Appendix S1.

MATERIALS AND METHODS

Design and setting

This study was performed at the Department of Dermatology at the University Medical Center Groningen. The local institutional review board approved the study.

Patients

Patients who were scheduled for surgical treatment of HS with the de-roofing technique or the skin-tissue-sparing excision with electrosurgical peeling (STEEP) procedure (S1), were included after written informed consent was obtained. Healthy volunteers were considered eligible for participation when they had no skin disease in the armpits and had given written informed consent. The age of all individuals was 18 years or older.

Collection procedure

Up to 17 perilesional samples were obtained from axillary or inguinal HS skin by 4-mm punch biopsy, immediately frozen in liquid nitrogen, and subsequently stored at –80°C. In addition, after injection with local anaesthesia consisting of 1 ml 1% lidocaine/adrenaline (1:200,000), 4-mm punch biopsies were obtained from axillary skin of 8 healthy volunteers that served as controls. Skin samples lacking an associated sebaceous gland with the hair follicle were excluded.

Staining procedure

PAS staining of the skin samples was performed. Briefly, after periodic acid solution oxidation, tissue sections were immerged in Schiff's reagent and counterstained with haematoxylin. Immunofluorescence (IF) staining for type XVII collagen, type VII collagen, laminin 332, integrin α6 and β4 was performed. The procedures for IF staining and image collection have been described in detail previously (S2). The following monoclonal antibodies were used: VK1 against type XVII collagen (Dr H. H. Pas, Groningen, The Netherlands), LH7: 2 against collagen type VII (gift from Dr I. Leigh, London, UK), K140 against laminin β3 (gift from Dr M. Marinkovich, Stanford, USA), 58xβ4 against integrin β4 (gift from Dr Sonnenberg, Amsterdam, The Netherlands) and GOH3 against integrin α6 (gift from Dr Sonnenberg, Amsterdam, The Netherlands). Fluorescence-conjugated goat anti-rat IgG (Southern Biotechnology Associates, Birmingham, USA) and Alexa 488-conjugated goat anti-mouse IgG (Molecular Probes, Eugene, USA) were used as secondary steps.

Assessments and statistical analysis

The intensity of the stainings was measured at the 5 segments of the FPSU: (*i*) the interfolliclar epidermis (IFE), (*ii*) the superior segment of the hair follicle, (*iii*) the inferior segment (IS) of the hair follicle, (*iv*) the sebofollicular junction (SFJ), and (*v*) the sebaceous gland. The superior segment was defined as the part of the hair follicle extending from the IFE to the SFJ. The IS was defined as the part extending from the SFJ to the bulb. The SFJ was defined as the transition zone from the hair follicle to the sebaceous gland. The SFJ of skin samples stained with IF was identified by the presence of fat globules characteristic of the sebaceous gland and by comparison of the skin sample with the PAS staining of that same biopsy. For each skin sample the intensity of all performed PAS and IF stainings at the aforementioned individual segments were analysed using Image J software. Subsequently, the ratio of individual segments to the IFE was calculated for both the PAS and IF stainings, with the IFE serving as an internal control for each skin sample. The Mann-Whitney U test was performed to compare the differences in these ratios between patients and controls.

SUPPLEMENTARY REFERENCE LIST

S1. Blok JL, Spoo JR, Leeman FW, Jonkman MF, Horváth B. Skin-tissue-sparing excision with electrosurgical peeling (STEEP): a surgical treatment option for severe hidradenitis suppurativa Hurley stage II/III. J Eur Acad Dermatol Venereol 2015; 29: 379–382.

S2. Vodegel RM, Jonkman MF, Pas HH, de Jong MC. U-serrated immunodeposition pattern differentiates type VII collagen targeting bullous diseases from other subepidermal bullous autoimmune diseases. Br J Dermatol 2004; 151: 112–118.