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The association of *LPCAT1*-rs9728 polymorphism with cord blood IL-10, MIF, and VEGF levels in neonatal respiratory distress syndrome: a case–control study

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Abstract

Background Lysophospholipid acyltransferase (LPCAT) is crucial for surfactant biosynthesis. It is encoded by *LPCAT* genes. We investigated the *LPCAT1*-rs9728 genotypes in neonatal respiratory distress syndrome (NRDS) cases and their possible association with the cord arterial serum interleukin-10 (IL-10), macrophage migration inhibitory factor (MIF), and vascular endothelial growth factor (VEGF) levels.

Methods The study included 160 neonates grouped into G1: 60 healthy neonates and G2: 100 NRDS cases. IL-10, MIF, and VEGF levels were measured by their corresponding kits. The Gene JETTM Whole Blood Genomic DNA Purification Mini Kit was used to extract the DNA from the newborn venous blood. The quantitative real-time polymerase chain reaction was carried out for *LPCAT1*-rs9728 genotyping.

Results The IL-10 and MIF levels were significantly higher, while VEGF levels were significantly lower in G2 than in G1. The percentages of *LPCAT1*-rs9728 AA and *LPCAT1*-rs9728 AG genotypes were significantly higher in G2 than in G1. The IL-10 and MIF levels were significantly higher, while the VEGF levels, birth weight, and APGAR score at 1 and 5 min were significantly lower in neonates with *LPCAT1*-rs9728 AA genotype than in neonates with *LPCAT1*-rs9728 AG and *LPCAT1*-rs9728 GG genotypes and in neonates with *LPCAT1*-rs9728 AG genotype than in neonates with *LPCAT1*-rs9728 GG genotype.

Conclusion There is an association between the LPCAT1-rs9728 AA genotype and its A allele and the NRDS development and severity. Further research may provide a better understanding of this association to help future management.

Keywords NRDS, *LPCAT1*-rs9728, IL-10, MIF, VEGF

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Background

Neonatal respiratory distress syndrome (NRDS) is the most frequent cause of respiratory failure in the first few days after delivery, leading to neonatal mortality [1]. It is a result of the lack of lung surfactant that negatively impacts normal lung function [2]. Pulmonary surfactant is a surface tension-lowering component that comprises lipids (90%) and proteins (10%). Phospholipids represent 80–85% of lipids' overall mass and are produced and secreted by alveolar type II cells [3]. About 80% of phospholipids are phosphatidylcholine, and the majority of it is dipalmitoyl phosphatidylcholine [4]. The biosynthesis of the lung surfactant and its function are affected by many factors, including genetic, developmental, and environmental ones [5, 6].

Many enzymes contribute to surfactant metabolism. One of them is lysophospholipid acyltransferase (LPCAT), which is crucial for surfactant biosynthesis [7]. The LPCAT1 isozyme is significant for membrane biogenesis and impacts the physiological response of the lung epithelium to damage [5]. It is encoded by the *LPCAT1* gene that is placed on chromosome 5p15.33 and is comprising 18 exons [7].

Interleukin-10 (IL-10) is an inhibitory and immunoregulatory cytokine that has a known function in regulating surfactant biosynthesis and metabolism [8]. Its serum levels have been reported as an important indicator of the severity of NRDS [9].

The macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that is found in the amniotic fluid and is expressed in the placenta and neonatal lungs (alveolar endothelium and macrophages) [10]. It is also expressed in the bronchial epithelial cells, monocytes, and eosinophils [11]. The MIF promotes the synthesis of lots of cytokines such as interleukins 1, 6, and 8, each of which has been related in one way or another to the development of NRDS [12]. Also, high levels of cord blood MIF were reported in cases with NRDS [13].

Vascular endothelial growth factor (VEGF), a crucial regulator of endothelial cells proliferation and angiogenesis, plays an essential role in the development of the lungs and the release of its surfactant. It also augments surfactant protein synthesis. Its cord blood levels were inversely related to the incidence of NRDS [14–16].

The current work aimed to investigate the *LPCAT1*-rs9728 genotypes in cases with NRDS and its possible association with the cord arterial serum levels of IL-10, MIF, and VEGF.

Methods

The current case–control study was carried out in the neonatal intensive care unit, and the woman's health hospital, Assiut University. It was authorized by the Ethics Board, College of Medicine (IRB: 17,200,578). The study included 160 neonates that were grouped into the following: G1: 60 normal healthy control neonates and G2: 100 neonates with RDS.

The diagnosis of NRDS depended on patient's clinical signs (tachypnea, retraction, flaring of the nasal alae, grunting, and cyanosis), arterial blood gas analysis (hypoxemia and hypercapnia), and chest x-ray findings (low volume lung with a diffuse reticulogranular pattern and visible air bronchograms) [17].

Complete medical and maternal and family histories were taken from the neonates' mothers. These included the parents' age, consanguinity, and maternal diseases, especially diabetes and hypertension, and medications taken by the mother during pregnancy. For each neonate, a full medical examination was taken, and anthropometric measurements were done. APGAR scores were observed at 1, 5, and 10 min after delivery.

The neonate was disqualified from the current research when his guardian refused to contribute or when the neonate exhibited neonatal sepsis, pulmonary infections, congenital anomalies, or any other systemic diseases. Written informed consent was taken from the guardians of all neonates.

Sampling and laboratory assays

The umbilical cord was properly washed with an antiseptic solution before being clamped. Then, 4 ml of arterial cord blood was withdrawn in plain tubes, allowed to clot for 20 min, and centrifuged at 3000 rpm for 5 min. The sera were collected and stored at − 70 °C until the measurement of IL-10, MIF, and VEGF using the human IL-10, MIF, and VEGF ELISA Kits (Thermo Fisher Scientific, USA, cat nos. BMS215-2, EHMIF, and KHG0111, respectively).

Genetic work

Two milliliters of venous blood were withdrawn from each neonate and kept at − 70 °C in an EDTA-containing tube for DNA extraction and the genetic work.

Single-nucleotide polymorphism (SNP) selection

Gene database supplied by the NCBI and the Human Genome Project and the Haploview tool was used to choose the SNP of *LPCAT1* [7].

Purification of DNA

According to the manufacturer's guidelines, the GeneJET™ Whole Blood Genomic DNA Purification Mini Kit (Thermo-Fisher Scientific, USA; Cat. No. 0781) was used to extract the genomic DNA from the venous blood of newborns.

Real-time polymerase chain reaction (RT-PCR) for SNP genotyping

An Applied Biosystems 7500 Fast Real-Time PCR System with version 2.3 software was used for the SNP genotyping. Purified genomic DNA, Pre-Designed 20X TaqMan® SNP Genotyping Assay, and TaqMan™ Universal Master Mix II were the three components essential for the genotyping.

A 96-well optical reaction plate was used for the amplification. Each well contained 12.5 ml of TaqMan™ Universal Master Mix II with no UNG Cat. No. 4440043; 1.25 ml of TaqMan® Pre-Designed SNP Genotyping Assays 20 X, Cat. No. 4351379; 5 ml of pure genomic DNA; and 6.25 ml of 1X TE buffer. Using the default parameters, all analyses were done automatically. The RT-PCR protocol for each reaction was as follows: polymerase activation step for 10 min at 95 °C, denaturation stage for 15 s at 95 °C, and 40–45 annealing/extension cycles at 60 °C for 1 min.

The main components of TaqMan® Pre-Designed SNP Genotyping Assays are two TaqMan® minor groove binder probes, one labeled with VIC® dye to detect the allele 1 sequence and the other with FAM™ dye to detect

Table 1 Sociodemographic data, clinical history, and findings of two studied groups

		G1 (healthy control) (*N* = 60)	G2 (NRDS) (*N* = 100)	*p*-value
Neonatal gender	**Male**	19 (31.67%)	33 (33%)	0.862
	Female	41 (68.33%)	67 (67%)	
Birth weight (kg)		2.6 ± 0.7	1.7 ± 0.8	< 0.001
Gestational age (GA)	**Pre-term**	12 (20%)	70 (70%)	< 0.001
	Full-term	48 (80%)	30 (30%)	
Consanguinity	**+Ve**	23 (38.33%)	45 (45%)	0.409
	−Ve	37 (61.67%)	55 (55%)	
Mode of delivery	**Emergency CS**	32 (53.33%)	55 (55%)	0.973
	Elective CS	24 (40%)	39 (39%)	
	Normal delivery	4 (6.67%)	6 (6%)	
APGAR Scores	**At 1 min**	6.42 ± 1.19	4.44 ± 1.39	< 0.001
	At 5 min	8.35 ± 0.92	6.99 ± 1.14	
	At 10 min	9.28 ± 0.59	8.78 ± 0.85	
Antenatal care	**Yes**	37 (61.67%)	44 (44%)	0.030
	No	23 (38.33%)	56 (56%)	
Previous fetus with NRDS	**Yes**	12 (20%)	32 (32%)	0.100
	No	48 (80%)	68 (68%)	
Use of antenatal steroids	**Yes**	42 (70%)	17 (17%)	< 0.001
	No	18 (30%)	83 (83%)	
Maternal risk factors (hypertension, diabetes mellitus, anemia, eclampsia, etc.)	**Yes**	22 (36.66%)	68 (68%)	< 0.001
	No	38 (63.33%)	32 (32%)	
Residence area	**Urban**	19 (31.67%)	44 (44%)	0.262
	Residence Semi-urban	19 (31.67%)	29 (29%)	
	Rural	22 (36.67%)	27 (27%)	
Education level of the mother	**Ignorant**	8 (13.33%)	13 (13%)	0.720
	Primary	10 (16.67%)	12 (12%)	
	Secondary	32 (53.33%)	52 (52%)	
	High education	10 (16.67%)	23 (23%)	
Working of mother	**Employee**	27 (45%)	54 (54%)	0.270
	Nonemployees	33 (55%)	46 (46%)	

NRDS neonatal respiratory distress syndrome, *GA* gestational age, *CS* cesarean section, *APGAR* appearance, pulse, grimace, activity, and respiration

Table 2 Serum IL-10, VEGF, and MIF levels in G1 and G2

	Mean ± SD		*p*-value
	G1 (healthy control) (*N* = 60)	G2 (NRDS) (*N* = 100)	
IL-10 (pg/ml)	7.63 ± 1.44	14.9 ± 2.44	< 0.001
MIF (ng/ml)	13.9 ± 5.27	22.8 ± 4.77	< 0.001
VEGF (pg/ml)	30.1 ± 12.7	17.8 ± 11.9	< 0.001

NRDS neonatal respiratory distress syndrome, *IL-10* interleukin-10, *MIF* macrophage migration inhibitory factor, *VEGF* vascular endothelial growth factor, *SD* standard deviation

the allele 2 sequence, along with sequence-specific forward and reverse primers. The TaqMan™ Universal Master Mix II with no UNG contains AmpliTaq Gold® DNA Polymerase, ultra-pure, deoxyribonucleotide triphosphates, ROX™ passive reference dye, and components of an optimized buffer. The Pre-Designed TaqMan® SNP Genotyping Assays and the TaqMan™ Universal Master Mix II with no UNG were manufactured by ThermoFisher Scientific (USA). The SNP ID of *LPCAT1*-rs9728 is at Chr.5:1,461,983 on Build GRCh38.

The type of polymorphism is transversion substitution (A/G), and the relevant sequence is [VIC/FAM]: CAACAC GCCAAGAGCCCTGAAATTG[A/G] CTTCGGTTTACT CCATCCCTGTCG.

Statistical analysis

The gathered data were analyzed by SPSS (v.26). A *chi-square* test was used for the comparison of qualitative data. Student *t*-test and one-way ANOVA were applied for the comparison of continuous variables. The receiver operating characteristic (ROC) curve was done to investigate the capability of the cord arterial serum levels of IL-10, MIF, and VEGF to differentiate cases with NRDS from healthy neonates. The test was considered significant when its *p*-value was ≤ 0.05 [18].

Results

The mean of birth weight; APGAR scores at 1, 5, and 10 min; and the percentages of mothers who reported antenatal care and antenatal steroids were significantly lower in G2 than in G1. On the other hand, the percentages of preterm neonates and mothers who had complicated pregnancies were significantly higher in G2 than in G1 (Table 1).

The cord arterial serum IL-10 and MIF levels were significantly high, while the levels of VEGF were significantly low in G2 compared to G1 (Table 2).

The cord arterial serum IL-10 and MIF levels correlated negatively, while VEGF levels correlated positively with birth weight values and APGAR scores at 1, 5, and 10 min (Table 3).

The use of antenatal steroids did not significantly affect the cord IL-10, MIF, or VEGF levels measured in this study, either in the cases or control groups.

From the ROC curve analysis, the cord arterial serum levels of IL-10 showed an excellent ability to differentiate cases with NRDS from healthy neonates. The cord arterial serum levels of MIF and VEGF showed a good ability to do this (Fig. 1).

The *LPCAT1*-rs9728 AA and *LPCAT1*-rs9728AG genotypes were significantly high in the G2 compared to G1 (Table 4 and Fig. 2).

There was significantly lower birth weight and APGAR score at 1 and 5 min in the *LPCAT1*-rs9728 AA genotype than in the *LPCAT1*-rs9728 AG and *LPCAT1*-rs9728 GG genotypes and in the *LPCAT1*-rs9728 AG genotype than in the *LPCAT1*-rs9728 GG genotype. There was a significantly lower APGAR score at 10 min in the *LPCAT1*-rs9728 AA genotype than in the *LPCAT1*-rs9728 GG genotype (Table 5).

The *LPCAT1*-rs9728 AA genotype group exhibited significantly higher values for both days of hospitalization (33.04 ± 3 days) and mortality percentage (53.1%) compared to the *LPCAT1*-rs9728 AG (30.54 ± 3.4 days, 45.4%) and *LPCAT1*-rs9728 GG (19.44 ± 1.9 days, 22.2%) genotypes, with *p*-value < 0.001.

Table 3 Correlations between serum of IL-10, MIF, and VEGF levels and birth weight and APGAR score at 1, 5, and 10

		Birth weight (kg)	APGAR score at 1 min	APGAR score at 5 min	APGAR score at 10 min
IL-10 (pg/ml)	*rho*	−0.405	−0.539	−0.466	−0.235
	P	**< 0.001**	**< 0.001**	**< 0.001**	**0.003**
MIF (ng/ml)	*rho*	−0.327	−0.372	−0.359	−0.222
	P	**< 0.001**	**< 0.001**	**< 0.001**	**0.005**
VEGF (pg/ml)	*rho*	0.269	0.317	0.350	0.197
	P	**0.001**	**< 0.001**	**< 0.001**	**0.012**

APGAR appearance, pulse, grimace, activity, and respiration, *IL-10* interleukin-10, *MIF* macrophage migration inhibitory factor, *VEGF* vascular endothelial growth factor, *rho* Spearman correlation

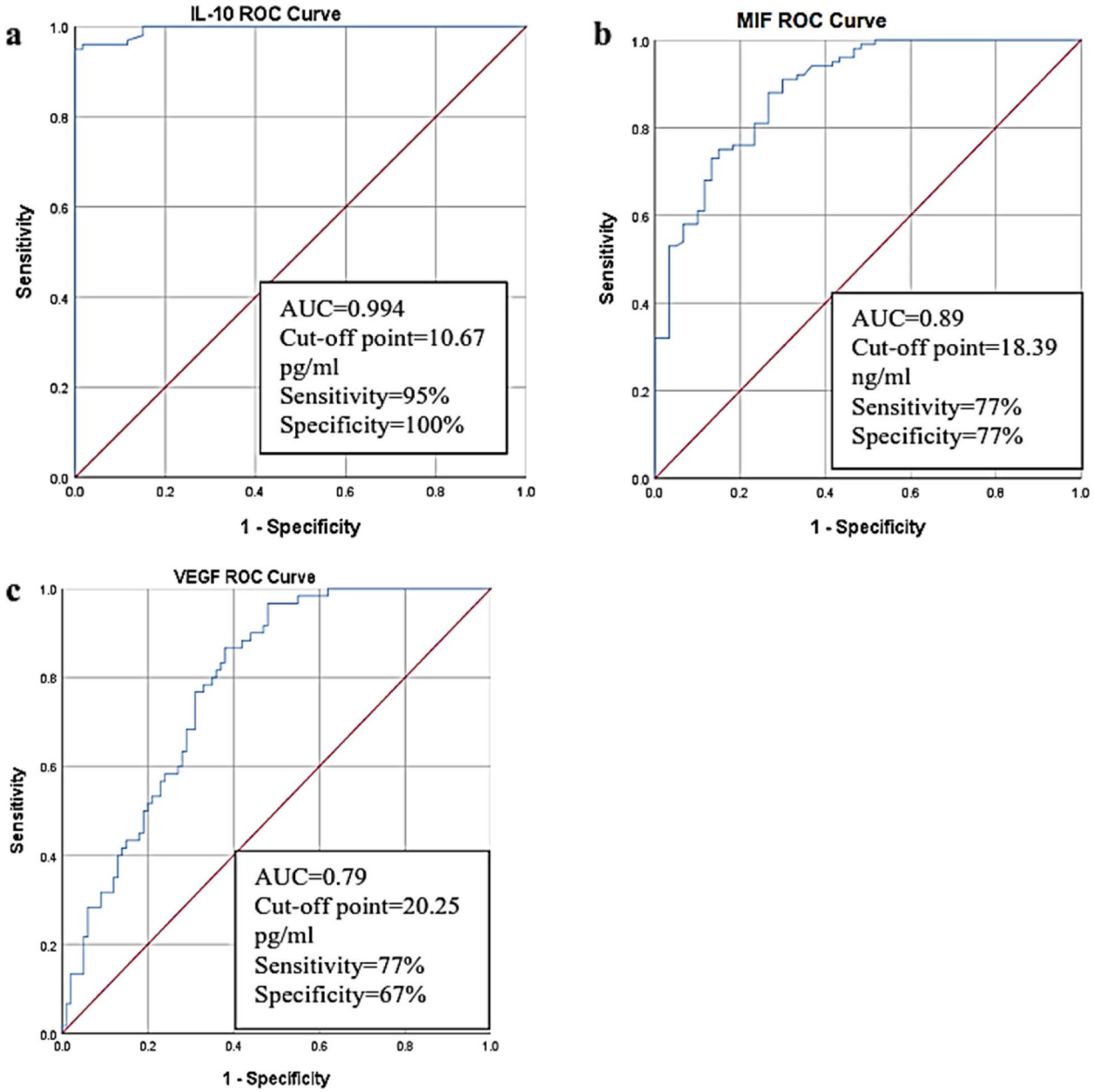

Fig. 1 Receiver operating characteristics (ROC) curve analysis for the capability of cord arterial serum levels of (**a**) IL-10, (**b**) MIF, and (**c**) VEGF to differentiate neonates with RDS from healthy neonates. AUC, area under the ROC curve

The cord arterial serum IL-10 and MIF levels were significantly higher, while the VEGF levels were significantly lower in the neonates with *LPCAT1*-rs9728 AA genotype than the neonates with *LPCAT1*-rs9728 AG and *LPCAT1*-rs9728 GG genotypes and in the neonates with *LPCAT1*-rs9728 AG genotype than the neonates with *LPCAT1*-rs9728 GG genotype ($p < 0.001$ for all) (Fig. 3).

Table 4 *LPCAT1*-rs9728 genotypes in G2 and G1

Genotypes	G1 (healthy control) ($N = 60$)	G2 (NRDS) ($N = 100$)	*p*-value
AA genotype	10 (16.7%)	47 (47%)	< 0.001
AG genotype	20 (33.3%)	44 (44%)	
GG genotype	30 (50%)	9 (9%)	

NRDS neonatal respiratory distress syndrome

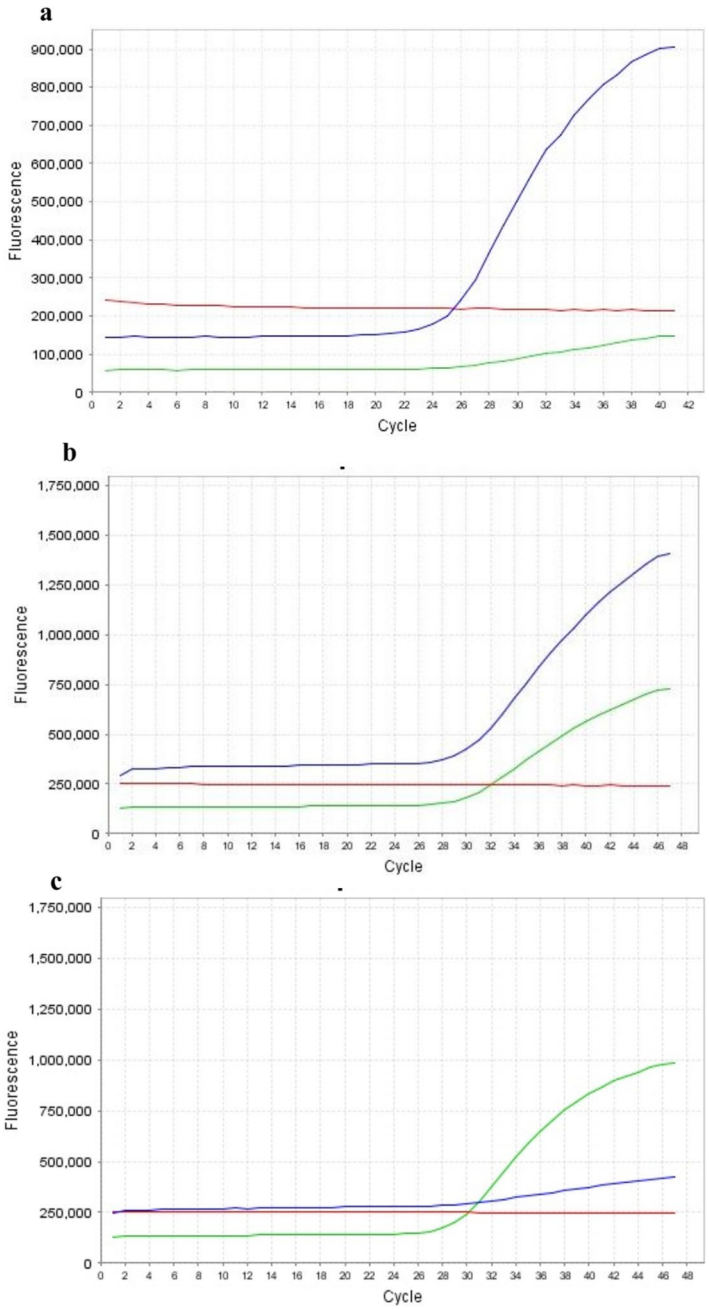

Fig. 2 The multicomponent plot for the *LPCAT1*-rs9728 genotyping. (**a**) Homozygous GG, (**b**) heterozygous AG, and (**c**) homozygous AA genotypes

Table 5 Birth weight, APGAR score at 1, 5, and 10 min in different *LPCAT1*-rs9728 genotypes

	Genotypes			***p*-value**			
	GG genotype (*N* = 39)	**AG genotype (*N* = 67)**	**AA genotype (*N* = 54)**	**GG vs AG vs AA**	**GG vs AG**	**GG vs AA**	**AG Vs AA**
Birth weight (kg)	2.5 ± 1.0	2.0 ± 0.7	1.7 ± 0.8	< 0.001	0.012	< 0.001	0.038
APGAR score at 1 min	5.8 ± 1.6	5.0 ± 1.6	4.8 ± 1.4	0.009	0.017	0.003	0.409
APGAR score at 5 min	8.0 ± 1.2	7.3 ± 1.2	7.2 ± 1.2	0.003	0.003	0.002	0.768
APGAR score at 10 min	9.1 ± 0.7	9.0 ± 0.7	8.8 ± 0.8	0.115	0.478	0.047	0.132

APGAR appearance, pulse, grimace, activity, and respiration

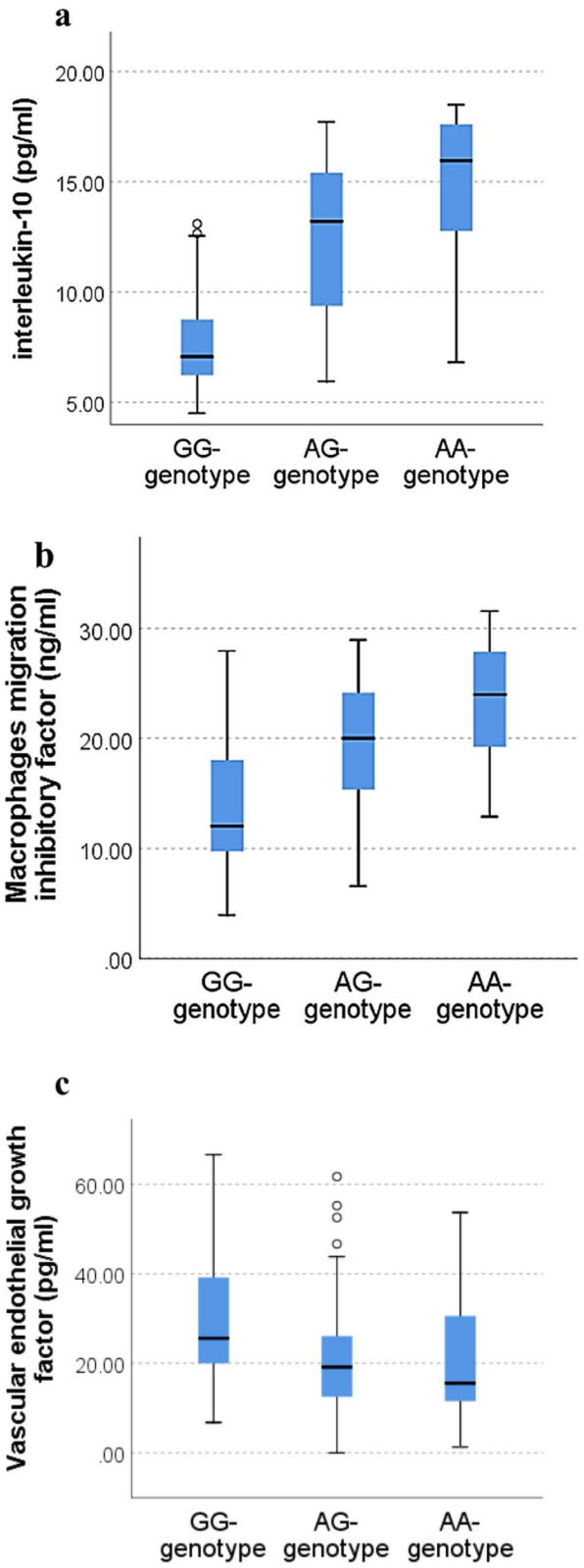

Fig. 3 The mean ± SD of cord arterial serum (**a**) Interleukin-10 (IL-10), (**b**) macrophage migration inhibitory factor (MIF), and (**c**) vascular endothelial growth factor (VEGF) according to the *LPCAT1*-rs9728 genotypes

Discussion

The findings of the current work emphasize the previously reported associations of the cord arterial serum IL-10, MIF, and VEGF levels with the development and severity of the NRDS [13–15, 19–22]. Also, our findings are the 1st to report an association between the *LPCAT1*-rs9728 AA genotype and its A allele with the severity of the NRDS as indicated by their accompanying low APGAR score values at 1, 5, and 10 min and VEGF and the high levels of IL-10 and MIF.

The exact causes of NRDS are yet unknown, but it is believed to be multi-factorial including both genetic and nongenetic causes [23]. Of these factors, pro-inflammatory and anti-inflammatory cytokines are particularly involved in the pathogenesis of RDS [24]. Indeed, cytokines regulate surfactant metabolism in preterm neonates [25]. IL-10 is one of these cytokines that is synthesized by monocytes, macrophages, and T and B lymphocytes [26]. It has inhibitory and immunoregulatory effects on macrophages and plays a role in the pathogenesis of many inflammatory conditions including RDS [9]. The significantly high levels of IL-10 found in the cases with NRDS, the current study, emphasize the results of many previous studies. Capasso et al. reported that the increased IL-10 production was correlated with the RDS severity in preterm neonates [24]. Other studies have reported similar results with an inverse correlation between the cord blood IL-10 and the gestational age at birth [19–21].

Macrophage migration inhibitory factor (MIF) is a cytokine released by various cell types and involved in diverse physiological and immunological responses [27]. Its expression begins before birth and has been detected in serum, urine, amniotic fluid, human milk, and cord blood [28]. The MIF role in lung development appears complex and potentially context dependent. In agreement with our findings, Park et al.'s found an association between increased MIF in preterm infants and the development of prematurity-related disorders [29]. Bayraktar et al.'s reported a positive correlation between high cord blood MIF levels and the severity of the RDS in newborns, suggesting a detrimental effect [13]. Interestingly, in vitro experiments contradict these findings, suggesting a positive role for MIF in surfactant production by lung epithelial cells, and even reporting negative impacts

of reduced intrapulmonary MIF levels on lung development, including the development of RDS [11].

The VEGF is a significant angiogenic factor that regulates endothelial cell proliferation. Type II alveolar cells are a major source of pulmonary VEGF [30]. VEGF contributes to the release of lung surfactants and pulmonary development, particularly pulmonary circulation [14, 30]. The variations in the VEGF levels in cord plasma help the early detection and treatment of RDS in preterm neonates [22]. Our results are in accordance with many previous studies that reported declines in cord blood VEGF levels in infants with RDS, and these declines correlated with the severity of RDS [14, 15, 22]. In contrast to our findings, Lassus et al. reported a significant increase in the levels of VEGF in tracheal aspirate fluid in the lungs of premature infants with RDS [31].

Genetics and individual variability and heterogeneity play crucial roles in NRDS pathogenicity [32]. The genetic basis of NRDS involves the polymorphism of many genes, including *LPCAT1* [7].These genetic polymorphisms are also essential in the mapping, development, and outcomes of NRDS [7, 33]. The increased risk of NRDS in preterm newborns has been associated with mutations and polymorphisms in several genes, particularly in surfactant-associated genes [7]. In the current study, the percentage of *LPCAT1*-rs9728 AA and *LPCAT1*-rs9728 AG genotypes were significantly higher in cases suffering from NRDS than in the control group. These results go with Shen et al. who reported a significant difference in the *LPCAT1*-rs9728 genotypes and its allelic distribution between the NRDS cases and the healthy neonates. Also, they found a protective role of the GG genotype and the G allele of *LPCAT1*-rs9728 against the development of NRDS. In the same study, RDS cases who had the G allele had a lower risk of intraventricular hemorrhage and a shorter stay in the hospital than those with the A allele [7]. On the other hand, some studies declined the contribution of *LPCAT1* polymorphism in the incidence of RDS [34].

The current study showed a significantly lower gestational age and a higher percentage of complicated pregnancy in the NRDS cases than the healthy neonates. As RDS is a complex and multigenic disorder, it is influenced by maternal diseases, gender, ethnicity, and the degree of prematurity [35].

Glucocorticoids significantly enhance the expression of *LPCAT1* in the late stages of embryonic development and promote phospholipid biogenesis in alveolar type II cells [33, 36]. The antenatal use of steroids in high-risk pregnancies was found to decrease the incidence and severity of NRDS [37, 38] which goes with our findings.

Conclusion

This study identified a positive association between the *LPCAT1*-rs9728 AA genotype and both the development and severity of NRDS. Neonates with NRDS and the AA genotype experienced longer hospital stays, higher mortality rates, and higher cord blood levels of IL-10 and MIF, but lower cord blood levels of VEGF, compared to the other two *LPCAT1*-rs9728 genotypes. This suggests that the A allele may be a potential risk factor for NRDS development.

Recommendations

Further research investigating *LPCAT1* genetic polymorphisms in conjunction with other relevant genes, particularly those involved in lung surfactant proteins and lipids, could provide a deeper understanding of the mechanisms underlying NRDS and contribute to developing improved management strategies for this condition.

Abbreviations

LPCAT1	Lysophosphatidylcholine acyltransferase 1
IL-10	Interleukin-10
MIF	Macrophage migration inhibitory factor
VEGF	Vascular endothelial growth factor
NRDS	Neonatal respiratory distress syndrome
RDS	Respiratory distress syndrome
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-8	Interleukin-8
IRB	Institutional review board
APGAR	Appearance, pulse, grimace, activity, and respiration
ELISA	Enzyme-linked immunosorbent assay
EDTA	Ethylenediamine tetraacetic acid
SNP	Single-nucleotide polymorphism
NCBI	National Center for Biotechnology Information
RT-PCR	Real-time polymerase chain reaction
UNG	Uracil-N-glycosylase
VIC	Victoria
FAM	Fluorescein amidites
GA	Gestation age
CS	Cesarian section
ANOVA	Analysis of variance
SD	Standard deviation
ROC curve	Receiver operating characteristic curve
AUC	Area under the curve

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Authors' contributions

Conceptualization and study design, KMM, OME, and MAE. Supervision, KMM, OME, and MAE. Data curation, KMM, AAS, YFA, and AMF. Investigation, KMM and AAS. Methodology, KMM and AAS. Software, KMM. Validation, KMM and YFA. Visualization, AMA and AMF. Writing — initial draft, KMM, AAS, and YFA. Writing — review and editing, KMM, AAS, and YFA. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets analyzed during the current study are available in the European Variation Archive repository, Assembly accession GCA_000001405.27(GRCh38.p12), Chromosome/Contig accession CM000667.2, and The European Bioinformatics Institute < EMBL-EBI.

Declarations

Ethics approval and consent to participate
The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board at the Faculty of Medicine, Assiut University (IRB: 17200578). A written informed consent was obtained from the parents.

Consent for publications
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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