Genomic copy number analysis of a spectrum of blue nevi identifies recurrent aberrations of entire chromosomal arms in melanoma ex blue nevus

May P Chan^{1,2}, Aleodor A Andea^{1,2}, Paul W Harms^{1,2}, Alison B Durham², Rajiv M Patel^{1,2}, Min Wang¹, Patrick Robichaud², Gary J Fisher², Timothy M Johnson² and Douglas R Fullen^{1,2}

¹Department of Pathology, University of Michigan, Ann Arbor, MI, USA and ²Department of Dermatology, University of Michigan, Ann Arbor, MI, USA

Blue nevi may display significant atypia or undergo malignant transformation. Morphologic diagnosis of this spectrum of lesions is notoriously difficult, and molecular tools are increasingly used to improve diagnostic accuracy. We studied copy number aberrations in a cohort of cellular blue nevi, atypical cellular blue nevi, and melanomas ex blue nevi using Affymetrix's OncoScan platform. Cases with sufficient DNA were analyzed for *GNAQ*, *GNA11*, and *HRAS* mutations. Copy number aberrations were detected in 0 of 5 (0%) cellular blue nevi, 3 of 12 (25%) atypical cellular blue nevi, and 6 of 9 (67%) melanomas ex blue nevi. None of the atypical cellular blue nevi displayed more than one aberration, whereas complex aberrations involving four or more regions were seen exclusively in melanomas ex blue nevi. Gains and losses of entire chromosomal arms were identified in four of five melanomas ex blue nevi with copy number aberrations. In particular, gains of 1q, 4p, 6p, and 8q, and losses of 1p and 4q were each found in at least two melanomas. Whole chromosome aberrations were also common, and represented the sole finding in one atypical cellular blue nevus. When seen in melanomas, however, whole chromosome aberrations were invariably accompanied by partial aberrations of other chromosomes. Three melanomas ex blue nevi harbored aberrations, which were absent or negligible in their precursor components, suggesting progression in tumor biology. Gene mutations involving *GNAQ* and *GNA11* were each detected in two of eight melanomas ex blue nevi. In conclusion, copy number aberrations are more common and often complex in melanomas ex blue nevi compared with cellular and atypical cellular blue nevi. Identification of recurrent gains and losses of entire chromosomal arms in melanomas ex blue nevi suggests that development of new probes targeting these regions may improve detection and risk stratification of these lesions.

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Blue nevi are a group of dermal pigmented melanocytic proliferations clinically characterized by a blue-black color owing to Tyndall effect. The prototype, conventional blue nevus, was first described by Jadassohn-Tieche in 1906.¹ Different morphologic variants have since been described. In conventional blue nevi, the melanocytes are dendritic with long and finely melanized cytoplasmic processes percolating between dermal

collagen bundles. Cellular blue nevi are characterized by a bulbous or 'dumbbell' silhouette with plump spindle melanocytes organized in broad fascicles.² Atypical cellular blue nevi are cellular blue nevi with significant atypia concerning for but short of a definitive diagnosis of malignancy. They may display infiltrative margins, asymmetry, hypercellularity, nuclear pleomorphism, hyperchromasia, increased mitotic activity, and even necrosis;³ however, clear diagnostic criteria are lacking. These lesions are commonly regarded as ambiguous or 'borderline' tumors of uncertain biologic potential. Finally, 'malignant blue nevi' are rare tumors that constitute the malignant end of the blue nevus spectrum. This term has been applied to melanoma resembling cellular blue nevi, as well as melanoma arising in a

Correspondence: Dr MP Chan, MD, Department of Pathology, University of Michigan, Medical Science I, M3261, 1301 Catherine Street, Ann Arbor, MI 48109, USA.
E-mail: mpchan@med.umich.edu

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conventional, cellular, or atypical cellular blue nevus ('melanoma ex blue nevus'). These lesions frequently exhibit destructive growth, nuclear pleomorphism, prominent nucleoli, atypical mitoses, and necrosis.⁴

Several studies have alluded to the highly aggressive and often lethal clinical course of melanoma ex blue nevus and blue nevus-like melanoma, including frequent metastases to the lung and liver.^{4–8} In contrast, the vast majority of lesions classified as cellular blue nevus did not recur or metastasize.^{2,9,10} Although a few studies suggested that atypical cellular blue nevus generally behave in a manner similar to that of cellular blue nevus,^{3,10,11} the data are rather limited. The classification of these lesions also tends to be subjective, as no clear-cut morphologic criteria exist for atypical cellular blue nevus. As a result, its distinction from cellular blue nevus and melanoma ex blue nevus, albeit important in predicting clinical outcome, often proves to be challenging even among experts.¹⁰ A more accurate and refined classification is therefore needed to better stratify the risk of these lesions and improve cohort homogeneity for future analysis.

Cytogenetic and molecular tools are increasingly used to aid in the diagnosis of challenging and ambiguous melanocytic lesions. A study showed that fluorescence *in situ* hybridization (FISH) assay targeting 6p25 (*RREB1*), 6q23 (*MYB*), 11q13 (*CCND1*), and centromere of chromosome 6 (Cep6) was able to discriminate 12 cellular blue nevi from 5 blue nevus-like melanomas with 100% sensitivity and 100% specificity.¹² Another study used comparative genomic hybridization (CGH) to examine 11 morphologically benign, 11 ambiguous, and 7 morphologically malignant blue nevi and related dermal melanocytic proliferations, and found that copy number aberrations were absent in all benign lesions, absent or few (no more than 3 aberrations) in the ambiguous group, and invariably present (3 or more aberrations) in all malignant lesions.¹¹ A recent study that examined 23 dermal melanocytic lesions histologically diagnosed as benign or ambiguous cellular blue nevus *versus* deep penetrating nevus by CGH demonstrated chromosomal aberrations in 9 lesions, including 3 that recurred or progressed.¹³ All of these studies provide valuable genomic information, and support the utilization of these tests as helpful ancillary tools in the diagnosis and risk management of this spectrum of lesions.

To further characterize these lesions at a molecular level, we sought to examine a series of cellular blue nevi, atypical cellular blue nevi, and melanomas ex blue nevi using a newer genomic microarray in which copy number aberrations are detected by molecular inversion probe technology. This microarray is superior to traditional CGH in that it performs well with degraded DNA in formalin-fixed, paraffin-embedded tissues, and requires significantly less DNA to generate high-quality copy number data.¹⁴ Application of this technology in melanoma

research is growing but has not yet been widely adopted. One study found excellent sensitivity (89%) and specificity (100%) of this microarray in distinguishing melanoma from benign nevi, whereas the results on histologically ambiguous lesions were less satisfactory.¹⁵ A shortcoming of this particular study, as admitted by Chandler *et al*,¹⁵ was the limited clinical follow-up. It was our aim to gain experience with this relatively new platform, and to explore its utility in predicting the clinical outcome of atypical cellular blue nevus and melanoma ex blue nevus. Given the low DNA requirement by the molecular inversion probe microarray, we also aimed to analyze separately any precursor blue nevus component from the malignant component in melanomas ex blue nevi, to better understand tumor progression in these lesions.

Materials and methods

Case Selection and Clinicopathologic Data

This study is approved by the Institutional Review Board at University of Michigan. The Multidisciplinary Melanoma Program database and the Surgical Pathology database at University of Michigan were searched for 'cellular blue nevus', 'atypical blue nevus', and 'malignant blue nevus' between years 1996 and 2014. The hematoxylin- and eosin-stained slides were reviewed to confirm the original histopathologic diagnoses. The following histopathologic features were evaluated and recorded for each case: tumor thickness, ulceration, necrosis, nuclear pleomorphism, prominent nucleoli, mitotic rate (number of dermal mitoses per 1 mm² 'hot spot'), atypical mitoses, neurotropism (melanocytes tracking or encircling peripheral nerves), and lymphovascular invasion. Clinical data obtained from the electronic medical record and the Multidisciplinary Melanoma Program database include: age at diagnosis, sex, anatomic site, sentinel lymph node status (if performed), outcome (recurrence, metastasis, and survival), and length of clinical follow-up. Additional follow-up data were gathered from the patients' primary physicians or dermatologists. Comparison of continuous data was performed by two-tailed *t*-tests. Comparison of categorical data was performed by χ^2 tests. A *P*-value of < 0.05 was considered statistically significant.

DNA Extraction

A representative tissue block was selected from each case for genomic copy number analysis. Ten-micron sections were cut, and the lesional tissue was either macro- or microdissected from the normal tissue. For melanoma ex blue nevus, any presumed precursor blue nevus component of decent size was microdissected from the malignant component for separate analysis. Microdissection was performed

using laser capture technique (Leica ASLMD system; Leica Microsystems, Wetzlar, Germany) as described previously¹⁶ after staining the tissue sections with hematoxylin. DNA was extracted and purified from the samples using the QIAamp DNA FFPE Tissue Kit (Qiagen, Germany) according to the manufacturer's protocols. The extracted DNA was quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) as per the manufacturer's procedure.

Genomic Copy Number Analysis

Genomic microarray hybridization was performed either at the Affymetrix Research Services Laboratory through the OncoScan FFPE Express 2.0 Service (Affymetrix, Santa Clara, CA, USA) or at the Dermatopathology Molecular Laboratory at University of Michigan using the OncoScan FFPE Express 3.0 platform (Affymetrix). Technical documentation is available on the Affymetrix website (http://media.affymetrix.com/support/technical/datasheets/oncoscan_ffpe_express_service.pdf). The microarray uses more than 335 000 molecular inversion probes targeting single-nucleotide polymorphisms spanning the genome to identify changes in copy number and LOH. The design and performance of the molecular inversion probe genomic microarray have been described previously.^{13,17–20} The data generated on OncoScan were analyzed at the University of Michigan using Nexus Copy Number v.3 (BioDiscovery, El Segundo, CA, USA).

GNAQ, GNA11, and HRAS Gene Mutation Analysis

Cases with sufficient remaining PCR-suitable DNA were studied for *GNAQ*, *GNA11*, and *HRAS* gene mutations. *GNAQ* exons 4 and 5, *GNA11* exons 4 and 5, and *HRAS* exons 2 and 3 were amplified using primer pairs tagged with M13 forward sequence (5'-TGTAACGACGCGCCAGT-3') or M13 reverse sequence (5'-CAGGAAACAGCTATGACC-3'). The following primers were used: *GNAQ* exon 4 forward (5'-TGGTGTGATGGTGTCACTGACAT-3'), *GNAQ* exon 4 reverse (5'-AAGGCATAAAAGCTGGGAAAT-3'), *GNAQ* exon 5 forward (5'-TTTCCCTAAGTTTGTAAGTAGTC-3'), *GNAQ* exon 5 reverse (5'-CCACACCCTACTTTCTATCATTTAC-3'), *GNA11* exon 4 forward (5'-GTGCTGTGTCCCTGTCTG-3'), *GNA11* exon 4 reverse (5'-GGCAAATGAGCCTCTCAGTG-3'), *GNA11* exon 5 forward (5'-CTGGGATTGCAGATTG-3'), *GNA11* exon 5 reverse (5'-CCACCGGACTTGGTCGTAT-3'), *HRAS* exon 2 forward (5'-AGGAGACCCTGTAGGAGGA-3'), *HRAS* exon 2 reverse (5'-CCTATCCTGGCTGTGTCTCTG-3'), *HRAS* exon 3 forward (5'-AGAGGCTGGCTGTGTGAAC-3'), and *HRAS* exon 3 reverse (5'-TCACGGGGTTCACCTGTACT-3').²¹ PCR products were purified using QIAquick PCR Purification Kit and analyzed by Sanger sequencing at the University of

Michigan Sequencing Core using the M13 primers. Chromatograms were visualized using Sequence Scanner 2 software (Applied Biosystems, Carlsbad, CA, USA).

Results

Sample Selection

Histologic slides and formalin-fixed, paraffin-embedded tissue blocks were available for 7 cellular blue nevi, 12 atypical cellular blue nevi, and 10 melanomas ex blue nevi identified in our databases. In six of the melanomas ex blue nevi, the associated conventional/cellular blue nevus was microdissected and separately extracted. Another melanoma ex blue nevus (case 23) had been previously biopsied and diagnosed as atypical cellular blue nevus (presumed precursor) without further treatment 3 years before the diagnosis of melanoma ex blue nevus. Both the prior biopsy and the subsequent melanoma of this case were analyzed. After DNA extraction, five samples (including two cellular blue nevi and three presumed precursor blue nevi) did not meet the minimal DNA requirement for molecular inversion probe genomic microarray and were excluded. An additional melanoma ex blue nevus was excluded because of uninterpretable copy number data. The final cohort comprised 30 samples (5 cellular blue nevi, 12 atypical cellular blue nevi, 9 melanomas ex blue nevi, and 4 presumed precursor blue nevi) from 26 patients.

Clinical Data

The patient demographic and clinical data are summarized in Table 1. The mean age at diagnosis was 33 years for cellular blue nevus, 36 years for atypical cellular blue nevus, and 49 years for melanoma ex blue nevus. Among the melanomas, those with copy number aberrations were associated with a significantly older mean age (59 years) compared with those without copy number aberrations (28 years) ($P=0.0028$). There was no sex predilection in any category. The most common location of melanomas ex blue nevi was the scalp (6 of 9 cases; 67%), whereas the buttock/sacrum was the most common site of cellular and atypical cellular blue nevi (6 of 17 cases; 35%).

Histopathologic Findings

The histopathologic features are summarized in Table 1. The majority of cases demonstrated a dumbbell or bulbous silhouette with pushing borders (Figures 1–3). All melanomas ex blue nevi consisted of a relatively bland blue nevus component at least focally. The tumor thickness was greatest among melanomas ex blue nevi (median, 12.5 mm), followed by atypical cellular blue nevi (median,

Table 1 Summary of clinical data and histopathologic findings of all cases

Case	Age (years)	Sex	Site	Diagnosis	Tumor thickness (mm)	Ulcer	Nuclear pleomorphism ^a	Prominent nucleoli	Mitotic rate (/mm ²)	Atypical mitosis	Necrosis	Neurotropism	LVI	SLN	Recurrence/metastasis
1	53	F	Leg	CBN	3.1	–	0	–	0	–	–	–	–	NP	Unknown
2	7	F	Buttock	CBN	4.75	–	–	–	0	–	–	+	–	NP	None in 4 years
3	33	F	Sacrum	CBN	8.5	–	0	–	0	–	–	+	–	NP	None in 3 years
4	12	M	Neck	CBN	2.8	–	1	–	1	–	–	–	–	NP	None in 1 year
5	61	M	Scalp	CBN	4.75	–	0	–	0	–	–	–	–	NP	None in 3 years
6	15	F	Foot	ACBN	8.5	–	1	–/+	5	–	–	–	–	NP	None in 1 year
7	16	M	Foot	ACBN	7.75	–	0	–	3	–	–	–	–	–	None in 1 year
8	11	M	Buttock	ACBN	10.5	–	1	–/+	2	–	–	+	–	NP	None in 1 year
9	71	M	Back	ACBN	5	–	1	–	0	–	–	–	–	NP	None in 4 years
10	20	M	Foot	ACBN	2.9	–	1	–	0	–	–	+	–	–	None in 5 years
11	72	F	Buttock	ACBN	>5.65	–	2	–	0	–	–	+	–	NP	None in 9 years
12	19	F	Scalp	ACBN	6.1	–	0	–	0	–	–	–	–	–	None in 5 years
13	83	M	Buttock	ACBN	>6.45	–	2	–	1	–	–	+	–	NP	None in 1 month
14	16	M	Hand	ACBN	>11.8	–	1	–	1	–	–	–	–	NP	Unknown
15	57	F	Buttock	ACBN	>8	–	1	–/+	1	–	–	–	–	NP	Unknown
16	37	M	Thigh	ACBN	8	–	1	–	2	–	–	–	–	NP	None in 4 years
17	20	M	Finger	ACBN	3.48	+	1	–	7	–	–	–	–	Unknown	Unknown
18	32	M	Scalp	MBN	4	+	1	–	6	–	–	–	–	–	Unknown
19	34	M	Buttock	MBN	12.5	–	2	–	1	–	–	+	–	Unknown	Unknown
20	19	M	Temple	MBN	>6	NA	2	+	2	–	–	–	–	NP	None in 18 years
21	67	F	Flank	MBN	>14.1	NA	2	–	1	–	–	–	–	NP	None in 7 years
22	49	M	Scalp	MBN	23	–	2	+	1	–	+	–	–	–	Metastases to brain, spinal cord; DOD in 4 years
23	55	F	Scalp	MBN	>15.5	–	2	+	4	–	–	–	–	–	Liver metastasis; DOD in 2 years
				Precursor ACBN	2.48	–	1	–	1	–	–	–	–	NP	
24	67	M	Scalp	MBN	4.75	–	1	–	4	–	–	+	–	–	None in 5 years
25	46	F	Scalp	MBN	10	–	1	–	17	+	–	+	–	Unknown	Unknown
26	69	F	Scalp	MBN	>17	–	1	+	10	+	+	+	–	+	Metastasis to neck LN in 1 year; no other in 2 years

Abbreviations: ACBN, atypical cellular blue nevus; CBN, cellular blue nevus; DOD, died of disease; F, female; LN, lymph node; LVI, lymphovascular invasion; M, male; MBN, melanoma ex blue nevus; NA, not applicable; NP, not performed; SLN, sentinel lymph node.

^aNuclear pleomorphism was graded as 0 = none, 1 = mild, and 2 = prominent.

7.10 mm) and cellular blue nevi (median, 4.75 mm). Ulceration was an uncommon feature observed in one atypical cellular blue nevus and two melanoma ex blue nevus. All melanomas exhibited nuclear pleomorphism and mitotic activity. Average mitotic rates were 0.2/mm² among cellular blue nevi, 1.8/mm² among atypical cellular blue nevi, and 5.1/mm² among melanomas ex blue nevi. Despite this trend of increasing mitotic activity, the differences between groups did not reach statistical significance. Atypical mitotic figures and necrosis were each observed in two melanomas ex blue nevi. Neurotropism was noted in two (40%) cellular blue nevi, four (33%) atypical cellular blue nevi, and four (44%) melanomas ex blue nevi (Figure 2). None of the cases showed lymphovascular invasion. All four presumed precursor blue nevi (cases 20–23) demonstrated significantly lower cellularity, fewer mitoses, and less cytologic atypia compared with their malignant counterparts (Figures 2 and 3).

No significant difference was identified with regard to tumor thickness, nuclear pleomorphism, prominent nucleoli, mitotic rate, atypical mitoses, necrosis, and neurotropism when comparing

melanomas ex blue nevi with and without copy number aberrations.

Genomic Copy Number and LOH Analysis

The results of copy number and LOH analysis are listed in Table 2. Copy number aberrations were found in 3 of 12 (25%) atypical cellular blue nevi and 6 of 9 (67%) melanomas ex blue nevi. None of the cellular blue nevi showed aberrations. All atypical cellular blue nevi with positive results had no more than one aberration in each case. One of the atypical cellular blue nevi showed gain of whole chromosome 20, whereas the remaining two cases showed copy number gain involving a segment of 15q.

As for melanomas ex blue nevi, each of the six positive cases demonstrated at least four aberrations involving multiple chromosomes, with the exception of case 21, which showed an isolated gain of the entire short arm of chromosome 6. Five of these cases showed gain and/or loss of at least one entire chromosomal arm. Of these, loss of 1p and gain of 1q were most common and tended to occur simultaneously in the same lesions; this combination

Figure 1 An atypical cellular blue nevus without copy number aberrations (case 6). (a) The tumor presents as an expansile, bulbous nodule in the dermis pushing into the subcutaneous fat (hematoxylin and eosin (H&E), $\times 20$). (b) It consists of broad fascicles and sheets of plump spindle melanocytes with mild nuclear pleomorphism. A mitotic figure is present in the center of this field. Up to 5 mitoses per mm^2 are identified in this lesion (H&E, $\times 400$). (c) No copy number aberrations are detected by molecular inversion probe microarray.

was observed in three melanomas ex blue nevi. Other recurrent chromosomal arm aberrations included gains of 4p, 6p, and 8q, and loss of 4q, each found in two cases. Whole chromosome (numerical) aberrations were also identified in three melanomas, including loss of whole chromosome 3 in two cases. All whole chromosome aberrations were accompanied by additional partial (structural) aberrations of other chromosomes in melanomas ex blue nevi.

The three presumed precursor blue nevi in cases 20–22 showed no copy number aberrations. In case 23, the prior biopsy diagnosed as atypical cellular blue nevus and the subsequent melanoma displayed almost identical aberrations, with the exception of the loss of 4q, which was only present in the latter. In addition, the gain of 4p was of much lower magnitude in the precursor compared to the melanoma (Figure 4).

Two melanomas ex blue nevi (cases 22 and 25) demonstrated LOH involving multiple chromosomes. No other cases showed LOH. A diagrammatic summary of the genomic copy number changes and LOH results is shown in Figure 5.

GNAQ, GNA11, and HRAS Gene Mutation Analysis

One atypical cellular blue nevus and eight melanomas ex blue nevi had remaining PCR-suitable DNA that were analyzed by Sanger sequencing for *GNAQ*, *GNA11*, and *HRAS* mutations. *GNAQ* mutation was detected in two melanomas ex blue nevi (cases 19 and 20), both of which lacked genomic copy number aberrations by molecular inversion probe microarray. Another two melanomas displayed *GNA11* mutation. None of the cases showed *HRAS* mutations. A summary of the mutation analysis is shown in Table 3.

Figure 2 A melanoma ex blue nevus without detectable copy number aberrations (case 19). (a) The lesion is asymmetric and extends into the deep dermis in a tongue-like manner (hematoxylin and eosin (H&E), $\times 20$). (b) The superficial and peripheral aspects of the lesion consist of bland dendritic spindle cells consistent with a blue nevus (H&E, $\times 400$). (c) In contrast, the central and deep aspects of the lesion are hypercellular and consist of highly pleomorphic and hyperchromatic epithelioid cells (H&E, $\times 400$). (d) Neurotropism is identified in the hypercellular, malignant component (H&E, $\times 200$).

Follow-Up Data

Clinical follow-up data were available for 19 of 26 (73%) patients and included in Table 1. The mean follow-up periods were 2.8, 3.4, and 6.3 years for cellular blue nevi, atypical cellular blue nevi, and melanomas ex blue nevi, respectively. Sentinel lymph node biopsy was performed for three atypical cellular blue nevi and five melanomas ex blue nevi. Of these, only one patient with melanoma

(case 26) had a positive sentinel lymph node. Another two patients with melanoma (cases 22 and 23) had distant metastases despite negative sentinel lymph nodes, and died of disease in 4 and 2 years, respectively. All three cases with subsequent metastatic disease were associated with complex copy number aberrations. Of these, loss of whole or part of chromosome 3 was the only aberration shared by all three cases, and was significantly associated with metastatic disease ($P=0.0143$).

Figure 3 A presumed precursor originally diagnosed as atypical cellular blue nevus and the subsequent melanoma (case 23). **(a)** The lesion biopsied 3 years ago demonstrates an intradermal pigmented melanocytic proliferation with an area of increased cellularity (hematoxylin and eosin (H&E), $\times 20$). **(b)** The spindle melanocytes contain melanized dendritic cytoplasmic processes and mildly pleomorphic nuclei, morphologically most in keeping with an atypical cellular blue nevus (H&E, $\times 400$). **(c)** The lesion recurred at the same site and was excised 3 years later. The recurrent lesion presented as an asymmetric, expansile nodule centered in the subcutaneous fat (H&E, $\times 20$). **(d)** The superficial aspect demonstrates bland spindle cells percolating between dermal collagen, compatible with a background blue nevus (H&E, $\times 400$). **(e)** In contrast, the deep nodular component consists of sheets of malignant epithelioid cells with prominent nucleoli and mitotic activity consistent with melanoma (H&E, $\times 400$).

Discussion

Blue nevi may display a spectrum of atypia. Although the unequivocally benign and the overtly malignant cases are easy to recognize, diagnosis of ambiguous lesions in the middle of this spectrum is notoriously difficult. These ambiguous lesions,

commonly referred to as atypical cellular blue nevi, may deviate from benign cellular blue nevi with regard to their architecture, cytology, and/or mitotic activity. Various atypical features have been described in both atypical cellular blue nevus and melanoma ex blue nevus, including asymmetry, expansile nodular silhouette, infiltrative borders,

Figure 3 Continued.

hypercellularity, pleomorphism, hyperchromasia, prominent nucleoli, and necrosis.^{3–6,8,9} Owing to the lack of clear histopathologic criteria, distinction of these entities is often challenging. To improve our understanding and ability to stratify the risk of these lesions, we retrospectively reviewed the histopathology and the clinical outcome of a cohort of cellular blue nevi, atypical cellular blue nevi, and melanomas ex blue nevi, and performed genomic analysis to identify any correlation between copy number changes, histopathologic features, and clinical prognosis.

We found that melanomas ex blue nevi were more likely to harbor copy number aberrations involving multiple chromosomes compared with those classified as atypical cellular blue nevi by histomorphology. As expected, aberrations were absent in all cellular blue nevi. Similar to the results reported by Maize *et al*,¹¹ a small subset of our atypical cellular blue nevi demonstrated copy number aberrations involving a single region in each case. However, unlike the study by Maize *et al*¹¹ in which each blue nevus-like melanoma and melanoma ex blue nevus had three or more aberrations, three of nine (33%) melanomas in our series lacked detectable copy number changes. It is possible that these cases may harbor other forms of genetic aberrations. Indeed, *GNAQ* mutation was identified in two of our melanomas ex blue nevi without copy number aberrations (cases 19 and 20). Somatic mutation in codon 209, as seen in these two cases, is known to result in constitutive activation of *GNAQ* and mitogen-activated protein kinase activation.²² This oncogenic mutation is common in blue nevi and has been reported in a subset of histopathologically benign and ambiguous cellular

blue nevi.¹³ As *GNAQ* mutation alone is insufficient for full progression to melanoma, these cases likely have acquired additional mutations or translocations not detected in this study. Several factors may have contributed to the higher rates of copy number changes reported by Maize *et al*¹¹ compared with our study. Given the significant diagnostic challenge associated with the spectrum of blue nevi, it is not surprising that various institutions may have adopted somewhat different thresholds in classifying these lesions, thus yielding slightly different results. It is also noteworthy that the ambiguous group in the study by Maize *et al*¹¹ comprised ‘atypical cellular blue nevus with features of Spitz nevus,’ ‘cellular blue nevus *versus* desmoplastic melanoma,’ and ‘epithelioid melanocytic proliferation.’ These morphologic descriptors suggest greater heterogeneity in the types of ambiguous lesions included in their cohort, which may have possibly led to a wider range of genomic abnormalities.

With the exception of one melanoma ex blue nevus, which showed an isolated gain of 6p (case 21), all other melanomas with copy number changes in our series had complex aberrations involving at least four regions. The latter included all three cases with adverse outcomes, whereas case 21 showed no evidence of disease at 7 years follow-up. These findings further support the correlation between increased tumor genomic instability and more aggressive clinical course.

An interesting finding is the frequent gains and losses of entire chromosomal arms in melanomas ex blue nevi, including recurrent gains of 1q, 4p, 6p, and 8q, and recurrent losses of 1p and 4q. These aberrations were also reported in some of the ambiguous and malignant lesions in two prior CGH

Table 2 Copy number and loss of heterozygosity data of all cases

Case	Diagnosis	Copy number gain	Copy number loss	Loss of heterozygosity
1	CBN	None	None	None
2	CBN	None	None	None
3	CBN	None	None	None
4	CBN	None	None	None
5	CBN	None	None	None
6	ACBN	None	None	None
7	ACBN	None	None	None
8	ACBN	None	None	None
9	ACBN	None	None	None
10	ACBN	None	None	None
11	ACBN	None	None	None
12	ACBN	None	None	None
13	ACBN	None	None	None
14	ACBN	None	None	None
15	ACBN	20	None	None
16	ACBN	15q11.2–26.3	None	None
17	ACBN	15q21.2–25.3	None	None
18	MBN	None	None	None
19	MBN	None	None	None
20	MBN	None	None	None
	Precursor	None	None	None
21	MBN	6p	None	None
	Precursor	None	None	None
22	MBN	7q11.21–21.11, 7q21.3–31.33, 8p23.3–q24.3, 9q33.2, 19p13.3–13.2, 19p13.12–13.11, 19q12, 19q13.41–13.43, 21q11.2–22.3, Xq28	3q21.1–21.2, 3q26.31, 10q11.22, 10q23.31	1p36.33–q44, 3p26.3–q29, 8p12–11.23
	Precursor	None	None	None
23	MBN	1q, 4p, 8q	1p, 3, 4q	None
	Precursor	1q, 4p ^a , 8q	1p, 3	None
24	MBN	1q32.3–q44, 6p, 8q21.3–24	1p, 3p, 9p	None
25	MBN	1q, 7, 15q11.2–26.3, 20	1p, 2, 4, 5q31.2–35.3, 9, 16, 17q11.2–q21.31, 18	2, 6, 8
26	MBN	1q, 4p, 8q	1p, 3, 4q, 8p, 9p21.3–p22.2	None

Abbreviations: ACBN, atypical cellular blue nevus; CBN, cellular blue nevus; MBN, melanoma ex blue nevus, p, short arm; q, long arm.

^aGain of 4p was of miniscule magnitude in the presumed precursor lesion of case 23.

studies on a spectrum of blue nevi, although chromosomal arm aberrations were not a dominant finding in these studies.^{11,13} Recurrent chromosomal arm aberrations have been associated with a number of malignancies. For example, gain of 3q is common in vulvar carcinomas,²³ and gain of 20q is frequently detected in pancreatic, prostatic, breast, and gastric carcinomas.^{24–27} Characteristic chromosomal arm aberrations have also been demonstrated in other types of melanocytic lesions including Spitz nevi, sinonasal melanomas, and uveal melanomas. In Spitz nevi, gain of entire short arm 11p is present in about 12% of cases and is frequently coupled with *HRAS* mutation;^{28,29} this finding is almost invariably absent in melanomas.^{30,31} In sinonasal melanomas, recurrent gains of 1q, 6p, and 8q were found in 100%, 93%, and 57% of 14 cases in a single series.³² These changes overlap with those detected in uveal melanomas, in which recurrent losses of 1p, 6q, and 8p and gains of 6p and 8q have been reported as tumor-specific cytogenetic aberrations.^{33,34}

The above recurrent changes involving the entire short or long arms of chromosomes 1, 6, and 8 in uveal and sinonasal melanomas are also identified in melanomas ex blue nevi. Interestingly, 1p loss (–1p) and 1q gain (+1q) consistently accompanied each

other in all three melanomas ex blue nevi harboring these aberrations in our series, a novel finding that has not been reported in uveal melanomas, sinonasal melanomas, or previous series of blue nevi-like melanomas and melanomas ex blue nevi. Interestingly, concomitant –1p/+1q have been described in a subset of hepatocellular carcinomas and gliomas.^{35–37} When found in gliomas, these changes were associated with better response to chemotherapy and longer survival.^{36,37} Of our three cases with concomitant –1p/+1q, one patient died of distant metastases (case 23), one patient had regional (nodal) metastasis (case 26), and one patient had no available follow-up information (case 25). Although no definitive conclusion can be drawn from this small number of cases, our data at least suggest that concomitant –1p/+1q may portend an increased risk of metastasis in melanomas ex blue nevi.

A similar phenomenon was also observed on chromosome 4, with concomitant +4p/–4q detected in two melanomas ex blue nevi. Interestingly, one of these cases (case 23) was preceded by a biopsy originally diagnosed as atypical cellular blue nevus 3 years prior showing the absence of –4q and almost negligible +4p (Figure 4), and the patient subsequently died of distant metastatic disease.

Figure 4 Copy number plots of case 23. (a) The precursor atypical cellular blue nevus demonstrates gains of 1q and 8q, and losses of 1p and whole chromosome 3. A minute gain of 4p is also noted (red arrow). (b) The subsequent melanoma shows a gain of 4p of much higher magnitude (black arrow), as well as an additional loss of 4q.

Morphologically, the malignant lesion clearly showed more expansile and asymmetric growth, increased cellularity and cytologic atypia, and higher mitotic rate compared with its presumed precursor in the previous biopsy (Figure 3). These findings suggest that +4p/−4q may have an important role in promoting tumor aggressiveness. Unlike −1p/+1q, concomitant +4p/−4q have not been reported as a recurrent pattern in any tumor type in the literature. Although the exact mechanism of concomitant gain and loss of the opposite arms on a given chromosome is unclear, it may involve a breakpoint on the lost arm close to the centromere, followed by a gain of the remaining arm as a result of aneuploidy. Another possible mechanism is via the formation of isochromosome, in which one arm is lost and replaced with an exact copy of the other arm secondary to misalignment of the affected chromosome during metaphase.³⁷

Other recurrent arm aberrations identified in this study include gain of 6p and gain of 8q. Additionally, loss of 6q has been reported in three 'malignant blue nevi' in the prior studies.^{11,13} These regions contain genes that are frequently involved in melanoma, including *RREB1* (6p25), *MYB* (6q23), and *MYC* (8q24). The former two genes on chromosomes 6, together with *CCND1* (11q13) and the *Cep6*, are targets of the standard 4-probe FISH panel commonly used in discriminating malignant and benign melanocytic neoplasms.^{38,39} Although this standard panel was previously found to have a sensitivity and specificity of 100% in distinguishing cellular blue nevus from blue nevus-like melanoma,¹² our data predict a low sensitivity (33%) of this panel in detecting melanoma ex blue nevus with genomic copy number aberrations. It is possible that some of our melanomas may contain small subclones with aberrations that fall below the

detection limit of the genomic microarray, which may be demonstrable only by FISH.⁴⁰ A follow-up FISH study is therefore necessary to explore this possibility and to reconcile the discrepancy between the high sensitivity reported by Gammon *et al*¹² and

the low sensitivity predicted from our data. Meanwhile, based on the current evidence in our study, an expanded FISH panel including probes that target *MYC* (8q24) and *CDKN2A* (9p21) will be required to detect the rest of our malignant cases with known copy number aberrations.

In this series, the loss of whole or part of chromosome 3 was the only aberration shared by all three malignant cases with subsequent metastatic disease, and this association was found to be statistically significant. This parallels the finding of Prescher *et al*,⁴¹ in which monosomy 3 was identified as a negative predictor of relapse-free survival in patients with uveal melanoma.⁴¹ In addition to the morphological similarities between melanoma ex blue nevus and uveal melanoma, our finding further supports a link between these two entities on a molecular level.

To our knowledge, only scarce prior case reports have separately examined the melanoma component and the precursor blue nevus component in similar lesions using CGH. North *et al*⁴² reported two cases of melanoma ex blue nevus morphologically resembling large plaque-type blue nevus with subcutaneous cellular nodules. In both cases, they found concomitant +6p/-6q in the nodules of melanoma but not in the background blue nevus. Another case report by Gerami *et al*⁴³ identified copy number gains involving the distal arm of 1q, 6p, the distal arm of 9q, the distal arm of 8q, and loss of 6q in the areas of melanoma, whereas the background nevus of Ota and other areas resembling cellular blue nevus were devoid of these changes.⁴³ Our study describes three more cases of melanoma ex blue nevus in which additional copy number changes were acquired in the morphologically malignant component, providing molecular evidence of tumor progression in these lesions.

In conclusion, our study demonstrated more frequent copy number aberrations involving multiple chromosomes among melanomas ex blue nevi compared to cellular and atypical cellular blue nevi. Complex copy number aberrations involving four or more chromosomal regions are indicative of malignancy in this spectrum of lesions. Detection of

Figure 5 Diagrammatic summary of copy number changes detected in three atypical cellular blue nevi (cases 15–17) and six melanomas ex blue nevi (cases 21–26). Copy number gains (green), copy number losses (red), and copy-neutral losses of heterozygosity (yellow) are indicated according to the chromosomal arms being affected. Those involving an entire chromosomal arm are marked with an asterisk (*). B, blue nevus (including atypical cellular blue nevus and presumed precursor blue nevus); Chr, chromosome; M, melanoma; p, short arm; q, long arm.

Table 3 Results of *GNAQ*, *GNA11*, and *HRAS* gene mutation analysis

Case	Diagnosis	<i>GNAQ</i> exon 4	<i>GNAQ</i> exon 5	<i>GNA11</i> exon 4	<i>GNA11</i> exon 5	<i>HRAS</i> exon 2	<i>HRAS</i> exon 3
16	ACBN	WT	WT	WT	WT	WT	WT
18	MBN	WT	WT	ND	WT	ND	WT
19	MBN	WT	Q209P	WT	WT	ND	WT
20	MBN	ND	Q209L	WT	WT	WT	WT
21	MBN	WT	ND	WT	WT	WT	WT
23	MBN	WT	WT	WT	Q209L	WT	WT
24	MBN	WT	WT	ND	WT	WT	WT
25	MBN	WT	WT	WT	WT	WT	WT
26	MBN	ND	WT	WT	Q209L	ND	ND

Abbreviations: ACBN, atypical cellular blue nevus; MBN, melanoma ex blue nevus; ND, no data; WT, wild type.

copy number aberrations may be viewed as a fair predictor of worse clinical outcome, as all cases with known ensuing metastatic disease in this series tested positive for copy number aberrations. We also identified common and recurrent gains and losses of entire chromosomal arms in melanomas ex blue nevi, including +1q, -1p, +4p, -4q, +6p, and +8q. Based on these findings, an expanded FISH panel with additional probes targeting 1q, 1p, 4p, and 4q may improve detection and risk stratification of melanoma ex blue nevus.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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