

The Role of Asprosin and Ceramides in the Development of Growth Hormone Deficiency in Children

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Abstract

Background: Growth hormone deficiency (GHD) is an endocrine condition, which is defined as a reduction in growth hormone (GH) synthesis; this study aims to evaluate the level of asprosin and ceramides in children (patients) with idiopathic isolated GHD. **Materials and Methods:** The present study was designed as a case-control study; 43 patients (24 males and 19 females) with idiopathic isolated GHD were involved in this study. Forty-five subjects (26 males and 19 females) who were apparently healthy control that enrolled in this study were matched with patients of the same sex and age. **Results:** The present study revealed a significant decrease ($P < 0.05$) in the levels of asprosin, ceramides, and glucose in patients with GHD when compared with its control group. **Conclusions:** Asprosin may be one of the underlying causes of GHD through its indirect role in releasing GH. GHD may decrease the level of ceramides by the effect of GH on lipid metabolism.

Keywords: Asprosin, ceramides, GHD, growth hormone deficiency

INTRODUCTION

Growth hormone (GH), known as “somatotropin,” is an anabolic hormone produced and secreted from somatotroph cells, which is found in the anterior lobe of the pituitary gland^[1]; GH regulates various metabolic aspects, including glucose homeostasis, fat mobilization, and oxidation; it also promotes protein synthesis and cell proliferation, as well as tissue and body growth.^[2] Growth hormone deficiency (GHD) is an endocrine condition, which is defined as a reduction in GH synthesis that results in a reduction in the production of GH-dependent hormones and growth factors, such as insulin-like growth factor-I (IGF-I), IGF-II, and their binding proteins.^[3] Children with GHD have appropriate body proportions when compared with peers of the same age and gender, although they are generally chubbier, shorter, and appear younger than their age.^[4,5] GHD may be congenital or acquired and may be isolated or combined with other pituitary hormone deficiencies (combined pituitary hormone deficiency).^[6]

Asprosin is a gluconeogenic hormone synthesized and released mainly by white adipose tissue, which was discovered and first identified as a novel glucogenic

protein adipokine by Romere *et al.* in a study of neonatal progeroid syndrome patients in 2016.^[7] The liver is asprosin's primary target organ, which it activates to create and release glucose.^[7] Asprosin stimulates glucose synthesis in the liver via the Olfactory 734 (Olfr734) receptor in both fasting and obese people.^[8] In addition, asprosin has a role in appetite regulation. According to Duerschmid *et al.*,^[9] asprosin penetrates the blood-brain barrier and influences appetite stimulation directly by triggering orexigenic agouti-related peptide (AgRP) neurons in the hypothalamus, as well as indirectly by inhibiting arcuate proopiomelanocortin anorexigenic neurons. Asprosin's impact has been demonstrated to be associated with the activation of the Gs-cAMP-protein kinase A (PKA) axis; AgRP+ neurons regulate energy homeostasis and food intake. The orexigenic neuropeptide AgRP as well as neuropeptide Y and gamma-aminobutyric acid (GABA) transmitters are all secreted by this specific subset of

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arcuate nucleus neurons, all of which are crucial for the promotion of food.^[10] A particular growth hormone secretagogue (GHS) is ghrelin, which produced primarily by the stomach's endocrine cells and also by the intestinal system and the hypothalamus, and it acts in the arcuate nucleus of the hypothalamus and in somatotrophs in the pituitary to release GH.^[11,12] GHS work by activating the GHS-receptor (GHS-R), a G protein-coupled receptor that triggers the release of GH.^[13] In the hypothalamic arcuate nucleus, where ghrelin colocalizes with growth hormone-releasing hormone (GHRH), ghrelin can directly cause GHRH secretion.^[14] Ghrelin is a orexigenic hormone, whose effects are mediated by activating both hypothalamic and extrahypothalamic areas involved in the control of both homeostatic and hedonistic eating. In addition to its capacity to promote GH secretion,^[15] the arcuate nucleus of the hypothalamus contains AgRP neurons, which are interestingly activated by both ghrelin and asprosin. Asprosin deficiency reduces the responsiveness of these neurons to ghrelin-mediated activation.^[9,16] Insulin-resistant individuals and mice both have pathologically high asprosin levels.^[17] Besides, asprosin leads to islet β -cell secretion dysfunction and apoptosis.^[18] Through inflammatory mechanisms, it lowers skeletal muscle insulin sensitivity.^[19,20]

Ceramides (Cer) is a hydrophobic backbone and the precursor for all complex sphingolipids.^[21] Sphingolipids are involved in many physiological processes, such as the development of the skin barrier, maintenance of the myelin sheath, immunity, blood vessel stabilization, detection of bacteria, viruses, and bacterial toxins, spermatogenesis, and the development of the auditory sense.^[22-25] They accomplish these tasks by interfering with cellular activities such as lipid microdomain formation, apoptosis, organelle and membrane structural organization, survival, migration, signaling, intracellular protein trafficking, autophagy, adhesion, stress response, and metabolic regulation.^[24,26] Ceramides contributes significantly to the development of the skin barrier by producing multilamellar lipids (lipid lamella) in the stratum corneum of the epidermis.^[27]

This study aims to evaluate the level of asprosin and ceramides in children with idiopathic isolated GHD (IGHD) and to determine the correlations between asprosin, ceramides, and glucose.

MATERIALS AND METHODS

The present study was designed as a case-control study. The period of this study was from October 2021 to May 2022. This work was done at the Department of Biochemistry, College of Medicine, University of Babylon and Pediatric Endocrine Clinic, Babylon Teaching Hospital for Maternity and Children in Hilla City, Iraq. Sample size was calculated according to Fisher sample size formula equation^[28]; 43 patients (24 males and 19 females) with idiopathic IGHD

who attended Pediatric Endocrine Clinic in Babylon Teaching Hospital for Maternity and Children were involved in this study. Forty-five subjects (26 males and 19 females) who were apparently healthy control that enrolled in this study were matched with patients of the same sex and age. The patients group includes 29 children treated with recombinant human growth hormone (rhGH) and 14 children without treatment. The exclusion criteria were many cases of GHD, and we selected only idiopathic IGHD.

IGHD is defined as a condition of GHD not associated with other pituitary hormone deficiencies, with or without an organic lesion.^[29] The term "idiopathic," which mean a clear cause for pituitary GHD, is often not identified, although some of these patients may actually have undiagnosed gene defects in GH production/secretion or have first manifestations of combined pituitary hormone deficiencies.^[30]

Inclusion criteria

The following were the inclusion criteria:

- Idiopathic IGHD (diagnosis was made by specialist endocrinologist depending on patient's history, examination, and clonidine stimulation test)
- Prepubertal children (as determined by specialist endocrinologist)
- Age range between 4 and 14 years.

Exclusion criteria

The following were the exclusion criteria:

- Any subject with diabetes mellitus or other chronic diseases
- Any subject with autoimmune diseases
- Any subject with other endocrinopathy.

Body mass index (BMI) was calculated using the formula $BMI = \text{weight (kg)} / \text{height (m)}^2$ and then BMI percentile, BMI Z-score, and height Z-score achieved according to the CDC chart using digital calculator. BMI below the fifth percentile and above the 95th percentile are regarded as underweight and obese, respectively. Overweight is defined as a child's BMI falling between the 85 and 95 percentile.^[31]

Using a disposable syringe (5mL), venous blood samples were obtained from the controls and patients. The levels of asprosin and ceramides in the patients and control groups were determined by using BT lab enzyme linked immunosorbent assay (ELISA) kits and following the manufacturer's instructions.^[32,33] The glucose level was determined by enzymatic-based spectrophotometric method.^[34]

Data analysis

Statistical analysis was carried out using Statistical Package of Social Science (SPSS) version 21. Continuous variables were given as mean $\pm$ standard deviation (SD),

whereas categorical variables were provided as frequencies and percentages. Student t-test was used to compare means between two groups. The correlation test (Pearson test) was performed to find the association between variables. Receiver operating characteristic (ROC) curve was used to give the sensitivity and specificity of biochemical parameter and calculate the optimal cutoff according to "Youden index" by selecting the point that is closest to the top-left corner of the ROC curve giving equal weight to sensitivity and specificity when picking a cut-off point is a typical practice. This idea is often referred to as the Youden index.^[35] *P* value less than 0.05 was considered as significant.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were re-viewed and approved by a local ethics committee according to the document number 1009 (including the number and the date in 1/11/2021) to get this approval.

RESULTS

General characteristics

Patients included in this study were in the age range of 4–14 years, with mean $\pm$ SD of 10.4 ± 3.0 years. Control group was with an age range of 4–14 years and mean $\pm$ SD of 9.3 ± 2.6 years.

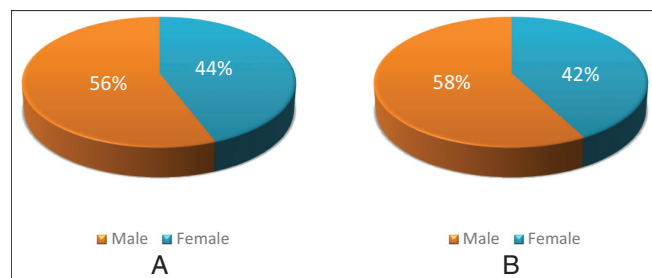


Figure 1: Gender distribution in patients and control: (a) rate of male and female among patients, (b) rate of male and female among control group

Distribution of the studied subjects according to gender

Among 43 patients with GHD who contributed to this study, there were 24 (56%) males and 19 (44%) females, and among 45 controls who contributed to this study, there were 26 (58%) males and 19 (42%) females [Figure 1].

Anthropometric measurements for GHD patients and the control

The data presented in Table 1 show the anthropometric measurements of GHD patients and the control. The results revealed a significant decrease in the height and height Z-score of the patients (121.9 ± 19.32 cm and -2.9 ± 1.48) compared with the control (131.4 ± 14.65 cm and -0.2 ± 1.27), respectively. There were nonsignificant differences in the weight of the patients (27.6 ± 11.86 kg) compared with their values in the control (31.6 ± 11.34 kg). Regarding BMI, the results shown nonsignificant difference between the patients (17.6 ± 3.82) and the control (18.3 ± 4.31). Also there is a nonsignificant difference in BMI percentile ($47.2\% \pm 33.56\%$) and BMI Z-score (-0.144 ± 1.48) in the patients compared with their values in the control ($58.8\% \pm 34.97\%$ and 0.214 ± 1.48 , respectively).

Biochemical parameters

Asprosin

The results revealed a significant decrease in the asprosin level of the patients (27.7 ± 18.81 ng/mL) compared with the control (42.1 ± 27.33 ng/mL). Also there are nonsignificant differences in asprosin level between patients treated with recombinant GH and patients without rhGH as shown in Table 2.

Ceramides

The results shown a significant decrease in the ceramides level of the patients (106.0 ± 65.04 ng/mL) compared with the control (149.3 ± 91.88 ng/mL). On the other hand, the mean of ceramides level was higher in patients treated with rhGH (110.5 ± 65.57 ng/mL) than the other without rhGH (92.1 ± 62.36 ng/mL), but this difference was statistically not significant (*P* value = 0.385) as shown in Table 3.

Table 1: Anthropometric measurements of GHD in patients and the control

Anthropometric measurements	Mean $\pm$ SD		<i>P</i> value
	Patients	Control	
Age (years)	10.4 ± 3.0	9.3 ± 2.6	0.071
Height (cm)	121.9 ± 19.32	131.4 ± 14.65	0.011
Height Z score	-2.9 ± 1.48	-0.2 ± 1.27	<0.001
Weight (kg)	27.6 ± 11.86	31.6 ± 11.34	0.108
BMI (kg/m ²)	17.6 ± 3.82	18.3 ± 4.31	0.428
BMI percentile (%)	47.2 ± 33.56	58.8 ± 34.97	0.117
BMI Z score	-0.144 ± 1.48	0.214 ± 1.48	0.26

Table 2: Comparison of asprosin level in patients and the control group

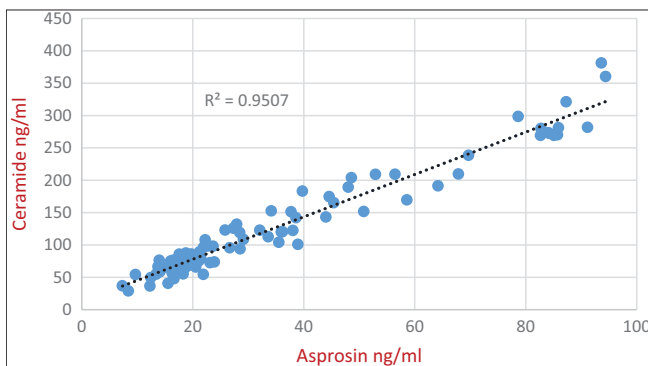
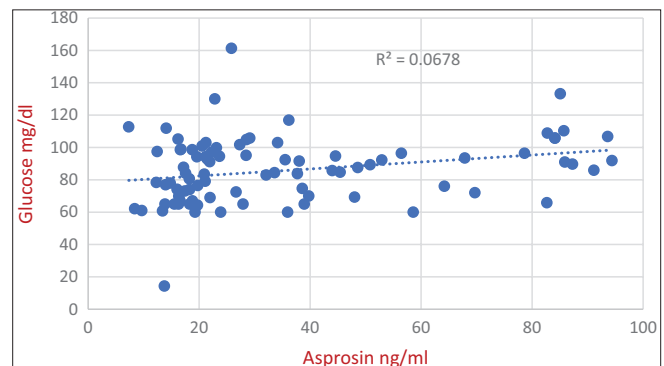
Study group	N	Asprosin level (ng/mL), mean $\pm$ SD	P value
Patient	43	27.7 $\pm$ 18.81	0.005
Control	45	42.1 $\pm$ 27.33	
rhGH	29	29.8 $\pm$ 18.65	0.298
No rhGH	14	26.4 $\pm$ 19.09	

Table 3: Comparison of ceramides level in patients and the control group

Study group	N	Ceramides level (ng/mL), mean $\pm$ SD	P value
Patient	43	104.5 $\pm$ 64.39	0.01
Control	45	149.3 $\pm$ 91.88	
rhGH	29	110.5 $\pm$ 65.57	0.385
No rhGH	14	92.1 $\pm$ 65.36	

Table 4: Comparison of glucose level in patients and the control group

Study group	N	Glucose level (mg/dL), mean $\pm$ SD	P value
Patient	43	79.2 $\pm$ 19.8	0.003
Control	45	91.8 $\pm$ 18.9	
rhGH	29	87.9 $\pm$ 29.93	0.042
No rhGH	14	74.9 $\pm$ 10.74	

**Figure 2: Correlations between asprosin and ceramides****Figure 3: Correlations between asprosin and glucose**

Glucose

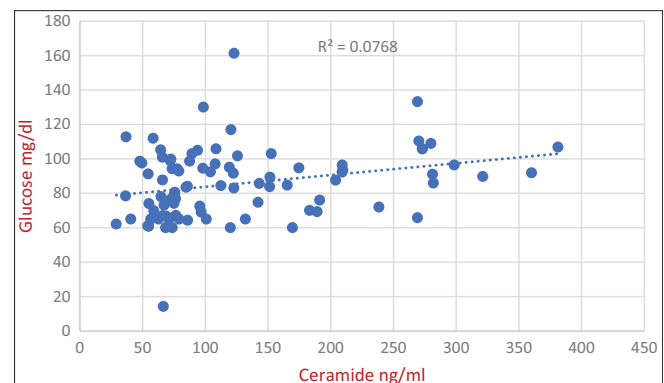
The results exposed a significant decrease in the glucose level of the patients (79.2 $\pm$ 19.8 mg/dL) compared with the control (91.8 $\pm$ 18.9 mg/dL). Also the results revealed a significant decrease ($P = 0.042$) in the glucose level in patients treated with rhGH (87.9 $\pm$ 29.93 mg/dL) compared with patients without rhGH (74.9 $\pm$ 10.74 mg/dL), as shown in Table 4.

Correlations between asprosin and ceramides

The results exposed a positive correlation between serum asprosin and ceramides ($r = 0.975$, $P < 0.001$), as in Figure 2.

Correlations between asprosin and glucose

The results exposed a positive correlation between serum asprosin and glucose ($r = 0.260$, $P = 0.014$), as in Figure 3.

**Figure 4: Correlations between ceramides and glucose**

Correlations between ceramides and glucose

The results exposed a positive correlation between serum ceramides with glucose ($r = 0.277$, $P = 0.009$), as in Figure 4.

Receiver operating characteristic curve for asprosin

Receiver operating characteristic curve for the sensitivity and specificity of serum asprosin (ng/mL) for the diagnosis of GHD disease (cut-off point was ≥ 21.144 [ng/mL]), area under the curve (AUC) = 0.686, P value = 0.003, 95% confidence interval (CI) = 0.576–0.797; the sensitivity and the specificity were 58.1% and 75.6% respectively, as shown in Figure 5.

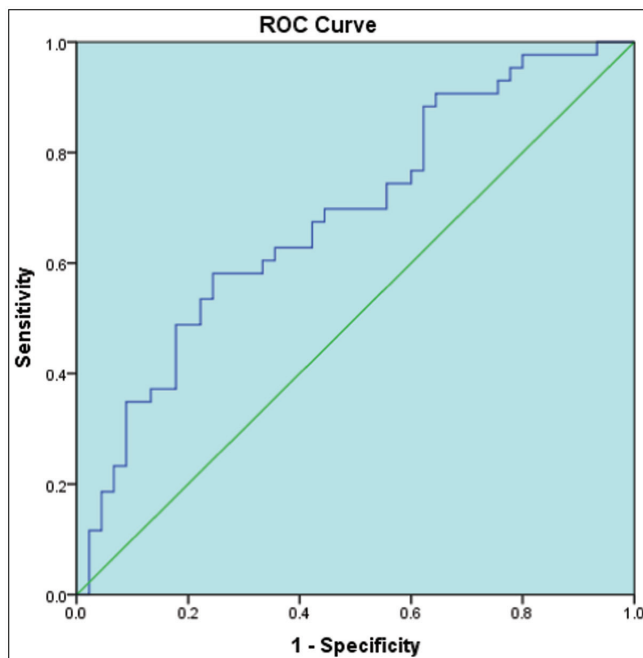


Figure 5: ROC curve for asprosin

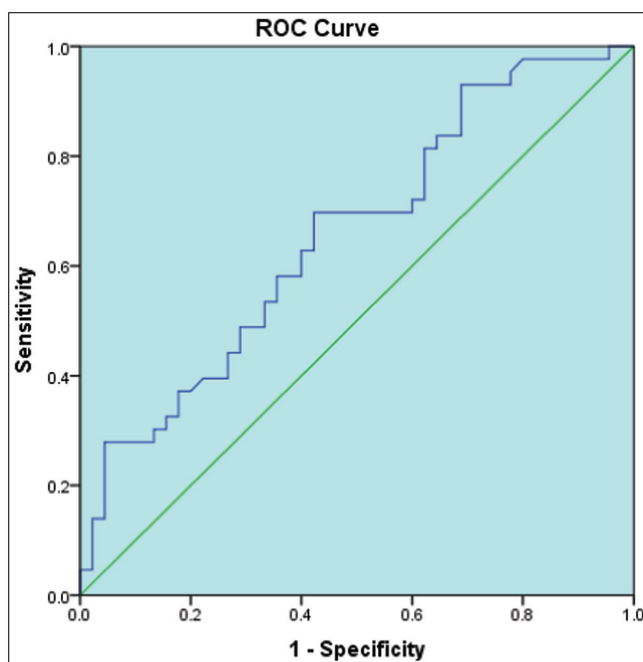


Figure 6: ROC curve for ceramides

Receiver operating characteristic curve for ceramides

ROC curve for the sensitivity and specificity of serum ceramides (ng/mL) for the diagnosis of GHD disease (cut-off point was ≥ 21.144 [ng/mL]), AUC = 0.656, P value = 0.012, 95% CI = 0.543–0.770; the sensitivity and the specificity were 93% and 31.1%, respectively, as shown in Figure 6.

DISCUSSION

The two studied groups were well matched regarding gender (P value = 0.855). This study finds that more males than females were referred to endocrine clinics due to the growth disturbances but without statistical significance. Short stature in female is often not detected, or it is reported late, which may be due to bad social habits or the parents' belief that it is normal and perhaps shortened because it is a female. For the correct care of short girls, a worldwide understanding of gender biases is essential.^[36] A similar result has also been observed in a review by Ranke *et al.* in Europe, USA, and Japan.^[36] In Iraq, a similar result has also been observed by Aldabagh *et al.*^[37]

In spite of that, the causes of GHD are idiopathic, and between 3% and 30% of the cases of GHD are suggested to have a genetic cause^[38]; the present study suggests that asprosin's overlapping with ghrelin in AgRP neurons is the same in GHS-R, and a decrease in asprosin level may lead to reduction in the responsiveness of these receptors to GHSs and may lead to deficiency of GH.

Also there are nonsignificant differences in asprosin level between patients treated with recombinant GH and patients without rhGH. This strengthens that asprosin is more likely to be a cause for GHD than to be a result.

The results revealed a significant ($P < 0.05$) decrease in the ceramides level of the patients (106.0 ± 65.04 ng/mL) compared with the control (149.3 ± 91.88 ng/mL); this may be due to the fact that GH stimulates lipolysis via the activation of the hormone-sensitive lipase, primarily in the visceral adipose tissue, which results in free fatty acid (FFA) flux from adipose tissue to circulation.^[39] In contrast to the GH effects on adipose tissue, GH promotes cellular uptake of FFA in skeletal muscle by increasing the activity of lipoprotein lipase.^[40] The re-esterification of triglycerides from FFA results in the accumulation of lipid intermediates such as diacylglycerol and ceramides in skeletal muscle.^[41,42] So, a decrease in ceramides level in patient with GHD may be due to deficiency of GH. On the other hand, the mean of ceramides level was higher in patients treated with rhGH (110.5 ± 65.57 ng/mL) than the other without rhGH (92.1 ± 62.36 ng/mL), but this difference was statistically not significant (P value = 0.385). This strengthens that ceramides are more likely to be a result for GHD than to be a cause.

The results revealed a significant ($P < 0.05$) decrease in the glucose level of the patients (79.2 ± 19.8 mg/dL) compared with the control (91.8 ± 18.9 mg/dL); this results may be due to low asprosin level cause lowered in blood glucose in patients than control. Also, there is a significant decrease in the glucose level in patients treated with rhGH (87.9 ± 29.93 mg/dL), compared with patients without rhGH (74.9 ± 10.74 mg/dL); this may be due to that GH is an insulin counter-regulatory hormone that increases glucose synthesis in the liver and kidneys while decreasing glucose uptake in peripheral tissues, making it a hyperglycemic hormone. Hypoglycemia is common in people with GHD, especially in early childhood.^[43]

The results exposed a positive correlation between serum asprosin and ceramides ($r = 0.975$, $P < 0.001$); this may be clarified through that asprosin may have an effect on GH secretion by GHS-R, and GH has an effect on lipid metabolism. So asprosin level indirectly affects ceramides level.

The results exposed a positive correlation between serum asprosin and glucose ($r = 0.260$, $P = 0.014$). This correlation can be explained that asprosin activating PKA in the liver via the Olfactory 734 (Olfr734) receptor promotes glucose release from hepatocytes.^[44] Also, asprosin levels are inversely proportional to glucose levels, with low glucose levels (fasting state) boosting asprosin production and high glucose levels inhibiting it (feeding state),^[45-48] and in spite of a significant decrease in the glucose level of the patients (79.2 ± 19.8 mg/dL) compared with the control group (91.8 ± 18.9 mg/dL), asprosin levels decrease in patients with GHD, which may be due to genetic defect in asprosin gene, so in summary, the initial problem is asprosin deficiency that lead to GH deficiency, and both of them cause lower blood glucose.

The results exposed a positive correlation between serum ceramides and glucose ($r = 0.277$, $P = 0.009$). GHD will cause decrease in blood glucose and GHD will lead to decrease in ceramides level, so the correlation between ceramides and blood glucose is related to GHD.

For asprosin and ceramides, our results for ROC curve stated that these two parameters have poor diagnostic value in the diagnosis of GHD depending on the AUC and the sensitivity and specificity.

CONCLUSIONS

Asprosin may be one of underlying causes of GHD through its indirect role in releasing GH. GHD may lead to decrease in the level of ceramides by the effect of GH on lipid metabolism. The presence of positive correlation between serum asprosin, ceramides, and glucose strengthens its relation to GHD.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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