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Effect of Tarcolimus on Skin Microbiome in Atopic Dermatitis

A Short running head: Tacrolimus and atopic dermatitis skin microbiome

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36

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38

Abstract

39 **Background:** Atopic dermatitis (AD) is a common allergic skin disease in which genetic and
40 environmental factors influence the development of skin barrier and immune system
41 dysfunction. Recently, evidence has emerged to support the notion that skin microbial flora can
42 modulate development and exacerbation of this disease. Our study is the first to characterise the
43 skin microbiome in Thai patients with atopic dermatitis before and after 4-week monotherapy
44 with tarcolimus.

45 **Methods:** Swab samples from skin lesions at volar forearm of 9 patients with atopic dermatitis
46 and normal skin samples of 12 healthy subjects were collected. The skin microbiome was
47 characterized using 16S ribosomal RNA gene sequencing.

48 **Results:** The diversity of skin microbes is significantly different between the control and AD
49 subjects. Lower prevalence of Actinobacteria and Gammaproteobacteria, but higher prevalence
50 of Firmicutes was observed in the AD group. A significant increase in *Staphylococcus* spp. but
51 decrease in several commensals such as *Coryebacterium* spp. and *Dermacoccus* spp. Was
52 detected in AD compared to healthy subjects. After treatment with tacrolimus, the skin
53 microbiota composition of AD individuals was comparable to the control group.

54 **Conclusion:**. Our unique study in Thai patients provides unequivocal proof of the positive
55 impact tacrolimus has on skin microbiome in AD.

56 Keywords: atopic dermatitis, skin microbiome, tacrolimus

57

58 1. **Introduction**

59 Atopic dermatitis (AD) is a common inflammatory skin disease, the prevalence of which
60 varies from 5-30% worldwide., and it is clear that incidence of AD has increased recently in
61 industrial countries[1,2]. The disease is chronic and often heralds other atopic diseases such as
62 asthma and allergic rhinitis.[3] Therefore, AD has become one of the most burdensome skin
63 diseases amongst people of all ages and ethnic backgrounds. The disease is characterised by a
64 dysfunctional skin barrier and associated immune response leading to chronic eczematous skin
65 eruptions. Both genetic and environmental inputs play roles in the development and
66 maintenance of the disease. In particular, several factors have been found to promote AD
67 including exposure to irritant substances, and recently the advance in metagenomics coupled
68 next generation sequencing has specifically identified dysbiosis of skin microbiome as being a
69 major factor. Excessive hyginene associated with urban lifestyle may lead to altered microbial
70 skin contact especially in early life, which results in dysbiosis and immune dysregulation in
71 AD.

72 Recent research has revealed the role of dysbiosis of the skin microbiome in
73 pathogenesis of AD. A reduction in antimicrobial peptides, defects of epidermal barrier and
74 dysregulation of the adaptive immune response results in a corresponding increase in skin
75 colonization by *Staphylococcus aureus*, which leads to a loss of skin bacterial diversity and
76 increases in specific IgE antibodies against bacterial toxins in the patients' serum.[4] Meta-
77 analysis reports estimated that pool prevalence of *S. aureus* colonization in AD skin lesion was
78 70 % and the prevalence of colonization correlated with disease severity.[5] Furthermore, *S.*
79 *aureus* has been reported to facilitate skin inflammation and barrier dysfunction via several
80 mechanisms.[6-9] Beside the conolization of *S. aureus* in AD skin lesions dysbiosis of the skin
81 microbiome via reduction of commensal microbes such as *Staphylococcus epidermidis*,
82 *Propionibacterium* spp. and *Corynebacterium* spp. has been evident in AD. In normal life, *S.*

83 *epidemidis* could inhibit rare colonization and biofilm formation by *S. aureus* and augment
84 human beta-defensin (HBD) expression by human keratinocyte via toll-like receptor 2 (TLR2)
85 signalling[10,11] Propionibacterium and Corynebacterium can diminish *S. aureus* infection via
86 porphyrin metabolism.[12]

87 Several therapeutic approaches exist for AD and these can act by specifically by
88 restoring the skin barrier, diminishing skin imflammation and reversing dysbiosis of skin
89 microbiome. Topical corticosteroids have been used alone or in combination with topical
90 antibiotics due to their cost-effectiveness. Nowadays, topical caucineurin inhibitors (TCIs)
91 have been recommened as a maintenance therapy as they are low risk of triggering adverse
92 events. To date, there is a paucity of information on the effect of TCIs on skin microbiome in
93 AD and our study seeks to address this. Our aim is to report the findings of a comprehensive
94 comparison of the healthy and AD skin microbiome following the introduction of tacrolimus.
95 We report for the first time that the anti-inflammatory effect of tacrolimus is sufficient to
96 restore the skin barrier leading to reversed dysbiosis of the skin microbiota in a Thai cohort
97 with AD

98

99

100 **2. Methods**

101 2.1 Patients and healthy controls

102 Nine patients diagnosed with atopic dermatitis according to Hanifin and Rajka criteria at
103 King Chulalongkorn Memorial Hospital (4 males, 5 females) and 12 normal subjects (4 male, 8
104 females) were enrolled in the study. The severity of AD was classified according to the Scoring
105 of Atopic Dermatitis (SCORAD), Eczema Area and Severity Index (EASI) and Investigators'
106 Global Assessment (IGA). Patients with other chronic inflammatory skin diseases were

107 excluded from the study. All patients were free from systemic skin therapies for at least 4
108 weeks, systemic antibiotics for at least 6 months or topical skin therapies and topical antiseptics
109 for at least 2 weeks prior to sample collection. Patients were allowed to use only mild liquid
110 soap and 10% urea cream for 2 weeks and avoid all washing 24 hours prior to sampling. The
111 study was approved by the ethical committee of the King Chulalongkorn University. All
112 participants provided informed consent. The demographic as well as the severity scales of AD
113 (before and after tarcolimus treatment) data are shown in Table 1. For abbreviation, 'D' denotes
114 disease, 'Bf' or 'Before' and 'Af' or 'After' denote AD-before and AD-after treatment, and the
115 number in the middle denotes individual patient in random order. Similarly, 'C' denotes control
116 followed by the number that denotes individual normal volunteer in random order.
117
118 2.2 DNA extraction
119 Samples were collected by rubbing the skin using a sterile cotton tipped applicators and
120 transferred into microcentrifuge with 200 µl of ST solution (0.15 MNaCl with 0.1% Tween
121 20).[13] Then, samples were centrifuged at 10,000g for 5 minutes, and supernatant was removed.
122 The sample pellet was kept at -80 °C. Total genomic DNA was extracted from the pellet by
123 GenElute bacterial genomic DNA kit (Sigma). Finally, genomic DNA was kept at -80°C.
124
125 2.3 16S rRNA gene library preparation and next generation sequencing
126 Universal prokaryote primers (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-
127 GGACTACHVGGGTWTCTAAT-3′) for 16S rRNA gene V3-V4 with the 5′ Illumina adapter
128 and 3′ Golay barcode sequences were used as previously discribed.[14] To prevent PCR stochastic
129 bias, the template quantity and quality was adequate, and a minimum of three independent PCR
130 reactions were performed per sample.[15] Paired-end sequencing, 2 × 150 was performed using

131 Illumina MiSeq platform (Illumina, San Diego, CA, USA) following the manufacturer's
132 protocols at Chulalongkorn Medical Research Center (Bangkok, Thailand).

133

134 2.4 Quality screening, taxon classifications and community comparison

135 All nucleic acid sequences in this study were deposited at the NCBI Sequence Read
136 Archive (SRA) database (accession number SRP155450). The raw sequences (FASTQ files)
137 were categorized individuals based on the 5′ barcode sequences. The sequences were processed
138 following mothur's MiSeq Standard Operating Procedures.[16] The pre-processing steps included
139 removal of (i) short read lengths of $\leq$ 100 nucleotides (excluding the primer and adaptor
140 sequences), (ii) long homopolymers of $\geq$ 8 nucleotides, (iii) ambiguous nucleotides and (iv)
141 chimera. Passing sequences were aligned to Greengenes[17] to remove contaminate sequences
142 such as mitochondria and chloroplast. The clean sequences were classified to the operational
143 taxonomic unit (OTU) using the Ribosomal Database Project (RDP) Classifier.[18] A minimum
144 bootstrap confidence score of 80 % was used as a cutoff for taxonomic assignment. Genus and
145 specie of OTU (GLOTU and SLOTU) were followed the phylotype-based methods.[19] Good's
146 coverage index to estimate the data coverage of a community, and the alpha diversity by
147 number of OTUs, Shannon and Chao bacterial community richness, were computed using
148 mothur.[16,20] Data normalization was performed to normalize the varying sequencing depth
149 among individuals.[16] The relative abundance of bacterial genera was visualized as Heatmap
150 using R statistics package. Venn diagram, and the beta diversity by Morisita-Horn community
151 dissimilarity index and non-metric multidimensional scaling (NMDS) based on Morisita-Horn
152 dissimilarity indices, along the analysis of molecular variance (AMOVA) and a homogeneity of
153 molecular variance (HOMOVA) statistics to determine significant differences between or
154 among the structures of the comparing communities (p-value < 0.05), were also computed
155 using mothur.[16] AMOVA determines whether the diversity is greater than their pooled

156 diversity, while HOMOVA determines whether the diversity in each is significant different. In
157 addition, differentially abundant genus detection by Metastats and the linear discriminant
158 analysis effect size (LEfSe) to find biomarkers between two or more groups from relative
159 abundances[21] were performed using mother.

160 2.6 Correlation analyses

161 Spearman correlation to evaluate the order and the directions of the species that drive
162 the microbiota structures, and Pearson correlation to evaluate the direction and p-value statistics
163 of the clinical data on the AD severity scales (Table 1: SCORAD, EASI and IGA) against the
164 microbiota, were performed using mothur.[16] The results were visualized by R package ggplot2
165 (https://cran.r-project.org/package=ggplot2).

166

167 **3. Results**

168 3.1 Skin microbiota in AD compared to healthy control

169 AD patients aged between 16-39 years and healthy subjects aged between 23-54 years,
170 participated in this study. The demographic data of the participants are summarized in Table 1.
171 All patients with AD reported significant improvement of all clinical scores (SCORAD, EASI
172 and IGA) after 4-week monotherapy with Tacrolimus.

173 The 16S rRNA gene sequencing yielded an average of 146,922 clean sequences for
174 OTU classification (Supplemental Table 1), and thus yielded high Good's coverage indices of
175 98.45-99.93% (avg. 99.26%) at genus level (Supplemental Table 2). The number of GLOTUs
176 vary from 6 to 162; hence, the diversity assessment within each microbiota was assessed
177 (OTUs, Chao and Shannon) and the the variance box plot analysis showed that the species
178 richness (Chao) and species richness and evenness (Shannon) were relatively high for the
179 healthy controls than the AD groups (especially the Bf group). Several samples in the Bf group

180 had poor diversity (low number of OTUs, Chao and Shannon) (Figure 1 and Supplemental
181 Table 2).

182 Taxonomic profiles demonstrated the diversity that might differ among the control and
183 the AD: phylum Actinobacteria (class Actinobacteria) was relatively high in control followed
184 by Proteobacteria (class Gammaproteobacteria), whereas phylum Firmicutes (class Bacilli) was
185 generally higher in the AD, in particular the Bf group (Figures 2A and 2B). In detail, the Af
186 group showed closer relative abundances of Actinobacteria by increasing from the matched Bf
187 subjects, the moderate abundances of Firmicutes from the matched Bf subjects where a few
188 were with minute and many with over high abundance, and likewise for the Proteobacteria. The
189 number of the overlapping OTUs between the control and Af group was thereby greater than
190 that between the control and Bf group (Figure 2C: Control-Bf overlapped 71.62%, Control-Af
191 overlapped 81.66%). In continuation, the NMDS was constructed to visualize the relative
192 dissimilarity among the microbiota structures, and the control and the disease groups were
193 discrete, although the D8 data were an exception showing close to C11, C12 and C7, in orderly.
194 When analysis without the D8 showed even more prominent the community structure
195 difference between the control and the disease groups with the AMOVA statistic of $p < 0.001$
196 (Figure 3A and B).

197

198 3.2 Effect of Tacrolimus on skin microbiome in AD

199 To determine the microbiota structural differences within the disease group, before and
200 after Tacrolimus treatment, AMOVA and HOMOVA statistical analyses among the three
201 groups (Control, Bf and Af) were computed and both demonstrated significant differences of
202 0.003 and 0.04, respectively. Additionally, the statistical difference between the Control-Bf
203 (AMOVA $p = 0.003$) was suggested greater than between the Control-Af (AMOVA $p = 0.15$).
204 This is supported by the NMDS illustration in Figures 3C and D, particularly in Figure 3D

205 where D8 data were exempted. Nevertheless, the *p*-value statistic between the Bf and Af groups
206 remained non-significant (AMOVA $p = 0.164$).

207 Metastats analysis highlighted species that were differentially statisticaly different
208 between the comparing groups. Consistently, compared to the control group, there were a fewer
209 number of species differences in the Af than the Bf groups (Supplemental Table 3).
210 Supplemental Table 3A describes the species whose presence or absence might be associated
211 with AD, Supplemental Table 3B describes the species that remained different even after the
212 treatment, and Supplemental Table 3C highlighted the species that might be associated with the
213 positive effect of Tacrolimus, for example the increases of *Dermacoccus*, *Pseudomonas*,
214 *Corynebacterium*, *Proteus*, *Micrococcus luteus*, and *Lactococcus* in AF group. This effect of
215 Tacrolimus caused the Bf community to become close to the Control.
216

217 3.3 Association of bacterial species and severity of AD

218 Spearman correlation analysis allowed determination of the associated direction of the
219 certain bacteria species to the microbiota structures representing control and disease groups,
220 given that the Af microbiota were found scatter around the middle between the Bf and the
221 Control (Figure 4). Many taxa (such as *Dermacoccus* and *Corynebacterium*) were associated
222 with the Control, and as well the Af since the communities of the Af, as displayed by the
223 positions of the green dots, are closer to the Control. For *S. epidermidis* and *Staphylococcus*
224 *lugdunensis*, both shared the directions for the majority of Af (5/7 samples equal 71.43%) and
225 half of the Bf (4/8 samples equal 50%). Moreover, the association with AD severity scales
226 were analyzed. The AD severity scales vectors were found scattered around the Bf and Af
227 groups, and no significant correlation could be depicted between the AD indicators and the Bf
228 groups (*p*-values of Scorad = 0.26, EASI = 0.59, IGA = 0.78). In parallel, sex and age factors
229 were considered. AMOVA analysis between the male and female microbiota reported no

230 statistical difference ($p > 0.05$).Pearson correlation analysis against age showed the vector
231 direction of the microbiota among control samples, however with insignificant p-value ($p >$
232 0.05) (Supplemental Figure 1).

233 As statistically differentially abundant species were observed, LEfSe analysis for
234 species biomarker was performed to identify the species that separate the control from the AD
235 (Figure 5: blue bar), and on ther other hand the species that signature the disease groups (Bf and
236 Af) (Figure 5: green and red bars). A total of 29 taxa were pointed as biomarkers for the control
237 for the AD, and included *Corynebacterium* with the highest LDA scores followed by
238 *Acitnomycetales* and *Micrococcaceae*. 3 taxa were pointed biomarkers for the Af, and
239 *Staphylococcus* has the highest LDA score.[22] 1 genus (Veillonella) was pinpointed for the Bf
240 biomarker, with minor LDA score.
241
242

243 4. Discussion

244 Skin microbes participate in innate defense of the sin by several mechanisms. Restricted
245 cutaneous microbial diversity and colonization of pathogenic bacteria are crucial biologic
246 characteristics that drive in atopic dermatitis. As expected, we found that the bacterial diversity
247 was relatively higher in the healthy controls than the AD groups and correlation analysis
248 determined the associated direction of the certain bacteria species to the microbiota structures
249 representing control and disease groups. Several previous reports from various countries
250 demonstrated decreased prevalence of Actinobacteria and Gammaproteobacteria as well as
251 increase colonization of *S. aureus* and *S. epidermidis* in AD and the involved site.[22] In addition,
252 allergy-defensive action of these commensals and allergy-provocation of *S. aureus* related to
253 AD has been observed.[23,24] Our data is unique in the fact that it highlights lower prevalence of
254 phylum Actinobacteria (class Actinobacteria) and Proteobacteria (class Gammaproteobacteria),

255 but higher prevalence of phylum Firmicutes (class Bacilli) in the AD group. Interestingly, this
256 study revealed significant increases in *Staphylococcus* spp. but decrease in several commensals
257 (such as *Coryebacterium* spp., *Dermacoccus* spp. and *Lactobacillus* spp) in the AD. This
258 finding is consistent with the recent metagenome analysis of skin microbiome in Singapore
259 (similar tropical status to Thailand), which demonstrated that *Dermacoccus* spp. are also
260 significantly diminished in patients with AD.[25] The similarity of findings in both studies
261 underpin the concept that dysbiosis of skin microbiome is one of important features of AD in
262 the Thai population. Nevertheless, we could not demonstrate any correlation among skin
263 microbiome and desease severity (either SCORAD, EASI and IGA) probably because of the
264 limited number of patients.

265 Several therapies for AD aim to reduce the bacterial load leading to attenuated
266 inflammation, restored skin barrier and reversed dysbiosis of skin microbiome. Tacrolimus, a
267 TCI, has been widely used as a effective and safe treatment in AD. To the best of our
268 knowledge, the effect of TCIs on skin microbiome has never before been reported. We
269 discovered that after treatment with tacrolimus, the skin microbiota structure of AD returned to
270 be comparable to control group. Furthermore, the fewer number of species differences in the Af
271 group than the Bf group when compared to control. This finding refected that tacrolimus could
272 reverse some dysbiosis in AD. Nonetheless, there are some remaining species that may still
273 persist to promote AD after treatment with tacrolimus. These species may require additional
274 treatment either to equilrerate those species to the relative abundances representing the control
275 subjects.

276 Tacrolimus can restore the skin barrier by several mechanisms. It acts as an
277 immunosuppressive agent by inhibiting the activation of T cellsn and suppressing scytokines
278 production by them. Additionally, tacrolimus has been report to alleviate pruritis by suppressing

279 sensory nerve activation.[26] Therefore, it is possible that the influence of tacrolimus in restitution
280 of the skin microbiome might be a consequence of its anti-inflammatory effect and potential to
281 restore the skin barrier.

282 To date, various methods have been used for skin microbiome analysis. Our study
283 analyzed skin microbiome in AD using 16S rRNA gene sequence. It should be note that the
284 power of species and genus classification is in part limited by the partial 16S rRNA gene
285 sequence. For future experiments of this nature, the unclassified and classified isolates of
286 interest might be full-length sequenced to confirm the species annotation.

287 In conclusion, this study for the first time characterises the skin microbiota in healthy
288 and patients with AD in Thailand (a tropical country). Several mechanisms of tacrolimus
289 efficacy in treatment of AD have been suggested. This study is the first original research study
290 to describe the effect of tacrolimus on the skin microbiome in AD, and it may further influence
291 the use of tacrolimus as a strategy toi alleviate AD in the future.

292

293
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305 ## References

306 **1.** Shaw TE, Currie GP, Koudelka CW, Simpson EL. Eczema prevalence in the United
307 States: data from the 2003 National Survey of Children's Health. *J Invest Dermatol*.
308 2011;131(1):67-73.
309 **2.** Odhiambo JA, Williams HC, Clayton TO, Robertson CF, Asher MI. Global variations in
310 prevalence of eczema symptoms in children from ISAAC Phase Three. *J Allergy Clin*
311 *Immunol*. 2009;124(6):1251-1258.e1223.
312 **3.** Boguniewicz M, Leung DYM. Recent insights into atopic dermatitis and implications
313 for management of infectious complications. *J Allergy Clin Immunol*. 2010;125(1):4-13.
314 **4.** Mark B, M. LDY. Atopic dermatitis: a disease of altered skin barrier and immune
315 dysregulation. *Immunol Rev*. 2011;242(1):233-246.
316 **5.** Totté JEE, Feltz WT, Hennekam M, Belkum A, Zuuren EJ, Pasmans SGMA.
317 Prevalence and odds of *Staphylococcus aureus* carriage in atopic dermatitis: a
318 systematic review and meta-analysis. *Br J Dermatol*. 2016;175(4):687-695.
319 **6.** Schlievert PM, Case LC, Strandberg KL, Abrams BB, Leung DYM. Superantigen
320 profile of *Staphylococcus aureus* isolates from patients with steroid-resistant atopic
321 dermatitis. *Clin Infect Dis*. 2008;46(10):1562-1567.
322 **7.** Travers JB. Toxic interaction between Th2 cytokines and *Staphylococcus aureus* in
323 atopic dermatitis. *J Invest Dermatol*. 2014;134(8):2069-2071.
324 **8.** Nakatsuji T, Chen TH, Two AM, Chun KA, Narala S, Geha RS, et al. *Staphylococcus*
325 *aureus* exploits epidermal barrier defects in atopic dermatitis to trigger cytokine
326 expression. *J Invest Dermatol*. 2016;136(11):2192-2200.
327 **9.** Laborel-Préneron E, Bianchi P, Boralevi F, Lehours P, Fraysse F, Morice-Picard F, et
328 al. Correction: effects of the *Staphylococcus aureus* and *Staphylococcus epidermidis*

329 secretomes isolated from the skin microbiota of atopic children on CD4+ T cell
330 activation. *PLoS One*. 2015;10(11):e0144323.

331 **10.** Pasparakis M, Haase I, Nestle FO. Mechanisms regulating skin immunity and
332 inflammation. *Nat Rev Immunol*. 2014;14:289.

333 **11.** Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada K, et al. *Staphylococcus*
334 *epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal
335 colonization. *Nature*. 2010;465:346.

336 **12.** Orenstein A, Klein D, Kopolovic J, Winkler E, Malik Z, Keller N, et al. The use of
337 porphyrins for eradication of *Staphylococcus aureus* in burn wound infections. *FEMS*
338 *Immunol Med Microbiol*. 1997;19(4):307-314.

339 **13.** Paulino LC, Tseng C-H, Strober BE, Blaser MJ. Molecular analysis of fungal
340 microbiota in samples from healthy human skin and psoriatic lesions. *J Clin Microbiol*.
341 2006;44(8):2933-2941.

342 **14.** Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-
343 high-throughput microbial community analysis on the Illumina HiSeq and MiSeq
344 platforms. *ISME J*. 2012;6(8):1621-1624.

345 **15.** Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, et al. Geroscience:
346 linking aging to chronic disease. *Cell*. 2014;159(4):709-713.

347 **16.** Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al.
348 Introducing mothur: open-source, platform-independent, community-supported software
349 for describing and comparing microbial communities. *Appl Environ Microbiol*.
350 2009;75(23):7537-7541.

351 **17.** McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, et al. An
352 improved Greengenes taxonomy with explicit ranks for ecological and evolutionary
353 analyses of bacteria and archaea. *ISME J*. 2012;6:610.

354 **18.** Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve bayesian classifier for rapid
355 assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ*
356 *Microbiol*. 2007;73(16):5261-5267.
357 **19.** Schloss PD, Handelsman J. Status of the microbial census. *Microbiol Mol Biol Rev*.
358 2004;68(4):686-691.
359 **20.** Claesson MJ, O'Sullivan O, Wang Q, Nikkilä J, Marchesi JR, Smidt H, et al.
360 Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring
361 microbial community structures in the human distal intestine. *PLoS One*.
362 2009;4(8):e6669.
363 **21.** Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic
364 biomarker discovery and explanation. *Genome Biol*. 2011;12(6):R60.
365 **22.** Bjerre RD, Bandier J, Skov L, Engstrand L, Johansen JD. The role of the skin
366 microbiome in atopic dermatitis: a systematic review. *Br J Dermatol*.
367 2017;177(5):1272-1278.
368 **23.** Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, et al. Temporal shifts in
369 the skin microbiome associated with disease flares and treatment in children with atopic
370 dermatitis. *Genome Res*. 2012.
371 **24.** Fyhrquist N, Ruokolainen L, Suomalainen A, Lehtimäki S, Veckman V, Vendelin J, et
372 al. Acinetobacter species in the skin microbiota protect against allergic sensitization and
373 inflammation. *J Allergy Clin Immunol*. 2014;134(6):1301-1309.e11.
374 **25.** Chng KR, Tay ASL, Li C, Ng AHQ, Wang J, Suri BK, et al. Whole metagenome
375 profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare.
376 *Nat Microbiol* 2016;1:16106.

377 **26.** Takeshi N, Hiroshi M, Naofumi M, Masutaka F. Mechanistic insights into topical
378 tacrolimus for the treatment of atopic dermatitis. *Pediatr Allergy Immunol*
379 2018;29(3):233-238.

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