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The relationship between bifidobacteria and allergic asthma and/or allergic dermatitis: A prospective study of 0–3 years-old children in Turkey

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ABSTRACT

Bifidobacteria are beneficial bacteria for humans. These bacteria are particularly effective at protecting against infectious diseases and modulating the immune response. It was shown that in newborns, the fecal distribution of the colonizing *Bifidobacterium* species influences the prevalence of allergic diseases. This study aimed to compare the faecal *Bifidobacterium* species of allergic children to those of healthy children to detect species level differences in faecal distribution. Stool samples were obtained from 99 children between 0 and 3 years of age whose clinical symptoms and laboratory reports were compatible with atopic dermatitis and allergic asthma. Samples were also obtained from 102 healthy children who were similar to the case group with respect to age and sex. Bifidobacteria were isolated by culture and identified at the genus level by API 20 A. In addition, 7 unique species-specific primers were used for the molecular characterization of bifidobacteria. The McNemar test was used for statistical analyses, and $p < 0.05$ was accepted as significant. *Bifidobacterium longum* was detected in 11 (11.1%) of the allergic children and in 31 (30.3%) of the healthy children.

Statistical analysis revealed a significant difference in the prevalence of *B. longum* between these two groups (X^2: 11.2, $p < 0.001$). However, no significant differences in the prevalence of other *Bifidobacterium* species were found between faecal samples from healthy and allergic children. ($p > 0.05$). The significant difference in the isolation of *B. longum* from our study groups suggests that this species favors the host by preventing the development of asthma and allergic dermatitis. Based on these results, we propose that the production of probiotics in accordance with country-specific *Bifidobacterium* species densities would improve public health. Thus, country-specific prospective case–control studies that collect broad data sets are needed.

1. Introduction

The flora of the human gut is one of the most complex ecosystems in nature, and the number of bacteria in the lumen of the gut is approximately 10 times larger than the number of eukaryotic cells in the human body [1–3]. The human gut is sterile at birth but exhibits a rich microflora that contains more than 500 species and approximately 1×10^{14} bacteria one week after birth; the

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establishment of this microflora depends on a number of factors, such as the mode of delivery, genetics, environmental contact and nutrition [4]. Within this microflora, bifidobacteria are the most extensively studied lactic acid-producing anaerobic bacteria, comprising approximately 95% of the newborn gut and 25% of the adult gut [5,6].

Bifidobacteria, facilitating the absorption of milk proteins, producing B_1, B_2, B_6, and B_{12} vitamins, and additionally nicotinic acid and folic acid, contribute to protein and vitamin metabolism [7–10]. A number of experiments demonstrated that bifidobacteria are effective not only at activating the immune system but also at enhancing lactose digestion, preventing diarrhea and constipation, and reducing the serum cholesterol level. Most importantly, these bacteria are integral to the daily diet and to therapy due to their antimicrobial and anti-carcinogenic activities [7,8,10].

As a chronic and recurrent inflammatory skin disease, atopic dermatitis is induced by various allergens and genetic factors, particularly in children between the ages of 0 and 6 years [11]. Allergic asthma is a chronic inflammatory disease in patients with bronchial hypersensitivity; a number of cells and cell products play important roles in this disease [12]. This study aimed to identify the *Bifidobacterium* species in stool samples from asthmatic and/or allergic children and in those from healthy children to detect species level differences in fecal distribution.

2. Materials and methods

2.1. Case-control group

This study was a case-controlled, cross-sectional study performed between September 2011 and October 2012. The case group included 99 children between 0 and 3 years of age. Of these children, 57 (57.5%) were boys and 42 (42.4%) were girls.

Clinical symptoms and laboratory reports (i.e., immunological, histological and biochemical reports) compatible with atopic dermatitis or allergic asthma were present in all subjects; 50.5% of the children were diagnosed with asthma, 38.3% were diagnosed with atopic dermatitis and 11.1% were diagnosed with asthma and atopic dermatitis.

In addition, the children in both the case and control groups had been maternally breastfed during their lives and had not taken any antibiotics in the two weeks prior to sample collection. The case group included randomly selected patients who applied to the Department of Pediatric Infectious Disease, the Department of Allergy and Immunology and the Department of Infant Care Service at Kanuni Sultan Suleyman Education and Research Hospital. The control group included 102 healthy children who applied to the same hospital during the same time period. The control group resembled the case group with respect to age and sex and included 58 boys (56.8%) and 44 girls (43%).

2.2. Collecting the samples

One gram of the stool sample obtained from each of the 99 children in the case group and from each of the 102 children in the control group was cultured. As discussed above, we only included children who had not taken any antibiotics in the two weeks prior to sampling and who had been breastfed during their lives.

The stool samples were collected in plastic containers and transferred to liquid Tripticase Phyton Yeast (TPY) medium, they were then kept in anaerobic jars and transported to the laboratory.

2.3. Microscopy and culture

In the laboratory of Cerrahpasa School of Medecine, Department of Medical Microbiology, the specimens were incubated at 37 °C under anaerobic conditions provided with AnaeroGen (Oxoid and Mitsubishi Gas Company) for 48 h. After incubation, 0.5 mL were removed from the liquid TPY medium and transferred to Tripticase Phyton Yeast Agar (TPYA), Wilkins Chalgren Agar (WCA), Man Rogosa Sharpe Agar (MRSA) and Modified Columbia Agar (DP) [13–16]. These media were incubated at 37 °C under anaerobic conditions for 72 h to properly isolate bifidobacteria. At the end of the incubation period, colonies with different morphologies were collected from the media and Gram-stained. The Gram-stained smears were examined, and colonies that exhibited a branched morphology typical of bifidobacteria were selected. The anaerobic characteristics of these colonies were confirmed using the aerotolerance test [17,18]. These anaerobic non-spore-forming Gram-positive rods were identified using API 20 A (BioMérieux, Marci L'etoile, France) to ensure that they belonged to a *Bifidobacterium* species.

2.4. Molecular tests

Throughout the PCR procedure, we isolated the DNA's from PBS suspensions bearing the cultivated colonies of bifidobacteria and we also used the selective liquid mTPY medium containing the stool samples. The High Pure PCR Template DNA isolation kit (Roche Diagnostics, Switzerland) was used for the isolation of bacterial DNA according to the manufacturer guidelines. Species-specific primers for *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium infantis* and *Bifidobacterium catenulatum* group were selected according to the research of Matsuki T et al. [19]. Species-specific primers for *Bifidobacterium pseudocatenulatum* was

Table 1
Bifidobacterium species-specific primers used in the study [19 20].

Target species	Name of primers	Sequence	Primer concentration (nM)	Product size (bp)
Bifidobacterium longum	BILON-1f	TTCCAGTTGATCGCATGGTC	200 nmol	831
	BILON-2r	GGGAAGCCGTATCTCTACGA		
Bifidobacterium adolescentis	BIADO-1f	CTCCAGTTGGATGCATGTC	200 nmol	279
	BIADO-2r	CGAAGGCTTGCTCCCAGT		
Bifidobacterium breve	BIBRE-1f	CCGGATGCTCCATCACAC	200 nmol	288
	BIBRE-2r	ACAAAGTGCCTTGCTCCCT		
Bifidobacterium bifidum	BIBIF-1f	CCACATGATCGCATGTGATTG	200 nmol	278
	BIBIF-2r	CCGAAGGCTTGCTCCCAAA		
Bifidobacterium infantis	BINF-1f	TTCCAGTTGATCGCATGGTC	200 nmol	828
	BINF–2r	GGAAACCCCATCTCTGGGAT		
Bifidobacterium catenulatum group	BICATg-1f	CGGATGCTCCGACTCCT	200 nmol	285
	BICATg-r	CGAAGGCTTGCTCCCGAT		
Bifidobacterium pseudocatenulatum	B-pcat f	AGCCATCGTCAAGGAGCTTATCGCAG	250 nmol	325
	B-pcat r	CACGACGTCCTGCTGAGAGCTCAC		

selected according to the research of Junick J et al. [20] (Table 1). Primer sequences were also examined for specificity by discrimination with known gene sequences in GenBank using the blast program at the NCBI BLAST website (http://www.ncbi.nlm.nih.gov/BLAST/). Primers were provided from Integrated DNA Technologies. (IDT, Coralville, IA). Amplification was carried out in a ABI 9700 Thermal Cycler (Applied Biosystems, CA, U.S.A.), and the program on the table was running as well (Table 1). Following the amplification, PCR products (10 μl DNA per sample) and 50-1000 bp. size marker (Promega, USA) were loaded, running in Agarose Gel. We used the 0.5xTBE electrophoresis buffer and 1.7% agarose. Finally, researched bands were interpreted and compared to positive controls (Fig. 1).

2.5. Statistical analyses

We used the McNemar test to compare the *Bifidobacterium* species in the gut flora of the children with atopic dermatitis and/or allergic asthma to those in the gut flora of the healthy control group. $p < 0.05$ was accepted as statistically significant.

3. Results

Among the 99 children in the patient group, *B. pseudocatenulatum* and *B. catenulatum* group were identified in 29 (29.2%) fecal samples, *B. breve* and *B. bifidum* were identified in 23 (23.2%) fecal samples and *B. infantis* was identified in 22 (22.2%) fecal samples; in contrast, *B. longum* was detected in only 11 (11.1%) samples and *B adolescentis* was detected in 3 (3%) samples. In the fecal samples from the 102 healthy children included in this study, *B. pseudocatenulatum* and *B. catenulatum* were detected in 35 (34.3%) samples, *B. breve* was detected in 29 (28.4%) samples, *B. bifidum* was detected in 26 (25.4%) samples, and *B. infantis* was detected in 22 (21.5%) samples; in addition, *B. longum* was detected in 31 (30.3%) samples and *B. adolescentis* detected in 7 (6.8%) samples.

A comparison of the distribution of *Bifidobacterium* species among the children in the patient group and the healthy children in the control group revealed that *B. longum* was detected in 11 (11.1%) of the allergic children and in 31 (30.3%) of the healthy children. Statistical analysis indicated that this difference in the prevalence of *B. longum* between the two groups was statistically significant (X^2: 11.2, $p < 0.001$). However, a comparison of the fecal samples from the healthy and allergic children with respect to the prevalence of other *Bifidobacterium* species revealed no statistically significant differences between the two groups ($p > 0.05$) (Table 2).

Moreover a gender-based comparison of the prevalence of *Bifidobacterium* species in the healthy control group and in the patient group revealed that the prevalence of *B. longum* among the allergic girls and boys was significantly lower then the prevalence of *B. longum* among the healthy girls and boys ($p < 0.05$) (Table 3). Similarly, *B. pseudocatenulatum* was also significantly less prevalent among allergic girls and boys than among healthy girls and boys ($p < 0.05$). In contrast, no significant gender-related difference in the prevalence of other *Bifidobacterium* species were observed between the samples from allergic childrens and the samples from healthy childrens.

Finally, when we compared the prevalence of *Bifidobacterium* species in the patient and healthy control groups according to age, *B. longum* was significantly less prevalent in samples from children aged 0–1 years and 1–2 years. However, this species did not exhibit significant differences between the two groups in other age groups ($p > 0.05$) (Table 4). Additionally, no significant age-related differences in the distribution of other *Bifidobacterium* species were detected between the healthy controls and the patients.

4. Discussion

Anaerobic bacteria constitute the largest proportion of the bowel microbiota, which represents one of the most important complex human ecosystems. In addition to the beneficial metabolic

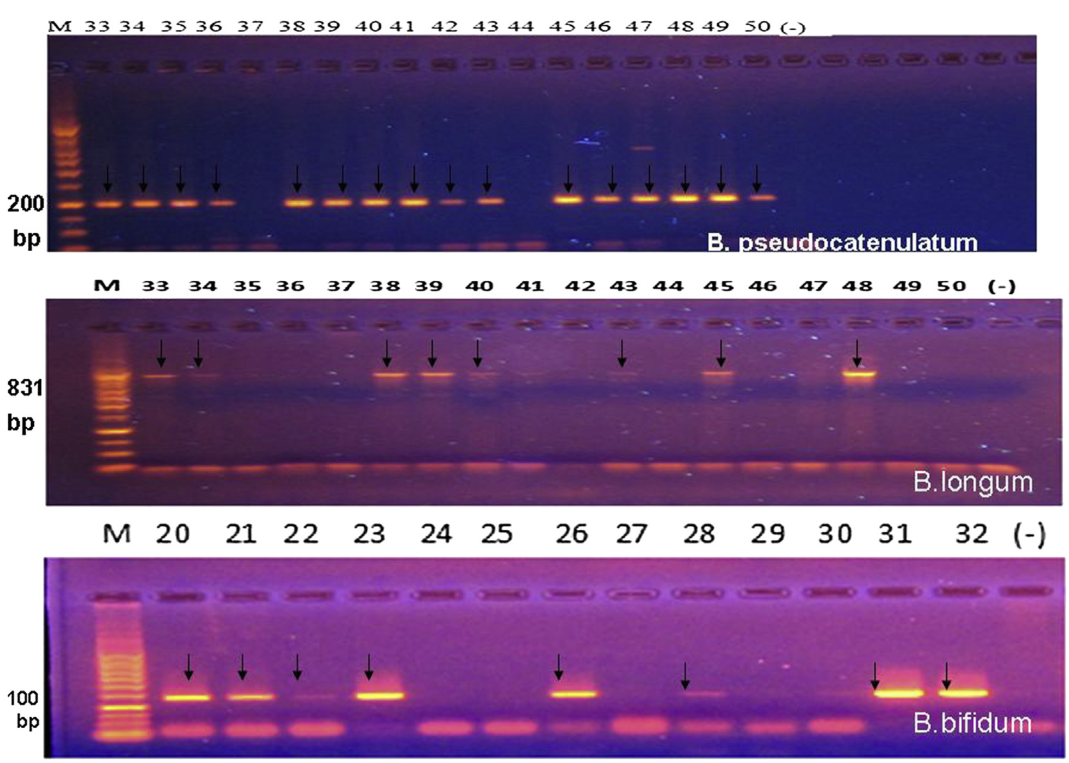

Fig. 1. Multiplex PCR results showing the gene region of *B. pseudocatenulatum*, *B. longum*, and *B. bifidum* detected from stool samples of the patients group.

Table 2
Comparison of patient and control groups in terms of isolated *Bifidobacterium* species.

Bifidobacterium species	Patients	Controls	X^2	*P*
	(+) (−)	(+) (−)		
B. longum	(11) (88)	(31) (71)	11.29	0.001
B. adolescentis	(3) (96)	(7) (95)	1.56	0.21
B. pseudocatenulatum	(29) (70)	(35) (67)	0.58	0.44
B. catenulatum group	(29) (70)	(35) (67)	0.58	0.44
B. breve	(23) (76)	(29) (73)	0.70	0.400
B. bifidum	(23) (76)	(26) (76)	0.139	0.709
B. infantis	(22) (77)	(22) (80)	0.013	0.911

(+): isolated, (−): not isolated.

functions of these bacteria, the fecal distribution of colonizing species that are reported to play a role in the initial regulation of the immune response in newborns influences the prevalence of allergic diseases [10,21,22]. Previous investigations of the hygiene hypothesis revealed an inverse relationship between the prevalence of allergic diseases and childhood infections. This situation was reported to occur due to the primarily undefined nature of Th2 response during allergic diseases and the primarily specific nature of the protective Th2 response during infectious diseases in newborns. The cause of this situation was stated as the alteration in balance of Th subsets toward a polarized Th2 response in the newborn child's immune system, in allergic diseases [23]). As a result, increased attention was focused on environmental factors, particularly on the structure of the intestinal microbiota and its effect on the development of the postnatal immune system [23]. This effect varies depending on the prevalence of anaerobes, such as *Lactobacillus*, *Clostridium*, and *Bifidobacterium*, among the organisms colonizing the intestine during the first few months of a baby's life. In addition it was indicated that, in the newborn gut, the distribution of these species can differ across geographical regions and the presence or absence of greater numbers of particular species of *Bifidobacterium* in the newborn gut is associated with the geographical distribution of allergic diseases [23–25]. The number of studies investigating the regulation of the immune response in the context of effective probiotic use has increased in recent years [26]. In 2004, Young et al. [26] found mostly *B. infantis* (23/32) in the stools of children living in countries with a low prevalence of allergic diseases; in contrast, in countries with a high prevalence of allergic diseases, such as the United Kingdom and New Zealand, *B. infantis* was not observed.

This study was performed in and around Istanbul, which is a city that harbors a population rich in different socio-economic and socio-cultural characteristics and exhibits a moderate prevalence of allergic diseases. In this population, the most frequently isolated *Bifidobacterium* species from patients with allergic dermatitis and/or asthma were *B. pseudocatenulatum* and *B. catenulatum*. In contrast, *B. pseudocatenulatum, B. catenulatum, B. longum* and *B. breve* were the most frequently isolated bifidobacteria from patients in the control group. Additionally, *B. adolescentis, B. bifidum,* and *B. infantis* were isolated from the intestinal flora of both diseased and healthy children with similar prevalence. We found that only *B. longum* was present at significantly lower levels in the patient group than in the healthy controls ($p < 0.01$). In 2007, Gonlund et al. [27] mentioned to *Bifidobacterium* species in the intestinal flora of healthy month-old babies from different countries; similar to the results of our study, those authors concluded that, *B. longum* was the most prevalent *Bifidobacterium* species in babies fecal samples, in reports from Germany in 1995, from Japan in 1999 and from Finland in 2007 [28–30]. In contrast, in 2001, He et al. [31] reported that in the intestinal flora of healthy children, *B. bifidum* was the most frequently isolated *Bifidobacterium* species and that in the intestinal flora of children with allergic dermatitis, *B. adolescentis* and *B. infantis* were the most frequently isolated *Bifidobacterium* species. In 2011, Walligora-Dupriet et al. [24] reported that *B. breve* was the most prevalent *Bifidobacterium* species in the intestinal flora of healthy babies.

The results of our study and the results of international studies suggest that in different geographic regions, the structure of the microbiota of both healthy children and children with allergic disease varies with respect to the fecal distribution of *Bifidobacterium* species. However, the species-specificity of Bifidobacterium colonization density in sick and healthy children is quite interesting.

Table 3
Comparison of patient and control groups in terms of gender on the basis of investigated *Bifidobacterium* species.

Bifidobacterium species		Patients	Controls	X^{2a}	P^a	X^{2b}	P^b
		$M^a - F^b$	$M^a - F^b$				
B. longum							
	(+)	(6) (5)	(17) (14)	6.34	0.012	4.95	0.026
	(−)	(51) (37)	(41) (30)				
B. adolescentis							
	(+)	(2) (1)	(3) (4)	0.19	0.662	1.7	0.184
	(−)	(55)(41)	(55)(40)				
B. pseudocatenulatum							
	(+)	(16) (13)	(29)(23)	5.80	0.016	4.01	0.04
	(−)	(41) (29)	(29)(21)				
B. catenulatum group							
	(+)	(18) (11)	(17)(18)	0.07	0.792	2.08	0.14
	(−)	(39)(31)	(41)(26)				
B. breve							
	(+)	(12)(11)	(16)(13)	0.66	0.414	0.12	0.729
	(−)	(45)(31)	(42)(31)				
B. bifidum							
	(+)	(12)(11)	(13)(13)	0.03	0.86	0.12	0.72
	(−)	(45)(31)	(45)(31)				
B. infantis							
	(+)	(12)(10)	(13)(9)	0.03	0.86	0.14	0.70
	(−)	(45)(32)	(45)(35)				

M: Male, F: Female, a: Males of the patient and control groups, b: Females of the patient and control groups. (+): isolated, (−): not isolated.

Table 4
Comparison of patient and control groups in terms of age on the basis of investigated *Bifidobacterium* species.

Bifidobacterium species/Age groups	Patients	Controls	X^2	*P*
	(+) (−)	(+) (−)		
B. longum/(0–1)	(4) (33)	(15) (19)	10.029	0.002
B. longum/(1–2)	(2) (32)	(9) (27)	4.82	0.028
B. longum/(2–3)	(5) (23)	(7) (25)	0.15	0.69
B. adolescentis/(0–1)	(1)(36)	(2)(32)	0.44	0.506
B. adolescentis/(1–2)	(1)(33)	(3)(33)	0.94	0.33
B. adolescentis/(2–3)	(1)(27)	(2)(30)	0.226	0.635
B. pseudocatenulatum/(0–1)	(12)(25)	(12)(22)	0.065	0.79
B. pseudocatenulatum/(1–2)	(10)(24)	(11)(25)	0.011	0.91
B. pseudocatenulatum/(2–3)	(7) (21)	(12)(20)	1.07	0.29
B. catenulatum/(0–1)	(9)(28)	(11) (23)	0.56	0.45
B. catenulatum/(1–2)	(10)(24)	(14) (22)	0.69	0.40
B. catenulatum/(2–3)	(10)(18)	(10) (22)	0.13	0.71
B. breve/(0–1)	(8)(28)	(11) (25)	0.643	0.422
B. breve/(1–2)	(8)(29)	(8) (22)	0.232	0.630
B. breve/(2–3)	(7)(19)	(10) (25)	0.020	0.887
B. bifidum/(0–1)	(7)(25)	(9) (25)	0.190	0.663
B. bifidum/(1–2)	(9)(26)	(8) (25)	0.020	0.889
B. bifidum/(2–3)	(7)(25)	(9) (26)	0.190	0.663
B. infantis/(0–1)	(7)(25)	(8) (27)	0.017	0.897
B. infantis/(1–2)	(7)(25)	(7) (27)	0.028	0.866
B. infantis/(2–3)	(8)(27)	(7) (26)	0.048	0.827

(+): isolated, (−): not isolated.

In the etiopathogenesis of this interesting multifactorial allergic disease, which is believed to be developed on the basis of multiple species, bifidobacteria exert immunomodulatory effects by initially stimulating IL-10 or IL-12 synthesis by dendritic cells. By interacting with T cells, these factors have the capacity to activate the Th1 or Th2 subsets, respectively. During this process, molecules such as thymic stromal lymphopoietin (TSLP), granulocyte-macrophage colony stimulating factor (GM-CSF), IL-25, and IL-33 activate dendritic cells to prime Th2 responses. Dendritic cells can accomplish this task by inhibiting the production of the Th1-polarizing cytokine IL-12, by inducing chemokines that attract Th2 cells or by favoring the development of Th2 cells via the upregulation of OX40L [32]. In addition, previous studies reported that single nucleotide polymorphisms of TLR-1, TLR-6, and TLR-10 exert a protective effect against allergic asthma. It was also shown that, specifically, genetic variants of TLR-6 (i.e., 5249p) increase IFN-gamma expression, which subsequently increases Th1 responses, decreases IL-4 levels and inhibits Th2 responses [33]. In a study conducted in 2004, in accordance with the hygiene hypothesis, the protective role of the genetic variants of TLR-6 was reported in European, Asian and African people [34]. In recent years, the role of TLR-9, which recognizes CpG DNA, in the immunomodulation of allergic asthma has been emphasized and it was proposed that by stimulating DCs, activating NK cells and inducing the synthesis of IFN-gamma, the balance between IL-10 and IL 12 tips in favor of a Th1 response [35–37]. The effect of Th1 responses was assumed to be essential to the etiopathogenesis of allergic diseases. However, the effect of defects in the suppressor function of IL-17- and IL-19-synthesizing CD4 cells and in the number and function of immunomodulator or immunosuppressor Treg cells (i.e., CD4-, CD28- and FoxP3-positive cells) on allergic asthma reveals that these factors play important roles in the maintenance of immunological tolerance. Additionally, the lack of IL-10 and TGF-beta secretion that occurs as a result of these defects was reported to complicate the control of the allergic inflammatory response [38]. Thus, Young et al. [26] reported that the presence of *B. longum* and other species in the intestinal flora of sensitive children living in countries with a high prevalence of allergic disease, such as the United Kingdom and New Zealand, is associated with the synthesis of IL-10 by dendritic cells. In contrast, the presence of *B. infantis* in the intestinal flora of children living in societies with a low prevalence of allergic disease, such as Ghana, was associated with inadequate secretion of IL-10 by dendritic cells; although CD8 expression was upregulated, indicating dendritic cell activation, this upregulation was not sufficient to maintain Th1 or Th2 responses. The resulting incomplete maturation downregulates effective immune responses and immunological tolerance. Similarly, in our study, the intestinal prevalence and variability of major *Bifidobacterium* species in healthy and allergic children suggests that structural differences exist among bifidobacterial PAMPs that interact with TLRs. Although the cell walls of all species contain basic polysaccharide molecules (i.e., glucose, galactose, and mannose), polyglycerol- phosphatidyl and associated lysol derivatives, we believe that differences in amino acid sequences that occur as a result of different geographic, environmental and nutritional factors play a role in the observed species variability [39]. Although differences among *Bifidobacterium* species were reported to be associated with geographical regions, in our study, *B. longum* was found to have a significantly lower prevalence in the patient group than in the healthy controls, consistent with an experimental animal study performed in Japan by Takahashi et al. [40]. The authors reported that in a murine model, the $BL_{07}S$ oligonucleotide obtained from the BB536 genomic region of *B. longum* blocked antigen-induced Th2 immune responses *in vivo*. Based on this finding, the authors stated that *B. longum* can serve as an useful probiotic for the prevention of allergic diseases. We believe that the accordance between the results of this experimental animal study and our *B. longum*-specific results is important for probiotic preparation. In our study, age and gender-based comparisons of the fecal distribution of *Bifidobacterium* species between patients and healthy control groups revealed statistically significant differences in the prevalence of *B. longum* among patients between the ages of 0 and 1 years and in the prevalence of *B. longum* and *B. pseudocatenulatum* among male and female patients ($p < 0.01$). Although diseases such as allergic asthma and dermatitis have been associated primarily with genetic sensitivity, these diseases are also thought to be associated with environmental factors and dietary habits that affect the microbiota during the first years of life [41]. In addition, the hygiene hypothesis posits that due to the lack of microbial warnings in early infancy, the formation of abnormal immune responses against harmless microbial antigens occurs later in life. Thus, during the newborn period, the development of Th1-based immunological tolerance stimulated by bacterial antigens is believed to be important in preventing the development of allergic diseases [41,42]. Although the protective effect of Th1 responses during this period is controversial, a study that investigated the stools of 25- to 30-day-old Ghanaian, British and New Zealander babies reported that the most frequently isolated *Bifidobacterium* species were *B. infantis* in Ghanaian babies' stool samples and *B. longum* in British and New Zealander babies' stool samples [26]. These findings emphasized the importance of the relationship between the geographical prevalence of allergic diseases and the fecal distribution of *Bifidobacterium* species during infancy. In a cohort study performed in 2008, Vael et al. [43] studied the relationship between the intestinal colonization of *Bacteroides* species in early infancy and the development of asthma in the first three years of life. The results of this study indicated that intestinal colonization by *Bacteroides fragilis* in 3-week-old babies may be an early indicator for the development of asthma later in life. This finding highlighted the importance of the modulation of the microbial flora during early infancy. Moreover, Walligora-Dupriet et al. [24] investigated 20 allergic children between 3 and 24 months of age and reported that regardless of age, the observed *Bifidobacterium* species did not differ between the patient and control groups. Thus, the relationship between allergic disorders and Bifidobacterium colonization is complex and cannot be explained by a single mechanism related to a single species.

5. Conclusion

To conclude, the data obtained in this study are similar to data obtained in a number of international studies that investigated the relationship between Bifidobacterium and allergic diseases in different geographical regions. The data presented here not only confirm the importance of the intestinal microflora of newborns in the development and prevention of allergic dermatitis and asthma but also demonstrate that these species can vary based on differences that exist among geographic locations 'The significant difference in the prevalence of *B. longum* among allergic and healthy children from the Istanbul region of Turkey reveals a complex relationship in which environmental factors, eating habits, and different cultural identities influence the host, allowing the host to avoid the development of asthma and allergic dermatitis. Based on these results, we propose that the production of probiotics in accordance with country-specific *Bifidobacterium* species densities would improve public health. Thus, country-specific prospective case–control studies that collect broad data sets are needed.

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