ASSOCIATIONS BETWEEN VASPIN RS2236242 GENE POLYMORPHISM, WALKING TIME AND THE RISK OF METABOLIC SYNDROME

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ABSTRACT

The associations between serum vaspin levels and metabolic or coronary artery disease (CAD) and polycystic ovary syndrome (PCOS) is under the scope of current researchers. Therefore, this adipokine can be considered as a biomarker of metabolic syndrome (MetS). The aim of the study was to analyze the associations between the vaspin rs2236242 polymorphism and physical activity in relation to MetS and its components. The analysis involved the genetic material and clinical data of 108 individuals with MetS and 110 controls. Vaspin rs2236242 polymorphism was detected using the tetra-primer amplification-refractory mutation system polymerase chain reaction (T-ARMS PCR) method. The TA genotype of vaspin rs2236242 was associated with a greater risk of MetS and its components compared with the TT genotype. The analysis of interactions between genotype and walking time revealed that a walking time longer than 60 min./day significantly decreased the risk of MetS in the TA carriers ($p = 0.007$). The obtained results suggest that any unfavorable effect of the TA genotype of the vaspin rs2236242 polymorphism can be essentially reduced, or even reversed, in a case of indi-

viduals walking longer than 60 min. a day. The analysis of the interaction between vaspin rs2236242 polymorphism and walking showed that a walking time of longer than 1 hour a day significantly reduced the risk of MetS, elevated blood pressure and triglycerides concentration.

Keywords: Abdominal; Adipokines; Exercise; Metabolic syndrome (MetS); Obesity.

INTRODUCTION

Vaspin (visceral adipose tissue-derived serine protease inhibitor) was isolated for the first time from visceral adipose tissue in Otsuka Long-Evans Tokushima Fatty rats, which comprises an animal model of abdominal obesity, accompanied by type 2 diabetes mellitus (T2DM) [1]. Human vaspin mRNA has been found both in the visceral and in subcutaneous adipose tissue. It is coded by the gene SERPNA12 located on the long arm of chromosome 14 (14q32.13) and consists of five introns and six exons. According to the literature review, there is a positive association between the vaspin concentration in the blood serum and the indicators of obesity, T2DM, polycystic ovary syndrome (PCOS) and coronary artery disease (CAD) [1,2]. Feng *et al.* [2] after the meta-analysis of the scores of six studies involving 1826 participants, revealed that the vaspin levels were approximately 0.52 ng/mL higher in obese patients than in controls. However, some studies showed a lack of associations between the level of vaspin and obesity [3,4]. Several researchers suggest that this adipokine increases the insulin sensitivity of adipose tissue in the condition of obesity. The increase of vaspin expression acts like a compensatory mechanism and being a reaction to growing obesity and insulin resistance. Several studies have revealed that vaspin concentration

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is related to a better metabolic profile and that its levels in the blood serum are significantly higher in individuals with metabolic syndrome (MetS) than in control [5,6]. So far, only a few studies devoted to the analysis of the relationships between vaspin gene polymorphism and the risk of MetS have been published [6-8]. Studies conducted by Hashemi *et al.* [7] and Mehanna *et al.* [8] confirmed that the risk of MetS was significantly lower in allele A rs2236242 carriers. On the other hand, Alnory *et al.* [6] in the studies involving the Egyptian population, did not find any differences between the frequency of vaspin rs2236242 genotypes and the risk of MetS. Therefore, it was stated that there are only a few studies concerning association between vaspin gene polymorphism and MetS, and their results are ambiguous. No such study has been conducted in the Polish population. According to our current knowledge, none of the previously published studies considered relationship between vaspin rs2236242 polymorphism and the risk of MetS including physical activity or walking. Therefore, the aim of the study was the analysis of the associations between vaspin rs2236242 polymorphism and physical activity in relation to MetS and its components.

MATERIALS AND METHODS

Study Population. Genomic DNA isolated from 108 participants with MetS and 110 controls of the Polish-Norwegian Study (PONS) project were used in this study. All participants were Caucasians. The study included blood pressure measurements, the analysis of collected fasting-blood samples, anthropometric measurements and a questionnaire interview. Detailed information regarding the project and research procedures were described in a previously published study [9]. In 108 participants, MetS was diagnosed on the basis of the International Diabetes Federation criteria [10].

Ethics. The study was approved by the Ethics Committee from the Cancer Centre and Institute of Oncology in Warsaw (data collection) and by the Committee on Bioethics at the Faculty of Health Sciences, Jan Kochanowski University in Kielce, Poland (data analysis) (decision number: 45/2016).

Genotyping. Determination of the vaspin rs2236242 polymorphism was conducted by the tetra-primer amplification-refractory mutation system polymerase chain reaction (T-ARMS PCR) method, according to the protocol described by Hashemi *et al.* [7]. The external primers VasFO (5'-GGA GGC AGA CCA GGC ACT AGA AA-3') and VasRO (5'-ACC ATC TCT CTG GCT TCA GGC TTC-3') were applied for the amplification of 378 bp DNA of vaspin gene fragment. Inter primers were specific to each genotype: Vas FI primer (5'-AAG ACG CCG CTT CTG

TGC ACT-3') for the T allele and Vas RI primer (5'-CAC AGG GAC CCA GGA TAA CTT GCT 3') for the A allele.

The commercially available PCR premix (PCR Mix Plus; A&A Biotechnology, Gdynia, Poland) was prepared according to the manufacturer's instructions. Briefly, 1 μ L of each primer (10 pmol/ μ L), 1 μ L of template DNA (~25 ng/ μ L), 12.5 μ L of PCR Pre Mix and made up to 25 μ L with DNase-free water. The procedure of T-ARMS PCR was optimized and final conditions were: denaturation at 95 °C for 3 min., amplification for 30 cycles at 95 °C for 1 min., 60 °C for 1 min., and 72 °C for 1 min., and a final extension at 72 °C for 8 min. After DNA amplification, the gel documentation system (InGenius; Syngene, Cambridge, Cambridgeshire, UK) was used for product visualization. The specific genotypes were characterized, respectively: 174 and 378 bp for homozygous T, 248 and 378 bp for homozygous A, and 174, 248 and 378 bp for heterozygous TA (Figure 1).

Figure 1. Electrophoresis patterns of T-ARMS PCR for the detection of SNPs in vaspin rs2236242. The product sizes were 174 bp for T allele, 248 bp for A allele, and 378 bp for control. M; DNA marker.

Meta-Analysis of the Vaspin Genotypes. The meta-analysis of the vaspin rs2236242 polymorphism included the data from Iran [7], Egypt [6,11] and Poland (this study). The distribution of the genotype was examined in MetS volunteers ($n = 459$) and controls ($n = 459$).

Statistical Analyses. All data were analyzed using the Statistical Package Statistica software (version 13.1) (TIBCO Software Inc., Palo Alto, CA, USA). Mean $\pm$ standard deviation (SD) and medians with interquartile ranges (Me; Q1-Q3) for continuous variables and proportions expressed as percentages for categorical variables, were used to describe the baseline characteristic of the group. Differences between MetS participants and the control group were assessed through the χ^2 test and the U Mann-Whitney-test. The χ^2 test was also used to analyze

genotype and allele frequencies and to determine whether the genotype distribution was congruent with Hardy-Weinberg equilibrium expectations. The multivariate logistic regression analyses were used to estimate the odds ratio (OR) and 95% confidence interval (95% CI) for MetS and its five components. The TT was a reference genotype in the analysis. In the second model, the risk for MetS and its components was adjusted for age, gender, walking time ($\leq$ or >60 min./day) and smoking. The choice of confounders in models II and III was based on the scores obtained from the cluster analysis. It was performed on raw values by means of a single linkage method. A distance measurement was the Euclidean distance. In the third model, body mass index (BMI), was added (as a continuous variable). Additionally, in models II and III, interactions between vaspin rs2236242 gene polymorphism and walking, in relation to MetS and its components, were considered. A reference point for walking was time ≤ 60 min./day. In all analyses, a p value of <0.05 was considered to be statistically significant.

RESULTS

The study involved 108 individuals with MetS and 110 participants representing the control group. The group with MetS was similar to the control group regarding sex and age. Participants with MetS were more often overweight (BMI >25.0 m/kg²), with abdominal obesity,

elevated blood pressure, glucose and triglyceride (TG) concentration and decreased concentration of high density lipoprotein (HDL) cholesterol, compared to the control group (all $p < 0.001$). No significant differences were found between the percentages of current and former smokers in both groups ($p = 0.456$). However, the individuals with MetS were characterized by a significantly shorter time of a total physical activity (PA) during the day (232.2 ± 144.4 min./day vs. 270.6 ± 145.6 min./day; $p = 0.039$), including mainly, a shorter walking time (79.6 ± 71.1 min./day vs. 109.2 ± 85.5 min./day; $p = 0.007$), compared to the control group.

There was no statistically significant difference in distribution of alleles and genotypes of the vaspin rs2236242 polymorphism between individuals with MetS and controls (Table 1). The meta-analysis of genotype distribution in Egyptian, Iranian and Polish populations showed that the TT genotype was identified more frequently in MetS patients than in controls ($p = 0.001$). The A allele occurrence was higher in the control group ($p = 0.001$) (Table 2).

However, a two-fold more frequent prevalence of the AA genotype in individuals from the control group compared to the group with MetS was found (15.45 vs. 8.33%). There was also a tendency to a slightly more frequent prevalence of the TA genotype in individuals with MetS, compared to the control group (51.86 vs. 45.45%). The genotype distribution showed no deviation from the Hardy-Weinberg equilibrium in the whole study sam-

Table 1. Genotype and allele distribution of the vaspin rs2236242 polymorphism in the control group and MetS patients.

Genotypes	Total <i>n</i> (%)	Control Group <i>n</i> (%)	MetS Patients <i>n</i> (%)	χ^2 (df = 2)	<i>p</i> Value
TT	86 (39.45)	43 (39.10)	43 (39.81)		
TA	106 (48.62)	50 (45.45)	56 (51.86)	2.783	0.250
AA	26 (11.93)	17 (15.45)	9 (8.33)		
Alleles				χ^2 (df = 1)	<i>p</i> Value
T	278 (63.76)	126 (61.82)	142 (65.74)	0.730	0.394
A	158 (36.24)	84 (38.18)	74 (34.26)		

MetS: metabolic syndrome.

Table 2. The meta-analysis of the the vaspin rs2236242 polymorphism in the MetS patients ($n = 469$) and the control group ($n = 459$) from Iran, Egypt and Poland.

Genotypes	MetS Patients <i>n</i> (%)	Control Group <i>n</i> (%)	χ^2 (df = 2)	<i>p</i> Value
TT	210 (44.78)	149 (32.46)		
TA	215 (45.84)	240 (52.59)	24.20	<0.0001
AA	34 (7.25)	70 (15.25)		
Alleles			χ^2 (df = 1)	<i>p</i> Value
T	635 (67.70)	538 (58.61)	33.21	<0.0001
A	283 (30.17)	380 (41.39)		

MetS: metabolic syndrome.

ple ($\chi^2 = 0.536$; $p = 0.464$), in subjects with MetS ($\chi^2 = 2.467$; $p = 0.116$), and in the control group ($\chi^2 = 0.200$; $p = 0.655$). The A allele frequency of the vaspin rs2236242 polymorphism was 0.360 in total, 0.343 in MetS and 0.378 in the control group.

The TA, AA and TA+AA genotypes showed no association with MetS when compared to the TT genotype ($p = 0.638$; $p = 0.187$; $p = 0.979$, respectively). The results of the conducted study also revealed that the vaspin rs2236242 polymorphism was not significantly associated with any of the MetS components. Despite a lack of statistically significant associations, having the AA genotype (compared to TT) was related to quite a clear tendency to a lower risk of MetS and its components (OR varied from 0.54 to 0.80) (Table 3, model I).

In the model adjusted for age, gender, smoking and total PA, no statistically significant correlations have been found between the vaspin rs2236242 gene polymorphism

and the risk of MetS and its components (data not shown). The differences of PA between MetS-subjects and the control group resulted mainly from the differences of walking time. The group of variables of small linkage distances included all MetS components, some demographic variables and lifestyle elements, along with walking time (Figure 2). Total PA and moderate-to-vigorous PA were characterized by greater Euclidean distances than the others. Taking this fact into consideration, in subsequent models walking time instead of total PA was used as a modifying variable. In the model adjusted for age, gender, smoking and walking (model II), there were also no significant associations between the vaspin rs2236242 polymorphism and MetS components. However, it was noted that having the TA genotype was related to a greater risk of MetS, compared to the TT genotype. The analysis of interaction 'genotype $\times$ walking time' showed that a longer walking time (>60 min./day) statistically significantly decreased the risk of

Table 3. Associations between the vaspin rs2236242 polymorphism a risk of MetS and its components [OR (95% CI)]. I: unadjusted model; II: model adjusted for gender, age, smoking, walking and interactions between the vaspin rs2236242 polymorphism and walking in relation to MetS and its components; III: model adjusted for gender, age, smoking, BMI, walking and interactions between the vaspin rs2236242 gene polymorphism and walking in relation to MetS or its components.

Components of MetS		AA (TT ref.)	p Value	TA (TT ref.)	p Value	TA+AA (TT ref.)	p Value	AA (TT+TA ref.)	p Value
MetS	I	0.54 (0.22-1.35)	0.187	0.15 (0.65-2.02)	0.638	0.99 (0.58-1.71)	0.979	0.50 (0.21-1.18)	0.115
	II	1.24 (0.31-4.97)	0.759	2.54 (1.07-60.5) ^a	0.035	2.14 (0.96-4.77) ^a	0.064	0.75 (0.20-2.83)	0.674
	III	0.91 (0.19-4.36)	0.911	2.96 (1.07-7.40) ^a	0.020	2.37 (1.01-5.57) ^a	0.047	0.60 (0.14-2.48)	0.480
Abdominal obesity	I	0.80 (0.32-2.04)	0.647	1.13 (0.60-2.12)	0.699	1.05 (0.58-1.91)	0.861	0.75 (0.32-1.79)	0.521
	II	1.61 (0.28-9.36)	0.593	0.91 (0.34-2.43)	0.846	1.02 (0.40-2.62)	0.961	1.41 (0.26-7.72)	0.695
	III	1.21 (0.11-12.77)	0.874	1.84 (0.45-7.57)	0.398	1.59 (0.43-5.09)	0.483	0.50 (0.07-3.78)	0.506
Elevated BP	I	0.71 (0.26-1.94)	0.503	0.89 (0.45-1.78)	0.744	0.85 (0.44-1.64)	0.627	0.75 (0.30-1.92)	0.554
	II	0.60 (0.20-1.77)	0.356	1.88 (0.64-5.52) ^b	0.251	2.38 (0.84-6.77) ^b	0.103	0.61 (0.23-1.64)	0.332
	III	0.44 (0.13-1.49)	0.187	1.86 (0.63-7.04) ^b	0.265	2.42 (0.83-7.04) ^b	0.104	0.62 (0.22-1.72)	0.360
Increased glucose concentration	I	0.80 (0.31-2.06)	0.648	1.14 (0.63-2.05)	0.663	1.07 (0.61-1.87)	0.823	0.75 (0.41-1.80)	0.517
	II	1.02 (0.24-4.33)	0.978	0.41 (0.60-3.30)	0.424	1.30 (0.58-2.91)	0.527	0.76 (0.19-2.97)	0.696
	III	0.78 (0.17-3.58)	0.748	1.46 (0.61-3.47)	0.395	1.29 (0.56-2.96)	0.544	0.65 (0.16-2.62)	0.546
Decreased HDL concentration	I	0.56 (0.19-1.64)	0.290	1.06 (0.57-1.96)	0.852	0.95 (0.52-1.172)	0.861	0.54 (0.19-1.50)	0.238
	II	1.44 (0.31-4.78)	0.642	1.84 (0.74-4.60)	0.189	1.72 (0.72-4.11)	0.219	1.10 (0.26-4.67)	0.899
	III	1.26 (0.25-6.21)	0.778	1.91 (0.76-4.79)	0.170	1.72 (0.71-4.16)	0.224	0.99 (0.22-4.36)	0.990
Increased TG concentration	I	0.60 (0.22-1.66)	0.324	1.42 (0.79-2.56)	0.245	1.22 (0.69-2.15)	0.493	0.49 (0.19-1.28)	0.148
	II	2.34 (0.57-9.64) ^c	0.237	2.02 (0.85-4.78)	0.110	2.09 (0.92-4.75)	0.076	1.66 (0.44-6.24) ^c	0.456
	III	1.92 (0.42-8.09) ^c	0.399	2.12 (0.88-5.11)	0.095	2.15 (0.93-5.00)	0.075	1.49 (0.37-5.97) ^c	0.569

MetS: metabolic syndrome; OR (95% CI): odds ratio (95% confidence interval); BMI: body mass index; BP: blood pressure; HDL: high density lipoprotein; TG: triglycerides.

^a Statistically significant interaction gene $\times$ walking, in relation to MetS.

^b Statistically significant interaction gene $\times$ walking, in relation to blood pressure.

^c Statistically significant interaction gene $\times$ walking, in relation to TG concentration.

Figure 2. Schematic diagram for 14 variables; linkage distances.

MetS in the TA genotype carriers ($OR = 0.24$; $p = 0.022$) and TA+AA ($OR = 0.25$; $p = 0.016$), as well as the risk of elevated blood pressure in the TA genotype carriers ($OR = 0.17$; $p = 0.039$ and TA+AA ($OR = 0.11$; $p = 0.007$), compared to the TT carriers. It was also found that a longer walking time decreased the risk of higher TG concentration in the AA genotype carriers ($OR = 0.04$; $p = 0.017$), compared to TT and the AA genotype carriers, compared to TT+TA ($OR = 0.60$; $p = 0.025$).

Including BMI in the model did not significantly change the obtained scores (model III). Similar to the previous model, it was observed that longer walking time (>60 min./day) significantly decreased the risk of MetS in TA and TA+AA genotype carriers ($OR = 0.16$; $p = 0.007$ and $OR = 0.17$; $p = 0.007$, respectively) as well as elevated blood pressure ($OR = 0.09$; $p = 0.012$ and $OR = 0.05$; $p = 0.002$ for TA and TA+AA genotypes, respectively) as well as the risk of increased TG concentration in AA genotype carriers, compared to TT ($OR = 0.06$; $p = 0.038$) and AA genotype, compared to TT+TA ($OR = 0.07$; $p = 0.043$).

DISCUSSION

In our study, the possible association between the vaspin rs2236242 gene polymorphism and the risk of developing MetS in a group of Polish men and women was investigated. To the best of our knowledge, this is the first time such an association has been studied in the Polish population. Both in the unadjusted and adjusted models, there have been no statistically significant associa-

tion between the vaspin rs2236242 polymorphism and the risk of the occurrence of individual components of MetS. However, in the model adjusted for known confounders (smoking, BMI, walking), it was noted that carrying the TA genotype was related to a greater risk of MetS, compared to the TT genotype.

Results of other studies are ambiguous in this respect. Hashemi *et al.* [7] in a study conducted on Iranian population observed that the A allele of the vaspin rs2236242 polymorphism plays a protective role against MetS. The risk of MetS was decreased significantly by being a carrier of the AA and TA genotypes compared to the TT genotype. The associations remained almost unchanged after adjusted for gender and age [7]. Mehanna *et al.* [8] in a study of Egyptian women, also found that the rare A allele of the vaspin rs2236242 polymorphism and the TA and AA genotypes appeared with higher frequencies among the control group individuals than in MetS subjects. A similar tendency, related to the protective function of the AA genotype against MetS, was shown in the results of our study, in the unadjusted model. The tendency to a slightly more frequent occurrence of TA genotype in MetS-subjects, compared to control-subjects (51.86 vs. 45.45%), as well as a higher risk of MetS in TA genotype carriers. However, these results did not reach a level of statistical significance. Different results were achieved on Egyptian women by Alnory *et al.* [6] who did not observe any significant associations between the A allele of the vaspin rs2236242 polymorphism and risk of MetS when compared to the T allele. The AA and AT genotypes also showed no association with MetS when compared to the TT genotype. Moreover, the authors did not find any associations between genotypes of the vaspin rs2236242 polymorphism and different serum vaspin levels [6].

In the Iranian population, the analysis of the associations between the vaspin rs2236242 gene polymorphism and health conditions such as obesity, PCOS and T2DM, showed ambiguous results. Kohan *et al.* [11] observed a decreased risk of PCOS in the A allele carriers when compared to the T carriers. However, this relationship was not statistically significant after adjusting genotypes for BMI [12]. Abdel Ghany *et al.* [12] concluded that the minor (A) allele of the vaspin rs2236242 gene polymorphism occurred less frequently in obese women compared to control group. It played a protective role against obesity in dominant, codominant, additive, and recessive inheritance models. However, after adjusting genotypes for T2DM there were no significant associations between polymorphism of this gene and obesity. According to the authors, the vaspin rs2236242 gene polymorphism shows protective effects in obesity, but this association results mainly from its effect on insulin sensitivity [12]. The

analysis of the German population conducted by Kempf *et al.* [13] revealed that the AA genotype carriers had an increased risk of T2DM, compared with the TT genotype carriers, but contrary to all other studies they analyze the vaspin rs2236242 polymorphism in the reverse strand of the vaspin gene. The results of the meta-analysis of three populations only enforce the theory that A allele plays a protective role against MetS occurrence and TT genotype increase the risk of syndrome progression.

It has been shown that lifestyle factors, such as physical activity, can modify associations between variants of several genes and the risk of developing obesity, T2DM and cardiovascular disease in various populations [14-16]. Metabolic syndrome is also treated as a result of interactions between genetic factors and unhealthy lifestyle. It is thought that despite the significant role of genetic factors, a lack of regular PA and obesity are the main causes of the increase of occurrence of metabolic disorders, especially in developed countries [17,18]. According to our current knowledge, none of the previously published studies on the relationship between the vaspin rs2236242 polymorphism and the risk of MetS included PA or walking. Thus, our study provides the first vaspin rs2236242 gene polymorphism $\times$ PA/walking time interaction data on MetS and its components. Studies of the influence of other genes and PA on adiposity and metabolic characteristics provide diverse results. Brito *et al.* [19] evaluated the effect of 'genes-physical activity' interaction on impaired glucose regulation risk for 17 loci related to T2DM and showed that three polymorphisms were statistically significant: CDKN2A/B rs10811661, HNF1B rs4430796 and PPARG rs1801282. Similar to our analysis, the inclusion or exclusion of BMI as a covariate in the SNP $\times$ physical activity interaction models made no essential difference to the interaction results. Thus the authors concluded that the genetic predisposition to hyperglycemia is partially dependent on a person's lifestyle [19]. Kilpeläinen [14] found that the effect of increasing of BMI by rs9939609 gene FTO polymorphism was weakened by 27.0% in physically active individuals compared to non active ones. However, Graff *et al.* [20] did not find any evidence of the interaction with PA for loci other than FTO.

The analysis of interactions revealed that in our study population, longer walking (>60 min./day) lowered the risk of MetS and elevated blood pressure in the TA and TA+ AA genotype carriers and the risk of increased TG concentration in the AA genotype carriers, results were statistically significant. Walking is the most frequent form of physical activity. Results of several studies show that despite its low intensity, it is associated with lower risks of cardiovascular disease, T2DM and all cause mortality in men and women [21,22]. In a prospective study of

Japanese workers aged 30-69, walking less than 60 min./day comprised one of the crucial lifestyle elements increasing the risk of MetS, during 1 year of observation [23]. A decrease of MetS occurrence related to longer walking time, was also observed in several cross-sectional studies [24] and meta-analysis of cohort studies [25].

The strength of the conducted study is the inclusion of confounding factors in statistical analysis, which may have a modifying effect on the risk of MetS occurrence and its components. The limitations are considered to be the small number of participants and the fact that PA was assessed with the use of questionnaires, which are prone to bias and errors.

Conclusions. In the unadjusted model, no associations between the vaspin rs2236242 polymorphism and the risk of MetS and its components have been observed. Only the tendency to a decreased risk of MetS and its components in the AA genotype carriers has been noted, but the result was statistically insignificant. The results of the conducted study suggest that any unfavorable effect of the TA genotype of the vaspin rs2236242 polymorphism can be essentially reduced, or even reversed, in a case of individuals walking longer than 60 min. The analysis of interaction between the vaspin rs2236242 gene polymorphism and walking has revealed that walking time longer than 60 min./day considerably reduces the risk of MetS, elevated blood pressure and TG concentration. It is necessary to conduct further studies on a bigger group of participants, which will allow us to determine whether these results will be useful in MetS prophylaxis.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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