






Therapeutic effects elicited by the probiotic *Lacticaseibacillus rhamnosus* GG in children with atopic dermatitis. The results of the ProPAD trial

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Abstract

Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease affecting up to 20% of the pediatric population associated with alteration of skin and gut microbiome. Probiotics have been proposed for AD treatment. The ProPAD study aimed to investigate the therapeutic effects of the probiotic *Lacticaseibacillus rhamnosus* GG (LGG) in children with AD.

Methods: In total, 100 AD patients aged 6–36 months were enrolled in a randomized, double-blind, controlled trial to receive placebo (Group A) or LGG (1×10^{10} CFU/daily) (Group B) for 12 weeks. The primary outcome was the evaluation of the efficacy of LGG supplementation on AD severity comparing the Scoring Atopic Dermatitis (SCORAD) index at baseline (T0) and at 12-week (T12). A reduction of ≥ 8.7 points on the SCORAD index was considered as minimum clinically important difference (MCID). The secondary outcomes were the SCORAD index evaluation at 4-week (T16) after the end of LGG treatment, number of days without rescue medications, changes in Infant Dermatitis Quality Of Life questionnaire (IDQOL), gut microbiome structure and function, and skin microbiome structure.

Results: The rate of subjects achieving MCID at T12 and at T16 was higher in Group B ($p < .05$), and remained higher at T16 ($p < .05$). The number of days without rescue

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medications was higher in Group B. IDQOL improved at T12 in the Group B ($p < .05$). A beneficial modulation of gut and skin microbiome was observed only in Group B patients.

Conclusions: The probiotic LGG could be useful as adjunctive therapy in pediatric AD. The beneficial effects on disease severity and quality of life paralleled with a beneficial modulation of gut and skin microbiome.

KEYWORDS

butyrate, gut microbiome, infant dermatitis quality of life questionnaire (IDQOL), scoring atopic dermatitis (SCORAD) index, skin microbiome

1 | INTRODUCTION

Atopic dermatitis (AD) is a significant worldwide health problem in early life, affecting up to 20% of the pediatric population.¹ It is a multifactorial, chronic inflammatory skin disorder deriving from defective skin barrier function.² Emerging evidence suggest the potential role of alteration in gut and skin microbiome in AD.³

Specific probiotic strains could modulate inflammatory response counteracting microbiome dysbiosis and immune dysfunction in AD.^{4,5} Indeed, above and beyond balancing the gut microecology and regulating immune response, specific probiotics might further aid in controlling the microbial colonization of the skin.^{6,7} Despite these premises, the probiotics role as part of therapeutic interventions for pediatric AD is still questioned, with major limitations deriving from different study designs and populations, strains and doses used, and treatment duration.⁸ *Lactocaseibacillus rhamnosus* GG (LGG) is one of the most investigated probiotic strains in the pediatric allergy field,^{9,10} with a wide range of beneficial actions on microbiome structure, immune system, and epithelial cells.¹¹ Previous studies suggested a potential role for LGG in treating pediatric AD.¹²⁻¹⁴ The Probiotic for Pediatric Atopic Dermatitis (ProPAD) trial was designed to evaluate the efficacy of LGG in children affected by AD.

2 | METHODS

2.1 | Study design

The ProPAD study was a randomized, double-blind, placebo-controlled trial aimed to evaluate the efficacy of a 12-week treatment with the probiotic LGG in AD children aged 6–36 months. The therapeutic efficacy was also assessed at 4 weeks after the end of the treatment (Figure 1).

2.2 | Study outcomes

The primary study outcome was the evaluation of the therapeutic efficacy of LGG supplementation on AD severity comparing SCORAD index at baseline (T0) and at 12-week (T12).

Key Message

Gut and skin microbiome alterations have been detected in children with atopic dermatitis (AD). Specific probiotic strains could modulate disease severity counteracting gut dysbiosis and immune dysfunction in AD, and they have been suggested as therapeutic strategy in AD children. *Lactocaseibacillus rhamnosus* GG (LGG) could be useful as adjunctive therapy in pediatric AD. The beneficial effects on disease severity and quality of life paralleled with a beneficial modulation of gut and skin microbiome, mediated at least in part by the increase of the well-known immunomodulatory microbiome-derived metabolite butyrate.

The secondary outcomes were the evaluation of SCORAD index at 4-week after the end of the LGG treatment (T16), number of days without rescue medications, changes in Infant Dermatitis Quality Of Life questionnaire (IDQOL), specific serum IgE level to food and environmental allergens, gut microbiome structure and function (butyrate production), skin microbiome structure, and occurrence of common infectious diseases. The safety of the LGG supplementation was also evaluated.

2.3 | Study subjects

All patients aged 6–36 months, both sexes, consecutively observed at a tertiary Center for Pediatric Allergy because a diagnosis of AD, were evaluated for the study. Only subjects who met the inclusion criteria were invited to participate in the trial. The exclusion criteria were age < 6 months or > 36 months; concomitant presence of acute or chronic infectious diseases, autoimmune diseases, immunodeficiencies, chronic systemic, pulmonary, gastrointestinal and/or cardiac diseases, malignancies, genetic and metabolic diseases, ichthyosis, cardiovascular, respiratory or gastrointestinal malformations; administration of pre-/pro-/synbiotics, systemic immunomodulators, corticosteroids or calcineurin antagonists, phototherapy, antibiotics or anti-mycotic drugs

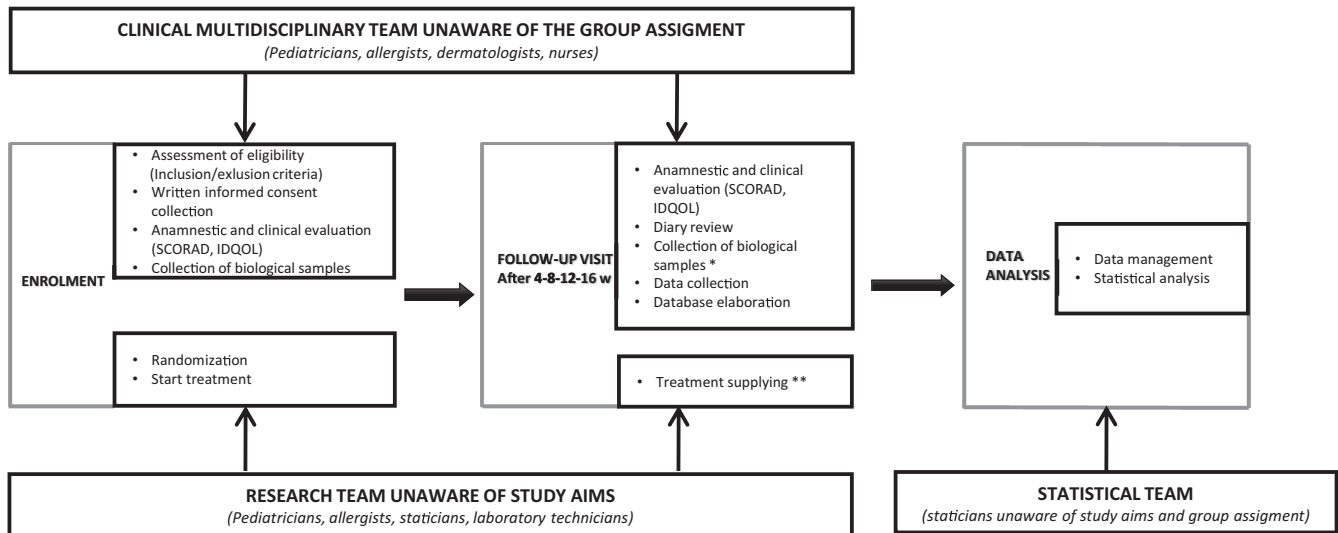


FIGURE 1 The design of PROPAD Trial. * At T12. ** At T4 and T8

during the 4 weeks before enrolment; treatment with topical immunomodulators (Tacrolimus or Pimecrolimus) over the 3 months prior to enrolment; investigator's uncertainty about the willingness or ability of the subject to comply with the protocol requirements; participation in any other studies involving investigational or marketed products concomitantly or within 2 weeks prior to entry into the study; hypersensitivity to components contained in study product. In addition, we also excluded patients with concomitant presence of food allergies and other allergic diseases because the possible use of other therapeutic strategies that could influence the AD severity, such as exclusion diet, steroids, immunomodulators, and biologics during the trial.

2.4 | Study procedures

At the baseline (T0), the diagnosis of AD was confirmed in all study subjects according to validated criteria^{15,16} by a clinical multidisciplinary team composed by pediatricians, allergists, and dermatologists, and written informed consent was obtained from the parents/tutors of each subject. The team assessed the anamnestic, demographic, anthropometric and clinical features of the patients, including information on socio-demographic factors, living conditions, parental history of allergic diseases, smoking exposure, number of siblings, and pet ownership. The severity of AD was assessed using the Scoring Atopic Dermatitis (SCORAD) index. The score, ranging from 0 to 103, defines three classes of AD severity (mild: <25, moderate: 25–50, and severe: >50).¹⁷ All anamnestic and clinical data were recorded in a dedicated clinical chart. At the same visit, a peripheral venous blood sample (4 ml), 2 stool samples (≥ 3 g/each) and 2 skin swab samples (1 from affected and 1 from unaffected skin area) were collected from each study subjects. The samples were immediately processed and stored at -80°C . Written information regarding the common therapeutic measures for pediatric AD, according

to the actual guidelines,^{15,16} were provided to the parents/tutors of all study subjects. In the event of AD deterioration, parents were allowed to use topical hydrocortisone butyrate 0.1% ointment as a rescue medication, according to the international guidelines.^{15,16}

2.4.1 | Randomization and intervention

The randomization performed by the research team was based on a list of consecutive numbers with an allocation ratio of 1:1 to one of two groups of 12-week intervention:

- Group A, received placebo capsule once a day.
- Group B, received isocolar and isosmell capsule containing 1×10^{10} CFU LGG once a day.

Each treatment was numbered according to the randomization scheme without any reference to the group assignment, which was known only to the statistician who generated the list and to the laboratory technician who prepared the packages. The packages and content of treatments were indistinguishable.

The parents received an anonymous paper box containing 30 capsules of placebo or LGG and were instructed to store the study products at room temperature by the research team. Parents were instructed about the daily amount of the assigned study product, to maintain the habitual child diet, and to avoid other probiotics, prebiotics, symbiotics, during the entire study period (from T0 to T16 weeks). For patients who could not swallow capsules, parents were instructed to mix the powder in water, milk, or foods.

At the end of the baseline visit, the parents were instructed to complete the IDQOL assessing the dermatitis severity and life quality index in the previous week, based on parents perception.^{18,19} In addition, parents received a diary with instructions to report the daily consumption of the study products, the use of emollients and

drugs for AD, and occurrence of the adverse events. The parents were instructed to contact the Center in the presence of respiratory and/or gastrointestinal symptoms and were invited to a monthly visit to assess the AD course.

The diagnosis of common infectious diseases was assessed as previously reported.²⁰ All clinical data were recorded in the dedicated clinical chart.

2.4.2 | Evaluation of the study outcomes

For each study subject, a monthly clinical examination was planned for the following 4 months after enrolment. At each visit the patients were assessed by the clinical multidisciplinary team unaware of the group assignment. Unscheduled visits were made as required. During each visit, a full clinical evaluation was performed, SCORAD index was assessed, diaries were checked, the empty study product boxes were collected to measure the subjects' compliance, and the study products were provided to the parents for the next 4 weeks until T12. At each visit, the IDQOL regarding the previous week was completed by the parents.^{18,19}

Compliance was defined as the consumption of $\geq 80\%$ of the assigned treatment during the study and was evaluated by counting the returned capsules and by reviewing the notes on the diary recorded by parents.

A new peripheral venous blood sample (4 ml), 2 stool samples (≥ 3 g/each) and 2 skin swab samples (1 from affected and 1 from unaffected skin area) were collected from each study subject at T12. All samples were immediately processed and stored at -80°C (Figure 1).

2.5 | Samples size calculation

A reduction of ≥ 8.7 points on the SCORAD index has been suggested as a clinically important difference (MCID) change for AD patients.²¹ We estimated that at least 20% of subjects in the placebo group and at least 50% in the active group could achieve this clinical result at the end of treatment. This estimation was based on the results of previous pilot trial performed at our Center. Thus at least 45 children per group were needed with a power of 0.85 at an alpha level of 0.05. Assuming a possible drop out up to 10%, the total number of enrolled subjects was planned as 50 per group.

2.6 | Data management and analysis

All data were recorded in the clinical chart by the multidisciplinary clinical team. An independent clinical trial monitor, blinded to the treatment assignment, was involved in the research. Study monitoring included on-site visits, to ensure that the investigation was conducted according to the protocol. The clinical trial monitor collected clinical charts, ensured compliance with the clinical trial

protocol, reviewed the clinical charts for completeness, clarity, and consistency, and communicated with the clinical research coordinators before the final analysis. Using a single data-entry method, all data recorded in the clinical charts were entered anonymously in the study database. Then, a statistical team unaware of study aims and treatment allocation, reviewed the study database and performed data cleaning and verification according to standard procedures. Finally, a biostatistician blinded to the treatment allocation and unaware of the study aims performed the statistical analysis using SPSS 27.0 (IBM Corporation) and Stata 16 (Stata Corporation).

2.7 | Statistical analysis

Descriptive statistics are reported as means and standard deviations or as medians and interquartile ranges (IQR) for continuous variables and as numbers and proportions for dichotomous variables.

The primary outcome, that was the rate of subjects achieving MCID for SCORAD index after 12-week treatment, was evaluated using a binomial regression model. We performed an intention-to-treat analysis (ITT) of the primary outcome by considering the children lost after randomization as missing values of the primary outcome set to the worst outcome in both Groups. The worst outcome was defined as a < 8.7 SCORAD index reduction after 12-week treatment. The primary outcome was also assessed using per-protocol analysis. The secondary outcomes were evaluated using per-protocol analysis.

The level of significance for all statistical tests was two-sided, $p < .05$.

2.8 | Adverse events

Adverse events were recorded throughout the study period. They were assessed based on inquiries to the parents and on daily records. An adverse event was defined as any event that was not consistent with the information provided in the consent form, or that could not reasonably be expected to accompany the natural history and progression of the subject's condition throughout the study. All adverse events were evaluated by the clinical multidisciplinary team for causal relationship to the study feeding and for severity. Adverse events were considered serious if they were fatal or life-threatening, required hospitalization or surgical intervention, resulted in persistent or significant disability/incapacity, or were considered medically relevant by the multidisciplinary team. All other adverse events were categorized as non-serious.

2.9 | Ethics

The study design was approved by the Ethics Committee of our Institution (Protocol n.184/19). The trial was registered on [ClinicalTrials.gov](https://www.clinicaltrials.gov) (NCT03863418) and was conducted in accordance with the Helsinki

Declaration (Fortaleza revision, 2013), the Good Clinical Practice Standards (CPMP/ICH/135/95), the Italian Decree-Law 211/2003 regarding personal data, and the European regulations on this subject.

2.10 | Laboratory procedures

All procedures were made by expert biologists unaware of the group assignment.

2.10.1 | Specific IgE serum levels

Four ml of peripheral venous blood were collected at T0 and at T12. Serum was collected using microcontainer serum separator tubes and was obtained by centrifugation for 15 min and then was flash frozen and stored at -80°C until analysis. Specific IgE serum levels to main food (milk, egg white, egg yolk, rice, wheat, cod, peanuts) and environmental allergens (*Dermatophagoides farinae*, *Dermatophagoides pteronissynus*, *Parietaria Judaica*, *Olea europea*, *Aspergillus*, *Lolium*) were analyzed with enzymatic immunoassay (ImmunoCAP ISAC multiplex system, ThermoFisher Scientific). Data were expressed as kilounits per liter (kU/L) and were considered as positive if level ≥ 0.35 .

2.10.2 | Gut and skin microbiome analyses

Two stool samples (≥ 3 g/each) were collected and stored at -80°C until analyses according to the Standard Operating Procedures (SOP 04) of the International Human Microbiome Standard Consortium, at the enrolment (T0) and at the end of 12-week treatment (T12). Fecal samples were brought frozen to the laboratory and stored at -80°C within 24 h.

Total genomic DNA (gDNA) was isolated from fecal samples following the Standard Operating Procedure 07 by the International Human Microbiome Standard Consortium (IHMS SOP P7 V2), and gut microbiome composition was evaluated by amplicon sequencing of the hypervariable regions V3-V4 of the 16S rRNA gene, as previously described.²²

Fecal butyrate concentration was measured as previously described by our group.²²

Skin microbiome was sampled using aseptic techniques under sterile airflow generated by a portable hood according to the Human Microbiome Project procedures,²³ at the T0 and at T12. Briefly, single use sterile cotton-tipped swabs (COPAN Ref.165KS01) pre-moistened with a sterile solution of deionized water containing 0.15M NaCl and 0.1% Tween 20 were used. Swabs were rubbed firmly for 20 s over 1 cm² area identified as being the most representative of affected skin. Similarly, samples were also collected from the closest unaffected skin area. The cotton tip samples were immediately stored at -80°C until analysis.

Genomic DNA was extracted from each swab using the Qiagen PowerSoil DNA isolation kit following the manufacturer's

instructions.²⁴ Amplification of the V1-V3 regions of the 16S rRNA gene was carried out using primers 27f 5'-AGAGTTTGATCCTGGCTCAG and 534r 5'-ATTACCGCGGCTGCTGG, considered as the best choice for skin microbiome.²⁵ PCR conditions used were as follows: an initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, 50°C for 1 min, 72°C for 1.30 min; a final elongation at 72°C for 10 min. Illumina sequencing was carried out on a MiSeq instrument, leading to 2 x 300 bp reads.

Raw sequences from fecal and skin samples were imported into QIIME2²⁶ and were evaluated as previously described.²²

3 | RESULTS

From September 2019 to December 2020, a total of 112 subjects were evaluated for eligibility. Twelve subjects were excluded because the presence of at least one exclusion criteria. A total of 100 subjects were enrolled and randomly allocated to either Group A or to Group B. Nine subjects were lost during the follow-up: 5 in group A and 4 in group B. Therefore, 91 patients, 45 in Group A and 46 in Group B, completed the study without any protocol violations and with a consumption of at least 80% of the assigned treatment (Figure 2). Treatment with LGG or placebo was well tolerated by all subjects. No adverse events were reported during the study period. Baseline anamnestic, demographic, and clinical features of the two study populations were similar (Table 1).

3.1 | Results of the primary study outcome

At baseline the SCORAD index was similar in the two study groups. The rate of subjects achieving MCID at T12 was significantly higher in Group B (Figure 3). Also, under per protocol analysis the results remained significant: 0.24 (95% CI: 0.11 to 0.37) for Group A vs. 0.63 for Group B (95% CI: 0.48 to 0.77; $p < .05$). To evaluate the effect of potential confounders on the main outcome, we added each of them separately to a binomial regression model using the presence of a SCORAD decrease of ≥ 8.7 at T12 as outcome and evaluated the changes in the estimated absolute risk change. The evaluated potential confounders were sex, age, caesarean delivery, breastfed for at least 2 months, exposed to passive smoking, mother smoked during pregnancy, exposed to pets, familial risk of allergy, parental risk of AD and urban setting. Although being breastfed was associated with the outcome (absolute risk reduction = 0.31, 95% CI: 0.17–0.45; $p < .001$), it left virtually unchanged the effect of the treatment on the outcome (absolute risk reduction of the treatment changing from 0.39 (0.20 to 0.57) to 0.40 (0.23 to 0.57) after inclusion of being breastfed as predictor).

3.2 | Results of the secondary outcomes

Intervention resulted in a significant improvement in SCORAD index in both groups starting from T4. However, a faster and higher

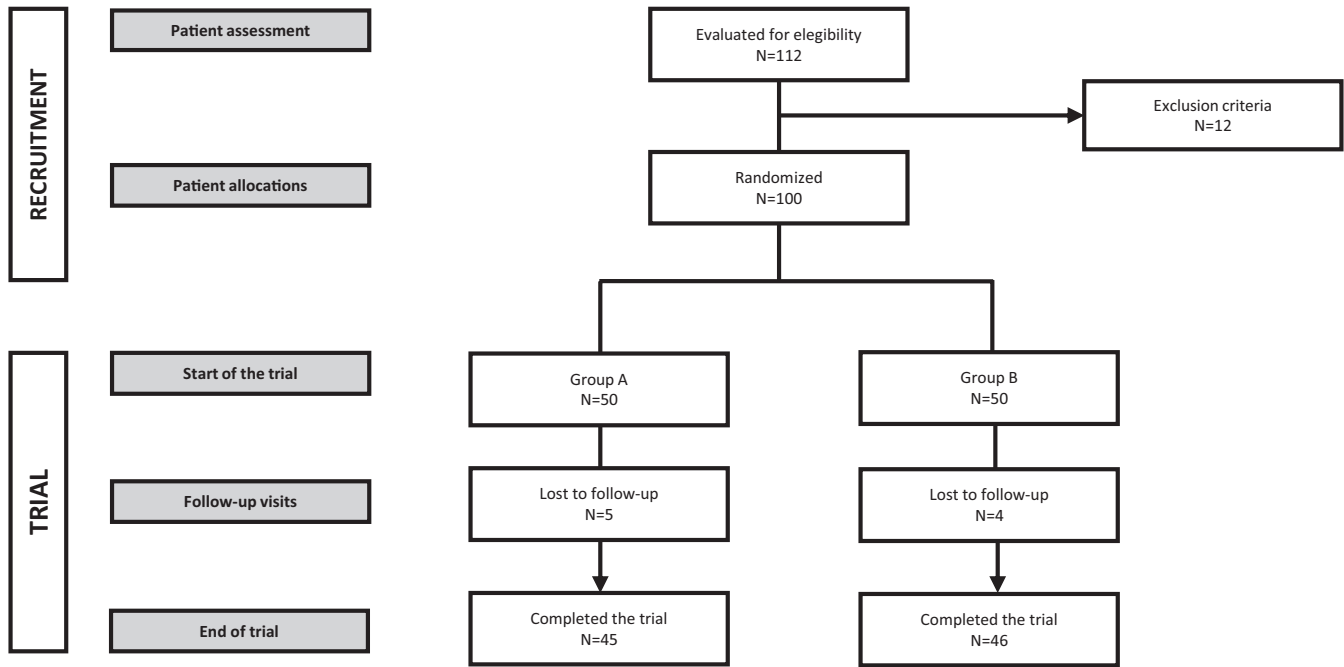


FIGURE 2 The flow of patients evaluated for the PROPAD Trial

SCORAD index decrease was observed in Group B throughout the study period (Figure 4, Panel A). The higher rate of subjects achieving MCID remained stable in Group B also at T16 (63.0% vs. 37.8%, $p < .05$). Also considering the AD severity (i.e., mild, moderate, and severe) the rate of subjects showing an improvement of SCORAD index was greater in Group B (Figure 4, Panel B).

The mean number of days without rescue medications during the study period was 98.7 in Group A and 101.8 in Group B, with a significant difference comparing Group A and Group B between T8 and T12 (24.8 vs. 26.6) and T12 and T16 (24.8 vs. 26.4; $p < .05$). A regular daily use of the emollients was observed in all study subjects without differences compared the two study groups.

The total number of common infections during the study period was similar between the two study groups (24 in Group A and 21 in Group B). At T12, there was an improvement in the quality-of-life score in both groups. The median value of IDQOL (IQR) resulted significantly lower in Group B compared with Group A [3 (6) vs. 2 (5) $p < .05$]. This result was sustained also at T16 [2 (5.5) in Group A vs. 1 (3) in Group B, $p < .05$].

All study subjects at baseline and then at T12 showed normal sIgE serum levels (≤ 0.35 kU/L) against most common food and inhalant allergens.

3.3 | Gut microbiome structure and function

No difference in the overall gut microbiome structure between the two groups was observed at baseline and at the end of the intervention, as shown by ADONIS based on Bray Curtis distance matrix. In addition, the alpha-diversity indices did not change

according to the treatment. However, at T12 we observed a significant increase of *Akkermansia*, *Ruminococcus* and a decrease of the families *Porphyromonadaceae*, *Enterobacteriaceae* and *Haemophilus* in the LGG group compared with placebo group ($p < .05$). The increase of these bacterial species paralleled with an increase in fecal butyrate concentration at 12-week only in subjects treated with LGG (Figure 5, Panel A). Moreover, we found evidence on a possible association between butyrate fecal levels and AD outcome. Comparing “responders” (patients achieving the minimum clinically important SCORAD index reduction of ≥ 8.7 units) and “non responders” (patients did not reach the outcome) we observed a significant higher increase in fecal butyrate concentration (mM) from T0 and T12 in “responders” patients: difference in change (mean \pm SD) of 2.1 ± 2.4 in “responders” versus 0.25 ± 2.1 in “non responders”, $p < .05$.

3.4 | Skin microbiome structure

No differences in the skin microbiome structure were observed at baseline between the two groups, as shown by PCoA based on Bray Curtis distance matrices and ADONIS test. However, we observed a significant difference between the two sampled skin area (affected and unaffected; ADONIS $p < .05$) at baseline. At T12, skin microbial community changed only in subjects receiving LGG and resulted different if compared with the placebo group (ADONIS $p < .05$). Interestingly, the skin microbiome composition of affected and unaffected skin area became more similar at the end of the treatment in the LGG group (ADONIS $p = .056$). Comparing the skin microbiome composition at genus level in the two groups at

TABLE 1 Main features of the study population at baseline

	Group A N = 50	Group B N = 50
Male	33 (66%)	31 (62%)
Age	16.4 (7.4)	18.9 (8.6)
Spontaneous delivery	21 (42%)	21 (42%)
Born at term	46 (92%)	44 (88%)
Weight at birth, kg (SD)	3.21 (0.5)	3.17 (0.5)
Breastfed for at least 2 months	36 (72%)	35 (70%)
Weaning age, months (SD)	5 (0.9)	4.9 (0.9)
Siblings, n (IQR)	1 (1)	1 (1)
Exposure to passive smoking	23 (46%)	24 (48%)
Mother smoked during pregnancy	7 (14%)	6 (12%)
Exposure to pets	9 (18%)	11 (22%)
Parental schooling > 10 years	46 (92%)	46 (92%)
Urban setting	36 (72%)	37 (74%)
Familial risk of allergy	37 (74%)	38 (76%)
Parental risk of atopic dermatitis	8 (16%)	8 (16%)
SCORAD index severity		
Mild	21 (42%)	19 (38%)
Moderate	26 (52%)	27 (54%)
Severe	3 (6%)	4 (8%)
SCORAD index, (SD)	29.8 (12.1)	30.8 (12.8)

Note: Discrete variables are reported as the number and proportion of subjects with the characteristic of interest. Continuous variables are reported as means and standard deviation or as median and interquartile range.

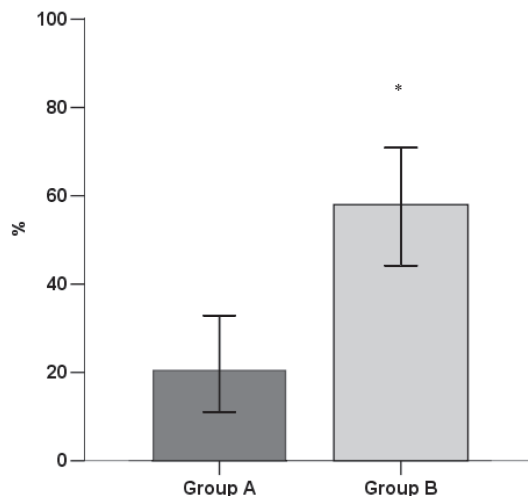


FIGURE 3 The result of the main study outcome: the rate of children with atopic dermatitis achieving the minimum clinically important difference of ≥ 8.7 units for the SCORAD index. Placebo = Group A; LGG = Group B. Error bars: 95.00% CI. * Group A vs. Group B, $p < .05$.

T12, higher levels of *Prevotella*, *Veillonella* and *Ralstonia* and lower of *Stenotrophomonas* and *Microbacterium* were found in the LGG group (Figure 5, Panel B).

4 | DISCUSSION

Probiotics have been proposed for preventing and treating AD.²⁷ Despite the increasing number of clinical trials reporting strain-specific effects, the results are still controversial, and to date there is no strong evidence supporting their effectiveness in clinical practice.^{15,16}

We explored the therapeutic effect of LGG as adjuvant treatment in children with AD. We found that a 12-week LGG daily supplementation was effective in reducing the SCORAD index, topical steroids use, and in improving the quality of life in pediatric AD patients. The rate of AD patients achieving MCID at T12, was higher in the LGG group, confirming previous data on the same probiotic strain.¹⁴ The beneficial effect on AD severity was sustained also at T16, suggesting a persistent modulation of AD in subjects treated with LGG. We observed that LGG was also able to reduce the topical steroid use. Also in this case, the beneficial effect was sustained at T16.

Other studies exploring the therapeutic efficacy of LGG in pediatric AD were unable to provide positive results. Differences in study design, AD clinical features (i.e., exclusion of subjects with mild AD severity, or inclusion of patients with food allergy-induced AD), duration and doses of the probiotic treatment could be responsible for these discrepancies.^{8,28–31} In addition, meta-analyses confirmed the greatest benefit of a longer treatment duration (>8 weeks) in improving AD course in children.^{32,33}

In our study, the improvement of AD severity was associated with an improvement of parental perception disease in both groups with a significant higher impact on IDQOL score in the LGG group. Other trials adopting the IDQOL score showed that the improvement of QOL reflected the objective improvement of AD, underlying the usefulness and reliability of this score.^{34,35}

The number of infectious diseases resulted similar in the two study groups. It should be underlined that the preventive measures for the COVID-19 pandemic applied during the study period could have influenced this outcome.

Also, regarding the specific IgE serum levels we did not find a modulation by LGG. It should be considered that we excluded patients affected by allergies, and this aspect of the trial design could impact this outcome.

The mechanisms of action elicited by LGG in modulating the AD clinical course are still poorly defined. The results of our study suggested a potential involvement of a parallel modulation of gut and skin microbiome, as also suggested by others.³⁶ Gut and skin dysbiosis have been described in patients with AD.³⁷ The possibility to modulate gut colonization through probiotic supplementation in childhood, has been long proposed. LGG is one of the most studied probiotics for the prevention and treatment of different atopic diseases with multiple mechanisms of action.^{9,10} Emerging evidence on gut and skin metabolomic features in AD patients suggest the microbiome role in modulating AD occurrence and disease course and support the relevance of the "Gut-Skin Axis."³⁸ Immunological and metabolic pathways have been postulated at the core of this bi-directional crosstalk.

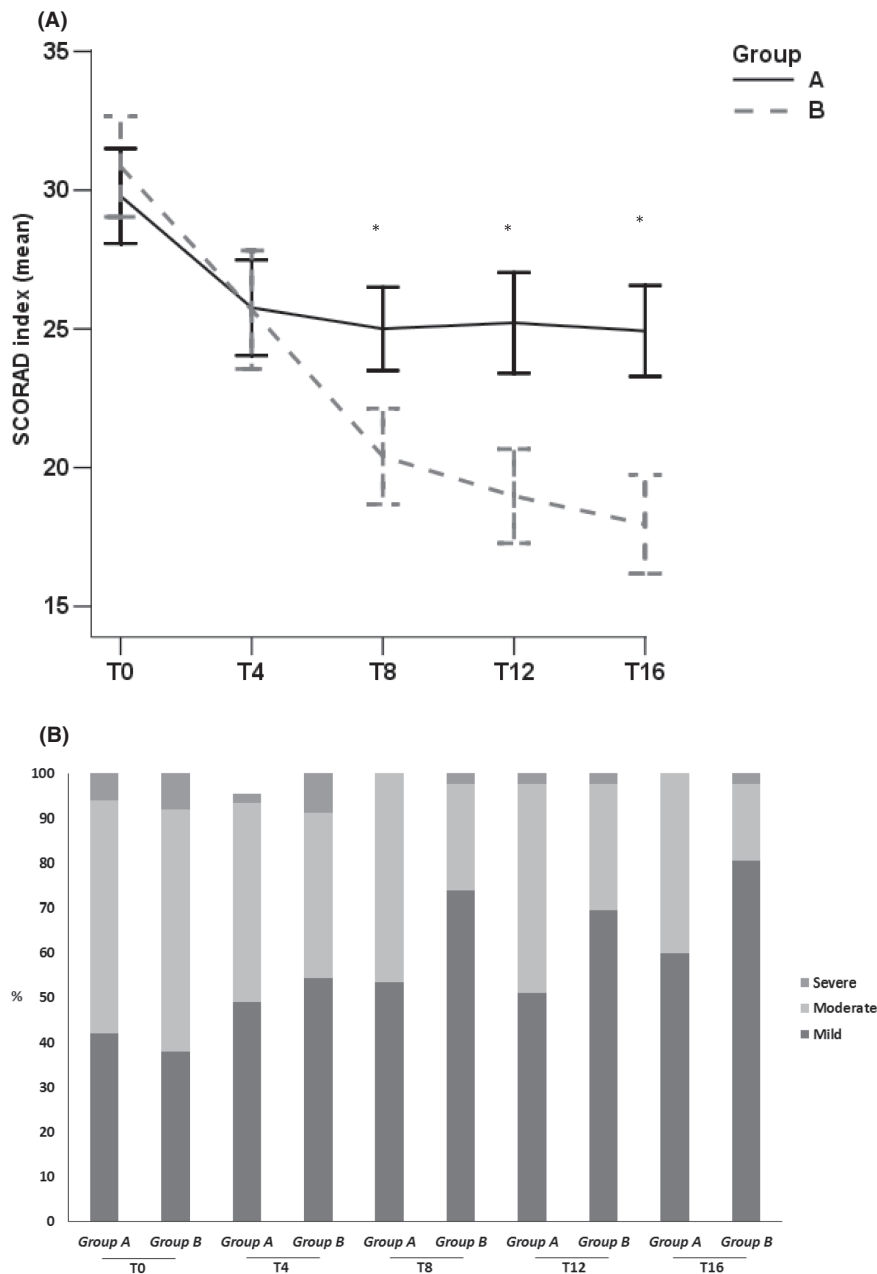


FIGURE 4 The SCORAD index pattern during the trial. (Panel A) Change in SCORAD index from baseline (T0) to the end of study period (T16) in the two study groups. Group A = placebo, group B = LGG. Error bars: ± 1.00 SE. *Group A vs. Group B, $p < .05$. Other significant differences: T0 vs. T8 Group A, $p < .05$; T0 vs. T8 Group B, $p < .05$; T0 vs. T12, Group B, $p < .05$; T0 vs. T16 Group A, $p < .05$; T0 vs. T16 Group B, $p < .05$; T4 vs. T12 Group B, $p < .05$; T4 vs. T16 Group B, $p < .05$. (Panel B) The atopic dermatitis severity pattern, evaluated through the SCORAD index, into the two study groups.

A major pathway is related to the SCFAs production.³⁹ SCFAs modulate several immune and non-immune mechanisms involved in AD pathogenesis.^{40–42} The SCFA butyrate exerts a pivotal role in preserving skin health.⁴³ Gut dysbiosis with decreased butyrate production have been reported to precede the AD onset.^{41,44–46} Low fecal butyrate levels have been detected in AD infants, and higher level of butyrate-producing bacteria have been reported in healthy infants if compared with AD pediatric patients.^{37,47,48} We demonstrated that LGG supplementation could result in the increase of butyrate fecal level together with an increase of well know butyrate-producer bacteria, which have been previously associated with a more favorable outcome in AD patients.⁴⁹ Similar results have been observed in children with cow milk allergy receiving LGG.⁵⁰

A positive modulation of gut microbiome has been also suggested by the increase of *Akkermansia*, a mucin-degrading bacteria involved

in the butyrate production though direct and indirect mechanisms, serving as the keystone species supporting a syntrophic network with butyrate-producing bacteria in the mucus layer.⁵¹ Lower levels of *Akkermansia* were previously reported in infants with higher risk of atopy, such as AD and asthma,⁵² and were significantly associated with stunted immune development in the AD patients compared with healthy controls.⁵³

The SCFAs could play a role in modulating the abundance of certain skin microbiome profiles which subsequently influence the cutaneous immune mechanisms.³⁹ We detected a change in the skin microbiome features in children receiving 12-week LGG supplementation with increased levels of *Prevotella* and *Veillonella* and reduced levels of *Stenotrophomonas*. Consistently, previous study reported reduced levels of *Prevotella* in skin microbiome of AD children,⁵⁴ while high levels of *Stenotrophomonas* have been previously

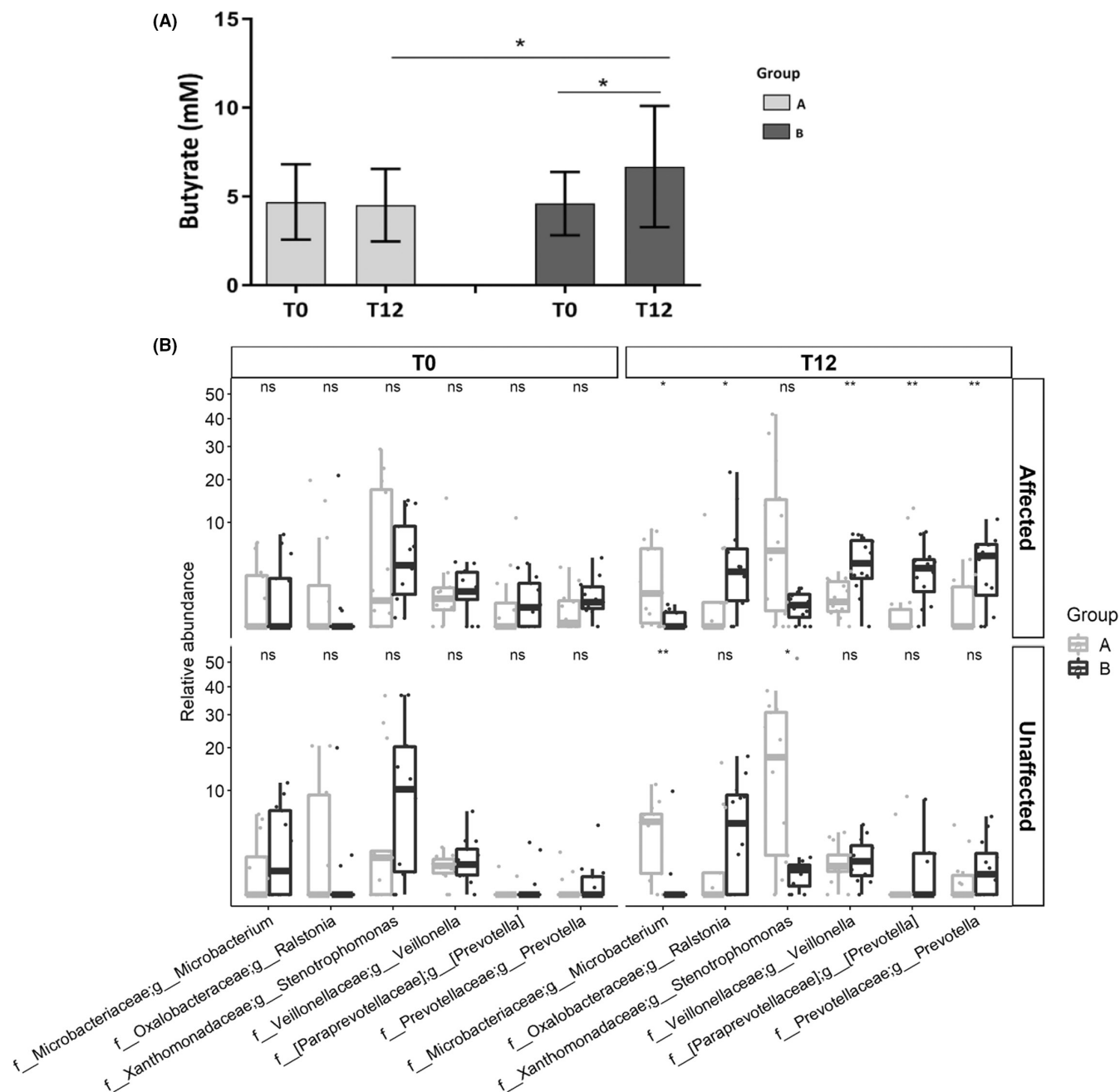


FIGURE 5 The modulation of the gut and skin microbiome in the two study groups. (Panel A) Butyrate concentration in fecal samples of subjects enrolled in the two study groups at baseline (T0) and after 12-week (T12). Placebo = Group A, LGG = Group B. (Panel B) Skin microbiome composition at genus level in children receiving placebo (Group A) and in children receiving LGG (Group B) in the affected and unaffected skin area. ns, not significant difference; * $p < .05$; ** $p < .001$.

described in AD patients.^{55,56} These results suggest the efficacy of LGG supplementation in restoring skin microbiome eubiosis.

5 | CONCLUSIONS

Emerging evidence underline the pivotal role of the “Gut-skin-axis” in AD patients. We found that the LGG supplementation as adjunctive treatment in AD pediatric patients could improve clinical severity, quality of life and could reduce topical steroid use. These

clinical improvements paralleled with a positive modulation of the skin and gut microbiome and with the increase of the well-known immunomodulatory SCFA butyrate. Further studies are needed to elucidate the underlying immune mechanisms and the potential long-term benefits for patients with AD.

AUTHOR CONTRIBUTIONS

Laura Carucci: Conceptualization (equal); investigation (lead); writing – original draft (equal); writing – review and editing (equal). **Rita Nocerino:** Conceptualization (equal); investigation

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ETHICAL APPROVAL AND TRIAL REGISTRATION STATEMENTS

The study design was approved by the Ethics Committee of our Institution (Protocol n.184/19). The trial was registered on ClinicalTrials.gov (NCT03863418) and was conducted in accordance with the Helsinki Declaration (Fortaleza revision, 2013), the Good Clinical Practice Standards (CPMP/ICH/135/95), the Italian Decree-Law 211/2003 regarding personal data, and the European regulations on this subject. Written informed consent was obtained from the parents/tutors of each subject.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/pai.13836>.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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