

Sloan, Philip, Husain, Akhtar, Lye, Jonathan, Best, Katie, Walker, Laura, Richardson, Grant, Armstrong, Jane and Lovat, P E (2026) Utility of Combined Immunohistochemical Staining for AMBRA1 and Loricrin for Improving the Pathologic Diagnosis of High-Grade Dysplastic Nevi. The American Journal of Dermatopathology, 48 (9). pp. 691-697.

Version: AAM

Available under license: Creative Commons: Attribution 4.0

Downloaded from: https://sure.sunderland.ac.uk/id/eprint/20859/

Which version am I reading (articles, book chapters and books)

Pre-print (or submitted version): the version of an article or chapter before any peer-review. Can be submitted to a publisher or added to a pre-print server.

AAM: Author Accepted Manuscript - The version of the article formally accepted for publication after the peer-review process. It includes the changes made by the author(s) in line with peer-reviewers' comments. It has not undergone the publisher's formatting, type-setting and copyediting.

VOR: Version of Record - The version published on the publisher's website which includes formatting, typesetting and copyediting.

Usage guidelines

Please refer to the usage guidelines at http://sure.sunderland.ac.uk/policies.html or alternatively

contact sure@sunderland.ac.uk.

Utility of Combined Immunohistochemical Staining for AMBRA1 and Loricrin for Improving the Pathologic Diagnosis of High-Grade Dysplastic Nevi.

Am J Dermatopathol. 2026 Sep 1;48(9):691-697. doi: 10.1097/DAD.0000000000003330.

Epub 2026 May 28. PMID: 42358006.

Authors and affiliations

Philip Sloan[1,2], Akhtar Husain[1], Jonathan Lye[2], Katie Best[1] Laura Walker[1], Grant Richardson[1], Jane Armstrong[3], Penny Lovat[1]

[1] Translation and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, United Kingdom

[2]Cellular Pathology, Newcastle upon Tyne Hospitals NHS Trust, Newcastle upon Tyne

[3]John Dawson Drug Discovery and Development Research Institute, University of Sunderland, Sunderland, United Kingdom

Name and address for correspondence

Philip Sloan, Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, United Kingdom

philip.sloan@newcastle.ac.uk

Conflict of interest

None

Source of funding

The study was supported by an Innovate UK Investor Partnership award, AMLo Biosciences Limited and Cancer Research UK award to Professor Penny Lovat and Dr Katie Best.

For the purpose of open access, the author has applied a Creative Commons Attribution (CC BY) licence to any Author Accepted Manuscript version arising from this submission.

Acknowledgements

The assistance of Claire Jones and Tom Ness and the staff of NovoPath, Newcastle upon Tyne is gratefully acknowledged

Abstract

High grade dysplastic nevi (HGDN) can present a diagnostic challenge for the pathologist. The utility of combined AMBRA1 and loricrin staining for the diagnosis of HGDN was evaluated using PRAME as a comparator.

A cohort of 145 cases of HGDN were selected from a consecutive series of 409 dysplastic nevi (DN) ascertained in a single pathology service between 2015 and 2019. Combined immunohistochemistry for AMBRA1 and loricrin expression in the epidermis overlying the HGDN was consensus scored by two experienced pathologists. PRAME immunohistochemistry was also consensus scored in the melanocyte population using a five-point scale (0-4+).

All HGDN showed maintenance of either one or both AMBRA1 and loricrin epidermal expression with three exceptions. In one instance a focus of melanocytes within the HGDN was associated with loss of both markers in the overlying epidermis and the melanocytes in the focus were strongly positive (4+) for PRAME. On expert review the diagnosis was changed to thin melanoma. In the other two cases showing loss of both markers, absence of staining was focal and restricted to mounds of parakeratosis. The majority of HGDN were negative for PRAME although 48% were positive to some degree and 4% showed 4+ expression.

Clinical follow up showed no evidence of progression or metastasis. We found that 23 patients (16%) had previous, concurrent or subsequent early-stage melanomas and 2 (1%) had previous melanoma in situ. One HGDN recurred after 3 years, and the further excision sample showed recurrent HGDN only.

We observed mild epidermal hyperplasia and parakeratosis were often found in HGDNs suggesting an active melanocyte-epidermal interaction in the lesions. We speculate that epidermal hyperplasia resulted in some of the HGDN showing single cell loricrin gaps (SCG) or thinning of the loricrin band. AMBRA1, loricrin and PRAME staining was additionally performed on 39 benign melanocytic nevi covering a range of types for comparison. Epidermal hyperplasia was generally not seen and almost all cases showed maintenance of both epidermal AMBRA1 and loricrin, with loricrin gaps and thinning seen only in three Meyerson nevi where there was parakeratosis (3/5 Meyerson nevi).

The study suggests that AMBRA1 and loricrin staining is a useful biomarker to confirm the diagnosis and/or to signal low-risk behavior in HGDN, particularly in cases where 4+ PRAME staining could potentially point to a melanoma diagnosis.

Introduction

Dysplastic nevus (DN) was initially described in families with multiple melanocytic lesions having distinctive clinical and pathological features with increased melanoma risk.[1] The term ‘dysplastic nevus’ has since been described in non-syndromic settings and the term remains controversial, with varying criteria for diagnosis and grading proposed.[2] Current diagnostic criteria for dysplastic nevus are provided by the WHO classification.[3] However, there is overlap of histopathological features between DN and other melanocytic lesions with atypia, including ‘special site nevus’, MELTUMP (melanocytic tumors of uncertain malignant potential), SAMPUS

(superficial atypical melanocytic proliferations of uncertain significance), MANIAC (melanocytic acral nevus with intraepidermal ascent of cells), DNIEMD (de novo intraepidermal epithelioid melanocytic dysplasia) and THIMUMP (THIn Melanoma of Uncertain Metastatic Potential [2]. The Melanocytic Pathology Assessment Tool and Hierarchy for Diagnosis (MPATH-Dx V2) proposes four categories of melanocytic lesion: I–low-grade, including DN with mild atypia; II–high-grade, including DN with severe atypia; III–melanoma pT1a; IV–melanoma ≥ pT1b.[4] The MPATH-Dx V2 system provides a useful guide for clinical management, and underscores the recognised low risk of progression in DN.

The histological diagnosis of high-grade dysplastic nevus (HGDN) can nevertheless be problematic and the distinction between melanoma and HGDN can sometimes be challenging for the pathologist. This is particularly the case with late onset HGDN that can resemble naevoid melanoma. [5]

Preferentially expressed antigen in melanoma (PRAME) immunohistochemistry is often utilised to aid distinction of benign and malignant melanocytic proliferations. In a recent single institution series, 80% of malignant melanomas were reported to show diffuse PRAME positivity but around 13% of dysplastic nevi also proved to be diffusely PRAME positive, with junctional proliferations, severe atypia, male gender, and older age being associated with PRAME positive staining. [6] Further, Knittel et al (2025) [7] found that PRAME was positive in 7.1% of benign nevi. Interestingly, when a cohort of severely dysplastic nevi diagnosed with the aid of PRAME by non-specialist pathologists were reviewed by an expert dermatopathology panel masked to the PRAME result, confirmed cases reduced from 54% to 7%, providing evidence for 'PRAME bias' that could lead to overdiagnosis. [7]

We have previously validated combined immunostaining for AMBRA1 and loricrin in the epidermis overlying primary AJCC stage I and II cutaneous melanomas as a prognostic biomarker. [8,9] The aim of the present study was to evaluate the possible diagnostic utility of

combined AMBRA1 and loricrin epidermal staining in a cohort of HGDN in combination with standard PRAME immunohistochemistry.

Materials and Methods

Cohort identification

Cases were identified from a database that was prospectively created following a weekly consensus meeting of specialist dermatopathologists at Newcastle upon Tyne NHS Hospitals Trust (NuTH), United Kingdom. As part of the dermatopathology service, all routine suspicious and borderline melanocytic lesions and melanomas were discussed around a multiheaded microscope by three specialist consultant dermatopathologists with trainees and non-specialist consultant pathologists also present. The consensus diagnosis was recorded. Search of the database between October 2015 and December 2019 revealed 409 cases of DN, of which 147 had been recorded as 'severely dysplastic nevus'. Fresh haematoxylin and eosin-stained sections were prepared from the cases where a consensus of 'severely dysplastic nevus' had been recorded and were reviewed by the study pathologists (AH and PS) with selection of 145 cases using the WHO 5th edition criteria and features of the Duke's classification.[10] as HGDN. Cases were excluded where features suspicious for melanoma in situ were present.

Release and use of the samples for this study was approved by the Newcastle Hospitals Biobank ethics committee.

Immunohistochemistry

Consecutive 4 µm sections prepared from formalin-fixed, paraffin-embedded tissue blocks were subjected to automated immunohistochemical analysis using proprietary antibodies for AMBRA1 and loricrin (AMLo Biosciences Ltd). IHC was performed using a Ventana Benchmark ULTRA automated IHC staining instrument (Ventana Medical Systems Inc.). Briefly, slides were deparaffinised, and antigen retrieval was performed using ULTRA Cell Conditioning (ULTRA

CC1) for 36 minutes. Primary antibodies were diluted in Da Vinci Green Diluent (Cell Path, PBC-PD900L) to a final concentration of 0.34 µg/ml for AMBLor anti-AMBRA1 and 14 µg/ml for AMBLor anti-Loricrin and incubated for 32 minutes at 36 °C. Primary antibody binding was detected with the Ventana UltraView DAB IHC Detection Kit. PRAME immunohistochemistry was performed using the Ventana Benchmark ULTRA automated IHC platform utilising rabbit-derived monoclonal antibody (PRAME Clone number: EPR20330 product number 790-7149, prediluted, Ventana, Roche). Briefly, slides were deparaffinised and antigen retrieval was performed using CC-1 (Ventana, Roche). Sections were incubated for 16 min at ambient temperature. Detection utilised the OmniMap red HRP system with haematoxylin employed for counterstaining.

IHC was performed by NovoPath (Newcastle Hospitals NHS Foundation Trust) using United Kingdom Accreditation Service (UKAS)-approved proprietary primary antibodies. Following the quality control assessment of immunostaining, slides were scanned using a Leica Aperio GT450 DX at x40 to produce high-resolution digital images for archiving and image capture.

Controls and non-dysplastic nevi

Normal human skin was used as a positive and isotype negative control tissue for AMBRA1 and loricrin immunohistochemistry and primary cutaneous malignant melanoma was used a positive and isotype negative control tissue for PRAME staining. For scoring AMBRA1 and loricrin, the normal epidermis in the marginal tissue was used as a positive internal control. AMBRA1, loricrin and PRAME immunohistochemistry was also undertaken on a control cohort of 39 benign melanocytic nevi of various types (Supplementary table 1).

Scoring

The stained sections were viewed using a double-headed microscope and consensus scoring of the immunohistochemistry was performed by the study pathologists (AH and PS). AMBRA1 and loricrin immunohistochemistry was scored as previously described. [9] Briefly, AMBRA1 and loricrin were scored as 'maintained' or 'lost'. For AMBRA1, a 'maintained' (M) score was given if the epidermal pattern over the melanocytic lesion was equivalent to the normal skin in the marginal tissue and 'loss' (L) was recorded when there was reduction of staining in any area of the epidermis overlying the melanocytic lesion. 'Maintained' (M) loricrin was defined as a continuous band of staining in the granular layer across the entire sample and 'Lost' (L) loricrin staining was defined as any discontinuity greater than the length of a single cell, also termed single cell gap (SCG). PRAME was scored on a five-point scale (0-4+) using the criteria of Lezcano (2018). [11] Melan A immunohistochemistry had been performed as part of the original diagnostic work up and these slides were used to aid diagnosis and PRAME scoring.

Clinical follow up

The hospital electronic databases were searched to ascertain clinical follow up. The majority of patients (89/145, 61%) were discharged from the dermatology clinic to primary care after excision of the HGDN. The remaining patients (56/145, 39%) attended dermatology for various reasons and were seen between 1 and 9 years (median 3 years) after initial excision. In December 2024 the database was searched for outcome at a minimum of 5 or more years (5-9 years, median 6 years). Data relating to the clinical suspicion of a melanoma diagnosis were obtained from the pathology request and report forms (2016 onwards) and included scores for history (H), clinical appearances (C) and dermatoscopic features (D). Each parameter was scored on a scale of 1-4 by the clinical assessor who examined the patient that day. (Supplementary table 2).

Results

Histopathology

Control tissues and benign melanocytic nevi

AMBRA1 showed cytoplasmic expression in normal skin in the epidermis and blood vessels, as previously described. [9] In epidermal keratinocytes, there was a gradient of increasing intensity from basal cells to the granular layer, where maximal expression was observed. Expression was also present in endothelial cell cytoplasm. Loricrin expression in normal skin was restricted to the granular layer. Both nuclear and cytoplasmic expression was observed and a continuous band of staining 1-3 cell layers in thickness was typically present. Single cell discontinuities were occasionally present in the loricrin band, as previously described. [9]

All types of benign melanocytic nevi studied showed maintenance of the normal pattern of epidermal expression of both AMBRA1 and loricrin, except three out of five cases of Meyerson (halo) nevus that showed parakeratosis with extensive multifocal loricrin losses only (3/39 benign nevi). PRAME staining was present in 15% (6/39) of benign nevi. Five nevi scored 1+ and one scored 2+ with none staining at 3+ or 4+ levels (Supplementary table 1).

High grade dysplastic nevi

98% of HGDN (142/145) showed maintained expression of either one or both of AMBRA1 or loricrin within the epidermis overlying the nevus (Figure 1). Loss of AMBRA1 staining alone was seen in 3/145 (2%) while loss of loricrin staining alone was seen in 11/145 (7%). Whilst PRAME positivity was seen in 69/145 (48%), a PRAME score of 4+ was only found in 11/145 (8%) of HGDNs (Supplementary table 2, Figures 2,3,4). In 37/145 (26%) cases, the epidermis overlying the nevus cells showed mild hyperplasia and occasionally focal or generalised parakeratosis when compared to the normal epidermis in the surrounding marginal skin.

In three HGDN, loss of both epidermal AMBRA1 and loricrin expression was observed, with loss in one case restricted to an area overlying a focus of atypical melanocytes that there were also 4+ PRAME positive (Figure 5). After review by a third specialist dermatopathologist familiar with interpretation of AMBRA1 and loricrin staining, the consensus diagnosis was agreed as a focus of developing thin melanoma in a HGDN. In the two other HGDN cases with loss of both AMBRA1 and loricrin expression, PRAME was negative. Here, the focal losses of AMBRA1 and loricrin were considered (after discussion with the third pathologist) to result from epidermal hyperplasia and proximity to a hair follicle ostium respectively rather than indicating developing 'at-risk' melanoma.

Clinical characteristics

In all HGDN cases, there was no evidence for the development of metastatic disease and there were no melanoma specific deaths. Three patients had died from unrelated causes. Re-excisions had been performed in 18/145 (12%) of the patients. None showed residual DN except in one case where the margin had been reported as involved in the initial biopsy and where complete excision was achieved by the second procedure. 24/145 (16%) of the patients had previous melanomas or subsequent melanomas following excision of the HGDN, but these occurred at different sites to the HGDN itself. Three patients had a previous diagnosis of melanoma in situ and one subsequently developed a Spitz melanocytoma four years after excision of the DN, all at different sites to the index HGDN. One HGDN recurred after 14 months but was re-excised with clear margins.

Discussion

The diagnosis of benign melanocytic nevus, even with low grade dysplasia, rarely presents a challenge in routine pathology practice. It is interesting to note, however, that 15% showed

positive staining by PRAME though the highest score seen was 2+ which was well below the threshold for concern. This broadly confirms the observations of Lezcano (2018) [11], who found only one case of 4+ PRAME staining out 140 benign melanocytic nevi of various types studied. Several studies have confirmed that benign nevi are either negative for PRAME or do not show diffuse (4+) staining patterns. [12] The benign nevi in our cohort showed maintenance of either or both epidermal AMBRA1 and loricrin staining, confirming their benign nature. Only three benign nevi showed any losses, and these were 3/5 Meyerson (halo) nevi that showed extensive reactive hyperkeratosis and parakeratosis with corresponding loricrin loss. AMBRA1 was maintained in all cases. This finding underscores that although loricrin loss characterises pre-ulcerative epidermis overlying high risk melanoma, loricrin loss may also result from inflammatory or traumatic stimuli that can cause parakeratosis. Clinical correlation is needed when interpreting loricrin immunostaining in such situations, particularly in cases where co-existing inflammatory dermatosis is present. However, combined AMBRA1 and loricrin staining scored 'low risk' in all benign nevi, with no false positive results.

In contrast to benign melanocytic nevi with no or only low-grade dysplasia, HGDNs can pose a significant diagnostic challenge to the pathologist. In the HGDNs studied here, the combined AMBRA1 and loricrin staining showed greater utility in confirming a diagnosis of benign or low risk melanocytic lesion in HGDNs (98%) than PRAME 4+ staining (92%). Moreover, it is likely that suspicion of melanoma as a differential diagnosis would also be raised by PRAME 3+ staining, lowering the threshold of confirming benignity in a HGDN to 87%. Although some degree of PRAME staining was observed in 48% of the HGDNs studied here, PRAME remains a very useful immunohistochemical marker in melanocytic pathology diagnosis. Gassenmaier et al (2021) [13] found that 21/35 (60%) of severely dysplastic nevi (HGDN) were positive for PRAME but only 2 cases showed 4+ staining and it was agreed that in one of these, melanoma could not be excluded. Importantly, diffuse (4+) PRAME was useful for distinguishing between thin melanomas and HGDN when critically applied. The findings underscore the need to use strict

PRAME scoring criteria when diagnostic decisions are being made. In our cohort there was a single case initially diagnosed as severely dysplastic nevus that showed focal loss of both AMBRA1 and loricrin, which combined with PRAME 4+ expression, led to changing the diagnosis from HGDN to thin melanoma on expert review. Clinical correlation is also advisable when a diagnosis of HGDN is challenging, because dermatoscopic findings, patient age, lesion location and history may help to decide on the further management strategy.

Only 3/145 (2%) cases in our HGDN cohort showed loss of both AMBRA1 and loricrin. One of these was reclassified as a focus of malignant melanoma within a HGDN after review including a third specialist dermatopathologist. The second case occurred on the left upper thigh in a 35-year-old female patient. Scattered cells were PRAME positive but less than 25% of melanocytes were stained. This patient had two concurrent pT1a superficial spreading malignant melanomas removed at the same time, one from the back (Breslow thickness 0.6mm) and one from the left abdomen (Breslow thickness 0.8mm). In this patient the further management related to the concurrent melanomas, but the finding of loss of both AMBRA1 and loricrin may indicate that the HGDN was also a high-risk lesion. The third case was from the lower left leg of a 61-year-old female patient. No PRAME staining was present and loss of both AMBRA1 and loricrin was restricted to a short strip of epidermis where parakeratosis was present. On review by the third specialist dermatopathologist, the diagnosis of HGDN was unchanged and it was considered likely that the AMBRA1 and loricrin staining could have resulted from incidental florid epidermal hyperplasia with parakeratosis and should not be used to assess prognosis.

It was also of interest to note that in a case where a HGDN had been re-excised from the right shoulder of a 39 year old male, both the primary and re-excision samples showed maintained AMBRA1 and loricrin and were negative for PRAME, in keeping with the findings of Kucharil et al (2025)[14] who found that in a series of 22 paired recurrent nevi all cases were PRAME negative on composite scoring.

A retrospective cross-sectional study of the use of immunohistochemistry for melanocytic lesions showed a rise from 11% in 2000 to over 50% by 2017, not matched by increase in mortality[15]. The clinical utility of immunohistochemistry is not always clear and increased testing may even contribute to melanoma overdiagnosis. The use of inappropriate immunohistochemistry can also add to delay in reporting and has economic implications, although for epidermal AMBRA1 and loricrin immunohistochemistry, it is likely that AI/ machine learning approaches will supplant this in the future[16]. In our view, the vast majority of HGDNs should be signed out on the basis of an H&E stain, without supplementary immunohistochemistry. Where immunohistochemistry can truly aid decision making, there may be significant economic implications in savings from reduced clinical follow up if the benign or low-risk nature of an equivocal melanocytic lesion can be established.

Single cell loricrin gaps are a recognised feature of normal skin and are likely to represent clonal heterogeneity in epidermal differentiation. [9] We noted that single cell loricrin gaps were found in 7/37 (19%) eligible benign melanocytic nevi with no or only low-grade dysplasia, whereas such gaps were found in 45/145 (31%) of HGDNs. Further, parakeratosis was found only in some Meyerson nevi in DNs with no or only low-grade dysplasia whilst parakeratosis, often in the form of mounds, was seen in 9/145 (6%) in HGDNs where it was often associated with loricrin loss. A variable degree of epidermal hyperplasia was noted in many of the HGDNs and this was not a feature in benign nevi with no or only low-grade dysplasia. Epidermal hyperplasia may result from interaction with the active melanocytic population in HGDN, possibly driving cell proliferation not seen in melanocytic nevi with no or only low-grade dysplasia.

In our previous study of epidermal loricrin and AMBRA1 expression in AJCC stage I and II melanomas[9] we observed instances where loss of one or other of the markers appeared to be related to modifiers of epidermal differentiation other than signalling from the underlying melanocytic population. These were mostly related to loricrin loss where there was focal or

generalised parakeratosis, often involving the overlying epidermis and surrounding non-lesional marginal skin. When the clinical findings were explored there was frequently a history of trauma or associated halo phenomenon.

The use of epidermal changes as a surrogate for predicting the biologic behavior of an underlying melanocytic population is intrinsically limited. When halo phenomenon or trauma is present, AMBRA1 and loricrin immunohistochemistry should not be used. In our view, histological interpretation remains paramount for diagnosis and when using immunohistochemistry as a guide, the pathologist must be aware of its limitations and pitfalls.

None of the HGDNs in this study showed progression, which accords with the low-risk status inferred by maintenance of either or both AMBRA1 and loricrin in the overlying epidermis. Review of clinical records showed that 18 patients had re-excisions performed. Of these 16 showed no evidence of residual lesion. In one case the pathologist advised re-excision because the peripheral margin was involved and residual HGDN was found in the re-excision specimen. In the other case the HGDN recurred four years after the initial excision and the re-excision specimen showed recurrent HGDN that was now completely removed. In retrospect, the majority of re-excisions were probably not necessary. Clinical practice regarding re-excision of HGDN varies and a treatment decision aid for patients eligible for re-excision has been proposed.[17] A recent review concludes that whilst observation is likely to be appropriate for dysplastic nevi with low or moderate dysplasia, HGDNs should be considered for re-excision.[18] The findings from our study suggest that combined AMBRA1 and loricrin staining could have been used to confirm benignity in HGDN and reduce the re-excision rate.

All of the cases in our cohort of HGDNs were re-evaluated after the initial diagnosis to avoid inclusion of cases that may have been melanoma in situ. In a separate ongoing study, we have stained 300 cases of melanoma in situ with AMBRA1 and loricrin and found loss of both markers occurred in around 10% of cases. This contrasts with the rate in HGDNs which is 1.3%. In some

cases of melanoma in situ, loss of both markers in the punch biopsy predicted invasive melanoma in the subsequent resection specimen, but this preliminary observation requires further research and validation.

Dermatoscopy had been performed on all the HGDNs in the cohort. The findings were recorded descriptively and in cases ascertained from 2016 onwards a dermatoscopic score of suspicion of melanoma was recorded on a scale of D1-D4 (D1 low suspicion, D4 high suspicion). The data have been included in Supplementary table 2 along with score of melanoma suspicion from the history (H1-H4) and clinical features (C1-4). The scale used was locally devised has not been validated. Therefore, limited conclusions can be drawn. It may be noteworthy that whilst only two HGDNs were given a dermatoscopic score of D4, 45/130 (35%) were given a score of D3, 78/130 (60%) D2 and 5/130 (4%) D1. Thus, there was reasonable clinical suspicion of melanoma by dermatoscopy in the majority of the HGDNs prior to excision.

We conclude that combined AMBRA1 and loricrin staining provides a useful adjunctive biomarker that, when used in conjunction with PRAME, may aid the assessment of worrisome HGDNs and help reduce unnecessary re-excisions, investigations, follow up and both patient and clinician anxiety. Pathologic diagnosis of HGDN should be based primarily on morphological interpretation but immunohistochemistry can provide helpful supplementary information, aiding the busy pathologist and reducing clinical workload.

References

1. Clark WH Jr, Reimer RR, Greene M, Ainsworth AM, Mastrangelo MJ. Origin of familial malignant melanomas from heritable melanocytic lesions. 'The B-K mole syndrome'. *Arch Dermatol*. 1978;114:732-738

2. Menzinger S, Merat R, Kaya G. Dysplastic Nevi and Superficial Borderline Atypical Melanocytic Lesions: Description of an Algorithmic Clinico-Pathological Classification. *Dermatopathology (Basel)*. 2025;12:3.

3. Skin Tumours. *WHO Classification of Tumours*, 5th Edition, Volume, 12 IARC publications 2025

4. Barnhill RL, Piep korn MW, Duncan LM, Knezevich S, Elmore JG, Elder DE. MPATH-Dx version 2.0 schema for melanocytic lesions: A robust tool for standardized diagnostic reporting. *Clin Dermatol*. 2025;43:306-314.

5. Kossard S, Sharifi S, Calvey L. Utilizing PRAME Expression and a Meta-Analytic Framework for iSALT to Explore Atypical Late-Onset Nevi of the Elderly and Their Relationship With Lentiginous and Nested Nevoid Melanomas. *Am J Dermatopathol*. 2024;46:825-832.

6. Turner N, Ko CJ, McNiff JM, Galan A. Pitfalls of PRAME Immunohistochemistry in a Large Series of Melanocytic and Nonmelanocytic Lesions With Literature Review. *Am J Dermatopathol*. 2024;46:21-30.

7. Knittel RJ, Harvey NT, Ardakani NM, O'Brien B, Lee S, Wood BA. Clinicopathologic Features of PRAME-Positive Common Melanocytic Nevi: A Case-Control Study. *Am J Dermatopathol*. 2025;47:379-386.

8. Ellis R, Tang D, Nasr B, Greenwood A, McConnell A, Anagnostou ME, Elias M, Verykiou S, Bajwa D, Ewen T, Reynolds NJ, Barrett P, Carling E, Watson G, Armstrong J, Allen AJ, Horswell S, Labus M, Lovat PE. Epidermal autophagy and beclin 1 regulator 1 and loricrin: a paradigm shift in the prognostication and stratification of the American Joint Committee on Cancer stage I melanomas. *Br J Dermatol*. 2020;182:156-165.

9. Ewen T, Husain A, Stefanos N, Barrett P, Jones C, Ness T, Long A, Horswell S, Bosomworth H, Lowenstein J, Richardson G, Swan D, McConnell A, Rose A, Andrew T, Reynolds N, Malvehy J,

Carrera C, Alos L, Mailer S, Helm T, Ding L, Bogner P, Podlipnik S, Puig S, McArthur GA, Paragh G, Labus M, Sloan P, Armstrong JL, Lovat PE. Validation of epidermal AMBRA1 and loricrin (AMBLor) as a prognostic biomarker for nonulcerated American Joint Committee on Cancer stage I/II cutaneous melanoma. *Br J Dermatol*. 2024;190:549-558.

10. Shea CR, Prieto VG, Shachaf CM, Florell SR. Grading Melanocytic Dysplasia: Updated Histopathologic Criteria. *J Cutan Pathol*. 2026;53:29-37

11. Lezcano, C; Jungbluth, A, Nehal, Kishwer S, Hollmann, Travis J, Busam, Klaus J. PRAME Expression in Melanocytic Tumors. *Am J Surg Pathol*. 2018;42:1456-1465

12. Lezcano, C, Jungbluth, A, Busam, Klaus J. Immunohistochemistry for PRAME in Dermatopathology. *Am J Dermatopathol*. 2023;45:733-747

13. Gassenmaier M, Hahn M, Metzler G, Bauer J, Yazdi AS, Keim U, Garbe C, Wagner NB, Forchhammer S. Diffuse PRAME expression Is highly specific for thin melanomas in the distinction from severely dysplastic nevi but does not distinguish metastasizing from non-metastasizing thin melanomas. *Cancers (Basel)*. 2021;13:3864.

14. Kucharik AH, Ghali H, Milani D, Warbasse E, Li P, Ravichandran S, Rodriguez-Waitkus P, Chen WS. PRAME Expression in Recurrent/Persistent Melanocytic Nevi. *Am J Dermatopathol*. 2025;47:834-839.

15. Ojukwu K, Eguchi MM, Adamson AS, Kerr KF, Piepkorn MW, Murdoch S, Barnhill RL, Elder DE, Knezevich SR, Elmore JG. Immunohistochemistry for Diagnosing Melanoma in Older Adults. JAMA Dermatol. 2024:60:434-440.

16. Andrew TW, Combalia M, Hernandez C, Grant S, Paragh G, Puig S, Mc Arthur G, Richardson G, Sloan P, Shalhout SZ, Plummer R, Lovat PE. Multimodal AI and tumour microenvironment integration predicts metastasis in cutaneous melanoma. Nat Commun. 2025;16:10095.

17. Roland-McGowan JN, Freeman SC, Baghoomian W, Carroll BT, Kim CC, Curiel-Lewandrowski C, Chu EY, Foster EL, Nelson KC, White KP, Ming ME, Hartman RI, Leachman SA, Mengden-Koon S, Chen SC, Swetter SM, Prieto VG, Berry E, Yu WY. Development of a treatment decision aid for patients with dysplastic nevi who are candidates for re-excision. *J Am Acad Dermatol*. 2025;92:1112-1114

18. Miles JA, Ming ME, Chu EY. Do all dysplastic nevi need re-excision? *Current Dermatology Reports*. 2025;14:19

Figures

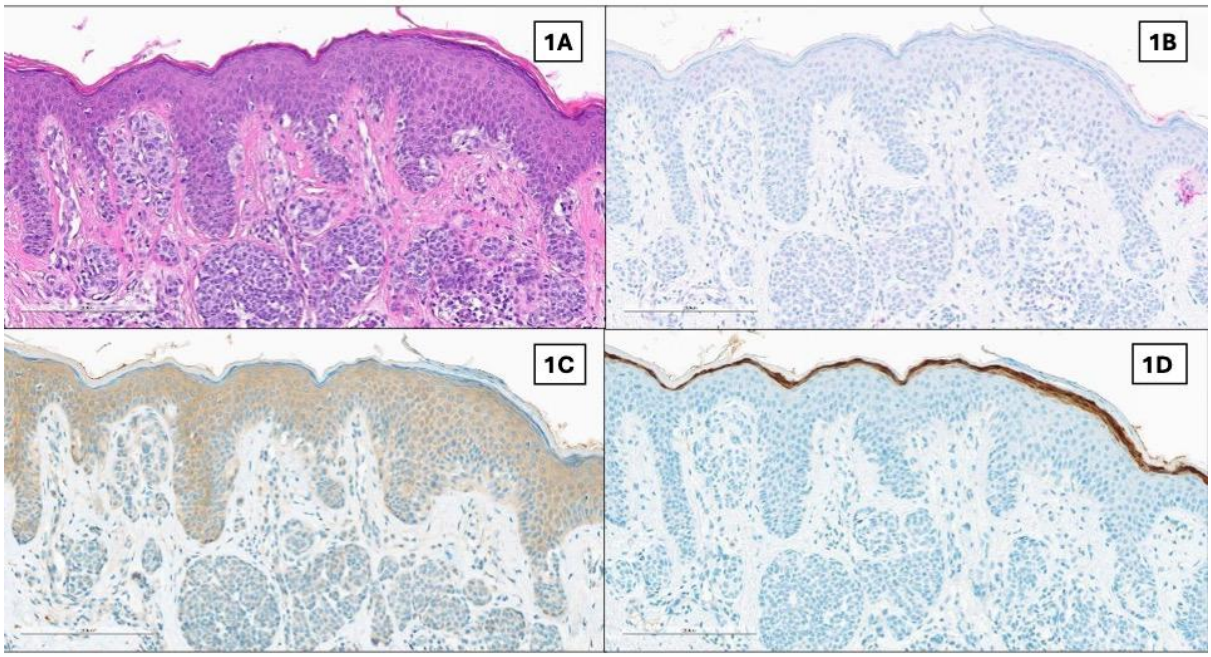


Figure 1. The most common immunohistochemical pattern encountered in this cohort of HGDN was retention of epidermal AMBRA1 and loricrin with PRAME negative. Note that AMBRA1 staining is cytoplasmic and increases in intensity from basal to granular layer. Loricrin staining is nuclear and cytoplasmic and forms a continuous band 2-3 cell layers in thickness in the granular layer. 1A H&E stain, 1B PRAME, 1C AMBRA1, 1D loricrin.

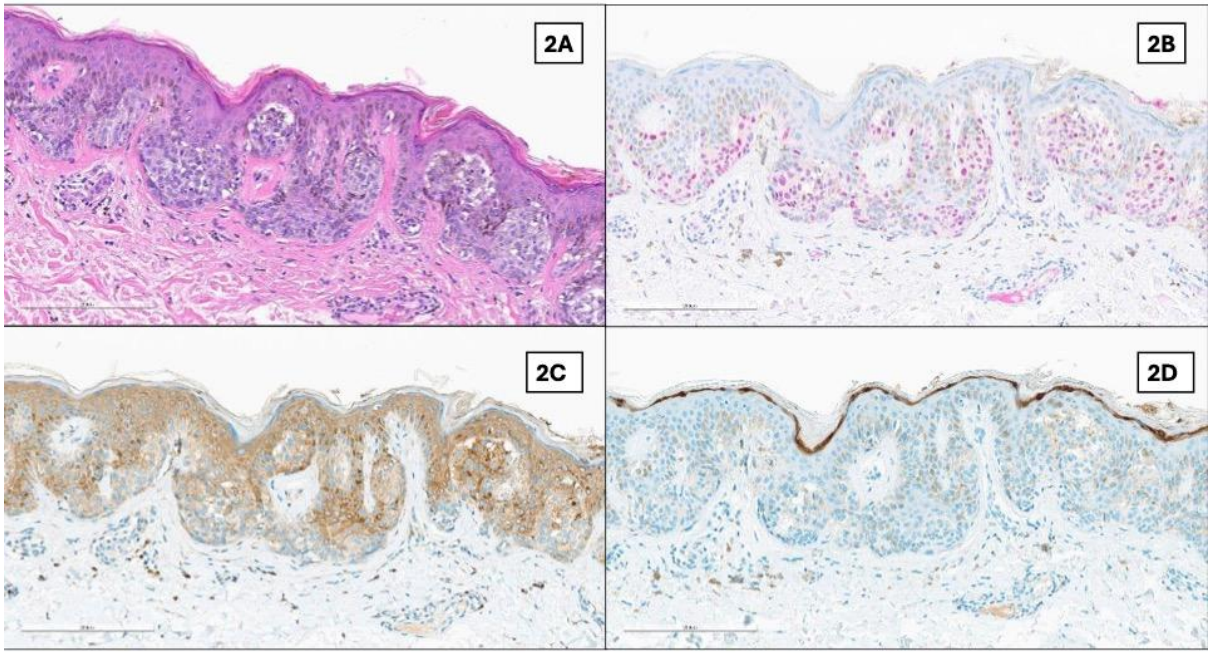


Figure 2. A HGDN (2A) where diffuse PRAME staining was seen in the melanocyte population (2B), but both epidermal AMBRA1 (2C) and loricrin (2D) were maintained, favoring a low-risk melanocytic lesion.

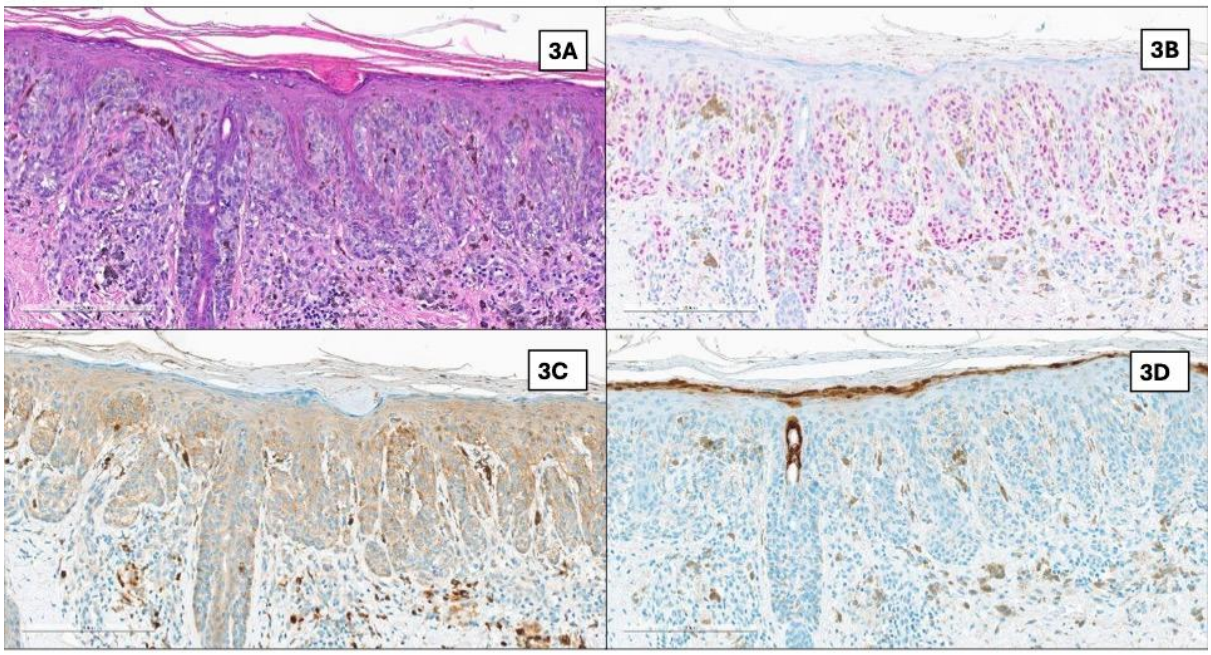


Figure 3. This HGDN showed concerning cytological features (3A) and diffuse PRAME staining (3B) raised suspicion of melanoma. However epidermal AMBRA1 staining showed only early patchy loss (3C) and loricrin was maintained (3D), favoring a low-risk melanocytic lesion.

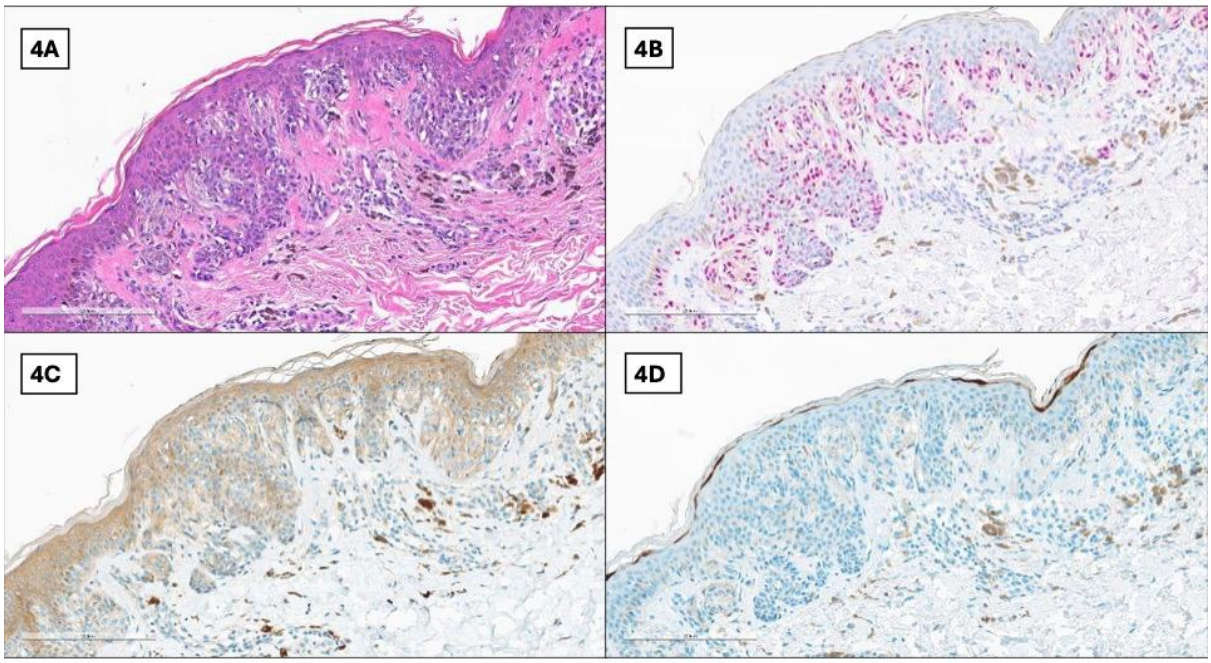


Figure 4. This HGDN showed morphological atypia (4A) and diffuse PRAME staining (4B). Epidermal AMBRA1 staining (4C) was maintained and the loricrin band was thinned and showed focal gaps (4D). The retention of one of the epidermal markers favors a low-risk melanocytic lesion.

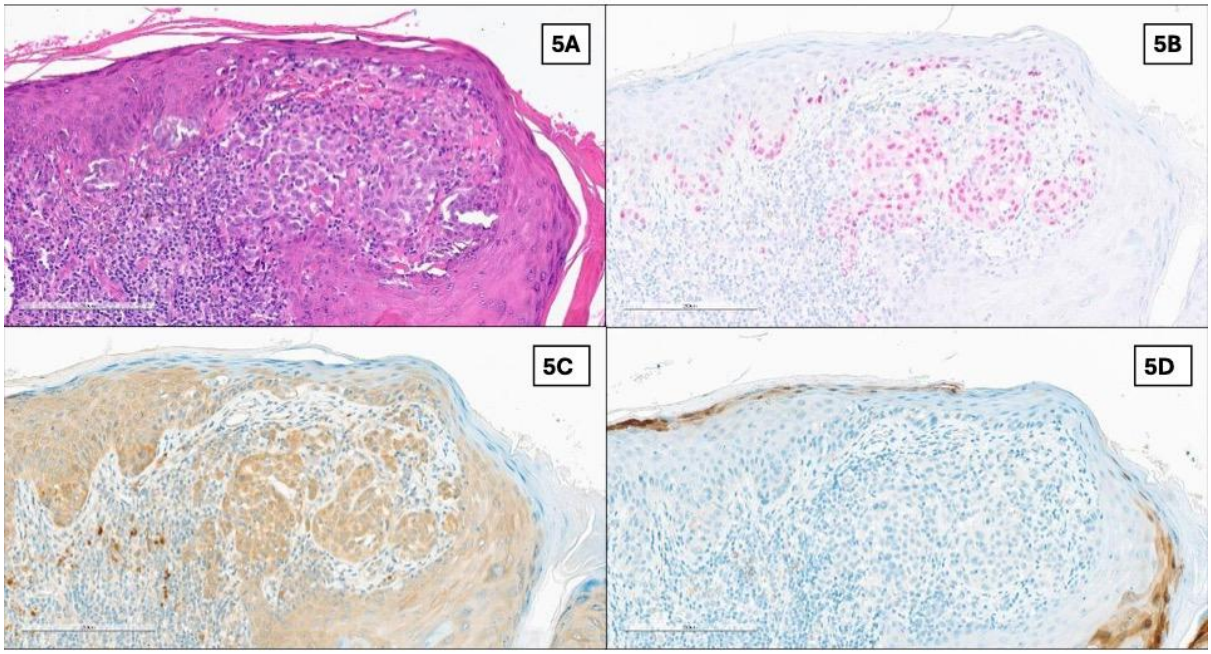


Figure 5. The case had originally been diagnosed as severe dysplastic nevus (5A), but showed focal PRAME staining with associated loss of both AMBRA1 and loricrin. On review the diagnosis was revised to early at-risk melanoma.