Importance Accurate diagnosis of choroidal melanoma is challenging and has important implications for both physicians and patients. We assessed the utility of quantification of fundus autofluorescence in the evaluation and follow-up of choroidal nevomelanocytic tumors.

Objective To assess the utility of autofluorescence quantification in distinguishing clinically diagnosed choroidal nevi, melanoma, and indeterminate nevomelanocytic lesions.

Design, Setting, and Participants A retrospective observational study from 2006 to 2012 of patients with choroidal nevomelanocytic lesions who had digital autofluorescence and color fundus imaging performed at the University of Michigan Kellogg Eye Center.

Intervention ImageJ software was used to output autofluorescence gray-scale values for each pixel of a 500 × 50-pixel region within each lesion and a corresponding adjacent control region.

Main Outcome and Measure A single value was generated, termed the Index of Retinal Autofluorescence (IRA), to represent the total difference in gray-scale values between the 2 regions in each affected eye.

Results Thirteen of the 14 clinically diagnosed nevi exhibited an IRA less than 150 gray-scale intensity squared (gsi²). Eight of 9 clinically diagnosed melanomas exhibited an IRA more than 150 gsi². An IRA of 150 gsi² distinguished nevi from melanomas with a sensitivity of 0.89 and specificity of 0.93. Fifteen of 19 patients with indeterminate nevomelanocytic lesions underwent clinical assessment and initial imaging with clinical follow-up at a median of 10 months. All 3 patients with an IRA less than 150 gsi² showed no evidence of clinical progression and 6 of 12 lesions with an IRA more than 150 gsi² showed clinical progression to melanoma. An IRA of 150 gsi² identifies indeterminate lesions that progressed to melanoma with a sensitivity of 1.00 and specificity of 0.33.

Conclusions and Relevance Quantification of digital autofluorescence images can differentiate between clinically benign and malignant choroidal nevomelanocytic lesions and may be predictive for clinical progression of indeterminate lesions.
Choroidal nevi are benign nevomelanocytic lesions with a prevalence of about 6.5% in white individuals. Approximately 1 of 9000 choroidal nevi convert to melanoma annually. At presentation, choroidal nevomelanocytic lesions are classified as nevi, melanomas, or indeterminate tumors. Differentiating indeterminate tumors from choroidal melanomas remains challenging as does the early detection of their conversion to melanoma. Combinations of tumor growth, thickness, and diameter as well as the presence of subretinal fluid, orange pigmentation, visual symptoms, tumor margin within 3 mm of the optic disc, and the absence of drusen and retinal pigment epithelial alteration all have been reported to indicate malignancy. A lesion with no risk factors is a nevus while the presence of risk factors alone or in combination is found in indeterminate lesions and melanomas. To our knowledge, there is currently no reliable quantitative method to further stratify the risk of progression of indeterminate lesions.

Autofluorescence of lipofuscin that accumulates in the retinal pigment epithelium and macrophages over choroidal tumors has been correlated with orange pigmentation seen in color fundus photographs. This phenomenon has been described qualitatively for benign and malignant choroidal nevomelanocytic tumors and found to correlate with malignancy. Subsequently, studies were designed in which experts classified autofluorescence changes and attempted to compare them with fundus color photograph features of clinically diagnosed nevi and melanomas. However, to our knowledge, quantification of autofluorescence in choroidal lesions has not been previously attempted.

Because variations in autofluorescence have been shown to correlate with malignancy, we hypothesized that quantification of autofluorescence may be useful as an objective measure of the malignant potential or malignancy of choroidal nevomelanocytic tumors. We anticipated that nevi and melanomas would exhibit relatively homogeneous and heterogeneous autofluorescence images, respectively. Accordingly, indeterminate choroidal nevomelanocytic lesions with autofluorescence profiles similar to nevi would not progress while those with profiles similar to melanoma would be more likely to progress to malignancy.

Methods

This study was performed at the University of Michigan Kellogg Eye Center after institutional review board approval. Autofluorescence images were obtained using the Heidelberg Spectralis (Heidelberg Engineering) with excitation at 488 nm to illuminate the fundus and detection of emitted fluorescence between 500 and 700 nm. Autofluorescence images were taken by 1 of 4 trained photographers after pupillary dilation. A database of patients with clinical diagnoses of choroidal nevus, melanoma, or indeterminate choroidal nevomelanocytic lesion and color and autofluorescence imaging was generated. Only patients with images centered on the lesion and of good clarity were enrolled. The clinical diagnosis was determined using a combination of conventional risk factors. These risk factors included thickness more than 2 mm, presence of subretinal fluid, visual symptoms, presence of orange pigment, lesions within 3 mm of the optic disc, absence of drusen, and low internal reflectivity on A-scan ultrasonography. Lesions were defined as follows: thickness less than 2 mm with no other risk factors = nevus; thickness greater than 2.5 mm with 2 other risk factors = melanoma; and not otherwise indicated = indeterminate. Patients with indeterminate lesions were observed regularly after the initial evaluation. Progression of an indeterminate lesion to melanoma was determined by conventional criteria.

The autofluorescence photograph and corresponding digital file for each lesion were analyzed with ImageJ (http://rsbweb.nih.gov/ij/). The color fundus photograph was referenced to ensure proper determination of the borders of each lesion (Figure 1). The 8-bit gray-scale intensity values of a 500 × 50-pixel area of the lesion demonstrating maximal autofluorescence were objectively determined by summing all 25,000 values of each unique 500 × 50-pixel area contained within the lesion. The highest summed area was then extracted for further analysis. An adjacent unaffected area was also extracted. The extracted gray-scale values were averaged across each column to yield 500 values that generated a unique autofluorescence signature (Figure 2). To obtain the Index of Retinal Autofluorescence (IRA), the signatures of the tumor and adjacent control area were then compared using a modification of a sum of the differences squared technique. The IRA is measured in gray-scale intensity squared (gsi²). Statistical analysis was performed using the Mann-Whitney U test as well as generating odds ratios; P < .05 was considered statistically significant. We quantified the statistical spread of the data with median average deviation.

Results

Forty-two patients were identified as having a unilateral choroidal nevomelanocytic lesion. Fourteen (33%) were classified as nevi, 19 (45%) were classified as indeterminate choroidal nevomelanocytic lesions, and 9 (21%) were classified as choroidal melanomas (eTable in Supplement). Twenty-three patients had IRA values more than 150 gsi² and 19 had IRA values less than 150 gsi². For nevi, the median IRA value was 67 gsi² (median average deviation, 45 gsi²) and 13 of the 14 nevi had IRA values less than 150 gsi². The 9 melanomas had a median IRA value of 226 gsi² (median average deviation, 98 gsi²), with 8 of the 9 having an IRA more than 150 gsi². The P value obtained by comparing IRA values of nevi and melanomas was <.001. The median IRA value for the 19 indeterminate lesions was 359 gsi² (median average deviation, 221 gsi²), of which 14 had IRA values more than 150 gsi² and 5 had IRA values less than 150 gsi².

Tumor progression was assessed by sequential clinical examination of 15 of the 19 patients (79%) with indeterminate lesions (median follow-up, 10 months; range, 4.4-12.6 months). Included in the 15 patients with indeterminate lesions were 12 patients (80%) with IRA values more than 150 gsi² and 3 (20%) with IRA values less than 150 gsi². Of
the 3 patients with initial IRA values less than 150 gsi², none showed clinical progression at follow-up (median, 10.0 months; range, 7-10 months), while 6 of the 12 patients (50%) with IRA values more than 150 gsi² exhibited clinical progression at follow-up (median, 8.8 months; range, 4.4-11.4 months) and 6 of the 12 patients (50%) did not exhibit progression at follow-up (median, 10.2 months; range, 5.6-11.0 months). The median duration of follow-up for those lesions that progressed did not differ from those that did not progress (P = .84).

An IRA value of 150 gsi² distinguished melanoma from nevus with a sensitivity of 0.89 and a specificity of 0.93. The IRA values more than 150 gsi² were predictive of indeterminate lesions progressing to melanoma with a sensitivity of 1.00 and a specificity of 0.33. Accepted clinical parameters predicted progression of the indeterminate lesions to melanoma as follows: thickness more than 2.0 mm with a sensitivity of 0.83 and a specificity of 0.93, subretinal fluid with a sensitivity of 0.33 and a specificity of 0.67, orange pigment with a sensitivity of 0.33 and a specificity of 0.67, visual symptoms with a sensitivity of 0.83 and a specificity of 0.67, within 3.0 mm of the optic disc with a sensitivity of 0.33 and a specificity of 0.67, and low internal reflectivity with a sensitivity of 0.00 and a specificity of 1.00.

To determine how IRA correlates with accepted clinical parameters for nevi and melanomas, we determined the odds ratio for each parameter predicting categorical IRA more than 150 gsi². We found that an IRA more than 150 gsi² was highly associated with most accepted clinical parameters: the odds ratio between an IRA more than 150 gsi² and thickness more than 2.0 mm was 10.4 (95% CI, 1.8 to 113.7; P = .003); with subretinal fluid was 9.4 (95% CI, 1.9 to 65.8; P = .002); with orange pigment was 10.4 (95% CI, 1.8 to 113.7; P = .003); with visual symptoms was more than 100 (95% CI, 6.1 to >200; P < .001); and with a lesion within 3.0 mm of the optic disc was 7.4 (95% CI, 1.3 to 80.7; P = .02). The odds ratio between an IRA more than 150 gsi² and low internal reflectivity was 4.8 (95% CI, 0.5 to 249.0; P = .20).
Discussion

Choroidal nevomelanocytic tumors exist on a spectrum from nevus to melanoma and are classified based on a variety of previously reported subjective and objective risk factors. In this study, we sought to develop a novel approach that quantifies autofluorescence images of nevomelanocytic lesions to identify both malignancy and malignant potential. We developed a quantification method that generates a single value, the IRA, that reflects autofluorescence variability of the lesion relative to an adjacent fundus area. We found that an IRA value of 150 gsi² best differentiates nevomelanocytic lesions. Interestingly, quantifying indeterminate choroidal nevomelanocytic lesions with the same method that separated nevi from melanomas also was predictive of progression for indeterminate lesions. None of the indeterminate choroidal nevomelanocytic lesions with IRA values of 150 gsi² or less progressed while half of indeterminate lesions with IRA values more than 150 gsi² did progress to melanoma. The sensitivity for IRA of 150 gsi² in indeterminate choroidal nevomelanocytic lesions was 100% suggesting this method may serve as a screening test to indicate the propensity for malignant progression. In addition, an IRA more than 150 gsi² demonstrated higher sensitivity than any of the accepted clinical parameters, suggesting that indeterminate lesions with an IRA more than 150 gsi² should be closely monitored for clinical evolution to melanoma.

We carefully designed our quantification technique to produce a single value representing the variability of a standardized sample region. Sampling the entire lesion would lead to a single averaged value unlike determining variations in autofluorescence within the lesion compared with variations in autofluorescence in an area of normal fundus outside of the lesion. This is a much more powerful quantification technique because averaging may obscure regions with hyperfluorescence and hypofluorescence and portray them as no change.

The IRA values of nevomelanocytic lesions do not simply reflect the presence of orange pigment that has been shown to be present in macrophages overlying most melanomas to varying degrees and is a known risk factor for malignancy. The autofluorescence used to calculate IRA values is also derived from other fluorophores, including the retinal pigment epithelium overlying the tumor, which demonstrates hypofluorescence and hyperfluorescence due to atrophy and proliferation in response to the neoplasm. Therefore, some lesions that do not have a substantial amount of orange pigment can still have high IRA values based on variability of these fluorophores. The IRA values also reflect local autofluorescence reductions due to hypofluorescent tumor seen through the atrophic retinal pigment epithelium layer. Conversely, orange pigment is never present in nevi but its presence alone is insufficient to establish a diagnosis of melanoma. Therefore, the IRA value is not a replacement for the orange pigment risk factor alone. Accordingly, several lesions with high IRA did not have orange pigment on clinical examination and several lesions with orange pigment on clinical examination did not have high IRA (eTable in Supplement).

Limitations of our pilot study include the small patient population, the length of follow-up for indeterminate lesions, and the small number of patients lost to follow-up as well as tumor and patient factors that can affect autofluorescence variability. Despite the fact that the follow-up period of these patients was short, half of the indeterminate lesions with IRA values more than 150 gsi² progressed, indicating the clinical utility of this method. Although 4 indeterminate lesions did not have follow-up, their clinical characteristics and IRA values did not differ from the other indeterminate lesions and their clinical course was likely to be similar to those we did follow up. Some indeterminate lesions with IRA values of 150 gsi² or less may eventually progress to higher IRA values and convert to melanoma, but we did not rigorously evaluate this possibility in this study. Tumor factors that can affect autofluorescence variability include the presence of hemorrhage as well as tumors sufficiently thick to prevent adequate focusing while patient factors include the presence of a dense cataract and inadequate dilation of the pupil. We attempted to minimize these...
potential causes of autofluorescence variability, as described in the Methods section.

In conclusion, we have developed an objective method to quantify autofluorescence in choroidal nevomelanocytic tumors, which has thus far only been assessed subjectively. The IRA values can be used to distinguish melanomas from nevi and identify the malignant potential of indeterminate lesions. Application of this method provides additional information for diagnosis, prognosis, and treatment decisions for what is currently a challenging clinical entity.

ARTICLE INFORMATION
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REFERENCES