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A distinctive melanocytic lesion associated with melanoma-prone dysplastic naevus syndrome: the hybrid naevus

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Abstract Clinically and histologically, the concept of dysplastic nevi remains controversial. To elaborate more precise criteria for the nevi of patients with dysplastic naevus syndrome (DNS), we examined 58 nevi from seven DNS patients who developed one or several malignant melanomas. Clinical presentation and histomorphology were evaluated, and immunohistochemistry was performed using proliferation marker Ki-S5 and antibody DO-7 to the p53 protein. Sixty nevi from individuals without history of melanoma served as controls. Of the DNS nevi, 21 (36.2%) exhibited no morphological particularities. The remaining 37 nevi presented distinctive histological features consisting of a slight epidermal acanthosis, spitzoid vertically oriented nests of dyscohesive nevus cells, and single-standing atypical melanocytes in the basal cell layer of the epidermis. Immunohistochemical analysis revealed an average proliferation index of 2.5%, which significantly surpassed the mean growth fraction of conventional dysplastic nevi (<1%). No increase in p53 expression was observed. Characteristically, active proliferation was found in junctional single-standing melanocytes with or without nuclear atypia rather than in nest-shaped compounds. In conclusion, certain moles of patients with DNS possess distinctive features. The newly characterized criteria may provide a basis for the diagnosis of DNS and might help to identify patients at increased risk for malignant melanoma by examination of a single biopsy.

Keywords Dysplastic nevus sydrome · Melanocytic nevus · Histomorphology · Melanoma · Precursor · Proliferation · P53 · Immunohistochemistry

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Introduction

Dysplastic nevus syndrome (DNS) is characterized by the occurrence of multiple acquired nevi, especially in non-sunexposed areas of the body and is associated with an increased risk for malignant melanoma [4, 5, 11, 14, 17, 23, 25, 37, 39, 40]. Nevi of such patients are claimed to have a clinically atypical appearance [5, 11, 17, 18], and corresponding histomorphological criteria have been proposed [5, 7, 9, 10, 11, 36]. However, there is little unanimity concerning both the clinical presentation and the histopathology of these melanocytic proliferations [1, 2, 3, 24, 31], suggesting that the concept of dysplastic nevi needs to be revisited.

In an attempt to elaborate more consistent criteria for the diagnosis of nevi that might be associated with the development of malignancy, we selected young patients presenting with numerous (generally >100) moles and a history of one or several cutaneous malignant melanomas. Conventional microscopic examination of the nevi was supplemented by the assessment of the tumor cell growth fraction using monoclonal antibody Ki-S5 [22, 33, 34] directed against a formalin-resistant epitope of the Ki-67 antigen [12], and the immunohistochemical determination of p53 expression by means of the antibody DO-7. The p53 protein is a key regulator of the cell cycle (for a recent review, see [27]), and its inactivation by point mutations or deletions of the encoding gene is considered an initial or at least early event in the development of various malignant tumors [16]. Mutations generally result in a stabilization of the transcribed protein, which accumulates in the nucleus and thus crosses the threshold for immunohistochemical detectability.

Using this approach, we will show that a substantial percentage of the nevi of DNS patients exhibit, in terms of both morphology and biology, characteristic features that are distinct from those previously described for familial and sporadic dysplastic nevi.

Patients, materials, and methods

Four of the melanoma-prone DNS patients were female, and three were male. Their age ranged from 17 years to 28 years (median 22 years). All of these patients had multiple moles located mainly on the back and buttocks, of which a total of 58 were available for histological examination. One patient developed three, and another patient developed two primary cutaneous malignant melanomas within 18 months; all others incurred a single melanoma before the age of 28 years. The nine melanomas were also included in the study. The controls consisted of 60 clinically atypical nevi [18] from 23 non-melanoma patients (15 females and 8 males, aged 16–30 years; median 23 years). Of these nevi, 32 were from five patients with numerous (more than 60) moles and a clinical diagnosis of DNS, and 28 nevi clinically corresponded to the type of lesion described as sporadic dysplastic nevus [10]. Ultraviolet exposure, sun damage, and significant mechanical irritation prior to excision could be ruled out on the basis of clinical information and morphological features. The patients were followed for a median time of 10 years (6–16 years). During this observation period, none of the control patients developed a malignant melanoma.

After fixation for approximately 20 h in 10% formalin, specimens were cut through the center of the lesion, embedded in paraffin, and routinely processed. Histological examination was done on sequential sections stained hematoxylin and eosin. Immunohistochemistry was carried out as described before [26, 33] on at least two sections consecutive to the most representative planes, as determined using hematoxylin and eosin microscopy. Briefly, 3- to 5-µm thick sections from formalin-fixed, paraffin-embedded specimens were mounted on APES (3-aminopropyl-triethoxy-silane)-coated slides and dried overnight at room temperature. Subsequently, the specimens were dewaxed in xylene and rehydrated in a graded ethanol series. After rinsing with phosphate-buffered saline (PBS), they were immersed in 0.01 M citric acid titrated to pH 6.0 with 0.1 M NaOH and heated twice for 10 min (Ki-S5) or 20 min (DO-7) in a microwave oven (Toshiba) at the highest power setting (750 W). Primary antibodies Ki-S5 (undiluted cell culture supernatant) and DO-7 (Dianova, Hamburg Germany, diluted 1:50) were then incubated on the sections for 30 min. Following enhancement of the immunoreaction by means of the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique [8] using a rabbit-anti mouse secondary antibody, the slides were briefly counterstained with Mayer's hematoxylin. To assess the tumor growth fraction or p53 overexpression, the cells of the entire tumor area of each slide were counted at high magnification, and the number of positive nuclei was calculated as a percentage of the total tumor cell population.

Results

Clinical presentation

The total number of clinically conspicuous nevi was highly variable between patients. These nevi also displayed considerable diversity in size, shape, and coloration, some of them resembling atypical nevi as defined previously [18]. More than half of the nevi examined histologically, however, exhibited characteristic features that shall be detailed in the following. A typical lesion from one female patient who developed two primary cutaneous melanomas is shown in Fig. 1a, b. Most nevi from this patient were characterized by a nearly symmetrical contour, slightly ill-defined borders, and a relatively flat surface. Their color consisted of shades from tan–brown to dark brown arranged in a haphazardly variegated pattern, and their size came close to 12 mm in most instances.

Figure 2a represents a macular nevus as the prototype of the lesions observed in another female patient who incurred three primary cutaneous melanomas. These nevi were characterized by some degree of asymmetry, irregular and ill-defined borders and, again, a relatively flat surface. Interestingly, all lesions displayed a uniform tan pigmentation except one, in which a dark brown dot arose (Fig. 2b). This lesion was identified as a malignant melanoma by means of histological examination (Fig. 3d). The size of these nevi ranged from 4 mm to 12 mm in diameter. Similarities with either type of lesion described above were also consistently observed in a major percentage of the nevi of the remaining five melanoma-prone DNS patients and correlated with a distinctive histomorphology.

Histological features

Of the nevi from the DNS patients who incurred malignant melanomas, 21 (36.2%) appeared as inconspicuous nevi of either dermal (n=4) or compound/junctional (n=15) type. The remaining 37 nevi (63.7%), of which 20 were junctional and 17 of the compound type, nevertheless displayed well recognizable histomorphological peculiarities (Fig. 3a–c). These consisted of a prominent intraepidermal component in association with slight psoriasiform acanthosis and only a minor tumor cell compartment confined to the papillary dermis in the case of compound lesions. The intraepidermal tumor cells were grouped in sharply circumscribed oval and vertically oriented nest-shaped aggregates, extending from the junctional zone into the upper layers of the stratum Malpighii. They were symmetrically distributed and somewhat reminiscent of the tumor cell nests of Spitz's nevi. By contrast to the latter, however, the naevus cells in our cases were of medium size, round to slightly oval, and often contained finely granular cytoplasmic melanin; they were arranged in a slightly dyscohesive pattern and had monomorphous nuclei with homogeneous chromatin and small but accentuated basophilic nucleoli. Another salient feature was the regular presence of randomly distributed single-standing melanocytes with often strik-

Fig. 1a, b Clinical features of one type of hybrid nevus: the lesions display slight asymmetry, a flattish surface, ill-defined borders, and a variegated pigmentation, ranging from light to dark brown ▶

Fig. 2 a Clinical aspect of the second type of hybrid nevus presenting as a flat macular lesion with irregular contours, ill-defined borders, and a pale tan pigmentation. **b** The sole *dark dot* seen in one of these nevi turned out to be a melanoma in situ (corresponding histology appears in Fig. 3d)

Fig. 3 a, b Histologically, hybrid nevi are characterized by irregularly spaced, vertically oriented, and sharply circumscribed nevus cell nests in the middle and upper layers of the epidermis and slightly atypical melanocytes arranged as solitary units in the junctional zone (hematoxylin and eosin, original magnification ×100). **c** Higher magnification of the lesion shown in **b** (×200). **d** Histological picture of the *dark dot* shown in Fig. 2b. Features of melanoma in situ are evident in contiguity with elements of a hybrid nevus (×100)

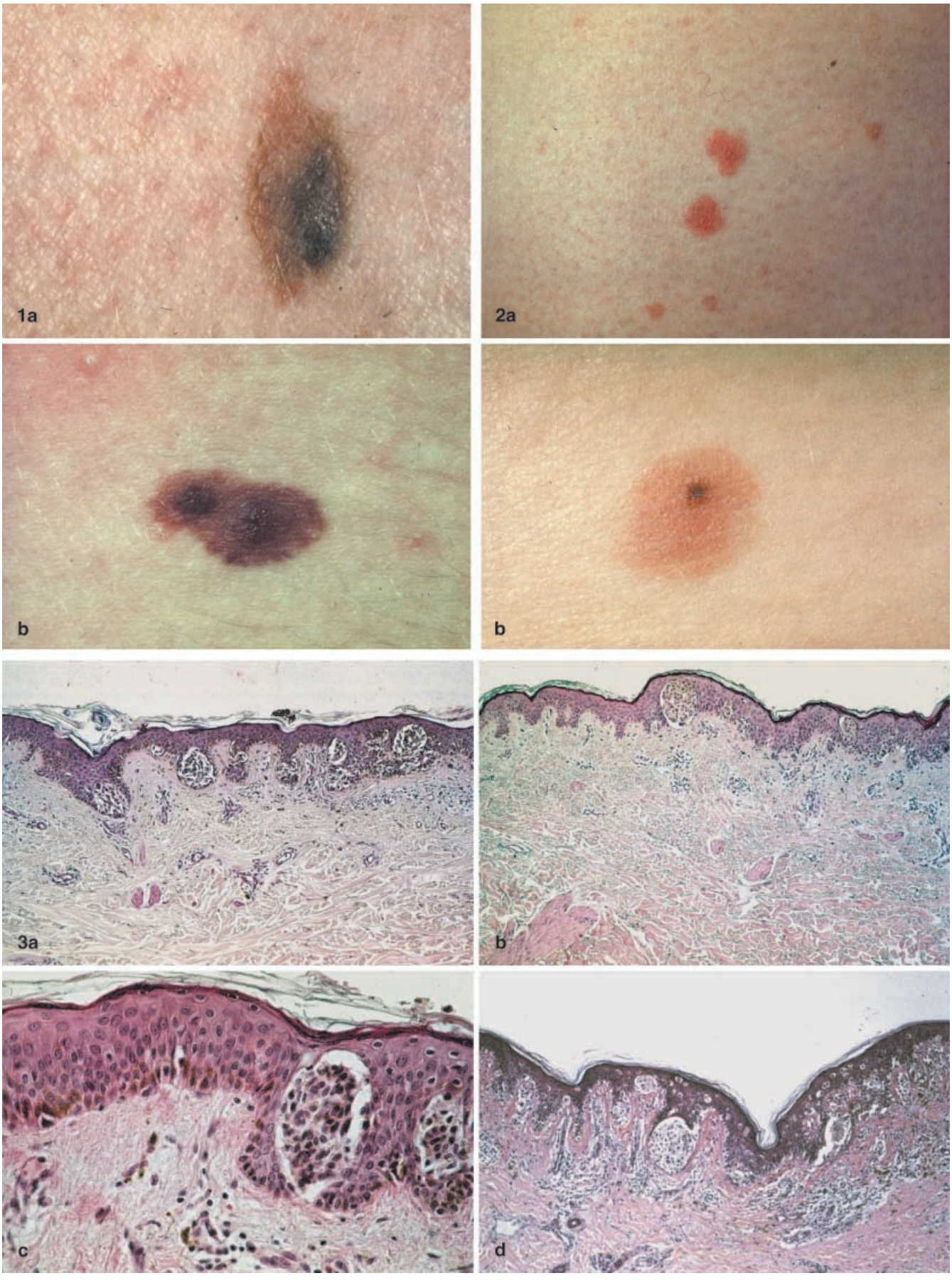

Fig. 1–3 Legends see page 167

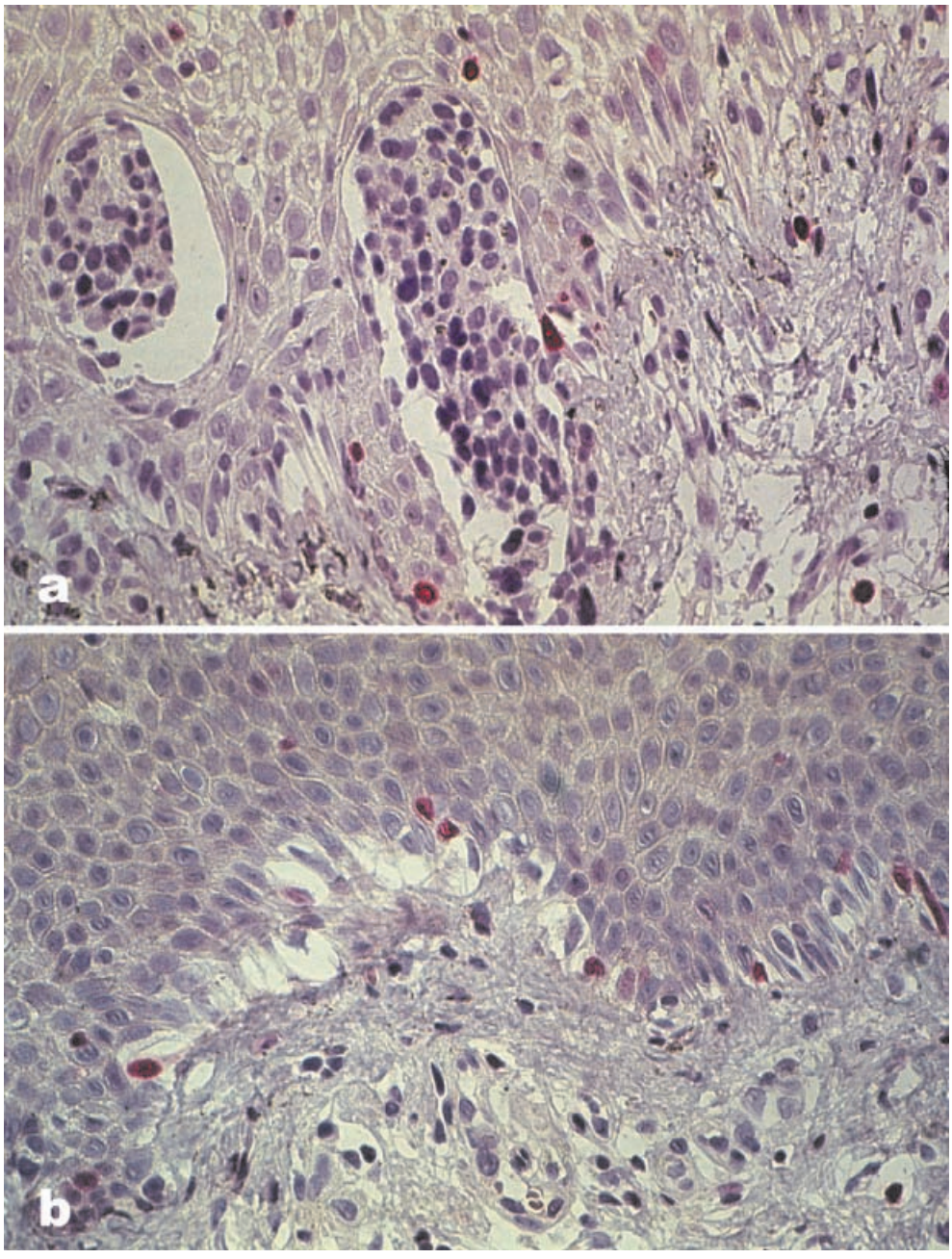

Fig. 4 Ki-S5 immunostaining, alkaline phosphatase anti-alkaline phosphatase (APAAP) technique, hematoxylin counterstain, original magnification ×350 **a** Proliferating melanocytes (*clear cytoplasm* and *red nuclear labeling*) are rarely found within nest-shaped compounds; *a single Ki-S5 positive cell* is seen on the right side in a small group of junctional melanocytes becoming confluent, the remainder are proliferating parabasal keratinocytes. **b** Proliferation of junctional melanocytes arranged as solitary units mainly accounts for the increased average tumor cell growth fraction of 2.5%

ingly pleomorphic enlarged hyperchromatic nuclei at the epidermo-dermal junction. The dermal tumor cells that were mostly disseminated in a band-like pattern were smaller than those in the epidermis, had roundish nuclei with dense homogeneous chromatin and no apparent nucleoli. It has to be noted that these characteristics, which appear to be unrelated to the diameter of the lesions, were consistently observed in at least one the nevi of each of the seven melanoma-prone DNS patients.

The 60 nevi of the control group comprised six nevi of the papillomatous intradermal type, 10 lentiginous junctional nevi, and 44 compound nevi. In the latter, step sections almost regularly revealed changes claimed to be diagnostic of dysplastic nevi [10], i.e., lentiginous hyperplasia with moderate random cellular atypia, concentric or lamellar fibroplasia, and perivascular inflammatory infiltrates. Conversely, none of the control nevi presented the singular features described above. Of the nine melanomas from our DNS patients, eight were of the superficial spreading type (SSM), and one appeared to be a primary nodular melanoma. Remnants of a DNS-associated naevus were detected in one case of SSM.

Proliferation and p53 expression

For the assessment of cellular proliferation, the junctional/intraepidermal and dermal tumor cell fractions were

evaluated separately. The growth fraction in the dermal tumor cell compartment was virtually identical in the DNS nevi and the control group, ranging from 0% to 0.5% (median 0.2%). In the intraepidermal compartment, however, the proliferative activity of the DNS naevus cells averaged 2.5% (from 1.0% to 3.6%; median 2.2%), whereas the growth fraction in the control group rarely exceeded 1% (range 0.1–1.3%; median 0.4%). The difference was statistically significant according to the Kruskal–Wallis test ($P<0.001$). In the control group, KiS5 positive cells were strictly confined to the junctional nests, whereas in the DNS nevi, they often occurred as isolated cells in the junctional zone, in addition to a slight increase of the proliferation index in the intraepidermal nest-shaped naevus cell compounds (Fig. 4a, b). Nuclear Ki-S5 positivity was not necessarily concurrent with nuclear atypia. No augmentation in the proliferative activity was observed in the 21 "banal" nevi from the DNS collective compared with the control group. However, even in these nevi, occasional single-standing melanocytes in the junctional layer exhibited nuclear KiS5 positivity. The presence of segregated proliferating cells was also consistently observed in all eight SSMs, in which the growth fractions ranged from 4% to 12% (median 6%).

Neither the DNS nevi nor the control lesions contained any melanocytic cells with detectable immunoreactivity for the p53 protein, although a few parabasal keratinocytes (less than 5%) had positive nuclei. In two of the nine melanomas, a minor percentage of cells (<2%) reacted with the antibody DO-7, whereas the remaining seven were entirely negative.

Discussion

The dramatic rise in the incidence of malignant melanoma worldwide over the past decades [13, 20, 21] has motivated the search for precursor lesions and indicators of an increased tumor risk. Because initial stages of malignant melanoma are curable by simple excision, there is an indisputable need for screening methods aiming at an early recognition of the disease. By their description of six melanoma-prone families, Clark et al. [5] established the notion of a phenotype linked to a high melanoma risk, the DNS, that was subsequently extended by Elder [10] to so-called sporadic cases with analogous features. However, the clinical features of DNS appear to be poorly reproducible [24], and the histopathological criteria considered to define dysplastic nevi were found at high prevalence in nevi of the normal control population [19, 28]. Some authors even regard these nevi as the most common type of melanocytic neoplasia in Caucasians [1, 2]. The ensuing controversy persists and, curiously, the discussion appears to be concerned with terminology rather than with a more precise characterization of this phenotype on a clinical or histological basis [6, 31, 39]. Nevertheless, it emerges that a large number of nevi, regardless of their clinical or histological aspect, is associated with an increased risk of malignant melanoma [24, 37, 38, 39, 42] even in extracutaneous sites [42]. In this setting, it is well conceivable that the nevi known as dysplastic, being the most prevalent type overall, should account for an important percentage of the nevi in melanoma patients.

The continuity of malignant melanomas with residual elements of naevus tissue has been advocated as a strong argument in favor of a precursory role of dysplastic nevi in the development of malignant melanocytic skin tumors [30, 38]. However, in line with previous observations [41], we failed to detect signs of a progressive malignant transformation in nevocellular lesions or a transition from sporadic dysplastic nevi to malignant melanoma [32, 33].

For this study, we therefore selected seven cases of young adults with a clinical DNS phenotype and a history of one or several malignant melanomas and compared their nevi with clinically atypical nevi from non-melanoma patients matched for age and gender. By this approach, we were able to recognize unique histological features in more than 60% of the nevi of the DNS patients, which were not encountered in the control group. These consist of a slight psoriasiform acanthosis of the epidermis and symmetrically distributed, vertically oriented nests of monomorphous medium-sized nevus cells in the spinous layer and isolated atypical melanocytes irregularly scattered along the epidermo-dermal junction. In addition to a significant overall increase of the nevocellular growth fraction in these lesions, a most salient trait was the consistent finding of actively proliferating single-standing melanocytes with or without nuclear atypia in the junctional or basal layer. Proliferation of isolated melanocytic cells, as assessed using Ki-67 nuclear positivity, appears to be a consistent attribute of superficial spreading melanoma, whereas we never noticed it in common or so-called dysplastic nevi [33, 35]. The mean growth fraction of the particular nevi seen in DNS patients is comparable to that of Spitz's nevi [33, 35], and their architectural pattern is also strongly reminiscent, but there are clear-cut cytological differences. In fact, the newly described lesions appear to be composed of two distinct elements, namely, nest-shaped aggregates of small cells and single-standing atypical melanocytes in the junctional zone. Neither meet the criteria for Spitz's nevi nor those for dysplastic nevi, given the absence of lentiginous melanocytic hyperplasia. Because of the characteristic coexistence of these two features, we would like to propose the designation of "hybrid naevus" for this distinctive entity.

Clinically, the features of nearly 40% of the nevi of melanoma-prone DNS patients did not markedly differ from those described for classical dysplastic nevi [5, 11, 14]. The nevi of two patients who developed more than one primary cutaneous melanoma were exceptions (Fig. 1 and Fig. 2). The hybrid nevi of the other DNS patients were also of comparatively large size, but their shape was more asymmetrical, approaching the features of classic dysplastic nevi. However, nearly all of these

nevi were flat, homogeneously and rather lightly pigmented, and had regular but variably ill-defined borders.

The enhanced proliferative activity in these nevi is consistent with the rapid development of multiple lesions [5, 11], indicating that a subset of their cells are likely to possess a growth advantage compared with common nevi. In line with other studies [29], we found no evidence for a primary p53 deficiency being the cause of this altered growth behavior. It is more probable that immunological factors, i.e., a reduced immune surveillance, may be responsible for a lessened growth control [15]. This would also be congruent with our finding of single-standing proliferating junctional melanocytes in otherwise inconspicuous nevi of DNS patients. A spatial association of melanoma with a hybrid nevus was seen in one case, indicating that a progression to malignancy can occur in these lesions. However, although their increased proliferative activity may imply a deficiency in growth control, this is not sufficient evidence to characterize them as premalignant states. Indeed, rather than a particular type of nevus, every melanocyte may be considered as a potential precursor of malignant melanoma [2], provided that either environmental or genomic factors allow for its transformation [27].

A cohort of seven patients may appear somewhat limited for drawing conclusions, but it also reflects the low frequency at which malignant melanomas actually occur in DNS patients. Probably, multicentric studies would be required to substantiate our findings, histologically and by means of further molecular analyses, such as the complex field of inactivations of the cyclin-kinase inhibitor $p16^{INK4a}$ [27]. In this way, our observations may be regarded as preliminary. For the time being, however, the selection of patients presenting a multiple mole phenotype in concert with the early occurrence of one or several primary cutaneous melanomas enabled us to characterize a distinctive type of nevus that appears to be significantly associated with this clinical setting. Rather than being precursors of malignant melanoma, these nevi may be considered as markers of an increased melanoma risk. In this regard, it appears that an augmented proliferative activity is an even more sensitive indicator, because it may reveal changes in the growth behavior of melanocytes undetectable using routine microscopy. However, awareness of the characteristic histological features of hybrid nevi may facilitate the identification of individuals at risk for malignant melanoma.

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