Table 2
Results of the neuropsychological tests (ITT)

	Baseline		16 weeks			
	Placebo	Probiotic	Placebo	Probiotic	Difference (95% CI)	*p*
RBANS total score	32.4 (7.5)	30.5 (10.6)	38.3 (13.0)	47.9 (13.4)	11.3 (6.7 to 15.8)	<0.0001
Immediate memory	36.4 (8.4)	36.9 (10.5)	38.7 (9.9)	48.2 (11.2)	9.2 (5.1 to 13.3)	<0.0001
Visuospatial/Constructional	34.4 (14.4)	32.1 (13.2)	35.8 (13.5)	46.2 (10.0)	11.4 (6.8 to 16.0)	<0.0001
Language	47.3 (7.8)	49.9 (10.7)	50.1 (8.8)	54.2 (8.1)	3.5 (–0.2 to 7.2)	0.064
Attention	49.2 (10.0)	45.7 (11.0)	53.3 (11.8)	51.1 (10.2)	0.5 (–2.7 to 3.8)	0.74
Delayed memory	31.1 (12.3)	31.1 (12.0)	34.6 (13.5)	45.6 (14.2)	11.0 (6.6 to 15.3)	<0.0001
JMCIS score	63.2 (7.5)	61.3 (9.2)	59.9 (9.9)	62.6 (8.4)	3.5 (0.2 to 6.9)	0.052

Baseline (Placebo: $n = 40$, Probiotic: $n = 40$), 16 weeks (Placebo: $n = 39$, Probiotic: $n = 40$). Values are indicated as mean (SD). Differences are indicated by changes of LSM between Placebo and Probiotice at 16 weeks. Effect of Probiotic was indicated in intergroup difference (95% CI) and p value by ANCOVA in intention-to-treat (ITT) analysis. RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; JMCIS, The Japanese version of the MCI Screen.

Table 3
Results of the neuropsychological tests (PP)

	Baseline		16 weeks			
	Placebo	Probiotic	Placebo	Probiotic	Difference (95% CI)	*p*
RBANS total score	32.4 (7.5)	30.4 (10.7)	38.3 (13.0)	48.0 (13.6)	11.5 (6.9 to 16.1)	<0.0001
Immediate memory	36.4 (8.4)	36.9 (10.6)	38.7 (9.9)	48.5 (11.2)	9.5 (5.4 to 13.6)	<0.0001
Visuospatial/Constructional	34.4 (14.4)	32.0 (13.4)	35.8 (13.5)	46.0 (10.1)	11.3 (6.6 to 15.9)	<0.0001
Language	47.3 (7.8)	49.8 (10.8)	50.1 (8.8)	53.9 (8.0)	3.2 (–0.5 to 6.9)	0.085
Attention	49.2 (10.0)	45.6 (11.1)	53.3 (11.8)	51.1 (10.4)	0.7 (–2.6 to 4.0)	0.67
Delayed memory	31.1 (12.3)	31.3 (12.0)	34.6 (13.5)	45.9 (14.3)	11.1 (6.6 to 15.5)	<0.0001
JMCIS score	63.2 (7.5)	61.4 (9.3)	60.5 (9.9)	63.0 (8.2)	3.5 (0.2 to 6.9)	0.036

Baseline (Placebo: $n = 40$, Probiotic: $n = 39$), 16 weeks (Placebo: $n = 39$, *Probiotic*: $n = 39$). Values are indicated as mean (SD). Differences are indicated by changes of LSM between Placebo and *Probiotic* at 16 weeks. Effect of Probiotic was indicated in intergroup difference (95% CI) and p value by ANCOVA in per-protocol (PP) analysis. RBANS, Repeatable Battery for the Assessment of Neuropsychological Status, JMCIS, The Japanese version of the MCI Screen.

RESULTS

Out of 80 final study participants, 79 subjects completed the study. The rate of consumption of the supplements for 16 weeks was considerably high in the 79 participants (>99.9%). Baseline characteristics of the participants were pretty much identical, and there were no significant baseline differences between groups (Table 1). Besides having mild cognitive function impairment, all participants were physically healthy, non-obese, older adults with no blood pressure abnormalities.

Cognitive function primary outcomes

Table 2 shows the results of the neuropsychological tests from baseline to 16 weeks after probiotic or placebo consumption as for ITT analysis. RBANS total score was significantly improved by 11.3 points by *B. breve* A1 (95% CI 6.7 to 15.8, $p < 0.0001$). Each memory domain score was analyzed. As shown in Table 2, compared to placebo group, immediate memory, visuospatial/constructional score, and delayed memory were improved by 9.2 points (95% CI 5.1 to 13.3, $p < 0.0001$), 11.4 points (95% CI 6.8 to 16.0, $p < 0.0001$), and 11.0 points (95% CI 6.6 to 15.3, $p < 0.0001$), respectively, by *B. breve* A1 consumption. Language shows a trend of improvement over placebo in the probiotic group (95% CI –0.2 to 7.2, $p = 0.064$). For the attention parameter, no improvement was observed after consumption (95% CI –2.7 to 3.7, $p = 0.74$). RBANS results as for PP analysis were very similar to ITT analysis (Table 3). Figure 2 shows the changes of RBANS scores at 16 weeks from baseline. Significant inter-group difference was observed in RBANS total scores and the domain scores of visuospatial/constructional and delayed memory and immediate memory (Fig. 2A, B). The secondary outcome (JMCIS) was also improved in the probiotic group over placebo in ITT analysis ($p = 0.052$, Table 2) and PP analysis ($p = 0.036$, Table 3).

Safety evaluation

Results of the hematological and biological blood parameters comparison between baseline and after 16 weeks of consumption did not show any signifi-

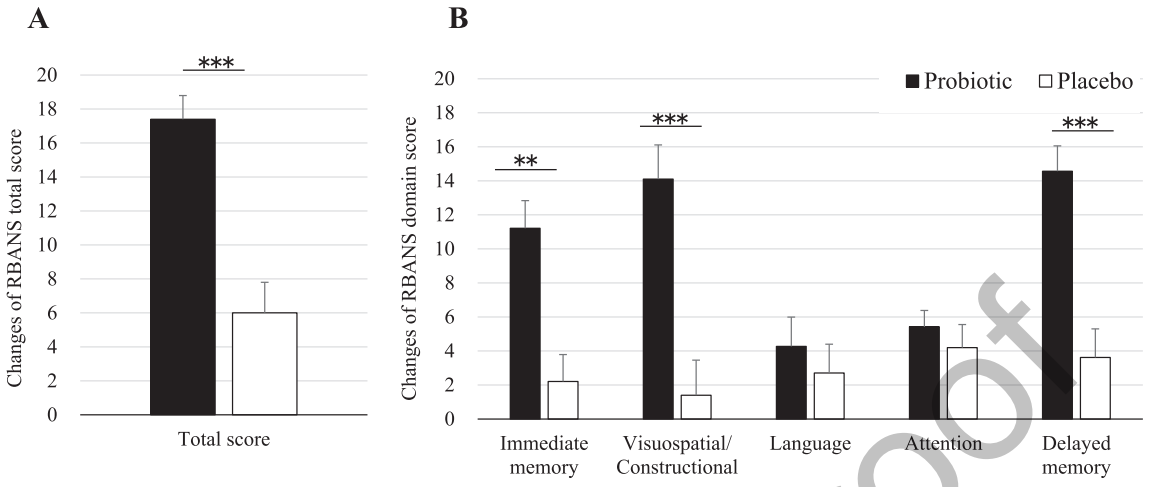

Fig. 2. Changes of RBANS scores at 16 weeks from baseline. Values are indicated as mean with error bars as SE. $^{**}p < 0.001$, $^{***}p < 0.0001$, inter-group difference, Student's *t*-test. RBANS, Repeatable Battery for the Assessment of Neuropsychological Status.

cant changes. Vital signs that include blood pressure and heart rate were also unchanged. Reported adherence was 97.5% and 100% in placebo and probiotic groups, respectively. No study related adverse events occurred.

DISCUSSION

The present study is the first double-blind, placebo-controlled study in humans to show the cognitive function enhancement benefit of the probiotic *B. breve* A1 in subjects with suspected MCI. Primary (RBANS) and secondary endpoints (JMCIS) were both met after 16 weeks of consumption in this population. The treatment was well-tolerated with no reported side-effects. We used RBANS in our study because, since its introduction in 1998, it has been proven sensitive at both detecting and characterizing very mild cognitive impairment, to distinguish dementia of different etiologies and to characterize MCI due to AD [17]. It has also been used in previous clinical studies as a sensitive measure of cognitive improvement after treatment with food products such as PUFA in the MCI population [18].

A significant improvement of cognitive functions was observed for treated participants over placebo in our study. RBANS score showed a significant 11.3-point improvement with *B. breve* A1 compared to placebo ($p < 0.0001$). RBANS domain scores were also improved: immediate memory ($p < 0.0001$), visuospatial/constructional ($p < 0.0001$), and delayed memory ($p < 0.0001$). Only language and attention domain scores saw no improvement over placebo. These data indicate that RBANS seems to be a sensitive and useful neuropsychological test to evaluate the central effects of probiotics on the memory of suspected MCI subjects and the effect of the probiotic strain in improving memory functions such as the awareness of who, when and where. The 11.3-point improvement seen after 16 weeks of *B. breve* A1 is remarkable. In comparison, dietary supplementation of arachidonic and docosahexaenoic acids in a similar MCI population showed a significant improvement of around 6 points in RBANS immediate memory score after 13 weeks [18]. Another clinical study that evaluated *Lactobacillus* fermented milk consumption effect on memory found a small but significant change of four points on the domain score of attention but no significant difference on RBANS total score [19]. Future longer longitudinal studies with *B. breve* A1 may reveal further tangible memory improvement.

A reduction of medial temporal lobe (MTL) volumes and a significantly smaller hippocampus have been previously demonstrated in MCI individuals [20]. MCI subjects also have a larger inferior lateral ventricle volume than usual. MTL volumes are significantly related to the RBANS immediate and delayed memory scores in a previous study [21], and both scores were significantly improved after *B. breve* A1 consumption. The MTL and the hippocampus are critical for short-term and long-term memory, which suggests that *B. breve* A1 is causing positive changes to the MCI subjects hippocampus, something that we also observed in our previous pre-clinical study [14]. In that study, *B. breve* A1 improved mouse memory and suppressed the hippocampal expressions of

inflammation and immune-reactive genes that are induced by amyloid-β, and this has been reported recently also using other *Bifidobacterium* strain [22]. While we cannot investigate gene expression alteration in the hippocampus of living individuals, we speculate that *B. breve* A1 may have caused similar changes in treated study participants. Future positron emission tomography imaging studies (PET) using TranSlocatorPrOtein (TSPO), a marker of brain inflammation that was used in MCI subjects [23] to study microglial activation/inflammation, would be helpful to visualize *B. breve* A1 extent effect on the brain non-invasively. Taking into account the possibility that MCI is closely associated with the immune system and inflammation, it will be valuable in future studies to assess the effectiveness of our probiotic in MCI patients with underlying conditions that include vascular impairment or cancer.

Bifidobacterium, including its metabolite acetate, a short-chain fat acid, has been shown to modulate the gut microbiota and the immune system [24, 25]. In our previous pre-clinical study, we found that non-viable components of the bacterium or its metabolite acetate partially ameliorated the cognitive decline observed in an AD mouse model [14]. In that study, the administration of *B. breve* A1 increased the plasma acetate levels in treated animals. Although our present study did not compare the alteration of the gut microbiota after treatment, there is a possibility that the cognitive improvement we observed comes from a change of the gut microbiota towards less pro-inflammatory gut bacteria species, many of which are known to release lipopolysaccharides (LPS) and other metabolites leading to microglia activation in the brain [12]. Gut microbiota alteration via microbial-derived indole derivatives production is also associated with intestinal epithelial barrier integrity and modulation of intestinal inflammation [12], which may result in microglia modulation in the brain of MCI subjects after *B. breve* A1 consumption. Recently, we demonstrated that human-residential bifidobacteria, including strain *B. breve* A1, are potential producers of indole-3-lactic acid, a metabolite that has an anti-inflammatory effect and that is also possibly involved in host-microbiota crosstalk and neuronal developmental processes [26–28].

Changes in brain BDNF have been reported for probiotics such as *Bifidobacterium* in rodent experiments [29] and have a beneficial effect on genes and inflammation pathways involved in neurological disorders [30]. BDNF serum levels are elevated in MCI and AD compared to healthy individuals [31], so it is not clear if future studies could measure serum BDNF to understand how *B. breve* A1 consumption leads to memory improvement in MCI subjects. Fecal microbiota comparison after treatment in humans, as well as LPS production by the gut microbiota, will help in future studies to shed light on the mechanism of action of this probiotic strain on cognition and inflammation. This is of interest since manipulations of pre-clinical mouse models of AD using germ-free conditions and alterations of the gut microbiota with antibiotics have shown the importance of gut bacteria to influence amyloid deposition in the brain as well as stimulating microglia activation, a significant contributor to brain inflammation and cognitive impairment in MCI [32].

In our next clinical trials, we will conduct exploratory biomarker studies using blood, cerebrospinal fluid, and feces to fully understand how *B. breve* A1 is causing an amelioration of memory in the MCI population as well as evaluate its potential to treat AD dementia. The identification of the precise mechanism would also shed much-needed light on what is causing dementia and related CNS disorders and potentially help justify further intervention studies in various neuropathologies.

No drugs are currently approved for treating MCI, as opposed to dementia. Approved symptomatic drugs for AD such as donepezil, rivastigmine, and galantamine were thought to potentially help with symptoms of MCI, or slow its progression to dementia, but clinical trials data turned negative. The identification of effective treatment of MCI subjects is, therefore, a pressing unmet medical need. In our study, *B. breve* A1 showed a clear and significant improvement of RBANS total score, in particular for domain scores of immediate memory, visuospatial/constructional and delayed memory, and JMCIS after only 16 weeks in the suspected MCI population. This finding, although seeking subsequent confirming studies, could signal a profound shift as to how MCI can be treated and perhaps, as a result, prevent the development of cognitive impairment by an affordable and safe solution to the general population.

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