

# Probiotics and the development of very low birthweight infants: follow-up study of a randomised trial

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## Abstract

**Objective** To investigate the effect of *Bifidobacterium bifidum* OLB6378 on the development of very low birthweight (VLBW) infants at 18 months of corrected age.

**Design** Long-term follow-up study of a cluster-randomised, placebo-controlled trial.

**Patients** VLBW infants (birth weight <1500 g) born between January 2010 and March 2011 and managed at 19 neonatal intensive care unit facilities assigned to two groups to account for the effect of probiotic cross-contamination within facilities.

**Interventions** For VLBW infants, administration of OLB6378 as a probiotic was started within 48 hours of birth and continued until the body weight reached 2000 g.

**Main outcome measures** At 18 months of corrected age, physical status and developmental quotient (DQ18) were assessed. The distribution of DQ18 scores was categorised into four levels of development: <70, significant developmental delay; 70–84, moderate developmental delay; 85–99, without developmental delay; ≥100, average development or better.

**Results** Among 153 infants assigned to the OLB6378 administration group and 130 assigned to the placebo administration group, 102 and 105 infants, respectively, underwent the 18-month medical examination. The distribution of developmental levels (DQ18 scores <70, 70–84, 85–99 and ≥100) was significantly more favourable for OLB6378 administration (12, 12, 25 and 40 infants, respectively) than for placebo administration (15, 17, 23 and 24 infants, respectively) (ordered logistic regression analysis: partial correlation coefficient, 0.589; P value, 0.038).

**Conclusions** Although limited by assessment rates, result suggests that OLB6378 may have a beneficial effect on the psychological development in VLBW infants.

**Clinical trial registration** UMIN000002543.

## INTRODUCTION

Many clinical studies have investigated the effectiveness of early *Bifidobacterium* administration in establishing a normal intestinal microbiota in preterm infants,<sup>1–4</sup> but without focusing on the time to establishment of enteral feeding or on psychological development. In our first clinical study of preterm infants who received *Bifidobacterium bifidum* OLB6378 as a probiotic, we found that the

## What is already known on this topic?

- Probiotics have an effect in gut-related illnesses.
- Probiotics administration can help very low birthweight infants achieve enteral feeding sooner.
- The gut-brain axis links the enteric and central nervous systems.

## What this study hopes to add?

- Early administration of *Bifidobacterium bifidum* OLB6378 may improve psychological development (developmental quotient at 18 months of corrected age) among very low birthweight infants.

rate of weight gain was significantly higher in infants started on OLB6378 within 48 hours after birth than in those started on OLB6378 at a later time.<sup>5</sup> A subsequent multicentre study initially reported that OLB6378 accelerated the establishment of enteral feeding without increasing morbidity, and reduced the incidence of late-onset sepsis in very low birth weight (VLBW) infants.<sup>6</sup> A long-term study was thus initiated based on the hypothesis that, by improving enteral feeding, OLB6378 administration can improve psychological development in VLBW infants. The aim of the present investigation was to report the physical status and developmental quotient of VLBW infants, who participated in the previous study, at 18 months of corrected age.<sup>6</sup>

## METHODS

### Study design and participants

It was speculated that probiotics could be easily spread among infants admitted in the same neonatal intensive care unit (NICU) according to our previous study.<sup>6</sup> Therefore, in order to completely avoid infant-to-infant dissemination of OLB6378 within the same NICU, the study was conducted as a

cluster-randomised trial. The present study is a follow-up investigation of a large cluster-randomised clinical trial that included 19 NICU facilities in Japan.<sup>6</sup> Detailed protocols were described in the initial evaluation.<sup>6</sup> Briefly, infants born between January 2010 and March 2011 with a birth weight <1500 g were enrolled. The exclusion criteria were: lack of parental consent, presence of major congenital malformation, systemic infection or failure to start OLB6378 or placebo administration within 48 hours of birth due to a clinical condition that precluded oral administration. To account for the effect of potential cross-contamination and infant-to-infant dissemination of OLB6378 within the same NICU, the study was conducted as a cluster-randomised trial that divided the 19 participating facilities into OLB6378 administration facilities and placebo administration facilities, none of which had any prior experience in the administration of probiotics to new borns.

The primary outcomes were the age (in days) at which the volume of enteral nutrition reached 100 mL/kg/day, as well as body weight and head circumference at discharge. The secondary outcomes were: incidence of necrotising enterocolitis and sepsis; physical and mental development at 18 months of age and intestinal microbiota colonisation (evaluated based on stool samples) at birth, at 1 week after birth and at 1 month after birth. All outcome measures were chosen prior to initiating the study.

### Randomisation and masking

The 19 NICU facilities were cluster-randomised to one of two study groups. Briefly, the facilities were paired according to the number of infants hospitalised in 2009. A computer program was used to generate a random number for each facility. Subsequently, each facility was allocated to one of two study groups based on whether the assigned number was higher or lower than the number assigned to the paired NICU facility.

The randomisation allocation sequence was concealed by Tokyo Women's Medical University. OLB6378 powder and placebo powder (masked) were provided by Meiji, and the intervention was concealed by Meiji. Assessments were performed at each facility, and all data were sent to Tokyo Women's Medical University.

After all infants were discharged from the NICUs and their short-term outcomes were recorded, the randomisation allocation sequence was revealed and the results were reported.<sup>6</sup> However, the 18-month medical examination was performed by blinded examiners. Because of the study design, individual data were compared based on the initial treatment, and not based on the clustering scheme.

### Procedures

For infants born at OLB6378 administration facilities (the OLB6378 administration group; B-group), 0.25 g of OLB6378 powder (Meiji) consisting of dextrin as the vehicle and approximately  $5 \times 10^9$  cfu/g of OLB6378

dissolved in 0.5 mL of breast milk, infant formula or warm water (if enteral feeding had not been introduced) were delivered into the stomach through an enteral feeding tube. Administration was started within 48 hours after birth and performed twice daily until the infant's weight reached 2000 g. For infants born at placebo administration facilities (the placebo administration group; P-group), 0.25 g of placebo powder consisting of dextrin dissolved in 0.5 mL of breast milk, infant formula or warm water was administered. Treatment and nutrition were otherwise given according to conventional practice in each NICU.

### Assessment of psychological development

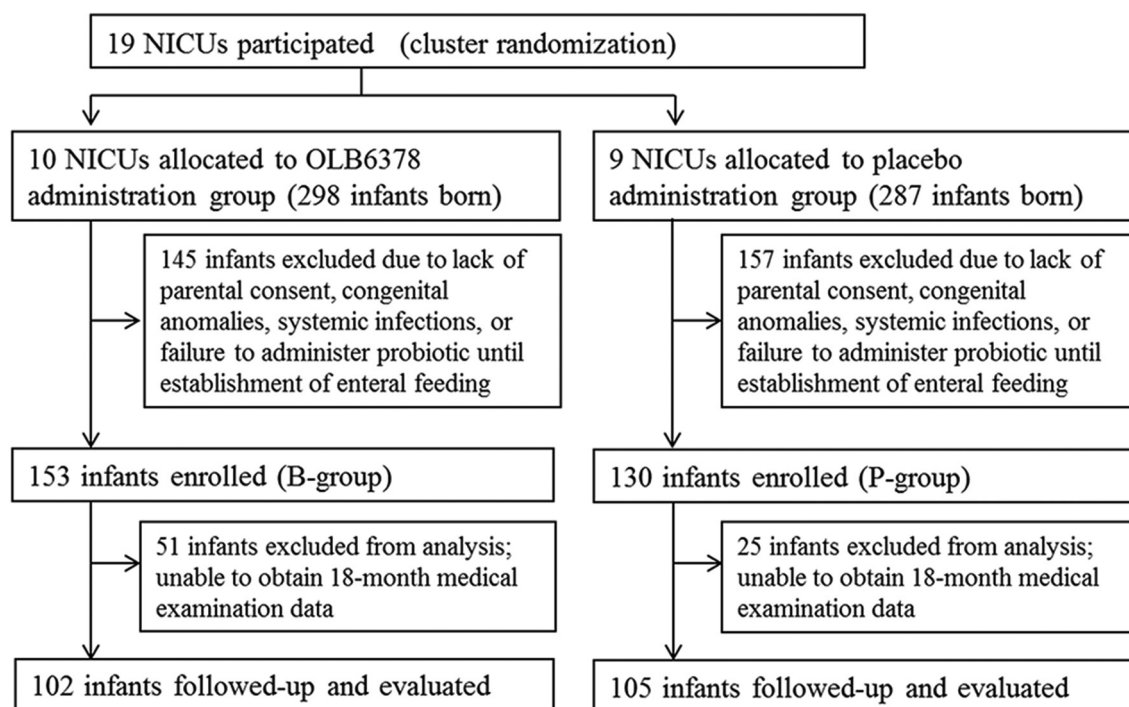
The Kyoto Scale of Psychological Development 2001 (KSPD) is the primary method used for developmental assessment of children in Japan, and has recently been shown to correlate with the Bayley III scale.<sup>7,8</sup> The developmental age is determined based on the score calculated for each of the three KSPD domains (posture and movement; cognition and adaptation; language and social ability). In the present analysis, the developmental quotient at 18 months of corrected age (DQ18) was calculated as the percentage ratio between developmental and chronological age. The distribution of developmental levels was defined in terms of the DQ18 scores: <70, significant developmental delay; 70–84, moderate developmental delay; 85–99, without developmental delay;  $\geq 100$ , average development or better. At the examination, infants with severe neurological damage, for whom developmental testing was not feasible, were considered to have DQ18 <70. On the other hand, for infants with obviously favourable psychological development, per the checklist included in the Maternal and Child Health Handbook issued by the Japan Ministry of Health, Labour and Welfare, developmental testing was considered unnecessary; these infants were considered to have DQ18  $\geq 100$ .

### Outcome measures

Body weight, body length, head circumference and prevalence of physical impairment at the 18-month medical check-up were compared in infants who underwent medical examination between September 2011 and March 2013. Cerebral palsy was defined for Gross Motor Function Classification System level II or greater.<sup>9</sup> Hearing impairment was defined if hearing aids were required, while visual impairment was defined if vision in both eyes was lost. Additionally, the two groups were compared in terms of the distribution of developmental levels (DQ18 values).

### Statistical analysis

The target sample sizes of 153 infants for the treatment group and 130 infants for the placebo group were described previously.<sup>6</sup> Student's t-test was used to compare variables that demonstrated normality and equal variance of the distribution, with results presented as mean  $\pm$  SD.



**Figure 1** Study flow chart. NICU, neonatal intensive care unit.

The Mann-Whitney U test was used to compare variables for which normality and equal variance of the distribution could not be confirmed, with results presented as median (IQR). To compare categorical data, Fisher's exact test was used. To evaluate treatment effect size, linear regression and logistic regression models were used for quantitative and categorical variables, respectively, and the results were expressed as the partial regression coefficient and OR with 95% CI, respectively. Another ordered logistic regression model was established to calculate a partial correlation coefficient between the intervention and distribution of developmental levels. All tests were conducted using SPSS V.22.0 (SPSS, Chicago, Illinois, USA). Statistical significance was set at a threshold of 0.05.

## RESULTS

### Study participants

Of 19 NICU facilities included in the study, 10 were assigned to administer OLB6378, and 9 were assigned to administer placebos (figure 1). The total number of infants enrolled was 153 in the B-group and 130 in the P-group; of these, 102 and 105 infants, respectively, underwent the 18-month medical examination and were analysed (see online supplementary table 1). Adverse events that occurred during the study period were described previously.<sup>6</sup> There were no side effects related to the intervention.

Background data and characteristics were found to be broadly similar between the two groups of infants who underwent the 18-month medical examination (table 1), which corresponds to the findings of the initial

evaluation of this trial.<sup>6</sup> However, Apgar scores at 1 and 5 min, the rate of caesarean delivery and the incidence of intraventricular haemorrhage differed significantly between the groups (table 1). Background characteristics including neonatal morbidities and interventions were not different between infants evaluated and not evaluated at 18-month medical examination among four groups (see online supplementary table 2).

Following the same approach as in the initial evaluation of this trial,<sup>6</sup> a between-group comparison of the time to establishment of enteral feeding was performed considering only infants who achieved enteral feeding by 21 days of age. The time until establishment of enteral feeding was significantly shorter in the B-group (n=80; median age, 10 days; IQR for age, 8–13 days) than in the P-group (n=91; median age, 11 days; IQR for age, 9–14 days) (Mann-Whitney U test; P=0.032), which was also noted in the previous report.<sup>6</sup>

### Outcomes at the 18-month medical check-up

At the 18-month follow-up (table 2), there were no significant differences between the groups in body weight, body length, head circumference, oxygen use, incidence of cerebral palsy or mean DQ18 scores (among the infants whose scores were obtained, not inferred). However, the range of DQ18 scores was very wide. We thus calculated the distribution of developmental levels in each group; in this analysis, we also included the infants whose scores were only inferred, not calculated (because developmental testing was either not feasible or considered unnecessary). We found significant between-group differences in the distribution of developmental levels, with more favourable development among infants in the

**Table 1** Background and characteristics of infants who underwent the 18-month medical check-up

Characteristic	OLB6378 administration group (n=102)	Placebo administration group (n=105)	P value
At enrolment			
Gestational age, weeks	28.7±3.1	28.4±3.0	0.568*
Birth weight, g	1036±289	994±283	0.297*
Light-for-dates, n (%)	35 (34)	41 (39)	0.564†
Head circumference at birth, cm	25.5±2.6	25.6±2.7	0.773*
Use of antenatal steroids, n (%)	37 (36)	49 (47)	0.158†
Caesarean section delivery, n (%)	47 (46)	85 (81)	<0.001†
Male sex, n (%)	60 (59)	58 (55)	0.674†
Apgar score at 1 min	6.0 (4.0, 8.0)	5.0 (3.0, 7.0)	0.003‡
Apgar score at 5 min	8.0 (8.0, 9.0)	7.0 (6.0, 9.0)	0.001‡
Respiratory distress syndrome, n (%)	68 (67)	68 (65)	0.769†
Chronic lung disease, n (%)	56 (55)	49 (47)	0.267†
Intraventricular haemorrhage, n (%)	10 (10)	23 (22)	0.022†
Grade III or IV, n (%)	2 (2)	3 (3)	1.000†
Periventricular leukomalacia, n (%)	3 (3)	5 (5)	0.721†
Late-onset sepsis, n (%)	6 (6)	12 (11)	0.218†
Use of total parenteral nutrition, n (%)	83 (81)	94 (90)	0.115†
Treatment for retinopathy of prematurity, n (%)	15 (15)	21 (20)	0.365†
Age at enteral feeding exceeding 100 mL/kg/day, days	11.0 (9.0, 17.0)	12.0 (9.5, 16.0)	0.654‡
Infants who achieved it by 21 days of age, n (%)	80 (78)	91 (87)	0.143†
Infants who achieved it by 21 days of age, days	10.0 (8.0, 13.0)	11.0 (9.0, 14.0)	0.032‡
At the 18-month medical check-up			
Age, years	1.71±0.16	1.71±0.15	0.686*
Corrected age, years	1.51±0.17	1.51±0.13	0.943*

Values are expressed as mean±SD, median (first quartile, third quartile) or number (frequency).

\*Student's t-test.

†Fisher's exact test.

‡Mann-Whitney U test.

B-group (partial correlation coefficient, 0.589;  $P=0.038$ ) (see [table 2](#), online supplementary figure 1).

### Subgroup analyses

To evaluate the treatment effect in subgroups of infants defined by certain baseline characteristics, we chose several background risk factors including gestational age, birth weight, light-for-dates status, delivery mode, Apgar scores, antenatal steroid use, sex, establishment of total parenteral nutrition and age at full enteral feeding. The differences between subgroups were calculated, as well as the partial correlation coefficient between the distribution of developmental levels and each risk factor, based on the ordered logistic regression model ([table 3](#)). Significantly more favourable development was noted in the B-group than in the P-group subgroups, among the infants with a birth weight  $\geq 1000$  g, gestational age  $\geq 28$  weeks, caesarean delivery, antenatal steroid use, female sex or  $\geq 13$  days until full enteral feeding (see [table 3](#), online supplementary figure 1).

### DISCUSSION

Among this population of VLBW infants, the distribution of developmental outcomes at 18 months of age was significantly more favourable after OLB6378 administration, with a more pronounced benefit among infants with certain characteristics. The consistency between our present findings and those of the initial evaluation<sup>6</sup> suggests that VLBW infants administered *Bifidobacterium* during the NICU stay retained the potential beneficial effects of the treatment well past the 18-month follow-up visit.

Cormack *et al* reported that enteral protein intake in the 2 weeks after birth was positively correlated with neurological development,<sup>10</sup> but speculated that healthy infants received more nutritional support because they were deemed to be less sick and better able to tolerate higher nutritional intake. Although *Bifidobacterium* administration clearly accelerated the establishment of enteral feeding, we found no between-group difference in body weight, body length or head circumference at the 18-month medical

**Table 2** Outcomes at the 18-month medical check-up

Outcome	OLB6378 administration (n=102)		Placebo administration (n=105)		Regression analysis*		
	n	n (%) Mean±SD	n	n (%) Mean±SD	Partial correlation coefficient	OR	P values
Body weight, kg	98	9.3±1.7	103	9.2±1.2	0.177†		– 0.277 to 0.581 0.390
Body length, cm	97	77.1±4.3	103	77.2±4.2	–0.148†		–1.333 to 1.038 0.806
Head circumference, cm	80	46.3±2.2	93	46.5±1.8	–0.259†		–0.864 to 0.347 0.401
Use of O <sub>2</sub> , n (%)	102	6 (6)	102	6 (6)		1.000‡	0.311 to 3.210 1.000
Cerebral palsy, n (%)	100	4 (4)	100	10 (10)		0.375‡	0.114 to 1.238 0.108
Developmental test	89		79				
DQ18 score	54	90.6±12.5	65	91.1±14.4	–0.443†		–5.384 to 4.499 0.859
Distribution of developmental levels§	89	3.0±1.1	79	2.7±1.1	0.589¶		0.034 to 1.144 0.038
DQ18 score <85 or developmental test was unfeasible, n (%)	89	24 (27)	79	32 (41)		0.542‡	0.283 to 1.038 0.065

Use of O<sub>2</sub>, use of oxygen postdischarge (this include all discharged for home oxygen).

\*Treatment effect.

†Linear regression analysis.

‡Logistic regression analysis.

§Developmental levels were determined in terms of the DQ18 scores, which were either computed or inferred (if developmental testing was unfeasible or unnecessary).

¶Ordered logistic regression analysis.

DQ18, developmental quotient at 18 months of corrected age.

examination, which suggests that OLB6378 may simply promote the absorption of nutrients in the early postnatal period. Future research should examine the exact relationship between OLB6378-induced acceleration of enteral feeding in the early postnatal period (in particular, the absorption of nutrients such as docosahexaenoic acid and arachidonic acid) and potentially related acceleration of physical and neurological development.

Subgroup analyses with different background risk factors showed significantly better development for OLB6378 administration than for placebo administration among the infants with birth weight ≥1000 g or gestational age ≥28 weeks. Both birth weight and gestational age are known to be strong risk factors for impaired neurodevelopmental outcomes. Interestingly, in this investigation, relatively bigger infants showed more beneficial effects, probably due to the fact that such infants were generally less sick compared with others. In very sick infants, we expect it would be difficult to promote enteral nutrition even with *Bifidobacterium*. The probiotic benefit was also noted for the infants who achieved full enteral feeding within <13 days from birth. As antenatal steroid use is known to promote the maturation of intestinal function, combining antenatal steroids with postnatal probiotics may have a synergic effect on intestinal maturation among VLBW infants.

We found that the distribution of developmental levels was more favourable for probiotic administration than

for placebo administration among female infants, which was reflected in the incidence of cerebral palsy in these subgroups (B-group subgroup, 0 of 42 infants; P-group subgroup, 6 of 38 infants; P=0.030). It was previously reported that, in term small-for-dates infants, male sex was associated with a greater incidence of severe developmental disability, which was correlated with the IQ score at 4, 6 and 8 years of age.<sup>11</sup> Lucas *et al* reported major loss of cognitive potential only in preterm male infants who drank standard milk formula.<sup>12</sup> Interestingly, our present investigation found that neurological development is not affected by OLB6378 administration in male infants. Our results may support a previous study reported that, of 75 full-term children who predominantly harboured *Bifidobacterium* in the intestine during infancy, all 6 children diagnosed with a neuropsychiatric disorder were male.<sup>13</sup>

Maintaining a beneficial intestinal microbiota by administration of OLB6378 may contribute to neurological development through the brain-gut interaction, which is supported by our finding that this benefit is more prominent in infants delivered by caesarean section. Certain strains of intestinal bacteria have been associated with the development of autism,<sup>14</sup> and faecal levels of *Bifidobacterium* in children with autism are known to be low.<sup>15 16</sup> Mouse models of autism have also demonstrated the effectiveness of probiotic treatment with certain bacterial strains.<sup>17</sup> Interestingly, plasma adrenocorticotrophic hormone and corticosterone elevation in response

**Table 3** Subgroup analysis of outcomes at the 18-month medical check-up

DQ18 scores					Distribution of developmental levels*									
OLB6378 administration		Placebo administration		Mean difference	95% CI	P value†	OLB6378 administration		Placebo administration		Partial correlation coefficient	95% CI	P value‡	
n	Mean±SD	n	Mean±SD				n	Mean ± SD	n	Mean ± SD				
Gestational age														
<28weeks	30	89.4±11.6	28	90.8±12.3	-1.4	-7.7 to 4.9	0.657	43	2.8±1.1	34	2.7±1.0	0.23	-0.585 to 1.046	0.580
≥28weeks	24	92.1±13.6	37	91.3±15.9	0.9	-7.0 to 8.8	0.825	46	3.3±1.0	45	2.7±1.2	0.939	0.158 to 1.720	0.018
Birth weight														
<1000g	28	87.4±12.7	35	88.6±13.6	-1.1	-7.8 to 5.6	0.740	40	2.7±1.1	40	2.7±1.0	-0.006	-0.795 to 0.783	0.988
≥1000g	26	94.0±11.5	30	94.0±14.9	0.1	-7.2 to 7.3	0.986	49	3.4±0.9	39	2.7±1.2	1.083	0.275 to 1.891	0.009
Light-for-dates														
Yes	16	89.9±12.9	25	88.7±15.5	1.2	-8.2 to 10.6	0.796	30	3.0±1.1	28	2.6±1.1	0.696	-0.250 to 1.642	0.149
No	38	90.9±12.5	40	92.6±13.6	-1.6	-7.5 to 4.3	0.584	59	3.1±1.1	51	2.8±1.1	0.532	-0.154 to 1.218	0.129
Delivery														
Caesarean	17	92.1±13.8	55	90.4±13.8	1.8	-5.9 to 9.4	0.648	37	3.2±1.1	68	2.6±1.1	1.093	0.324 to 1.861	0.005
Vaginal	37	89.9±12.0	10	94.9±17.3	-4.9	-14.4 to 4.6	0.301	52	2.9±1.0	11	3.1±0.9	-0.26	-1.454 to 0.934	0.669
Apgar score at 5min														
≥8	36	90.7±13.0	27	94.1±14.6	-3.4	-10.3 to 3.6	0.339	66	3.1±1.1	31	2.9±1.1	0.518	-0.268 to 1.305	0.197
<8	18	90.4±11.8	38	88.9±14.0	1.5	-6.2 to 9.1	0.698	22	2.7±1.0	48	2.6±1.1	0.191	-0.720 to 1.102	0.681
Antenatal steroid use														
Yes	19	96.8±11.0	24	89.2±13.3	7.5	-0.1 to 15.2	0.054	31	3.2±1.0	31	2.7±1.1	1.108	0.161 to 2.055	0.022
No	35	87.3±12.1	41	92.1±15.0	-4.9	-11.2 to 1.4	0.129	58	2.9±1.1	48	2.8±1.1	0.331	-0.363 to 1.025	0.350
Sex														
Male	31	88.6±13.3	38	92.6±14.5	-4.1	-10.8 to 2.7	0.234	52	2.9±1.1	44	2.8±1.1	0.159	-0.566 to 0.884	0.667
Female	23	93.4±11.0	27	88.9±14.1	4.5	-2.8 to 11.8	0.218	37	3.3±0.9	35	2.6±1.1	1.219	0.333 to 2.104	0.007
Total parenteral nutrition														
Yes	53	90.2±12.2	62	90.6±14.3	-0.4	-5.4 to 4.5	0.862	75	2.9±1.0	70	2.7±1.0	0.376	-0.216 to 0.968	0.213
No	1	114	3	100.3±15.5	-	-	-	14	3.6±1.1	9	2.4±1.5	1.886	-0.070 to 3.842	0.059
Age at enteral feeding of 100mL/kg/day														
<13days	30	92.0±11.5	37	95.3±13.5	-3.3	-9.5 to 2.9	0.287	49	3.1±1.0	44	3.0±1.1	0.267	-0.483 to 1.017	0.485
≥13days	24	88.9±13.7	28	85.5±13.8	3.5	-4.2 to 11.2	0.369	40	2.9±1.1	35	2.4±1.1	0.996	0.154 to 1.837	0.020

\*Developmental levels were determined in terms of the DQ18 scores, which were either computed or inferred (if developmental testing was unfeasible or unnecessary).

†Student's t-test.

‡Ordered logistic regression analysis.

DQ18, developmental quotient at 18 months of corrected age.

to restraint stress was substantially higher in germ-free mice than in specific pathogen-free mice. However, the exaggerated hypothalamic-pituitary-adrenal stress response of germ-free mice was reversed by reconstitution with *Bifidobacterium infantis*.<sup>18</sup> Taken together, these findings clearly demonstrate that intestinal microbiota can act on the nervous system.

The present study is a follow-up investigation of a large cluster-randomised clinical trial, a post hoc safety type of study. A limitation of the study is that the sample size was not predecided via a power analysis specifically meant to facilitate detecting differences in neurodevelopmental outcomes between the groups. The primary outcome of the randomised controlled trial was the establishment of enteral feeding among VLBW infants. Another limitation is that we could only evaluate infants who were followed-up at the participating hospitals, although background characteristics were not different between infants evaluated and not evaluated.

Despite these limitations, the present report is the first to describe a clinical trial demonstrating that probiotics affect psychological development in the long term, suggesting that probiotics might be a candidate for neuroprotection, as predicted by Keunen *et al.*<sup>19</sup>

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**Competing interests** MT is employed by Meiji Co., Ltd.

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**Ethics approval** The study protocol and follow-up analysis were approved by the Ethics Committee of the Tokyo Women's Medical University (registration number 1675).

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**Data sharing statement** All the data in the study are available to researchers via a data request to the corresponding author.

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## REFERENCES

1. Kitajima H, Sumida Y, Tanaka R, *et al.* Early administration of *Bifidobacterium breve* to preterm infants: randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed* 1997;76:F101-7.
2. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev* 2014;CD005496.
3. Rao SC, Athalye-Jape GK, Deshpande GC, *et al.* Probiotic supplementation and late-onset sepsis in preterm infants: a meta-analysis. *Pediatrics* 2016;137:e20153684-16.
4. Costeloe K, Hardy P, Juszczak E, *et al.* *Bifidobacterium breve* BBG-001 in very preterm infants: a randomised controlled phase 3 trial. *Lancet* 2016;387:649-60.
5. Yamasaki C, Totsu S, Uchiyama A, *et al.* Effect of *Bifidobacterium* administration on very-low-birthweight infants. *Pediatr Int* 2012;54:651-6.
6. Totsu S, Yamasaki C, Terahara M, *et al.* *Bifidobacterium* and enteral feeding in preterm infants: cluster-randomized trial. *Pediatr Int* 2014;56:714-9.
7. Ishii N, Kono Y, Yonemoto N, *et al.* Outcomes of infants born at 22 and 23 weeks' gestation. *Pediatrics* 2013;132:62-71.
8. Kono Y, Yonemoto N, Kusuda S, *et al.* Developmental assessment of VLBW infants at 18 months of age: a comparison study between KSPD and Bayley III. *Brain Dev* 2016;38:377-85.
9. Palisano R, Rosenbaum P, Walter S, *et al.* Development and reliability of a system to classify gross motor function in children with cerebral palsy. *Dev Med Child Neurol* 1997;39:214-23.
10. Cormack BE, Bloomfield FH, Dezoete A, *et al.* Does more protein in the first week of life change outcomes for very low birthweight babies? *J Paediatr Child Health* 2011;47:898-903.
11. Fitzhardinge PM, Steven EM. The small-for-date infant. II. Neurological and intellectual sequelae. *Pediatrics* 1972;50:50-7.
12. Lucas A, Morley R, Cole TJ. Randomised trial of early diet in preterm babies and later intelligence quotient. *BMJ* 1998;317:1481-7.
13. Pärty A, Kalliomäki M, Wacklin P, *et al.* A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. *Pediatr Res* 2015;77:823-8.
14. Finegold SM, Downes J, Summanen PH. Microbiology of regressive autism. *Anaerobe* 2012;18:260-2.
15. Wang L, Christophersen CT, Sorich MJ, *et al.* Low relative abundances of the mucolytic bacterium *Akkermansia muciniphila* and *Bifidobacterium* spp. in feces of children with autism. *Appl Environ Microbiol* 2011;77:6718-21.
16. Adams JB, Johansen LJ, Powell LD, *et al.* Gastrointestinal flora and gastrointestinal status in children with autism--comparisons to typical children and correlation with autism severity. *BMC Gastroenterol* 2011;11:22.
17. Hsiao EY, McBride SW, Hsien S, *et al.* Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* 2013;155:1451-63.
18. Sudo N, Chida Y, Aiba Y, *et al.* Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol* 2004;558:263-75.
19. Keunen K, van Elburg RM, van Bel F, *et al.* Impact of nutrition on brain development and its neuroprotective implications following preterm birth. *Pediatr Res* 2015;77:148-55.