The contribution of the arrector pili muscle and sebaceous glands to the follicular unit structure

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Background: The evidence of hairs grouped into well-defined follicular units has given a new vision of hair anatomy and pathology. The sebaceous glands and the arrector pili muscle, as part of the pilosebaceous unit, should be viewed as important parts of this organized follicular unit structure.

Objective: This study was performed to define the morphology and the relationships between the sebaceous glands, muscles, and follicles within the context of the follicular unit structure.

Methods: This study analyzes horizontal, microscopic serial sections of large areas of normal human scalp skin stained with hematoxylin and eosin, Masson's trichrome, and desmin. The course of the arrector pili muscles from their superficial origins to their follicular attachments was followed in each section, which enabled us to match each muscle with its corresponding follicular unit.

Results: Serial, horizontal sections show that, at the upper isthmus, the arrector pili muscle is arranged as a muscular unit structure at the periphery of each follicular unit. Then, at a lower level, the muscle divides and encircles the sebaceous gland, and penetrates between the sebaceous lobules towards the follicular attachment zone.

Conclusion: Microphotographic evaluation of large areas of scalp is important for assessing the anatomical relationships between the sebaceous glands, arrector pili muscles, and hair follicles as components of the follicular unit. We introduce the anatomical concept of a follicular unit served by a muscular unit, which can be identified in horizontal sections made at the upper level of the isthmus. This muscular unit results from the merging of the arrector pili muscles that originate from the hair follicles contained in that particular follicular unit. This anatomical disposition suggests that the arrector pili muscles could play an important role in the integrity of the follicular unit as well as in the secretion of the sebum contents. (J Am Acad Dermatol 2004;51:217-22.)

Hair shafts exit the skin of the scalp as groupings, which appear to maintain a certain distance between each other.1 There is a significant variation in the number of terminal hairs per hair grouping between individuals of different races.2 For example, in the normal scalp of white individuals, two-hair groupings are the most abundant, followed by three-hair groupings.1 These hair groupings correlate with well-defined histological structures known as follicular units (FUs) (Fig 1). Using horizontal sections of scalp biopsies, Headington3 described the FU as a well-circumscribed structure composed of two to four terminal follicles, and one or, very occasionally, two vellus follicles, the associated sebaceous lobules, and the insertions of the arrector pili (AP) muscles of the terminal follicles.

We recently reported evidence suggesting that the AP muscles are shared by the hair follicles contained in the FU.4 Therefore, the traditional anatomic concept of “one hair follicle associated independently with one arrector pili muscle” was challenged, favoring instead the concept of “one arrector pili muscle unit shared by all the hair follicles contained...
within the follicular unit.” In an effort to further demonstrate our anatomical model, we designed this study in which we have analyzed serial horizontal microscopic sections of large samples of scalp skin, tracking the course of the arrector pili muscles from their upper dermal origin to their insertions in the hair follicles at the lower isthmus level. In this study we have selected large areas of skin (2 × 1 cm) for microscopic observation because we realized that these are necessary to study the anatomic distribution of all the components of the FUs, including the sebaceous glands and the AP muscles. In contrast, punch biopsies only permit the observation of a few complete FUs and preclude establishing conclusions about the interrelation and anatomic distribution of adjacent FUs.

In this study we have observed that the AP muscles and the sebaceous glands participate intimately in the grouping of the hair follicles, thus contributing to the compartmentalization of skin adnexa. Digital, microphotography of large areas of the scalp followed by a mosaic-type reconstruction enabled us to figure out a three-dimensional image that confirms our previous hypothesis, and points towards functional implications of the FU as a highly organized structure.

**MATERIALS AND METHODS**

Healthy human scalp skin was obtained from 14 adult cadavers. There was no ethnic differences, all being white. Two by one centimeter segments of scalp were excised. In 4 cases, the tissue was obtained from the occipital region, and in 10 cases, from the temporal region. The excisions were carried out down to the deepest part of the subcutaneous fat in order to include the hair bulbs of the terminal hair follicles. Fixation of tissue was carried out in 10% neutral-buffered formaldehyde solution.

After fixation, serial sequences of horizontal sections were cut from the uppermost dermis down to the junction of the dermis and subcutaneous fat. The slides were consecutively numbered in order to record the different section levels. Sections numbered with odd numbers were stained with hematoxylin and eosin (H&E). After studying the H&E sections, some of the paired sections were selected for additional staining with either Masson’s trichrome or immunohistochemically with anti-desmin antibody (DER-11, monoclonal, DAKO). The immunohistochemistry was performed with the standard streptavidin biotin-peroxidase complex, using aminoethylcarbamazole as the chromogen.

Each serial horizontal section was micro-photographed at 100× magnification. At this magnification, the muscular fascicles could be clearly differentiated from other histological structures. Bundles of the AP muscles were identified at the upper dermal level and then tracked down to their insertions in the external root sheaths of the hair follicles. For each section, multiple fields were microphotographed with a digital camera (Nikon Coolpix 995), in order to make a complete computer-assisted
RESULTS

Arrector pili muscles and follicular units

Horizontal sections made at the most superficial level (papillary dermis) fail to correlate the AP muscles fibers with their corresponding FU. At this level, the AP muscles can be located far from the FU to which it is going to be anatomically related (Fig 2). This distance was estimated to be approximately 0.25 millimetres from the most external sheath of perifolliculum (the adventitial collagen that surrounds each FU).

At the level of the upper isthmus, the muscle fibers converge into a thick muscular structure, which can easily be seen at the periphery of each follicular unit. At this depth, a muscular unit is associated with its corresponding FU regardless of the number of follicles contained in each FU. It is characteristic to observe that the AP muscles of adjacent FUs maintain the same orientation, localizing at the same pole of the unit.

Sebaceous glands and follicular units

The sebaceous glands are localized between the AP muscle and the external hair sheaths. The distribution of the sebaceous glands varies depending on the depth level. At superficial levels, the sebaceous glands surround the follicles and appear not only at the periphery, but also in the interior of FU, between the hair follicles. The entrance of the sebaceous ducts into the hair follicles appears to be localized at the periphery. Deeper levels show how sebaceous glands become polarized between the hair follicles and the AP muscles (Figs 3 and 4).

DISCUSSION

The physiologic significance of the characteristic distribution of the hair follicles in follicular units remains unclear, even more so if we consider that each hair follicle acts independently of its neighbours, undergoing cyclical transitions through the anagen, catagen, and telogen phases. The analysis of the possible role that the FU may play in hair physiology should be preceded by a description of several aspects of the follicular microanatomy that have not been studied in detail, such as the topographic relationship between the AP muscles and the FU.

The AP muscle is a smooth muscle that rises from the uppermost part of the dermis, just beneath the epidermis, and inserts into the hair follicle. The distal end of the AP muscle shows multiple muscle fibers splaying into multiple branches at the level of the papillary dermis. The zone of the AP muscle follicular attachment, the bulge, is thought to contain epithelial stem cells responsible for regenerating follicles, playing a critical role in the hair growth cycle. Recent evidence shows that fascicles of the AP muscle may surround the entire circumference of some hair follicles at the bulge area.

Studying the anatomy of FUs in horizontal sections, we recently reported that the AP muscle displays a clear relationship to the follicular unit, forming a common thick bundle which is always oriented towards the same pole in different FUs. Based on these findings, we hypothesised that the AP muscles form a single structure that is shared by the follicles contained in each follicular unit. In order to...
confirm our hypothesis, we followed the complete course of the AP muscle and its associated follicular unit, from its origin in the superficial dermis to its insertion into the bulge area. To achieve this goal, we developed a method of serial horizontal sectioning of large scalp samples, because hair follicles are not completely straight and the AP muscle usually originates at a considerable distance from their associated FU. After analyzing serial horizontal sections, we were able to demonstrate that the AP muscles converge into a muscular unit that is associated with each follicular unit. At the mid and lower isthmus level, this muscular unit divides in fascicles that insert in each of the hair follicles contained in the FU.

The anatomical relationship between the FUs and the AP muscles described above was observed in every biopsy analyzed. However, although we believe that this anatomic pattern (an FU served by a muscular unit) is always the rule, this study was meant to be a descriptive one not a quantitative one; thus we cannot provide specific data on the frequency of our observations.

The three-dimensional representation that we propose is different from the classical diagram of the pilosebaceous apparatus; instead of the classical picture of one arrector pili muscle emerging from one hair follicle, our findings support the concept of an AP muscular unit structure per follicular unit (Fig 5).

The analysis of this new anatomical scheme enables us to speculate on several aspects of hair physiology. Some of these functional considerations appear to be obvious, such as the fact that the hair erection, which is the only proven function of the AP muscle known to date, should simultaneously affect all the hairs of the same follicular unit. Other possible functions of the AP muscle, such as its contribution to the anatomic integrity of the follicular unit, the role in the secretion of the sebum contents, or the protective role over the stem cells located in the bulge region, are still hypotheses that need further scientific validation.

The grouping of hairs is said to be maintained and supported by a common dermal matrix with characteristics of papillary dermis, known as the perifolliculum. This is a prolongation of the papillary dermis around the cluster of hairs of the FU and can be easily recognized in H&E-stained sections or, even better, in sections stained with Masson’s trichrome. Given our results, we have reasons to believe that the AP muscles could contribute to the anatomic integrity of the FU by holding together each of the hair follicles contained in the follicular unit at the isthmus level. Therefore, we imagine the AP muscle acting as a string that ties all the hair follicles of each FU together, like a ribbon on a bunch of flowers. This is supported by the fact that the distribution in follicular units is only evident above the muscle insertion. The confluence of the AP muscle fibers in a muscular unit structure per FU is probably the only way to efficiently maintain the integrity of the FU, because it would be impossible to do so if single and independent muscles pulled their corresponding follicles in different directions. On the other hand, the orientation of the AP muscles at the same pole in adjacent follicular units would explain why all the erected hair shafts follow the same orientation.

Finally, the role of the AP muscle in the secretion of the sebum is an old theory that has been postulated in the past but which has still not been demonstrated.5,11 Sebaceous glands are composed
of acini, which are attached to a common excretory duct of cornifying squamous epithelium that is continuous with the wall of the hair canal. As mature sebocytes differentiate, they accumulate quantities of lipid that will eventually migrate to the central duct. The sebaceous glands are located between the external follicular sheaths and the AP muscle. In our study we have observed that AP muscle fascicles embrace the sebaceous lobules in their journey to the follicular insertion. This work is not a functional confirmation but certainly the model that we propose suggests that the contraction of the muscle should induce the excretion of sebum from the sebaceous lobules into the follicular canal.

**Fig 4.** Tangential serial sections through a follicular unit of three hairs (two in anagen and one in telogen). Sections are ordered sequentially from the surface (1) down to the lower isthmus (8). The arrector pili muscle that appears at level 4 (upper isthmus) surrounds the sebaceous gland lobules on their journey to the hair follicle insertion zone. The arrector pili muscles are shown in red and the sebaceous glands in yellow (100×).

**Fig 5.** Drawing A shows the traditional anatomy of human scalp hair. Drawing B shows a more accurate three-dimensional representation of a four-hair follicular unit based on horizontal serial sections. The epidermis is drawn in yellow, the arrector pili muscle in red, and the sebaceous glands in green.

**REFERENCES**