

ORIGINAL ARTICLE

Efficacy and safety of the probiotic *Lactobacillus paracasei* LP-33 in allergic rhinitis: a double-blind, randomized, placebo-controlled trial (GA2LEN Study)

DJ Costa¹, P Marteau², M Amouyal¹, LK Poulsen³, E Hamelmann⁴, M Cazaubiel⁵, B Housez⁵, S Leuillet⁵, M Stavnsbjerg⁶, P Molimard⁷, S Courau⁷ and J Bousquet^{8,9}

BACKGROUND/OBJECTIVES: An imbalance between Th1 and Th2 cells is involved in allergic rhinitis (AR) that may be improved by probiotics. To test the efficacy of the probiotic *Lactobacillus paracasei* subsp. *paracasei* LP-33, a double-blind, placebo-controlled, randomized trial was carried out in patients with AR to grass pollen treated with loratadine and presenting altered quality of life.

SUBJECTS/METHODS: Subjects with persistent AR, symptomatic during the grass pollen season, and a positive skin test or specific immunoglobulin E to grass pollens were included by general practitioners (GPs). All received loratadine for 5 weeks. The primary end point was the improvement in Rhinitis Quality of Life (RQLQ) global score at the fifth week of LP-33 consumption compared with placebo (in addition to loratadine). Secondary end points included nasal and ocular symptoms (individual and total symptom scores), visual analogue scale and time of first exacerbation of the symptoms when loratadine was stopped.

RESULTS: A total of 425 subjects were included. Using intent-to-treat analysis, the RQLQ global score decreased significantly more in the LP-33 group than in the placebo group ($P=0.0255$, difference = -0.286 (95% confidence interval (CI): -0.536 ; -0.035)). No significant differences were noted for the change of the rhinitis total symptom score 5 global score between groups ($P=0.1288$, difference = -0.452 (95% CI: -1.036 ; 0.132)). Significant differences in ocular symptoms (RQLQ) were observed between groups ($P=0.0029$, difference = -0.4087 (95% CI: -0.6768 ; -0.1407)).

CONCLUSIONS: This study performed by GPs shows that LP-33 improves the quality of life of subjects with persistent AR who are currently being treated with an oral H₁-antihistamine. Whereas nasal symptoms had not changed, ocular symptoms had consistently improved.

European Journal of Clinical Nutrition advance online publication, 26 February 2014; doi:10.1038/ejcn.2014.13

INTRODUCTION

Allergic rhinitis (AR) is a very common disease worldwide.¹ It is characterized by nasal and, often, ocular symptoms that impact quality of life (QOL), including social life, work and school performance.¹ It is subdivided into intermittent and 'PER' (persistent) disease based on the time of allergen exposure. Treatment-resistant phenotypes² have been described, and a significant proportion of patients continue to experience symptoms^{3,4} or are dissatisfied.⁵ Ocular symptoms are the most bothersome⁶ and are often difficult to control. AR results from immunoglobulin E (IgE)-mediated immune responses and a complex inflammatory network of cells, mediators and cytokines. An imbalance between Th1 and Th2 cells is involved in IgE-allergic inflammation.¹ The gut microbiota interacts with the gut-associated lymphoid tissue, and modulates inflammation and immune reactions in AR.^{7–9} Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'.¹⁰ They may improve health by several mechanisms including immune modulation resulting from the recognition of specific microbial molecules or patterns by

toll-like receptors and nod-like receptors.¹¹ Some probiotics can regulate local and systemic immunity and, as a consequence, may reduce allergic responses.¹² *In vitro* studies show that probiotic lactic acid bacteria stimulate interferon-alpha production influencing a Th1 phenotype.^{12–14} Moreover, allergy was associated with change in faecal microbiota composition.¹⁵ A restricted biodiversity of the intestinal microbiota was directly related to a risk of allergy, and probiotics may have a preventive effect on allergy.^{16,17} The consumption of probiotic strains can also stabilize the microbiota.^{18–20} Probiotic lactic acid bacteria may be a promising approach in the management of AR,^{21,22} with or without concomitant pharmacologic treatment. A meta-analysis described the results of 12 randomized clinical trials with probiotics in AR:²³ in nine of the 12 trials, there were some improvements in clinical outcomes including nasal or ocular symptoms. Two studies with *Lactobacillus paracasei* subsp. LP-33 showed an improvement of QOL and symptoms in AR.^{24,25} Other studies showed the efficacy of probiotics in AR related to house dust mite allergy,²⁶ in addition to levocetirizine in children²⁷ or during nasal allergen challenges.²⁸ However, the number of

¹Primary Care Department, University of Medicine, Montpellier I University, Montpellier, France; ²University Paris Diderot, Sorbonne Paris Cité, APHP, Department of Hepato-gastro-enterology, Hôpital Lariboisière, Paris, France; ³Allergy Clinic, Copenhagen University Hospital at Gentofte, Copenhagen, Denmark; ⁴University Children's Hospital, Ruhr-University Bochum, Department of Pediatrics, Bochum, Germany; ⁵Biofortis, Mérieux NutriSciences Company, Saint-Herblain, France; ⁶Global Regulatory and External Affairs, Chr. Hansen A/S, Hørsholm, Denmark; ⁷Merck Consumer Health, Merck Médication Familiale, Dijon, France; ⁸Department of Respiratory Diseases, University Hospital, Hôpital Arnaud de Villeneuve, Montpellier, France and ⁹Inserm, CESP Centre for Research in Epidemiology and Population Health, Respiratory and Environmental Epidemiology team, Villejuif, France. Correspondence: Professor J Bousquet, Service Maladies Respiratoires, Hôpital Arnaud de Villeneuve, 34295 Montpellier Cedex 5, France. E-mail: jean.bousquet@inserm.fr

Received 2 May 2013; revised 2 November 2013; accepted 29 November 2013

patients enrolled in these randomized controlled trials was usually low, most studies involved children and a confirmation of the data in a large randomized controlled trial in adults was still warranted. Moreover, as the effects of probiotics are strain-specific,^{29,30} a larger scaled study with LP-33 was missing.

In order to test the efficacy of the probiotic LP-33, we carried out a randomized trial in patients with demonstrated AR to grass pollen. The primary end point was the improvement in QOL at the fifth week of LP-33 consumption compared with placebo (as assessed by the Rhinitis Quality of Life (RQLQ) global score).³¹ RQLQ is a validated scale and was the end point of the previous studies with LP-33. Secondary end points included nasal and ocular symptoms (individual and total symptom scores), visual analogue scale (VAS) and time of first exacerbation of the symptoms.^{32,33} Exploratory outcomes included individual RQLQ scores.

SUBJECTS AND METHODS

Study design

A double-blind, randomized, parallel, placebo-controlled study was conducted in France. Subjects included adults with persistent AR sensitized to grass pollens. The trial was scheduled for the pollen season of 2010 but a second year of recruitment was necessary in 2011 because of insufficient number of randomized subjects in 2010.

Subjects. Subjects were included after written informed consent. The study conformed to good clinical practices and was first approved by the Montpellier (France) ethics committee in January 2010. All subjects fulfilled the following inclusion criteria: (i) 18–60 years of age; (ii) of either sex; (iii) with at least a 2-year history consistent with symptoms of AR defined according to the International Consensus on Rhinitis;³⁵ and (iv) who met the criteria for PER according to the ARIA classification.³⁶ Allergy had to be confirmed by a positive skin prick test or by serum-specific IgE to grass pollen (current or historical: 5 years). Subjects had to have an RQLQ global score ≥ 2 after 7–10 days of loratadine 10 mg once daily treatment³⁷ to be randomized. Subjects had to consent to stop consumption of probiotics (food or food supplement containing probiotics) and vitamins and/or minerals throughout the study.

Intervention. The study was conducted in 164 general practitioners (GPs) in France. As the study lasted for 5 weeks, it was not ethical to leave subjects without treatment in the placebo group. Thus, during the first two periods of the study, all subjects were treated with loratadine 10 mg once daily (Qualimed, France), oral H1-antihistamines being the recommended AR treatment.¹

Active product (containing at least 2.0×10^9 colony forming units of LP-33), microcrystalline cellulose, dicalcium phosphate and magnesium stearate or placebo (microcrystalline cellulose, dicalcium phosphate and magnesium stearate) were administered orally once daily in identical capsules and had to be consumed with a meal. Active and placebo capsules were manufactured by Chr. Hansen A/S (Hørsholm, Denmark). GPs provided the products (LP-33 or placebo and loratadine) to the subjects.

The study consisted of three consecutive periods (Figure 1). The run-in period lasted for 7–10 days, and subjects received loratadine alone. At the end of the run-in period, subjects having a RQLQ global score ≥ 2 were randomized to LP-33 or placebo treatment for 5 weeks in addition to loratadine. Loratadine was then discontinued and the subjects continued only LP-33 treatment (placebo or active product) for two additional weeks, in order to test the effect of LP-33 alone. Each subject was followed up by his/her GP during five visits (V0–V4) scheduled in the protocol. V0 was the pre-inclusion visit, V1: inclusion and randomization visit, V2: follow-up visit,

V3: follow-up visit and V4: end-of-study visit. Concomitant medications for the treatment of AR were coded according to the WHO Drug Dictionary and categorized by an Anatomical Therapeutic Chemical Classification code.³⁸

Outcomes

The primary outcome was the improvement in the RQLQ global score at the fifth week (V3) of LP-33 consumption compared with placebo (in addition to loratadine). The RQLQ is a validated scale for evaluating the QOL on the basis of how symptoms affect the subject. It includes 28 questions related to 7 domains (activities, sleep, general practical problems, nasal problems, eyes problems and emotional state) All the items are averaged in an overall score ranging from 0 (no at all) to 6 (extremely).³¹ The questionnaire was filled in at V1 and V3.

Secondary outcomes. The improvement was measured by the change from V1 to V3 of the RTSS-5 (Rhinitis Total Symptoms Score) global score. RTSS includes five individual AR symptoms (nasal congestion, sneezing, rhinorrhea, nasal pruritus and eye itching) noted from 0 (none symptom) to 3 (severe symptom). Each AR symptom was recorded in the evening in subject daily diaries from V1 to V4. The RTSS-5 global score is the sum of the ratings for individual scores.^{32,33} For analysis, it was averaged weekly.

The improvement was measured by the change from V1 to V3 of the score of nasal and ocular symptoms of the RQLQ (RQLQ sub-domains), nasal (nasal obstruction, sneezing, rhinorrhea and nasal itching) and ocular symptoms (ocular itching) of the RTSS-5.

The time to onset of the first exacerbation of symptoms measured as intolerable symptoms (use of rescue medication) or RTSS-5 increased by at least 3.

The 24-h reflective VAS rating on well-being was recorded in subject daily diaries. Subjects were asked to indicate the severity of their symptoms: scores range from not at all bothersome (0 mm) to very bothersome (100 mm).^{39–41}

Exploratory outcomes. The improvement, measured by the change from V1 to V3 of the score of individual RQLQ domains, other than ocular and nose symptoms, and individual scores of nasal symptoms of RTSS-5.

Compliance was assessed by comparing the number of capsules dispensed at the baseline visit with the number returned at the end of the trial. Subjects were considered non-compliant, if they had taken <80% or $\geq 120\%$ of the study product.

Adverse events were recorded in the diary at each visit.

Randomization

Products were randomly allocated to the subjects, according to a randomization table generated by Biofortis using the software SAS 9.1.3 Service Pack 4 (SAS Institute Inc., Cary, NC, USA). Centres received a block of six randomization numbers (named NR for the corresponding French acronym). Each NR was assigned when a screened patient became eligible at the end of the baseline assessments (V1). Active or placebo allocation was dependent only on the time sequence in which subjects entered the study. Subjects, investigators and other study personnel were blinded to the treatment the subjects received.

Statistical methods

A sample size of 418 subjects was calculated to provide at least 92.5% power to detect a 0.5-product difference in a change from baseline reflective RQLQ score at V3, with a 5% two-sided level of significance and an assumed s.d. of 1.5. In order to account for a drop-out rate of 15%, 500 subjects were planned.

Statistical analyses were performed using the software SAS 9.2 Service Pack 4 (SAS Institute Inc.). The significance level of statistical tests was set to 5%. An ITT (intent-to-treat) analysis was performed for efficacy analysis. Baseline characteristics were compared between groups by a Student's *t*-test or χ^2 -test for quantitative or qualitative parameters, respectively. Comparison of the proportion of subjects in each group who took at least one concomitant medication was conducted using a χ^2 -test. Analysis of covariance was applied on the primary end point, that is, the RQLQ score of V3, and was expressed as the change from V1 (baseline), with product as a factor and baseline value as a covariate of adjustment. Secondary end points were ranked according to clinical relevance, and the multiplicity issue was handled according to a hierarchical strategy for testing these pre-ordered

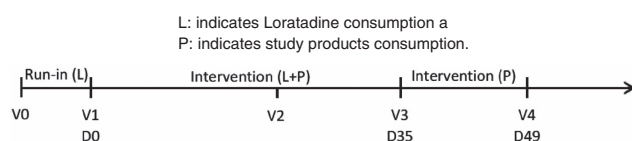


Figure 1. Experimental plan. L indicates loratadine consumption and P indicates study products consumption.

hypotheses, avoiding the need for any formal adjustment of type I error rates. The same analysis (analysis of covariance) was planned for all secondary and exploratory quantitative end points. The time of the first exacerbation of rhinitis symptoms (use of rescue medication or RTSS-5 increased by at least 3) was analysed with a log-rank test and a survival curve plotted using the Kaplan–Meier method. Intragroup analysis comparing V3 and V1 on the RQLQ global score used the paired Student's *t*-test. The difference between RQLQ at V3 between groups was analysed by analysis of variance.

RESULTS

Participant flow and compliance

A total of 602 subjects were screened (LP-33 group: 96 in 2010, 119 in 2011; placebo group: 95 in 2010, 115 in 2011) and 425 were randomized: 215 subjects in the LP-33 group and 210 in the placebo group (Figure 2). The data are presented for the ITT population, which corresponds to all subjects randomized whether they completed the study or not ($N=425$). The demographic characteristics of the randomized subjects are given in Table 1. There were no statistically significant differences between the two groups at baseline. Nearly the same numbers of subjects were recruited in 2010 and 2011. For LP-33 and the placebo products, a mean compliance of 101.6% and 100.8% was observed in the placebo group and the active group, respectively. For loratadine, a mean compliance of 106.5% and 113.3%, respectively, was observed in the placebo group and in the active group (not statistically different $P=0.2303$). Extreme values of above 100% are mainly explained by subjects who prematurely discontinued the study and did not return any capsules at the final visit. The numbers of subjects non-compliant to the study product (placebo or LP-33) according to the definition of returned product were the following: compliance $<80\%$, 9 subjects in the placebo group and 5 in the probiotic group; compliance $>120\%$, 12 subjects in the placebo group and 10 in the probiotic group. The

numbers of subjects non-compliant to loratadine according to the definition of returned product were the following: compliance $<80\%$ for loratadine, 6 subjects in the placebo group and 6 in the probiotic group; compliance $>120\%$ for loratadine, 29 subjects in the placebo group and 34 in the probiotic group.

Outcomes

Primary outcome. The RQLQ global score were comparable at inclusion (placebo 3.25 ± 0.91 , LP-33 3.25 ± 0.85 , $P=1.0$). At V3, the score was significantly lower in the LP-33 group vs the placebo group (1.40 ± 0.98 vs 1.65 , $P=0.04$). Intragroup analysis highlights a significant decrease in the RQLQ global score between V1 and V3 in both groups, however, the decrease in the RQLQ global score between V1 and V3 was significantly greater in the LP-33 group compared with the placebo group (Table 2) (-1.89 ± 1.22 vs -1.61 ± 1.31 ; $P=0.0255$) which corresponds to a difference of 0.286 (95% confidence interval (CI): -0.536 ; -0.035), and an additional effect of 17.7% of LP-33 over the placebo group.

Secondary outcomes. No significant differences were noted with regards to the change of the RTSS-5 global score between the active and placebo groups ($P=0.1288$, difference = -0.452 (95% CI: -1.036 ; 0.132)) and the hierarchical strategy was thereafter stopped. Other secondary parameters were therefore analysed for complementary information. Significant differences in ocular symptoms assessed by the RQLQ 'eye symptoms' domain (V3 – V1 change) were observed between the active and placebo groups, in favour of the active group ($P=0.0029$, difference = -0.4087 (95% CI: -0.6768 ; -0.1407)) (Table 3). No statistically significant differences were noted between the two groups for nasal symptoms ('nose symptoms' domain of the RQLQ), time of the first exacerbation of the symptoms and the VAS score (Table 3).

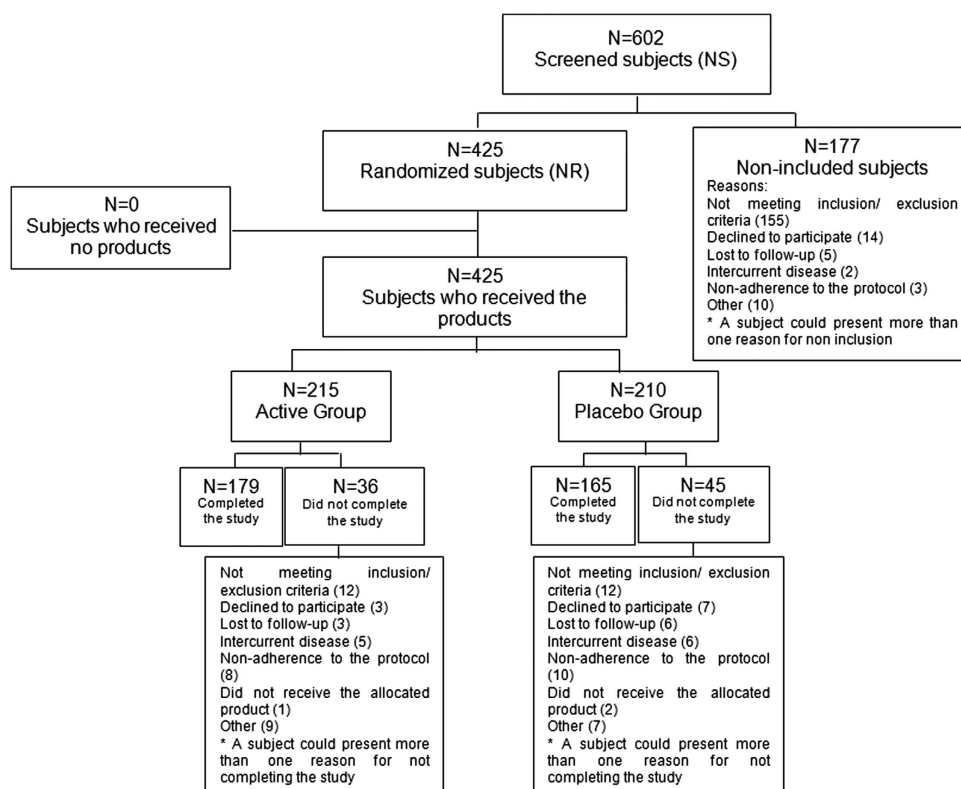


Figure 2. Disposition of the subjects.

Table 1. Baseline characteristics of subjects (ITT population)

	Placebo (N = 210)	Probiotic LP-33 (N = 215)
Gender (F/M)	106/104	123/92
Age (years)	37.19 (11.82)	38.05 (12.25)
Height (cm)	169.12 (9.07)	170.49 (9.18)
Weight (kg)	73.65 (16.44)	73.71 (15.61)
BMI (kg/m ²)	25.65 (4.99)	25.28 (4.66)

Abbreviations: BMI, body mass index; ITT, intent-to-treat. Values are means (s.d.).

Table 2. Primary parameter: RQLQ global score

Parameter	Groups	V1	V3	V3 – V1 variation
RQLQ global score	Placebo	3.25 (0.91)	1.65 (1.24) [#]	– 1.61 (1.31)
	LP-33	3.25 (0.85)	1.40 (0.98) [#]	– 1.89 (1.22) [†]
	P (ANOVA)	NS	0.046	0.0255

Abbreviations: ANOVA, analysis of variance; NS, not significant; RQLQ, rhinitis quality of life. Values are means (s.d.). Within-group comparisons between V3 and V1, [#] $P < 0.0001$. Between-group comparison on V3 – V1 variation, [†] $P < 0.05$.

Table 3. Changes in secondary outcomes (V3 – V1)

Secondary parameters	Placebo	LP-33	P-value
RTSS-5 global score	– 2.46 (3.57)	– 2.70 (3.48)	0.1288
RQLQ—'Nose symptoms' domain	– 1.80 (1.61)	– 1.89 (1.66)	0.4622
RQLQ—'Eye symptoms' domain	– 1.36 (1.77)	– 1.75 (1.63)	0.0029 [†]
Time to first exacerbation of symptoms (d)	10.63 (4.63)	10.50 (5.04)	0.6473
Well-being VAS	– 2.81 (3.03)	– 3.17 (2.97)	0.4958
RTSS-5—nasal symptoms	– 1.95 (2.86)	– 2.15 (2.89)	0.1783
RTSS-5—ocular symptoms	– 0.50 (0.92)	– 0.56 (0.85)	0.0700

Abbreviations: RQLQ, rhinitis quality of life; RTSS, rhinitis total symptom score; VAS, visual analogue scale. Values are means (s.d.). Between-group comparison on V3 – V1 variation, [†] $P < 0.05$.

Exploratory outcomes. Statistically significant differences were noted, in favour of the active product, for two domains of the RQLQ: the 'emotional function' domain, ($P = 0.0095$, difference = – 0.335 (95% CI: – 0.587; – 0.082)), the 'non-nose/eye symptoms' domain ($P = 0.0151$, difference = – 0.275 (95% CI: – 0.496; – 0.053)). In addition, analysis revealed statistically significant differences in favour of LP-33 in the change of individual score of rhinorrhea at V3/V1 in favour of the active product ($P = 0.0320$, difference = – 0.154 (95% CI: – 0.294; – 0.013)).

Concomitant medications

No statistically significant association was observed between this concomitant medication consumption and the study groups (22.9% and 29.8% of subjects took at least one concomitant medication in the placebo and active groups, respectively) ($P = 0.1059$).

Safety

Dietary supplementation with LP-33, in addition to loratadine, was considered safe. Adverse events were reported by 56 patients (27.1%) of the placebo group vs 64 of the probiotic group (30.2%)

(difference not significant). Only 13.7% of the adverse events were estimated by the GPs as being related to the study (10 in the placebo group, 10 in the active group), and a large part was exacerbation of AR symptoms. Two severe adverse events were reported, both in the placebo group: a case of acute appendicitis and a case of periartthritis of the shoulder.

DISCUSSION

This multi-center, randomized, double-blind, placebo-controlled, parallel study performed by GPs shows that LP-33 significantly improves the QOL of subjects with persistent AR (PER) currently being treated according to medical guidelines with an oral H₁-antihistamine. Whereas nasal symptoms were not changed, ocular symptoms were consistently improved. To our knowledge, this study is the first to show that a probiotic is effective in AR as an add-on therapy to the recommended medicinal treatment.

The diagnosis of PER was established in all subjects using a standardized questionnaire, evaluation of QOL and a positive skin prick test or serum-specific IgE to grass pollen. All subjects received loratadine during the first phase of the study (from days minus 7–10 to 35) for the following reasons: (i) it is not acceptable that symptoms should be experienced during a long period of time without medicinal treatment with established efficacy; (ii) in real life, subjects receive treatment, and our study aimed to mimic real life as closely as possible; (iii) oral H₁-antihistamines moderately potentiate other AR treatments.³⁷ An add-on design was, for example, used in a study of a probiotic given to adolescents treated with levocetirizine.²⁷

The primary outcome was the improvement in RQLQ in the LP-33 group compared with the placebo group. RQLQ is the most widely used tool for the assessment of QOL in subjects suffering from AR.³¹ Previous studies on LP-33 showed an effect on this parameter,^{24,25} and our group is well trained to use it.^{42–44} Secondary and exploratory outcomes were also based on validated tools (RTSS-5, nasal and ocular symptoms and VAS).^{39–41} Baseline levels of global RQLQ scores were comparable between the two groups and in keeping with usual results in literature for subjects with moderate-to-severe PER.⁴⁵

The intragroup analysis showed significant and important decreases from the baseline of RQLQ global score at V3 in both groups. The effect in the placebo group is high and can largely be ascribed to loratadine.^{32,33,46} We planned our study to be able to detect a difference in the score of 0.5 and observed a difference of 0.28 points. This difference is statistically significant and we consider it as an interesting improvement in the QOL. Compliance to loratadine and to the study products was high. Some results with a compliance of loratadine above 100% are likely to be owing to the fact that patients did not return loratadine pills as well as they returned the tested product. Compliance did not differ between the study groups and it is unlikely that they could influence the results. The pollen season may also have some impact on rhinitis symptoms. Given the high number of subjects, and the rather short duration of the pollen season, it was not possible to recruit all the subjects in the time frame in a single year. It is known that the seasonal variation in pollen counts may be considerable from one year to another and dictates symptoms. We do not believe that this biased our results, as the same tendencies were observed in the effect in both years and they were equally represented. At last, the loss of subjects for evaluation is largely explained by partly unfilled questionnaires. We did not (and could not) use any substitution method of missing data for this primary criterion.

Secondary objectives focused on the analysis of the change of AR symptoms at the fifth week of product consumption and were analysed using the hierarchical method. The change in RTSS-5 global score was the second outcome analysed. Significant improvement in symptom intensity was observed in both LP-33

and placebo groups at V3, but the difference between the two groups was not significant. This result should not be interpreted as contradictory as those of the primary end point, as impairment of QOL is usually only poorly related to symptoms.^{31,41} Analysis of the secondary and exploratory outcomes was conducted from an informative point of view. Significant differences were observed between active and placebo groups in change from baseline to V3 in the RQLQ domains 'eye symptoms', 'emotional function' and 'non-nose/eye symptoms', and in the RTSS domain 'rhinorrhea'. A trend was found in the RTSS with regards to improvement of ocular symptoms. These results suggest an effect of LP-33 on eye symptoms, the most bothersome symptoms of AR.^{6,47}

No immunological parameters were measured in the present study. The level of serum-specific IgE varies largely during the pollen season and there is no evidence of a correlation between IgE levels during any treatment and clinical efficacy.

The study confirmed previous studies and the effect of LP-33 consumption on the QOL of subjects with rhinitis symptoms.^{24,25} In these studies, positive significant results were obtained on QOL, which was their primary end point. Our findings are also in line with previous studies showing that some probiotic strains are effective in AR.^{12,19,20,23,26–30} However, as probiotic strains have strain-specific effects, results cannot be extended to all probiotics.

Probiotic foods or food supplements seem to be popular and widely used by subjects suffering from AR, however, a study under real-life conditions and in subjects receiving a medicinal treatment was needed. We believe that our data showing a significant improvement in the QOL of subjects with moderate/severe AR uncontrolled by oral H1-antihistamines is of high relevance, as the disease is recognized as a public health issue in view of its numerous negative effects on day-to-day activities and social life as well as the corresponding economical impact.

CONFLICT OF INTEREST

DJC received fees for consultancy from Merck Medication Familiale. PM received fees for consultancy, and lectures for Biocodex, Danone Merck Medication Familiale. LKP received fees for consultancy from Merck Medication Familiale. EH received fees for consultancy, and lectures for Milupa, Danone Merck Medication Familiale. MC, BH and SL are employees of Biofortis. MS is an employee of Chr. Hansen A/S. PM is an employee of Merck Medication Familiale. SC is an employee of Merck Medication Familiale. JB received fees for participation in scientific and advisory boards, giving lectures and press engagements from Actelion, Almirall, AstraZeneca, Chiesi, GlaxoSmithKline, Meda, Merck Sharpe & Dohme, Merck Medication Familiale Novartis, oM Pharma, Sanofi-Aventis, Schering Plough, Stallergènes, Takeda, Teva and Uriach. MA declares no conflict of interest.

ACKNOWLEDGEMENTS

Biofortis managed all administrative and logistic procedures. The study was funded by Merck Medication Familiale and Chr Hansen A/S. The investigators who collaborated in this study.

REFERENCES

- Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A *et al*. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008; **63**(Suppl 86): 8–160.
- Bousquet J, Bachert C, Canonica GW, Casale TB, Cruz AA, Lockey RJ *et al*. Unmet needs in severe chronic upper airway disease (SCUAD). *J Allergy Clin Immunol* 2009; **124**: 428–433.
- Canonica GW, Bousquet J, Mullol J, Scadding GK, Virchow JC. A survey of the burden of allergic rhinitis in Europe. *Allergy* 2007; **62**(Suppl 85): 17–25.
- Schatz M. A survey of the burden of allergic rhinitis in the USA. *Allergy* 2007; **62**(Suppl 85): 9–16.
- Valovirta E, Ryan D. Patient adherence to allergic rhinitis treatment: results from patient surveys. *Medscape J Med* 2008; **10**: 247.

- Virchow JC, Kay S, Demoly P, Mullol J, Canonica W, Higgins V. Impact of ocular symptoms on quality of life (QoL), work productivity and resource utilisation in allergic rhinitis patients—an observational, cross sectional study in four countries in Europe. *J Med Econ* 2011; **14**: 305–314.
- Hakansson A, Molin G. Gut microbiota and inflammation. *Nutrients* 2011; **3**: 637–682.
- Morita H, He F, Kawase M, Kubota A, Hiramatsu M, Kurisaki J *et al*. Preliminary human study for possible alteration of serum immunoglobulin E production in perennial allergic rhinitis with fermented milk prepared with *Lactobacillus gasseri* TMC0356. *Microbiol Immunol* 2006; **50**: 701–706.
- Ciprandi G, Vizzaccaro A, Cirillo I, Tosca MA. *Bacillus clausii* exerts immunomodulatory activity in allergic subjects: a pilot study. *Eur Ann Allergy Clin Immunol* 2005; **37**: 129–134.
- Food and Agriculture Organization and World Health Organization expert consultation. Consultation of Health and Nutritional Properties of Powder Milk and Live Lactic Acid Bacteria. Cordoba, Argentina. http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf 2001.
- Magrone T, Jirillo E. The interplay between the gut immune system and microbiota in health and disease: nutraceutical intervention for restoring homeostasis. *Curr Pharm Des* 2013; **19**: 1329–1342.
- Singh A, Hacini-Rachinel F, Gosoni ML, Bourdeau T, Holvoet S, Doucet-Ladeveze R *et al*. Immune-modulatory effect of probiotic *Bifidobacterium lactis* NCC2818 in individual suffering from seasonal allergic rhinitis to grass pollen: an exploratory, randomized, placebo-controlled clinical trial. *Eur J Clin Nutr* 2013; **67**: 161–167.
- Pochard P, Gosset P, Granelle C, Andre C, Tonnel AB, Pestel J *et al*. Lactic acid bacteria inhibit TH2 cytokine production by mononuclear cells from allergic patients. *J Allergy Clin Immunol* 2002; **110**: 617–623.
- Ghadimi D, Folster-Holst R, de Vrese M, Winkler P, Heller KJ, Schrezenmeier J. Effects of probiotic bacteria and their genomic DNA on TH1/TH2-cytokine production by peripheral blood mononuclear cells (PBMCs) of healthy and allergic subjects. *Immunobiology* 2008; **213**: 677–692.
- von Mutius E. A fascinating look at the world with a new microscope. *J Allergy Clin Immunol* 2012; **129**: 1202–1203.
- Jensen MP, Meldrum S, Taylor AL, Dunstan JA, Prescott SL. Early probiotic supplementation for allergy prevention: Long-term outcomes. *J Allergy Clin Immunol* 2012; **130**: 1209–1211.
- Prescott S, Nowak-Węgrzyn A. Strategies to prevent or reduce allergic disease. *Ann Nutr Metab* 2011; **59**(Suppl 1): 28–42.
- Ivory K, Chambers SJ, Pin C, Prieto E, Arques JL, Nicoletti C. Oral delivery of *Lactobacillus casei* Shirota modifies allergen-induced immune responses in allergic rhinitis. *Clin Exp Allergy* 2008; **38**: 1282–1289.
- Kawase M, He F, Kubota A, Hiramatsu M, Saito H, Ishii T *et al*. Effect of fermented milk prepared with two probiotic strains on Japanese cedar pollinosis in a double-blind placebo-controlled clinical study. *Int J Food Microbiol* 2009; **128**: 429–434.
- Ouweland AC, Nermes M, Collado MC, Rautonen N, Salminen S, Isolauri E. Specific probiotics alleviate allergic rhinitis during the birch pollen season. *World J Gastroenterol* 2009; **15**: 3261–3268.
- Kalliomaki M, Antoinette JM, Herz U, Rijkers GT, Wells JM, Mercenier A. Guidance for substantiating the evidence for beneficial effects of probiotics: prevention and management of allergic diseases by probiotics. *J Nutr* 2010; **140**: 7135–7215S.
- Rijkers GT, Bengmark S, Enck P, Haller D, Herz U, Kalliomaki M *et al*. Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research. *J Nutr* 2010; **140**: 6715–6765S.
- Vliagoftis H, Kouranos VD, Betsi GI, Falagas ME. Probiotics for the treatment of allergic rhinitis and asthma: systematic review of randomized controlled trials. *Ann Allergy Asthma Immunol* 2008; **101**: 570–579.
- Peng GC, Hsu CH. The efficacy and safety of heat-killed *Lactobacillus paracasei* for treatment of perennial allergic rhinitis induced by house-dust mite. *Pediatr Allergy Immunol* 2005; **16**: 433–438.
- Wang MF, Lin HC, Wang YY, Hsu CH. Treatment of perennial allergic rhinitis with lactic acid bacteria. *Pediatr Allergy Immunol* 2004; **15**: 152–158.
- Lin TY, Chen CJ, Chen LK, Wen SH, Jan RHA. Randomized prospective double blind controlled trial of the effect of probiotics on allergic rhinitis confined to Df, Dp or dust-sensitive children. *Indian Pediatr* 2013; **50**: 209–213.
- Lue KH, Sun HL, Lu KH, Ku MS, Sheu JN, Chan CH *et al*. A trial of adding *Lactobacillus johnsonii* EM1 to levocetirizine for treatment of perennial allergic rhinitis in children aged 7–12 years. *Int J Pediatr Otorhinolaryngol* 2012; **76**: 994–1001.
- Wassenberg J, Nutten S, Audran R, Barbier N, Aubert V, Moulin J *et al*. Effect of *Lactobacillus paracasei* ST11 on a nasal provocation test with grass pollen in allergic rhinitis. *Clin Exp Allergy* 2011; **41**: 565–573.
- Snel J, Visser YM, Smit BA, Jongen JM, van der Meulen ET, Zwijsen R *et al*. Strain-specific immunomodulatory effects of *Lactobacillus plantarum* strains on birch-pollen-allergic subjects out of season. *Clin Exp Allergy* 2011; **41**: 232–242.

- 30 Vißers YM, Snel J, Zuurendonk PF, Kleerebezem M, Wichers HJ, Savelkoul HF. Lactobacillus strains differentially modulate cytokine production by hPBMC from pollen-allergic patients. *FEMS Immunol Med Microbiol* 2011; **61**: 28–40.
- 31 Juniper EF. Measuring health-related quality of life in rhinitis. *J Allergy Clin Immunol* 1997; **99**: S742–S749.
- 32 Bousquet J, Bachert C, Canonica GW, Mullol J, Van Cauwenberge P, Bindslev Jensen C et al. Efficacy of desloratadine in intermittent allergic rhinitis: a GALEN study. *Allergy* 2009; **64**: 1516–1523.
- 33 Bousquet J, Bachert C, Canonica GW, Mullol J, Van Cauwenberge P, Jensen CB et al. Efficacy of desloratadine in persistent allergic rhinitis—a GA(2)LEN study. *Int Arch Allergy Immunology* 2010; **153**: 395–402.
- 34 International Consensus Report on Diagnosis and Management of Rhinitis. International Rhinitis Management Working Group. *Allergy* 1994; **49**(19 Suppl): 1–34.
- 35 Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *The J Allergy Clin Immunol* 2001; **108**(5 Suppl): S147–S334.
- 36 Bachert C, Maspero J. Efficacy of second-generation antihistamines in patients with allergic rhinitis and comorbid asthma. *J Asthma* 2011; **48**: 965–973.
- 37 Babre D. Medical coding in clinical trials. *Perspect Clin Res* 2010; **1**: 29–32.
- 38 Bousquet PJ, Combescure C, Neukirch F, Klossek JM, Mechin H, Daures JP et al. Visual analog scales can assess the severity of rhinitis graded according to ARIA guidelines. *Allergy* 2007; **62**: 367–372.
- 39 Bousquet PJ, Fabbro-Peray P, Janin N, Annesi-Maesano I, Neukirch F, Daures JP et al. Pilot study assessing the impact of smoking on nasal-specific quality of life. *Allergy* 2004; **59**: 1015–1016.
- 40 Bousquet PJ, Combescure C, Klossek JM, Daures JP, Bousquet J. Change in visual analog scale score in a pragmatic randomized cluster trial of allergic rhinitis. *J Allergy Clin Immunol* 2009; **123**: 1349–1354.
- 41 Bousquet J, Bodez T, Gehano P, Klossek JM, Liard F, Neukirch F et al. Implementation of guidelines for allergic rhinitis in specialist practices. a randomized pragmatic controlled trial. *Int Arch Allergy Immunol* 2009; **150**: 75–82.
- 42 Ciprandi G, Canonica WG, Grosclaude M, Ostinelli J, Brazzola GG, Bousquet J. Effects of budesonide and fluticasone propionate in a placebo-controlled study on symptoms and quality of life in seasonal allergic rhinitis. *Allergy* 2002; **57**: 586–591.
- 43 Walter Canonica G, Bousquet J, Van Hamme G, Bachert C, Durham SR, Klimek L et al. Levocetirizine improves health-related quality of life and health status in persistent allergic rhinitis. *Respir Med* 2006; **100**: 1706–1715.
- 44 Brozek JL, Bousquet J, Baena-Cagnani CE, Bonini S, Canonica GW, Casale TB et al. Allergic Rhinitis and its impact on Asthma (ARIA) guidelines: 2010 revision. *J Allergy Clin Immunol* 2010; **126**: 466–476.
- 45 Bachert C, Bousquet J, Canonica GW, Durham SR, Klimek L, Mullol J et al. Levocetirizine improves quality of life and reduces costs in long-term management of persistent allergic rhinitis. *J Allergy Clin Immunol* 2004; **114**: 838–844.
- 46 Ciprandi G, Klersy C, Cirillo I, Marsegli GL. Quality of life in allergic rhinitis: relationship with clinical, immunological, and functional aspects. *Clin Exp Allergy* 2007; **37**: 1528–1535.
- 47 Juniper EF, Guyatt GH, Willan A, Griffith LE. Determining a minimal important change in a disease-specific Quality of Life Questionnaire. *J Clin Epidemiol* 1994; **47**: 81–87.