Primary Dietary Intervention Study to Reduce the Risk of Islet Autoimmunity in Children at Increased Risk for Type 1 Diabetes

The BABYDIET study

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OBJECTIVE—To determine whether delaying the introduction of gluten in infants with a genetic risk of islet autoimmunity is feasible, safe, and may reduce the risk of type 1 diabetes–associated islet autoimmunity.

RESEARCH DESIGN AND METHODS—A total of 150 infants with a first-degree family history of type 1 diabetes and a risk HLA genotype were randomly assigned to a first gluten exposure at age 6 months (control group) or 12 months (late-exposure group) and were followed 3 monthly until the age of 3 years and yearly thereafter for safety (for growth and autoantibodies to transglutaminase C [TGCAs]), islet autoantibodies to insulin, GAD, insulinoma-associated protein 2, and type 1 diabetes.

RESULTS—Adherence to the dietary-intervention protocol was reported from 70% of families. During the first 3 years, weight and height were similar in children in the control and late-exposure groups, as was the probability of developing TGCAs (14 vs. 4%; $P = 0.1$). Eleven children in the control group and 13 children in the late-exposure group developed islet autoantibodies (3-year risk: 12 vs. 13%; $P = 0.6$). Seven children developed diabetes, including four in the late-exposure group. No significant differences were observed when children were analyzed as per protocol on the basis of the reported first gluten exposure of the children.

CONCLUSIONS—Delaying gluten exposure until the age of 12 months is safe but does not substantially reduce the risk for islet autoimmunity in genetically at-risk children.

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Type 1 diabetes is an autoimmune disease with a preclinical phase characterized by the presence of islet autoantibodies (1). Genetic susceptibility for islet autoimmunity is well documented (2), and environmental factors are assumed to modify the genetically defined risk of developing islet autoantibodies (1,3).

Data from mouse models of autoimmune diabetes support a role for gluten in modifying autoimmune diabetes risk, with deprivation of gluten or even delayed introduction resulting in later and less frequent development of diabetes (4,5). In humans, prospective studies (6,7) show that the age at introduction of solid food, such as gluten-containing foods or cereals, affects the development of islet autoimmunity in children who are genetically susceptible to type 1 diabetes. Two studies report increased risk of islet autoimmunity in children who are exposed to gluten before the 4th month, and one of the studies also demonstrates increased risk when gluten exposure is delayed beyond age 6 months (6,7). Furthermore, intervention in islet autoantibody–positive children indicates that β-cell function may be improved by a deprivation of gluten for 6 months (8). Gluten is a driving antigen of celiac disease. There is no association between early gluten exposure and risk for autoantibodies to transglutaminase C (TGCAs), which is a marker of celiac disease (9,10), but one study reports an increased risk for TGCAs in children who were first exposed to cereals after the age of 7 months (10).

We performed a dietary primary pilot intervention study to determine whether delaying the introduction of gluten to the diet may be beneficial in reducing the risk of type 1 diabetes–associated islet autoimmunity in children with a predetermined genetic risk of islet autoimmunity, which was ~15% of children (11). We specifically assessed the feasibility of such an intervention, the safety with respect to growth, the development of gluten-driven celiac disease, and, as a pilot efficacy measure, the cumulative frequency of islet autoimmunity by age 3 years.

RESEARCH DESIGN AND METHODS

Eligibility criteria

Children from Germany were eligible to participate in the BABYDIET study if they were younger than 2 months of age, not yet exposed to gluten, and had at least two first-degree relatives with type 1 diabetes or one first-degree relative with type 1 diabetes and one of the following type 1 diabetes–associated HLA genotypes:

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*DRB1*03-DQA1*0501-DQB1*0201/DRB1*04-DQA1*0301-DQB1*0302;*
*DRB1*04-DQA1*0301-DQB1*0302/DRB1*04-DQA1*0301-DQB1*0302;*
*DRB1*03-DQA1*0501-DQB1*0201/DRB1*03-DQA1*0501-DQB1*0201;*
*DRB1*04-DQA1*0301-DQB1*0302/DRB1*08-DQA1*0401-DQB1*0402; or*
*DRB1*04-DQA1*0301-DQB1*0302/DRB1*01-DQA1*0101 -DQB1*0501*

Written informed consent for genetic screening as well as for enrollment into the intervention trial was provided by the infant's primary caretakers. Infants were excluded from the study if they had an illness or birth defect that precludes long-term follow-up. After inclusion, children were followed in 3-monthly intervals until the age of 3 years and yearly thereafter for efficacy and safety assessment. The trial was conducted at the Diabetes Research Institute (Munich, Germany) and was approved by the ethics committee of the Ludwig-Maximilian University, Munich, Germany (Ethikkommission der Medizinischen Fakultät der Ludwig-Maximilians Universität no. 329/00).

Intervention

Children were randomly assigned to be introduced to gluten at the age of 6 months (n = 77, control group), which corresponds to the national recommendations for early infant feeding, or to delay the introduction of gluten until the age of 12 months (n = 73, late-exposure group). Random assignment was performed independently using the minimization method and included stratification for HLA genotype, a family member with type 1 diabetes, and the sex of the participating child to achieve balance between both groups. At inclusion, each participating family was visited by a nutritionist who explained the gluten-free diet. Lists of the most common foods introduced during the first year of life were examined, and gluten-containing products were indicated. Details of gluten-free commercial infant products were provided to the parents. Until the age of 1.5 years, daily food records were used to assess compliance to the intervention, to estimate the dose of gluten at first exposure, and to record the age at introduction of other food items.

Safety parameters

Safety parameters included the analysis of celiac disease–associated IgA autoantibodies to tTGCA and data on growth (height and weight). tTGCAs were determined in samples at the age of 6, 12, 18, 24, 30, and 36 months and yearly thereafter. Positivity was defined as positive for tTGCAs in two consecutive samples. Data on weight and height were obtained at the age of 3, 6, 12, 24, and 36 months by a physician during the clinical study visit.

End-point assessment

The primary end point was the development of persistent autoantibodies to one or more of the antigens insulin, GAD65 or insulinoma-associated protein 2 (IA-2A). Persistence was defined as being positive in at least two consecutive samples and in the last available sample. Islet autoantibodies were measured in venous blood samples from all scheduled visits. Diabetes development was monitored and diagnosed according to the American Diabetes Association Expert Committee criteria (12).

Confounding variables

Data on breastfeeding (yes or no), the duration of breastfeeding (weeks), and the introduction of solid food (gluten-free and gluten-containing cereals, vegetables, and fruits) were taken from daily food records completed by the child's parents.

Laboratory testing

HLA-DRB1, *HLA-DQA1*, and *HLA-DQB1* were determined using PCR-amplified DNA and nonradioactive sequence-specific oligonucleotide probes, as described previously (13). Islet autoantibodies and antibodies to tissue transglutaminase also were measured, as described previously. Insulin autoantibodies (IAAs) were measured by protein A/G-radiobinding assays using ^{125}I-labeled recombinant human insulin labeled at tyrosine aa 14 (14), and GAD antibodies and insulinoma-associated protein 2 antigens (IA-2As) were measured by protein A radiobinding assays using [^{35}S]methionine-labeled in vitro transcribed/translated recombinant antigen (15). tTGCAs were measured using the enzyme-linked immunosorbent assay (Eurospital, Trieste, Italy) and confirmed by the radiobinding assay (9). Samples with values above the 99th percentile of control children were defined as positive. The interassay coefficient of variation values for samples with low autoantibody levels were 11% (IAAs), 18% (GAD antibodies), and 16% (IA-2As).

Statistical analysis

The study was designed to primarily test the feasibility of a dietary-intervention study in infants at high genetic risk for islet autoimmunity and type 1 diabetes. The study was not powered to examine efficacy other than for major differences between intervention and control groups. With the assumption that 15% of the control group will develop islet autoantibodies and 8% will develop tTGCAs, the power of the study was 40% to detect a halving of the probability for islet autoantibodies (α = 0.05) and 45% to detect a doubling of the tTGCA autoantibody probability.

The probability of developing islet autoantibodies or tTGCAs was estimated by Kaplan-Meier analysis. Hazards ratios (HRs) were determined using the Cox proportional hazards model. The age of onset of islet autoantibodies or tTGCA positivity was defined as the age at the first positive sample. Groups were compared on the intention-to-treat principle (control versus late-exposure groups), as well as according to the true gluten exposure date by per-protocol principle using the following categories: first gluten exposure between 4.5 and 7.4 versus 10.5 and 13.5 months or by using age at first gluten exposure (months) as a continuous variable. HRs were adjusted for the following confounding variables: *1*) duration of breastfeeding (0–3.0 vs. >3.0 months); *2*) still breastfeeding at first gluten exposure (yes or no); *3*) age at first exposure to solid food (≤5.5 vs. >5.5 months); and *4*) number of days with gluten exposure in the 4 weeks after the first gluten exposure (<13 vs. ≥13 days). All P values were two-tailed. Statistical analyses were performed using the Statistical Package for Social Science (PASW Statistics 18.0; SPSS, Inc., Chicago, IL).

RESULTS

Feasibility and compliance

Between 2000 and 2006, 1,168 offspring or siblings of patients with type 1 diabetes were screened for eligibility (Fig. 1). Of those, 169 were eligible and 150 agreed to participate in the study and were randomly assigned to the control (n = 77) or late-exposure (n = 73) groups. Subject characteristics are shown in Supplementary Table 1.

Of 150 participating children, 120 (63 control group children and 57 late-exposure group children) completed the follow-up to at least age 3 years or had reached the study end point (persistent islet autoantibody-positive) before the age of 3 years (follow-up range 3.0–10.0). Eight children (three in the control group

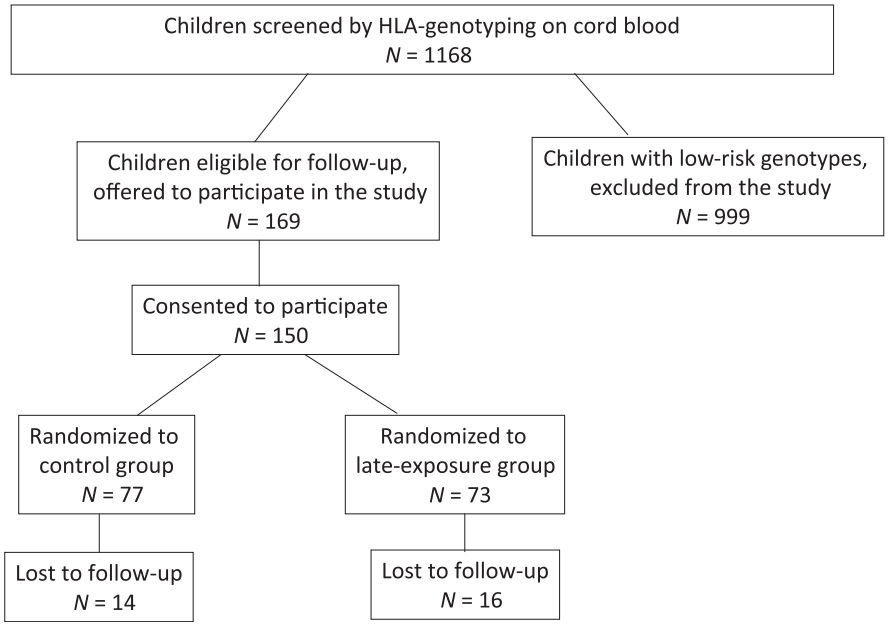

Figure 1—*Flowchart of the BABYDIET study population.*

and five in the late-exposure group) withdrew from the study during the intervention period (up to12 months of age).

The age at first gluten exposure was provided for 140 children (68 children from the late-exposure group and 72 children from the control group). The overall median age of gluten exposure was 10.0 and 7.0 months (interquartile range 6.0–9.9) for those in the control group and 11.9 months (10.8–12.0) for those in the late-exposure group ($P < 0.0001$) (Fig. 2*A*). A total of 23 (32%) children in the control group were not introduced to gluten within the specified time interval of 4.5–7.5 months (4 were introduced to gluten earlier, and 19 were introduced later), and 18 (26%) children in the late-exposure group were not introduced to gluten in the specified 10.5- to 13.5-month time interval (15 were introduced to gluten earlier, and 3 were introduced later). Data on the age at introduction of any solid food and the duration of full breastfeeding were available in 136 and 144 children, respectively (Fig. 2*B* and *C*). The age of introducing solid food was similar in the control (median 5.8 months) and late-exposure (median 5.6 months) groups ($P = 0.65$). The median duration of full breastfeeding was 2 weeks (interquartile range 0–22) in the control group and 10 weeks (0–25) in the late-exposure group ($P = 0.09$).

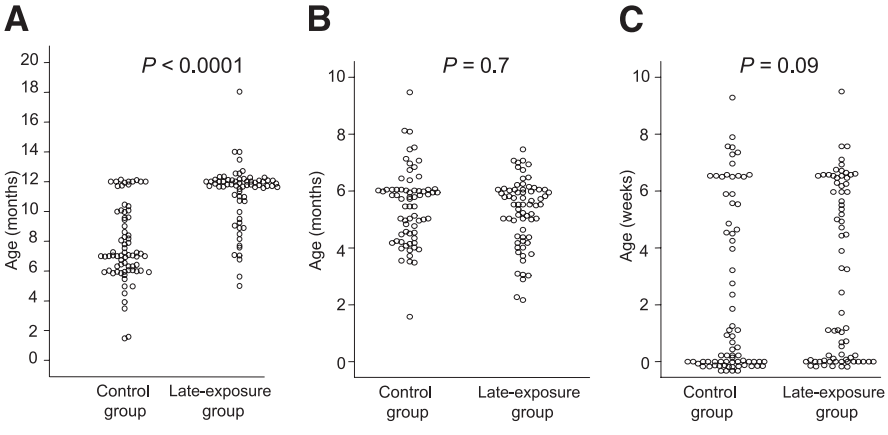

Figure 2—*Age at first exposure to* (A) *gluten-containing food,* (B) *any solid food, and* (C) *full breastfeeding duration in children randomly assigned to the late-exposure and control groups.*

Safety assessments

Effect of delayed gluten introduction on celiac disease–associated tTGCAs. By the age of 3 years, eight children in the control group and three children in the late-exposure group developed persistent tTGCAs. The cumulative risk of developing tTGCAs at age 3 years was 14% (95% CI 4.2–23.8) in the control group and 4% (0.1–9.9; $P = 0.1$) in the late-exposure group (Fig. 3*A*). An additional six children developed tTGCAs after age 3 years (two children in the control group and four in the late-exposure group). Three of the tTGCA-positive children of the control group underwent a biopsy, and celiac disease was diagnosed in two of them. In the late-exposure group, four children had a biopsy and all had celiac disease.

Effect of delayed gluten introduction on growth. The development of height and weight during the first 3 years of life did not differ between the control and late-exposure groups (Fig. 3*B*). Weight gain during the first year of life (control group: 5,630 g vs. late-exposure group: 5,640 g; $P = 0.6$) and from age 12 to 48 months (control group: 7,070 g vs. late-exposure group 7,230 g; $P = 0.9$) was comparable between groups. The prevalence of overweight (BMI percentile ≥90 as determined by the German reference system [16]) was similar between groups (age 2 years: 4 of 56 children in the control group vs. 4 of 49 children in the late-exposure group; $P = 1.0$; data not shown).

Effect of delayed gluten introduction on islet autoimmunity outcome and type 1 diabetes: intention-to-treat analysis. A total of 16 children developed islet autoantibodies during the first 3 years, including 8 in the control group and 8 in the late-exposure group; 11 of 16 children developed more than one islet autoantibody (6 in the control group and 5 in the late-exposure group). An additional eight children developed islet autoantibodies after the age of 3 years (five children in the late-exposure group). Six children developed autoantibodies prior to their first reported gluten exposure. The probability of developing any islet autoantibody (IAAs, GAD antibodies, and/or IA-2As) by the age of 3 years in the total cohort was 12% (95% CI 6.1–17.9) and was 12% in the control (4.2–19.8) and 13% in the late-exposure (5.2–20.8; $P = 0.6$) groups (Fig. 4*A*). The probability of developing multiple islet autoantibodies also was similar between groups (control group: 9.0% by age 3 years; late-exposure group:

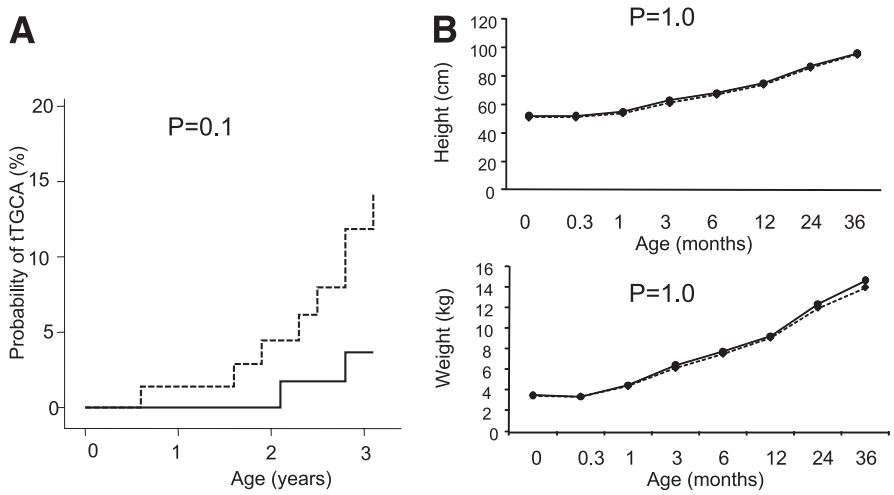

Figure 3—*Safety assessments.* A*: Probability of tTGCAs by Kaplan-Meier analysis in offspring randomly assigned to the BABYDIET late-exposure group* (solid line*) and the BABYDIET control group* (broken line*).* B*: Height (centimeters) and weight (kilograms) development from birth to 36 months of age in offspring randomly assigned to the BABYDIET late-exposure group* (solid line*) and the BABYDIET control group* (broken line*).*

8.0%; $P = 0.7$) (Fig. 4*B*), as was the probability of developing IAAs and GAD antibodies (Supplementary Fig. 1). The adjusted HR for developing islet autoantibodies was 1.3 (95% CI 0.6–3.0) for the late-exposure group after adjusting for confounding variables (breastfeeding duration, breastfeeding during first gluten exposure, introduction of solid food, and gluten dosage after the first exposure). Seven children developed diabetes (three in the control group and four in the late-exposure group).

Per-protocol analysis. Approximately 30% of families did not follow protocol guidelines and introduced gluten outside the time period assigned by randomization. We therefore analyzed the risk to develop islet autoantibodies and tTGCAs according to the reported gluten exposure age (Supplementary Fig. 2*A* and *B*). Children who were exposed to gluten between 10.5 and 13.5 months of age ($n = 63$) had a similar 3-year islet autoantibody risk (12%) compared with children who were exposed to gluten between 4.5 and 7.5 months of age ($n = 44$; 13%; $P = 0.7$). tTGCA risk by 3 years also was nonsignificantly different in children exposed to gluten late (6%) versus early (18%; $P = 0.08$). When age at first gluten exposure was considered as a continuous variable, the age at gluten exposure did not influence the probability of developing islet autoantibodies (per month delay in gluten exposure: HR 1.08 [95% CI 0.9–1.3]; $P = 0.4$) or tTGCAs (0.92 [0.73–1.16]; $P = 0.5$).

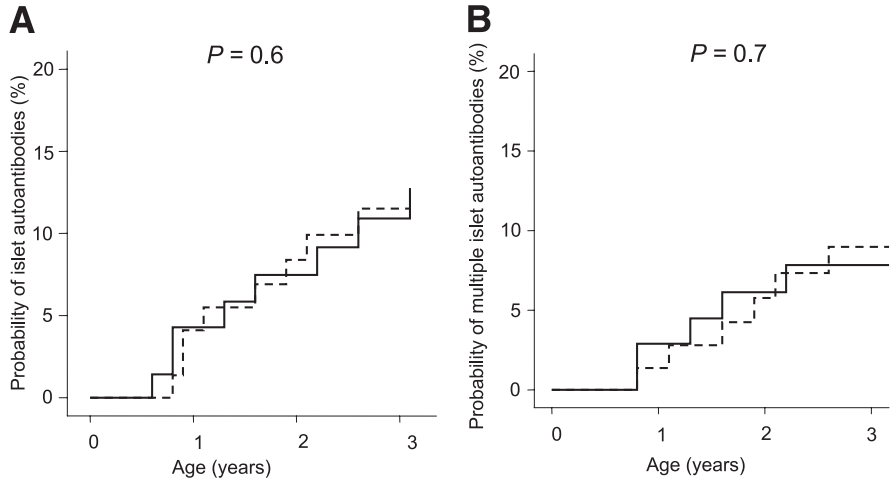

Figure 4—*Outcome analysis. Probability of* (A) *any islet autoantibody (IAAs, GAD antibodies, and/or IA-2As) and* (B) *multiple islet autoantibody frequency by Kaplan-Meier analysis in offspring randomly assigned to the BABYDIET late-exposure group* (solid line*) and the BABYDIET control group* (broken line*).*

CONCLUSIONS—The BABYDIET study is a pilot study to determine whether delaying the introduction of gluten to the diet of neonates until 12 months of age is a feasible strategy to reduce the risk of islet autoimmunity in children with a high genetic risk for type 1 diabetes. The study found that 30% of families reported that they did not adhere to the gluten introduction protocol for their randomization group. Nevertheless, late gluten introduction, whether by randomization group or as reported by families, did not increase the risk for tTGCAs and did not affect growth, suggesting that it is relatively safe. Although the study was not powered to examine efficacy other than for major differences between intervention and control groups, the number of islet autoantibody–positive children and the number of cases of diabetes in the late-exposure group and the control group were very similar. These data suggest that delaying gluten exposure until the age of 12 months is safe but will not substantially reduce the prevalence of islet autoimmunity in genetically at-risk children.

Previous observations showed an increased risk of islet autoimmunity when gluten-containing cereals (6) or any cereals (7) were introduced during the first 3 months of life in children with increased genetic risk for type 1 diabetes. Because Germany and many other countries recommend introducing gluten at 6 months of age, we were unable to design a study to formally compare intervention with 3 months versus later gluten introduction. Hence, our design was to extend the gluten-free exposure to 12 months of age. Recruitment was not problematic, with the majority of eligible families consenting to participate in the intervention. Retention, despite the intensive follow-up of the protocol, also was high (80%). Families were well instructed as to the foods that contain gluten. In addition, study members were in contact with families weekly to discuss issues related to feeding during the study. However, randomization was not blinded, and ~30% of families did not adhere to the protocol for their assigned randomization

group. Over half of the lack of compliance was attributed to families opting for a delayed gluten introduction, potentially as a result of the perceived positive effects of intervention over control. Thus, dietary intervention in early infancy appears feasible but will likely be affected by a relatively high rate of noncompliance, unless blinding of diet is possible.

Safety was a potential issue in the current study because previous reports had suggested a link between delayed gluten introduction and an increase in celiac disease incidence (17). Moreover, and in contrast to our previous findings (6,9), the DAISY (Diabetes Autoimmunity Study in the Young) study found that delaying gluten introduction to later than age 7 months was associated with an increase in the risk for tTGCAs (10) and islet autoantibodies (7). Our current study found no increase in tTGCA risk in children of the late-exposure group also when the analysis was performed on a per-protocol basis. Indeed, although not significant, the risk of tTGCAs in children within the late-exposure group was around one-third that of children in the control group. Growth also was unaffected by the intervention. Thus, we conclude that delay of gluten to the diet is safe and we cannot confirm previous findings suggesting that it will increase the risk for celiac disease.

We found no evidence of a benefit with respect to reducing the risk for islet autoantibodies. Similar to the TRIGR (Trial to Reduce Insulin-Dependent Diabetes Mellitus in the Genetically at Risk) pilot study (18), the BABYDIET study was not powered for efficacy. The power of the study was ~40% to detect a 50% reduction in the probability of islet autoantibodies. Increasing the power to 80% would require at least three times the number of participants, and in view of the difficulty with compliance, it appears justified to have conducted a pilot feasibility study first. Nevertheless, even with the limited power of the study, we do not recommend altering current pediatric guidelines with respect to the introduction of gluten into the diet of children who are genetically at risk for type 1 diabetes.

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S.H. researched data, contributed to the discussion, and wrote the manuscript. M.P. and M.H. researched data and contributed to the discussion. E.B. and A.-G.Z. researched data, contributed to the discussion, and reviewed and edited the manuscript.

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