

## ACKNOWLEDGMENTS

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## Café-au-lait Patches and Senile Plaques: How APPT the Connection?

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Neurofibromatosis type 1 (NF1) is a genetic disease caused by mutations in the *NF1* gene, which encodes the protein neurofibromin. Patients exhibit characteristic hyperpigmented patches called café-au-lait patches. Melanocytes of NF1 patients differ from normal human melanocytes, but no differences account completely for lesional hyperpigmentation. An association between  $\beta$ -amyloid precursor protein (APP) and neurofibromin, and their localization to the melanosome, may help explain the development of café-au-lait patches.

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The nature of café-au-lait lesions in neurofibromatosis type 1 (NF1) has interested investigators for decades. NF1 is an autosomal dominant genetic disease characterized by a number of different abnormalities affecting various organ systems and tissues in addition to the characteristic hyperpigmented — or café-au-lait, as they will be referred to here — macules and patches. These abnormalities include benign neoplasms such as dermal neurofibromas and optic gliomas, as well as a predisposition to malignancies such as pheochromocytomas and juvenile myeloid leukemia. NF1 is associated with mutations in the human *NF1* gene. *NF1* encodes the protein neurofibromin, a large, 260-kilodalton tumor suppressor protein containing a Ras-GTPase-activating protein (RasGAP) domain. Perhaps the best-understood biochemical function of neurofibromin is its activity as a negative regulator of Ras, accelerating the conversion of active, guanosine triphosphate-bound

Ras to inactive, guanosine diphosphate-bound Ras (DeClue *et al.*, 1992). The cellular abnormalities that result in various clinical manifestations of NF1 have been attributed to hyperactive Ras activity due to loss-of-function mutations or deletions of one or both *NF1* alleles.

Early studies of café-au-lait patches focused on characterizing their density and morphology and revealed two surprising observations: (1) not only did the café-au-lait patch often have a somewhat higher number of melanocytes than the normal skin of historical controls, but so did the rest of the skin in these patients; and (2) virtually all of the melanocytes visualized in the epidermis of NF1 patients, whether lesional or non-lesional, contained enlarged pigment granules (Benedict *et al.*, 1968; Martuza *et al.*, 1985). Both melanocyte and keratinocyte numbers were quantified in lesional and non-lesional skin of NF1 patients as well as the skin of normal

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control individuals. NF1 patients had an increased density of melanocytes in both their lesional and their non-lesional skin, effectively demonstrating that they possessed a smaller epidermal melanin unit than unaffected controls. However, only half of NF1 patients were found to have a statistically significant, modest increase in the numbers of melanocytes within café-au-lait lesions compared with unaffected skin (Benedict *et al.*, 1968; Frenk and Marazzi, 1984).

Ultrastructural analysis of giant pigment granules, variously called macromelanosomes (Jimbow *et al.*, 1973) and melanin macroglobules (Martuza *et al.*, 1985), within NF1 melanocytes revealed that they are several times the diameter of normal melanosomes, contain varying amounts of electron-dense and electron-lucent globular bodies, and coexist with ultrastructurally normal melanosomes within cells. In melanocytes, macromelanosomes also differ from normal melanosomes in that they are surrounded by a membrane. Large pigment granules transferred to the keratinocytes in café-au-lait patches contain singly distributed macromelanosomes surrounded by a membrane similar to that surrounding aggregates of normal melanosomes also found in these cells (Jimbow *et al.*, 1973).

Taken together, these studies documenting melanocyte number and giant pigment granules in NF1 skin do not provide an explanation for a specific defect restricted to melanocytes that results in the hyperpigmentation of the café-au-lait patch. Both the café-au-lait patches and the non-lesional skin of NF1 patients contain macromelanosomes, which unfortunately are not specific to NF1, having been described in melanocytes of a variety of pigmentary disorders (Martuza *et al.*, 1985). Both hyperpigmented and normal-appearing skin also contain increased numbers of melanocytes when compared with the skin of unaffected individuals. The increased density of melanocytes that is consistently observed in the skin of NF1 patients is nonetheless interesting and may be in part a result of diminished RasGAP activity in affected melanocytes. Consistent

with this hypothesis, expression of stem cell factor/Kit ligand, the ligand for the Kit receptor tyrosine kinase, which conveys intracellular signals via Ras, results in increased numbers of murine intrafollicular melanocytes in a transgenic mouse model (Kunisada *et al.*, 1998). Additionally, the abnormalities in melanocyte density and organelle composition that extend to the non-lesional skin of these patients correlate with the description by Riccardi (1992) that even the non-lesional skin of NF1 patients tends to be more pigmented than the skin of their unaffected siblings.

The report by De Schepper *et al.* (2006, this issue), demonstrating an interaction between neurofibromin and  $\beta$ -amyloid precursor protein (APP) in human melanocytes, may extend our knowledge about the activity of neurofibromin in melanocytes to lead eventually to an explanation of the hyperpigmentation observed in café-au-lait patches. APP is a ubiquitously expressed protein that is best known as the precursor protein to the 4-kilodalton peptide amyloid  $\beta$  protein, linked with Alzheimer's disease. Amyloid  $\beta$  protein accumulates extracellularly as a component of senile plaques, one of several characteristic histopathologic findings in the brain tissue of AD patients. The mammalian APP belongs to a highly conserved family of type 1 integral membrane proteins with large extracellular and short intracellular domains. APP itself has interesting biochemical properties as a proteolytic substrate. Most APP is cleaved in its extracellular domain by an  $\alpha$ -secretase, which releases a secretory N-terminal domain (sAPP $\alpha$ ) into the extracellular environment. Several members of the ADAM (a disintegrin and metalloproteinase) family, including ADAM17, ADAM10, and ADAM9, may possess this enzymatic activity. In contrast, the activity of a distinct protease  $\beta$ -secretase, that cleaves APP N-terminal to the previous site, initiates the release of the amyloid  $\beta$  protein peptide, whose subsequent aggregation is thought to be pathogenic in Alzheimer's disease. The human APP gene is alternatively spliced, involving exons 7, 8, and

15, to three major isoforms, APP695, APP757, and APP770 (Herzog *et al.*, 2004; Tanzi and Bertram, 2005).

However, the biology of APP extends beyond its expression and putative functional role within neurons of the central nervous system. High levels of APP expression have also been described in human epidermis, with the main site of expression localized to the basal layer (Hoffmann *et al.*, 2000). The analysis of APP expression within the basal layer of whole-mount preparations of human epidermis revealed that APP expression was significantly higher in melanocytes, identified by their co-expression of the melanogenic enzyme tyrosinase, than in neighboring keratinocytes (Quast *et al.*, 2003).

De Schepper *et al.* (2006) identified neurofibromin as an interacting partner of APP by yeast two-hybrid screening and confirmed this interaction by co-immunoprecipitation in cultured human melanocyte lysate. Further exploration of the co-expression of these two proteins in melanocytes confirmed these results and revealed some interesting findings. APP, as demonstrated previously (Quast *et al.*, 2003), was expressed in a perinuclear distribution as well as at the dendrite tips. Neurofibromin was present in a perinuclear distribution, colocalizing with the melanosomal marker NKI-beteb, but was not present in the dendrite tips. On the ultrastructural level, immunogold electron microscopy was used to localize both APP and neurofibromin to the melanosome, using NKI-beteb as a known melanosomal marker. Interestingly, melanocytes cultured from the unaffected, non-lesional skin of an NF1 patient showed a marked reduction in the presence of both APP and neurofibromin on the melanosome. This finding is not only consistent with an expected reduction of neurofibromin protein in an NF1 melanocyte but also may imply that neurofibromin is functionally important for maintaining the melanosomal localization or stability of APP. Even without these patient-based data, the localization of both neurofibromin and APP on melanosomes should stimulate interest in the potential role of these proteins in melanosomal transport.

In their discussion, De Schepper *et al.* (2006) note how both APP and neurofibromin interact with the microtubule motor protein kinesin in neurons, and how APP is required for the transport of an axonal membrane compartment in these cells. As kinesin is expressed in human melanocytes (Hara *et al.*, 2000; Vancoillie *et al.*, 2000), their findings are likely to prompt inquiries about whether analogous interactions and mechanisms exist in melanocytes for mediating the transport of melanosomes via microtubules. It is tantalizing to speculate that a macromolecular complex including neurofibromin, kinesin, and APP is involved in the tethering of the melanosome to the microtubule network to promote melanosome transport down the dendrite, in much the same way that a complex including the small GTPase Rab27a, melanophilin, and the actin-based vesicle motor myosin-Va is essential for capture of the melanosome at the dendritic periphery (Wu *et al.*, 2002).

Similarly, the localization of neurofibromin to the melanosomal membrane should inspire some questions regarding its activity on the melanosome — especially whether it regulates Ras activity in this location. The three ubiquitously expressed Ras isoforms H-, K-, and N-Ras exhibit distinct trafficking routes to the plasma membrane. They have been shown to signal both from the plasma membrane and from internal membranes, such as those of the Golgi apparatus and endosomes. Does the localization of neurofibromin to the melanosomal membrane imply that this membrane, of a specialized subcellular organelle normally restricted to pigment cells and keratinocytes, is also a site of Ras-dependent signaling? In addition, the small guanosine triphosphate-binding protein Rab27a, and perhaps other small guanosine triphosphate-binding proteins such as the Rho family members, which mediate melanosome phagocytosis in keratinocytes (Scott *et al.*, 2003), might be regulated on the melanosome as well. This regulation could be mediated either by neurofibromin, or by other GTPase-activating proteins, such as p190, which regu-

lates the GTPase activity of Rho family members and associates with RasGAP (Settleman *et al.*, 1992). Another possible avenue for exploration may be the role of sAPP $\alpha$  in the melanosomal environment. Membrane-associated  $\alpha$ -secretase activity might catalyze the intracellular release of sAPP $\alpha$ . In melanocytes, sAPP $\alpha$  has been shown to stimulate activity of dendritic lamellipodia and enhance melanin release (Quast *et al.*, 2003). It is not unreasonable to speculate that aspects of these processes might be regulated by intracellular release of sAPP $\alpha$  from the melanosomal membrane, as opposed to extracellular release from the plasma membrane or even the membranes of neighboring keratinocytes.

In a manner reminiscent of the changes in melanocyte density and pigment granules previously described with non-lesional NF1 melanocytes, De Schepper *et al.* found that both neurofibromin and APP were severely reduced in melanosomes of melanocytes cultured from non-lesional skin of a patient with NF1. Hence, observation of these changes alone is insufficient to provide a complete explanation for the distinct hyperpigmentation found within café-au-lait patches. To explain the hyperpigmentation of café-au-lait patches, it may be necessary to look beyond the melanocyte. In one interesting study, secretion of the growth factors hepatocyte growth factor and stem cell factor/Kit ligand from fibroblasts underlying café-au-lait patches was significantly greater than that from fibroblasts underlying non-lesional skin from either NF1 patients or normal individuals (Okazaki *et al.*, 2002). Both hepatocyte growth factor and stem cell factor/Kit ligand can modulate melanocyte survival, proliferation, and differentiation. In the absence of well-defined numerical, morphological, or subcellular differences between melanocytes on the lesional and on the non-lesional skin of these patients, it seems reasonable to propose that some complex interplay between abnormal growth factor secretion from regional populations of fibroblasts and enhanced responsiveness of overlying melanocytes to their signals may in part account for the

hyperpigmentation observed in café-au-lait patches. Perhaps the loss of melanosomal neurofibromin and APP in NF1 melanocytes is a determinant of enhanced melanocyte responsiveness to paracrine growth factor stimulation. In any case, the proximity of neurofibromin and APP on the melanosome, their physical interaction, and the wealth of knowledge about APP already obtained from neuronal studies should make it possible to decipher the functional significance of these unique interactions in the melanocyte in the near future.

#### CONFLICT OF INTEREST

The authors state no conflict of interest.

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