APPENDIX S1

SUPPLEMENTARY PATIENTS AND METHODS

Patients and skin sampling

Specimens from 13 patients with histopathologically confirmed parapsoriasis were included in this study (10). Six of the patients had SPP and 7 had LPP. The sample material was collected during January–May and September 2014 at the Department of Dermatology and Allergology, Helsinki University Hospital, Helsinki, Finland. The ethics committee of the University of Helsinki approved the study protocol (approval number 12/13/03/01/2012). The clinical characteristics of the patients have been described in detail previously (10). None of the patients had received antibiotic treatment or ultraviolet (UV) phototherapy within 12 months prior to sampling.

To compare the prevalence, quantity and sequence diversity of Chlamydiales DNA in healthy and lesional skin, we investigated samples taken from both lesional parapsoriasis skin and from healthy skin of these 13 patients (10). Both skin swabs and skin biopsies were taken from the same body sites (lesional skin and contralateral healthy skin). Swabs were collected by a Copan Flocked Swab® (Copan Diagnostics Inc., Murrieta, CA, USA) that was immersed into a buffer solution (sterile 0.15 M NaCl with 0.1% Tween 20) and then wiped bidirectionally over the target skin area of 3×3 cm, as described previously (10). Each skin area was sampled twice, so that from each patient we had 2 lesional swabs and 2 swabs of healthy skin. One biopsy from each side was carried out as incisional biopsies, fixed in formalin and embedded in paraffin.

PCR methods and sequence analysis

DNA from swab samples was extracted earlier in Dr Petri Auvinen's laboratory in the University of Helsinki, Finland, by FastDNA Spin Kit for Soil (MP Biomedicals , LLC, Santa Ana, CA, USA) following the manufacturer's instructions (10). DNA from biopsy samples was extracted from 10 sections of 20 µm by the QIAamp DNA FFPE Tissue Kit (Qiagen , Hilden Germany) as recommended by the manufacturer. All samples were examined with a Chlamydiales-specific amplification method (PanChlamydiales-PCR) as described earlier (7, S1). In brief, PanChlamydiales-PCR was performed in a final volume of 25 ml. Extreme precautions were taken to avoid contamination. DNA extraction and preparation of PCR reaction mixes were conducted in separate laboratories with separate equipment, dedicated to pre-PCR activities only. In each run, 4 wells of distilled water were included as negative controls and 4 wells were used as nucleic acid extraction negative controls. Cycling conditions were 10 min at 95°C, followed by 45 cycles of 15 s at 95°C and 60 s at 60°C, all carried out in an Applied Biosystems® 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). All 78 samples were tested in duplicate. Samples with Ct ≤37 (S2) or with Ct 37–40 and a Chlamydiales family levels sequence (>90% similarity with established Chlamydiales sequence (12)) were considered positive.

To approximate the quantity of Chlamydiales DNA in relation to the amount of sample material, the human house-keeping gene betaglobin was amplified from all skin samples in same runs (S3). Threshold cycle (Ct) was used as a relative measure of the concentration of target DNA in the reaction. In order to estimate of the relative quantities of Chlamydiales DNA in swabs and in biopsies, the ΔCt ($Ct_{panchlamydiales} - Ct_{betaglobin}$) value for each sample was calculated (S4). The 2-sample *t*-test was performed to estimate the significance of the detected differences.

The amplicons from Chlamydiales-specific PCRs were purified by Illustra ExoProStar 1-Step (GE Healthcare, Bucks, UK) as instructed by the manufacturer. Sequencing was then performed in the sequencing unit of Institute for Molecular Medicine Finland. Sequences were trimmed for primers, aligned using MUSCLE and quality controlled manually by eye by inspecting the chromatogram using Geneious 6.1 (S5, S6). The sequences were: (*i*) compared with all sequences in the GenBank nt database using BLAST (S7); (*ii*) identified to species using SINTAX with RDP classifier (S8) with probability cut-off 0.8; and then (*iii*) compared with human skin microbiome produced in a previous study, as described in the following section.

Analysis of the previous microbiome data

Raw data from a previous microbiome study for these patients had been uploaded to the European Nucleotide Archive with accession number PRJEB15287 (10). These sequences were searched for Chlamydiales sequences, as follows. Shortly, we downloaded all the raw (5 664 717) reads into Finnish IT Center for Science server (www.csc.fi). Then, paired reads were merged (USEARCH fastq_mergepair), quality-filtered+primer-trimmed (cutadapt with Q30 threshold), de-replicated to unique reads (USEARCH fastx_uniques), and finally clustered into operational taxonomic units (OTUs) (97% similarity) (S5, S9). These OTUs were used as a local BLAST database, and trimmed PanChlamydiales-PCR sequences (see previous section "PCR methods and sequence analysis") were queried against this newly created database.

SUPPLEMENTARY REFERENCES

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