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Molecular Profiling of Noncoding Mutations Distinguishes Nevoid Melanomas From Mitotically Active Nevi in Pregnancy

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西京医院病理科

徐梦微

WHO classification of skin tumors

Melanocytic tumours

Melanocytic tumours in intermittently sun-exposed skin	
Low-CSD melanoma (superficial spreading melanoma)	8743/3
Simple lentigo and lentiginous melanocytic naevus	8742/0*
Junctional naevus	8740/0
Compound naevus	8760/0
Dermal naevus	8750/0
Dysplastic naevus	8727/0
Naevus spilus	8720/0
Special-site naevi (of the breast, axilla, scalp, and ear)	
Halo naevus	8723/0
Meyerson naevus	8720/0
Recurrent naevus	
Deep penetrating naevus	8720/0
Pigmented epithelioid melanocytoma	8780/1*
Combined naevus, including combined *BAP1*-inactivated naevus/melanocytoma	8720/0
Melanocytic tumours in chronically sun-exposed skin	
Lentigo maligna melanoma	8742/3
Desmoplastic melanoma	8745/3
Spitz tumours	
Malignant Spitz tumour (Spitz melanoma)	8770/3
Spitz naevus	8770/0
Pigmented spindle cell naevus (Reed naevus)	8770/0
Melanocytic tumours in acral skin	
Acral melanoma	8744/3
Acral naevus	8744/0*
Genital and mucosal melanocytic tumours	
Mucosal melanomas (genital, oral, sinonasal)	8720/3
Mucosal lentiginous melanoma	8746/3
Mucosal nodular melanoma	8721/3
Genital naevus	8720/0
Melanocytic tumours arising in blue naevus	
Melanoma arising in blue naevus	8780/3
Blue naevus NOS	8780/0
Cellular blue naevus	8790/0
Mongolian spot	
Naevus of Ito	
Naevus of Ota	
Melanocytic tumours arising in congenital naevi	
Melanoma arising in giant congenital naevus	8761/3
Congenital melanocytic naevus	8761/0*
Proliferative nodules in congenital melanocytic naevus	8762/1
Ocular melanocytic tumours	
Uveal melanoma	
Epithelioid cell melanoma	8771/3
Spindle cell melanoma, type A	8773/3
Spindle cell melanoma, type B	8774/3
Conjunctival melanoma	
Melanoma NOS	8720/3
Conjunctival primary acquired melanosis with atypia/melanoma in situ	8720/2
Conjunctival naevus	8720/0
Nodular, naevoid, and metastatic melanomas	
Nodular melanoma	8721/3
Naevoid melanoma	8720/3
Metastatic melanoma	8720/6

痣样黑色素瘤
Nevoid Melanomas

【定义】痣样黑色素瘤是皮肤恶性黑色素瘤的一个亚型，特点是当病变由小细胞构成时，组织学特点类似于混合痣和皮内痣；当瘤细胞为中等大小或大细胞时，似Spitz痣。因此类病变可累及真皮并有转移潜能，因此不认为其是非典型性痣，而是黑色素瘤。有些文献也曾用“微小偏离性黑色素瘤”的名称。

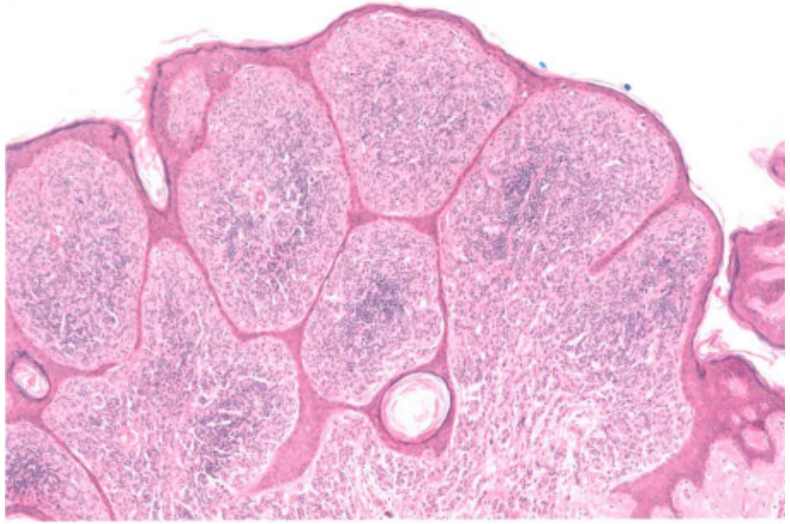
【ICD-O编码】 8720/3

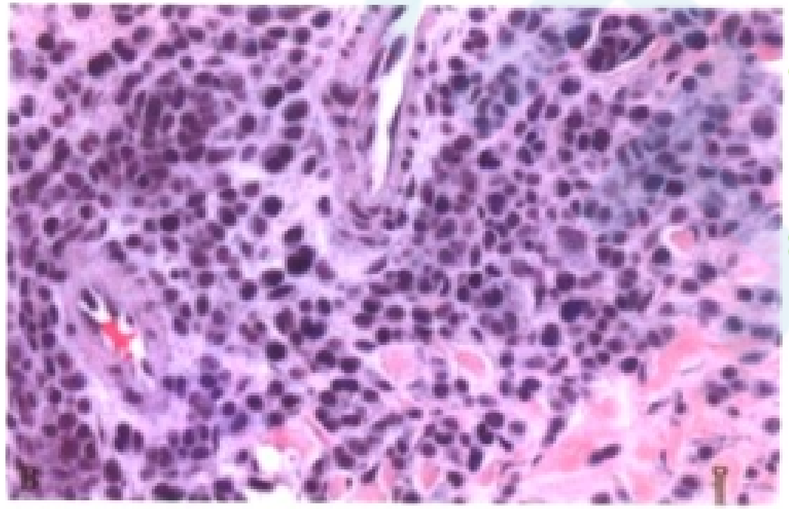
【流行病学】可发生于任何年龄，中位年龄56岁（范围：16-89岁），男女均可受累；病变主要位于躯干部和近侧肢体。

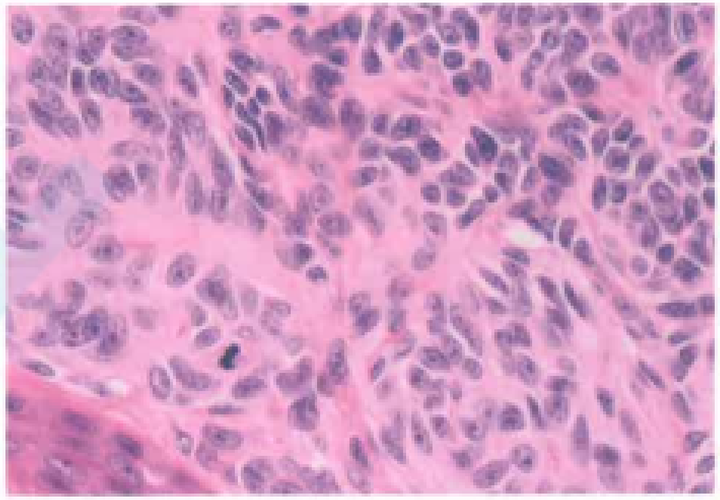
【临床特点】小的丘疹、结节或呈疣状，棕褐色到深棕色，直径约5~10mm，临床上无明显炎症。病变质软而无触痛，常为孤立性病变。

痣样黑色素瘤
Nevoid Melanomas

【组织病理学】大体为半球形、息肉状或疣状。表皮内派杰样播散较少，低倍镜下轮廓相对对称的一种病变。病变侧缘境界清楚，真皮内常有增生的黑色素细胞融合成片的区域，多数病例中真皮内可见核分裂象。可以由相对较一致的小细胞构成，细胞核深染，也可由中等大小或大细胞构成，胞浆浅染，胞核较空。炎症反应一般很轻或缺如。







ELSEVIER

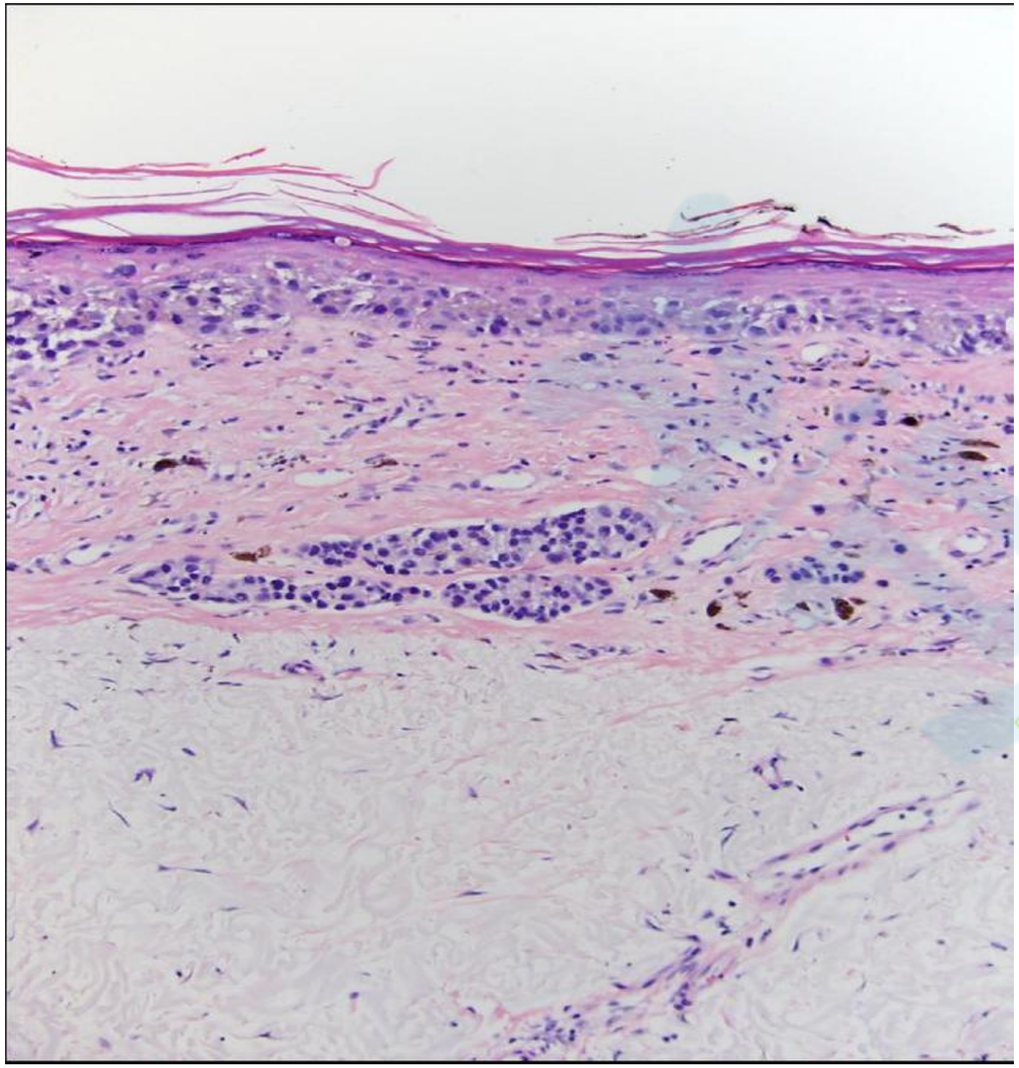
Human Pathology

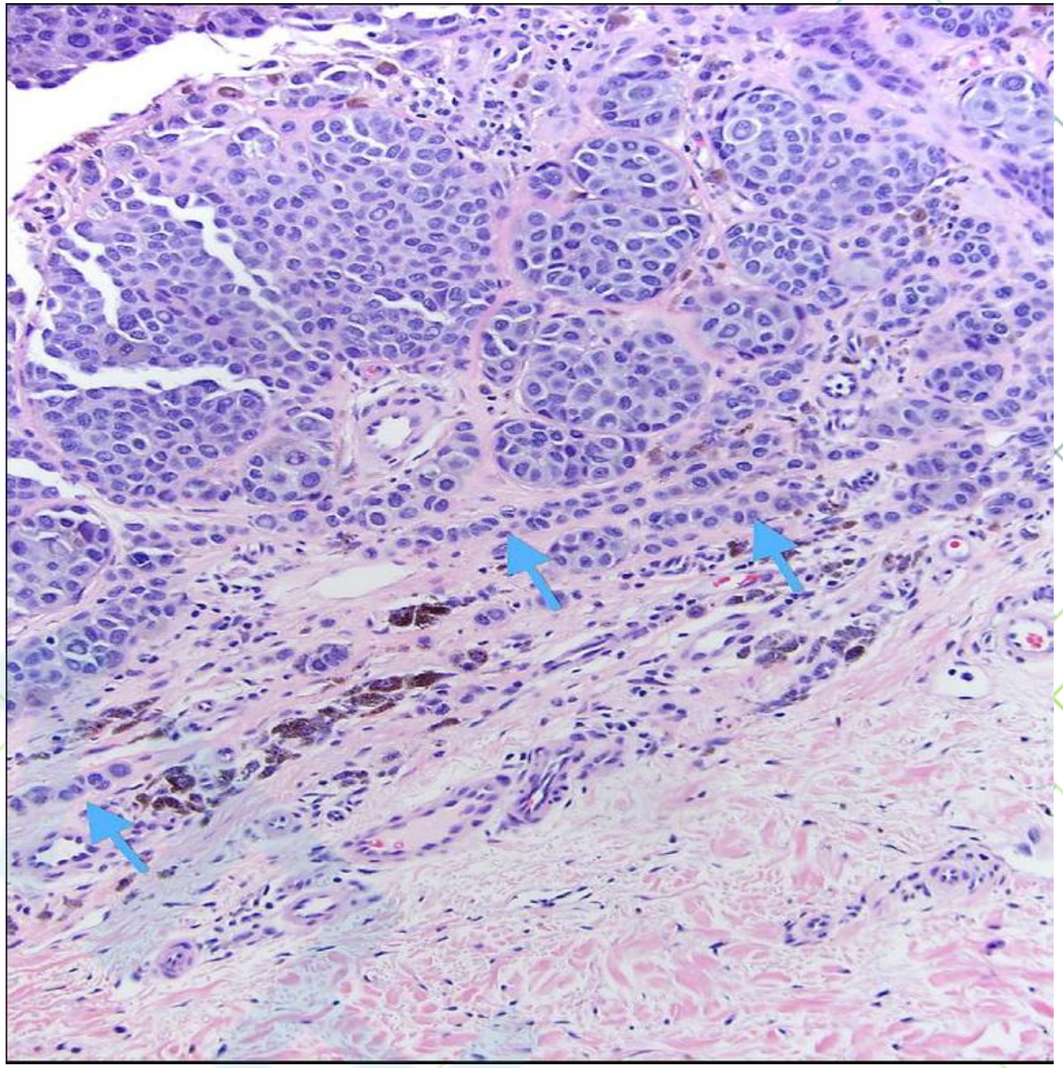
Volume 26, Issue 2, February 1995, Pages 171-179

Original contribution

Nevoid melanoma: A clinico… …en cases … mimic… …oid cell ne… …l nevus

报告了七例恶性黑色素瘤的独特类型，其特征是具有良性组织学外观，类似于黑色素细胞痣；临床随访显示，间隔5个月至5年的3例患者局部复发，2年后的1例患者发生局部转移。





◆痣样黑素瘤的标准包括：（1）轮廓相对对称（2）黑素细胞的细胞学外观与小圆形或多角形 A 型痣细胞（泡状核和胞浆丰富）相似（3）存在成熟的证据（4）极少的交界成分

Idriss MH . J Am Acad Dermatol

妊娠期“痣”改变

◆色素沉着是妊娠期最常见的皮肤改变，雌孕激素、促黑素细胞激素水平升高均可刺激黑素形成。妊娠期色素沉着主要累及深肤色妇女和正常色沉的部位，痣、雀斑和近期疤痕颜色均可变黑。

◆有研究表明，恶性黑素瘤是妊娠期最常见的恶性肿瘤之一，妊娠期妇女的免疫系统改变可能降低了肿瘤免疫监视，还发现有些黑色素瘤存在激素受体。

中国黑色素瘤规范化病理诊断专家共识（2017版）

•病理报告内容及规范

•建议常规病理组织学报告内容包括：肿瘤部位、标本类型、肿瘤大小或范围、组织学类型、Breslow厚度、有无溃疡、浸润深度（ Clark水平分级）、分裂活性、切缘状况（包括各切缘与肿瘤的距离以及切缘病变的组织学类型）、有无微卫星或卫星转移、有无脉管内瘤栓、有无神经侵犯等。报告辅助诊断结果（相关免疫组化和/或FISH检测）和靶向治疗相关分子检测结果（BRAF、c-KIT等）。前哨淋巴结和区域淋巴结需报告检见淋巴结的总数、转移淋巴结个数以及有无淋巴结被膜外受累。

•Clark水平分级（Clark level）：指皮肤黑色素瘤的浸润深度，分为5级。1级表示肿瘤局限于表皮层（原位黑色素瘤）；2级表示肿瘤浸润真皮乳头层但尚未充满真皮乳头层；3级表示肿瘤细胞充满真皮乳头层到达乳头层和网状层交界处；4级表示肿瘤浸润真皮网状层；5级表示肿瘤浸润皮下组织。

•Breslow厚度（Breslow thickness）：指皮肤黑色素瘤的肿瘤厚度，是T分期的基本指标。非溃疡性病变指表皮颗粒层至肿瘤浸润最深处的垂直距离；溃疡性病变指溃疡基底部至肿瘤浸润最深处的垂直距离。

Breslow厚度（Breslow thickness）

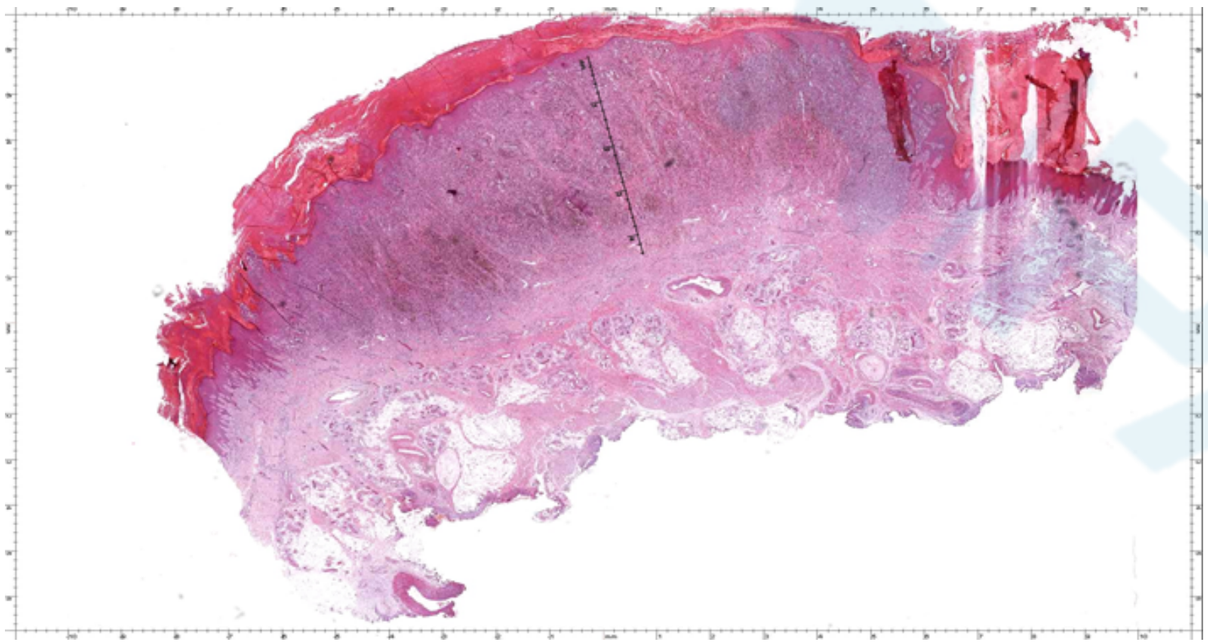


图1 非溃疡性病变厚度：从表皮颗粒层垂直测量至肿瘤浸润最深处

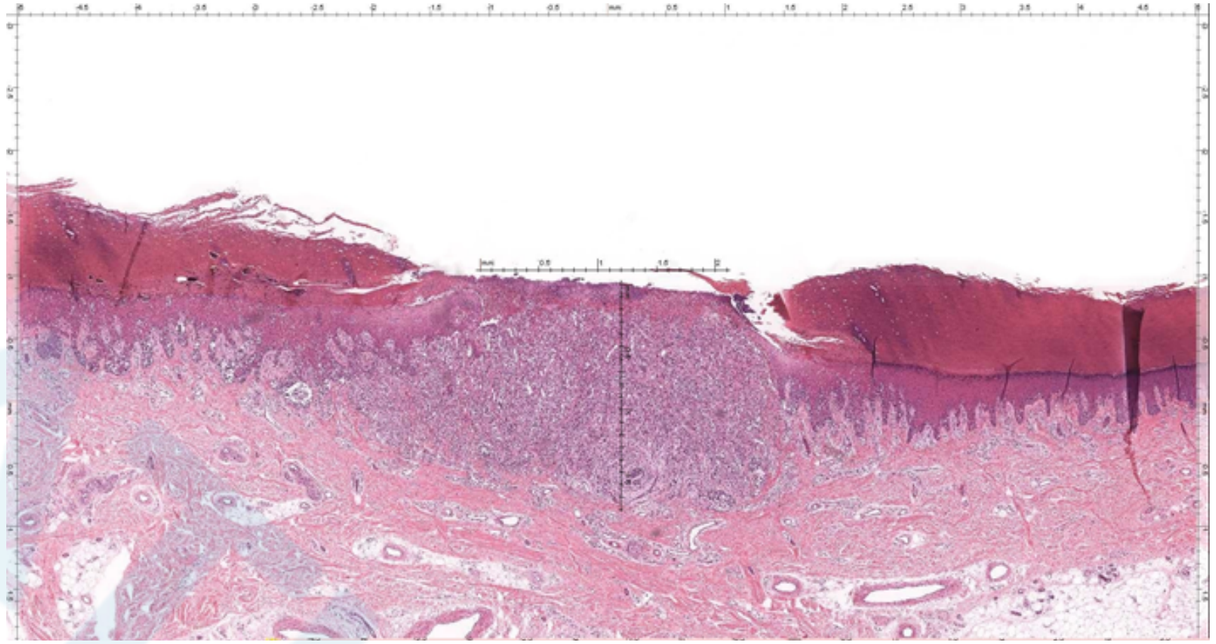


图2 溃疡性病变厚度：从溃疡基底部垂直测量至肿瘤浸润最深处

研究目的

- Accurate diagnosis of NM and MANP is of great practical importance not only to reduce the risk of overtreatment or undertreatment, but also because it is essential if optimal patient outcomes are to be achieved.
- This study sought to identify differences in clinical, pathologic,and molecular characteristics between NM and MANP,which may assist in their accurate pathologic diagnosis.

材料与方法

◆Patients:

- Department of Tissue Pathology and Diagnostic Oncology at Royal Prince Alfred Hospital, Sydney.
- NM and melanocytic lesions from women who were either pregnant or up to 6 weeks postpartum(2003 – 2016).

◆DNA Extraction

- Tumor-enriched DNA was extracted from archival formalin-fixed biopsies using the High Pure FFPET DNA Isolation Kit following the manufacturer’s protocols (Roche).

材料与方法

◆Next-generation Custom Amplicon Sequencing

- **Coding :** *ARID2, BAP1, BRAF, CDKN2A, EZH2,GNA11, GNAQ, HRAS, KIT, KRAS, MAP2K1, MAP2K2, NF1, NRAS, PPP6C, PTEN, RAC1, RAF1, RB1, SF3B1, TERT, and TP53*
- **Noncoding :** *AP3D1, ARHGEF18, BLCAP, C16ORF59, CDC20, CHCHD2, DHX16, DPH3, ERGIC3, FTH1, HSBP1, KBTBD8,MRPS31, MRPS33, NFKBIE, NSUN6, PES1, RALY, RNF185, RPL13A, RPL18A, RPL29, RPL34, RPS14, RPS27, SLC30A6, SMUG1, SWI5, SYF2, TERT, UBXN8, YAE1D1, and ZNF778*

◆Sequence Alignment and Variant Calling

结　果

结果

TABLE 1. Key Clinicopathologic Characteristics of Nevoid Melanomas That Underwent Mutational Analysis

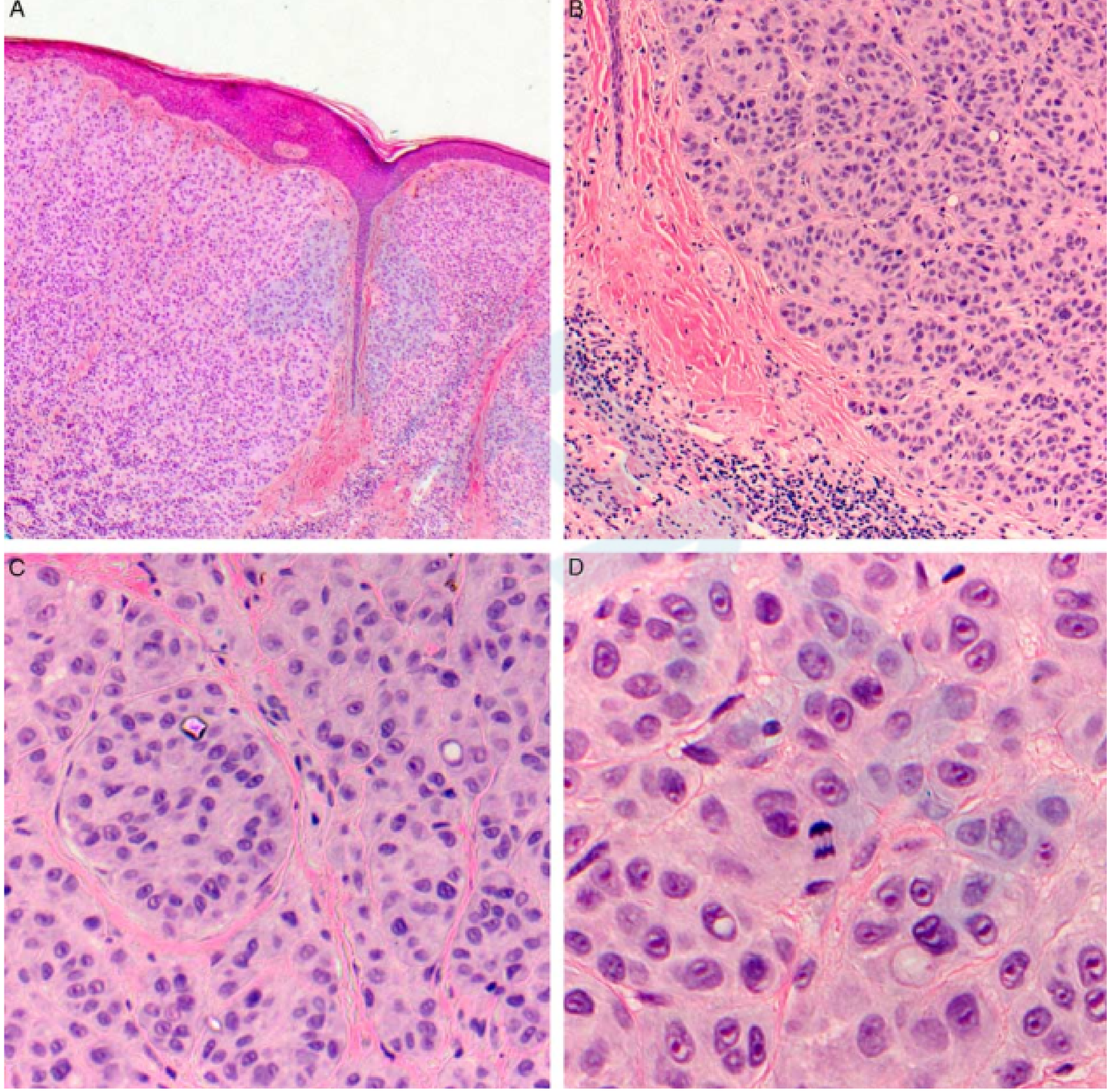
Case No.	Age/Sex	Site	Associated Nevus	Breslow Thickness (mm)	Clark Level	Dermal Mitotic Rate	Location of Mitoses	Symmetry	Regression	Pagetoid Spread	Lentiginous Proliferation	Inflammatory Infiltrate	Cytologic Atypia	Maturation
1	55/female	Left shoulder	Incom									ent	Mild	Absent
2	34/male	Right cheek	Abs									ent	Mild	Present
3	47/female	Left leg	Abs									ent	Moderate	Absent
4	64/female	Right arm	Dyspl comp nev									mal	Mild	Absent
5	19/male	Right lower leg	Den nev									ent	Moderate	Present
6	62/male	Left upper back	Comp nev									ent	Mild	Incomplete
7	83/female	Scalp	Absent	1.5	4	3	Superficial	Symmetrical	Absent	Absent	Minimal	Absent	Mild	Incomplete
8	47/male	Right anterior shoulder	Absent	0.7	3	1	Superficial and deep	Mild asymmetry	Absent	Absent	Minimal	Absent	Mild	Present

- 平均年龄为46岁（17-83岁）
- 病变位于上肢、头颈部、下肢和躯干；4例解剖部位未知
- Breslow厚度中位数1.4mm（0.6-2mm)
- 核分裂象3 / mm^2

结果

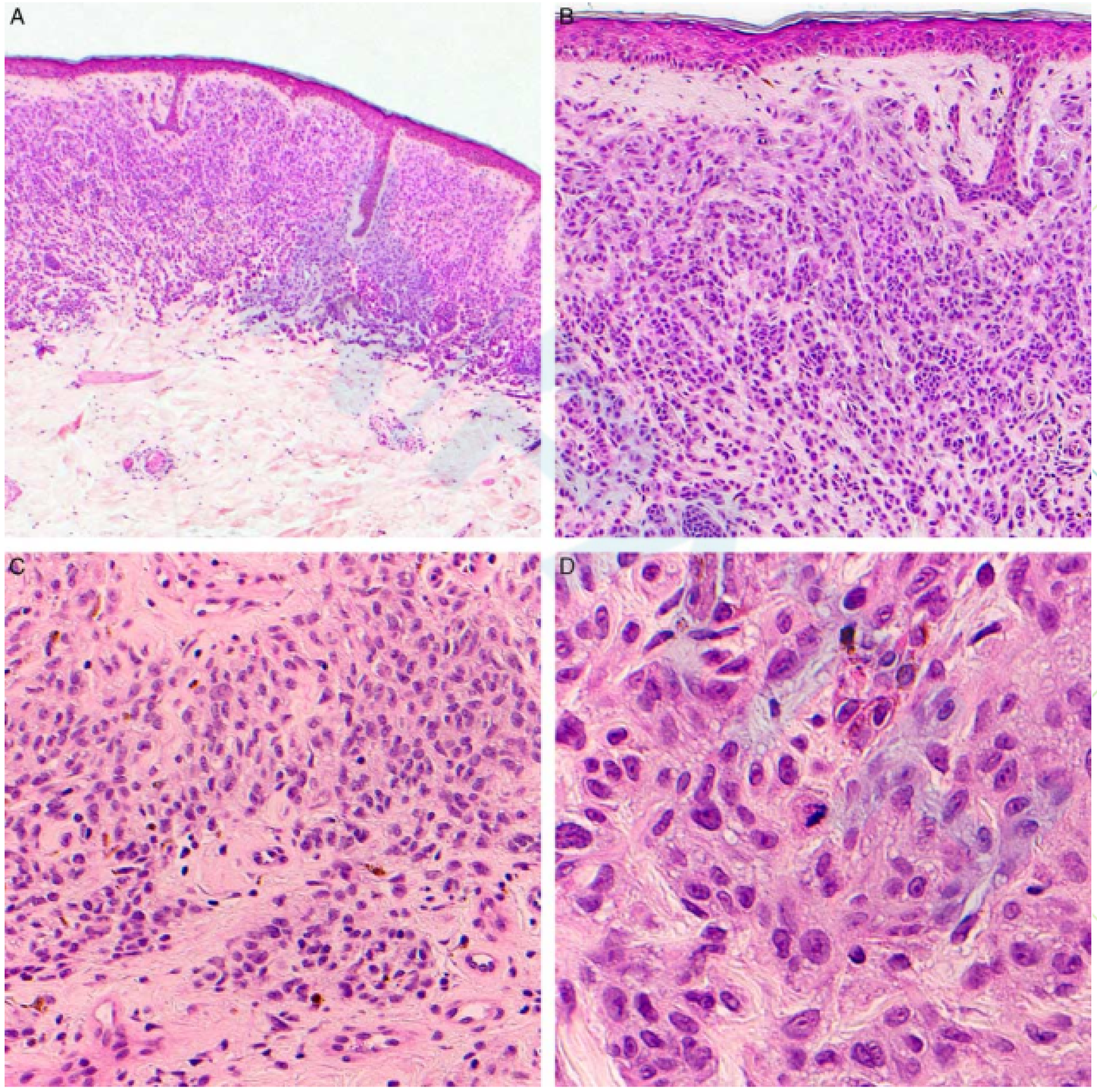
TABLE 1. Key Clinicopathologic Characteristics of Nevoid Melanomas That Underwent Mutational Analysis

Case No.	Age/Sex	Site	Associated Nevus	Breslow Thickness (mm)	Clark Level	Dermal Mitotic Rate	Location of Mitoses	Symmetry	Regression	Pagetoid Spread	Lentiginous Proliferation	Inflammatory Infiltrate	Cytologic Atypia	Maturation
1	55/female	Left shoulder	Incomplete	1.5	4	5	Superficial and deep	Mild asymmetry	Absent	Minimal	Minimal	Absent	Mild	Absent
2	34/male	Right cheek	Absent	1.8	4	2	Superficial and deep	Asymmetrical	Absent	Absent	Absent	Absent	Mild	Present
3	47/female	Left leg	Absent	1.5	4	3	Superficial and deep	Symmetrical	Absent	Absent	Absent	Absent	Moderate	Absent
4	64/female	Right arm	Dysplastic compound nevus	1.4	4	3	Superficial and deep	Symmetrical	Absent	Minimal	Minimal	Minimal	Mild	Absent
5	19/male	Right lower leg	Dermal nevus	1.4	4	4	Superficial and deep	Mild asymmetry	Absent	Minimal	Minimal	Absent	Moderate	Present
6	62/male	Left upper back	Compound nevus	1.5	3	2	Superficial	Symmetrical	Absent	Absent	Absent	Absent	Mild	Incomplete
7	83/female	Scalp	Absent	1.5	4	3	Superficial	Symmetrical	Absent	Absent	Minimal	Absent	Mild	Incomplete
8	47/male	Right anterior shoulder	Absent	0.7	3	1	Superficial and deep	Mild asymmetry	Absent	Absent	Minimal	Absent	Mild	Present



Case1-NM

55岁，女性， 左肩



Case5-NM

19岁，男性；右小腿

结果

TABLE 2. Key Clinicopathologic Characteristics of MANP That Underwent Mutational Analysis

Case No.	Age/Sex	Sit	Diagnosis	Dermal Mitotic Rate (/mm^2)
9	19/female	Vul	ombined nevus	3
10	27/female	Right	ombined nevus	2
11	27/female	Mid sternu	Dermal nevus	3
12	27/female	Lower ab	ompound nevus	2
13	31/female	Abdo	ompound nevus	2
14	33/female	Right b	ompound nevus	1
15	31/female	Bac	ompound nevus	2
16	33/female	Right fou	ompound nevus	1
17	27/female	Right b	ompound nevus	3
18	30/female	Abdo	ompound nevus	1
19	39/female	Left fo	ompound nevus	1
20	32/female	Right fo	Dermal nevus	2

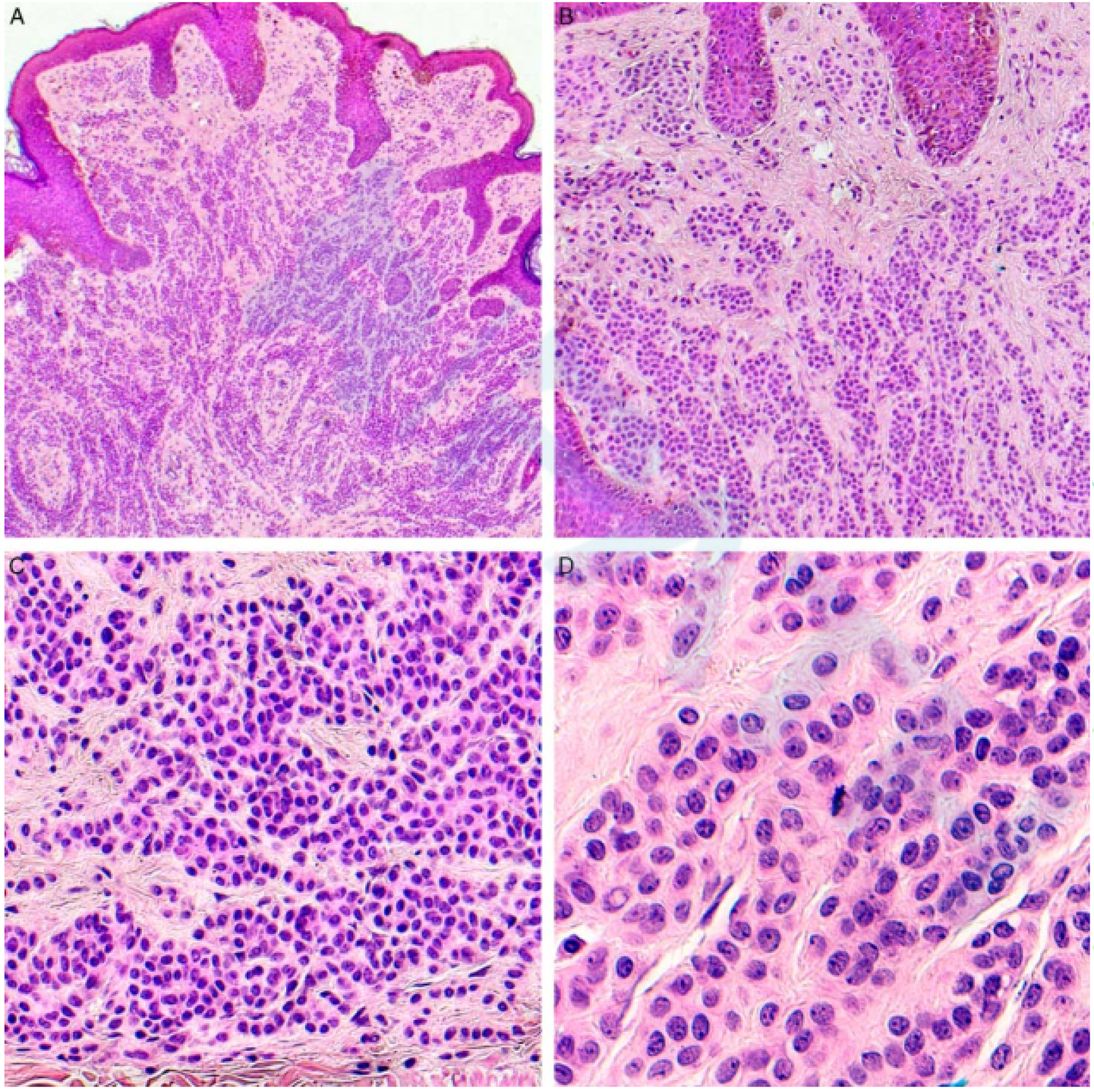
- 平均年龄为31岁（范围：18到45岁）
- 中位妊娠年龄为26周（范围：6至38周）；3例患者在产后6周以内
- 在15名接受随访的患者中，没有复发或转移性疾病（随访期：2-14年）
- 病变部位是躯干（53%），下肢（13%），上肢（13%），头颈（10%），外阴（7%）

结果

TABLE 2. (*continued*)

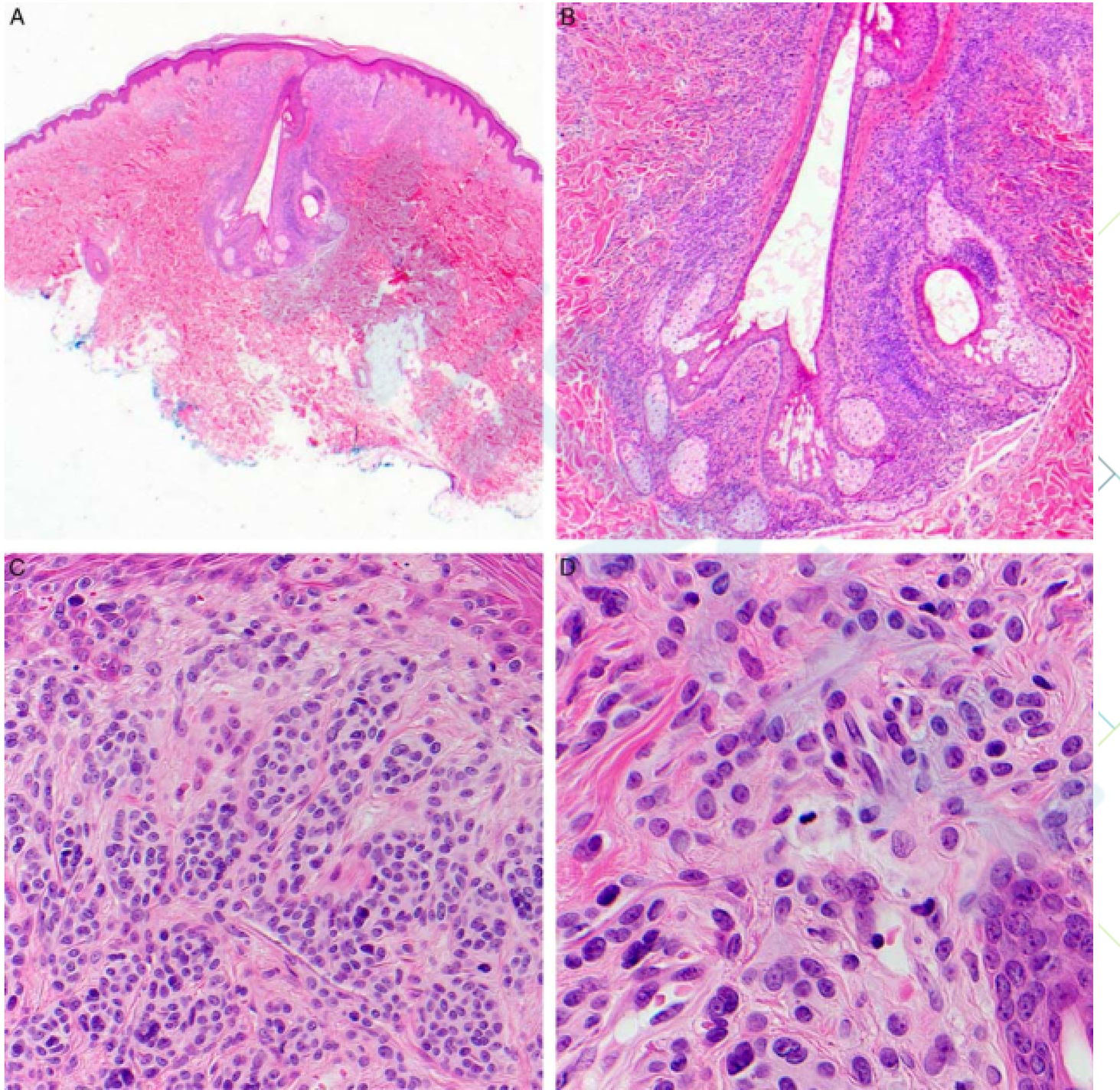
Location of Mitoses	Symmetry	Regression	Pagetoid Spread	Lentiginous Proliferation	Inflammatory Infiltrate	Cytologic Atypia
Superficial and deep	Symmetrical				bsent	Absent
Superficial only	Mild asymmetry				atchy	Spitzoid population
Superficial and deep	Symmetrical				bsent	Minimal
Superficial and deep	Symmetrical				bsent	Mild
Superficial and deep	Symmetrical				bsent	Absent
Superficial	Symmetrical				bsent	Absent
Superficial and deep	Symmetrical				bsent	Absent
Superficial and deep	Symmetrical				bsent	Absent
Superficial and deep	Symmetrical				bsent	Absent
Superficial	Symmetrical				bsent	Absent
Superficial	Symmetrical				bsent	Absent
Superficial and deep	Symmetrical				bsent	Absent

MANP形态特征包括对称性，成熟性，轻度至中度的细胞异型性，无派杰样播散，浅表和深部可见核分裂



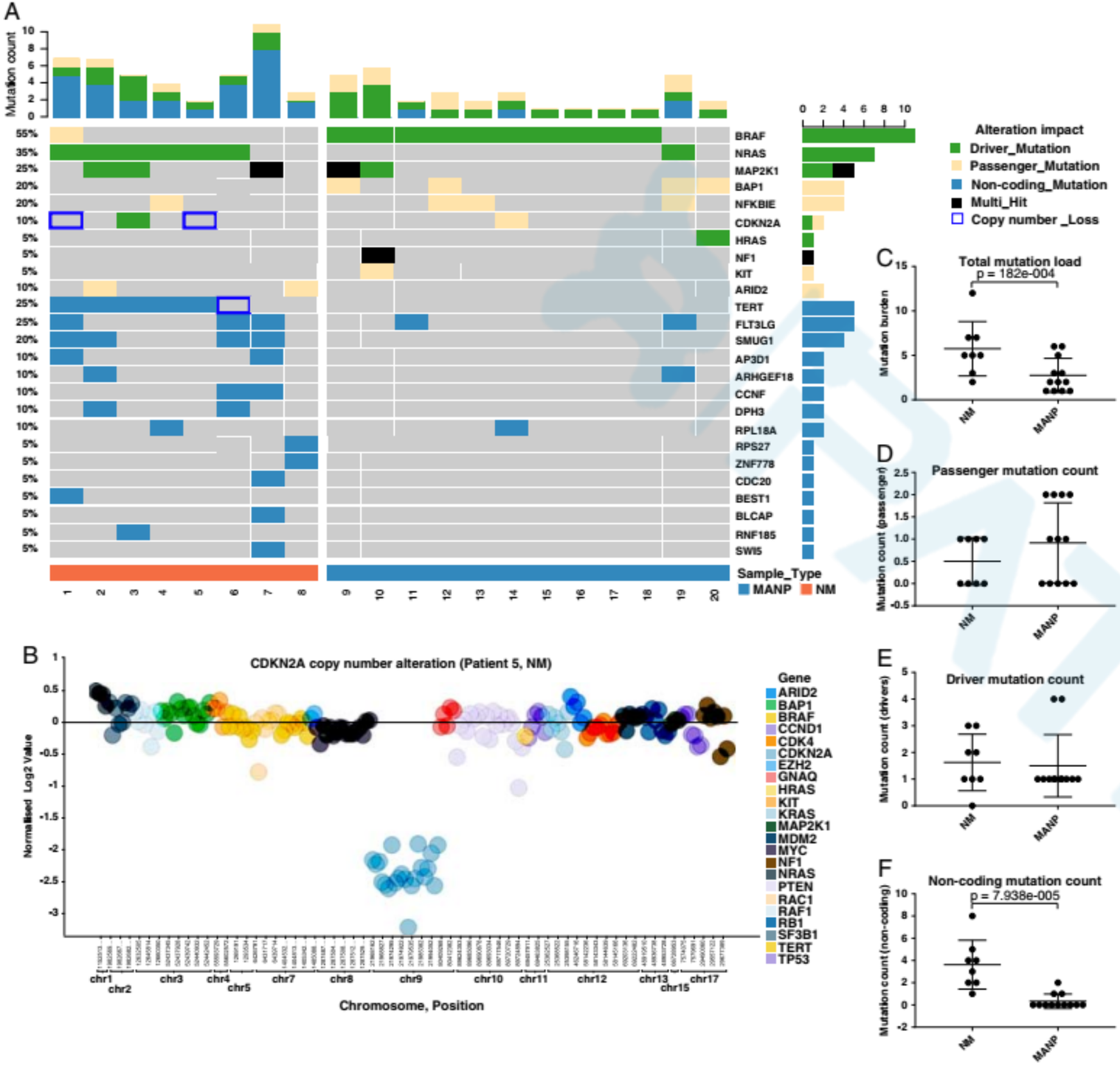
Case17-MANP

27岁，女性，右乳



Case19-MANP

39岁，女性，左前臂



- 8例NM有6例*NRAS*突变（Q61R 3例，Q61K 2例，G13R 1例），其中一例同时发生的*BRAF*突变
- 检测到三个拷贝数改变，所有拷贝数丢失，并且全部发生于NM的*CDK2NA*或*TERT*
- 12例MANP有10例*BRAF V600E*突变。所有MANP中*TERT*启动子均为野生型

讨论

- The diagnosis of NMs and MANP remains challenging in both clinical and histopathologic practice.
- In our cohort of MANP lesions, the most common reason for biopsy was a change in the size or pigmentation of a preexisting nevus. This is in line with other studies that have long concluded that pregnancy can induce a range of skin changes, including color variation, growth, or altered dermoscopic features of nevi.
- The investigators found no chromosomal copy number changes in any of the mitotically active nevi, but there were consistent aberrations of chromosome 6 and/or 11 in the NM group.

讨论

- There was also only 1 case of MANP with an *HRAS* mutation and a passenger mutation in BAP1 (case 20). In contrast to our findings, *HRAS* mutations are not commonly reported in conventional nevi but are noted in up to 25% of Spitz nevi.
- many recent studies have assessed the utility of *TERT* mutation detection to aid in the diagnostic and prognostic assessment of melanocytic lesions.
- We recognize several limitations to our study. Ours was a small cohort and quality tissue was only available for a limited number of histologically suitable cases for molecular analysis. Follow-up was limited.

总结

- In our study of NMs and MANP, *NRAS* was the most common mutation in NM and was present in the majority of cases .
- Noncoding mutations were largely restricted to NM, including *TERT* mutations that were present in the majority of NMs and absent in MANP. Noncoding mutations and copy number alterations were also rare in MANP.
- NGS analysis may have a potential ancillary role.

THANK YOU