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Genetic Diversity of Crossbred Cattle using Cytochrome Oxidase Subunit I (COI) Gene in South Sulawesi, Indonesia

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ABSTRACT

Background: The genetic diversity information of local cattle and crossbred in South Sulawesi, Indonesia is essential for the establishment of conservation and breeding strategies. This research targeted to evaluate the phylogenetic relationship and genetic diversity of local and crossbred (CB) cattle.

Methods: The present research organized at the Animal Laboratory, Professor Nidom Foundation, Surabaya, Indonesia from February to October 2021. The genome was isolated from 20 whole blood samples from seven purebreed belongs to Singosari National Artificial Insemination Center Malang, Indonesia, four crossbred in Gowa and nine crossbred in Sidrap South Sulawesi, Indonesia. Partial sequences of mtDNA cytochrome oxidase subunit I (COI) gene were amplified employing the polymerase chain reaction technique.

Result: This study revealed that a lot of single nucleotide polymorphism (SNP) found in local cattle (Bali and Peranakan Ongole) and CB cattle in Gowa and Sidrap indicated various genetic diversity can be used as a marker candidate and potential to be developed into future research knowing the correlation of the SNP with the growth rate of CB. Thus, this information will be helpful for further strategy in breeding development program.

Key words: COI, Crossbreed, Genetic diversity, Multidimensional scaling, Phylogenetics.

INTRODUCTION

Since livestock producers in Indonesia rely heavily on cattle for their revenue, cattle are one of the most essential livestock products (Dakhlan *et al*., 2022; Kuswati *et al*., 2022; Pakpahan *et al*., 2022; Prihandini *et al*., 2022; Sudrajad *et al*., 2022). The productivity of Indonesian livestock, especially local cattle, is still relatively low and is a major problem that must be resolved immediately (Mayulu *et al*., 2010). The program to increase the local cattle population is not yet optimal due to the extensive traditional rearing system, high production of livestock slaughter, limited feed, shrinking grazing area and decreased genetic quality (Kumar *et al*., 2017a; Pundir *et al*., 2019). The productivity of beef cattle can be increased through genetic and environmental quality improvements, in its application through a breeding program (Agung *et al*., 2019; Jobirov *et al*., 2022; Kibona *et al*., 2022). Moreover, some breeders' cross local cattle with Limousine semen or Simmental, Brahman to get a breed of cattle that have a better production performance than the local cattle and to get the proceeds of sale the cattle is high. In fact, the maintenance of crossbred also has constraints in reproduction for low heifers compared to local cows (Malau-Aduli *et al*., 2021).

The genetic diversity of native cattle breeds has recently been challenged by a lack of information on other cattle breeds, the development of native and local cow breeds, particularly in South Sulawesi, Indonesia and the import of exotic breeds (Sari *et al*., 2016; Sutarno and Setyawan, 2016). Making successful conservation plans is challenging due to their existing genetic variety, prevalence of inbreeding and genetic blood combination. Major risks include genetic erosion and the loss of breed-specific genetic variation. It is commonly recognized that local cattle, such as those from Bali, have a significant number of favorable features, including the capacity to withstand hot weather conditions, poor fodder quality and resistance to internal parasites and infectious illnesses (Mohamad *et al*., 2012; Bakae *et al*., 2022; Liu *et al*., 2022; Trujano-Chavez *et al*., 2022; Xu *et al*., 2020).

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The mtDNA COI is a gene that is often used as a DNA barcode to analyze the diversity of genetic in cattle (Hidayati *et al*., 2016). This gene was also used to reconstruct phylogenetics at the species level of evolution (Cai and Ma, 2016). Regarding the above points, thus, explored the COI gene to assess the genetic diversity and phylogenetic relationships of the local cattle and CB populations in Gowa and Sidrap District, Indonesia. Supplying information of genetic in crossbreds is vital for future breeding programs in order to support policies and programs to elevate the quality of beef cattle in South Sulawesi Province, Indonesia.

MATERIALS AND METHODS

Blood samples collection and DNA extraction

This study organized at the Animal Laboratory, Professor Nidom Foundation, Surabaya, Indonesia from February to October 2021. This study approved by the Institutional Animal Care and Use Committee - Professor Nidom Fundation (IACUC-PNF), Surabaya, Indonesia (Number: 010221/ IACUC/VII/2021). Twenty blood samples were isolated from three regions representing seven purebred cattle belongs to Singosari National Artificial Insemination Center, Malang, Indonesia and four CB cattle from Gowa District and nine CB cattle from Sidrap, South Sulawesi, Indonesia (Fig 1 and Table 1). Samples isolated from jugular vein using 3 mL syringe. Those blood samples were kept in room temperature and preserved in EDTA for laboratory analysis. The DNA was extracted using gSYNC™ DNA extraction kit and stored at -20°C before further analysis.

Table 1: Samples of purebreed and CB cattle.

Pure breed	CB cattle gowa district	CB cattle sidrap district
S1 : Ongole	Sp A : Lim - PO	Sp1 : Lim - Bali
S2 : Bali	Sp B : Angus - PO	Sp2 : Sim - Bali
S3 : Limousine	Sp E : Brah - PO	Sp5 : Bali - Lim - Lim
S4 : Madura	Sp F : Sim - PO	Sp6 : Bali - Lim - Brah
S5 : Angus		Sp7 : Bali - Brah
S6 : Brahman		Sp8 : Bali - Lim - Madura
S7 : Sim		Sp9 : Bali - Brah - Sim
		Sp10 : Bali - Madura
		Sp11: Bali - Sim - Lim

Note: Lim: Limousine; PO: Peranakang ongole; Sim: Simmental; Brah: Brahman.

Quantitative and qualitative test of DNA isolation

1.5% agarose gel electrophoresis in 1XTAE solution was used for a quantitative and qualitative evaluation of the extracted DNA. For 40 minutes, electrophoresis was carried out at 100 V. By using spectrophotometry on all samples, purity level measurement and concentration of the extracted DNA are achieved (Ansori *et al*., 2021).

PCR amplification

Gene COI HCO 2198 and LCO 1490 was amplified employing two primers: Forward 5′TAAACTTCAGGG TGACCAAAAAATCA 3′ and Reverse 5′GGTCAAAT CATAAAGA TATTGG 3′ referred to Poniente *et al*. (2022). The amplification conditions were as follows initial denaturation (95°C for 5 mins), denaturation (95°C for 45 sec with 35 cycles), annealing (60°C for 45 sec), extension (72°C for 60 sec) and final extension (72°C for 5 mins). The PCR products were sequenced employing ABI 310 xL Genetic Analyser (Applied Biosystems, Inc) referred to Ansori *et al*. (2021) and Prihandini *et al*. (2022).

Genetic, phylogenetic tree and statistical analysis

All samples (mtDNA COI gene sequences) were aligned using the ClustalW. MEGA 11 software was used to estimate the COI gene diversity metrics, such as the number of polymorphic sites genetic distances (Kimura two-parameter model algorithm) and the resulting distance matrices were used to create a neighbor-joining (NJ) tree (1000 bootstrapping repeats) (Ansori *et al*., 2021; Naseer *et al*., 2018; Tamura *et al*., 2021). In the present research, two

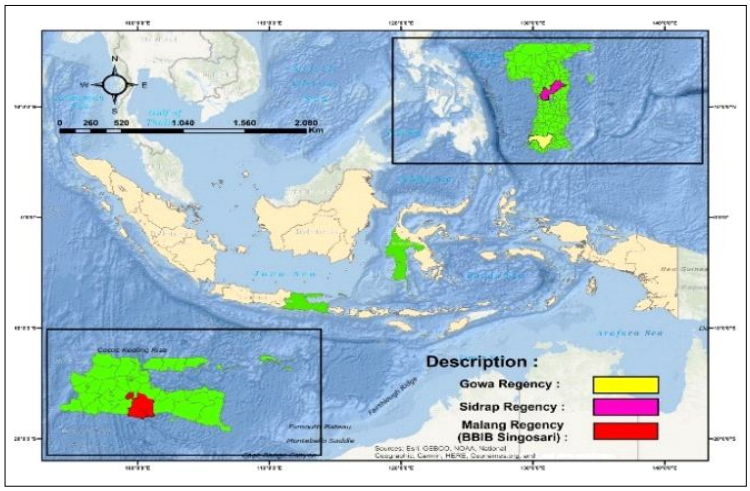


Fig 1: Sampling location of this study.

samples of *Bos taurus* (GenBank Accession Number: HM.102289.1 and HQ.860420.1) were used in the phylogenetic analysis. To obtain comparative data for genetic variation of studied cattle, sequence of *Canis lupus familliaris, Panthera leo* and *Felis catus* obtained from GenBank was used as an outgroup. Bivariat correlation anaylsis on SPSS version 22 was used to determine a significant relationship of nucleotide base and amino acid diversity to some parameters of hematology and blood chemistry description.

RESULTS AND DISCUSSION

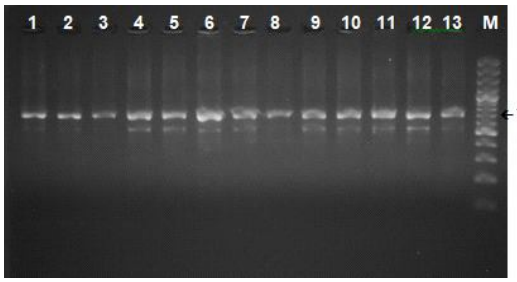
DNA isolation and PCR amplification

The precision and concentration of the DNA used as a template in the PCR procedure affected its success. The purity and concentration of the DNA ranged from 372.6 to 764.6 with the degree of purity reached from 1.8 to 1.96 µg/mL. The result of electrophoresis on a 1.5% agarose gel showed succesfully DNA amplification (Fig 2 and 3), generating a COI gene composing of 700 bp.

mtDNA sequence variation and genetic diversity

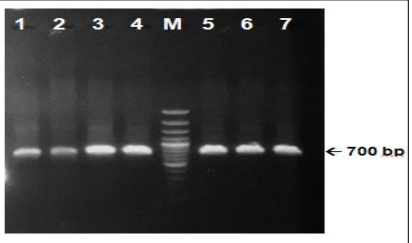
Considering result of alignment 770 nt COI gene, the base content of thymine (T) were 28.86%, cytocine (C) 26.78%, adenine (A) 27.38% and guanine (G) 16.99%. Total nucleotide A+T were 56.24% and G+C were 43.77%, therefore GC<AT. Multiple alignments obtained SNP (single nucleotide polymorphism) were 962 sites consist of 618 transition, 179 transvertion, 158 insertion and 7 deletion. Based on the polymorphic sites Bali cattle dominated among the purebred were found 56 situs, PO 33 and Madura cattle 8 sites. Among the crossbred (CB) cattle, the highest number of SNP found in (Angus×PO) were 139 sites, (Bali× Limousine×Brahman) 81 sites and (PO × Brahman) 72 sites (Fig 4). In addition, data obtained that specific nucleotide COI gene was 53.89% (415/770) conserved nucleotide and 42.2% (325/770) variable nucleotide. Variable nucleotide consist of 15.97% (123/770) parsimony informative and 22.7% (176/770) singlet on nucleotide.

The observed polymorphism number of SNP transition C>T in purebreed cattle (Bali, PO) and CB (Simmental-Bali), (Angus-PO), (Limousine-PO) were higher than T>C, A>G and G>A. Transvertion SNP A>T in (Angus-PO) cattle higher than another breed. Number of insertion detected in CB (Angus-PO), (Bali-Limousine-Brahman) and (Bali-Limousine-Limousine) more than another breed. The heterozygosity of Bali cattle, (Angus-PO), (Simmental-Bali), (Bali-Limousine-Brahman) and (Bali-Limousine-Limousine) were high, which means that cattle above have plenty of diversity of genetic (Kumar *et al*., 2017a; Pundir *et al*., 2019). The COI sequence analysis identified in Bali cattle dominant number a non-synonymous mutation among purebred cattle. Transition (C>T) at position 485, 500 and 631 of the translated sequence, resulting in an amino acid change serin to leusin. SNP C>T at 503, 533 resulting threonine change to isoleusin, at position 516, 539, 622 resulting prolin change to leusin. In addition, non-synonymous mutations in CB cattle are most commonly found in (Simmental-Bali), (Bali-Limousine-Limousine) and (Bali-Limousine-Brahman). Transition (C>T) at position 485, 500 and 631 of the translated sequence, resulting in an amino acid change serin to leusin found in CB (Simmental-Bali), (Bali-Limousine-Brahman), while in (Bali-Limousine-Limousine) found at 596. Transition (C>T) at base position 476 and 516 resulting amino acid prolin change to leusin in CB (Simmental-Bali) and (Bali-Limousine-Limousine) while at position 622 that *missense mutation* found in (Simmental-Bali) and (Bali-Limousine-Brahman). Some sites of the position of the asinonymal mutation in CB are the same as those found in



M=Marker 100 bp; 1=SpA; 2=SpB; 3=SpE; 4=SpF; 5=Sp1; 6=Sp2; 7=Sp5; 8=Sp6; 9=Sp7; 10=Sp8; 11=Sp9; 12=Sp10; 13=Sp11.

Fig 2: Ampification product of crossbred.



M=Marker 100 bp; 1=Sp_PO; 2=Sp_bali; 3=Sp-Lim; 4=Sp_Mdr; 5=Sp_angus; 6=Sp_brahman; 7=Sp_sim.

Fig 3: Ampification product of purebred.

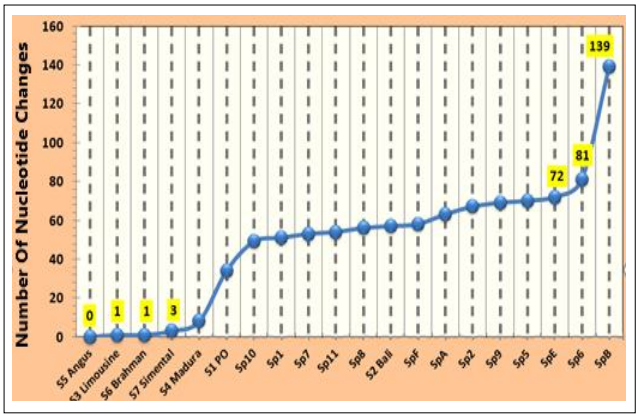


Fig 4: Number of SNP purebreed and CB cattle.

Balinese cattle, so that base positions of CB above have the potential to be used as markers.

Genetic distance and phylogenetic tree based on the COI gene

Results of the analysis of genetic distance between purebreed, CB cattle and *B. taurus* was in the range of 0.000 to 0.297. The result was in accordance with Kumar *et al*. (2017b) who stated that the genetic diversity of COI gene in the Bovidae was between 0.0 and 1.92%. In this study showed that seven pure breed cattle might be grouped into *B. taurus* because it had a genetic distance of 0.000 (Angus, Brahman and Simmental), while Limousine has a genetic distance of 0.002 and Madura with a value of 0.013. Bali and PO cattle have a longer genetic distance were 0.055 and 0.095. Among the CB (Angus-PO) is very far to the GenBank reference (0.248) and other CB and purebred. The closest genetic distance of CB (Bali-Brahman) and (Bali-Madura) is 0.077 each. We revealed that most of CB cattle should be grouped into *B. indicus* (Bali and PO) because it had a genetic distance closer than to *B. taurus*.

A Neighbor-joining (NJ) tree was generated in order to verify the phylogenetic relationship of 20 of the COI gene using MEGA 11 based on nucleotide diversity (Fig 5). The result showed that all these samples were grouped into three main clades (A, B and C) and outgroup (D). Clade A is consist of CB cattle (Angus-PO) with boostrap 94%. CB cattle (Angus-PO) which separated from other groups of CB cattle.

B clade was supported by 100% bootstrap value and consist purebreed cattle Angus, Brahman and Simmental with the closest genetic distance was 0.00 and 100% homolog to *B. taurus*, while Limousine has a genetic distance of 0.002 and Madura with a value of 0.013. Cluster C has a boostrap value of 48%, the members of this cluster are mostly CB cattle except PO and Bali. The genetic distance of PO and Balinese cows is 0.055 and 0.095, respectively. Considering the result, based on the DNA barcode, most of

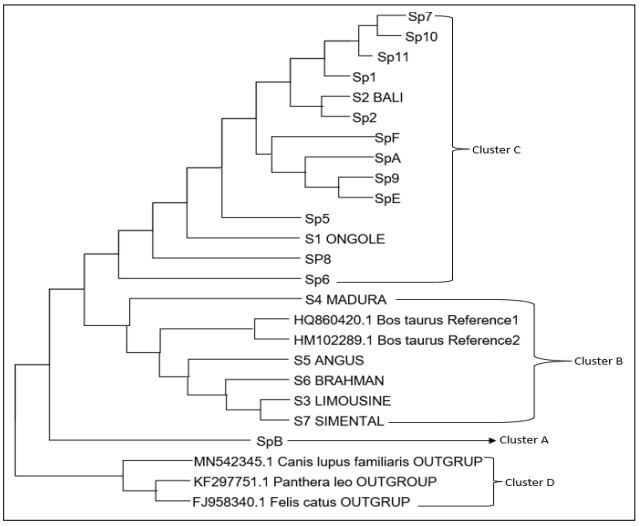


Fig 5: Phylogenetic tree purebreed and CB cattle.

Table 2: Pearson's corellation analysis of nucleotide (snp) to hematology and blood chemistry description.

Variable	Correlation to SNP	P value
SNP (Nucleotide)	1	
WBC	0.542	0.017*
RBC	-0.650	0.003**
HGB	-0.336	0.160
HCT	-0.619	0.005**
PLT	0.336	0.123
MCV	0.145	0.554
MCHC	0.541	0.017*
MCH	0.617	0.005**
Total protein	-0.211	0.386
Albumin	-0.407	0.084
Globulin	-0.076	0.757
SGOT	0.284	0.239
SGPT	-0.129	0.598
ALP	-0.088	0.720
Total cholesterol	-0.069	0.784

Description: *Significant (p-value <0.05). **Very Significant (p-value <0.01). RBC-Red blood cell, WBC-White blood cell, HGB-Hemoglobin, HCT-Hematokrit, PLT-Platelets, MCV-Mean corpuscular volume, MCH-Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration. SGOT- Serum glutamic oxaloacetic transaminase, SGPT-Serum glutamic pyruvate transaminase, ALP-Alkaline phospatase.

Table 3: Pearson's correlation analysis of amino acid (snp) to hematology and blood chemistry description.

Variable	Correlation to SNP	P value
SNP (Nucleotide)	1	
WBC	0.479	0.033*
RBC	-0.702	0.001**
HGB	-0.590	0.006**
HCT	-0.619	0.005**
PLT	0.310	0.183
MCV	-0.005	0.982
MCHC	0.479	0.032*
MCH	0.263	0.282
Total protein	-0.202	0.394
Albumin	-0.545	0.013*
Globulin	-0.007	0.977
SGOT	0.209	0.377
SGPT	-0.006	0.981
ALP	-0.081	0.735
Total cholesterol	-0.232	0.339

Description: *Significant (p-value <0.05). **Very Significant (p-value <0.01). RBC-Red blood cell, WBC-White blood cell, HGB-Hemoglobin, HCT-Hematokrit, PLT-Platelets, MCV-Mean corpuscular volume, MCH-Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration. SGOT-Serum glutamic oxaloacetic transaminase, SGPT-Serum glutamic pyruvate transaminase, ALP-Alkaline phospatase.

CB cattle were in the same cluster with *B. indicus* as well as Bali and PO cattle.

Statistical analysis

The result of Pearson's corrrelation analylsis could be shown on Table 2 and Table 3. Results of Pearson's correlation analysis that was illustrated in Table 2 showed that there was a very significant (p-value<0.01) and significant (p-value<0.05) correlation between SNP (nucleotide) and several hematological parameters. These hematological parameters were WBC, RBC, HCT, MCH and MCHC. Three parameters had a very significant correlation to SNP, namely RBC (0.003), HCT (0.005) and MCHC (0.005). Other parameters had a significant correlation to SNP were WBC (0.017) and MCH (0.017). Whereas Pearson's correlation analysis results in the following Table 3 described that several hematological parameters had a very significant correlation to SNP (amino acid). These parameters were RBC (0.001), HGB (0.006) and HCT (0.001).

Other parameters had a significant correlation to SNP were WBC (0.033) and MCHC (0.032). Only albumin (0.013) as a blood chemistry parameter had a significant correlation to SNP (amino acid). These hematological and blood chemistry parameters that had a very significant and significant correlation to SNP (nucleotide and amino acid) would be used as a dependent variable to construct multidimensional scaling (MDS) map. The results of multidimensional scaling map would be illustrated in the following figures.

Fig 6 above shows that all samples were divided into three groups. Angus-PO CB (SpB) was localized separately (A). The second group members were purebred (B). The last group members were crossbred (C), except S1 (Ongole)

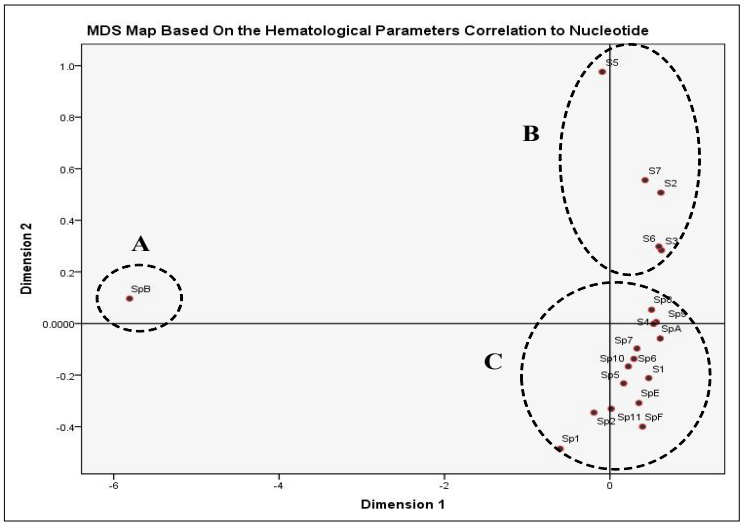


Fig 6: MDS map based on the hematological parameters correlation to SNP nucleotides.

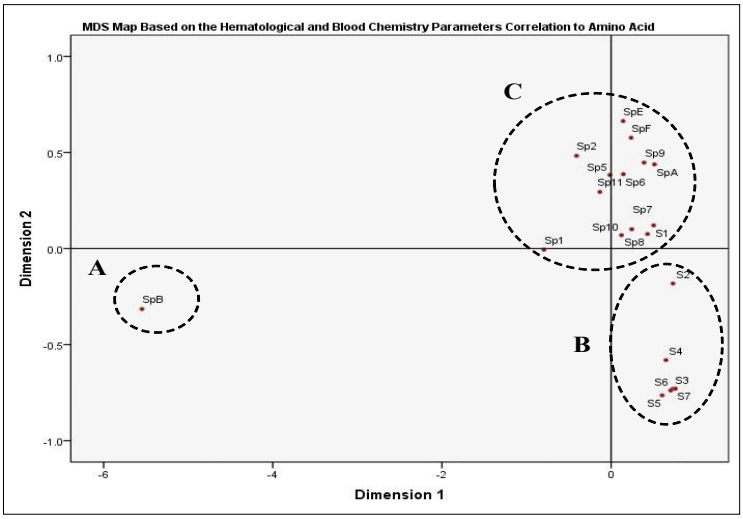


Fig 7: MDS map based on the hematological and blood chemistry parameter correlation to SNP amino acids.

was purebred. Results of the MDS map (Fig 6) shows that each group has mostly the same members configuration as the phylogenetic tree groups.

Fig 7 above described that the MDS map configuration based on the Amino Acid correlation to hematological and blood chemistry parameters was mostly the same as the MDS map based on the Nucleotide correlation to both variables (Fig 7), so both MDS maps had similar configuration members as the phylogenetic tree for describing the relationship among samples. MDS map method could be an appropriate technique to support and complete Neighbor-joining for constructing the phylogenetic tree.

CONCLUSION

This study revealed that a lot of SNP found in local cattle (Bali and PO) and CB cattle in Gowa and Sidrap indicated various genetic diversity could be a marker candidate and potential to be developed in future research knowing the correlation of the SNP with the growth rate of CB. This study also shows that the correlation of SNP to hematological and blood chemistry description can be an appropriate potential indicator and parameter to describe healthy animal status. This information will be helpful for further strategy in breeding development program.

Conflict of interest

All authors declared that there is no conflict of interest.

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