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Microbial dysbiosis and foot odor in Nagashima-type palmoplantar keratosis: Improvement with topical benzoyl peroxide

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Nagashima-type palmoplantar keratosis (also referred to as *SERPINB7*-related palmoplantar epidermal differentiation disorder) is the most common form of palmoplantar keratoderma in East Asia. It is characterized by erythematous hyperkeratosis, palmoplantar hyperhidrosis, and a distinctive foot odor that significantly impairs QOL. In this study, we aimed to clarify the etiology of this odor by investigating the plantar microbiome and evaluating the therapeutic effects of topical benzoyl peroxide. Compared with healthy controls, individuals with Nagashima-type palmoplantar keratosis exhibited significantly higher objective odor scores; increased bacterial load, especially in the intertoe area; and reduced microbial diversity. *Corynebacterium* and *Staphylococcus* were the predominant dysbiotic flora species. The topical application of benzoyl peroxide significantly reduced foot odor and bacterial load; increased microbial diversity; and selectively decreased *Corynebacterium* abundance, particularly that of *C. tuberculoearicum*. These changes correlate with the species-specific susceptibility of skin-resident bacteria to benzoyl peroxide. Our findings suggest that microbial dysbiosis, especially the overgrowth of *Corynebacterium* species, is central to the pathogenesis of foot odor in Nagashima-type palmoplantar keratosis. Topical benzoyl peroxide is a promising therapeutic intervention for mitigating dysbiosis and its associated foot odor. This study highlights the potential of microbiome-targeted therapies for symptom relief in inherited skin disorders such as Nagashima-type palmoplantar keratosis.

Keywords: *Corynebacterium* spp, *Corynebacterium tuberculoearicum*, Microbiome-targeted therapies, *Staphylococcus epidermidis*, *Staphylococcus* spp

INTRODUCTION

Nagashima-type palmoplantar keratosis (NPPK) (Mendelian Inheritance in Man 615598; also referred to as *SERPINB7*-related palmoplantar epidermal differentiation disorder) is the most prevalent form of palmoplantar keratoderma (PPK) in East Asia (Kubo, 2025; Sprecher et al, 2025). NPPK is an autosomal recessive PPK caused by loss-of-function variants of *SERPINB7*, a serine protease inhibitor (Kubo et al, 2013). Founder variants are common in East Asian populations and account for

higher rates of NPPK. In contrast, only a few cases have been reported in other regions of the world, except for Finland (Hannula-Jouppi et al, 2020). This is presumably attributable to the low prevalence of pathogenic variations in *SERPINB7* among non-East Asian and non-Finnish populations. Although the proteases inhibited by *SERPINB7* are not fully characterized, legumain protease was identified as a potential target (Li et al, 2025). Accelerated degradation of desmoglein 1 and desmocollin 1 in NPPK skin suggests that kallikreins may also be relevant target proteases (Cohen-Barak et al, 2022). Biallelic defects in *SERPINA12* have been associated with NPPK phenocopy in PPK (Mohamad et al, 2020). *SERPINA12* inhibits kallikrein 7, and desmoglein 1 and corneodesmosin—the substrates of kallikrein 7—have lower expression in the *SERPINA12*-related palmoplantar epidermal differentiation disorder (Mohamad et al, 2020; Sprecher et al, 2025).

The primary symptom of NPPK is erythematous hyperkeratosis of the palms and soles. Upon exposure to water, affected skin becomes whitish and swollen but returns to normal once dry (Kubo et al, 2013). This suggests that the water-repellent barrier of the stratum corneum is compromised in NPPK-affected skin, likely owing to overdegradation of structural proteins.

Other common NPPK symptoms include palmoplantar hyperhidrosis, easy peeling of the palmoplantar stratum corneum, and a distinctive odor of the palms and feet. The odor,

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Abbreviations: ASV, amplicon sequence variant; BPO, benzoyl peroxide; NPPK, Nagashima-type palmoplantar keratosis; PPK, palmoplantar keratoderma; rRNA, ribosomal RNA

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more prominent on the feet, especially between the toes, significantly impacts the QOL. Patients report that it diminishes after daily foot washing. Body odor arises when resident bacteria metabolize otherwise nonodorous secretions from eccrine, apocrine, and sebaceous glands into low-molecular-weight volatiles such as propionic and isovaleric acid; other volatile fatty acids; and sulphur-containing compounds, including thioalcohols (James et al, 2013; Kanda et al, 1990; Marshall et al, 1988). Foot odor has been less extensively studied than axillary odor; nevertheless, isovaleric acid is regarded as a major contributor, generated by *Staphylococcus* spp and *Corynebacterium* spp. (Amoore, 1977; Ara et al, 2006; Stevens et al, 2015). On the basis of these observations, we investigated palmoplantar dysbiosis in NPPK and evaluated whether topical benzoyl peroxide (BPO) could reduce bacterial burden and foot odor.

RESULTS

This cohort comprised 32 individuals with NPPK who exhibited the typical clinical symptoms of NPPK with compound heterozygous or homozygous pathogenic variants of *SERPINB7* (Figure 1 and Table 1). The cohort included 7 male and 25 female participants, with a median age of 27 years (range = 2–69 years). The healthy control group comprised 20 individuals, including 14 parents of individuals with NPPK who were heterozygous for the *SERPINB7* pathogenic variant and did not manifest cutaneous symptoms as well as 6 healthy individuals who did not have cutaneous diseases (Figure 1 and Table 1). The control cohort included 6 male

and 14 female participants, with a median age of 41 years (range = 5–57 years).

Elevated foot odor and bacterial load in NPPK

A substantial proportion of skin odor is produced by bacteria that reside on the skin and constitute part of its natural microbiota. Given that the number of bacteria residing on the skin is significantly lower than that on the intestinal tract, it is suspected that not only the alteration in bacterial composition but also the alteration in bacterial load exerts a substantial influence on skin odor. Accordingly, we initially investigated skin odor and the difference in bacterial load between the skin of individuals with NPPK and that of healthy controls by approximating the bacterial load on the basis of the copy number changes in 16S ribosomal RNA (rRNA) extracted from skin swab specimens. A limitation of this method is that the copy number of 16S rRNA varies among bacterial species.

The objective odors of the feet of 19 healthy controls (13 parents of individuals with NPPK and 6 others) and 30 individuals with NPPK were compared. The Mann–Whitney *U* test revealed a significant difference in odor scores between the NPPK group and healthy controls ($P < .001$) (Figure 2a). Among the NPPK group, 8 individuals had concomitant tinea pedis, but no difference in odor scores was observed (Supplementary Figure S1a).

Next, we selected the skin areas from which swab samples were collected (Figure 2b). The 16S rRNA copy number was calculated from the results of qPCR using bacterial DNA



Figure 1. Representative clinical phenotype of individuals with NPPK and healthy controls. (a) An individual (NG020) with NPPK with a biallelic pathogenic variant in *SERPINB7*. (b) A healthy control (NG010) with a heterozygous pathogenic variant in *SERPINB7*. (c) A healthy control (NG007) without the *SERPINB7* pathogenic variant. All patients provided written informed consent for publication of these clinical images. NPPK, Nagashima-type palmoplantar keratosis.

Table 1. Study Participants with NPPK and Healthy Controls

NPPK/ Control	Patient Number	Sample Number	Year/ Sex	<i>SERPINB7</i> (NM_001040147.3) Variant Alleles			Comorbidities
NPPK	1	NG008	3 y/M	c.796C>T (p.Arg266*)	c.830C>T (p.Pro277Leu)	Compound heterozygote	n.s.
	2	NG013	16 y/M	c.796C>T (p.Arg266*)	c.796C>T (p.Arg266*)	Homozygote	n.s.
	3	NG014	40 y/F	c.336+2T>G	c.796C>T (p.Arg266*)	Compound heterozygote	n.s.
	4	NG015	63 y/F	c.796C>T (p.Arg266*)	c.796C>T (p.Arg266*)	Homozygote	n.s.
	5	NG016	32 y/F	c.455-1G>A	c.796C>T (p.Arg266*)	Compound heterozygote	Tinea
	6	NG017	20 y/F	c.796C>T (p.Arg266*)	c.796C>T (p.Arg266*)	Homozygote	Tinea
	7	NG020	73 y/F	c.218_219delinsTAAACTTTACCT (p.Gln73Leufs*17)	c.636del (p.Lys213Serfs*12)	Compound heterozygote	n.s.
	8	NG021	36 y/F	c.796C>T (p.Arg266*)	c.796C>T (p.Arg266*)	Homozygote	n.s.
	9	NG023	13 y/F	c.796C>T (p.Arg266*)	c.830C>T (p.Pro277Leu)	Compound heterozygote	n.s.
	10	NG028	46 y/F	c.218_219delinsTAAACTTTACCT (p.Gln73Leufs*17)	c.455-1G>A	Compound heterozygote	n.s.
	11	NG029	31 y/F	c.218_219delinsTAAACTTTACCT (p.Gln73Leufs*17)	c.218_219delinsTAAACTTTACCT (p.Gln73Leufs*17)	Homozygote	n.s.
	12	NG031	46 y/F	c.796C>T (p.Arg266*)	c.796C>T (p.Arg266*)	Homozygote	Tinea
	13	NG033	22 y/F	c.796C>T (p.Arg266*)	c.796C>T (p.Arg266*)	Homozygote	n.s.
	14	NG034	40 y/F	c.636del (p.Lys213Serfs*12)	c.455G>T (p.Gly152Val)	Compound heterozygote	n.s.
	15	NG035	20 y/F	c.796C>T (p.Arg266*)	c.796C>T (p.Arg266*)	Homozygote	Tinea
	16	NG037	24 y/M	c.218_219delinsTAAACTTTACCT (p.Gln73Leufs*17)	c.796C>T (p.Arg266*)	Compound heterozygote	Atopic dermatitis
	17	NG041	54 y/F	c.218_219delinsTAAACTTTACCT (p.Gln73Leufs*17)	c.455-1G>A	Compound heterozygote	n.s.
	18	NG043	25 y/F	c.796C>T (p.Arg266*)	c.796C>T (p.Arg266*)	Homozygote	Tinea
	19	NG044	51 y/F	c.796C>T (p.Arg266*)	c.796C>T (p.Arg266*)	Homozygote	n.s.
	20	NG046	5 y/F	c.455-1G>A	c.796C>T (p.Arg266*)	Compound heterozygote	n.s.
	21	NG049	22 y/F	c.218_219delinsTAAACTTTACCT (p.Gln73Leufs*17)	c.796C>T (p.Arg266*)	Compound heterozygote	n.s.
	22	NG056	2 y/F	c.455-1G>A	c.796C>T (p.Arg266*)	Compound heterozygote	Atopic dermatitis
	23	NG058	56 y/F	c.455-1G>A	c.455-1G>A	Homozygote	Atopic dermatitis
	24	NG059	23 y/F	c.218_219delinsTAAACTTTACCT (p.Gln73Leufs*17)	c.796C>T (p.Arg266*)	Compound heterozygote	Tinea
	25	NG061	10 y/M	c.455-1G>A	c.796C>T (p.Arg266*)	Compound heterozygote	n.s.
	26	NG064	69 y/F	c.454+1del	c.796C>T (p.Arg266*)	Compound heterozygote	Tinea
	27	NG065	64 y/F	c.454+1del	c.796C>T (p.Arg266*)	Compound heterozygote	n.s.
	28	NG068	67 y/F	c.454+1del	c.796C>T (p.Arg266*)	Compound heterozygote	n.s.
	29	NG069	28 y/F	c.455-1G>A	c.796C>T (p.Arg266*)	Compound heterozygote	n.s.
	30	NG071	19 y/M	c.796C>T (p.Arg266*)	c.796C>T (p.Arg266*)	Homozygote	n.s.
	31	NG072	16 y/M	c.796C>T (p.Arg266*)	c.796C>T (p.Arg266*)	Homozygote	Tinea
	32	NG073	5 y/M	c.455-1G>A	c.830C>T (p.Pro277Leu)	Compound heterozygote	n.s.
Healthy control	1	NG004	39 y/M	c.796C>T (p.Arg266*)	No pathogenic variants	Heterozygote, a parent of NPPK*	n.s.
	2	NG007	48 y/F	No pathogenic variants	No pathogenic variants	No pathogenic variants, healthy Ct	n.s.
	3	NG009	30 y/M	c.796C>T (p.Arg266*)	No pathogenic variants	Heterozygote, a parent of NPPK#1	n.s.
	4	NG010	30 y/F	c.830C>T (p.Pro277Leu)	No pathogenic variants	Heterozygote, a parent of NPPK#1	n.s.
	5	NG011	49 y/M	c.796C>T (p.Arg266*)	No pathogenic variants	Heterozygote, a parent of NPPK#2	n.s.
	6	NG012	57 y/F	c.796C>T (p.Arg266*)	No pathogenic variants	Heterozygote, a parent of NPPK#2	n.s.
	7	NG018	52 y/F	c.796C>T (p.Arg266*)	No pathogenic variants	Heterozygote, a parent of NPPK#6	n.s.
	8	NG022	57 y/F	n.a.	n.a.	n.a., a parent of NPPK#8	n.s.
	9	NG024	41 y/M	n.a.	n.a.	n.a., healthy Ct	n.s.
	10	NG025	12 y/M	n.a.	n.a.	n.a., healthy Ct	n.s.

(continued)

Table 1. Continued

NPPK/ Control	Patient Number	Sample Number	Year/ Sex	SERPINB7 (NM_001040147.3) Variant Alleles			Comorbidities
	11	NG026	9 y/F	n.a.	n.a.	n.a., healthy Ct	n.s.
	12	NG027	5 y/M	n.a.	n.a.	n.a., healthy Ct	n.s.
	13	NG036	47 y/F	c.796C>T (p.Arg266*)	No pathogenic variants	Heterozygote, a parent of NPPK#15	n.s.
	14	NG038	53 y/F	c.796C>T (p.Arg266*)	No pathogenic variants	Heterozygote, a parent of NPPK#16	n.s.
	15	NG040	40 y/F	n.a.	n.a.	n.a., a parent of NPPK*	n.s.
	16	NG048	46 y/F	c.796C>T (p.Arg266*)	No pathogenic variants	Heterozygote, a parent of NPPK#20	n.s.
	17	NG053	42 y/F	No pathogenic variants	No pathogenic variants	No pathogenic variants, healthy Ct	n.s.
	18	NG057	39 y/F	c.796C>T (p.Arg266*)	No pathogenic variants	Heterozygote, a parent of NPPK#22	n.s.
	19	NG062	37 y/F	c.796C>T (p.Arg266*)	No pathogenic variants	Heterozygote, a parent of NPPK#25	n.s.
	20	NG074	41 y/F	c.830C>T (p.Pro277Leu)	No pathogenic variants	Heterozygote, a parent of NPPK#32	n.s.

Abbreviations: Ct, control; n.a., not analyzed; NPPK, Nagashima-type palmoplantar keratosis; n.s., nothing specific.

The asterisk indicates that the proband was not included in the study.

obtained from swab samples (Supplementary Tables S1 and S2 provide raw data). The intertoe sample exhibited the highest 16S rRNA copies/cm² in each individual among both healthy controls and the NPPK group. A comparison of 16S rRNA copies in each skin area between individuals with NPPK and healthy controls using the Mann–Whitney *U* test indicated that the 16S rRNA copy numbers were significantly higher in affected skin areas, including the intertoe, first metatarsal head region, center of the heel, and center of the palm, in individuals with NPPK (Figure 2c). The Mann–Whitney *U* test yielded $P < .001$ for the intertoe and first metatarsal head regions, $P = .033$ for the center of the heel, and $P = .003$ for the center of the palm. In contrast, no significant difference was observed between individuals with NPPK and healthy controls in nonlesion areas (Figure 2c). Notably, the coexistence of tinea pedis did not influence the 16S rRNA copy number in the feet of individuals with NPPK (Supplementary Figure S1b), suggesting that the increased bacterial load was independent of fungal infection.

BPO reduces foot odor and bacterial load

Given the hypothesis that an elevated bacterial load in the stratum corneum may contribute to odor in NPPK lesions, the next objective was to ascertain whether a reduction in bacterial load would result in a concomitant decline in odor. BPO gel was selected for topical application to reduce the bacterial load rather than an antibiotic cream because of the former's lack of induction of antibiotic-resistant bacteria and minimal impact on the test results when resistant bacteria were present. A total of 2.5% BPO gel (Maruho, Osaka, Japan) was administered once daily to the palmoplantar skin, including the intertoe area, of the individuals with NPPK. The objective odor and 16S rRNA copy number of swab samples were compared before and after BPO treatment.

In the NPPK group, the subjective odor of the feet was evaluated in 21 individuals before and after BPO treatment. The duration of the topical application of BPO was 3–21 weeks (median = 6 weeks). The Wilcoxon matched-pairs signed-rank test revealed a statistically significant reduction in foot odor intensity after topical BPO application ($P = .007$) (Figure 2d). In addition, a visual analog scale was used to assess subjective symptoms in 13 of the 21 individuals with NPPK. The duration of BPO treatment was 4–21 weeks (median = 5 weeks). The subjective symptoms that followed BPO application exhibited a clear tendency for reduced visual analog scale scores for hand odor (23%) and foot odor (33%); however, no notable changes were observed in the dryness and redness of the hands and feet (Table 2).

Next, the 16S rRNA copy numbers in specific areas of the hands and feet were compared before and after BPO treatment. The hands and feet of 16 and 22, respectively, of individuals with NPPK who did not meet the exclusion criteria (Materials and Methods) were evaluated. The durations of topical application were 4–21 and 3–21 weeks for the hands and feet, respectively, with a median of 6 weeks for both groups. Among the affected areas, the Wilcoxon matched-pairs signed-rank test identified a statistically significant reduction in bacterial numbers after the application of BPO at the intertoe ($P = .017$), first metatarsal head region ($P < .001$), and center of the palm ($P < .001$); however, no statistically significant difference was observed at the center of the heel ($P = .11$) (Figure 2e). No significant alterations in 16S rRNA copy number were observed in the nonaffected areas where BPO was not topically administered, including the dorsal aspects of the foot ($P = .56$), hand ($P = .50$), and inner forearm ($P = .78$). The raw data for the 16S rRNA copy numbers are provided in Supplementary Table S2.

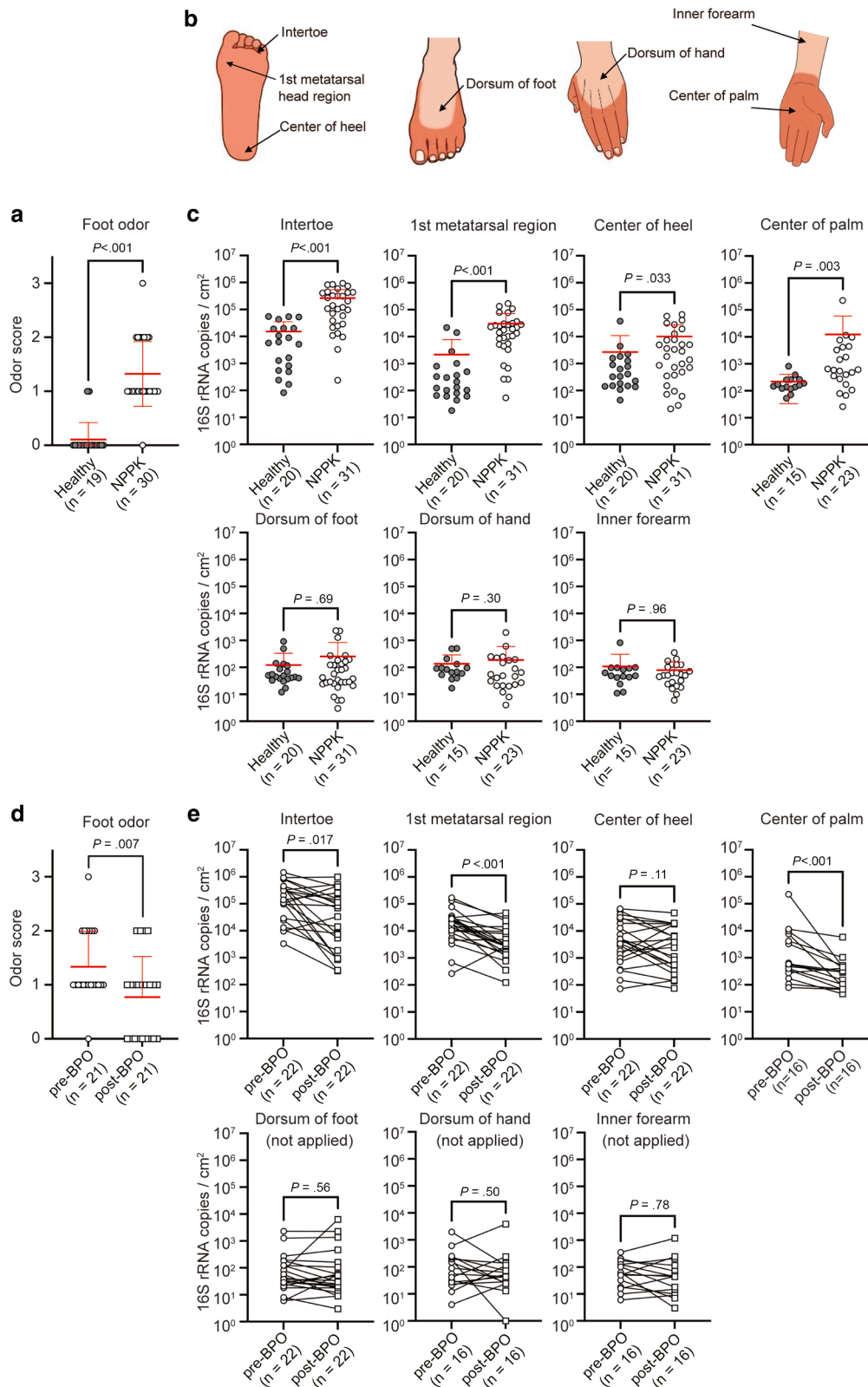


Figure 2. Odor and bacterial load on healthy skin and NPPK-affected skin before and after BPO treatment. (a) Foot odor scores in individuals with NPPK and healthy controls. **(b)** Schematic illustration of the sampling sites for microbiome analysis. Affected areas are shown in red. **(c)** The 16S rRNA copy numbers per cm² in swab samples collected from the indicated sites. **(d)** Foot odor scores in individuals with NPPK before and after BPO treatment (before BPO and after BPO). **(e)** The 16S rRNA copy numbers per cm² in swab samples collected from the indicated sites in individuals with NPPK before and after BPO treatment (before BPO and after BPO). BPO, benzoyl peroxide; NPPK, Nagashima-type palmoplantar keratosis; rRNA, ribosomal RNA.

Dysbiosis with reduced bacterial diversity in NPPK is partially restored by BPO

Given that the average bacterial load was highest in the intertoe samples, a comparative analysis of the microbial composition was conducted using intertoe samples from

healthy controls and individuals with NPPK. In the NPPK group, pre- and post-BPO intertoe samples were compared using the Wilcoxon matched-pairs signed-rank test. Initially, meta-16S rRNA analysis employing amplicon sequence variants (ASVs) was conducted on intertoe samples from 20

Table 2. Percentile Changes in the VAS Score of Foot Odor, Redness, and Dryness after Topical Application of BPO

Sample Number	Odor VAS (Hand)	Odor VAS (Foot)	Redness VAS (Hand)	Redness VAS (Foot)	Dryness VAS (Hand)	Dryness VAS (Foot)	BPO Application Period (wk)
NG023	55	53	100	100	60	57	21
NG043	0	5	100	100	100	100	9
NG044	50	48	100	100	100	100	6
NG049	0	30	90	90	95	95	4
NG058	52	52	100	100	50	50	5
NG061	3	24	100	100	100	100	4
NG064	3	3	100	100	19	100	6
NG065	0	5	100	100	81	79	5
NG068	86	14	100	100	100	100	12
NG069	0	25	100	100	100	90	5
NG071	32	100	100	100	100	100	4
NG072	1	47	100	100	100	100	5
NG073	13	24	100	100	51	63	6
Average	23	33	99	99	81	87	5 [†]

Abbreviations: BPO, benzoyl peroxide; VAS, visual analog scale.

[†]Median.

healthy controls and 22 individuals with NPPK. A total of 1689 bacterial ASVs in 64 samples were identified in healthy controls and before and after BPO in the NPPK group. To determine whether the microbial composition of the skin was altered in NPPK, we first compared individuals with NPPK with healthy controls by examining alpha diversity using 4 different metrics to measure community richness (observed ASV numbers and Chao1), evenness (Simpson diversity index), and overall diversity (Shannon index) (Supplementary Figure S2). Individuals with NPPK exhibited a significant decrease in ASV numbers and Chao1 compared with healthy controls ($P = .003$ and $P = .002$ with the Mann–Whitney U test, respectively), whereas no significant difference was observed in the Simpson diversity and Shannon indices. Next, we evaluated the effect of BPO on bacterial diversity. A patient-matched comparison between the pre- and post-BPO treatments revealed a tendency for an increased ASV number and Chao1 and a significant difference in the Shannon index ($P = .025$, Wilcoxon test). The results indicated that bacterial diversity was low in the high bacterial load intertoe skin of individuals with NPPK and that BPO treatment increased bacterial diversity with decreasing bacterial load.

BPO selectively suppresses *Corynebacterium tuberculoostearicum*

Next, changes in microbial composition were analyzed to evaluate the effect of BPO. The most abundant phylum in the intertoe skin was Firmicutes_D, followed by Actinobacteriota, in both healthy controls and individuals with NPPK (Supplementary Figure S3a). The proportion of Actinobacteriota but not Firmicutes_D was significantly reduced by BPO treatment (Supplementary Figure S3b). Analysis at the genus level revealed that *Staphylococcus* and *Corynebacterium* were the most prevalent genera in both healthy controls and individuals with NPPK (Figure 3a). To understand the changes in their absolute abundances, the total 16S rRNA copy numbers (Figure 2c and e) were multiplied by the

proportion of each genus to estimate both the changes in bacterial composition and bacterial load (Figure 3b). The results indicated that the substantial increase in bacterial load in individuals with NPPK was primarily attributable to the overgrowth of *Staphylococcus* and *Corynebacterium* without a significant change in their relative abundance. The Wilcoxon matched-pairs signed-rank test identified that BPO treatment not only reduced the bacterial load but also reduced the relative abundance of *Corynebacterium* ($P = .005$) and increased the abundance of *Staphylococcus* ($P = .092$) in the individuals with NPPK (Figure 2e and 3b and c). Although the relative abundance of *Staphylococcus* slightly increased, the estimated absolute abundances of both *Corynebacterium* and *Staphylococcus* decreased after BPO treatment (Supplementary Figure S4a).

To understand the alterations in microbial composition at the species level, the 20 most prevalent ASVs were identified, all of which belonged to the genera *Staphylococcus* or *Corynebacterium* (Figure 3d). Among these, *S epidermidis* (ASV0003) and *C tuberculoostearicum* (ASV0001) were predominant in the intertoes of healthy controls. In contrast, the NPPK group exhibited a shift in bacterial composition, characterized by an increased abundance of *C tuberculoostearicum* (ASV0001) and a decreased abundance of *S epidermidis* (ASV0003). After BPO treatment, the microbial composition in the intertoes of individuals with NPPK exhibited a decreased relative abundance of *C tuberculoostearicum* and an increased relative abundance of *Staphylococcus* species (Figure 3d). The estimated bacterial load of ASV0001 but not ASV0003 significantly decreased after BPO treatment, suggesting that BPO exerts differential antibacterial effects against these bacterial species (Supplementary Figure S4b). Consistently, an in vitro assay revealed that the growth of the type strain of *C tuberculoostearicum* (JCM 13389) was inhibited by lower concentrations of BPO than that of the standard strain *S epidermidis* (JCM 2414) (Figure 3e).

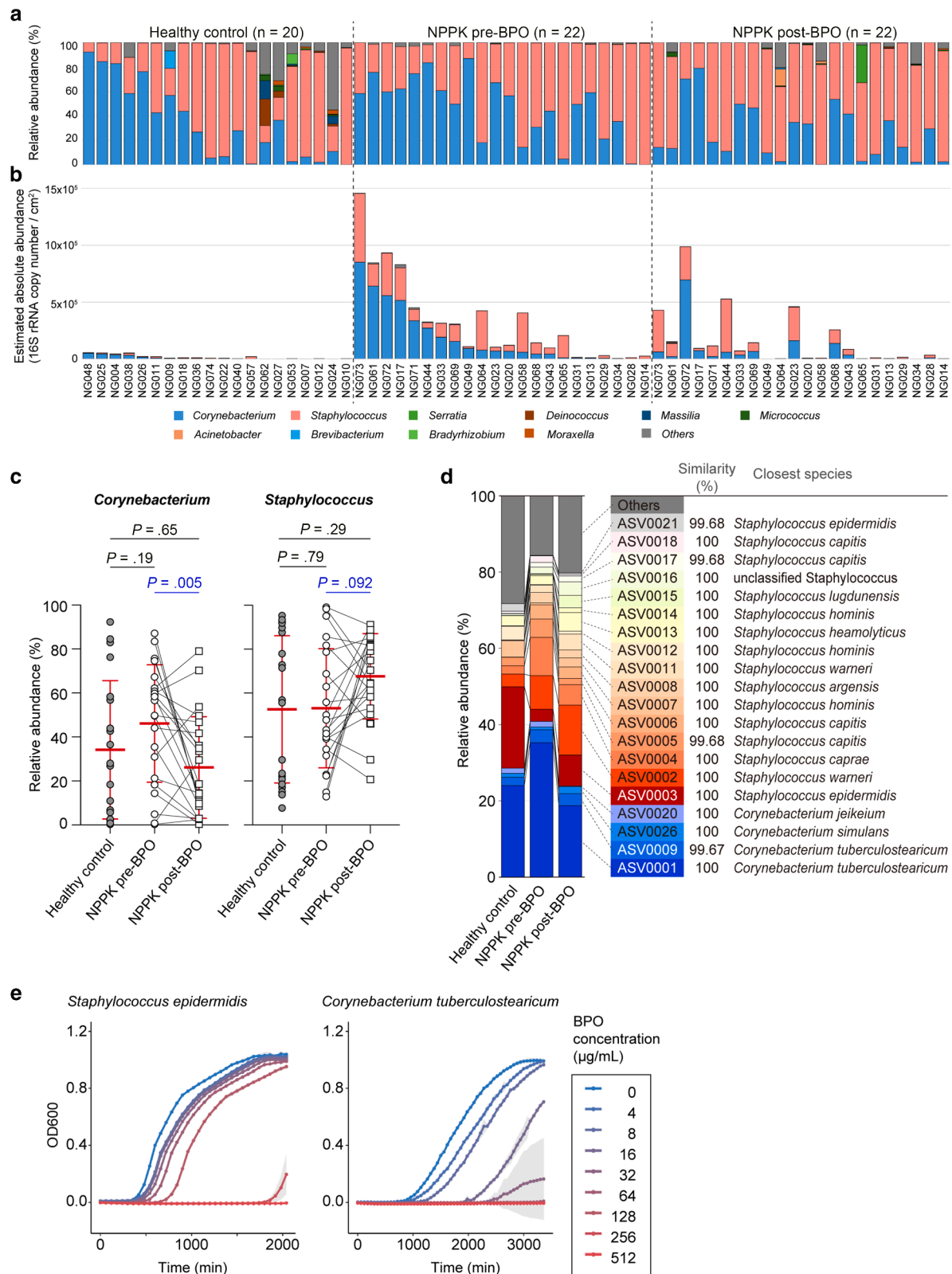


Figure 3. Microbial flora of the intertoe skin in healthy controls and individuals with NPPK and the effect of BPO treatment. (a) Relative genus abundance and (b) virtual bacterial load of each genus on the intertoe skin in healthy controls and individuals with NPPK before and after BPO treatment (before BPO and after BPO). (c) Relative abundance of *Corynebacterium* and *Staphylococcus* at the genus level on the intertoe skin in each indicated group. (d) Relative abundance of the 20 most prevalent species in each indicated group, shown with their sequence similarities to the standard strains. (e) The MIC of BPO on the standard strain of *C. tuberculostrictum* and *S. epidermidis*. BPO, benzoyl peroxide; MIC, minimum inhibitory concentration; NPPK, Nagashima-type palmoplantar keratosis.

DISCUSSION

Palmoplantar dysbiosis with an elevated bacterial load was identified in NPPK, with the most substantial bacterial load detected at the intertoe, where the most prevalent bacterium was identified as Firmicutes_D at the phylum level and *Corynebacterium* and *Staphylococcus* at the genus level. Topical application of BPO reduced the bacterial load and absolute abundance of *Corynebacterium* and decreased the distinctive odor. These findings suggest that microbial dysbiosis may be a contributing factor to the distinctive palmoplantar odor in NPPK and that antibacterial treatment could serve as an effective approach for addressing dysbiosis and foot odor.

The stratum corneum comprises a stack of dead corneocytes and intercorneocyte lipid lamellae, with corneocytes adhering to each other through corneodesmosomes and the adherence force of the lipid lamellae. Reduction in desmoglein 1 and desmocollin 1, the major structural components of corneodesmosomes, has been observed in the stratum corneum of individuals aged NPPK (Cohen-Barak et al, 2022). Furthermore, studies on *Serpinb7*^{-/-} mice have revealed a decrease in cytoskeletal proteins and an overactivation of legumain protease (Li et al, 2025). These observations suggest that overactivated proteases that escape SERPINB7 inhibition may contribute to the accelerated degradation of cell adhesions and cytoskeletons in the stratum corneum in NPPK. Such structural alterations in the stratum corneum may result in whitish changes and swelling of the stratum corneum after water exposure, as observed in the skin affected by NPPK. In conjunction with hyperhidrosis, which frequently occurs in NPPK (Kabashima et al, 2008) it was hypothesized that structural alterations of the stratum corneum and hyperhidrosis in NPPK provide a moist intrastratum corneum microenvironment that may contribute to the overgrowth of the plantar commensal bacteria observed in this study. In NPPK, co-occurrence of tinea pedis is frequent, but tinea status showed no impact on foot odor or on bacterial dysbiosis in this study.

This study demonstrated that *Corynebacterium* species with an abundance of *C. tuberculo-stearicum* and *Staphylococcus* species overgrew on the plantar skin of individuals with NPPK. Most bacteria detected on the skin surface are thought to be nonviable (Acosta et al, 2023); accordingly, the bacterial DNA that we collected may include genomes from dead cells. However, unlike the truncal skin, the plantar surface lacks pilosebaceous units; therefore, the increased bacterial DNA burden on the sole is more plausibly explained by in situ proliferation of viable microbes within the stratum corneum.

Bacterial overgrowth and hyperhidrosis can result in an increased abundance of odor-producing enzymes and substrates. Previous studies have identified isovaleric acid as the major odorant in human foot odor produced by microorganisms, including *S. epidermidis* and *Corynebacterium* species (Amoore, 1977; Ara et al, 2006; Stevens et al, 2015). Because foot odor in NPPK differs from typical human foot odor, additional as-yet-unidentified odorants—likely of *Corynebacterium* origin—are thought to contribute alongside isovaleric acid. Bothnian-type PPK shares with NPPK hyperhidrosis and water-induced whitening of the stratum corneum, and reports describe increased foot odor with

enrichment of *Corynebacterium* (Fertitta et al, 2022; Krøigård et al, 2016), suggesting that a structural alteration of the stratum corneum common to NPPK and Bothnian-type PPK may promote *Corynebacterium* overgrowth and odor production. By contrast, in Vörner-type PPK, the plantar skin is typically dry, and—on the basis of clinical experience—the odor appears different; future studies should investigate dysbiosis, causative odorants, and BPO effectiveness.

Study limitations include the small sample size, lack of fungal dysbiosis assessment, and constrained odor evaluation using a pragmatic 4-point odor score (Watanabe et al, 2016) instead of the European 10-point scale (ASTM E1207-14, 2022). Larger studies using standardized, validated olfactometric methods with molecular determination for odor molecules are needed in the future to clarify the contribution of the microbiota and the therapeutic effect of BPO on foot odor in NPPK and other PPKs.

In addition to foot odor, individuals with NPPK exhibit redness, hyperkeratosis, and exfoliation of the hands and feet. Although dysbiosis amelioration reduces odor, redness and hyperkeratosis persist, indicating that dysbiosis may not be the primary contributing factor to the pathogenesis of redness and hyperkeratosis in NPPK. Further elucidation of NPPK pathogenesis is required to develop effective therapeutic interventions.

MATERIALS AND METHODS

Individuals included in the study

Overall, 32 individuals with a clinical diagnosis of NPPK and known pathogenic homozygous or compound heterozygous variants of *SERPINB7* were included (Figure 1 and Table 1). As controls, 14 family members of individuals with NPPK who had a heterozygous pathogenic variant but no symptoms of NPPK and 6 other healthy individuals without any skin diseases were recruited (Figure 1 and Table 1). The off-label use of BPO was approved by the Medical Safety Committee of Keio University Hospital (2022-077). This study was approved by the Ethics Committee of Keio University School of Medicine (20120226, 20130384, and 20170394) and the Ethics Committee of Kobe University Graduate School of Medicine (B230011) in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

Genetic testing

Genomic DNA was extracted from peripheral blood leukocytes using the Maxwell RSC Instrument and Maxwell RSC Blood DNA Kit (Promega, Madison, WI). Sanger sequencing was performed for all exons and exon–intron boundaries of *SERPINB7*. Variants are described according to HGVS nomenclature using NM_001040147.3 (*SERPINB7*) as the reference sequence.

Evaluation of clinical phenotypes

Because trained sniffer panels are not routinely available in Japan, objective foot odor was assessed by a single clinician using a 4-point scale adapted from Watanabe et al (2016), that is, 0 representing the absence of odor, 1 representing a faint odor (odor discernible when the clinician's face was within 10 cm of the foot), 2 representing a modest odor (odor present when the patient was sitting on the bed facing the doctor without their socks), and 3 representing a strong odor (odor apparent as soon as the patient removed their socks from the room).

The subjective perception of odor, erythema, and scaling of the hands and feet was evaluated using a 100-mm visual analog scale. Patients were instructed to assess the condition of their lesions after treatment with BPO gel. The absence of symptoms was rated 0, whereas the average lesion status before BPO treatment was 100.

Exclusion criteria

Healthy controls and individuals with NPPK who had taken oral antimicrobials within 1 week of the assessment, had applied topical steroids or antimicrobials to the microbiota sampling site within 1 week, had washed their feet within 6 hours, had washed their hands within 3 hours, or were unable to consent to the objective evaluation of odor were excluded from the study.

Collection of skin swab samples

Skin microbiota was obtained using the swabbing method, as previously described (Grice et al, 2009; Obata et al, 2023). Briefly, the skin was rubbed 50 times with a swab moistened with PBS, and the swab was immersed in 1 ml of PBS, which was stored at -20°C . The sampling sites are shown in Figure 2b. The sampling area was 2×1 cm for the intertoes and 2×2 cm for the other sites. Consequently, samples obtained from the feet of 31 individuals with NPPK and 20 healthy controls and from the hands of 23 individuals with NPPK and 15 healthy controls were analyzed.

The 16S rRNA gene sequencing

Swab samples were centrifuged at 3000g for 10 minutes at 4°C . The resulting pellets were incubated with lysis buffer (25 mM Tris-hydrogen chloride [pH 8.0], 2.5 mM ethylene diamine tetraacetic acid, 1% Triton X-100, 20 mg/ml lysozyme; Sigma-Aldrich, St. Louis, MO) for 30 minutes at 37°C . Bacterial DNA was extracted using a Maxwell RSC instrument with a Maxwell RSC blood DNA kit (Promega) according to the manufacturer's instructions.

The V1–V2 region of the 16S rRNA gene was amplified by PCR using the universal primers 27F (5'-CGATAGAGTTTGATCCTGGCTCAG-3') and 354R (5'-TGCTGCCTCCCGTAGGAGT-3') for 30 cycles with TaKaRa ExTaq (Takara Bio, Shiga, Japan). PCR products were purified using AMPure XP (Beckman Coulter, Brea, CA). The final concentration of the library was adjusted to 10 pM by adding 20% PhiX Control v3 (Illumina, San Diego, CA), followed by quantification using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Carlsbad, CA).

The 16S rRNA gene was sequenced using the MiSeq platform (Illumina) with MiSeq Reagent Kits v2 (500 cycles, Illumina), in accordance with the manufacturer's instructions. ASVs were inferred from Illumina-sequenced paired-end reads using the DADA2 package (Callahan et al, 2016) (version 1.30.0) in R (version 4.3.3) according to the official DADA2 pipeline (<https://benjjneb.github.io/dada2/tutorial.html>) with slight modifications. The reads were subjected to quality filtering and trimming using the filterAndTrim function with the following parameters: maxN = 0, rm.phix = TRUE, maxEE = c(2,2), and truncQ = 2. ASVs that had the highest similarity to eukaryotes and *Lysobacter* sequences and hit the bacterial genome database with <90% coverage were further filtered out. Taxonomic assignment of each ASV was made through searching by similarity against the Greengenes2 (McDonald et al, 2024) database using the GLSEARCH program (version 36.3.8e). Rarefaction was performed by randomly subsampling sequences to 10,000 reads per sample.

Quantitative estimation of bacterial load

The standard value for the copy number of the 16S rRNA gene was determined using the genomic DNA extracted from *E. coli* DH5 α by measuring the copy number of the amplicon amplified with the universal primers 27F and 1344R (5'-TTACTAGCGATTCCGACTT CATGGAGT-3') using the QuantStudio 3D Digital Real-Time PCR system (Thermo Fisher Scientific). The copy number of the 16S rRNA gene in each sample was estimated by qPCR, which amplified the V1–V2 region of the 16S rRNA of each sample and the standard. Universal primers 27F and 354R were used in conjunction with the PowerUp SYBR Green Master Mix kit (Thermo Fisher Scientific) in a StepOnePlus Real-Time PCR system (Thermo Fisher Scientific) to amplify the V1–V2 region. The estimated copy number of the bacteria was converted to /cm² for comparison. The estimated absolute abundance of each bacterial genus was calculated by multiplying the 16S rRNA copy number by the relative abundance obtained from the microbial composition analysis.

Bacterial growth with or without BPO

The standard strain of *S. epidermidis* (JCM 2414) and the type strain of *C. tuberculoearicum* (JCM 13389), obtained from the Japan Collection of Microorganisms (RIKEN BioResource Research Center, Ibaraki, Japan), were precultured in brain–heart infusion broth (Sigma-Aldrich) with 0.1% glycerol and 3% Tween 80 to reach the logarithmic phase. The bacteria were then prepared to achieve a final cell concentration of approximately 10^5 colony-forming unit/ml and subsequently cultured in the medium supplemented with BPO (Sigma-Aldrich) at concentrations ranging from 0 to 512 $\mu\text{g}/\text{ml}$ at 37°C . Bacterial growth was quantified by measuring optical density at 600 nm.

Quantification and statistical analysis

Statistical significance was calculated using GraphPad Prism software (GraphPad Software, Boston, MA) with the Mann–Whitney U test and the Wilcoxon matched-pairs signed-rank test. Statistical significance was set at $P < .05$.

ETHICS STATEMENT

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Keio University School of Medicine (approval numbers 20120226, 20130384, and 20170394) and the Ethics Committee of Kobe University Graduate School of Medicine (approval number: B230011). The off-label use of benzoyl peroxide was approved by the Medical Safety Committee of Keio University Hospital (approval number 2022-077). Written informed consent was obtained from all participants for study participation and for the publication of clinical images.

DATA AVAILABILITY STATEMENT

The meta 16S ribosomal RNA sequencing dataset is available in the DNA Data Bank of Japan with the accession number PRJDB35509: <https://ddbj.nig.ac.jp/search/entry/bioproject/PRJDB35509>.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: AK; Formal Analysis: YI, RK, WS; Funding Acquisition: YI, AK; Investigation: NO, YI, SA, AS, TS, RK, WS, AK; Methodology: NO, YI, SA, AS, TS, RK, WS, AK; Project Administration: AK; Supervision: MA, AK; Validation: NO, YI, AK; Visualization: NO, YI, SA, AS, TS, RK, WS, AK; Writing - Original Draft Preparation: NO, AK; Writing - Review and Editing: YI, MA, AK

SUPPLEMENTARY MATERIAL

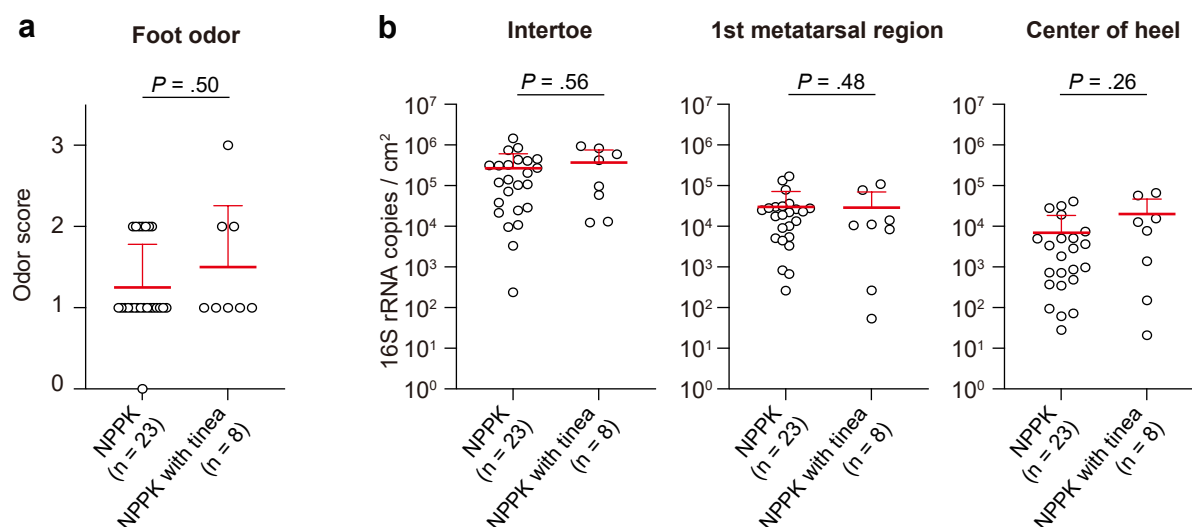
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at [10.1016/j.jid.2025.11.011](https://doi.org/10.1016/j.jid.2025.11.011).

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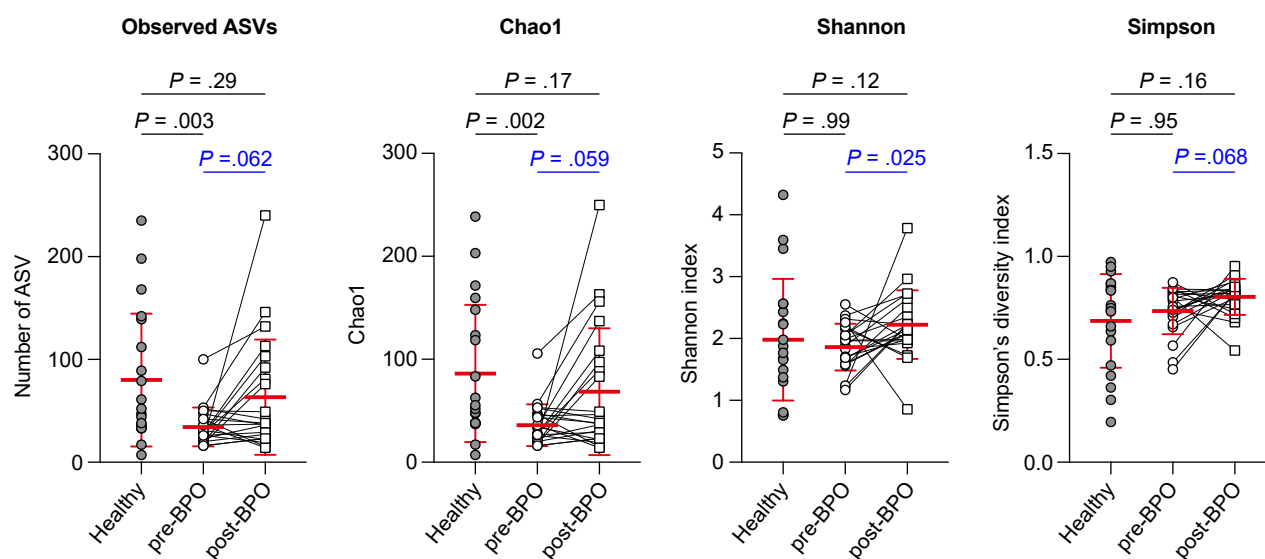
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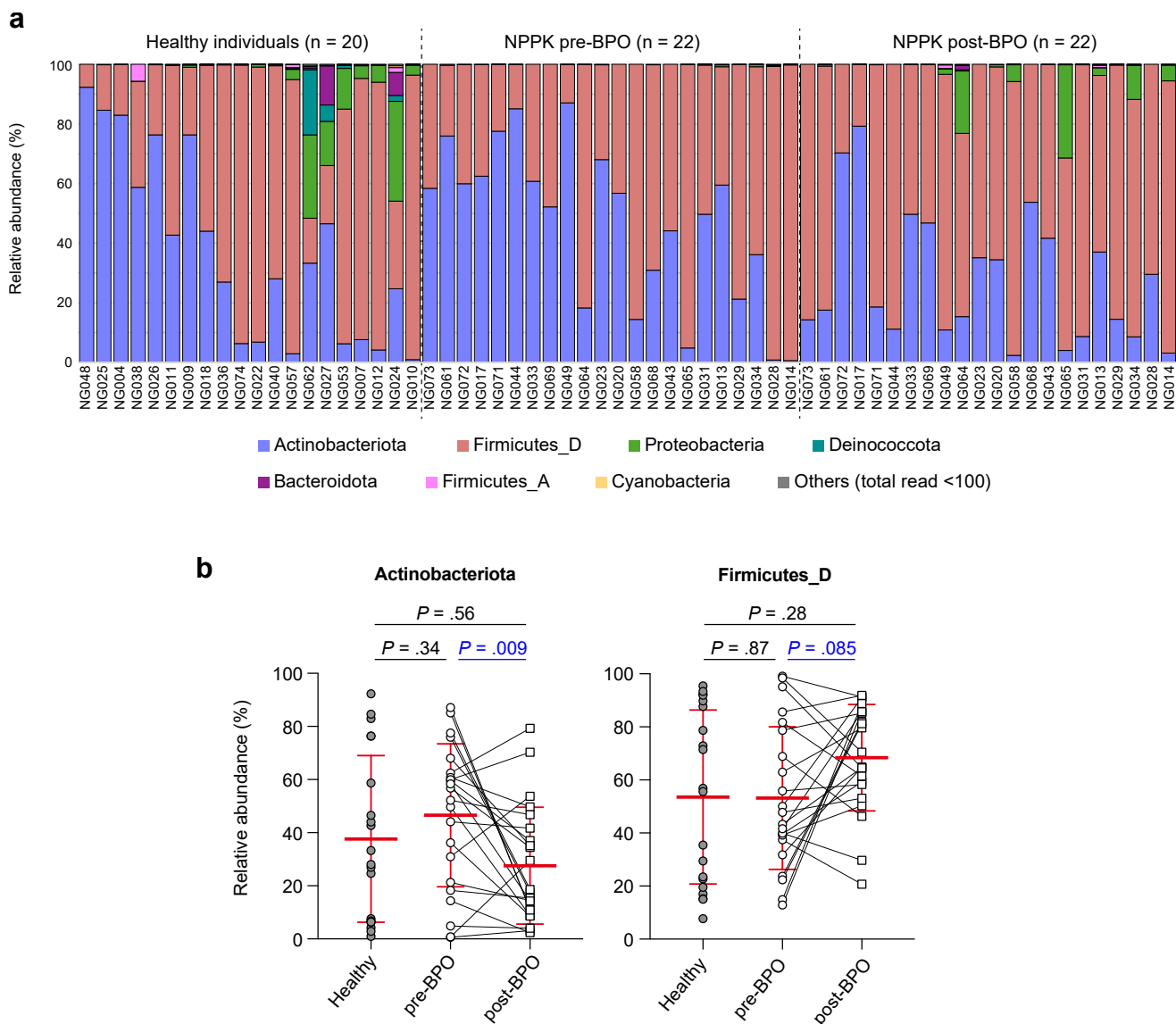
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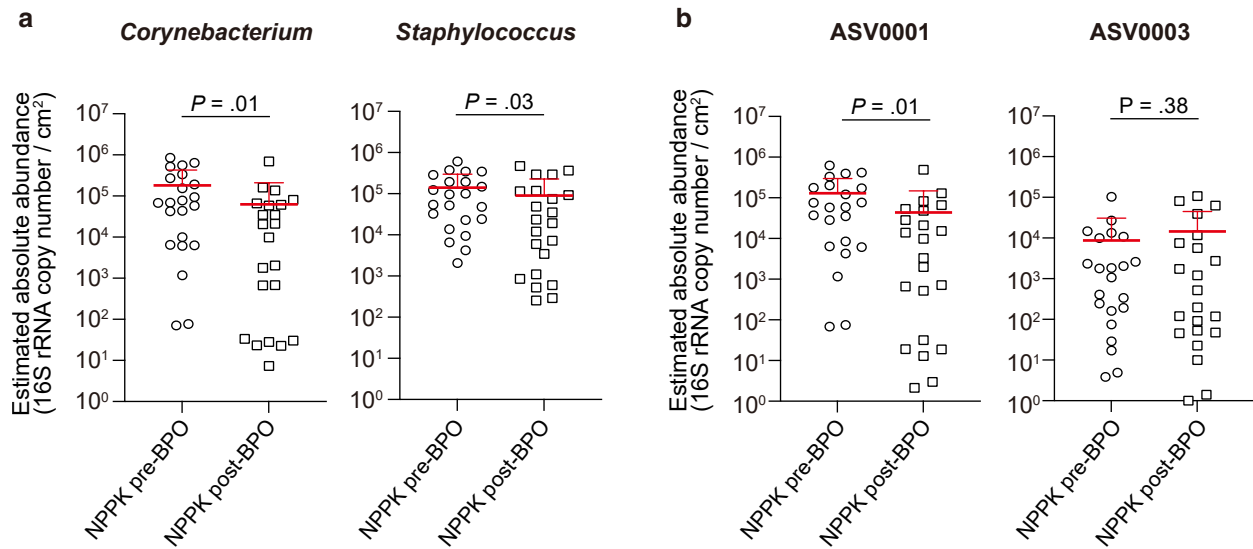
Supplementary Figure S1. Odor intensity and bacterial load on NPPK-affected foot skin with or without tinea infection. (a) Foot odor scores and (b) 16S rRNA copy numbers per cm^2 in swab samples collected from the foot skin of individuals with NPPK, with or without tinea infection. Data represent the mean $\pm$ SD. Statistical significance was determined using the Mann–Whitney U test. NPPK, Nagashima-type palmoplantar keratosis; rRNA, ribosomal RNA.



Supplementary Figure S2. Diversity of skin microbial flora in healthy controls and individuals with NPPK before and after BPO treatment. Alpha diversity, measured according to observed ASVs, the Chao1 index, Shannon's diversity index, and Simpson's index, is shown for healthy individuals (healthy) and individuals with NPPK before and after BPO treatments. ASV, amplicon sequence variant; BPO, benzoyl peroxide; NPPK, Nagashima-type palmoplantar keratosis.



Supplementary Figure S3. Microbial composition of NPPK-affected skin and the effect of BPO. (a) Relative abundance of skin microbiota at the phylum level on the intertoe skin of healthy individuals and individuals with NPPK before and after BPO treatment (before BPO and after BPO). (b) Relative abundance of Actinobacteriota and Firmicutes_D (phylum level) on the intertoe skin in each group. BPO, benzoyl peroxide; NPPK, Nagashima-type palmoplantar keratosis.



Supplementary Figure S4. Estimated bacterial load of major microbiota of individuals with NPPK before and after BPO treatment. (a, b) Estimated bacterial load on the intertoe skin at the (a) genus level and (b) ASV level. The bacterial load was calculated by multiplying the relative abundance of each taxon by the total 16S rRNA copy number obtained from each sample. ASV0001, *Corynebacterium tuberculostearicum*; ASV0003, *Staphylococcus epidermidis*. Data represent the mean $\pm$ SD. Statistical significance was determined using the Wilcoxon matched-pairs signed-rank test. ASV, amplicon sequence variant; BPO, benzoyl peroxide; NPPK, Nagashima-type palmoplantar keratosis; rRNA, ribosomal RNA.

Supplementary Table S1. 16S rRNA Copy Number in Skin Swab Samples from Healthy Controls

NG Number	16S rRNA (Copy Number/cm ²)						
	Intertoe	First Metatarsal Head	Center of the Heel	Dorsum of the Foot	Center of the Palm	Dorsum of the Hand	Inner Forearm
NG004	43,223	18	626	17	N.A.	N.A.	N.A.
NG007	240	330	774	39	N.A.	N.A.	N.A.
NG009	5727	103	44	44	N.A.	N.A.	N.A.
NG010	1626	61	150	134	138	60	92
NG011	19,019	2838	4477	87	266	132	97
NG012	549	66	109	69	122	67	100
NG018	8987	364	410	40	138	37	43
NG022	5178	44	148	41	N.A.	N.A.	N.A.
NG024	83	304	143	50	258	91	49
NG025	52,750	14,063	37,537	493	146	65	46
NG026	20,126	123	324	49	394	209	81
NG027	167	79	138	37	173	507	42
NG036	10,614	61	183	30	71	53	12
NG038	54,241	21,767	2260	115	189	95	63
NG040	823	102	332	12	53	17	11
NG048	54,990	211	226	45	161	84	24
NG053	1283	130	1253	33	254	94	49
NG057	19,931	1023	1586	910	817	495	816
NG062	495	499	1830	55	118	40	97
NG074	6263	795	904	142	N.A.	N.A.	N.A.

Abbreviations: N.A., not applicable; rRNA, ribosomal RNA.

N.A. is due to exclusion by the sample collection criteria.

Supplementary Table S2. The 16S rRNA Copy Number in Individuals with NPPK

Before BPO Application									After BPO Application							
16S rRNA (Copy Number/cm ²)									16S rRNA (Copy Number/cm ²)							
NG Number	Intertoe	First Metatarsal Head	Center of the Heel	Dorsum of the Foot	Center of the Palm	Dorsum of the Hand	Inner Forearm	Tinea Pedis	Intertoe	First Metatarsal Head	Center of the Heel	Dorsum of the Foot	Center of the Palm	Dorsum of the Hand	Inner Forearm	Tinea Pedis
NG008	21,476	27,778	28	24	N.A.	N.A.	N.A.	—	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	+
NG013	10,739	36,388	19,196	196	7610	183	205	—	1851	1142	18,561	162	87	1	70	—
NG014	24,560	24,747	373	34	N.A.	N.A.	N.A.	—	945	1593	718	19	N.A.	N.A.	N.A.	—
NG015	38,342	829	94	133	N.A.	N.A.	N.A.	—	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.F.
NG016	58,504	54	21	27	N.A.	N.A.	N.A.	—	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	—
NG017	829,157	77,873	56,932	273	644	147	55	+	92,326	2892	5937	465	150	26	44	+
NG020	120,333	5464	2878	42	¹	46	50	—	5361	1629	253	25	N.A.	N.A.	N.A.	—
NG021	237	262	61	28	67	21	45	—	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.F.
NG023	103,049	17,566	1833	80	544	55	18	—	459,614	2701	2424	16	293	44	10	—
NG028	9,635	3327	348	25	N.A.	N.A.	N.A.	—	33,238	774	141	12	N.A.	N.A.	N.A.	—
NG029	28,972	13,313	3654	6	26	8	24	—	14,060	2716	4900	56	N.A.	N.A.	N.A.	—
NG031	13,100	264	150	18	N.A.	N.A.	N.A.	+	7922	2745	75	18	461	¹	¹	—
NG033	314,356	29,944	28,192	21	N.A.	N.A.	N.A.	—	70,047	9967	19,556	59	N.A.	N.A.	N.A.	—
NG034	3,308	10,123	7467	6	357	4	6	—	324	7952	19,039	9	67	24	9	—
NG037	738,494	79,389	867	93	N.A.	N.A.	N.A.	—	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.F.
NG041	71,797	9084	486	3	N.A.	N.A.	N.A.	—	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	—
NG043	96,632	14,048	1383	28	3268	23	10	+	83,822	14,587	17,887	24	521	28	15	—
NG044	324,538	26,150	40,859	183	478	89	125	—	526,898	2680	19,144	58	297	63	208	—
NG046	432,755	5102	¹	276	612	20	28	—	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.F.
NG049	107,364	22,059	5076	29	173	21	62	—	342	2907	1132	21	46	52	3	—
NG056	272,780	22,961	713	2232	4470	49	71	—	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.F.
NG058	404,596	19,099	4949	81	276	196	51	—	7,365	351	382	6344	412	137	244	—
NG059	595,393	109,226	7776	163	1434	65	129	+	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.S.
NG061	846,063	4477	970	2291	9748	1956	172	N.S.	152,664	4905	6592	2273	423	116	116	N.S.
NG064	423,760	8351	12,716	57	1796	250	62	+	858	1335	841	9	N.A.	N.A.	N.A.	—
NG065	204,922	673	72	8	398	31	16	—	934	124	148	3	103	13	45	—
NG068	140,398	133,350	5015	38	79	27	32	—	255,313	29,883	542	34	68	43	7	N.S.
NG069	308,453	31,327	725	29	589	226	50	N.S.	141,385	46,811	3831	52	330	72	49	N.S.
NG071	450,370	28,081	31,722	122	224,577	247	125	—	113,860	3383	6238	30	455	24	44	—
NG072	933,251	10,576	66,170	1273	11,493	609	354	+	988,011	9635	46,687	1364	5810	3845	1175	+
NG073	1,456,272	169,715	3374	124	4176	41	102	N.S.	428,341	23,873	553	106	1051	237	18	N.S.

Abbreviations: BPO, benzoyl peroxide; N.A., not applicable; N.F., no follow-up; NPPK; Nagashima-type palmoplantar keratosis; N.S., no symptom; rRNA, ribosomal RNA.

N.A. is due to exclusion by the sample collection criteria, N.S. denotes no symptoms of tinea pedis, + denotes positive by microscopic examination, and — denotes negative by microscopic examination.

¹Undetermined.