

The relationship between Salusin- α , Salusin- β , and Klotho levels with subclinical atherosclerosis in ankylosing spondylitis

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Abstract. – OBJECTIVE: Subclinical atherosclerosis (SA) is often observed in ankylosing spondylitis (AS) patients; Salusin- α (Sal- α), Salusin- β (Sal- β), and Klotho hormones are thought to be associated with atherosclerosis. This study aims to evaluate the relationship between Sal- α , Sal- β , and Klotho levels with SA in AS.

PATIENTS AND METHODS: The study included patients older than 18 years who applied between August 1, 2019, and September 1, 2019. Patients with AS were included in the AS group, and patients without a known disease were included in the healthy group. Epicardial adipose tissue thickness (EATT) and carotid intima-media thickness (CIMT) measurements were used to assess SA.

RESULTS: The study group included 38 (40.9%) patients diagnosed with AS, and the control group included 55 (59.1%) participants. CIMT and EATT levels were higher in the AS group than in the healthy group [0.37 (0.17) vs. 0.54 \pm 0.18, p <0.001; 0.44 \pm 0.11 vs. 0.54 (0.18), p =0.004, respectively]. There was no significant difference in Sal- α , Sal- β , and Klotho levels between the AS and healthy groups (p >0.05). Furthermore, there was no observed relationship between EATT or CIMT and Klotho, Sal- α , or Sal- β in either group (p >0.05).

CONCLUSIONS: Although SA level was higher in AS patients, there was no relationship between SA and Sal- α , Sal- β , and Klotho levels.

Key Words:

Ankylosing spondylitis, Atherosclerosis, Carotid intima-media thickness, Klotho, Salusin- α .

Introduction

Ankylosing Spondylitis (AS) is a chronic inflammatory illness that particularly involves the sacroiliac joint and the axial skeleton, as well as extraarticular areas, including the eye, gastrointestinal, and cardiovascular systems¹. Patients with AS have 1.6-1.9-fold higher mortality rates compared with the general population and also a 20-40% increased rate of cardiovascular disease (CVD) related mortality compared to healthy controls². This increase has been attributed to numerous factors other than the classical CVD risk factors, including systemic inflammation, cardiovascular pathologies specific to AS, use of non-steroidal antiinflammatory medications, disease activity, and limited functional activity³. The first step of CVD, in most cases, is the development of atherosclerosis. The presence of chronic inflammation contributes to all stages of atherosclerosis, including early atheroma plate formation, thrombus formation, and the development of a cardiovascular event⁴. In patients with AS, the detection of subclinical atherosclerosis prior to the development of clinical signs will help to determine CVD risk and take the necessary measures.

In the early detection of atherosclerosis, non-invasive atherosclerosis markers that reflect the cumulative effects of the risk factors are used. Among various methods, carotid intima-media thickness (CIMT) and epicardial adipose tissue thickness (EATT) have gained wide acceptance

in the detection of early atherosclerosis^{1,5,6}. A study⁷ that included 5,000 healthy individuals showed that every 0.20 mm increase in CIMT was associated with a 30% increase in CVD.

Salusins, discovered by Shichiri et al⁸ in 2003, are endogenous bioactive peptides with strong hemodynamic and mitogenic properties. Salusin- α (Sal- α) and Salusin- β (Sal- β) are synthesized as preprosalusin and are secreted mostly from vascular smooth muscle cells and endothelial cells. Detection of salusin expression on coronary atherosclerotic plaques has led to the assumption that they play a role in the formation and progression of atherosclerosis⁹. In the literature, it was shown that the two isoforms of salusins have opposite roles in atherosclerosis and inflammation. Sal- α increases the expression of acetyl-coenzyme A acyl transferase, thereby suppressing macrophage foam cell formation and inducing anti-atherogenic effects. Sal- β upregulates the enzyme and induces pro-atherogenic effects¹⁰. Therefore, Sal- α and Sal- β are potential biomarkers for atherosclerosis.

The Klotho gene is an antiaging gene that encodes a single-pass transmembrane protein and is predominantly expressed in the distal tubular cells of the kidney, parathyroid glands, and the choroid plexus of the brain¹¹. There are two forms of Klotho: membrane and secreted. The membrane Klotho acts as an obligatory co-receptor for a fibroblast growth factor-23, which is produced in the bone and induces phosphate excretion by urine¹¹. The secreted Klotho serves various functions, including the regulation of nitric oxide production in the endothelium and kidney calcium homeostasis¹². Klotho gene-deficient mice have been shown to have a phenotype similar to human aging characterized by endothelial dysfunction, vascular calcification, progressive atherosclerosis, and shortened life span. In the atherosclerotic mice model, *in vivo* application of Klotho protected against endothelial dysfunction¹³. Studies¹⁴ also showed that a low serum Klotho concentration was associated with markers of vascular dysfunction, including increased CIMT, CVD development, and vascular stiffness. A study in 2019¹⁵ emphasized that Sal- β played a role in vascular calcification by down-regulating Klotho. As a result, the central role of Klotho in the development of CVD has made it a potential biomarker that can be used in the diagnosis of vascular disease.

To the best of our knowledge, there are no studies on the levels of Sal- α , Sal- β , and Klotho in AS patients. This study aims to evaluate the relationship between Sal- α , Sal- β , and Klotho levels with subclinical atherosclerosis.

Patients and Methods

The study included patients older than 18 years who were admitted to the Internal Medicine, Rheumatology and Family Medicine Polyclinics of Sakarya University Training and Research Hospital between August 1, 2019, and September 1, 2019. The patients who were admitted to the Internal Medicine and Rheumatology Polyclinics and were diagnosed with AS according to the modified New York criteria at least 5 years before were assigned to the AS group. The individuals who were admitted to the Family Medicine Polyclinics for periodic health examinations and had no known disease were included in the healthy group. Individuals who smoked or consumed alcohol, had no known disease, had an active infection during the last 3 months were excluded.

The demographic, clinical, and laboratory data of the subjects, including age, gender, duration of AS, and attack frequency, were retrieved from patient files and recorded. Insulin resistance was calculated using the homeostasis model assessment formula: Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [fasting insulin (mU/L) x glucose (mmol/dL)/22.5]¹⁶. Blood pressure measurement, waist circumference and height-weight measurements were made during admission, and body mass index was calculated in kg/m². In the AS group, disease activity status was determined by calculating the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score¹⁷. Patients with BASDAI scores ≥ 4 were considered active patients¹⁷. Moreover, subclinical atherosclerosis was evaluated with EATT and CIMT measurements.

At the start of the study, the data collected from AS patients and the healthy community were compared. In the follow-up, AS patients were compared with those who were in the active and remission stages.

Measurement of Serum Salusin- α , Salusin- β , and Klotho Levels

Fasting blood samples were collected from the subjects in the morning for the assessment of Sal- α , Sal- β , and Klotho levels, then centrifuged at 3000 rpm for 15 minutes. The supernatant plasma samples were kept at -80°C in eppendorphs until they were analyzed. Sal- α , Sal- β , and Klotho levels were analyzed by Human Sal- α enzyme-linked immunosorbent assay (ELISA) kit (Sanghai Yehua Biological Technology Co Ltd, Shanghai, China), Human Sal- β ELISA kit

(Sanghai Yehua Biological Technology Co Ltd, Shanghai, China) and Klotho ELISA kit (Sanghai Yehua Biological Technology Co Ltd, Shanghai, China). A 40 μ L sample was analyzed with the micro ELISA method. The test results were read by using (Thermo Scientific™) ELISA at 450 nm. Sensitivity for Sal- α was 2.34 pg/mL (assay range: 5-1,000 pg/mL), for Sal- β was 5.22 pg/mL (assay range: 10-1,800 pg/mL), and for Klotho was 0.01 ng/mL (assay range: 0.5-20 ng/mL)^{18,19}.

Carotid Intima-Media Thickness Measurement

CIMT measurement was performed via B-mode examination using the Toshiba Aplio 400 high-level color Doppler ultrasonography device (Toshiba Medical Systems Corporation, Minato, Tokyo, Japan) and a linear probe with a high-resolution central frequency of 7.5 (range 5-12) MHz. The measurements of all subjects were made by a single radiologist in an outside unit. The patients lay supine in a quiet and dark room. The probe was placed 1 cm proximal to the common carotid artery bifurcation. The CIMT was defined as the distance between the lumen intima and media-adventitia interface in the posterior wall. The right and left side CIMTs were determined and recorded after calculating the means. The average of right and left CIMT measurements was evaluated in the study.

Measurement of Epicardial Adipose Tissue Thickness

Measurements of all subjects were made by a single cardiologist in the outdoor unit. A transthoracic echocardiography (Vivid system S7, GE-Vingmed Ultrasound AS, Horten, Norway) was performed in all patients using the standard technique. The hypochoic area between the outer aspect of the myocardium and the visceral layer of the pericardium was defined as the EATT. The EATT was measured in the echoless area adjacent to the right ventricle in the parasternal long-axis window, perpendicular to the right ventricle, at the end of the systole, and in three cardiac cycles.

Ethical Approval

All procedures in this study were approved by the Sakarya University Local Ethics Committee on 12.07.2019 and 04.01.2021. (Ethics committee number: E-16214662/050.01.04/113 and E-16214662-050.01.04-269). This study was conducted in accordance with the Declaration of Helsinki. All participants signed the informed consent form before they were included in the study.

Statistical Analysis

The data of the study were analyzed with the SPSS ver. 29.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as frequency, percentage, mean, median, standard deviation, and interquartile range. Student's *t*-test was used for the analysis of variables showing normal distribution, and the Mann-Whitney U test was used for variables showing abnormal distribution. The assessment of the relationship between the analysis of constant variables was made using the Spearman correlation test. The Chi-square test was used to evaluate the categorical variables. A value of $p < 0.05$ was accepted significantly.

Results

The study included 38 patients diagnosed with AS and 55 healthy controls with similar demographic characteristics. The sociodemographic characteristics and cardiovascular risks of the participants are summarized in Table I. There were 18 males (47.4%) in the AS group and 28 (51.9%) males in the healthy group ($p=0.672$). Mean ages in the AS and healthy groups were 43.6 ± 11.9 and 42.8 ± 8.9 , respectively ($p=0.716$). The differences with respect to sex, smoking habits, waist circumference, BMI, and systolic and diastolic blood pressures between the AS group and the healthy group were not different ($p > 0.05$). In AS patients, the mean time from diagnosis was 103.7 ± 54.4 months, and the mean duration of symptoms was 156.0 (119.0) months. Ten (52.6%) patients were HLA B27 positive. The mean BASDAI was 4.4 ± 2.5 , 19 patients were in the active stage of the disease, and 19 were in remission. Assessment of biochemical parameters showed that sedimentation and C-reactive protein (CRP) levels were significantly higher in the AS group compared to the healthy group ($p < 0.001$). A comparison of CIMT and EATT measurements between the AS and healthy groups showed that both CIMT and EATT were higher in the AS group ($p < 0.001$ and $p < 0.001$, respectively) (Figure 1).

Sal- α , Sal- β , and Klotho levels were not significantly different between the AS and healthy groups ($p=0.265$, $p=0.227$ and $p=0.334$, respectively). Sal- α , Sal- β , and Klotho levels of the participants are summarized in Table II.

When the relationship between EATT and CIMT was analyzed, a relationship was observed between EATT and CIMT in both AS and healthy groups ($r=0.423$ and $p=0.008$ for AS group;

Table 1. Sociodemographic features and cardiovascular risk factors according to groups.

	Healthy group (n=55)	AS group (n=38)	p
Age (years)	42.8±8.9	43.6±11.9	0.716*
Gender			
Male	28 (51.9)	18 (47.4)	0.672**
Female	26 (48.1)	20 (52.6)	
Waist circumference (cm)	98.8±11.5	101.6±12.1	0.129*
Body mass index (kg/m²)	26.2±4.3	27.6±5.0	0.090*
Systolic BP (mmHg)	110.0 [30.0]	110.0 [30.0]	0.077***
Diastolic BP (mmHg)	70.0 [14.0]	70.0 [10.0]	0.106***
Disease duration (months)	-	103.7±54.4	-
Duration of compliants (months)	-	156.0 [119.0]	-
HLA-B27 positivity	-	10 (52.6)	-
BASDAI	-	4.4±2.5	-
FBG (mg/dL)	91.3±8.3	89.0 [12.0]	0.328***
HOMA-IR	1.86±0.78	1.67 [1.58]	0.566***
Creatinine (mg/dL)	0.8±0.2	0.7±0.2	0.007*
ALT (IU/L)	21.0 [12.0]	21.0 [15.0]	0.959***
Triglyceride (mg/dL)	117.0 [74.0]	102.8±44.7	0.183***
LDL cholesterol (mg/dL)	140.0±33.4	130.0 [39.0]	0.534***
HDL cholesterol (U/L)	48.5 [17.0]	46.5 [14.0]	0.453***
CIMT (mm)	0.37 [0.17]	0.54±0.18	<0.001***
EATT (mm)	0.44±0.11	0.54 [0.18]	0.004***
Sedimentation (mm/h)	7.0 [9.0]	13.5 [16.0]	<0.001***
C-reactive protein (mg/L)	0.0 [0.0]	3.6 [11.3]	<0.001***

The data are presented as mean±standart deviation, n (%), median [interquartile range]. *Student's *t*-test, **Chi-square test, ***Mann-Whitney U test. ALT: Alanine transaminase; BP: Blood pressure; BASDAI: Bath ankylosing spondylitis disease activity index; CIMT: Carotid intima-media thickness; EATT: Epicardial adipose tissue thickness; FBG: Fasting blood glucose.

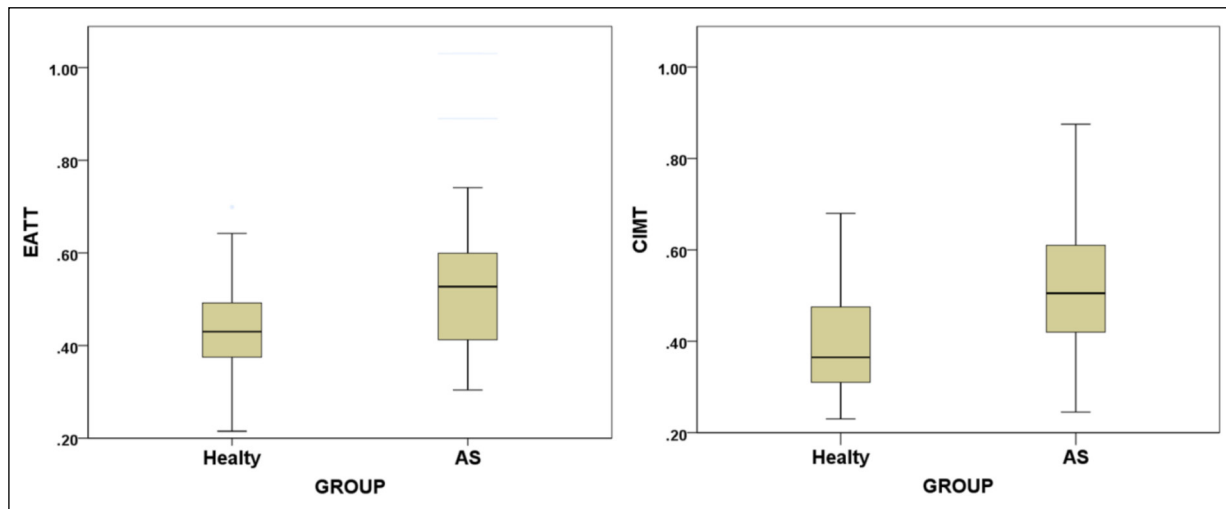


Figure 1. A comparison of EATT and CIMT measurements between the AS and healthy groups showed that both EATT and CIMT were higher in the AS group ($p<0.001$ and $p<0.001$, respectively). EATT: Epicardial adipose tissue thickness; CIMT: Carotid intima-media thickness; AS: Ankylosing spondylitis.

$r=0.507$ and $p<0.001$ for the healthy group). On the other hand, no correlation was found between EATT and Sal- α , Sal- β , and Klotho in both the AS group and the healthy group ($p=0.408$, $p=0.977$, $p=0.711$ for the AS group; $p=0.611$, $p=0.945$, $p=0.726$ for the

healthy group). Moreover, no correlation was found between CIMT and Sal- α , Sal- β , and Klotho in both the AS group and the healthy group ($p=0.328$, $p=0.576$, $p=0.398$ for the AS group; $p=0.212$, $p=0.236$, $p=0.095$ for the healthy group).

Table II. Klotho, Salusin- α , and Salusin- β levels according to groups.

	Healthy group (n=55)	AS group (n=38)	<i>P</i>
Sal- α (pg/mL)	16.68 [27.29]	14.78 [20.07]	0.265
Sal- β (pg/mL)	42.95 [94.26]	37.74 [49.10]	0.227
Klotho (ng/mL)	0.64 [2.0]	0.61 [0.45]	0.334

The data are presented as median [interquartile range]. Mann-Whitney U test. Sal- α : Salusin- α , Sal- β : Salusin- β .

Of the patients in the AS group, 19 (50.0%) were in the active stage of the disease, and 19 (50.0%) were in remission. EATT, CIMT, Sal- α , Sal- β , and Klotho levels according to the activation of AS disease are summarized in Table III.

Discussion

This study aims to evaluate the relationship between Sal- α , Sal- β , and Klotho levels with subclinical atherosclerosis. This present study found that compared to healthy controls, AS patients having no CVD or traditional risk factors had higher values for CIMT and EATT. On the other hand, no difference was observed in terms of disease activity in CIMT and EATT levels. Moreover, there was no significant relationship between CIMT and EATT with Sal- α , Sal- β , and Klotho.

Two large cohort studies^{20,21} found that rheumatologic diseases increase the risk of cerebrovascular and cardiovascular disease due to accelerated atherosclerosis. In a recent study²² including patients with psoriatic arthritis, endothelial dysfunction, which is a precursor to atherosclerosis, was found to be present. CIMT has been widely accepted in the detection of early atherosclerosis in asymptomatic patients because it is a reliable, noninvasive, relatively cheap, and easily repeatable marker²³. The literature so far has focused on the measurement of CIMT in AS; however, they found inconsistent results. In order to investigate this inconsistency, Yuan et al²⁴ analyzed

24 studies that included AS patients and published it as a review in 2019. This systemic review, similar to our study, emphasized that AS patients had higher CIMT levels compared to healthy controls, and regression analyses showed that CRP and BASDAI values significantly affected the CIMT.

EATT is a metabolically active fatty tissue that secretes the inflammatory hormones and cytokines participating in the pathogenesis of subclinical atherosclerosis¹. A study²⁵ published in 2012 on 190 asymptomatic patients found a correlation between EAT with coronary artery disease and negative cardiovascular events independent of other risk factors. There are only a few studies that have investigated the EATT in AS patients. These studies^{1,4}, similar to ours, found that patients with AS had significantly higher EATT values compared to healthy controls. Also, some of these studies found a positive correlation between EATT and CIMT, which are markers for subclinical atherosclerosis^{4,26,27}.

Salusins are multifunctional new bioactive peptides that have a regulating role in hemodynamics and atherogenesis¹⁰. Sal- α has anti-atherogenic, and Sal- β has pro-atherogenic effects. Studies²⁸ have shown that in coronary artery disease, the Sal- α level decreases, and it is negatively correlated with CIMT. A study²⁹ published in 2020 and analyzed 256 patients who underwent coronary angiography due to chest pain emphasized that Sal- β is a superior indicator than Sal- α in evaluating coronary atherosclerosis. Another study on chronic kidney disease patients found a negative

Table III. EATT, CIMT, Sal- α , Sal- β , and Klotho levels according to the activation of AS disease.

	Active stage (n=19)	Remission (n=19)	<i>P</i>
EATT (mm)	0.54±0.14	0.53±0.18	0.946*
CIMT (mm)	0.54±0.14	0.51 [0.33]	0.624**
Sal- α (pg/mL)	14.81 [30.17]	14.75 [18.30]	0.954**
Sal- β (pg/mL)	38.31 [72.78]	37.17 [38.01]	0.644**
Klotho (ng/mL)	0.61 [0.67]	0.62 [0.41]	0.840**

The data are presented as mean±standart deviation, median [interquartile range]. *Student's *t*-test, **Mann-Whitney U test. CIMT: Carotid intima-media thickness; EATT: Epicardial adipose tissue thickness; Sal- α : Salusin- α , Sal- β : Salusin- β .

correlation between Sal- α and CIMT, and a positive correlation between Sal- β /Sal- α ratio and CIMT30. There are few studies^{31,32} that aim to establish the relationship between subclinical atherosclerosis and Salusins in rheumatologic diseases. A study³¹ involving patients with systemic lupus erythematosus and systemic sclerosis found low serum Sal- α and high CIMT levels in patient groups, similar to the literature. In contrast,

another study³² involving patients with rheumatoid arthritis (RA) and Behçet's disease found that both Sal- α and CIMT were higher in the patient group compared to the healthy control group; however, there was no relationship between the two. In our study, because CIMT and EATT were thicker in AS patients compared to healthy controls, it was expected that Sal- α level would be low and Sal- β level would be high; however, there was no statistically significant difference between the two groups with respect to Sal- α and Sal- β levels. This suggests that in patients with AS, Salusins may not play a role in the development of atherosclerosis or a different pathophysiologic mechanism may be responsible.

The first study on serum Klotho levels in adults, published by Semba et al¹¹ in 2011, found that individuals with high serum Klotho levels independently had a lower risk of CVD. In the following years, a lot of evidence has been found that relates Klotho deficiency to CAD, atherosclerosis, myocardial infarction, and left ventricle hypertrophy³³. Studies¹⁴ have associated serum Klotho concentration with CIMT, a marker of subclinical atherosclerosis, and reported that low Klotho levels were related to increased CIMT. Few studies^{34,35} have investigated serum Klotho levels in autoimmune diseases. A study³⁴ in 2017 involved patients with SS and found lower serum Klotho levels compared to controls, while there was no significant relationship between Klotho levels and disease activity. In another study³⁵ performed on RA patients, serum Klotho levels in RA patients were found to be higher compared to controls and positively correlated with RF. Subgroup analyses showed higher serum Klotho levels in RA patients treated with biologic agents than those receiving conventional treatment; however, there was no relationship with CIMT³⁵. In our study, serum Klotho levels in the AS and the control group did not have a significant difference; both healthy individuals and AS patients had Klotho levels similar to those detected in studies performed on healthy individuals¹³. Also, the relationship between CIMT and EATT

with Klotho was not significant in either group. Similar to our study, a study³⁶ published in 2022 did not find a significant difference in Klotho levels between Systemic Lupus Erythematosus patients and healthy controls; multivariate analysis found a positive relationship between prednisolone intake and Klotho level.

Limitations

Our study had some limitations. Being a single-center study, the recruitment of a relatively small number of AS patients and the short duration of the disease were the major limitations. Because patients with CVD or patients who carried the risk factors were not included, a comparison of Sal- α , Sal- β , and Klotho levels between patients with and without CVD could not be made. Another limitation was that CIMT and EATT measurements were made manually.

Conclusions

In this study, CIMT and EATT levels, which are indicators of subclinical atherosclerosis, were found to be higher in AS patients compared to healthy controls. On the other hand, there was no significant relationship between CIMT and EATT with Sal- α , Sal- β , and Klotho levels. In order to investigate Sal- α , Sal- β , and Klotho levels in the prediction of CVD in AS patients, studies with larger patient populations and longer disease duration are necessary.

Authors' Contributions

Study Design: AGT, BD, ST, AEA, LTA, SY, EG, AT.
Data Collection: AGT, BD, EUA, LTA, SY, EG, ACG.
Statistical Analysis: ST, EUA, SY, ACG.
Data Interpretation: ST, AEA, EG, AT.
Manuscript Preparation: AGT, BD, ST, AET, LTA, ACG.
Literature Search: AGT, BD, EUA, LTA, EG, AT.
Funds Collection: AGT, BD, EUA, AEA, SY, AT.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

We would like to thank the participants who agreed to participate in this study for their contribution to science.

Availability of Data and Materials

The data and materials generated/analyzed in the present study are available from the corresponding author upon request.

Ethics Approval

All procedures in this study were approved by the Sakarya University Local Ethics Committee on 12.07.2019 and 04.01.2021. (Ethics committee number: E-16214662/050.01.04/113 and E-16214662-050.01.04-269). This study was conducted in accordance with the Declaration of Helsinki.

Informed Consent

All participants signed the informed consent form before they were included in the study.

Funding

This research received no specific grant from any funding agency.

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