**Methods**

*Patients*

Patients were enrolled at the Section of Dermatology of the University of Ferrara from January 1, 2017, to August 1, 2017. Eligible candidates did not receive any systemic or topical therapy for at least 2 months before surgical procedure. All subjects were Caucasian.

Hidradenitis suppurativa (HS) specimens were obtained from patients affected by HS that underwent surgery to excise single or multiple inflammatory nodules in HS-bearing skin areas. The diagnosis of HS was always made by a dermatologist specialized in HS management (V.B.) and confirmed by the pathology reports of the excised specimens. The healthy control (HC) samples consisted of normally pigmented skin obtained from the distal portion of a lozenge performed for the excision of a histologically proven melanocytic nevus in patients who were not affected by any previous or active dermatological disease. The minimum distance between the nevus border and the skin sample was 4 mm, in order to minimize the effect of nevus-associated inflammation [25]. Epidemiologic and clinical data comprising age, sex, body weight, smoking habit, comorbidities, Hurley stage, modified Sartorius score, and previous treatments were collected the day of the surgical procedure. A further control group consisted of 10 anonymous skin samples from abdominoplasties previously stored at the Pathological Anatomy Section of the University of Ferrara.

*Immunohistochemistry*

All skin samples were collected, fixed, and embedded according to standard procedures. Sections of 4-μm thickness were prepared. Slides were heated at 60°C for 20 min, de-paraffinized with xylene, and rehydrated by sequential passages through decreasing concentrations of ethanol. Endogenous peroxidase activity was blocked by 15 min of incubation at room temperature (RT) with 0.3% H2O2 in TBS (50 mM Tris, 150 mM NaCl, pH 7.6). After three rinses in wash buffer (0.025% Triton X-100 in TBS), tissue sections were kept for 2 h at RT with a blocking solution (1% BSA, 10% FBS in TBS), and then incubated overnight at 4°C in the presence of the antibody against the target molecule. For the IHC staining procedure, the following rabbit primary antibodies were used diluted in incubation solution (1% BSA in TBS): anti-P2X7R (1:100 dilution; Sigma-Aldrich, St. Louis, MO, USA) [26],anti-IL-1β (1:20 dilution; Novus Biologicals, Centennial, CO, USA), and anti-NLRP3 (1:20 dilution; Novus Biologicals). Normal rabbit serum (1:500 dilution; Thermo-Fisher Scientific, Rockford, IL, USA) was used as control for the rabbit primary antibodies. Sections were then rinsed twice in wash buffer, incubated for 60 min at RT with goat anti-rabbit HRP-conjugated secondary antibody (Dako, Glostrup, Denmark) (1:100 dilution in incubation solution), washed in TBS, and further incubated for 6–10 min at RT with DAB Substrate Chromogen System (Dako). Counterstaining was performed with Mayer’s haematoxylin. At least 9 slides per biopsy, 3 slides for each experimental condition, and 10 fields for each slide were examined with a Nikon Eclipse H550L microscope and analysed with NIS-Elements software (Nikon, Tokyo, Japan).

The intensity of immunostaining was graded semi-quantitatively for (1) keratinocytes, (2) lymphocytes and monocytes, and (3) neutrophils. Absent (0%), few (<10%), moderate (10–60%), numerous (60–70%), and abundant (>70%) positive cells were graded as 0, 1, 2, 3, and 4, respectively.

*Statistics*

The Mann-Whitney U test was applied to compare the HS and HC groups using SPSS v17.0 (SPSS Inc., Chicago, IL, USA). Benjamini-Hochberg correction was calculated for the *p* values using a false discovery rate <0.05. Cohen’s κ correlation coefficient was calculated to compare Hurley stage and P2X7R, NLRP3, and IL-1β protein expression in skin keratinocytes in HS lesions. Cohen’s κ correlation coefficient and X2 test were calculated to compare P2X7R, NLRP3, and IL-1β protein expression in skin keratinocytes of HS lesions, considering a positive score when more than 60% of the cells were stained by the antibodies. The X2 test and Student’s *t* test were used to compare HS and HC groups regarding epidemiological variables such as gender or tobacco smoking habits and age or BMI. Statistical analyses were considered significant with *p* < 0.05.