

## Case Report/Case Series

# Dark Homogeneous Streak Dermoscopic Pattern Correlating With Specific *KIT* Mutations in Melanoma

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**IMPORTANCE** Mutations driving melanoma growth have diagnostic, prognostic, and therapeutic implications. Traditional classification systems do not correlate optimally with underlying melanoma growth-promoting mutations. Our objective was to determine whether unique dermoscopic growth patterns directly correlate with driving mutations.

**OBSERVATIONS** We evaluated common driving mutations in 4 different dermoscopic patterns (rhomboidal, negative pigmented network, polygonal, and dark homogeneous streaks) of primary cutaneous melanomas; 3 melanomas per pattern were tested. Three of the 4 patterns lacked common mutations in *BRAF*, *NRAS*, *KIT*, *GNAQ*, and *HRAS*. One pattern, the dark homogeneous streaks pattern, had unique *KIT* mutations in the second catalytic domain of *KIT* in exon 17 for all 3 samples tested. Two tumors with the dark homogeneous streaks pattern turned out to be different primary melanomas from the same patient and had different sequence mutations but had an impact on the same *KIT* domain.

**CONCLUSIONS AND RELEVANCE** While future study is required, these results have multiple implications. (1) The underlying melanoma-driving mutations may give rise to specific dermoscopic growth patterns, (2) *BRAF*/*NRAS* mutations in early melanomas may not be as common as previously thought, and (3) patients may be predisposed to developing specific driving mutations giving rise to melanomas or nevi of similar growth patterns.

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Primary cutaneous melanomas are typically categorized by clinicohistopathologic features, namely, superficial spreading melanoma (SSM), lentigo maligna melanoma, acral lentiginous melanoma, and nodular melanoma (NM). They have also been subcategorized based on the degree of sun damage: chronic sun damage, intermittent sun damage, or nonchronic sun damage.<sup>1</sup> Molecular studies have revealed *BRAF*, *NRAS*, and *KIT* are the common and major growth-promoting mutated genes involved in melanoma pathogenesis.<sup>1-5</sup> *HRAS* and *GNAQ* mutations have been reported in cutaneous melanoma, but they are much less frequent.<sup>5,6</sup> Several publications have shown that *BRAF* and *NRAS* mutations are present in up to 50% and 20%, respectively, of cutaneous melanomas.<sup>2,3</sup> *BRAF* and *NRAS* are more prevalent in the vertical growth phase and in fast-growing cutaneous melanomas, especially *NRAS*.<sup>7,8</sup> These mutated genes can be partially correlated with the current clinicohistopathologic classification system in which *BRAF* mutation is more likely to be present in SSM, and *NRAS* in NM.<sup>2,3</sup> *BRAF* and *KIT* mutations have also been shown to predominate in melanomas arising in intermittently sun damaged and chronically sun damaged skin, respectively.<sup>1,4</sup> While there is a correlation of the driving mutation with the current classification scheme,

the correlation overall is modest, and for a considerable fraction of melanomas, the growth-promoting mutation is unknown.

Growth-promoting mutations activate the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway, which promotes DNA replication, cell cycle progression, and, ultimately, tumor growth. Only certain mutations seem to be able to activate these proteins. All the reported *NRAS* gene mutations are located in exon 2 and exon 3 (previously termed *exon 1* and *exon 2*); most are present in exon 3 at codon 61, where the common amino acid substitutions are Q61K and Q61R. *BRAF* gene mutations are located in codon 600, usually converting valine to glutamic acid.<sup>2,3</sup> Mutations in *KIT* are more widely distributed and frequently affect exons 11 and 13, but mutations in exon 17 and 18 have been reported. The usual amino acid substitutions for *KIT* are L576P, K642E, D816H, and A829P.<sup>1,4</sup> Although *HRAS* and *GNAQ* mutations are more likely to be found in Spitz nevi and blue nevi, respectively, there have been 3 reported cases of *HRAS* (Q61H/L/K) mutations and 2 reported cases of *GNAQ* (Q209L/P) mutations in cutaneous melanoma.<sup>5,6</sup> In particular, *GNAQ* mutations were present within melanomas with chronically sun-damaged areas.<sup>6</sup>

While the commonly identified melanoma growth-promoting mutations all drive the MAPK/ERK pathway, they also interact with other regulatory pathways, which may alter their growth pattern. Dermoscopy allows for improved visualization of tumor structures and increased diagnosis of thin or early cutaneous melanomas. We hypothesize that tumors with similar “dermoscopic” surface growth patterns will have similar underlining growth-promoting mutations and that ultimately dermoscopic growth patterns may serve to better segregate melanoma subtypes and genotypes than the current classification system.

## Reports of Cases

### Study Set

Our study was approved by the institutional review board of the University of Miami. A collection of 182 dermoscopic images of melanomas from the University of Miami Melanoma Program and Plantation Cutaneous and Surgery Clinic from 2000 to 2011 were reviewed by 3 of us (M.I.S., H.S.R., and J.M.G.). Primary cutaneous melanomas with similar dermoscopic features were grouped together based on dermoscopy features from Argenziano et al,<sup>9</sup> Pizzichetta et al,<sup>10</sup> Keir,<sup>11</sup> and our own experience. At least 10 different primary dermoscopic growth patterns were noted, including disorganized fine network,<sup>9</sup> epidermal crease sparing, gray-dotted regression,<sup>9</sup> homogeneous,<sup>9</sup> multicolor multicomponent,<sup>9</sup> perifollicular pigmented,<sup>9</sup> rhomboidal (grayish brown pigmentation surrounding the follicular ostia),<sup>9</sup> polygonal (irregular grayish brown pigmentation outlining the limits between hair follicles forming multiple-sided figures),<sup>11</sup> negatively pigmented network (light areas forming the “cords” of the network and darker areas filling the holes),<sup>10</sup> and dark homogeneous streaks patterns (structureless, brownish black pigmentation with bulbous projections or streaks at the edge)<sup>9</sup> (Figures 1, 2, and 3 and eFigure 1 and eFigure 2 in Supplement). Four of the 10 melanoma patterns were selected for genetic analysis based on the dermoscopic pattern being relatively uniform throughout the entire lesion (reducing the chance of different mutations in different areas of the lesion) and the availability of at least 3 cases with adequate tissue in the formalin-fixed, paraffin-embedded (FFPE) blocks. The 4 patterns selected for genetic analysis were the rhomboidal (eFigure 1 [Supplement]), negatively pigmented network (Figure 2), polygonal (eFigure 2 [Supplement]), and dark homogeneous streaks pattern (Figure 3). The Breslow thicknesses ranged from in situ to 1.1 mm in the lesions tested. Individually, the depths of the rhomboidal, polygonal pattern, negatively pigmented network, and dark homogeneous streaks patterns ranged from in situ to 0.55 mm, in situ to 0.32 mm, 0.4 to 0.46 mm, and 0.75 to 1.1 mm, respectively (eTable 1 [Supplement]). Histologic reports classified the lesions as melanoma in situ and melanoma for the rhomboid and polygonal patterns. For the negatively pigmented network and dark homogeneous streaks patterns, the histologic reports classified the lesions as melanoma and superficial spreading melanoma, respectively. An associated nevus precursor was not

noted for any of the tested melanomas. The specific comments on the morphologic characteristics for each pattern are noted in eTable 1 (Supplement).

### Genetic Testing

Three cases were analyzed for each of the 4 specific dermoscopic growth patterns chosen for study. All of the cases were from unique patients except for 2 of the cases of dark homogeneous streaks pattern, which turned out to be different primary tumors from the same patient. To maximally enrich the specimens for tumor DNA, the slides were deparaffinized and immunohistochemically stained with a cocktail of anti-melan A (Abcam) and anti-S100 (Biocare Medical). NovaRed (Vector Laboratories) was used as a chromagen. Melanoma cells and keratinocytes (as controls) from each case were specifically and separately captured within different 0.5-mL tubes using the Laser Capture microdissection system (Leica Microsystems). Each sample went through DNA extraction using a QIAamp kit (QIAGEN) and amplification with a REPLI-g kit (QIAGEN). Polymerase chain reactions (PCRs) for all the samples were performed using a KOD Hot Start Master Mix (Novagen), primers (Integrated DNA Technologies) (described in eTable 2 [Supplement]). Next, DNA was extracted and amplified on a Master Cycle Pro S device (Eppendorf) using the following parameters: 95°C for 2 minutes, 42 cycles of 95°C for 20 seconds, and an annealing temperature as described in eTable 2 for 17 seconds and at 70°C for 17 seconds. The final elongation was at 70°C for 20 seconds. The PCR products were analyzed on agarose gels, and bands were purified with a MinElute gel extraction kit (QIAGEN) if not sufficiently pure for sequencing. The products were directly sequenced in both forward and reverse directions in the Genewiz Laboratories. Sequence output was reviewed and visually analyzed for the presence of secondary peaks indicating mutations.

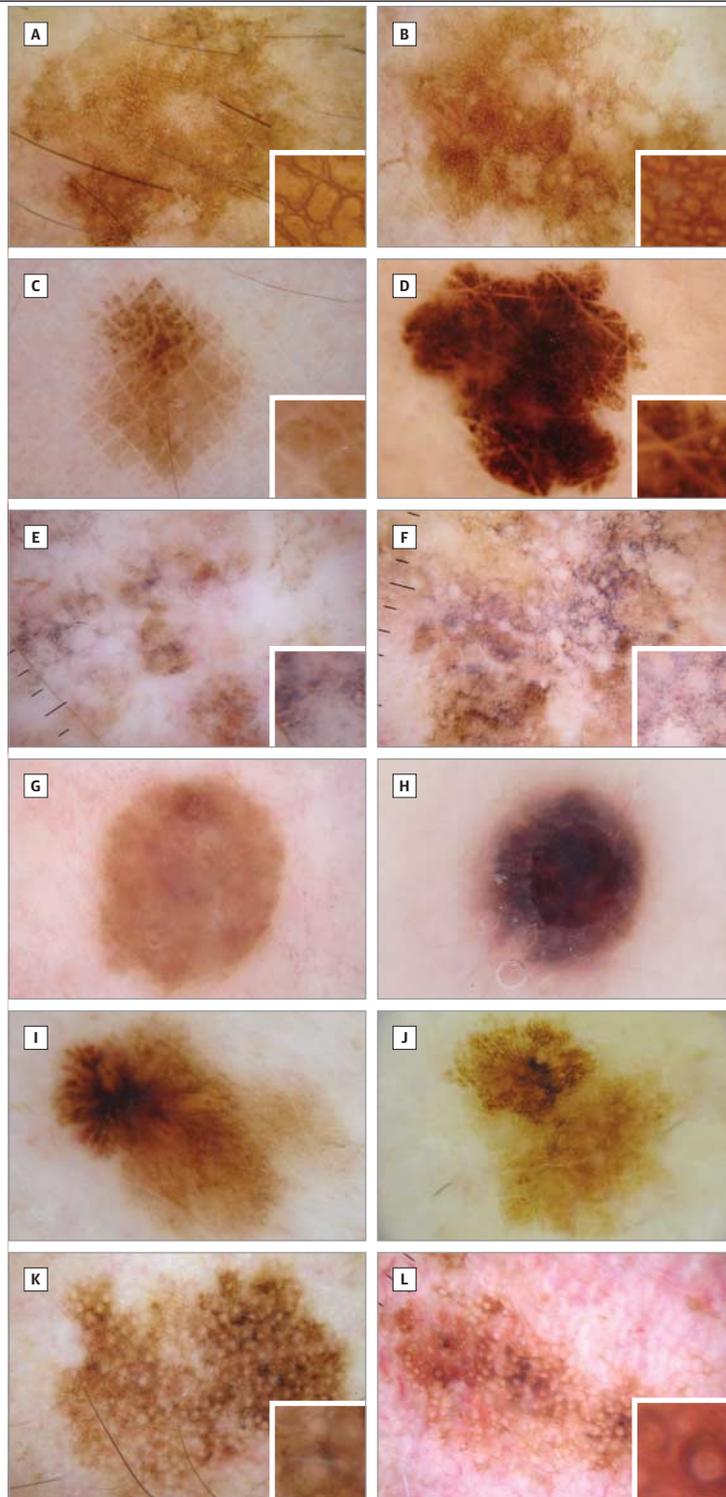
## Results

DNA isolated from rhomboid, negatively pigmented network and polygonal dermoscopic growth pattern groups were wild type for *BRAF*, *NRAS*, *HRAS*, *GNAQ*, and *KIT*. DNA isolated from the dark homogeneous streaks pattern group revealed *KIT* mutations in the catalytic loop of the distal kinase domains H790Q, A794V, and I798M (Figure 4) (eTable 3 [Supplement]). All 3 of these point mutations were novel. The dark homogeneous streaks pattern group was wild type for *BRAF*, *NRAS*, *HRAS*, and *GNAQ*.

## Discussion

Dermoscopy allows for visualization of melanoma growth patterns. Using a dermoscopy-based, pattern-matching approach, we have identified 3 melanoma growth patterns that lack mutations in *BRAF* (exon 15), *NRAS* (exons 2 and 3), *HRAS* (exons 2 and 3), *GNAQ* (exon 5), and *KIT* (exons 11, 13, 17, and 18). Despite the high prevalence of *BRAF* and *NRAS* mutations in melanomas as a whole, all of these tested patterns were

Figure 1. Melanoma Dermoscopic Growth Patterns

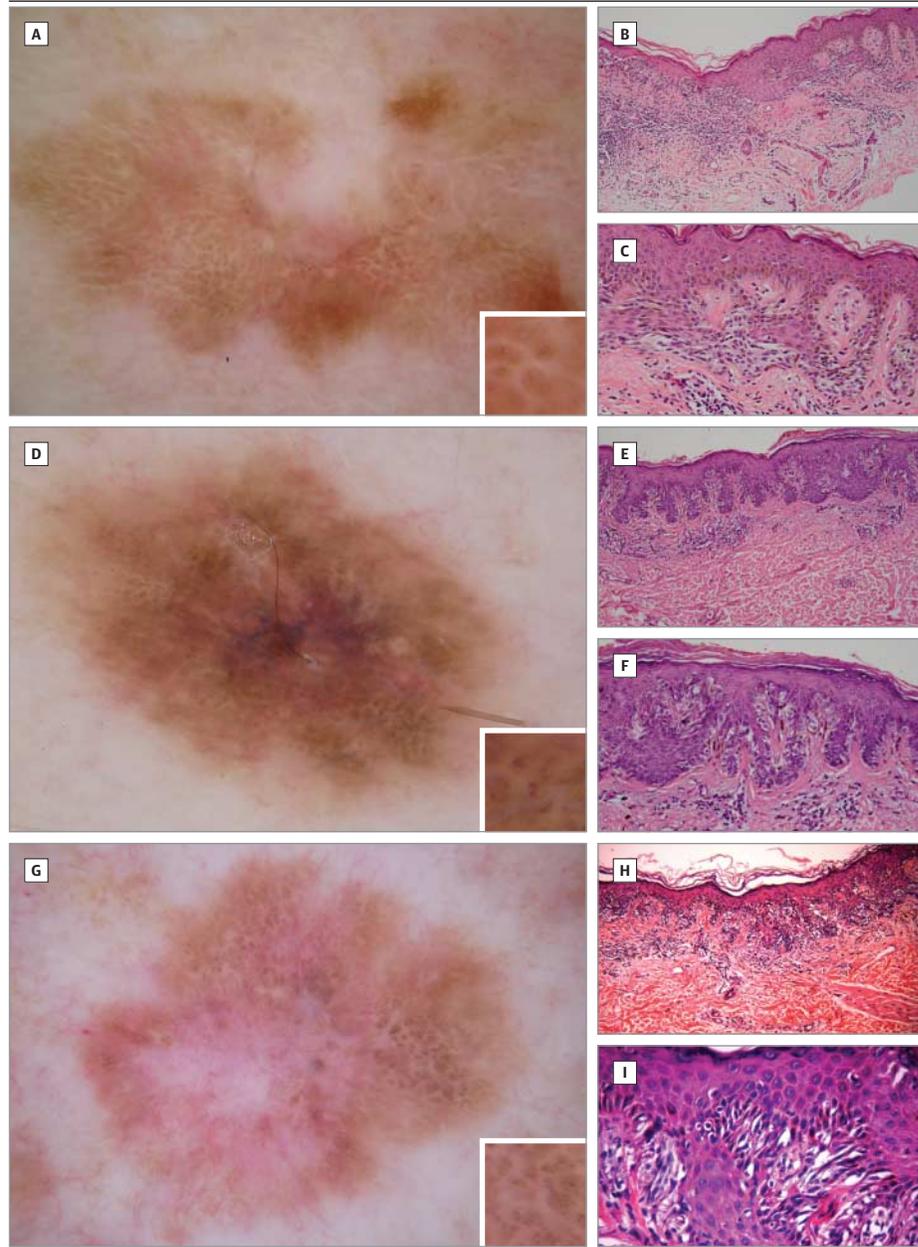


Shown are the 6 dermoscopic patterns that were not subjected to genetic analysis. A and B, Disorganized fine network pattern; the insets show a brown network structure with irregular holes and lines. C and D, Epidermal crease sparing pattern; insets show well-defined epidermal creases surrounded by pigmented structureless areas. E and F, Gray dotted-regression pattern; insets show gray dots and areas of regression. G and H, Homogeneous pattern; the images show light and dark brown irregular structureless areas. I and J, Multicolor multicomponent pattern; the images show light brown, dark brown, and black colors with structureless areas, an irregularly pigmented network, and asymmetrical fingerlike projections. K and L, Perifollicular pigmented pattern; inset shows eccentric annular pigmentation around follicular ostia.

wild type. This may merely be due to a selection bias, but it is also possible that *BRAF* and *NRAS* are overrepresented in published data sets owing to increased growth, tumor bulk and availability, and lethality. Reports have noted that fast-growing and vertical growth phase melanomas are more frequently mutated for *BRAF* and *NRAS*.<sup>8</sup> Ellerhorst<sup>12</sup> et al

have shown that the median Breslow thicknesses of *BRAF*- and *NRAS*-mutated melanomas were 1.28 mm and 1.4 mm, respectively, whereas for wild-type melanomas the median was 0.93 mm. Thus, it is possible that thinner and low-risk tumors will be found to have fewer *BRAF* and *NRAS* mutations than previously thought. Since the rhomboid, nega-

Figure 2. Negative Pigmented Network Pattern



Dermoscopic images with negative pigmented network features of the 3 melanomas (A, D, and G) with their corresponding histologic images at lower and high magnifications (B, C, E, F, H, I). All 3 have similar negative network pattern with light areas forming the "cords" of the network and darker areas filling the holes (insets). B, C, E, F, H, I: hematoxylin-eosin; B, E, and H, original magnification  $\times 4$ , C and F,  $\times 10$ , I,  $\times 20$ .

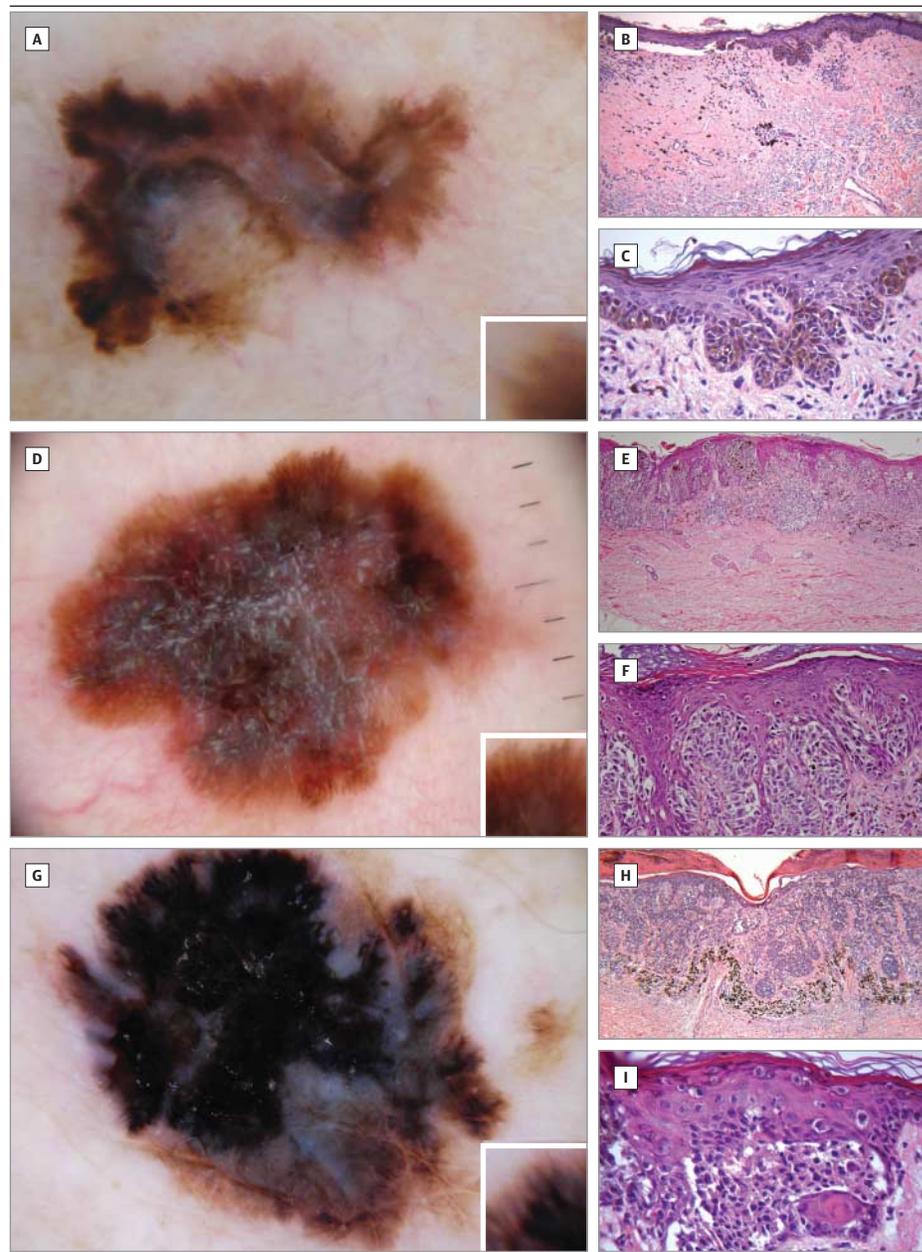
tive, and polygonal dermoscopic patterns of melanoma did not demonstrate common driving mutations, this pattern of tumors could be used to identify as-yet unknown melanoma growth-promoting gene mutations. They may also uncover a set of mutations that occur prior to the development of *BRAF* and *NRAS* mutations if *BRAF* and *NRAS* mutations occur later in progression.

The dark homogeneous streaks pattern was found to have a specific *KIT* gene mutation. *KIT* mutations have been previously reported to be present in 4.3% of the cutaneous melanomas<sup>1-4</sup>; of them, 4.4% were from exon 17, which suggests that only 0.19% of cutaneous melanomas will have a mutation in exon 17. Thus, the chance that 3 out of 3 melanomas would have mutations in *KIT* exon 17 is 6.8 in 1 billion; there-

fore, it is rational to speculate that this specific mutation gives rise to this specific dermoscopic growth pattern. Although all the other cases of melanomas studied were from unique patients, it is also of interest that 2 of the dark homogeneous streaks pattern were from different primary tumors on the same patient. While *KIT* was mutated in both primary tumors, the specific mutated sequence was different, confirming the different origins of the 2 tumors. Patients often have similar mole patterns, and it has also been noted that patients with multiple melanomas often have tumors with similar patterns.<sup>13</sup> Thus, it is possible that patients are predisposed to developing melanomas with mutations in specific pathways.

Our *KIT*-mutated tumors were dark, and this is consistent with the findings of Wu et al,<sup>14</sup> who previously reported

Figure 3. Dark Homogeneous Streak Pattern



Dermoscopic images with streaks and homogeneous features of the 3 melanomas (A, D, and G) with their corresponding histologic images at lower and high magnifications (B, C, E, F, H, and I). All 3 have a similar pattern of dark homogeneous areas with streaks at the margins (insets). B, C, E, F, H, I: hematoxylin-eosin; B, E, and H, original magnification  $\times 4$ , C, F, and I,  $\times 20$ .

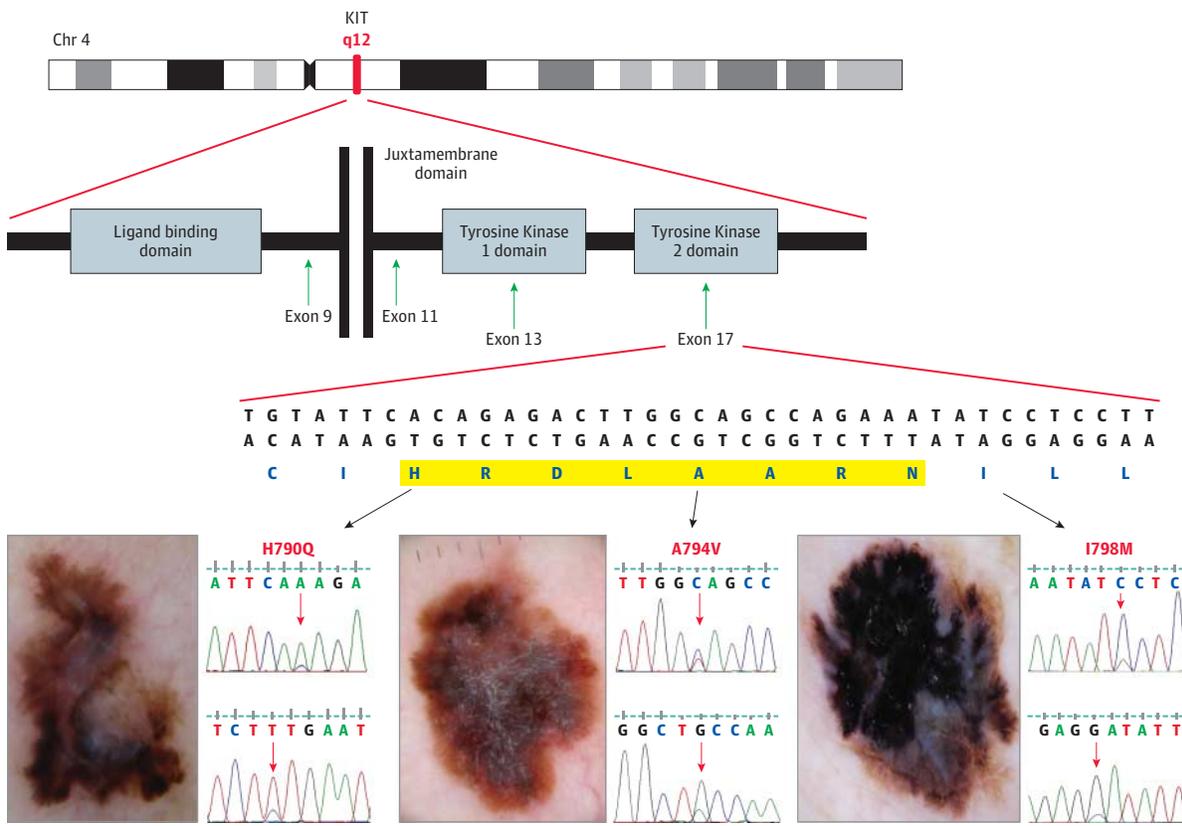
that *KIT* is mutated and/or overexpressed in darkly pigmented cutaneous melanomas. *KIT* mutations have been previously related to a histologic lentiginous pattern present in chronically sun-damaged areas.<sup>1</sup> Two of the *KIT*-positive melanomas presented herein were described histologically as lentiginous, but only 1 was in a chronically sun-damaged area.

The 3 mutations noted herein are novel and are all located in the second kinase domain, within or next to the catalytic loop. This domain is thought to maintain the enzyme in its active form.<sup>15</sup> Given that *KIT* also has mutations in other domains and interacts with a number of different pathways, the different *KIT* mutations may eventually be found to give rise to different subsets of melanoma growth patterns.

## Conclusions

In summary, one of the dermoscopic patterns, the dark homogeneous streaks pattern, seems to correlate with *KIT* mutations in or around the second kinase domain, suggesting the possibility that specific growth patterns are caused by specific driving mutations. Two of the similarly patterned primary tumors occurred in the same patient, and, while having different specific nucleotide changes, the mutations occurred in the same domain of the same gene, suggesting the possibility that patients may have a predisposition to develop and/or maintain certain mutations.

Figure 4. Dark Homogeneous Streak Pattern Revealing Unique Mutations in Exon 17



The location of the *KIT* gene on chromosome 4. Exon 17 includes the second tyrosine kinase domain. The highlighted bases are those included in the catalytic domain. All 3 mutations identified are in or directly next to the catalytic

domain and are shown in the sequences in the panel to the right of each tumor image.

Three of the 4 patterns tested lacked common melanoma-driving mutations suggesting the possibility that these patterns could be used to identify other novel driving genes and/or identify genes that are mutated in early melanoma

prior to the development of the common driving mutations. Further study is required, but it is possible that dermoscopic growth patterns will lead to improved molecular classification of melanocytic neoplasias.

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Study concept and design: Sanchez, Grichnik.

Acquisition of data: Sanchez, Rabinovitz, Oliviero, Elgart, Perez, Puig, Malveyh.

Analysis and interpretation of data: Sanchez, Grichnik.

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