Defining the Molecular Genetics of Dermoscopic Naevus Patterns

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Abstract

Melanocytic naevi are common melanocytic proliferations that may simulate the appearance of cutaneous melanoma. Naevi commonly harbour somatic mutations implicated in melanomagenesis but in most cases lack the necessary genomic alterations required for melanoma development. While the mitogen-activated protein kinase pathway and ultraviolet radiation strongly contribute to naevogenesis, the somatic mutational landscape of dermoscopic naevus subsets distinguishes some of the molecular hallmarks of naevi in relation to melanoma. We herein discuss the classification of naevi and theories of naevogenesis and review the current literature on the somatic alterations in naevi and melanoma. This review focusses on the clinical-dermoscopic-pathological and genomic correlation of naevi that shapes the current understanding of naevi.

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Introduction

Individuals at an increased risk of developing cutaneous melanoma have high naevus counts or have melanocytic naevi with atypical clinical features [1–5]. Specifically, the pooled relative risk for melanoma development in individuals with 16–40 naevi and 101–120 naevi is 1.47 and 6.89, respectively, relative to individuals with 0–15 naevi [3]. As naevi have been shown to co-exist in histopathological contiguity with cutaneous melanoma, these melanocytic proliferations have also been postulated to be the premalignant and direct precursors to melanoma [6]. Indeed, naevi commonly harbour the same somatic mutations implicated in melanoma development [7], and their molecular genetics are continually being explored to gain additional insight into the link between naevi and melanoma with regard to their morphology and biology [7]. Knowledge of the genetic properties of naevi, in relation to their phenotypic characteristics and evolution, aid in our understanding of the pathways leading to cutaneous melanoma.

In recent years, dermoscopy has unveiled a new dimension in the clinical assessment of melanocytic proliferations, allowing for more detailed observation of colour and structure [8]. The acquisition, storage and retrieval of

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digitalised dermoscopic images has facilitated the investigation into the epidemiology, morphology and evolutionary life cycle of naevi. As such it is now feasible to observe unique dermoscopic patterns of acquired melanocytic naevi which correspond to distinct microanatomical growth patterns [9–11], life cycle [12] and time of onset [12, 13]. Importantly, the ability to monitor naevi permits greater diagnostic precision on melanoma risk prediction [11–13]. In this review, we discuss the classification of naevi, theories of naevogenesis, somatic alterations in naevi and melanoma and the clinical-dermoscopic-pathological and genomic correlation of naevi that shape the current understanding of naevi.

Classification of Melanocytic Naevi

Clinical History

The traditionally used classification scheme of melanocytic naevi is based on clinical history and divides naevi into congenital or acquired depending on the time of onset.

Congenital Melanocytic Naevi

By definition, congenital melanocytic naevi (CMN) develop in utero, are present at birth and are classed as small (<1.5 cm), medium (1.5–10 cm), large (11–20 cm) and giant (≥ 20 cm), with the larger CMN conferring greater potential for melanoma development [14]. Somatic mutations in *NRAS* have been linked with CMN, with a higher prevalence of *NRAS* mutations in large and giant CMN as compared to small and medium CMN (94.7 vs. 70%, respectively) [15]. 30% of small to medium CMN harbour the *BRAF* mutation [15].

Some small acquired naevi (<10 mm in diameter) display histopathological features found in naevi present at birth and are typified by large, predominantly intradermal melanocytic nests often with a periadnexal distribution but lack the definitive history of presence at birth. As these develop during early childhood rather than in utero, they have been referred to as congenital pattern naevi, congenital naevus-like naevi, or “tardive” CMN to reflect their overlapping similarities to “true” CMN [16]. In order to indicate its onset more accurately, these naevi have also been occasionally labelled as early-acquired melanocytic naevi (early AMN). Bauer et al. [17] demonstrated differences between “true” CMN and congenital pattern naevi. Congenital pattern naevi harbour a *BRAF* mutation in ~70% of cases which is in contrast to “true” CMN where *NRAS* is frequently mutated (81% of cases) [17].

Therefore histopathologic criteria alone is unable to reliably distinguish between small ‘true’ CMN and congenital pattern naevi.

Acquired Melanocytic Naevi

Acquired melanocytic naevi (AMN) develop after birth and constitute the most common naevi. These naevi may be clinically typical in appearance (“common” or “ordinary” naevi) or clinically atypical but are essentially benign melanocytic proliferations. In 1990, Ackerman and Magana-Garcia [18] acknowledged the disagreement amongst dermatologists and pathologists in the classification of melanocytic proliferations and in the nomenclature surrounding naevi. As a first step towards an integrated approach, Ackerman and Magana-Garcia made a proposal for the eponymously named Unna’s naevus, Miescher’s naevus, Clark’s naevus and Spitz’s naevus in honour of the individuals who first recognised or popularised these AMN [18]. These naevi are recognised based on gross clinical appearance, distinct histopathology silhouette and biology. As opposed to CMN where size is the determinant of malignancy risk, the number of AMN has been found to be the strongest predictor of melanoma risk, with the risk of development of melanoma (de novo or within a pre-existing naevus) increasing almost linearly with naevus counts [17]. The number of clinically atypical AMN (Clark/dysplastic naevi) is also a considerable independent risk factor for melanoma in the general population, especially in individuals with the atypical naevus syndrome [19]. A high frequency of *BRAF* mutation has been detected in Clark/dysplastic naevi [20] and in several AMN dermoscopic subtypes which will be elaborated later [9, 10].

Histopathological Criteria

Histopathological classification of naevi is dependent on the anatomical location of melanocytes/naevus cells/naevomelanocytes (melanin-producing cells) within the layers of the skin, subcategorising naevi into three types: (1) junctional naevus (Fig. 1a) that consists of discrete nests of naevus cells at the dermal-epidermal junction (DEJ), particularly at the rete ridges; (2) compound naevus (Fig. 1b) that consists of nests of naevus cells at both the DEJ and within the papillary dermis; and (3) dermal naevus (Fig. 1c) that is composed of naevus cells confined wholly to the dermis and arranged in nests and cords (intradermal). Histopathology is regarded as the “gold” standard in the diagnosis of melanocytic proliferations, but it merely provides a cross-sectional view in the life of any given individual naevus.

Fig. 1. Histopathological classification of naevi. **a** Junctional naevus. **b** Compound naevus. **c** Dermal naevus.

Dermoscopic Pattern

Dermoscopy (dermatoscopy, epiluminescence microscopy or skin surface microscopy) is a clinical and bedside examination technique that has improved the diagnostic accuracy of malignant skin tumours such as melanoma, with a higher discriminatory power than naked-eye examination [8, 21–27]. It is a non-invasive and in vivo surface microscope technique that utilises an incident light magnification system with or without immersion fluid. This technique today involves viewing skin lesions using a hand-held device usually with a 10-fold magnification lens called a dermatoscope (dermoscope) or a video imaging system applied to the skin.

The surface reflection renders the stratum corneum to be translucent and enables visualisation of skin surface and subsurface anatomical structures at the level of the epidermis, DEJ and superficial papillary dermis, not visible to the naked eye. Studies to date demonstrate that morphological features and criteria seen using dermoscopy correlate well with histopathological substrates, and therefore it has been considered to be an in vivo representation of gross pathology [23–26, 28] that provides a clinicopathological classification approach towards naevi.

The dermoscopic assessment of naevi is dependent on (1) colour, (2) pattern, (3) pigment distribution and (4)

Table 1. Dermoscopic colours and their histopathological correlates

Colour	Histopathological correlate	Skin anatomical level
Black	Melanin present in keratinocytes or melanocytes	Stratum corneum
Brown	Melanin present in keratinocytes or melanocytes	Dermal-epidermal junction
Grey	Melanin present in melanocytes or melanophages	Superficial papillary dermis
Blue	Melanin present in melanocytes or melanophages	Papillary or reticular dermis

body-site-specific pattern. Colour can be observed as black, brown, grey and blue under the dermatoscope according to the location of melanin-containing melanocytes, keratinocytes or melanophages in the different skin layers (Table 1). Pattern can be perceived as globular, reticular, starburst, structureless/homogeneous and mixed. Pigment may be distributed in a uniform, central, eccentric and multifocal manner. Naevi on special sites such as the face, acral sites and nails have been observed to have site-specific patterns.

The Memorial Sloan-Kettering Cancer Centre (New York, NY, USA) adapted a dermoscopic pattern classification for naevi based on observations outlined by Hofmann-Wellenhof et al. [29] for clinically atypical naevi. Accordingly, the existence of 4 dermoscopic pattern groups – (1) globular pattern, (2) reticular pattern, (3) structureless/homogeneous brown pattern and (4) reticuloglobular pattern – has been used as the foundation for their published work [30–32].

In a second approach by Zalaudek et al. [12], AMN and “tardive” CMN/early AMN are distinguished based on 5 dermoscopic patterns: (1) globular, (2) reticular, (3) mixed pattern with central structureless brown-grey area/network surrounded by a peripheral rim of small brown globules, (4) mixed pattern with central globules/structureless area surrounded by a peripheral network and (5) unspecific pattern. Notably some of these observed patterns may not represent distinct dermoscopic naevus subgroups. For example, as longitudinal data have observed the structureless/homogeneous brown pattern to change to or from either the reticular or globular pattern [30], it is likely to represent a heterogeneous group of naevi [33]. Moreover, these naevi can be histopathologically junctional or compound naevi, therefore they demonstrate overlapping histopathological features with dermoscopically globular and reticular naevi [29]. It is also yet to be determined whether mixed pattern naevi represent a distinct entity or belong to globular or reticular naevi from a clinicopathological perspective [33]. Nonetheless, mixed pattern naevi with a peripheral rim of glob-

ules (PG) potentially constitute a unique subset due to its growth dynamics.

To add another layer of complexity, more recently, Argenziano et al. proposed a new classification scheme dividing the most common naevi encountered in clinical practice (<15 mm in diameter) into 7 groups: (1) globular “congenital” naevus, (2) reticular “acquired” naevus, (3) starburst “Spitz/Reed” naevus, (4) blue “homogeneous” naevus, (5) site-related naevi, (6) naevi with special features and (7) unclassifiable melanocytic lesions [34]. The first 4 types of naevi were categorised based on common epidemiological and morphological features with a distinct clinicopathological correlation. The latter 3 groups differ from the former 4 groups as these naevi either have site-specific features or unusual clinical features which have potentially conflicting diagnostic criteria from a clinicopathological and/or histopathological standpoint.

Theories of Naevogenesis

Unidirectional Melanocyte Migration

Unna’s Downward Migration “Abtropfung” Theory

In 1893, Paul Gerson Unna introduced the concept of naevus cells developing and proliferating from within the epidermis, then migrating downwards via the DEJ and into the dermis over time [16]. Unna’s theory therefore implies that naevi are first junctional in nature, then compound as it consists of both junctional and dermal components, and finally the naevus cells complete the migration into the dermis to become dermal naevi. Dermoscopically this movement of melanocytes from the epidermis to the dermis corresponds to reticular naevi in youth progressively becoming globular naevi later in life. However, the observed age-dependent shift from globular (81% in individuals <15 years of age) to reticular naevi (69% in individuals >60 years of age) in a cross-sectional dermoscopic study [13] seems to challenge Unna’s theory but favour Cramer’s upward migration theory described below.

Fig. 2. Globular pattern naevi. **a–c** These naevi have numerous differently sized round to oval brown globules on dermoscopy. Histopathologically these are often compound naevi as the globules correspond to nests of naevus cells both at the DEJ and in the upper papillary dermis (**d**) or nests of naevus cells that are entirely in the papillary dermis as in the papillomatous dermal naevus (**e**).

Cramer's Upward Migration "Hochsteigerung" Theory

In 1984, Cramer (in Marghoob [16]) challenged Unna's theory based on new knowledge that melanocytes originated from the neural crest and so must ascend along individual nerve fibres through the dermis in an upward migration fashion. Cramer's theory postulates that naevi start as dermal naevi with the globular pattern in youth, then become compound naevi as some of the naevus cells ascend into the epidermis, and finally later in life this process would result in junctional naevi with the reticular pattern when there are no residual melanocytes in the dermis.

Dispute towards the Concept of Unidirectional Melanocyte Migration

Longitudinal dermoscopic data from the Study of Naevi in Children (SONIC), a population-based study of 366 adolescent children, observed stability in the dermoscopic patterns of individual naevi, where globular

and reticular naevi in particular did not seem to cross over in pattern [30]. This study was conducted over a 3-year period with observational data supporting the stability of dermoscopic patterns during the life cycle of a naevus [30]. Overall, the authors suggested that specific dermoscopic patterns may represent distinct biological entities.

The Dual Pathway of Naevogenesis

Prior to the completion of the SONIC study, Zalaudek et al. [11] proposed a dual pathway of naevogenesis based upon dermoscopic observations that globular and reticular pattern naevi demonstrate age-related prevalence [13]. This alternative concept of naevus development also suggests that most naevi retain their original dermoscopic pattern throughout the life of the naevus and encompasses (1) the constitutive or endogenous pathway for globular naevi and (2) the acquired or exogenous pathway for reticular naevi.

Fig. 3. Reticular pattern naevi. **a–c** These naevi demonstrate a network of pigmented interconnected lines on dermoscopy. Histopathologically these are either compound or junctional naevi as depicted in **d** and **e**, where the pigment network corresponds to elongated and pigmented rete ridges accentuated by nests of naevus cells at the tips of the rete ridges.

Constitutive/Endogenous Pathway – The Globular Pattern

Globular naevi (Fig. 2a–c) are typically either compound or dermal naevi in histopathology as the globules correspond to nests of naevus cells at both the DEJ and papillary dermis, or entirely in the dermis (Fig. 2d, e). They may demonstrate a congenital pattern in histopathology and have been associated with CMN, hence being labelled as “tardive” CMN and referred to as “congenital” naevi by Argenziano et al. [34]. Globular naevi are prevalent in childhood [12] and tend to have a stable life cycle and lifelong persistence [11, 16].

Acquired/Exogenous Pathway – The Reticular Pattern

Reticular naevi (Fig. 3a–c) are usually junctional naevi on histopathology as the pigment network corresponds to a lentiginous/single layer of melanin-containing naevus cells and keratinocytes along the basal layer of regularly elongated rete ridges of the DEJ or nests of naevus cells accumulated at the tips of these elongated rete ridg-

es (Fig. 3d). The melanin-rich rete ridges appear as pigmented lines, and the less pigmented or non-pigmented suprapapillary plates appear as holes, conferring the network appearance. Occasionally the naevus cells are present at both the DEJ and the dermis on histopathology corresponding to a compound naevus (Fig. 3e). Reticular naevi have been referred to as acquired naevi, as this dermoscopic naevus type is commonly seen in adults [13] and has been found to be the pattern demonstrated in 47% of new naevi developing in adulthood [32]. Further epidemiologic evidence suggests reticular naevi increase in number from puberty until midlife and decrease in number later in life as these naevi eventually involute [11, 16, 35].

Conclusions from the Dual Pathway of Naevogenesis

The constitutive/endogenous pathway suggests globular naevi develop from dermal naevus cells during the prepubertal period, then persist and eventually acquire the stereotypical clinical appearance of dermal naevi (Unna or Miescher type) later in life [11]. Conversely, in

Fig. 4. Mixed pattern naevi with a peripheral rim of globules (PG naevi). **a, b** Dermoscopically these naevi demonstrate a peripheral rim of small brown globules with either a central network, or central globule brownish area (**c**). **d, e** Histopathologically all PG naevi have delicate pigmented junctional nests of melanocytes at the periphery of the lesions.

the acquired/exogenous pathway it is proposed that reticular naevi derive from epidermal naevus cells that proliferate at the DEJ (possibly in response to external factors such as intermittent ultraviolet exposure), causing an increase in naevus counts from puberty until midlife, and then regress later in life [11].

Indicator of Naevus Growth – The Mixed Pattern with a Peripheral Rim of Globules

Naevi with a peripheral rim of globules (PG; Fig. 4a–c) may be junctional naevi on histopathology (Fig. 4d) but are typically compound naevi with delicate pigmented junctional nests of melanocytes at the periphery of the lesions (Fig. 4e). This latter feature is thought to be the histopathological substrate for the rim of globules (Fig. 5a, b) [36]. By contrast, the histopathological substrate of globules in a papillomatous dermal naevus corresponds to cap-like pigmentation of naevus cells in the papillary dermis (Fig. 5c, d). Longitudinal dermoscopic studies found that PG naevi exhibit symmetrical enlargement

over time, which implies that growth does not always indicate malignant transformation [37, 38]. Naevi with a peripheral rim of globules constitute 49% of enlarging naevi, with the relative risk of enlargement to be 28-fold higher (95% confidence interval) for naevi with a peripheral rim of globules than naevi without a peripheral rim of globules [36]. Further, a study examining the growth pattern of PG naevi reported 96% ($n = 116$) of lesions enlarged at some point during sequential monitoring, but these lesions display a decrease in number of the PG over time [38]. After a period of growth, PG naevi lose their rim of globules as the larger peripheral junctional nests extend horizontally to manifest either a reticular pattern (e.g., reticular naevus) or a mixed pattern with central globules/structureless area with a peripheral network [35]. Without a longitudinal assessment, it is uncertain how long the period of stability is before involution occurs (or if involution occurs). Incidentally, this dermoscopic pattern was not found in individuals older than 60 years of age, has an onset during puberty and early ado-

Fig. 5. The histopathological substrate for globules on dermoscopy. **a** The peripheral rim of globules in a mixed pattern naevus with a peripheral rim of globules, i.e. PG naevus, correspond to junctional nests of melanocytes, which are shown at a higher magnification in **b**. **c** The globules of a globular pattern papillomatous dermal naevus correspond to cap-like pigmentation of naevus cells in the papillary dermis, which is demonstrated at a higher magnification (**d**).

lescence, a peak incidence at 11–30 years of age, with the number of PG naevi decreasing rapidly after the third decade of life [12]. Thus, these findings suggest that the transient nature of this dermoscopic pattern is a signature for the growth phase of naevi.

Somatic Alterations in Melanocytic Proliferations

The focus on the genetics of melanocytic naevi has been on identifying somatically acquired mutations found in cutaneous melanoma. This section summarises the molecular genetics of cutaneous, acral and mucosal melanoma and integrates this with the current genetics of melanocytic naevi.

Genomic Landscape of Melanoma

The genomic landscape of cutaneous melanoma has been studied most extensively and is characterised by driver mutations, ultraviolet radiation (UVR) mutational

signature and copy number aberrations [39, 40]. Cutaneous melanomas have been classified into 4 genomic subtypes based on the pattern of the most prevalent significantly mutated genes: *BRAF* subtype, *RAS* family (*NRAS/HRAS/KRAS*) subtype, *NF1* subtype and triple-WT (wild-type) subtype (i.e., non-*BRAF/NRAS/NF1* mutant) [39]. The *BRAF* subtype is the largest genomic subtype, followed by *RAS*, *NF1* and triple-WT [39].

In contrast to cutaneous melanoma, acral and mucosal melanomas demonstrate a markedly different genomic landscape with a lower mutation burden dominated by large-scale copy number aberrations and structural rearrangements [40]. Acral and mucosal melanomas often lack a UVR mutational signature but harbour alternate mutational signatures not commonly identified in cutaneous melanoma [40]. Furthermore, approximately 50% of acral and mucosal melanomas lacked *BRAF*, *RAS* or *NF1* mutations (i.e., triple-WT) but loss-of-function mutations in *CDKN2A*, *TP53* and *ARID2*, and activating hotspot mutations in *GNAQ* and *SF3B1*, were more common [40].

*Common Gene Mutations in
Melanocytic Proliferations
BRAF (Melanoma and Naevi)*

The *BRAF* oncogene on chromosome 7q34 encodes a serine/threonine kinase (v-raf murine sarcoma viral oncogene homolog B1) and is a key component of the mitogen-activated protein kinase (MAPK) pathway. Somatic mutations of the *BRAF* oncogene commonly occur in exons 11 and 15, and this leads to increased cell proliferation and survival due to overactivation of the MAPK pathway. Specifically, somatic mutations in the V600 codon of *BRAF* have been detected in 35–50% of melanomas [39, 41–44], most commonly in cutaneous melanomas arising in intermittently sun-exposed skin sites rather than on chronic sun-damaged skin. In particular, the amino acid substitution from a valine to a glutamic acid at codon 600 (V600E), accounts for 74–92% of the *BRAF* mutations in melanoma [41, 45]. Other less frequent somatic hotspot *BRAF* mutations include *BRAF* V600K, *BRAF* V600R and *BRAF* K601 [39]. Individuals with *BRAF* mutant cutaneous melanomas generally have a younger age of onset [39].

Remarkably, the *BRAF* V600E mutation has also been found in 73–82% of melanocytic naevi [46, 47] that include small to medium CMN [15], congenital-pattern naevi [17], Clark/dysplastic naevi [20] and globular, reticular and PG dermoscopic subtypes of AMN [9, 10] that will be elaborated in more detail below.

RAS Family (Melanoma and Naevi)

The *NRAS*, *HRAS* and *KRAS* oncogenes are situated on different chromosomes (chr1p13.2, chr11p15.5 and 12p12.1, respectively) and encode GTP-binding proteins located upstream of *BRAF* within the MAPK pathway. *NRAS* is more frequently somatically mutated compared to *HRAS* or *KRAS*. Overall, 28% of primary and metastatic melanoma samples have a somatic *NRAS* mutation [39] but are less frequently observed in acral and mucosal melanomas [40]. The *NRAS* mutation has also been linked to CMN, with a higher prevalence of *NRAS* mutations in large and giant CMN as compared to small and medium CMN (94.7 vs. 70%, respectively) as described above [15]. Only 4 hotspot *HRAS* mutations and 3 hotspot *KRAS* mutations have been found amongst 318 primary and metastatic cutaneous melanoma samples, and all were mutually exclusive with *NRAS* and *BRAF* mutations [39]. The *HRAS* mutation is linked to Spitz “starburst pattern” naevi [7] and is discussed later in this review.

NF1 (Melanoma)

The *NF1* (neurofibromin 1) gene on chromosome 17q11.2 is a tumour suppressor gene and encodes a GTPase-activating protein within the MAPK pathway that downregulates RAS activity. Somatic mutations in *NF1* have been observed in 14% of primary and metastatic cutaneous melanoma samples, and more than 50% of its mutations were predicted to be loss-of-function events [39]. Therefore *NF1* mutations serve as an alternative way to activate the MAPK signalling pathway. Somatic mutations in *NF1* have been found to be mutually exclusive with hotspot *BRAF* mutations but may exist concurrently with non-hotspot *BRAF*/*NRAS* cutaneous melanomas, particularly in samples with a UV signature [39]. Loss of function can also be due to deletion. Most acral and mucosal melanomas lacked *NF1* mutations [40].

GNAQ (Uveal Melanoma and Blue Naevi)

The *GNAQ* oncogene on chromosome 9q21.2 encodes a GTP-binding protein (guanine nucleotide-binding protein, q polypeptide) that mediates signal between the G-protein-coupled receptor and downstream effectors within its pathway. The hotspot mutations Q209L and R183Q are associated with uveal melanoma [48, 49], although both have also been associated with 83% of blue naevi with “homogeneous blue pattern” [7]. Mutation in *GNAQ* is rarely found in the triple-WT subtype of cutaneous melanoma [39].

GNA11 (Uveal Melanoma and Blue Naevi)

The *GNA11* oncogene on chromosome 19p13.3 is a *GNAQ* homologue and encodes a G protein α -unit (guanine nucleotide-binding protein, α 11) within the G-protein-coupled receptor pathway. Mutation in *GNA11* is rarely found in the triple-WT subtype of cutaneous melanoma and is more frequently found in metastatic uveal melanomas rather than primary uveal melanomas [50, 51]. It is also found in a minority of blue naevi [51].

CDKN2A (Melanoma)

The *CDKN2A* tumour suppressor gene on chromosome 9p21.3 encodes the cyclin-dependent kinase inhibitor p16 (p16INK4a) and nucleolar protein p14ARF that regulate the cell cycle. Although *CDKN2A* and p14ARF are familial melanoma susceptibility genes with high penetrance, sporadic somatic mutations in *CDKN2A* are less frequently detected in cutaneous melanoma [39] but are mainly attributed to loss of function due to homozygous deletion. Recently, biallelic loss (loss of both copies) of *CDKN2A* has been proven to be an early event in melanogenesis [52, 53].

TERT Promoter (Melanoma and Intermediate Melanocytic Proliferations)

The *TERT* gene on chromosome 5p15.33 encodes the reverse transcriptase component and the core catalytic subunit of telomerase, an RNA-dependent DNA polymerase that maintains telomere ends by synthesising telomeric DNA and preventing telomere erosion associated with cell divisions. Telomerase plays a role in cellular senescence and extends the cellular life span by lengthening telomeres. Overall, *TERT* promoter mutations were prevalent in 86% of cutaneous melanomas [40]. Importantly, *TERT* promoter mutations have also been detected in intermediate cutaneous melanocytic proliferations and cutaneous melanoma in situ and represent the earliest secondary alterations in melanoma development and progression [54, 55].

Mutational Signatures in Melanoma

The Catalogue of Somatic Mutations in Cancer (COSMIC) mutational signatures are based on analysis of 10,952 exomes and 1,048 whole genomes across 40 distinct types of human cancer. These signatures reflect the different mutational processes that generate unique combinations of mutation types including the intrinsic slight infidelity of the DNA replication machinery, exogenous or endogenous mutagen exposures, enzymatic modification of DNA and defective DNA repair [56]. The profile of each signature is generated using the 6 classes of base substitution (C>A, C>G, C>T, T>A, T>C, T>G) with each substitution incorporating the bases immediately 5' and 3' to each mutated base and displayed based on the observed trinucleotide frequency [56]. There is currently a total of 30 mutational signatures (signature 1–30) in the COSMIC database. Signature 7, a signature associated with UVR that is characterised by the predominance of C>T transitions and large numbers of CC>TT dinucleotide mutations at dipyrimidines [56], is a feature of >90% of BRAF/RAS/NF1 mutant cutaneous melanomas [39]. In contrast, only 30% of triple-WT cutaneous melanomas display a UV signature [39]. A subsequent study confirmed that signature 7 was the dominant mutational signature in 97% ($n = 136/140$) of cutaneous melanomas [40]. In acral and mucosal melanomas, as expected, UVR is not involved in their aetiology; instead, these melanoma subtypes frequently (93% or $n = 40/43$) have an age-related signature (signature 1) as well as signature 5 [40]. Signature 5 has recently been found to be associated with defective mismatch repair [57].

Copy Number Aberrations and Structural Rearrangements in Melanoma

Cutaneous melanomas have a high degree of copy number aberrations (CNA) [39]. Specifically, melanomas often have focal deletions in tumour suppressor genes (e.g., *CDK2NA*), focal amplifications in oncogenes (e.g., *BRAF*, *NRAS* and *TERT*) [39], along with extensive regions of copy number loss of heterozygosity often encompassing whole chromosomal arms [58]. Complex genomic rearrangements are a hallmark of non-UVR-induced acral and mucosal melanomas [40].

Overview of the Genetics in Melanocytic Naevi

The MAPK pathway is frequently mutated in melanoma, particularly in the *BRAF* and *NRAS* oncogenes, and as such these genes have been commonly assessed in naevi. In the earliest report investigating the *BRAF* mutation status in naevi, Pollock et al. [47] assessed exons 11 and 15 for the presence of common hotspot mutations. *BRAF* V600E (initially reported as V599E) was mutated in 82% ($n = 63/77$) of naevi, 80% of primary melanoma ($n = 4/5$) and 68% of metastatic melanoma [47]. This landmark study introduced the concept that activation of the MAPK pathway is an essential first step for a melanocytic proliferation but is not sufficient for malignant transformation. Other attempts to characterise the genomic make-up of melanocytic naevi have also involved mutational analyses of *TP53*, *MC1R* and targeting the high-penetrance familial melanoma susceptibility genes *CDKN2A* and *CDK4* [59, 60]. With increased dermal melanosis found in mutant mice that harbour germline mutations in *GNAQ* and *GNA11* [61], these genes were subsequently assessed in blue naevi due to the histopathological reminiscence of blue naevi in *GNAQ* and *GNA11* mutant mice [62].

Clinical-Dermoscopic-Pathological and Genomic Correlation of Melanocytic Naevi

Single Gene Targeted Mutation Detection

Due to the complexity of using clinical history and morphological criteria to reliably determine which naevi are at risk for melanoma, there is an unmet clinical need to identify naevi based on a clinicopathological and molecular approach. The investigation into biological subsets of naevi using the dermoscopic classification of naevi provides a correlation with microanatomical growth patterns [9–11], time of onset [12, 13], life cycle [12] and relative melanoma risks [11–13]. Somatic mutations in

Table 2. Summary of studies investigating the molecular genetics of dermoscopic naevus patterns

Reference	Dermoscopic pattern	Specimens	Assessed genes/ genetic characteristics	Mutation detection methods	Conclusion
Zalaudek et al. [10]	Globular (<i>n</i> = 5) Reticular (<i>n</i> = 17) Mixed pattern with central globules/ homogenous area with a peripheral network (<i>n</i> = 12) Peripheral rim of globules (<i>n</i> = 11)	FFPE	<i>BRAF</i>	Ultradeep pyrosequencing vs. Sanger sequencing	<i>BRAF</i> more frequent in globular and peripheral rim of globules naevi Discordant results for reticular naevi
Marchetti et al. [9]	Globular (<i>n</i> = 12) Reticular (<i>n</i> = 13)	FFPE	<i>BRAF</i> V600E	Immunohistochemistry	Globular naevi are 3 times more likely to harbour <i>BRAF</i> V600E than reticular naevi
Tan et al. [67]	Globular (<i>n</i> = 13) Reticular (<i>n</i> = 15) Peripheral rim of globules (<i>n</i> = 12)	Fresh naevus	<i>BRAF</i> V600E/K, <i>NRAS</i> codons 12/13/61	ddPCR	Mutually exclusive <i>BRAF</i> and <i>NRAS</i> mutations in 85 and 15% of naevi, respectively (100% MAPK pathway activation) Globular naevi are 92% <i>BRAF</i> V600E/K mutant and 8% <i>NRAS</i> mutant Peripheral rim of globules naevi are 100% <i>BRAF</i> V600E mutant Reticular naevi are 67% <i>BRAF</i> V600E/K mutant and 33% <i>NRAS</i> mutant
Stark et al. [69]	Globular (<i>n</i> = 12) Reticular (<i>n</i> = 14) Peripheral rim of globules (<i>n</i> = 4)	Fresh naevus and adjacent non-lesional skin	Exome-wide somatic mutation burden <i>TERT</i> promoter Mutation signatures Copy number aberrations	Whole-exome sequencing, <i>TERT</i> promoter analysis (nested PCR and Sanger sequencing)	Naevi have a low somatic mutation burden (3 mutations per megabase) Naevi have mutually exclusive <i>BRAF</i> and <i>NRAS</i> mutations The mutation profile between naevi in comparison with somatic mutations present in melanoma data sets reveals similarities between the C>T mutation frequency distribution of naevi and cutaneous melanoma Naevi have a prevalent proportion of UVR-related (97%), age-related (93%) and defective DNA repair-related signatures (30%) Globular naevi are largely genomically silent containing only minor copy number aberrations Reticular and peripheral rim of globules naevi had a significantly higher proportion of indels and genome-wide copy number aberrations involving combined loss of melanoma-related tumour suppressor genes and oncogenes

FFPE, formalin-fixed paraffin-embedded; ddPCR, droplet digital polymerase chain reaction; MAPK, mitogen-activated protein kinase; UVR, ultraviolet radiation.

HRAS have been linked with Spitz “starburst pattern” naevi whereas *GNAQ* is associated with blue naevi [7]. It is proposed that the benign and stable nature of Spitz and blue naevi is likely to be attributed to the non-existence of *BRAF* and *NRAS* mutations in these naevus types and alterations in *HRAS* and *GNAQ* not being key players in melanomagenesis [7]. Clinically, it has been observed that pigmented Spitz naevi in childhood often involute and blue naevi are very stable; however, their appearance often mimics melanoma.

Besides Spitz and blue naevi, most studies that have assessed other dermoscopic subtypes of AMN were limited to investigating *BRAF* status only (Table 2). In 2011, Zalaudek et al. [10] evaluated the frequency of *BRAF* mutation in melanocytic naevi of 4 dermoscopic pattern groups consisting of globular (*n* = 5), reticular (*n* = 17), mixed pattern with central globules/homogeneous area with a peripheral network (*n* = 12) and mixed pattern reticular/homogeneous naevi with a peripheral rim of globules (*n* = 11). The authors compared two different mutation detection methods (ultradeep pyrosequencing and Sanger sequencing) of formalin-fixed paraffin-embedded naevus specimens. *BRAF* mutation was more frequent in globular and mixed pattern naevi with a peripheral rim of globules, as well as more frequent in histopathologically diagnosed dermal and compound naevi. The authors

were unable to draw conclusions for reticular naevi as both sequencing methods revealed discordant results for this subtype.

Likewise, a subsequent study by Marchetti et al. [9] looked into *BRAF* V600E expression in melanocytic naevi using immunohistochemistry and presented statistically significant findings that *BRAF* V600E expression was detected in 92% (*n* = 11/12) of globular naevi and only in 30% (*n* = 4/13) of reticular naevi. The authors also investigated the association of *BRAF* mutation and microanatomical growth patterns, assessing formalin-fixed paraffin-embedded histopathological sections based on 4 criteria: (1) junctional, compound or intradermal naevi, (2) the predominant microanatomical growth pattern, (3) nest formation of junctional melanocytes and (4) presence or absence of architectural disorder and cytological atypia. Marchetti et al. [9] also revealed that globular naevi, most often corresponding to a predominantly dermal growth pattern and/or the presence of large junctional nests, are at least 3 times more likely to harbour *BRAF* V600E (87 vs. 20%, *p* = 0.002) than reticular naevi, characterised by smaller junctional or compound nests. Therefore, these findings support the discovery that intradermal naevi are more likely to harbour *BRAF* mutations than junctional naevi [63–65] and conversely *BRAF* V600E-positive naevi are less likely to have a junctional

component ($p = 0.04$) or show lentiginous growth ($p = 0.023$) [66].

In a follow-up study investigating common hotspot mutations in both *BRAF* and *NRAS* genes, we subsequently analysed 40 AMN with globular ($n = 13$), reticular ($n = 15$) and PG ($n = 12$) dermoscopic patterns [67]. Using the highly sensitive quantitative QX200 droplet digital™ PCR system, we observed mutually exclusive *BRAF* and *NRAS* mutations in 85 and 15% of naevi, respectively, constituting 100% MAPK pathway activation. The *BRAF* mutation was present in 92% ($n = 12/13$) globular (*BRAF* V600E/K) and 100% ($n = 12/12$) PG (*BRAF* V600E) naevi, whereas reticular naevi were 67% ($n = 10/15$) *BRAF* V600E/K and 33% ($n = 5/15$) *NRAS* mutant. Specifically, the *BRAF* mutations consisted of 70% *BRAF* V600E (c.1799T>A), 7.5% *BRAF* V600E (c.1799_1800delTGinsA) and 7.5% *BRAF* V600K. *NRAS* mutant naevi frequently had concurrent *NRAS* mutations and were present at very low fractional abundance compared with cellular content [67]. These data also confirmed that a predominantly dermal growth pattern or the presence of large junctional nests are significantly associated with a *BRAF* mutation [67].

Targeted Panel Sequencing of Melanocytic Naevi

It is now conclusive that naevi require additional events beside MAPK pathway activation for malignant transformation to occur. Direct evidence of this was observed in a study of primary melanomas that had histopathological evidence of an adjacent naevus [55]. Shain et al. [55] sequenced a targeted panel of 293 cancer-relevant genes in 150 areas of 27 primary melanomas and their adjacent precursor benign or intermediate melanocytic proliferation and found that unequivocally benign melanocytic proliferations (i.e., benign naevi) harboured *BRAF* V600E with the absence of additional driver mutations. Furthermore, when intermediate melanocytic proliferations (i.e., melanocytic proliferations with ambiguous malignant potential) were assessed, they were discovered to have additional somatic alterations (including *TERT* promoter mutations) besides *BRAF* and *NRAS* mutations [55]. It is important to distinguish that these so-called “intermediate melanocytic proliferations” are not necessarily “dysplastic” or “atypical” naevi in the general sense as this terminology is broadly used with limited consensus [57]. These “intermediate melanocytic proliferations” would be more associated with naevi with atypia as such and closely resemble melanoma but fall short of defining melanoma features. *TERT* promoter mutations were as such identified as the earliest secondary alterations required for melanoma development [55].

Whole-Exome Sequencing of Melanocytic Naevi

More recently, whole-exome sequencing of 19 naevi comprising 11 “dysplastic” naevi derived from patients with atypical naevus syndrome, 2 CMN and 6 AMN demonstrated a substantially lower mutational load than cutaneous melanoma and a different and lower frequency of UVR-associated mutational signature, together with an absence of known melanoma driver mutations [68].

In our recent study, we assessed 30 AMN based upon their dermoscopic pattern using whole-exome sequencing [69]. The somatic mutational landscape was determined in globular ($n = 12$), reticular ($n = 14$) and PG naevi ($n = 4$) as well as adjacent normal skin [69]. The findings from this study confirm the mutually exclusive nature of *BRAF* and *NRAS* mutations together with a wide range of somatic mutations (30–668 mutations) or 0–9 mutations per megabase present in each melanocytic naevus [69]. Other driver genes detected at a lower prevalence to *BRAF* V600 include the melanoma-associated genes (e.g., *GRIN2A*) and novel genes (e.g., *HDAC9*, *MYH11* and *DCC*) present at a similar mutation frequency to *BRAF* V600E and *BRAF* V600K – implying that they most likely occurred at the same time as *BRAF* during naevus formation [69]. There was a low median somatic mutation burden in the naevi (3 mutations per megabase) in comparison to other UVR-associated cutaneous malignancies such as cutaneous melanoma (38 mutations per megabase) [40] or primary and metastatic cutaneous squamous cell carcinoma (33 mutations to megabase) [70, 71]. C>T transitions were confirmed to be the most prevalent SNV class in the analysed naevi, and overall the analysed naevi shared many similarities to the mutation frequency distribution in cutaneous melanoma [69]. Notably, there was a prevalent proportion of UVR-related (97%), age-related (93%) and defective DNA repair-related signatures (30%). However, the discovery that two naevi had no age-related signature and a low or absent UVR-related signature but had evidence for defective DNA mismatch repair highlights an alternative mechanism for naevus development besides UVR. C>T transition was also the dominant base substitution in adjacent non-lesional skin, although this only culminated in a UVR-related mutation signature in 10% of adjacent non-lesional skin [69]. Remarkably, defective DNA repair signatures were more frequently observed in adjacent non-lesional skin than in the assessed naevi, in addition to 100% age-related mutation signature prevalence [69]. These data build upon the perception of UVR being the only pathogenic factor in naevogenesis [55].

Fig. 6. The clinical-dermoscopic-pathologic-genomic correlation of globular, reticular and peripheral rim of globules subtypes of acquired naevi. This figure provides a summary of the common clinical presentation of globular, reticular and peripheral rim of globules subtypes of acquired naevi and the corresponding histopathology. Following somatic mutation profiling, the common *BRAF* and *NRAS* mutation prevalence differs across the dermoscopic subtypes as does total mutations per megabase (exome li-

brary is approx. 75 megabases) and insertion/deletion (indels) mutations. Overall, somatic mutation signatures are commonly found in order of prevalence (age, UVR and defective DNA repair) in all dermoscopic subtypes. Strikingly, genome-wide copy number aberrations (CNA) are largely absent in globular naevi, whereas reticular naevi and naevi with a peripheral rim of globules have numerous CNA which are balanced events (loss of tumour suppressor genes and oncogenes).

In relation to dermoscopic patterns, mutational signature analysis identified globular naevi to have a significantly higher proportion of C>T transitions (specifically at T[C>T]A); but overall globular naevi are largely ge-

nomically silent containing only minor CNAs [69]. Reticular and PG naevi are more likely to have a higher proportion of indels and genome-wide CNAs [69]. These observations suggest that specific mutation types in different

dermoscopic patterns may contribute to the development of distinct dermoscopic and histopathological naevus phenotypes. However, it is also important to consider that other factors such as the individual's age at naevus onset may account for the somatic mutation differences seen between dermoscopic patterns since there are significant age-related differences in the onset of globular (childhood), reticular (puberty and adulthood) and PG naevi (puberty or early adolescence). This however is not possible to accurately determine without longitudinal monitoring. Furthermore, the minor CNA in globular naevi may play a role in the stable life cycle and lifelong persistence of this naevus.

Reticular and PG naevi with a predominantly epidermal growth pattern are typically characterised by junctional nests in the same data set [69] and had a significantly higher proportion of indels and genome-wide CNA involving combined loss of melanoma-related tumour suppressor genes and oncogenes [69]. It should be noted that reticular and globular naevi had similar total somatic mutation burdens and PG naevi had a many-fold lower mutation burden. This lower mutation burden may reflect the age of onset of the PG naevi. As focal regions' of loss of tumour suppressor genes and gains of oncogenes (i.e., "unbalanced") is a hallmark of cutaneous melanoma, the genome-wide CNA with balanced copy number events in reticular and PG naevi seem to keep these naevi in a state of equilibrium. Lastly, the clinically benign state of all analysed naevi is supported by the absence of *TERT* promoter mutations, as this mutation has been reported as early events in malignant transformation [55].

Summary of the Genomic Analysis of Dermoscopic Naevus Patterns

The development and maintenance of melanocytic naevi is comprised of a complex interplay of genomic factors that not only keeps them in a state of equilibrium, but also influences their evolution and life cycle (Fig. 6). The 100% mutually exclusive *BRAF* and *NRAS* mutation prevalence in globular, reticular and PG naevi is in keeping with the role of the MAPK pathway in early naevogenesis although additional initiating events are most likely involved in *NRAS* mutant AMN [67, 69]. Given that *BRAF* V600 mutations are the most prevalent, *BRAF* V600 mutations are likely to be the initiator in *BRAF* mutant naevi, and the other driver genes present at equal (e.g., *HDAC9*, *MYH11* and *DCC*) or lower prevalence

provide a permissive environment for naevus development [69]. UVR mutation signatures have been identified as the dominant mutation signatures in naevi in contrast to adjacent non-lesional skin which primarily consists of age-related and defective DNA repair signatures with minimal UVR signatures [69], despite being exposed to the same degree of sunlight.

The somatic mutation landscape of globular, reticular and PG naevi can be characterised by differences in *BRAF* and *NRAS* prevalence and somatic mutation burden [67, 69]. Differences in the mutation frequency suggest that specific mutation types may contribute to the development of distinct dermoscopic and histopathological naevus phenotypes. Overall, globular naevi are almost exclusively *BRAF* mutant and are largely genomically silent containing only minor CNA [67, 69]. Reticular naevi are mutated for both *BRAF* and *NRAS*, whereas PG naevi are solely *BRAF* mutant which supports the notion that *BRAF*-mutant clones contribute to the growth of naevi. Both reticular and PG naevi share a significantly higher proportion of indels and large regional genome-wide CNA, involving combined loss of melanoma-related tumour suppressor genes and oncogenes [67, 69]. These observations reveal that CNA events that are absent or balanced in clonally expanded melanocytes contribute to the stability of naevi. The stability of naevi can also be attributed to the common *BRAF* V600 mutations that drive the initial proliferative burst which then initiates oncogene-induced senescence [72]. Furthermore, the differences in CNA may play a role in the stable life cycle and lifelong persistence of globular naevi, and in influencing the more dynamic life cycle of reticular and PG naevi that subsequently leads to involution.

Overall, the molecular genetics of dermoscopic naevus patterns is starting to provide insight into naevogenesis and has distinguished some of the molecular hallmarks of naevi in relation to melanoma. Future studies on signalling pathways via transcriptome analysis are needed to elucidate the clinical significance of the somatically acquired mutations in melanocytic naevi and adjacent skin. Finally, future studies will hopefully shed more light on the mechanisms of naevogenesis, their maintenance in a state of equilibrium, their involution and their transformation into melanoma.

Key Message

The molecular hallmarks of benign naevi provide a greater understanding of early melanoma development.

Disclosure Statement

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