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Clinical Significance of Serum lncRNA Cancer Susceptibility Candidate 2 (CASC2) for Chronic Renal Failure in Patients with Type 2 Diabetes

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: lncRNA CASC2 has been established to have critical functions in tumorigenesis but, while its involvement in high-glucose-induced chronic renal failure remains unclear.

Material/Methods: We included patients with type 2 diabetes combined with chronic renal failure, as well as patients with diabetic retinopathy, diabetic ketoacidosis, diabetic foot infections or diabetic cardiomyopathy, and diabetic patients without any obvious complication, as well as healthy controls. Blood samples and renal tissues were obtained from each participant and expression of lncRNA CASC2 in those tissues was detected by qRT-PCR. Diagnostic value of lncRNA CASC2 for type 2 diabetes combined with chronic renal failure was evaluated by ROC curve analysis. All patients were followed up for 5 years and the occurrence of chronic renal failure was recorded.

Results: Compared with healthy controls, expression of lncRNA CASC2 in serum and renal tissue was specifically down-regulated in patients with type 2 diabetes combined with chronic renal failure but not in type 2 diabetic patients combined with other complications. Follow-up showed that patients with low serum level of lncRNA CASC2 had significantly higher incidence of chronic renal failure.

Conclusions: lncRNA CASC2 is a reliable diagnostic biomarker for type 2 diabetes combined with chronic renal failure and low serum level of lncRNA CASC2 predicts the occurrence of chronic renal failure in patients with type 2 diabetes.

MeSH Keywords: **Diabetes Mellitus, Type 2 • Diagnosis, Dual (Psychiatry) • Kidney Failure, Chronic**

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Background

As a common chronic disease, diabetes is a group of metabolic disorders caused by abnormally high glucose level in blood [1]. Diabetes affects about 10% of people during their lifetime, and the incidence of this disease is expected to significantly increase in the near future due to changes in lifestyle [2,3]. Besides the abnormal physiological conditions caused by diabetes itself, complications of this diseases also seriously affect human health [4]. Diabetes can be divided into 3 major subgroups: type 1, type 2, and gestational diabetes [5]. Progression of chronic renal failure, which is common in patients with type 2 diabetes, may eventually lead to end-stage renal failure or even death in diabetic patients [6]. Although a variety of treatment options, such as antihyperglycemic agents, have been developed to prevent the occurrence or inhibit the progression of chronic renal failure in patients with chronic renal failure, treatment outcomes are usually poor and adverse effects are common [7]. Therefore, the development of novel treatment targets is needed to improve the treatment of this disease.

The development of chronic renal failure is a complex process with various internal and external factors involved. Long non-coding RNAs (lncRNAs) are a group of RNA transcripts composed of more than 200 nucleotides and show no protein-coding capacity [8]. It has been reported that certain lncRNAs are involved in development of renal injury caused by high-glucose conditions [9]. CASC2 is a newly discovered lncRNA with pivotal roles in the development of various types of human malignancies, including renal cell carcinoma [10], but its involvement in high-glucose-induced chronic renal failure remains unclear. Therefore, we investigated the correlations between CASC2 expression and chronic renal failure in patients with type 2 diabetes.

Material and Methods

Subjects

A total of 66 patients with type 2 diabetes combined with chronic renal failure were selected in Cangzhou Central Hospital from January 2010 to January 2012. Those patients included 37 males and 29 females, and the age ranged from 31 to 72 years, with an average age of 49.2 ± 7.2 years. Patients with other complications of type 2 diabetes that affect other parts of the body, including diabetic cardiomyopathy, diabetic retinopathy, and diabetic foot infections, as well as healthy controls, were also included. During the same time period we also enrolled 296 patients with type 2 diabetes but without obvious complications in major organs, and those patients were followed up for 5 years to record the occurrence of complications of type 2 diabetes. In addition, 56 healthy controls were

included as the same time to serve as a healthy control group. All patients were diagnosed according to the diagnostic standard proposed by the Chinese Medical Association in 2014. Patients complicated with mental disorders or other severe diseases were excluded. There were no significant differences in age, sex, or BMI among groups. All participants provided written informed consent. This study was approved by the Ethics Committee of Cangzhou Central Hospital. See Table 1 for basic information on participants.

Specimen collection

Renal biopsy tissues were obtained from all participants. Whole blood (15 ml) was obtained from all participants on the day of admission. Blood samples were kept at room temperature for 2 h, followed by centrifugation at 1875 rpm for 25 min to collect serum.

Real-time quantitative PCR

Total RNA extraction from serum and renal biopsy tissues were performed using Trizol reagent (Invitrogen, USA). Renal tissues were ground in liquid nitrogen before the addition of Trizol. RNA samples with a ratio of A260/A280 between 1.8 and 2.0 were used to synthesize cDNA through reverse transcription. Sequences of primers used in PCR reactions were: 5'-GCACATTGGACGGTGTTC-3' (forward) and 5'-CCC AGTCTTCACAGGTCAC-3' (reverse) for lncRNA-CASC2; GACCTCTATGCCAACACAGT (forward) and AGTACTTGCCTCAGGAGGA (reverse) for β -actin. PCR reaction conditions were 95°C for 48 s, followed by 40 cycles of 95 °C for 12 s and 60°C for 38 s. The data were processed according to $2^{-\Delta\Delta CT}$ method, expression of lncRNA-CASC2 was normalized to endogenous control β -actin, and the tissue with the lowest expression level of CASC2 was set as 1.

Statistical analysis

SPSS19.0 (SPSS Inc., USA) was used to perform all statistical analysis. Comparisons of measurement data between 2 groups and among multiple groups were performed using the *t* test, and one-way analysis of variance and post-hoc Tukey HSD, respectively. Comparisons of count data were performed by chi-square test and $p < 0.05$ was considered to be statistically significant.

Results

Expression of lncRNA CASC2 in renal tissues and serum of different groups of participants

As shown in Figure 1A, no significant differences in the expression of lncRNA CASC2 in renal tissues were found between

Table 1. Basic information of all participants.

Groups	Cases	Sex		Mean age	BMI
		Male	Female		
T2D+CRF	66	37	29	49.2±7.2	22.1±1.1
T2D+DC	45	25	20	48.1±9.1	22.3±0.9
T2D+DR	33	19	14	50.6±7.6	22.2±1.0
T2D+DFI	35	19	16	47.3±6.9	21.9±1.3
Control	56	30	26	49.5±7.7	21.9±1.2
T2D	296	166	130	47.4±6.1	22.2±1.0

CRF – chronic renal failure; DC – diabetic cardiomyopathy; DR – diabetic retinopathy; DFR – diabetic foot infections; BMI – body mass index; T2D – type 2 diabetes.

Figure 1. Expression of lncRNA CASC2 in renal tissues and serum of different groups of participants. This figure shows the expression of lncRNA CASC2 in renal tissues (A) and serum (B) of different groups of participants. * $p < 0.05$; CRF – chronic renal failure; DC – diabetic cardiomyopathy; DR – diabetic retinopathy; DFR – diabetic foot infections; BMI – body mass index; T2D – type 2 diabetes.

patients only with type 2 diabetes and healthy controls, indicating that the development of type 2 diabetes may have no significant effects on the expression of lncRNA CASC2 in renal tissue. However, expression levels of lncRNA CASC2 in renal tissues were found to be significantly lower in patients with type 2 diabetes complicated with chronic renal failure ($p < 0.05$), while no significant differences were found between healthy controls and patients with other types of complications of type 2 diabetes (Figure 1A). Similar expressions of lncRNA CASC2 were found in serum of participants in each group (Figure 1B).

Diagnostic values of lncRNA CASC2 expression in serum and renal tissues for type 2 diabetes complicated with chronic renal failure

ROC curve analysis was performed. As shown in Figure 2A, the area under the curve (AUC) of CASC2 expression in renal tissue was 0.8646, with 95% confidence interval of 0.8023 to 0.9270 ($p < 0.0001$). As shown in Figure 2B, AUC of CASC2 expression in serum was 0.8467, with 95% confidence interval of 0.7810 to 0.9123 ($p < 0.0001$).

Figure 2. Diagnostic values of lncRNA CASC2 expression in serum and renal tissues for type 2 diabetes complicated with chronic renal failure. This figure shows the ROC curve analysis of the diagnostic values of lncRNA CASC2 expression in renal tissue (A) and serum (B) for type 2 diabetes complicated with chronic renal failure.

Table 2. Correlation between serum levels of lncRNA CASC2 and basic data of patients with type 2 diabetes complicated with chronic renal failure.

Items	Groups	Cases	High expression	Low expression	χ^2	p Value
Sex	Male	37	15	22	3.01	0.08
	Female	29	18	11		
Age	>50 (years)	32	14	18	0.97	0.32
	<50 (years)	34	19	15		
Drinking	Yes	31	14	17	0.26	0.61
	No	35	19	16		
Smoking	Yes	38	22	16	2.21	0.14
	No	28	11	17		
Duration of disease	>10 years	26	18	8	6.35	0.012
	<10 years	40	15	25		

Correlation between serum levels of lncRNA CASC2 and basic data of patients with type 2 diabetes complicated with chronic renal failure

Patients with type 2 diabetes complicated with chronic renal failure were divided into a high-level group and a low-level group according to the median serum level of lncRNA CASC2. Correlations between serum lncRNA CASC2 level and clinical data of those patients were subjected to chi-square test. As shown in Table 2, serum levels of lncRNA CASC2 showed no significant correlations with patient age, sex, or smoking and drinking habits. However, serum lncRNA CASC2 level was significantly correlated with the duration of disease.

Follow-up data

As mentioned in the Subjects section of this report, a total of 296 patients with type 2 diabetes but without obvious complications in major organs were also included; these patients were followed up for 5 years and the occurrence of complications of type 2 diabetes during follow-up was recorded. Those patients were divided into a high-level group and a low-level group according to the median serum level of lncRNA CASC2 on the day of admission. As showed in Figure 3 and Table 3, no significant differences in the occurrence of diabetic cardiomyopathy (Figure 3A), diabetic retinopathy (Figure 3B), and diabetic foot infections (Figure 3C) were found between the 2 groups, while incidence of chronic renal failure was significantly

Figure 3. (A–D) Occurrences of type 2 diabetes-related complications. This figure shows the number of patients with type 2 diabetes affected by diabetic cardiomyopathy (DC), diabetic retinopathy (DR), diabetic foot infections (DFR), and chronic renal failure (CRF) during 5-year follow-up.

Table 3. Correlation between the incidence of diabetes-related complications and serum levels of lncRNA CASC2.

Items	Groups	Cases	High expression	Low expression	χ^2	p Value
DC	Yes	115	58	57	0.014	0.91
	No	181	90	91		
DR	Yes	108	53	55	0.058	0.81
	No	188	95	93		
DFI	Yes	58	30	28	0.086	0.77
	No	238	118	120		
CRF	Yes	62	23	39	5.223	0.022
	No	234	125	109		

higher in the low-expression group than in the high-expression group ($p < 0.05$).

Discussion

Previous studies showed that several lncRNAs are involved in the development of renal injury caused by high blood glucose levels. Expression of lncRNA MIAT is upregulated in renal tubular epithelial injury caused by high glucose levels,

and increased lncRNA MIAT expression level is positively correlated with the severity of disease [12]. In another study, lncRNA MALAT1 was found to be downregulated in diabetic nephropathy and caused translocation of β -catenin to the nuclei and the enhanced expression of serine/arginine splicing factor 1, which in turn led to aggregation of disease [13]. CASC2 has pivotal roles in the development of various types of human malignancies, including renal cell carcinoma [10]. In the present study, expression of CASC2 in renal tissues and serum was found to be significantly lower in diabetic patients (type 2) complicated with chronic renal function but not in diabetic patients affected by other complications or in patients without obvious complications. These data suggest that downregulation of CASC2 is very likely to be involved in the pathogenesis of chronic renal failure in patients with type 2 diabetes.

Our ROC curve analysis showed that expression levels of CASC2 in renal tissues and serum are effective in diagnosing type 2 diabetes complicated with chronic renal failure. However, expression of lncRNA may be affected by various factors such as alcohol and tobacco consumption [14,15]. In addition, transcription profiles of lncRNAs may change with age [16] and are vary by sex [17], which in turn reduces the reliability of the use of certain lncRNAs as biomarkers for human diseases. We found that serum levels of lncRNA CASC2 were significantly correlated with course of disease.

However, serum levels of lncRNA were not significantly correlated with age, sex, or smoking and drinking habits, indicating the high reliability of serum lncRNA CASC2 as a diagnostic marker for chronic renal failure induced by high glucose. Our long-term (5 years) and large-sample-size (n=296) follow-up study showed that diabetic patients (type 2) with low serum level of lncRNA CASC2 were more likely to have chronic renal failure. Therefore, CASC2 expression may serve as a target for the treatment and prevention of this disease. However, the mechanism underlying the role of lncRNA CASC2 in high-glucose-induced chronic renal failure remains unclear.

Conclusions

Our study found that lncRNA CASC2 is downregulated in serum and renal tissue of patients with type 2 diabetes combined with chronic renal failure but not in patients combined with other diabetes-related complications. CASC2 expression is a promising diagnostic biomarker for high-glucose-induced chronic renal failure. Follow-up showed that patients with low serum levels of lncRNA CASC2 had significantly higher incidence of chronic renal failure. Therefore, we conclude that lncRNA CASC2 is a reliable diagnostic biomarker for type 2 diabetes combined with chronic renal failure, and low serum level of lncRNA CASC2 predicts the occurrence of chronic renal failure in patients with type 2 diabetes.

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