1AX* mutations are associated with favorable prognosis. *SF3B1* mutations are found in younger patients and are associated with development of late metastasis.

Genomic Factors and Melanoma Risk

Genetic Susceptibility to Melanoma

Familial aggregation. A family history of melanoma is a significant risk factor for melanoma (Gandini et al. 2005a). Between 1 and 12% of

melanoma cases are estimated to occur in those with a first-degree relative with melanoma (Ford et al. 1995). The relative risk of melanoma in individuals with one or more affected first-degree relatives is estimated to be about twofold (Olsen et al. 2010c). However, familial relative risk appears to differ by region, with higher familial relative risks in regions with lower ambient UV exposure and lower melanoma incidence (e.g., North America) than in regions with higher UV exposure (e.g., Australia), due to the presence of phenocopies, in which an affected family member has melanoma but does not have the susceptible genotype, in higher incidence regions (Begg et al. 2004; Olsen et al. 2010c). Familial relative risk may also be higher for relatives of people who experienced melanoma at a young age, or with known phenotypic risk factors such as higher mole count or red hair, or that have multiple relatives with melanoma, or with relatives that have multiple melanomas (Begg et al. 2004; Hemminki et al. 2003).

Familial aggregation may be partly due to the strong effect of UV radiation from sunlight on melanoma risk, exposure to which is likely to be correlated within families, or due to rare, highly penetrant mutations. However, the strongest epidemiological risk factors for melanoma, nevus count, and pigmentation are also heritable through common polymorphic variation and are likely to explain much of the familial clustering. This has been borne out by genetic studies that have uncovered both rare, high-risk genetic mutations that explain much of the familial clustering of melanoma and more common, low-risk variants that explain not only some of the risk associated with a family history of melanoma but also go some way toward explaining the influence of the above-mentioned heritable risk phenotypes.

High-risk variants. Familial clustering of melanoma has long been observed (Cawley et al. 1952; Greene and Fraumeni 1979; Norris 1820), suggesting the existence of rare, highly penetrant mutations. Twin studies suggest that genetic variation explains about 58% of the variance in risk of melanoma (Mucci et al. 2016), but other studies have estimated that only 3% (Hemminki et al. 2003) to 7% (Olsen et al. 2010c) of melanoma cases are attributable to familial risk. Segregation analysis has also found that high-risk variants are likely to explain familial aggregation in only a relatively small proportion of such families, suggesting that risk in most melanoma families is likely due to either shared environment and behaviors or multiple common lower-risk variants (Aitken et al. 1998). Unsurprisingly, then, high-risk genes have not been identified in most of these high-risk families (Aoude et al. 2015a).

By far the most common high-risk melanoma gene is *CDKN2A*, a tumor suppressor gene involved in cell cycle regulation, whose effect was first detected through loss or translocation of the 9p region in melanoma tumors (Cowan et al. 1988). The same region was identified as a germline risk factor through linkage analysis (Cannon-Albright et al. 1992), and the *CDKN2A* gene was subsequently cloned and identified (Kamb et al. 1994). In populations studied in detail to date, *CDKN2A* mutations have been observed in about 2% of melanoma cases (about 1 in 1000 people in the general population are carriers) but are more common in those with two or more affected relatives (25%), three or more primary melanomas (29%), or more than one primary melanoma and other affected relatives (27%) (Harland et al. 2014). Population-based estimates indicate that around 30–50% of *CDKN2A* mutation carriers will develop melanoma by age 80 years (Begg et al. 2005; Cust et al. 2011) and that risk of melanoma does not depend on the ambient UV irradiance of the region in which they live. However, lifetime risk estimates derived from clinic-based sampling of families with multiple cases of melanoma indicate that risk is higher in countries with higher ambient UV irradiance, with a lifetime risk of 58% in Europe, 76% in the United States, and 91% in Australia, although confidence intervals were large (across all regions combined the risk by age 80 was 67%, with a 95% confidence interval of 31–96%) (Bishop et al. 2002). A common accompanying phenotype to familial melanoma is the presence of large numbers of nevi and/or several atypical nevi. Carriage of a mutation in *CDKN2A* is also associated with this phenotype, but the correlation between the phenotype and presence of a

CDKN2A mutation is too weak for the phenotype to be a useful indicator of mutation presence within families (Wachsmuth et al. 1998).

Subsequent to the discovery of *CDKN2A*, a candidate screening approach was taken to finding other familial melanoma genes by looking at related cell cycle regulators. This resulted in the discovery of *CDK4* on 12q14 (Zuo et al. 1996), which has a similar risk profile to *CDKN2A*, but is associated with a greater number of nevi (Goldstein et al. 2000). However, melanoma-predisposing mutations in *CDK4* are much rarer than in *CDKN2A*, with only seven melanoma families worldwide having been found to carry mutations in *CDK4* (de Snoo and Hayward 2005).

Since the discovery of *CDK4*, a number of other high penetrance melanoma genes have been identified. *BAP1*, a tumor suppressor gene, was principally identified as predisposing to familial clustering of uveal melanoma, but such families were also found to include multiple cases of cutaneous melanoma among other cancers (Abdel-Rahman et al. 2011; Wiesner et al. 2011, 2012). Only 15% of *BAP1* mutation carriers are reported to have developed melanoma so is considered a medium penetrance mutation, despite being identified through multicase family studies (Carbone et al. 2012). A mutation in the promoter region of *TERT* was identified as a high-risk melanoma variant in a single multicase melanoma family (Horn et al. 2013), though more common low-risk variants at the same locus were subsequently discovered through genome-wide association analyses.

The only other high-risk mutations identified have all been members of the shelterin complex. Shelterin regulates the effect of telomerase on telomeres, which in turn protect the ends of chromosomes from degradation. The first such gene to be discovered was through exome sequencing of melanoma pedigrees, resulting in the identification of loss-of-function variants in *POT1* causing increased telomere length through disruption of protein-telomere binding (Robles-Espinoza et al. 2014; Shi et al. 2014). Carriers of *POT1* mutations tend to have an early age of onset and present with multiple primary tumors. Such loss-of-function variants were identified in 4% of familial melanoma pedigrees with no mutations in either *CDKN2A* or *CDK4*. This was followed up by sequencing of the remaining five members of the shelterin complex (*ACD*, *TERF2IP*, *TERF1*, *TERF2*, and *TINF2*) revealing nonsense mutations and point mutations co-segregating with melanoma in *ACD* and *TERF2IP* in a total of ten families (Aoude et al. 2015b). The germline frequency of shelterin mutations is too low to be currently estimable.

Medium- to-low penetrance melanoma genes. Even beyond the strong family clusters of melanoma cases, indicative of rare highly penetrant mutations, risk of melanoma is highly heritable through shared pigmentary and nevus (mole) phenotypes within families. Nevus count and pigmentary phenotype factors are well-established, strong risk factors for melanoma (Gandini et al. 2005a, b; Olsen et al. 2010a, b), and they are themselves heritable factors (Martin et al. 2017; Wachsmuth et al. 2001; Zhu et al. 1999), indicating that the heritability of melanoma may be intertwined with these phenotypes.

The first clear demonstration of this co-dependency between common genetic risk factors for melanoma and these heritable risk phenotypes was in *MC1R* (Frants et al. 1996; Valverde et al. 1996). Functional variants in *MC1R*, well known to be associated with pigmentary phenotypes (most notably red hair color), were shown to be conclusively associated with melanoma risk; this increased risk was, at least in part, mediated by the effect of skin pigmentation (Palmer et al. 2000). These genetic variants have been classified as having either a strong effect on pigmentation (the "R" variants with a combined minor allele frequency of 0.22 and estimated OR from 1.4–2.4) or a smaller effect (the "r" variants with a combined minor allele frequency of 0.27 and estimated OR from 1.15 to 1.42) (Duffy et al. 2004; Raimondi et al. 2008). At least 85 different variants in *MC1R* have been identified, 10 of which occur at a frequency $> 1\%$ (Kanetsky et al. 2006). Exome and genome sequencing studies have recently shown that carriage of *MC1R* variants is associated with a gene dose-dependent increase in subsequent melanoma mutation burden (Johansson et al. 2017; Robles-Espinoza et al.

2016). Each R allele and r allele increased single nucleotide variant (SNV) counts by 1.7-fold and 1.5-fold, respectively (Johansson et al. 2017), providing direct evidence for the impact of these risk genotypes on increased tumorigenesis.

Around the same time, a combination of theoretical predictions that common genetic variation may contribute significantly to the risk of common complex diseases (the "common disease, common variant" hypothesis) (Kruglyak 1999; Risch and Merikangas 1996), and technological developments, paved the way for genome-wide association studies (GWAS) comparing the frequencies of hundreds of thousands of common polymorphisms in case-control samples of tens of thousands of individuals. Following early success in complex diseases, most notably in the proof-of-principle Wellcome Trust-funded study of seven common diseases (Wellcome Trust Case Control Consortium 2007), this approach began to be applied to a wide range of cancers, including melanoma.

For the majority of complex diseases, GWAS produced associations with single nucleotide polymorphisms (SNPs) in regions with no clear indication of function. However, the first genome-wide association studies for melanoma identified loci near *ASIP* (Brown et al. 2008; Gudbjartsson et al. 2008), which interacts with *MC1R* and had previously been associated with variation in pigmentation (Kanetsky et al. 2002), and loci near *TYR*, which was previously associated with eye color and skin response to the sun (Sulem et al. 2008). In line with other cancer GWAS, these were low-penetrance variants with odds ratios estimated between 1.21 and 1.75. The next GWAS of melanoma conducted (Bishop et al. 2009) confirmed the *TYR* association and was powerful enough to pick up a polymorphism in linkage disequilibrium with the functional *MC1R* variants. Most interestingly, this study identified a variant near *CDKN2A*, despite the fact that carriers of germline *CDKN2A* mutations had been excluded, suggesting that the same functional locus harbors both rare highly penetrant mutations and common polymorphisms with a much smaller effect (here with an estimated odds ratio of 1.18) (Bishop et al. 2009), as has been seen for other traits (Rivas et al. 2011). Thus, initial GWAS of melanoma identified loci known to be associated with either pigmentation (*MC1R*, *TYR*) or nevus count (*CDKN2A*), confirming the importance of these traits for melanoma risk. A further genetic association study, concentrating solely on polymorphisms within known pigmentation-related genes, confirmed the association of several of these (*TYRP1*, *OCA2*, and *LSC45A2*) with melanoma risk for the first time (Duffy et al. 2010a). Subsequent research found that *IRF4*, another known pigmentation gene, was associated with both melanoma and nevus count but that both the magnitude and direction of effect were age-dependent (Duffy et al. 2010b; Gibbs et al. 2016).

Pigmentation and nevus count remain the predominantly genetically determined pathways associated with melanoma risk, although nevus genotype only explains a small proportion of the nevus risk for melanoma (Law et al. 2015; Newton-Bishop et al. 2010). Further studies began to identify genetic variants apparently unrelated to either pigmentation or nevus count, notably a polymorphism in the telomerase-related *TERT-CLPTM1L* region (Barrett et al. 2015; Rafnar et al. 2009) and SNPs in the region of *CASP8* (a gene involved in apoptosis), *CCND1* (a regulator of cell cycle progression), and *FTO* (Amos et al. 2011; Barrett et al. 2011; Iles et al. 2013; Macgregor et al. 2011). Some variants thought at the time to be unrelated to pigmentation or nevus count later turned out to be so (Law et al. 2015), such as variants near *ARNT/SETDB1* associated with ease of tanning and *ATM* associated with nevus count.

One potential candidate pathway was telomere length, known to be associated, when directly measured, with both melanoma risk and nevus count (Bataille et al. 2007; Han et al. 2009; Nan et al. 2011), particularly after mutations associated with a high risk of melanoma had been found in *POT1*. However, the observed association between telomere length and melanoma risk could be as a result of reverse causality (melanoma itself or treatment received affecting telomere length) or a shared environmental effect (a third factor independently affecting melanoma risk and telomere length, such as UV exposure).

Researchers tested polymorphisms at seven loci known to be associated with telomere length from a recent GWAS (*TERC*, *TERT*, *NAF1*, *OBFC1*, *RTEL1*, *ZNF208*, and *ACYP2*) (Codd et al. 2013). Four of these were found to be nominally associated with melanoma risk, and a polygenic risk score (Dudbridge 2013) predicting telomere length on the basis of these seven SNPs was associated with melanoma risk at a genome-wide significant level (Iles et al. 2014). Not only did this demonstrate that multiple genetic determinants of telomere length influence melanoma risk, this was the first time that this had been demonstrated for any cancer. Thus, a third genetically determined phenotypic risk pathway for melanoma was identified.

Slightly anomalous is *MITF*, which was identified as being related to melanoma risk after a novel variant was found to partially co-segregate with melanoma in one family (Yokoyama et al. 2011). Subsequent genotyping in both Australian and the United Kingdom melanoma case-control samples found that the variant was overrepresented in cases and associated with increased numbers of nevi and non-blue eye color. With an estimated odds ratio of 2.19 and a minor allele frequency just below 0.01, it is probably best considered a low-medium penetrance variant, though too rare to have yet been detected by GWAS.

Fine-mapping of 13 of the low-risk loci detected by GWAS suggested that, while the signal at most loci could be explained by a single SNP, in more than a third of regions multiple susceptibility variants existed (Barrett et al. 2015).

The largest GWAS to date (Law et al. 2015), consisting of almost 16,000 cases, brought the total number of common loci associated with melanoma to 20. Five of these loci are also associated with pigmentation (*SLC45A2*, *TYR*, *MC1R*, *ASIP*, and *OCA2*), four with nevus count (*CDKN2A*, *PLA2G6*, *TERT*, and *CCND1*), two suggesting a role for DNA repair (*PARP1* and *ATM*), and *OBFC1* with telomere length (though the *TERT* gene is also known to be associated with telomere length and the DNA repair functions of *PARP1* and *ATM* include telomere maintenance). The remaining eight loci (*ARNT*, *CASP8*, *FTO*, *MX2*, *RMDN2*, *CDKAL1*, *AGR3*, *TMEM38B*) are of unclear function. Subsequent work (Iles M et al., unpublished) has shown that many of these are in fact associated with either nevus count (*ATM*), aspects of pigmentation (*ARNT*, *CDKAL1*, *AGR3*), or both (*RMDN2*). The *FTO* gene is traditionally associated with body mass index, although the SNPs related to melanoma risk are from a part of the gene that appears unrelated to obesity (Iles et al. 2013). Another study showed that several SNPs in *FTO* were significantly associated with hair color, tanning ability, and melanoma risk but not obesity (Li et al. 2013). Recently a PGC1β variant was associated with tanning ability, nevus count, and melanoma risk as well as mortality (Li et al. 2017); this gene is related to mitochondrial biogenesis and other metabolic functions. Future GWAS studies are expected to detect new loci, by increasing the sample size but also through more targeted approaches in which samples will be carefully stratified by clinical or pathologic factors or by host characteristics.

Gene-Environment, Gene-Phenotype, and Gene-Gene Interactions

Analyses of gene-environment, gene-gene (epistasis), and gene-phenotype interactions can lead to new knowledge about biological processes involved in melanoma, identify those individuals for whom the risk factor of interest is most critical, and improve accuracy of prediction for risk and prognosis (Cole et al. 2017; Read et al. 2016). Gene-environment interaction is considered to be present when individuals with different genotypes are affected differently by exposure to the same environmental factors, and thus gene-environment interactions can result in different disease risks and phenotypes. Gene-gene interaction (epistasis) is considered to be present when genes have a joint effect on a trait. Xiao et al. developed and applied such a model to melanoma and found potential gene regions interacting with *HERC2* and *MC1R* genes (Xiao et al. 2014).

Gene-Gene Interactions and Melanoma Risk

Gene-gene interactions have not been very prevalent in the literature to date. One reason may be the need to have larger sample sizes in order to see an effect. Another may be the difficulty in assessing the underlying biology when there are likely more than two genes interacting in specific pathways or in multiple pathways. Furthermore, the interaction of the genetic factors with additional cell types, such as lymphocytes, macrophages, and other cells, is coming under scrutiny, and the methodology for conducting such studies is in its early stages.

Among people carrying a *CDKN2A* mutation in melanoma-prone families, *MC1R* variants significantly increased penetrance of *CDKN2A* mutations, with one *MC1R* variant associated with a doubling of melanoma risk compared to no *MC1R* variants in this high-risk group (Demenais et al. 2010; Fargnoli et al. 2010; Goldstein et al. 2005). In addition, among *CDKN2A* mutation carriers, the presence of *MC1R* variants was associated with melanoma diagnosis at a younger age and with development of multiple melanomas compared with *CDKN2A* mutation carriers with no *MC1R* variants (Fargnoli et al. 2010; Goldstein et al. 2005).

In a melanoma case-control study of 602 cases and 603 controls, Li et al. evaluated three SNPs in base excision repair genes (*ADPRT*, *XRCC1,* and *APE1*) and found that there was evidence for a gene-gene interaction between *XRCC1* and *APE1* variants with melanoma risk; however, they pointed out that larger studies are needed for verification (Li et al. 2006). In another small study with 130 melanoma patients and 707 healthy controls, Kosiniak-Kamysz et al. discovered significant gene-gene interactions between *MC1R* and *TYR*, *SLC45A2* and *VDR*, *HERC2* and *VDR*, *OCA2* and *TPCN2,* and *MC1R* and *VDR* (Kosiniak-Kamysz et al. 2014). Although there were significant findings separately for SNPs in univariable analyses, only the gene-gene interactions remained significant in multivariable analyses.

Dysfunction in the telomere pathway was demonstrated in much larger datasets evaluated by Brossard and colleagues, where 6803 subjects with GWAS data were evaluated using pathway analyses and then gene-gene interaction analyses (Brossard et al. 2015). One pair of SNPs stood out – in *TERF1* and *AFAP1L2* – as having an interaction and passing false discovery. Further validation and functional and experimental studies will be required to shed light on the biological pathways that underpin the interaction and to better characterize the risk.

Interactions Between Genes, Phenotype, and the Environment

There are complex interactions between host characteristics, environmental exposures, and genomic factors in causing melanoma due to the correlation between some of these factors as well as the potential for their biological interactions influencing risk. However, there are very few studies that have examined these potential interactions.

Some studies have shown that sunburns, high levels of sun exposure, and presence of nevi further add to melanoma risk for people with a *CDKN2A* mutation (Bishop et al. 2002; Chaudru et al. 2004), while others have suggested no further increased risk associated with sun exposure (Begg et al. 2005; Cust et al. 2011).

In a large study from the GenoMEL melanoma genetics consortium, Demenais and colleagues showed that *MC1R* variants, hair color, and number of nevi were jointly associated with melanoma risk among *CDKN2A* mutation carriers (Demenais et al. 2010). Increased numbers of UV radiation mutational signatures have also been observed for *MC1R* carriers, highlighting their increased sensitivity to UV exposure (Johansson et al. 2017; Robles-Espinoza et al. 2016; Yu et al. 2018). Other studies that combined germline and somatic genetic data showed that *MC1R*, *IRF4*, and *PLA2G6* inherited genotypes influence melanoma *BRAF* and *NRAS* subtype development (Thomas et al. 2017, 2018), which in turn are related to clinical and pathologic

characteristics and melanoma-specific survival (Thomas et al. 2015).

A novel molecular marker of risk is global DNA methylation. A recent case-control study with 540 melanoma cases and 540 controls showed that global hypomethylation in blood leukocyte DNA was associated with increased risk of melanoma, with a dose-response relationship, and that the level of methylation was influenced by pigmentation and sun exposure (Shen et al. 2017).

Interestingly, Kanetsky et al. found that *MC1R* variants were strongly predictive of risk for melanoma among those with a dark phenotype (Kanetsky et al. 2010). This finding was subsequently replicated by others (Cust et al. 2012; Pasquali et al. 2015). This phenomenon has also occurred with *MITF* E318K SNP, where carriage of the variant was more strongly associated with melanoma for people with dark hair than fair hair (P for interaction 0.03) and for those with no moles than some or many moles (P for interaction <0.01) (Berwick et al. 2014). These findings suggest that these genetic variants may assist in predicting risk of melanoma in people without classical risk factors.

Analyses of melanoma by anatomic site can also shed light on gene-environment interactions, as different patterns of sun exposure and different causal pathways to melanoma development are associated with different body sites (Armstrong and Cust 2017). Mauguen et al. (2017) conducted a cluster analysis using somatic genotypes for *BRAF* and *NRAS* and germline data for 580 SNPs in *CDKN2A, MC1R*, and in genes related to vitamin D and immune function (Mauguen et al. 2017). Twenty-six SNPs passed false discovery; the final clusters showed associations between *BRAF* and *NRAS* somatic mutations and anatomic sites but not with germline SNPs. This type of approach should help to identify risk factors for melanoma distinguished by specific anatomic sites and histologic subtypes.

People with a genetic susceptibility for disease generally have an earlier age at diagnosis (Wu et al. 2018), and specific variants or other risk factors might be more strongly related to earlier diagnosis. For example, a study of 322 cases and 3607 controls from The Cancer Genome Atlas (TCGA) reported an age of diagnosis of 35.5 years among *MTAP* carriers and 53.9 years among *MC1R* carriers, compared to 57.7 among noncarriers (Yu et al. 2018). An Australian study found that the mean age at diagnosis was 56 years for patients with a family history, 59 years for those with many nevi, and 69 years for those with a previous melanoma, and that body site of the melanoma also differed according to these risk groups (Watts et al. 2017).

Methodologic considerations. Attention to methodologic aspects of study design and analysis remains important for studies examining gene-environment interaction. Analyses of gene-environment interactions usually assume that the genetic factor and the environmental factor are independent; when there is "dependence" between the genetic factor and the environmental factor, results can be misleading (Vanderweele et al. 2012). An example in melanoma is when a redhead with freckles who has several genetic variants associated with their phenotype is strongly sun avoidant; thus the genetic variants are associated with sun exposure because of their effect on pigmentary phenotype characteristics. Similarly, confounding can arise due to inaccurate measurement (Keller 2014; Vanderweele et al. 2012). For example, VanderWeele et al. demonstrate that unmeasured confounding may lead to erroneous estimates of gene-environment effects when the unmeasured confounder interacts with the genetic factor (Vanderweele et al. 2012).

Imputation of genotypes in GWAS studies can also lead to misclassification insofar as the causal variants have not been measured accurately, as the causal variant frequency may differ from the marker (or imputed variant) (Dudbridge and Fletcher 2014). The misclassification would usually be non-differential, in that the errors do not differ by exposure or outcome groups, but it does lead to bias toward the null and necessitates a larger sample size. This may be one of the reasons that gene-environment and gene-gene studies are relatively few in number in comparison to genetic risk studies.

Clinical and Public Health Applications

The research discussed in this chapter has important clinical and public health applications. One application is better risk assessment of melanoma, both within families and at the population level. Improvements in the development and validation of melanoma risk prediction models provide a more accurate way of calculating and communicating melanoma risk and facilitate targeted prevention, screening, and surveillance strategies (Olsen et al. 2018; Vuong et al. 2014, 2016). These risk prediction models can be feasibly incorporated into clinical practice using web-based delivery (Vuong et al. 2018) or delivered at the population level (Smit et al. 2017, 2018). Recent studies have demonstrated that genomic variants improve the discriminatory performance of melanoma risk prediction models when added to traditional risk factors and can improve identification of people who are susceptible to melanoma despite not having a traditional phenotypic risk profile (Cust et al. 2018; Gu et al. 2018). Melanoma risk prediction models and risk assessment are discussed in more detail in chapters ▶ "Clinical Epidemiology of Melanoma" and ▶ "Clinical Genetics and Risk Assessment of Melanoma."

An improved understanding of the molecular basis of melanoma also enables better estimation of patient prognosis, more accurate staging of melanoma, and major implications for improving the treatment and ongoing management of melanoma. These advances are described in more detail in chapters ▶ "Biomarkers for Melanoma," ▶ "Melanoma Prognosis and Staging," and ▶ "Molecular Pathology and Genomics of Melanoma."

Future Studies

There have been major advances in our understanding of the molecular epidemiology of melanoma over the past decade. Some of the most exciting developments have come from novel approaches that combine different types of data, including somatic, germline, clinical, pathologic, phenotypic, and environmental risk factors. Future studies are likely to build on these approaches, in particular integrating data from multiple omics platforms including genomics, proteomics, and metabolomics enabled by high-throughput technologies. Combining these different types of data provides a much better understanding of the biological system and flow of information that underlies disease (Hasin et al. 2017).

Future studies of the genetics of melanoma will likely uncover further high-risk variants; these are expected to be very rare but may be important in understanding the etiology of melanoma. Perhaps more promising is the study of low-risk variants, with increased focus on the various biological pathways underlying melanoma risk and whether new loci are related to one of the traditional phenotypes such as nevus counts or pigmentation or to novel pathways. Joint analysis of melanoma and heritable risk phenotypes will facilitate the identification of shared genetic risk factors or of genetic risk factors beyond these pathways. More detailed investigation of the way these pathways work could demonstrate whether specific aspects of pigmentation have different effects on melanoma risk and whether they act differently dependent on melanoma subtype or anatomical site of the melanoma. The data relating *MCIR* to tumor mutation burden provide proof of principle that genetically defined risk pathways can directly influence the rate of tumorigenesis.

Further characterizing the risk of melanoma associated with gene variants in different pathways will hasten the development of polygenic risk scores and their application in clinical and public health practice. Risk prediction models may be used to more effectively triage patients for screening and surveillance but also for communicating personalized risk information and tailoring prevention, screening, and surveillance advice to the individual. Further research into the molecular basis of melanoma, and its interaction with UV radiation and host characteristics, will give us a deeper understanding of the mechanisms underlying melanoma with the anticipation that this will lead to improved prevention, screening, and treatment.

Cross-References

- ▶ Biomarkers for Melanoma
- ▶ Clinical Epidemiology of Melanoma
- ▶ Clinical Genetics and Risk Assessment of Melanoma
- ▶ Melanoma Prognosis and Staging
- ▶ Molecular Pathology and Genomics of Melanoma

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