**BRAF, NRAS, and GNAQ Mutations in Conjunctival Melanocytic Nevi**

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**PURPOSE.** To evaluate **BRAF, NRAS,** and **GNAQ** mutations in surgical specimens of common and blue conjunctival melanocytic nevi.

**METHODS.** Surgical specimens from 25 conjunctival melanocytic nevi (23 common and 2 blue) of 25 patients were evaluated. All common nevi were analyzed immunohistochemically for the expression of **BRAF** V600E or **NRAS** Q61R. One lesion with negative immunoreactivity for both **BRAF** and **NRAS** Q61R was found to harbor an **NRAS** Q61K mutation by sequence analysis. Patients with **NRAS**-mutated nevi were more likely to report occurrence of the lesion prior to 18-years old and more likely to have intrinsic cysts. The mean largest basal diameter was 6.0 and 3.5 mm for **NRAS** and **BRAF**-immunoreactive lesions, respectively (P = 0.003). **GNAQ** mutations were identified in each of the two blue nevi of this study.

**RESULTS.** Of common melanocytic nevi, 9 (39.1%) were immunoreactive for **NRAS** Q61R and 13 (56.5%) were immunoreactive for **BRAF** V600E. One common nevus, which was immunonegative for both **BRAF** V600E and **NRAS** Q61R was found to harbor an **NRAS** Q61K mutation by sequence analysis. Patients with **NRAS**-mutated nevi were more likely to report occurrence of the lesion prior to 18-years old and more likely to have intrinsic cysts. The mean largest basal diameter was 6.0 and 3.5 mm for **NRAS** and **BRAF**-immunoreactive lesions, respectively (P = 0.003). **GNAQ** mutations were identified in each of the two blue nevi of this study.

**CONCLUSIONS.** These findings document that common conjunctival melanocytic nevi have mutually exclusive mutations in **BRAF** and **NRAS.** The two conjunctival blue nevi harbored **GNAQ** mutations. This suggests the driver mutations of conjunctival nevi are similar to those of nevi of the skin. At the molecular level, conjunctival nevi appear more like cutaneous nevi than choroidal nevi.

Keywords: conjunctiva, nevi, genetics, melanoma, eye
Patient data included sex, age, and ethnicity. Clinical data included initial occurrence of conjunctival nevus (juvenile [18 years or younger] or adulthood), age at time of excision, ocular site (bulbar, palpebral, caruncle), site of ultraviolet light exposure (intrapalpebral fissure or otherwise), iris pigmenta-
tion, largest basal diameter, degree of pigmentation (amelanot-
ic, melanotic, deeply melanotic, or amelanotic/melanotic),
thickness (flat or raised), and presence of intrinsic cysts,
intrinsic vessels, or sentinel vessels. Location was defined
histopathologically (compound, subepithelial, etc.). Clinical
data was not available for the de-identified conjunctival blue
nevus specimens. Representative clinical images are shown in
Figures 1, 2, and 3.

![Figure 1](image1.png)

**Figure 1.** Conjunctival nevus occurring in childhood. (A) Clinical
photo demonstrating intrinsic cysts, diameter of 3.5 mm and
amelanotic appearance. (B) Histopathology of a compound melano-
cytic nevus with epithelial cysts and evidence of maturation (×10,
hematoxylin and eosin). (C) The junctional and subepithelial
melanocytes are immunohistochemically positive for NRASQ61R
(×10, peroxidase antiperoxidase).

For the single sample that did not reveal a mutation by the
above method and the blue nevi, the MSKCC IMPACT assay was
used as previously described, 16 on formalin-fixed paraffin
embedded tissue.

For statistical analysis, continuous variables (age, age at
excision, and largest basal diameter) were compared with a
Student’s t-test and all categoric variables (clinicopathologic
parameters) were compared with Fisher’s Exact Test. Analysis
was performed with Prism 7 (Graphpad Software, Inc., La Jolla,
CA, USA) and a P value < 0.05 was considered statistically
significant.

**RESULTS**

Of nevi, nine (40.9%) were immunoreactive for NRAS Q61R
and 13 (56.5%) were immunoreactive for BRAFV600E
mutations. One specimen lacked immunoreactivity for all immuno-
histochemistry targets, for which the MSK-IMPACT assay
identified an NRASQ61K mutation. Both blue conjunctival
nevii had a mutation in GNAQ (Q209L and Q209H) detected by
MSK-IMPACT.

For the common nevi, the median age at the time of
excision was 13.5 and 29 years for patients with an NRAS
and BRAF mutated lesion, respectively (P = 0.09). The Table shows
associations of NRAS and BRAF expression or mutational status
with clinicopathologic features. There were three parameters
that showed a statistical difference between patients with an
NRAS- and BRAF-immunoreactive lesion: patients with NRAS-
immunoreactive lesions were significantly more likely to report
occurrence of the lesion prior to 18-years old. In addition,
NRAS-immunoreactive lesions were significantly more likely to

either homogeneous immunoreactive for the respective marker
or completely negative.
have intrinsic cysts and have a largest basal diameter more than 5 mm. The mean largest basal diameter was 6.0 and 3.5 mm for NRAS- and BRAF-immunoreactive lesions, respectively ($P = 0.003$).

At a mean follow-up of 13.1 months, there were no events of recurrence or malignant transformation.

**DISCUSSION**

Ocular nevi differ in their genetic underpinnings depending on the anatomical derivation of their melanocytes. Nevi derived from uveal tract melanocytes harbor GNAQ/11 mutations. On the contrary, conjunctival nevi are more akin to their cutaneous counterpart and have been shown to have BRAF mutations. The present study confirms the similarity between cutaneous and conjunctival nevi by identifying further genetic aberrations and associations with clinical features that are found in cutaneous nevi.

Cutaneous nevi have NRAS and BRAF mutations. BRAF is a serine-threonine kinase and NRAS an isoform of the RAS family of GTPase proteins; and both activate the MAPK signaling cascade, which leads to cell cycle progression and cell proliferation. Similarly, for all the conjunctival nevi in this study, immunoreactivity and mutational analysis allowed for identification of either an NRAS or BRAF aberration. For this cohort, NRAS and BRAF mutations occurred in a mutually exclusive manner, with just over half the lesions exhibiting immunoreactivity with the antibody VE1, thereby indicating the presence of a BRAFV600E mutation.

For cutaneous nevi, the presence of either a BRAF or NRAS mutation has been associated with specific clinical features. For instance, congenital cutaneous nevi are more likely to harbor NRAS mutations, while acquired nevi have a propensity toward BRAF mutations. One prior study on conjunctival nevi, that evaluated for BRAF mutations only, found no difference in the proportion of children and adults with mutant lesions. On the contrary, in our series, NRAS mutations were statistically more frequent in patients reporting the occurrence of their conjunctival nevus prior to the age of 18 years. However, the retrospective nature of this study limited our understanding of each lesion’s occurrence to patient reporting. Furthermore, conjunctival nevi, particularly those that are congenital, are commonly amelanotic and difficult to fully appreciate overlying the white sclera; thereby, possibly influencing the recognition of their true occurrence date.

In cutaneous nevi, there is evidence to suggest that larger congenital lesions more commonly harbor NRAS mutations, while smaller lesions may have either BRAF or NRAS mutations. In line with this finding, in the present study, conjunctival nevi with a basal diameter greater than 5 mm

### Table. Associations of NRAS and BRAF Expression or Mutational Status With Clinicopathologic Features in Common Conjunctival Nevi

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All, $n = 23$</th>
<th>NRAS, $n = 10$</th>
<th>BRAF, $n = 13$</th>
<th>$P$ Value</th>
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<tr>
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<td>6</td>
<td>10</td>
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UV, ultraviolet; amel, amelanotic; mel, melanotic.
were statistically more likely to have NRAS-immunoreactivity (P = 0.006). Evaluated another way, the mean largest basal diameter was significantly greater (almost double) in NRAS-immunoreactive lesions compared with BRAF-immunoreactive nevi. Finally, intrinsic cysts were statistically more likely in NRAS-immunoreactive lesions; which may be a clinical marker for juvenile/congenital nature of these lesions.

The risk of malignant transformation of conjunctival nevi into melanoma is predicted to be 1% (Gerner et al.18), and more common in adult-onset lesions compared with those that appear in childhood. Like conjunctival nevi, up to 14% to 50% of conjunctival melanomas have been found to harbor BRAF mutations and 18% have NRAS mutations (Griewank et al.7 Lake et al.6 Goldenberg-Cohen et al.7 Gear et al.9 Riechardt et al.10). In one study, BRAF mutant conjunctival melanomas were associated with a caruncle location and in another study with young age, male sex, sun-exposed location, mixed/nonpigmented color and nevus origin (Larsen et al.19 Griewank et al.3). However, this latter study found no association between BRAF mutant status and prognosis. Along the same lines, Gear and colleagues9 found no correlation between BRAF mutations and location or other clinicopathologic characteristics (Gear et al.9). Therefore, the implications of BRAF mutations in conjunctival melanoma are controversial, and the potential of malignant transformation of nevi has an unknown association with BRAF or NRAS status.

It is proposed that lesions with GNAQ mutations possess the unique traits of being derived from extrapapithelial melanocytes and particularly those that originate from cranial neural crest cells.11 Therefore, it would be conceivable that conjunctival blue nevi would be GNAQ mutant. This theory is strengthened by the discovery that cutaneous, central nervous system, and oral cavity blue nevi have GNAQ mutations.10–15 In keeping with this prediction, the present study demonstrates GNAQ mutations in both conjunctival blue nevi. Ultraviolet light exposure is not thought to play a role in GNAQ mutations, and remains debatable in BRAF and NRAS mutations. This shared genetic aberration in both cutaneous and conjunctival derived nevi is another example of the similarity between these two lesions from differing (skin and conjunctiva) anatomic origins.

Immunohistochemistry techniques for BRAF V600E and NRAS Q61R are highly sensitive and specific.20–22 In our series, there was only one patient with negative immunohistochemistry for all targets. Instead, this specimen was evaluated with the MSK-IMPACT assay,23 which revealed a less common mutation in NRASQ61K, which is not recognized by the antibody SP174 The MSK-IMPACT assay is an invaluable tool with the advantage providing a combination approach for the detection of multiple categories of genetic alterations, and is particularly useful at identifying less common alterations that may not be easily detected through immunohistochemistry.

In summary, our findings confirm the parallels that exist between cutaneous and conjunctival melanocytic lesions. Our common conjunctival nevi cohort revealed data to support mutually exclusive genetic alterations of BRAFV600E and NRAS, with slightly higher presence of the former. Like cutaneous nevi, the NRAS-immunoreactivity was more common in larger lesions with an earlier occurrence in life. The similarities extend to blue nevi where we demonstrate a common genetic aberration in GNAQ, which were present in two blue nevi specimens. These findings would benefit from validation with a larger cohort study. Prior concerns of specialists in the field point to the semantic problems with classifying pigmented lesions of the conjunctiva.23 The association between genetics and histopathology of nevi was limited to blue versus nonblue nevi (and did not extend to distinguishing among common nevi); however, genetics did suggest an association with some clinical features of common nevi. These findings open up the possibility of organizing pigmented lesions molecularly or genetically, and this in turn may relate to pathogenesis and ultimately inform treatment approaches.

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References


