

# KIF9-AS1, LINC01272 and DIO3OS lncRNAs as novel biomarkers for inflammatory bowel disease

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**Abstract.** Long non-coding RNAs (lncRNAs) are a novel group of non-coding RNAs that are associated with inflammation and tumorigenesis. At present, the diagnostic efficacy of lncRNAs in inflammatory bowel disease (IBD) is unclear. The present study aimed to identify lncRNAs that may be used as potential biomarkers for IBD. The mRNA expression levels of various lncRNAs (KIF9-AS1, LINC01272 and DIO3OS) were detected in tissue and plasma samples from patients with IBD by reverse transcription-quantitative polymerase chain reaction. The results indicated that the mRNA expression levels of KIF9-AS1 and LINC01272 were significantly upregulated in tissue and plasma samples from patients with IBD compared with in the healthy controls; conversely, the mRNA expression levels of DIO3OS were significantly downregulated in tissue and plasma samples from patients with IBD compared with in the healthy controls. Subsequently, the specificity and sensitivity of KIF9-AS1, LINC01272 and DIO3OS were determined using a receiver operating characteristic (ROC) curve analysis. The results indicated that KIF9-AS1, LINC01272 and DIO3OS had potential diagnostic value for the detection of IBD. Furthermore, there were significantly positive correlations in KIF9-AS1, LINC01272 and DIO3OS expression between IBD tissue and plasma samples. Therefore, the present study indicated that KIF9-AS1, LINC01272 and DIO3OS may be potential diagnostic biomarkers for IBD.

## Introduction

Inflammatory bowel disease (IBD) refers to idiopathic intestinal inflammatory diseases that involve the ileum, rectum and

colon. The most common forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). IBD is a global disease, the incidence and prevalence of which are increasing worldwide. Growing evidence has suggested that the occurrence of IBD is closely associated with immunological, genetic and modifiable environmental factors in a genetically susceptible host, which results in immunological reactions against a subset of gut commensal microbiota (1-5).

CD involves any part of the gastrointestinal tract, and usually affects the colon or terminal ileum. The most important feature of CD is intestinal inflammation in a discontinuous fashion. The pattern of inflammation for CD is associated with pathophysiological complications, including intestinal fibrosis, strictures, non-caseation granulomas, thickened submucosa and fistulas (6). Conversely, UC involves only the rectum and colon; the most important feature of UC is superficial inflammation that is limited to the mucosa and submucosa. Symptoms of UC include rectal bleeding, diarrhea, abdominal pain and superficial mucosal ulceration (7). To specifically diagnose CD or UC, radiological tests, biopsy histology, endoscopic features and clinical symptoms are all taken into consideration (6). Unfortunately, there are no useful diagnostic markers for CD and UC, and the majority of patients with IBD present with serious disease, due to the lack of sensitive biomarkers for early diagnosis. Therefore, the identification of potential biomarkers for IBD is critical.

Noncoding RNAs (ncRNAs), which include microRNA (miRNA), long noncoding RNA (lncRNA), circular RNA (circRNA), transfer RNA, ribosomal RNA and small nucleolar RNA, affect every stage of gene expression from transcription and mRNA stability to mRNA translation. Previous studies have gradually uncovered the critical roles for ncRNAs in disease pathogenesis (8-11). Dysregulated expression or dysfunction of specific ncRNAs has been reported to initiate inflammation in human disease. The three forms of ncRNAs that are particularly important for the regulation of gene expression in cells are miRNAs, lncRNAs and circRNAs (12). To date, an increasing number of studies have demonstrated that ncRNAs may serve as novel biomarkers for disease. lncRNAs, which are a type of ncRNA >200 nucleotides in length, are able to regulate gene expression through transcriptional regulation,

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post-transcriptional regulation, chromatin modification and genomic imprinting (13,14). Therefore, lncRNAs may be potential diagnostic biomarkers for various diseases; however, the function and mechanism of lncRNAs requires further investigation. A previous study reported that there were 438 and 745 differentially expressed lncRNAs in inflamed CD and UC respectively, compared with healthy individuals (15). A recent study indicated that numerous lncRNAs were differentially expressed, including ENST00000522970.1, LINC01272, ENST00000522970.1, KIF9-AS1, DIO3OS in IBD (16). To the best of our knowledge, KIF9-AS1, LINC01272 and DIO3OS have not been studied yet. Therefore, the present study aimed to determine the expression levels and diagnostic value of KIF9-AS1, LINC01272 and DIO3OS in IBD.

The present study examined the expression levels of KIF9-AS1, LINC01272 and DIO3OS, using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), in tissue and plasma samples from patients with IBD and healthy controls. The specificity and sensitivity of KIF9-AS1, LINC01272 and DIO3OS were determined using a receiver operating characteristic (ROC) curve analysis. The potential diagnostic values of KIF9-AS1, LINC01272 and DIO3OS in IBD were determined. In addition, the correlations between IBD tissue and plasma expression levels of KIF9-AS1, LINC01272 and DIO3OS were analyzed using the Pearson Correlation Coefficient. The present study aimed to identify the lncRNAs that may be considered potential diagnostic biomarkers for IBD.

## Materials and methods

**Clinical specimens.** The present study collected samples from patients with CD or UC, and healthy controls, from The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (Hefei, China) between 2013 and 2016. This study was approved by the Ethics Committee of The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine, and informed consent was obtained from each individual. For CD or UC to be diagnosed, symptoms were required to meet the Copenhagen criteria (17). Tissue and plasma samples were collected from 252 individuals (84 patients with CD, 84 patients with UC and 84 healthy controls). Healthy control individuals had no symptoms of autoimmune diseases or IBD. For the extraction of plasma samples, 5 ml peripheral blood was collected from all 252 individuals, plasma was separated by centrifugation (3,000 × g at 4°C for 10 min); the supernatant plasma was maintained at -80°C until further analysis. According to the World Health Organization, the histological diagnosis was evaluated (18). All tissue samples were frozen at -80°C for the extraction of total RNA.

**RNA preparation and RT.** Total RNA was extracted from the tissue and plasma samples using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Similarly, QIAamp Circulating Nucleic Acid kit (Qiagen K.K., Tokyo, Japan) was used to extract total RNA from 800 µl plasma. RNA extraction was conducted according to manufacturers' protocols. cDNA was synthesized from RNA with random primers using a RevertAid First Strand cDNA Synthesis kit (cat. no. K1622; Thermo Fisher Scientific, Inc.) according to

the manufacturer's protocol; each RT reaction consisted of 1.0 µg RNA.

**RT-qPCR.** As described previously (19), RT-qPCR was performed using SYBR *Premix Ex Taq* (Takara Bio, Inc., Otsu, Japan). qPCR was performed on an ABI 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The mRNA expression levels of various lncRNAs (KIF9-AS1, LINC01272 and DIO3OS) were detected by RT-qPCR using the KAPA SYBR FAST qPCR Kit (KK4601, Kapa Biosystems, Inc. Wilmington, MA, USA) on Applied Biosystems Real-Time PCR system in tissue and plasma samples obtained from patients with IBD and healthy controls. The reaction system was in a 20 µl volume, and the program of RT-qPCR was set as following: 95°C for 10 min, 95°C for 10 sec, 60°C for 2 min, 72°C for 2 min, 72°C for 10 min, and 38 amplification cycles were performed from second step to fourth step. The primer sequences were as follows: KIF9-AS1, forward 5'-AGTCCT TCCCATTCACAGGG-3', reverse 5'-GCCCTCTTCTTCCTC CACAT-3'; LINC01272, forward 5'-TGTTCACTGCTGTAC ACCCA-3', reverse 5'-TGTGGAGAGGGGATTCTGG-3'; DIO3OS, forward 5'-ATACCTACCCCTCCCCAACT-3', reverse 5'-TACCTGCTCTGAGATGTGCC-3'; and GAPDH, forward 5'-TGTTTCGTCATGGGTGTGAAC-3' and reverse 5'-ATGGCATGGACTGTGGTCAT-3'. The mRNA expression levels were calculated using the  $2^{-\Delta\Delta C_q}$  method with GAPDH as the control (20).

**Statistical analysis.** All experimental data were analyzed using SPSS software 16.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Association between lncRNA expression and clinicopathological characteristics were analyzed using Chi-squared test. Diagnostic value was detected using a ROC curve analysis. The area under the curve was used to assess the predictive power and to determine the cutoff scores for the high-expression and low-expression of lncRNAs. The correlation between lncRNA tissue and plasma expression in IBD was analyzed using Pearson's correlation coefficient analysis. A Student's t-test was used for comparisons between two groups, such as the comparisons between healthy controls and CD or UC. All results are presented as the mean ± standard deviation, experiments were conducted in triplicate.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**lncRNA-KIF9-AS1 is highly expressed in IBD.** The expression levels of KIF9-AS1 in patients with CD (n=84) and in healthy controls (n=84) were determined by RT-qPCR. The results demonstrated that the mRNA expression levels of KIF9-AS1 were significantly higher in patients with CD compared with in the healthy controls ( $P < 0.001$ ; Fig. 1A). In addition, the mRNA expression levels of KIF9-AS1 were significantly higher in patients with UC (n=84) compared with in the healthy controls ( $P < 0.001$ ; Fig. 1B). Furthermore, the area under the ROC curve between KIF9-AS1 expression in patients with CD and healthy controls was 0.811 ( $P < 0.0001$ ; Fig. 1C). The area under the ROC curve between KIF9-AS1 expression in patients with UC and healthy controls was 0.872 ( $P < 0.0001$ ; Fig. 1D). The

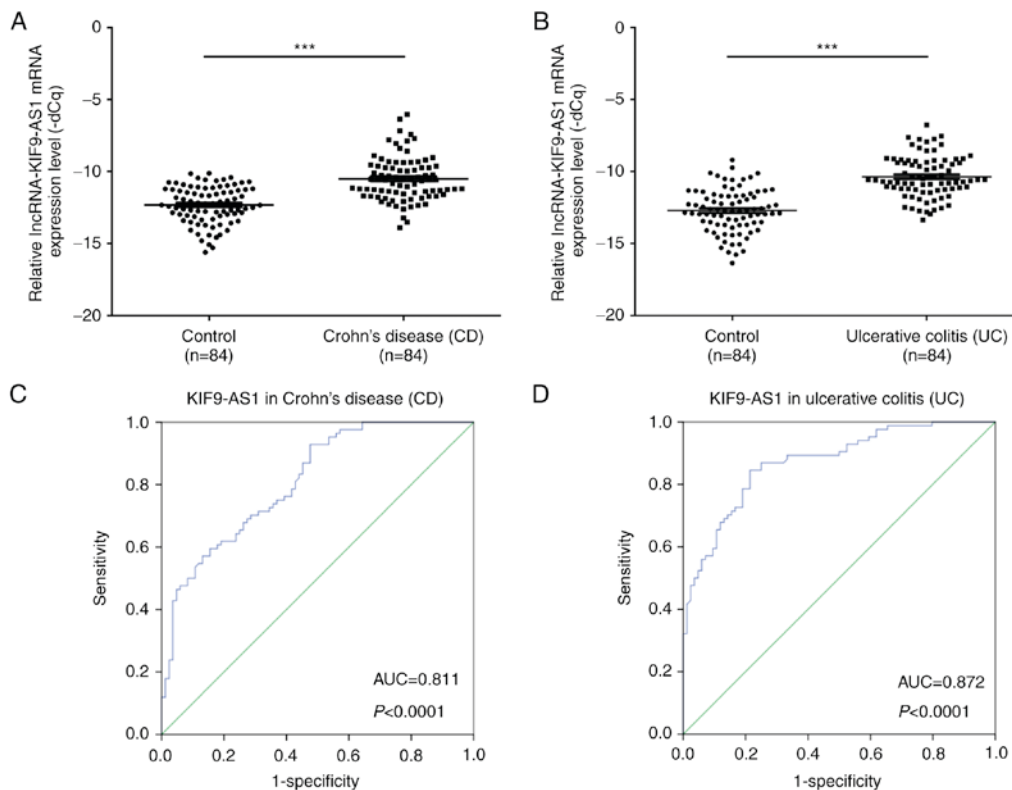


Figure 1. Long non-coding RNA KIF9-AS1 is highly expressed in patients with inflammatory bowel disease. (A) Relative expression levels of KIF9-AS1 in patients with CD (n=84) and healthy controls (n=84) were detected by RT-qPCR. (B) KIF9-AS1 expression levels in patients with UC (n=84) and healthy controls (n=84) were measured by RT-qPCR. GAPDH was used as an RNA loading control; KIF9-AS1 abundance was normalized to GAPDH mRNA expression. \*\*\*P<0.001. (C) ROC curve of KIF9-AS1 expression between patients with CD and healthy controls (P<0.0001; AUC=0.811). (D) ROC curve of KIF9-AS1 expression between patients with UC and healthy controls (P<0.0001; AUC=0.872). The y-axis indicated sensitivity, the x-axis indicated 1-specificity. AUC, area under the curve; CD, Crohn's disease; ROC, receiver operating characteristic; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; UC, ulcerative colitis.

associations between KIF9-AS1 expression ( $\Delta Cq$ ) and the clinicopathological characteristics of patients with IBD are presented in Table I. Patients were separated into high and low expression groups according to a cut off value (10.638 for CD; 11.313 for UC). A statistical significance between KIF9-AS1 expression and alcohol history was observed (P=0.023).

**lncRNA-LINC01272 is highly expressed in IBD.** The expression levels of LINC01272 in patients with CD (n=84) and in healthy controls (n=84) were determined by RT-qPCR. The results demonstrated that the mRNA expression levels of LINC01272 were significantly higher in patients with CD compared with in the healthy controls (P<0.001; Fig. 2A). In addition, the mRNA expression levels of LINC01272 were significantly higher in patients with UC (n=84) compared with in the healthy controls (P<0.001; Fig. 2B). Furthermore, the area under the ROC curve between LINC01272 expression in patients with CD and the healthy controls was 0.887 (P<0.0001; Fig. 2C). The area under the ROC curve between LINC01272 expression in patients with UC and the healthy controls was 0.777 (P<0.0001; Fig. 2D). The associations between LINC01272 expression levels ( $\Delta Cq$ ) and the clinicopathological characteristics of patients with IBD are presented in Table II. Patients were separated into high and low expression groups according to a cut off value (10.355 for CD; 11.593 for UC). There was a statistical significance between

LINC01272 expression and tobacco smoking (P=0.018) in CD. And there was a statistical significance between LINC01272 expression and alcohol history (P=0.044) in UC.

**lncRNA-DIO3OS expression is reduced in IBD.** The expression levels of DIO3OS in patients with CD (n=84) and in healthy controls (n=84) were determined by RT-qPCR. The results demonstrated that the mRNA expression levels of DIO3OS were significantly lower in patients with CD compared with in the healthy controls (P<0.001; Fig. 3A). The mRNA expression levels of DIO3OS were also significantly lower in patients with UC (n=84) compared with in the healthy controls (P<0.001; Fig. 3B). Furthermore, the area under the ROC curve between DIO3OS expression in patients with CD and the healthy controls was 0.794 (P<0.0001; Fig. 3C). The area under the ROC curve between DIO3OS expression in patients with UC and the healthy controls was 0.653 (P=0.001; Fig. 3D). The associations between DIO3OS expression levels ( $\Delta Cq$ ) and the clinicopathological characteristics of patients with IBD are presented in Table III. Patients were separated into high and low expression groups according to a cut off value (10.599 for CD; 12.069 for UC). A statistical significance between DIO3OS expression and gender was observed (P=0.025) in CD.

**KIF9-AS1, LINC01272 and DIO3OS expression levels were validated in the plasma of patients with CD and UC.** To further

Table I. Association between KIF9-AS1 expression and clinicopathological characteristics.

Characteristic	CD			UC		
	High expression (%)	Low expression (%)	P-value	High expression (%)	Low expression (%)	P-value
Age (years)			0.40			0.636
>60	36 (70.6)	15 (29.4)		19 (37.3)	32 (62.7)	
≤60	26 (78.8)	7 (21.2)		14 (42.4)	19 (57.6)	
Gender			0.356			0.141
Male	38 (77.6)	11 (22.4)		16 (32.7)	33 (67.3)	
Female	24 (68.6)	11 (31.4)		17 (48.6)	18 (51.4)	
Tobacco smoking			0.12			0.505
Never	17 (63.0)	10 (37.0)		12 (44.4)	15 (55.6)	
Past or current use	45 (78.9)	12 (21.1)		21 (36.8)	36 (63.2)	
Alcohol history			0.601			0.023 <sup>a</sup>
Never	27 (71.1)	11 (28.9)		20 (52.6)	18 (47.4)	
Past or current use	35 (76.1)	11 (23.9)		13 (28.3)	33 (71.7)	

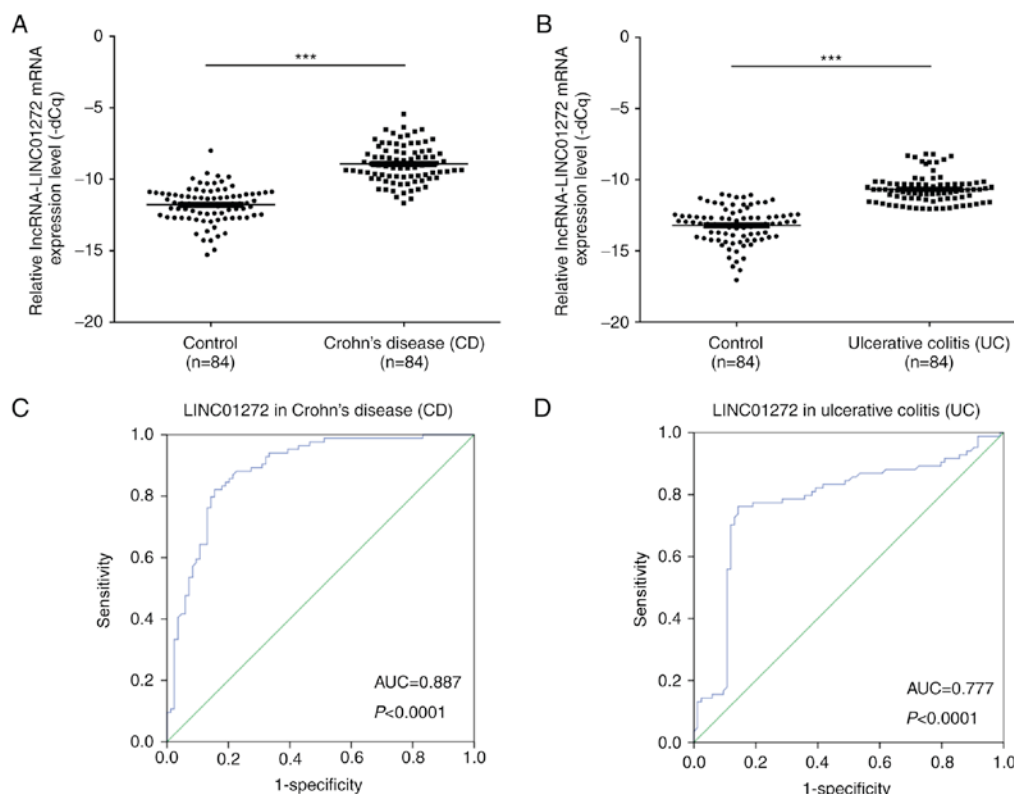
<sup>a</sup>P<0.05. CD, Crohn's disease; UC, ulcerative colitis.

Figure 2. Long non-coding RNA LINC01272 is highly expressed in inflammatory bowel disease. (A) Relative expression levels of LINC01272 in patients with CD (n=84) and healthy controls (n=84) were detected by RT-qPCR. (B) LINC01272 expression levels in patients with UC (n=84) and healthy controls (n=84) were measured by RT-qPCR. GAPDH was used as an RNA loading control; LINC01272 abundance was normalized to GAPDH mRNA expression. \*\*\*P<0.001. (C) ROC curve of LINC01272 expression between patients with CD and healthy controls (P<0.0001; AUC=0.887). (D) ROC curve of LINC01272 expression between patients with UC and healthy controls (P<0.0001; AUC=0.777). The y-axis indicated sensitivity, the x-axis indicated 1-specificity. AUC, area under the curve; CD, Crohn's disease; ROC, receiver operating characteristic; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; UC, ulcerative colitis.

confirm the expression levels of KIF9-AS1, LINC01272 and DIO3OS in patients with IBD, the mRNA expression levels of KIF9-AS1, LINC01272 and DIO3OS were detected in

plasma samples from patients with IBD. The results indicated that the mRNA expression levels of KIF9-AS1 were significantly upregulated in plasma samples from patients with CD



Table II. Association between LINC01272 expression and clinicopathological characteristics.

Characteristic	CD			UC		
	High expression (%)	Low expression (%)	P-value	High expression (%)	Low expression (%)	P-value
Age (years)			0.225			1.00
>60	27 (52.9)	24 (47.1)		17 (33.3)	34 (66.7)	
≤60	13 (39.4)	20 (60.6)		11 (33.3)	22 (66.7)	
Gender			0.555			0.87
Male	22 (44.9)	27 (55.1)		16 (32.7)	33 (67.3)	
Female	18 (51.4)	17 (48.6)		12 (34.3)	23 (65.7)	
Tobacco smoking			0.018 <sup>a</sup>			0.32
Never	18 (66.7)	9 (33.3)		11 (44.7)	21 (55.3)	
Past or current use	22 (38.6)	35 (61.4)		17 (29.8)	40 (70.2)	
Alcohol history			0.087			0.044 <sup>a</sup>
Never	22 (57.9)	16 (42.1)		17 (44.7)	21 (55.3)	
Past or current use	18 (39.1)	28 (60.9)		11 (23.9)	35 (76.1)	

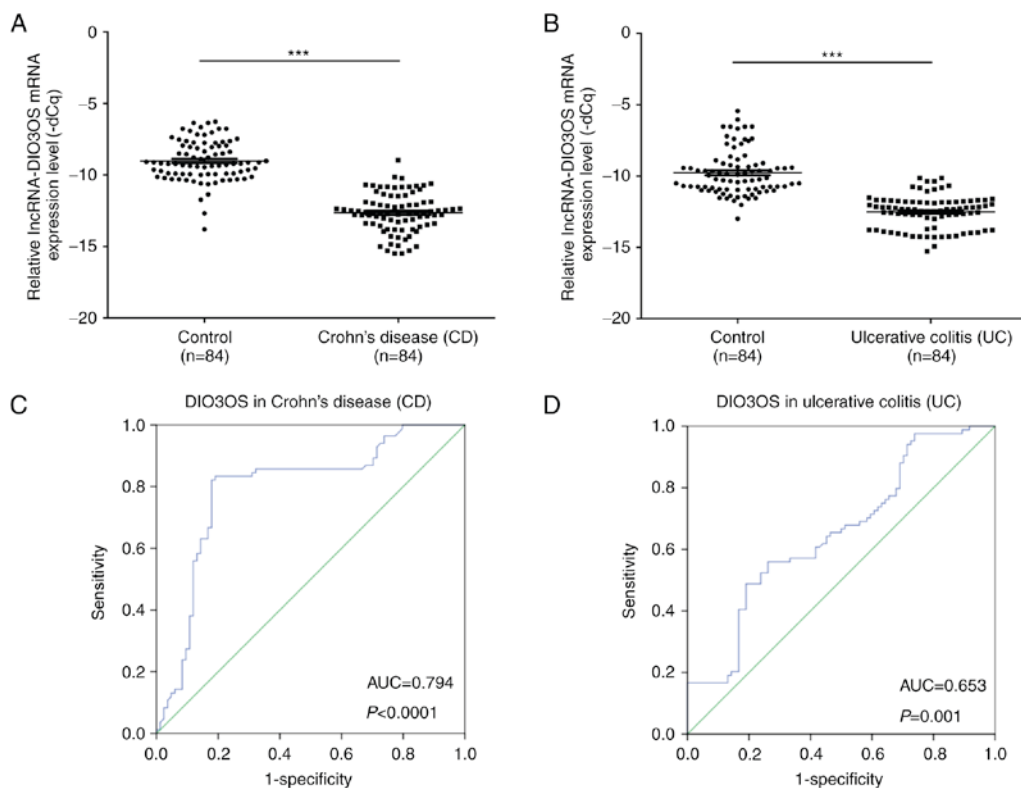
<sup>a</sup>P<0.05. CD, Crohn's disease; UC, ulcerative colitis.

Figure 3. Long non-coding RNA DIO3OS expression was reduced in inflammatory bowel disease. (A) Relative expression levels of DIO3OS in patients with CD (n=84) and healthy controls (n=84) were detected by RT-qPCR. (B) DIO3OS expression levels in patients with UC (n=84) and healthy controls (n=84) were measured by RT-qPCR. GAPDH was used as an RNA loading control; DIO3OS abundance was normalized to GAPDH mRNA expression. \*\*\*P<0.001. (C) ROC curve of DIO3OS expression between patients with CD and healthy controls (P<0.0001; AUC=0.794). (D) ROC curve of DIO3OS expression between patients with UC and healthy controls (P=0.001; AUC=0.653). AUC, area under the curve; CD, Crohn's disease; ROC, receiver operating characteristic; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; UC, ulcerative colitis.

compared with in the healthy controls (P<0.001; Fig. 4A). In addition, the mRNA expression levels of LINC01272 were significantly upregulated in plasma samples from patients with CD compared with in the healthy controls (P<0.001; Fig. 4B).

Conversely, the mRNA expression levels of DIO3OS were significantly downregulated in plasma samples from patients with CD compared with in the healthy controls (P<0.001; Fig. 4C). Similarly, the mRNA expression levels of KIF9-AS1

Table III. Association between DIO3OS expression and clinicopathological characteristics.

Characteristic	CD			UC		
	High expression (%)	Low expression (%)	P-value	High expression (%)	Low expression (%)	P-value
Age (years)			0.949			0.23
>60	22 (43.1)	29 (56.9)		17 (33.3)	34 (66.7)	
≤60	14 (42.4)	19 (57.6)		7 (21.2)	26 (78.8)	
Gender			0.025 <sup>a</sup>			0.62
Male	26 (53.1)	23 (46.9)		13 (26.5)	36 (73.5)	
Female	10 (28.6)	25 (71.4)		11 (31.4)	24 (68.6)	
Tobacco smoking			0.225			0.09
Never	9 (33.3)	18 (66.7)		11 (40.7)	16 (59.3)	
Past or current use	27 (47.4)	30 (52.6)		13 (22.8)	44 (77.2)	
Alcohol history			0.448			0.95
Never	18 (47.4)	20 (52.6)		11 (28.9)	27 (71.1)	
Past or current use	18 (39.1)	28 (60.9)		13 (28.3)	33 (71.7)	

<sup>a</sup>P<0.05. CD, Crohn's disease; UC, ulcerative colitis.

were significantly upregulated in plasma samples from patients with UC compared with in the healthy controls ( $P<0.001$ ; Fig. 4D). The mRNA expression levels of LINC01272 were also significantly upregulated in plasma samples from patients with UC compared with in the healthy controls ( $P<0.001$ ; Fig. 4E). However, the mRNA expression levels of DIO3OS were significantly downregulated in plasma samples from patients with UC compared with in the healthy controls ( $P<0.001$ ; Fig. 4F).

*KIF9-AS1*, *LINC01272* and *DIO3OS* expression is positively correlated between IBD tissue and plasma samples. According to the present study, the mRNA expression levels of *KIF9-AS1* and *KIF9-AS1* were increased in IBD tissue and plasma samples, whereas the mRNA expression levels of *DIO3OS* were decreased in IBD tissue and plasma samples. The present study further analyzed the correlation between lncRNA expression in IBD tissue and plasma samples. The results indicated that there was a positive correlation between *KIF9-AS1* expression in CD tissue and plasma samples ( $R^2=0.3788$ ,  $P=0.0002$ ; Fig. 5A), and UC tissue and plasma samples ( $R^2=0.3466$ ,  $P=0.0012$ ; Fig. 4B). In addition, a positive correlation was detected between *LINC01272* expression in CD tissue and plasma samples ( $R^2=0.7133$ ;  $P<0.0001$ ; Fig. 5C), and UC tissue and plasma samples ( $R^2=0.5326$ ,  $P<0.0001$ ; Fig. 5D). Furthermore, a positive correlation was detected between *DIO3OS* expression in CD tissue and plasma samples ( $R^2=0.2524$ ,  $P=0.0141$ ; Fig. 5E), and UC tissue and plasma samples ( $R^2=0.2707$ ,  $P=0.0083$ ; Fig. 5F). Therefore, these results indicated that *KIF9-AS1*, *LINC01272* and *DIO3OS* were aberrantly expressed in IBD tissue and plasma samples, and may be considered potential biomarkers for IBD.

## Discussion

At present, research into the potential biomarkers of IBD has focused on ncRNAs, particularly lncRNAs, most of which

are transcribed by RNA polymerase (Pol) II/Pol I, and some of which are transcribed by RNA Pol III (21). lncRNAs are able to regulate the expression of protein-coding genes at transcriptional and post-transcriptional levels, and may affect physiological processes (22,23). In addition, lncRNAs serve critical roles in the regulation of gene expression (24-27), and participate in cell cycle progression, cell differentiation (28) and apoptosis (29,30). A recent study indicated that lncRNAs are differentially expressed in IBD (15). Furthermore, associations among ncRNAs, cytokines and inflammation-associated diseases have been noted (31). Previous studies have also indicated that lncRNAs may regulate the lipopolysaccharide-induced inflammatory response in human monocytes (32,33), and that lncRNAs are associated with transforming growth factor- $\beta$ /Smad3-mediated renal inflammation and fibrosis (34). lncRNA DQ786243 has been revealed to affect regulatory T cell-related cAMP response element-binding protein and forkhead box P3 expression in CD (34). However, the specific functions of lncRNA are not entirely clear in inflammation. A deeper understanding of the lncRNA regulatory network is required, and the biological and molecular mechanisms underlying the effects of lncRNAs require further investigation.

In a previous study (15), a microarray platform was used to conduct genome-wide transcriptome profiling of lncRNAs in 96 inflamed and non-inflamed tissue samples extracted from numerous colonic locations of 45 patients (CD=13, UC=20, controls=12). The results indicated that there were 12 and 19 differentially expressed lncRNAs associated with CD and UC, respectively. In addition, the upregulated protein-coding genes included dual oxidase maturation factor 2, chitinase 3 like 1, dystonin, matrix metalloproteinase 12, lncRNAs RP11-731 F5.2 and AC007182.6, and the downregulated protein-coding genes included phosphoenolpyruvate carboxykinase 1, potassium two pore domain channel subfamily K member 10, serpin family B member

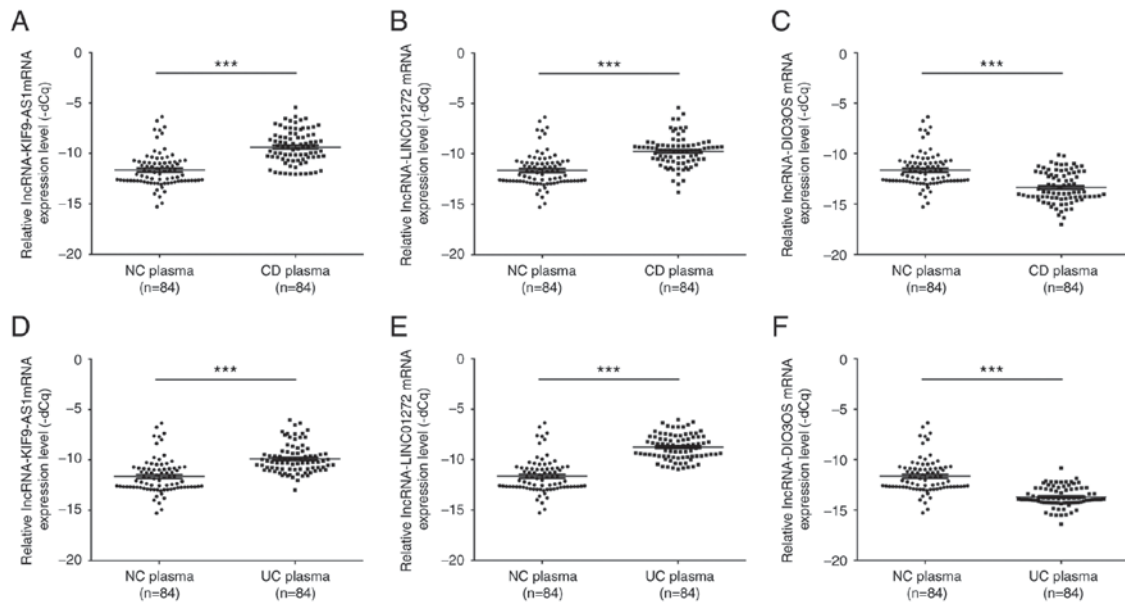


Figure 4. KIF9-AS1, LINC01272 and DIO3OS expression in inflammatory bowel disease was validated in plasma samples from patients with CD and UC. (A) KIF9-AS1, (B) LINC01272 and (C) DIO3OS mRNA expression levels were measured in plasma samples from patients with CD by RT-qPCR (n=84). \*\*\*P<0.001. (D) KIF9-AS1, (E) LINC01272 and (F) DIO3OS mRNA expression levels were measured in plasma samples from patients with UC by RT-qPCR (n=84). \*\*\*P<0.001. Relative expression levels were normalized to GAPDH and were quantified using the  $\Delta\Delta C_q$  method. CD, Crohn's disease; NC, healthy controls; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; UC, ulcerative colitis.

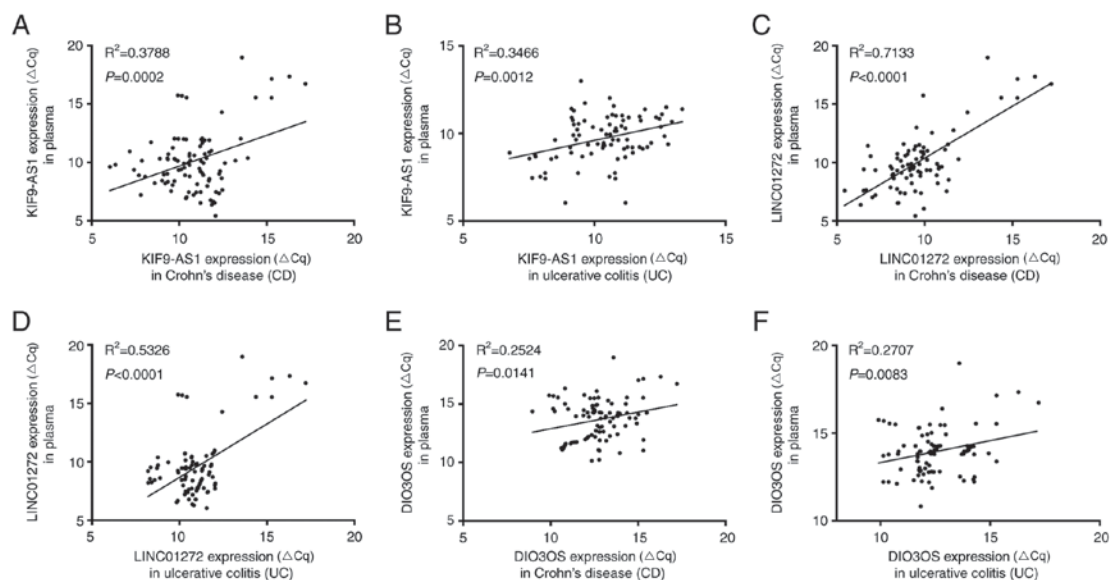


Figure 5. Correlations in long non-coding RNA expression between inflammatory bowel disease tissue and plasma samples. Correlation analysis of KIF9-AS1 expression (A) between CD tissue and plasma samples ( $R^2=0.3788$ ,  $P=0.0002$ , and (B) between UC tissue and plasma samples ( $R^2=0.3466$ ,  $P=0.0012$ ). Correlation analysis of LINC01272 expression (C) between CD tissue and plasma samples ( $R^2=0.7133$ ,  $P<0.0001$ ), and (D) between UC tissue and plasma samples ( $R^2=0.5326$ ,  $P<0.0001$ ). Correlation analysis of DIO3OS expression (E) between CD tissue and plasma samples ( $R^2=0.2524$ ,  $P=0.0141$ , and (F) between UC tissue and plasma samples ( $R^2=0.2707$ ,  $P=0.0083$ ). CD, Crohn's disease; UC, ulcerative colitis.

3, DPP10 antisense RNA 1, CDKN2B antisense RNA 1 and lncRNA AL928742.12 (15). The present study detected KIF9-AS1, LINC01272 and DIO3OS expression in tissue samples from patients with IBD and in healthy controls by RT-qPCR.

The present study demonstrated that KIF9-AS1 and LINC01272 were significantly increased, and DIO3OS was significantly decreased in IBD (n=168) compared with in the healthy controls (n=168). Subsequently, the specificity

and sensitivity of KIF9-AS1, LINC01272 and DIO3OS were determined using a ROC curve analysis. The results indicated that KIF9-AS1, LINC01272 and DIO3OS had potential diagnostic value for the detection of IBD. Furthermore, KIF9-AS1 and LINC01272 expression was significantly increased, and DIO3OS was significantly decreased in plasma samples from patients with IBD (n=168) compared with in the healthy controls (n=168). In addition, KIF9-AS1, LINC01272 and DIO3OS expression was significantly correlated between IBD

tissue and plasma samples. Therefore, these findings suggested that KIF9-AS1, LINC01272 and DIO3OS may be promising candidates for the diagnosis of IBD.

In conclusion, KIF9-AS1, LINC01272, and DIO3OS were differentially expressed in tissue and plasma samples from patients with IBD compared with in the healthy controls. In addition, KIF9-AS1, LINC01272 and DIO3OS had potential diagnostic value for the detection of IBD. Therefore, these findings indicated that KIF9-AS1, LINC01272 and DIO3OS may be potential diagnostic biomarkers for IBD.

## References

- Xavier RJ and Podolsky DK: Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 448: 427-434, 2007.
- Morrison G, Headon B and Gibson P: Update in inflammatory bowel disease. *Aust Fam Physician* 38: 956-961, 2009.
- Uslu N, Usta Y, Balamtekin N, Demir H, Saltik-Temizel IN and Yüce A: Inflammatory bowel disease in infancy. *Indian J Gastroenterol* 28: 224-225, 2009.
- Van Limbergen J, Radford-Smith G and Satsangi J: Advances in IBD genetics. *Nat Rev Gastroenterol Hepatol* 11: 372-385, 2014.
- Sartor RB: Genetics and environmental interactions shape the intestinal microbiome to promote inflammatory bowel disease versus mucosal homeostasis. *Gastroenterology* 139: 1816-1819, 2010.
- Abraham C and Cho JH: Inflammatory bowel disease. *N Engl J Med* 361: 2066-2078, 2009.
- Hanauer SB, Robinson M, Pruitt R, Lazenby AJ, Persson T, Nilsson LG, Walton-Bowen K, Haskell LP and Levine JG: Budesonide enema for the treatment of active, distal ulcerative colitis and proctitis: A dose-ranging study. U.S. Budesonide enema study group. *Gastroenterology* 115: 525-532, 1998.
- Avitabile C, Cimmino A and Romanelli A: Oligonucleotide analogues as modulators of the expression and function of noncoding RNAs (ncRNAs): Emerging therapeutics applications. *J Med Chem* 57: 10220-10240, 2014.
- Bayoumi AS, Sayed A, Broskova Z, Teoh JP, Wilson J, Su H, Tang YL and Kim IM: Crosstalk between Long Noncoding RNAs and MicroRNAs in Health and Disease. *Int J Mol Sci* 17: 356, 2016.
- Wang Y, Chen L, Chen B, Li X, Kang J, Fan K, Hu Y, Xu J, Yi L, Yang J, *et al*: Mammalian ncRNA-disease repository: A global view of ncRNA-mediated disease network. *Cell Death Dis* 4: e765, 2013.
- Xie N and Liu G: ncRNA-regulated immune response and its role in inflammatory lung diseases. *Am J Physiol Lung Cell Mol Physiol* 309: L1076-L1087, 2015.
- Hayes EL and Lewis-Wambi JS: Mechanisms of endocrine resistance in breast cancer: An overview of the proposed roles of noncoding RNA. *Breast Cancer Res* 17: 40, 2015.
- Guttman M and Rinn JL: Modular regulatory principles of large non-coding RNAs. *Nature* 482: 339-346, 2012.
- Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, *et al*: Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci USA* 106: 11667-11672, 2009.
- Mirza AH, Berthelsen CH, Seemann SE, Pan X, Frederiksen KS, Vilien M, Gorodkin J and Pociot F: Transcriptomic landscape of lncRNAs in inflammatory bowel disease. *Genome Med* 7: 39, 2015.
- Zacharopoulou E, Gazouli M, Tzouvala M, Vezakis A and Karamanolis G: The contribution of long non-coding RNAs in Inflammatory Bowel Diseases. *Dig Liver Dis* 49: 1067-1072, 2017.
- Jakobsen C, Bartek J Jr, Wewer V, Vind I, Munkholm P, Groen R and Paerregaard A: Differences in phenotype and disease course in adult and paediatric inflammatory bowel disease—a population-based study. *Aliment Pharmacol Ther* 34: 1217-1224, 2011.
- Peyrin-Biroulet L, Cieza A, Sandborn WJ, Coenen M, Chowers Y, Hibi T, Kostanjsek N, Stucki G and Colombel JF; International Programme to Develop New Indexes for Crohn's Disease (IPNIC) group: Development of the first disability index for inflammatory bowel disease based on the international classification of functioning, disability and health. *Gut* 61: 241-247, 2012.
- Jiang L, Lai YK, Zhang J, Wang H, Lin MC, He ML and Kung HF: Targeting S100P inhibits colon cancer growth and metastasis by Lentivirus-mediated RNA interference and proteomic analysis. *Mol Med* 17: 709-716, 2011.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
- Bierhoff H, Schmitz K, Maass F, Ye J and Grummt I: Noncoding transcripts in sense and antisense orientation regulate the epigenetic state of ribosomal RNA genes. In: Cold Spring Harbor symposium on quantitative biology. Cold Spring Harb Symp Quant Biol 2010: 357-364, 2011.
- Qiu MT, Hu JW, Yin R and Xu L: Long noncoding RNA: An emerging paradigm of cancer research. *Tumor Biol* 34: 613-620, 2013.
- Eades G, Zhang YS, Li QL, Xia JX, Yao Y and Zhou Q: Long non-coding RNAs in stem cells and cancer. *World J Clin Oncol* 5: 134-141, 2014.
- Wang KC and Chang HY: Molecular mechanisms of long noncoding RNAs. *Mol Cell* 43: 904-914, 2011.
- Nagano T and Fraser P: No-nonsense functions for long noncoding RNAs. *Cell* 145: 178-181, 2011.
- Mercer TR, Dinger ME and Mattick JS: Long non-coding RNAs: Insights into functions. *Nat Rev Genet* 10: 155-159, 2009. Loewer S, Cabili MN, Guttman M, Loh YH, Thomas K, Park IH, Garber M, Curran M, Onder T, Agarwal S, *et al*: Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. *Nat Genet* 42: 1113-1117, 2010.
- Liu X, Li D, Zhang W, Guo M and Zhan Q: Long non-coding RNA gadd7 interacts with TDP-43 and regulates Cdk6 mRNA decay. *EMBO J* 31: 4415-4427, 2012.
- Lakhotia SC: Long non-coding RNAs coordinate cellular responses to stress. *Wiley Interdiscip Rev* 3: 779-796, 2012.
- Paralkar VR and Weiss MJ: A new 'Linc' between noncoding RNAs and blood development. *Genes Dev* 25: 2555-2558, 2011.
- Marques-Rocha JL, Samblas M, Milagro FI, Bressan J, Martinez JA and Marti A: Noncoding RNAs, cytokines, and inflammation-related diseases. *FASEB J* 29: 3595-3611, 2015.
- Ilott NE, Heward JA, Roux B, Tsitsiou E, Fenwick PS, Lenzi L, Goodhead I, Hertz-Fowler C, Heger A, Hall N, *et al*: Corrigendum: Long non-coding RNAs and enhancer RNAs regulate the lipopolysaccharide-induced inflammatory response in human monocytes. *Nat Commun* 6: 6814, 2015.
- Ilott NE, Heward JA, Roux B, Tsitsiou E, Fenwick PS, Lenzi L, Goodhead I, Hertz-Fowler C, Heger A, Hall N, *et al*: Long non-coding RNAs and enhancer RNAs regulate the lipopolysaccharide-induced inflammatory response in human monocytes. *Nat Commun* 5: 3979, 2014.
- Zhou Q, Chung AC, Huang XR, Dong Y, Yu X and Lan HY: Identification of novel long noncoding RNAs associated with TGF- $\beta$ /Smad3-mediated renal inflammation and fibrosis by RNA sequencing. *Am J Pathol* 184: 409-417, 2014.
- Qiao YQ, Huang ML, Xu AT, Zhao D, Ran ZH and Shen J: LncRNA DQ786243 affects Treg related CREB and Foxp3 expression in Crohn's disease. *J Biomed Sci* 20: 87, 2013.



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