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The G894T Polymorphism of the Endothelial NO-Synthase Gene Influences Human Blood Oxygen Transport

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Abstract

Background: Nitric oxide (NO) interacts with haemoglobin to help blood carry oxygen to tissues. The polymorphism G894 T of endothelial nitric oxide synthase (G894T eNOS) affects NO synthase activity and expression. Polymorphism's effects on oxygen-dependent biological processes are currently unknown.

Methods: Study subjects were healthy young males aged 18 to 24 years (n = 172). Blood samples were drawn from the cubital vein at rest, 12 hours after the last food intake. G894T polymorphism and the blood oxygen indices (pO_2, CvO_2, SO_2, pH, p50 standard [temperature = 37 °C, pH = 7.4, pCO_2 = 40 mm Hg], and p50 actual [measured at actual temperature, pH, and pCO_2]) were determined.

Results: In persons with the TT-genotype, the oxygen content in venous blood was 48.7% (p=0.006, q=0.016) lower compared to subjects with the GT-genotype and 49.4% (p=0.012, q=0.017) lower compared to subjects with the GG-genotype. The saturation of blood in carriers of the TT-genotype was 32.4% (p=0.014, q=0.018) and 35.9% (p=0.007, q=0.016) lower as opposed to subjects with the GT-genotype and the GG-genotype, respectively. In the blood of subjects with the TT-genotype, the oxygen tension was 26.1% (p=0.008, q=0.016) lower as compared to subjects with the GT-genotype and 27.4% (p=0.012, q=0.017) lower as opposed to the GG-genotype. Volunteers with a common allele in their genotype (GG + GT) had an oxygen tension approximately 36.5 % higher than subjects with the TT genotype, whereas subjects with the TT genotype exhibited oxygen tension about 26.7 % lower than those carrying a common allele (p = 0.008). The blood pH values of the subjects having the recessive genotype were 0.022 units lower compared to the GG (p=0.038, q=0.038) and GT (p=0.034, q=0.036) genotypes. In volunteers with the TT-genotype, the p50stand parameter was 5.8% (p=0.019, q=0.023) lower compared to subjects with the GT-genotype and 6.8% (p=0.009, q=0.016) lower compared to volunteers with the GG-genotype. In persons with two T-alleles in their genotype, p50act was 5.4% (p=0.008, q=0.016) lower compared to subjects with the GT-genotype and 6.4% (p=0.005, q=0.016) lower compared to persons with the GG-genotype.

Conclusion: The T-allele of G894T polymorphism is associated with low values for oxygen content, oxygen tension, acidic pH shift and increased haemoglobin affinity for oxygen under standard and real conditions. The presence of a minor allele in the G894T polymorphism of the NOS3 gene contributes to the formation of the oxygen transport function of blood.

Introduction

Through its interactions with haemoglobin, nitric oxide (NO) plays a crucial role in the oxygen transport function of blood and tissue oxygenation. One significant element controlling the production and operation of the NO synthase enzyme is the G894T eNOS (eNOS) [1]. Although a lot of research has been done on the physiological effects of NO, such as its modulation of vascular tone and other organ systems, the precise effect of eNOS gene polymorphisms on oxygen-dependent activities remains unclear [2]. NO functions via several mechanisms, including modifying haemoglobin's affinity for oxygen and regulating vascular tone. Moreover, NO modifies haemoglobin's affinity for oxygen by producing various NO-haemoglobin complexes, which support blood's oxygen transport mechanism [3].

In addition to its physiological functions, NO is linked to nitrosative and oxidative stress, which are symptoms of an imbalanced production and neutralization of RNS and ROS. These kinds of stresses can harm cells by affecting proteins, lipids, and DNA. They are also associated with some clinical illnesses. The *eNOS* gene polymorphisms have drawn interest because NO deficiency is associated with endothelial dysfunction and the pathogenesis of cardiovascular diseases, diabetes, and other metabolic disorders. To fully understand the impact of specific *eNOS* genotypes on human health and disease susceptibility, more research is still required.

The purpose of the study was to look for the correlation between the G894T *eNOS* polymorphism and blood oxygen transport in healthy male participants. This study will provide light on the intricate relationships between oxygen delivery, NO generation, and genetic diversity.

Methods

172 young, healthy non-smoking males from the Republic of Iraq, aged 18 to 24, were included in the study. They engaged in 150–300 minutes of physical exercise each week on average. The study participants affirmed their voluntary participation by providing signed informed consent. Blood was drawn from the cubital vein when the subject was at rest, precisely 12 hours after the last meal, to analyse the blood oxygen levels and the *NOS3* gene polymorphism.

Determination of G894T polymorphism

G894T polymorphism was genotyped by allele-specific polymerase chain reaction real-time fluorescence detection [7]. Twelve hours after the previous meal, the cubital vein was punctured. Three millilitres of blood were collected in an EDTA K_3 vacuum tube, and leukocytes (white blood cells) were used for DNA extraction from whole blood using the Xpress™ DNA blood kit from Lytech Co. Ltd. (Russia). At room temperature, 100 microlitres of whole blood were spun for ten minutes at 3500 rpm. Plasma was separated from other components of the blood using centrifugation. After that, the plasma was removed from the tubes and frozen for one hour at -20°C. The tube was thawed at room temperature and prepared for use.

After that, a rotary shaker (vortex) was used to add the DNA-XPress™ reagent and fully mix it for 15 seconds. After that, the tube was heated for 15 minutes to a temperature of 98°C. Finally, 15 seconds of centrifugation at 15,000 revolutions per minute were applied to the contents of the tube. Utilizing the recovered supernatant, a DNA sample was extracted.

The G894T polymorphism was detected using Probes labelled with different fluorophores containing Taq DNA polymerase, diluent, and 2.5-fold reaction mixture used in this experiment (Syntol, Russia). The DNA region of interest was amplified using the Rotor Gene-Q technique (Qiagen, Germany). The software for the amplifier is called (Q-Rex Software) used for allele discrimination depending on the relationship between dye fluorescence intensity and gene copy number.

Blood oxygen measurement

Blood gases were measured using a 3-ml Heparin Vacuum Tube (VACUETTE®). Within three hours after blood collection, the test was performed. The measurements included the partial pressure of oxygen and carbon dioxide, as well as the standard base deficit/excess (ABE/SBD) and the standard base excess (SBE). The p50 value, which represents the blood oxygen tension at which haemoglobin is 50% saturated with oxygen, was used to assess the affinity of haemoglobin for oxygen. This assessment was conducted under both standard conditions (p50 stand: 37 °C, pH = 7.3, pCO_2 = 43 mm Hg) and actual conditions (p50 act: 37 °C, pH = 7.4, pCO_2 = 40 mm Hg). The Oxyhaemoglobin dissociation curve location was predicted using the p50 values obtained.

Stable NO nitrates and nitrites end products determination.

Serum nitric oxide concentrations were estimated using the amount of stable NO end products, (NO3/NO2). Blood was collected in a 2-ml Heparin (VACUETTE®). Blood plasma was deproteinized using Zinc sulphate and NaOH, and nitrates were converted to nitrites using cadmium granules.

The Griess reagent measured plasma NO2/NO3 levels at 540 nm.

Statistical data analyses

The genotype distribution of the polymorphism under study was checked using Pearson's chi-squared (χ^2) test to assess the Hardy–Weinberg equilibrium. The Statistic 10.0 application was used for conventional statistical analysis. The statistical significance of variations in numeric parameters with a normal distribution was investigated using the t-test for independent samples. For non-normal distributions, the statistical significance of the Mann-Whitney test was applied, with the median values being presented at the 25th and 75th percentiles. At the p-value⩽0.05 significance threshold, the differences were determined to be statistically significant. To account for multiple testing, the false discovery rate (FDR) approach was employed. The text contains the p and q values that have been adjusted for the FDR.

Results

The distribution of frequencies of the NOS3 "G894T polymorphism" allele in males was studied and is presented in Table 1. Based on the table, it is evident that the genetic structure of the sample is not affected by any external factors, such as mutations, Genetic drift, or non-random mating, in relation to the distribution of the polymorphism G894 genotype T. It was shown that 49.1% of the individuals had the homozygous common genotype (GG), whereas 44.2 % had the heterozygous genotype, and just 6.7 % of those people had the homozygous recessive genotype (TT). Using the NOS3 G894T polymorphism, Table 2 outlines the features of the blood's ability to transport oxygen in healthy people. Polymorphism allele and genotype frequencies were inconsistent in the study of blood oxygen transport characteristics. Respondents with TT and GT genotypes reported lower venous oxygen saturation levels than those with GG and GG (3.9 [3.1; 7.3] and 7.63.5, respectively, p=0.006 and q=0.016, respectively). It was shown that the TT genotype had a lower oxygen content (7.73.7 [3.1; 7.3]) than the GG+GT genotype, with a statistical significance (p = 0.007) and significance (q = 0.016).

In a blood oxygen saturation investigation, patients with the TT-genotype had lower blood oxygen saturation (5.5 ± 13.0 vs. 37.7 ± 15.3, p = 0.014, q = 0.018) than patients with the GT- and GG-genotypes. In the recessive model, blood oxygen saturation was lower in carriers of the recessive genotype (25.5 ± 13.0) compared to those with a common allele (38.8 ± 15.9, p = 0.008, q = 0.016). There was a statistically significant difference in oxygen tension between subjects with the TT and GT genotypes, respectively (17.0 ± 5.5 vs. 23.0 ± 7.0 mm Hg; p = 0.008, q = 0.016). Oxygen tension was substantially higher in volunteers with GG+GT genotypes (23.27.5 vs 17.0, p=0.008; q=0.016) than those with the TT genotype (17.0). In individuals with the common allele (GG + GT), the blood pH was 0.022 units higher than in those with the recessive genotype (7.351 [7.326; 7.377], p = 0.030, q = 0.034), which is suggestive of an acidic pH shift in subjects with the recessive genotype.

It should be noted that the p50 values differ according to genotype. Compared to patients with the GT genotype (25.9 [23.0; 27.6] versus 27.51.7, p = 0.019, q = 0.023) or the G genotype (25.9 [23.0; 27.6), patients with the TT genotype were shown to have lower stand metrics. Participants with recessive genotypes exhibited lower p50stand values (27.72.0 vs 25.9 [23.0; 27.6], p = 0.010, q = 0.016), according to studies using the recessive model. Compared to haemoglobin with the G allele, those with the TT genotype exhibited a greater affinity for oxygen. A comparison of the p50act values showed significant differences between genotypes. In comparison to individuals with the GT or GG genotypes, two T-alleles in the genotype were linked to a lower p50act (26.3 [24.5; 27.7] versus 27.81.5, p = 0.008, q = 0.016). Recessive carriers had lower p50act values (27.0 ± 17.3 vs. 26.3 [24.5; 27.6], p = 0.004 and q = 0.016) when GG+GT and TT were contrasted with each other. These results indicate that the TT causes the oxyhaemoglobin dissociation curve to move to the left (Figure 1).

The systolic and diastolic blood pressure measurements were considerably higher among carriers of the homozygous recessive genotype (Table 3).

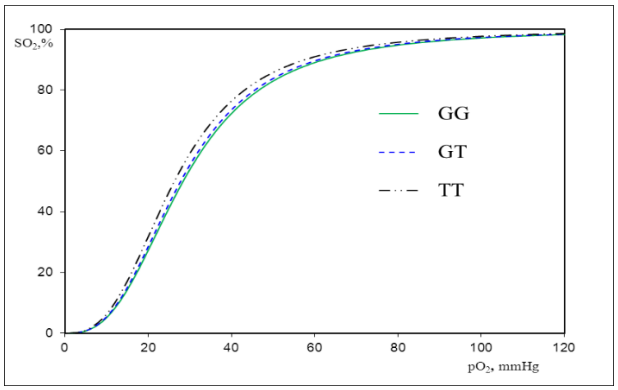

Figure 1: Oxyhaemoglobin dissociation curves for NOS3 G894T polymorphisms.
(Individuals with the TT genotype exhibited a lower p50act compared to those with GT or GG genotypes. Recessive carriers had reduced p50act values when GG+GT were contrasted with TT. These findings suggest that TT genotype shifts the oxyhemoglobin dissociation curve to the left).

Genotypes			Allele\frequency	The Hardy-Weinberg balance	
GG	GT	TT	G/T	χ^2	P
84	75	13	0.72/0.28	1.032	0.311

Table 1: NOS3 gene allele and genotype frequencies in males with the G894T polymorphism.

Parameters	Total N=172	GG + GT N=159	GT + TT N=88	GG N=84	GT N=75	TT N=13
Hb, g/L	146 ±10	146 ±10	145 [141; 154]	146 ±11	145 [140;153]	146 [141; 154]
CvO_2, mL/L	7.5 ±3.7	7.7* ±3.7	7.3 ±3.5	7.7* ±3.9	7.6* ±3.5	3.9 [3.1; 7.3]
OC, mL / L	20.6 ±1.6	20.6 ±1.6	20.7 ±1.7	20.5 [19.4;21.6]	20.7 ±7.1	20.7 [20.1;21.8]
SO_2, %	37.9 ±16	38.8* ±15.9	36.1 ±15.5	39.8* ±16.4	37.7* ±15.3	25.5 ±13.0
MetHb, %	2.0 ±0.6	2.0 ±0.6	2.1 ±0.7	2.0 ±0.4	2.0 ±0.7	2.2 [1.9; 2.7]
pO_2, mm Hg	22.8 ±7.6	23.2* ±7.5	22.2 ±7.1	23.4* ±8.0	23.0* ±7.0	17.0 ±5.5
pH, units	7.371 ±0.032	7.372*± 0.031	7.371 ± 0.028	7.372* ± 0.031	7.372* ± 0.028	7.352 [7.325; 7.378]
pCO_2, mm Hg	55.8 ±6.1	55.4 ±6.1	55.2 [52.9; 58.7]	57.1 ±7.3	56.0 [53.5; 59.0]	58.2 [53; 61.5]
HCO_3^-, (mmol/L)	32.4 ±1.9	32.4 ±1.9	32.4 ±1.9	32.6 [31.4;33.4]	32.4 ±1.9	32.9 [32.1;33.9]
TCO_2, (mmol/L)	34.1 ±2.0	34. 1±2.0	34.1 ±2.0	34.4 [32.9;35.2]	34.0 ±2.0	34.8 [34.0;35.6]
ABE, (mmol/L)	6.0 ±1.6	6.0 ±1.7	6.1 [4.9; 7.2]	6.0 ±1.7	6.2 [4.9; 7.2]	6.0 [5.2; 6.7]
SBE, mol/L	6.9 ±1.9	6.9 ±2.0	6.9 [5.7; 8.4]	6.9 ±2.0	6.8 [5.7; 8.4]	7.0 [6.7; 8.5]
SBC, (mmol/L)	27.8 ±1.3	27.9 ±1.3	28.0 [26.8; 28.8]	27.9 ±1.4	28.1 [26.8; 28.8]	27.4 [26.6; 28.2]
$P50_{stands}$, mm Hg	27.5 ±2.1	27.7* ±2.1	27.31 ±1.93	27.8* ±2.2	27.5* ±1.8	25.9 [23.1; 27.5]
$P50_{act}$, Mm Hg	27.9± 1.71	28.0* ±1.71	27.6 ±1.71	28.1* ±1.91	27.8* ±1.51	26.3 [24.5; 27.7]

Table 2: The G894T polymorphism's allele and genotype frequencies are connected to blood oxygen transport function measures.

Note: Compared to the T-genotype, the differences are statistically significant (FDR-corrected). The oxygen tension in the blood of volunteers with the TT-genotype was lower compared to subjects with the GT-genotype (17.0±5.5 vs. 23.0±7.0, p=0.008, q=0.016) and the GG-genotype (17.0±5.5 vs. 23.4±8.0, p=0.012, q=0.017). In their turn, volunteers having a common allele in their genotype (GG+GT) exhibited higher oxygen tension in comparison with bearers of the TT-genotype (23.2±7.5 vs. 17.0, p=0.008, q=0.016). The blood pH values of the subjects with the common allele (GG+GT) were 0.022 units higher as opposed to the recessive genotype (7.373±0.030 vs. 7.351 [7.326; 7.377], p=0.030, q=0.034), which is suggestive of some acidic pH shift in this subjects.It should be noted that the differences in p50 values depended on the genotype. Thus, subjects with the TT-genotype demonstrated lower p50stand parameters compared to volunteers with the GT-genotype (25.9 [23.0; 27.6] vs. 27.5±1.7, p=0.019, q=0.023) and GG-genotype (25.9 [23.0; 27.6] vs. 27.8±2.1, p=0.009, q=0.016). A comparison applying the recessive model showed lower p50stand values in subjects with the recessive genotype (27.7±2.0 vs. 25.9 [23.0; 27.6], p=0.010, q=0.016). As can be seen, the TT-genotype was responsible for higher haemoglobin affinity for oxygen compared to the genotypes with G-allele. A comparison of the p50act values showed similar changes. Subjects with two T-alleles in their genotype, had lower p50act compared to subjects with the GT-genotype (26.3 [24.5; 27.7] vs. 27.8±1.5, p=0.008, q=0.016) and the GG-genotype (26.3 [24.5; 27.7] vs. 28.1±1.9, p=0.005, q=0.016). A comparison of this parameter using GG+GT versus TT revealed that carriers of the recessive genotype have lower p50act values (28.0±1.7 vs. 26.3 [24.5; 27.7], p=0.004, q=0.016). These findings data show that the TT is responsible for the shift of the oxyhemoglobin dissociation curve to the left (Figure 1).

Parameters	Total N=172	GG+GT N=159	GT+TT N=88	GG N=84	GT N=75	TT N=13
BMi(kg/m²)	26±7	27±7	25±7.6	24±4	25±9	25 [23; 28]
SBp (mm Hg)	123.0±8.2	122.4±7.7*	123.1±8	122.1±8 *	122.9±7*	129.9±12.5
DBp (mm Hg)	81.2±7	80.6±6.4*	80.4±8	80.6±6*	78.5±7.1*	90.0 [81.0; 91.0]

Table 3: BMI and blood pressure correlated with G894T polymorphism allele and genotype frequencies.

Note: Compared to the T-genotype, the differences are statistically significant (FDR-corrected).

Discussion

Studies on the distribution of allele and genotype frequencies of G894T polymorphism have been conducted in different population samples. According to some findings, the prevalence of GG, GT and TT G894T polymorphism in Chinese subjects was 46.2%, 37.8% and 16.0%, respectively, and the frequency of G/T alleles amounted to 65.1%/34.9% In a study conducted on a homogeneous population of the Caucasian origin (Greeks), showed the distribution of GG, GT and TT G894T polymorphism to be 44.7%, 43.1% and 12.2%, respectively, and the frequency of G/T alleles to be 66.2%/33.8%. The data of our research into the distribution of allele and genotype frequencies of G894T polymorphism are, in general, comparable to the results of similar studies.

Recently, a significant number of papers have been published indicating that G894T polymorphism is associated with the development of cardiovascular disorders. For instance, an association between G894T polymorphism and the development of endothelial dysfunction was found. In the work of Guo X . an association between TT-genotype and increased risk of ischaemic stroke compared to genotypes with G-allele was detected, particularly in Asian subjects. A contribution of the TT-genotype to the development of angina was also revealed.

It was established that G894T polymorphism was associated with the development of myocardial infarction and an association of T-allele with the development of coronary heart disease was also determined (OR=1.52, 95% CI=1.37–1.69).

The above disorders are based on a few pathogenetic factors, with one of which being endothelial dysfunction. It is obvious that the low values for oxygen tension, oxygen content and oxygen saturation of blood, that were found in our study in subjects with the two T-allele genotypes of G894T polymorphism, reflected changes in oxygen transport function of blood which might contribute to the development of endothelial dysfunction.

The present study revealed a small (0.022 units) reduction of blood pH values in subjects with the recessive genotype and a simultaneous decrease of the p50stand and p50act. Reduction of pH is known to decrease haemoglobin affinity for oxygen and displace the oxyhaemoglobin dissociation curve to the right. However, human erythrocyte haemoglobin has a specific environment, which modifies its main functions. The position of the oxyhaemoglobin dissociation curve in vivo results from a combined interaction of many modulating factors. It is evident that other factors of intraerythrocytic mechanisms regulating blood oxygen-binding properties (pCO2, 2,3 diphosphoglycerate) are responsible for the compensation of the oxyhaemoglobin dissociation curve shift, observed in our study. At the same time, it should be noted that irrespective of the genotype, pH remains within the physiological range and therefore

there are no grounds to suggest an acid-base imbalance.

The versatile interaction between NO and haemoglobin should also be mentioned. Under physiological conditions, an increased level of NO is responsible for an elevated amount of oxidized and nitrosylated haem, which decreases the total amount of haemoglobin involved in oxygen transport, thus resulting in a reduced general oxygen transport function of blood The activity of the endothelial NO synthase decreases in the presence of the T-allele of G894T polymorphism. Under hypoxic conditions, erythrocytic NO synthase may exert a vasodilating effect in regions adjacent to blood vessels. A significant rise in the activity of erythrocytic NO synthase was observed with an increase in shear stress to 0.1 Pa.

NO synthesis in erythrocytes provides effective intracellular signaling and participates in the modification of haemoglobin affinity for oxygen. Our data show that the TT-genotype of G894T polymorphism is associated with a rather low content of O2 in venous blood. We believe these differences to be of a NO-dependent nature and to be based on the functional state of the endothelium and the activity of the L-arginine–NO pathway.

After binding to NO, the nature of haemoglobin interaction with ligands is changed. Bonaventura C. et al. suggested a model in which haemoglobin affinity for oxygen is related to the reduction of nitrites and formation of NO. By binding to the T-quaternary structure of haemoglobin, allosteric anion effectors (chloride, 2,3-diphosphoglycerate, inositol hexaphosphate) change the position of an allosteric balance between the low- (T) and high-affinity (R) structures of this protein, which significantly influences the reactions of haemoglobin with NO and O2 : the binding of NO with haemoglobin shifts the T-R-balance to the R-side and subsequent binding of oxygen to vacant sites of the tetramer occurs under increased affinity. In turn, the anions, that promote T-state (inositol hexaphosphate), contribute to the development of pentacoordinate geometry of NO-haem, increased haem oxidation and decreased haemoglobin affinity for.

In some of our studies, we have shown the participation of NO in the mechanisms of regulation of blood oxygen-binding capacity. Experiments in vitro using different concentration relationships showed that when blood is incubated with nitrosocysteine under oxygenation, the p50act was lower by 3.4±0.9 mm Hg ($p<0.05$). A shift of the oxyhaemoglobin dissociation curve to the left was observed in hypothermic L-arginine-treated rats compared to control animals (p50act = 17.45±0.60 vs. 21.03±0.35 mm Hg ($p<0.05$) In patients with arterial hypertension, endothelial dysfunction promotes impairment of blood oxygen transport processes while the use of products modulating NO synthesis (Nebivolol) normalises parameters of the oxygen transport function of blood.

Binding of NO to haemoglobin is known to influence its oxygen-binding capacity. The effect of L-arginine seems to result directly from the NO interaction with Hb and to be mediated through an oxygen-dependent mechanism for regulation of NO synthesis. The absence of changes in a number of blood gas values and circulatory buffers (pCO2, TCO2, $HCO3^-$, SBC, ABE, SBE, etc.) reflects a relatively specific influence of G894T polymorphism. The obtained results suggest that the differences in blood oxygen parameters in relation to the genotype are of a NO-dependent nature and can be explained by the functional state of the endothelium and the activity of the L-arginine-NO pathway. On the other hand, the influence of G894T polymorphism alone on systemic oxygen transport seems to be unlikely. It is obvious that the differences in blood oxygen parameters in different genotypes of G894T polymorphism are due to a number of factors: they arise directly through binding of NO to haemoglobin and indirectly through modulation of allosteric effectors and changes in the functioning of the cardiovascular system. Undoubtedly, other factors also play a role in the formation of total oxygen homeostasis. The findings of our study suggest that the differences in oxygen content, pH, and haemoglobin affinity for oxygen are associated with the distribution of allele and genotype frequencies of G894T polymorphism of the NOS3 gene.

Recommendations for the practical use of the results

The results show that the analysed population has similar allele and genotype distributions to data from other regions for the endothelium NO synthase gene's G894T and T786C polymorphism variations. Peculiarities of polymorphisms of this gene must be considered when evaluating a person's aerobic processes and physical abilities. Further study of genetic factors is essential for understanding the mechanisms that form the body's aerobic metabolism.

Author Contributions

Dr. Mustafa Aljaberi has designed the study and conducted the data analysis and edited the manuscript. Dr. Zain Alabdean Azeez Alnoor: collected the samples, Dr. Elyes Chabchoub supervised the research, guided the experiments, and revised the manuscript.

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