ACTN3/ EXON-19 GENE POLYMORPHISM AND RELATIONSHIP WITH LOCAL HORSES PERFORMANCE IN IRAQ

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ABATACT

This study was conducted to investigate the polymorphism of ACTN3/EXON-19 gene and their relationship the performance of local horses in Iraq. This study was carried out at the Iraqi Equestrian Club (15 km west from Baghdad city center) period during d from 1 February to 30 December 2022, to investigate the gene polymorphism of ACTN3 region EXON-19 and their relationship with performance of local horses in Iraq. Were found three variants (3 SNPs) in the ACTN3 gene - exon 19 and in different locations, namely rs1148960207, whose genotypes were CC, CT, and TT, as their distribution rates were significantly different (P≤0.05). The ratios of heterogeneity rs1144413495 with genotypes CC, TC, and TT were 34.21, 60.53, and 5.26% that differ significantly at (P≤0.01), with an allelic frequency of 0.64 and 0.36 for the two alleles C and T, respectively. The variant rs1148960207 had a significant (P≤0.05) effect on post-exercise breathing depth, speed rate and body length. Moreover a highly significant (P≤0.01) effect on heart girth and forefoot height for local horses. In conclusion ACTN3 gene has a distinct role in some physiological traits like the number and depth of respiration after exercise, rate of speed, and some body measurements, like body length, heart girth, and frontal height.

Keywords: genotype, body dimensions, philological traits, actn, horses.
***Part of Ph.D. of the 1st author.**

مجلة العلوم الزراعية العراقية- 2025 :56 (3):993-1000 تركي والانباري

المظاهر الوراثية في جين EXON-19 /*ACTN3* وعلاقتها بأداء الخيول المحلية في العراق

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المستخلص

هدفت الدراسة الى الكشف عن المظاهر الوراثية في جين ACTN3 منطقة EXON–19 وعلاقتها في اداء الخيول المحلية في العراق. تم اجراء البحث للمدة من 2021/10/1 ولغاية 2022/11/30 في نادي الفروسية العراقي. تبين أن هناك 3 تغايرات (5 SNPs) في جين ACTN3 فيما يخص اكسون 19وفي مواقع مختلفة وهي rs1148960207 مظاهرها CC و CT و TT أذ كانت نسب توزيعها ذات فروق معنوية (P≤0.05) والتغاير rs1144413495 ذات التراكيب الوراثية CC و TC وTT ونسبها 34.21 و 60.53 و 5.26 % (P≤0.01) وبتكرار اليلي بلغ 0.64 و 0.36 للاليلين C و T على التوالي والتغاير rs1143578253 مظاهرها TT و TA و AA أذ كانت نسب توزيعها ذات فروق معنوية. كان للتغاير rs1148960207 تأثيرا معنويا (P≤0.05) في عمق التنفس بعد التمارين ومعدل السرعة وطول الجسم وعالي المعنوية (P≤0.01) في محيط الصدر والارتفاع عند المقدمة. يمكن الاستنتاج إن جين ACTN3 له دورا مميزا في عدد وعمق التنفس بعد التمارين ومعدل السرعة وبعض قياسات الجسم مثل طول الجسم ومحيط الصدر والارتفاع من الامام.

الكلمات المفتاحية: التراكيب الوراثية ،ابعاد الجسم، الصفات الفسلجية، اكتين3 ، الخيول.
*البحث مستل من اطروحة الدكتوراه للباحث الاول.

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INTRODUCTION

Various criteria have been used to determine the optimal speed in racehorses by relying on accurate speed data obtained from different breeds (30, 33, 35). Nowadays, the living Iraqi purebred Arab horses present leveled type of horses with harmonious conformation and the economic cost of caring for pregnant mares is relatively high, especially since the mares' period is considered long (2, 3). Several studies detect a mathematical model that provides information about how horses regulate their speed and potential over a given distance (16, 21), as well as measuring of endurance performance, as horses can achieve an average speed in endurance races exceeding 25 km/h, especially in the final stage of the race (7, 8, 28). Gurgul *et al.* (19) reported that the identification of improvement programs in Arabian horses is most likely related to selection that focuses on improving riding and racing based on the genetic variants obtained using Illumina microarray technology in horses. The sarcomere α-actin proteins, encoded by the ACTN2 and ACTN3 genes, are major structural components of Z-line proteins and have high sequence similarity: α-actinin2 is found in all skeletal muscle fibers, while α-actinin3 has evolved as a result of specialized expression in type 2 fibers only which is rapidly glycogen lytic (23, 32). Functional and structural analysis of the ACTN3 gene has been performed in relation to its association with horse performance characteristics, and until now many studies have focused on identifying SNP differences in the ACTN3 gene for horses in different breeds (10). The study of genetic variation within and 4among individuals enables researchers to obtain important information about any organism, which may not be available in traditional methods. as well as help in finding and describing levels of genetic variation, and then obtaining basic information about the genetic composition of the population (4, 10, 20 , 22, 24). The Anglo-Arabian horse breed AA, is the results of crossing between Thoroughbred and Arabian horses with different percentages of the two breeds, and the modern AA is common in many European countries as a versatile horse both for showjumping and racing, with the two main nuclei reared in France and Italy (1, 5, 6, 11, 12, 13). Although harness racing is of high economic importance to the global equine industry, significant genomic resources have yet to be applied to mapping harness racing success (34, 36, 37). Iraqi possesses a wide variety of livestock for different type of farm animals (9). This study aims to determine the genetic polymorphism of the actin gene in a sample of local horses in Iraq and to detect variations in the ACTN3 /EXON-19 gene, and the relationship of the multiple polymorphisms of the gene on some physiological traits, body dimensions, rate of speed, and the concentration of the actin gene**.**

MATERIALS AND METHODS

The study was conducted at the Iraqi Equestrian Club located in Baghdad/ Al-Amriya region, on a sample of purebred Arabian horses participating in the races that take place in the club, the study was conducted during the period from 1 February to 30 December 2022, with the aim of DNA extracting and detecting the genetic polymorphism of ACTN3 gene/ EXON-19 region and its relationship to the performance of local horses in Iraq.

Blood collection

10 ml of blood was collected from the jugular vein of each animal, and the blood was divided into three tubes. The first tube was added with an EDTA anticoagulant produced by the Jordanian AFCO (Al-Hanoof Factory), and transferred to the laboratory for storage it at freezing at - 4 °C until the time of DNA extraction. Omeprazole was added to the sample to facilitate the knowing of gene expression, and then waiting for 15 minutes until clotting occurred, after which a centrifugation of samples were performed at a speed of 3000 rpm for 5 minutes, and the serum was separated and storage until assay for blood characteristics.

Molecular genetics

The analyzes were carried out initially by preparing kits, primers (primers) and tool at the laboratory. The primer was used on annealing according to Temp. C=63 and product by 916 bp as (Table 1).

F:GCAGATGCAGAGATGTGAT

R: TCCTCCTCCTGTTCCATATAC

DNA extraction: DNA was isolated from the blood sample according to the ReliaPrep™ Blood gDNA Miniprep System, Promega protocol. A quantitative fluorimeter was used to detect the concentration of extracted DNA in order to screen for sample quality for downstream applications. For 1 µl of DNA, 200 µl of diluted Quantifluor dye was mixed. After 5 minutes of incubation at room temperature, DNA concentration values were detected.

Table 1. PCR program used for DNA analysis

Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	
Annealing	63	00:30	30
Extension	72	00:30	
Final extension	72	07:00	1

After PCR amplification, agarose gel (3%) electrophoresis was performed to confirm the presence of amplification. The polymerase chain reaction (PCR) was completely based on the standards of the extracted DNA. Ethidium bromide (10mg / ml), DNA ladder marker (50 bp), 1 X TAE buffer Sequence analysis technology was performed to determine genotypes and detect the presence of mutations by sending samples to South Korea, by sending PCR products for Sanger sequencing using the ABI3730XL device, to know the sequences of automated DNA nitrogenous bases, by Macrogen Corporation - in Korea, and the results were 38 sample analyzed using genetic software (Macrogen Corporation - in Korea).

Statistical analysis: The data were analyzed statistically using the program Statistical Analysis System–SAS (30) to study the effect of the genetic polymorphism of the actin gene (ACTN3) of the EXON-19 region on the studied traits. The significant differences among the averages were compared using Duncan's test (18) by applying the least square means, and the Chi-square- $\chi 2$ test (30) was used to compare the distribution percentages of genotypes for each SNP in the ACTN3/EXON-19 gene.

RESJLTS AND DISCUSSION

Then, photographed the result to ensure the success of the doubling process and obtain the required piece, which its size were 960 base pairs, as (Figures (1-4) which represents the sequence forms for each SNP.

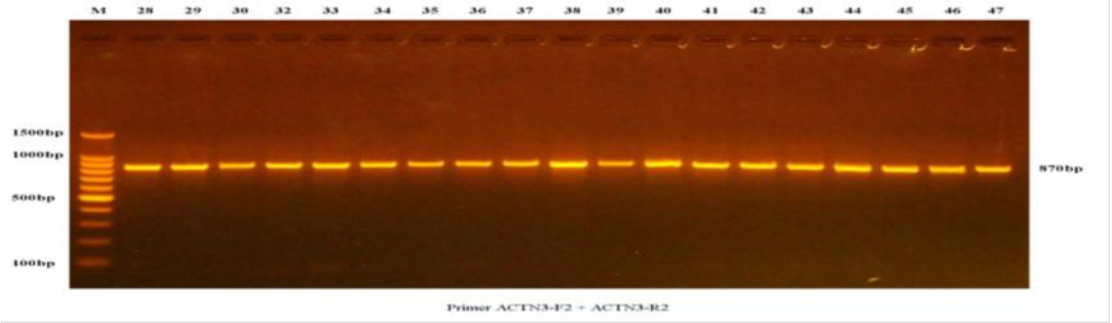

Figure 1. DNA extraction of ACTN3 gene/EXON 19 in Local Iraqi horses

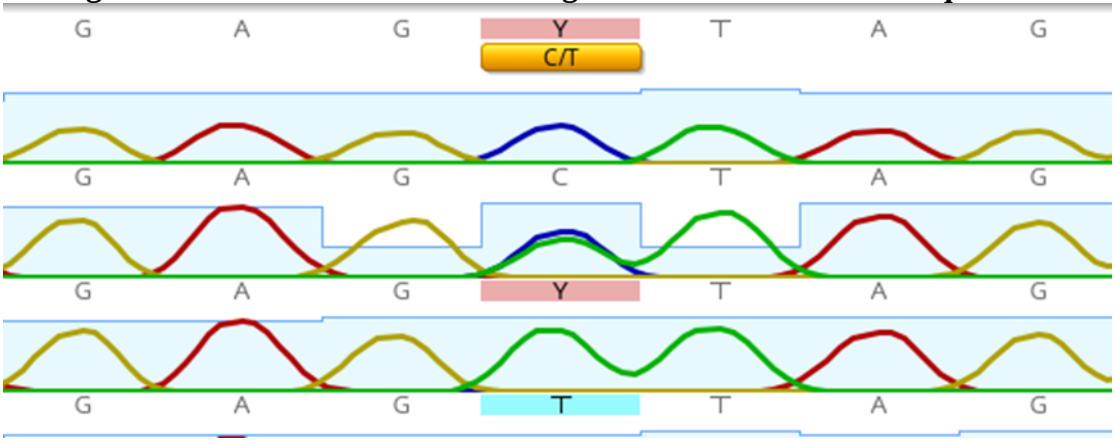

Figure 2. ACTN3 gene/EXON 19 / rs1148960207 SNP

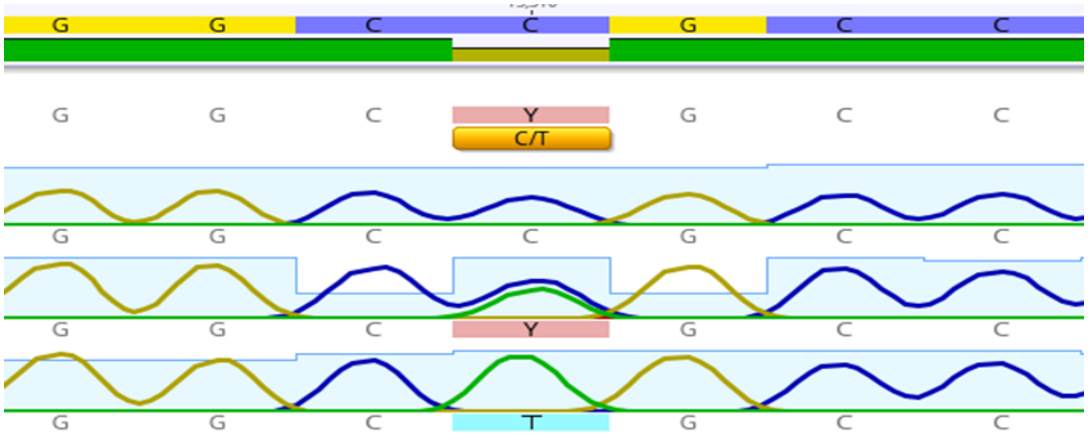

Figure 3. ACTN3 gene/EXON 19 / rs1144413495 SNP

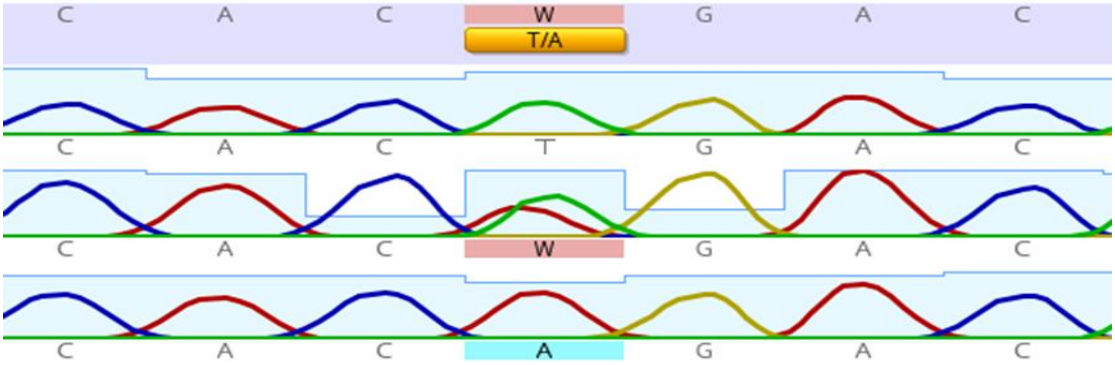

Figure 4. ACTN3 gene/EXON 19 / rs1143578253 SNP

Genotypes and allelic frequency

Table 2. revealed that the distribution ratios of the genotypes of the ACTN3/EXON19 gene at position rs1148960207/SNP1 for the horse sample. The, which its percentages were 13.16, 71.05, and 15.79% (P≤0.05) for genotypes CC, TC, and TT, and the allele frequency was 0.49 and 0.51 for the C and T alleles, respectively. The distribution ratios of CC, TC and TT genotypes of the ACTN3/EXON19 gene at position rs1144413495/SNP2 were 34.21, 60.53 and 5.26% (P≤0.01) and the allele frequency was 0.64 and 0.36 for the C and T alleles. The Table 2 related the distribution ratios of the ACTN3/EXON19 gene polymorphism at rs1143578253/SNP3, and the ratios were 13.16, 71.05, and 15.79% (P≤0.05) for the TT, TA, and AA polymorphism respectively. The variance among them was highly significant (P≤0.01), and the frequency of the allele was 0.49 and 0.51 for the T and A allele, respectively. The results of the distribution of genotypes and allelic frequency vary according to the regions of the gene, the size of the segment, the study site, the number or size of the sample included in the study, and the role of chance (15, 16, 27, 29, 31).

Table 2. Genotype distribution and allele frequency in ACTN3-EXON-19 gene in local horses

SNPs	Genotype	No	%	Allele	Frequency
	CC	5	13.16	C	0.49
rs1148960207 /SNP1	CT	27	71.05		
	TT	6	15.79	T	0.51
	Total	38	100%		
	χ^2	--	6.789 *	--	--
	CC	13	34.21	C	0.64
rs1144413495 /SNP2	CT	23	60.53		
	TT	2	5.26	T	0.36
	Total	37	100%		
	χ^2	--	8.052 **	--	--
	TT	5	13.16	T	0.49
rs1143578253 /SNP3	TA	27	71.05		
	AA	6	15.79	A	0.51
	Total	38	100%		
	χ^2	--	6.789 *	--	--

* (P≤0.05), ** (P≤0.01).

The relationship of the genetic polymorphism of the ACTN3/EXON19 gene with the studied traits

Heterozygosity rs1148960207/SNP1: It is noted from Table 3. the relationship of genotypes in the ACTN3-EXON19 rs1148960207/ gene with the physiological traits and body dimensions on local horses. There was a significant (P≤0.05) variation in the depth of respiration after exercise according to the different genotypes. The maximum respiration depth for wild-type horses (CC) was 65.00 ± 6.90 seconds, while the mutant genotype were (63.85 ± 6.74 s). The CT hybrid genotypes came in the lowest rate (56.85 ± 2.57 seconds). There is a significant (P≤0.05) variation in the rate of speed according to the genotype of local horses. The highest rate of speed for horses with the hybrid genotype CT was 2017.91 ± 479.08 m / min. There were significant (P≤0.05) differences in the body length of domestic horses according to the genotype of the ACTN3 gene segment, as the CT and then TT recorded an average of 154.21 ± 1.35 and 153.00 ± 5.19 cm respectively, which is higher than that of the wild genotype CC (144.72 ± 1.83 cm). The heart girth result was similar, but the differences were highly significant (P≤0.01). The two genotypes TT and then TC recorded an values of 169.50 ± 12.27 and 161.97 ± 4.15 cm, respectively, which is higher than that of the wild genotype CC (139.90 ± 1.90 cm). There were significant differences among the three genotypes of the ACTN3 gene segment, for height at foreleg (P≤0.01). The expression of α-actinin-3 in muscle fibers is fast glycolytic, which is necessary for rapid muscle contraction, as well as the variation in muscle contraction, stretching, and contractile sarcomere movement (26, 28). Therefore, actin has a role in many cellular functions, which may be reflected in some dimensions of body, including height, according to genotype (26).

Heterozygosity rs1144413495/ SNP2

From Table 4. that there is a significant (P≤0.05) variation in the rate of speed according to the genotype of local horses. The highest rate of speed was for the wild genotype CC at 2128.37 ± 74.35 m/min, while the lowest rate for horses with the TT genotype being 1202.60 ±22.94 m/min. There were significant (P≤0.05) differences in the body length of local horses according to the genotype of the ACTN3 gene segment, The TT genotype recorded the highest(P≤0.01) rate (157.47 ± 1.68 cm). The highest rate of heart girth (cm) was for the genotype TC (169.45 ± 4.58 cm) and the lowest for those of the wild type CC (144.28 ± 3.31 cm). The variation in the concentration of alpha-actin 3 according to the genotype in the ACTN3 gene was significant (P≤0.05), and the rates for the CC and TT were 13.66 ± 0.790.92 and 13.02 ± 1.06 ng / ml, respectively, and lower than that in the hybrid genotype CT (10.81 ± 0.82 cm). These results may be attributed to the difference in gene expression according to the genotype. Broos *et al,* (13) and Thomas *et al,* (30) reported that actinin-3-α protein evolved its role as a result of specialized expression in fibers which are rapidly glycogen lytic, the structure of the sarcomere of light and thick filaments acts on the stretching and contracting of muscles, and Jungbluth *et al.* (22) found that there are mutations in genes that encode proteins in skeletal muscle.

Table 3. Relationship of ACTN3-EXON-19 gene polymorphism /rs1148960207 SNP1 and parameters study in Local horses

Parameters	Mean ± SE			Level of Sig.
	CC	CT	TT	
Depth of respiration before exercise (sc)	28.00 ±3.69	24.42 ±1.08	26.14 ±3.73	NS
No of respiration before exercise (min.)	16.00 ±0.85	15.78 ±0.37	14.42 ±0.71	NS
Depth of respiration after exercise (sc)	65.00 ±6.90 a	56.85 ±2.57 b	63.85 ±6.74 ab	*
No of respiration after exercise (min.)	83.50 ±5.69	82.15 ±1.80	76.00 ±5.53	NS
Rate of speed (m/min.)	1088.34 ±16.05 b	2017.91 ±479.08 a	1002.95 ±83.40 b	*
Body length (cm)	144.72 ±1.83 b	154.21 ±1.35 a	153.00 ±5.19 a	*
Heart girth (cm)	139.90 ±1.90 b	161.97 ±4.15 a	169.50 ±12.27 a	**
Height from the front (cm)	153.66 ±1.44 b	161.54 ±1.21 ab	166.65 ±0.75 a	**
α-ACTN3 conc. (ng/ml)	12.07 ±1.14	11.64 ±0.85	11.19 ±0.81	NS

Means having with the different letters in same row differed significantly. * (P≤0.05), ** (P≤0.01).

Table 4. Relationship of ACTN3-EXON-19 gene polymorphism / s1144413495 SNP2 and parameters study in Local horses

Parameters	Mean ± SE			Level of Sig.
	CC	CT	TT	
Depth of respiration before exercise (sc)	25.56 ±2.13	25.39 ±1.23	24.72 ±1.50	NS
No of respiration before exercise (min.)	15.75 ±0.46	15.64 ±0.44	14.57 ±0.29	NS
Depth of respiration after exercise (sc)	61.93 ±4.03	56.75 ±2.92	64.50 ±2.61	NS
No of respiration after exercise (min.)	81.50 ±3.15	81.96 ±1.92	77.02 ±2.17	NS
Rate of speed (m/min.)	2128.37 ±74.35 a	1592.75 ±40.21 ab	1202.60 ±22.94	*
Body length (cm)	148.42 ±2.37 b	154.97 ±1.22 ab	157.47 ±1.68 a	*
Heart girth (cm)	144.28 ±13.31 b	169.45 ±4.58 a	162.65 ±4.05 a	**
Height from the front (cm)	157.74 ±1.74	162.78 ±1.27	163.76 ±1.02	NS
α-ACTN3 conc. (ng/ml)	13.66 ±0.79 a	10.81 ±0.82 b	13.02 ±1.06 a	*

Means having with the different letters in same row differed significantly.
* (P≤0.05), ** (P≤0.01).

Heterozygosity rs1143578253/ SNP3

From Table (5) that there is a highly significant (P≤0.01) variation in the rate of speed according to the genotype, and the highest rate was for the hybrid genotype TA (2017.91 ± 479.08 m / min). The body dimensions of the heart girth (P≤0.01) and the height from the front (P≤0.05) were affected, all of which were in favor of horses with two genotypes of mutant AA and hybrids AT. Dominguez and Holmes (17) suggest that actin is the most abundant protein in most eukaryotic cells, as it is highly available and participates in more protein-to-protein interactions than any known protein, these properties along with its ability to transition between the globular (G-actin) and filamentous (F-actin) state under the control of hydrolysis of nucleotides, ions, and a large number of actin-binding proteins, makes actin an important role in many cellular functions, ranging from cell motility.

Table 5. Relationship of ACTN3-EXON-19 gene polymorphism/ rs1143578253 SNP3 and parameters study in Local horses

Parameters	Mean ± SE			Level of Sig.
	TT	TA	AA	
Depth of respiration before exercise (sc)	28.02 ±3.69	24.42 ±1.08	26.14 ±3.73	NS
No of respiration before exercise (min.)	16.00 ±0.85	15.78 ±0.37	14.42 ±0.72	NS
Depth of respiration after exercise (sc)	65.13 ±6.90	56.84 ±2.57	63.85 ±6.74	NS
No of respiration after exercise (min.)	83.50 ±5.69	82.15 ±1.81	76.01 ±5.53	NS
Rate of speed (m/min.)	1088.34 ±16.05 b	2017.91 ±479.08 a	1002.95 ±83.40 b	**
Body length (cm)	148.72 ±1.73	154.21 ±1.35	153.01 ±5.19	NS
Heart girth (cm)	139.90 ±1.90 a	161.97 ±4.15 a	169.50 ±12.27 a	**
Height from the front (cm)	153.66 ±1.44 b	161.54 ±1.21 a	166.65 ±0.75 a	*
α-ACTN3 conc. (ng/ml)	12.91 ±1.02	11.78 ±0.94	11.61 ±0.75	NS

Means having with the different letters in same row differed significantly.
* (P≤0.05), ** (P≤0.01).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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