Int J Clin Exp Pathol 2017;10(10):10483-10488
www.ijcep.com /ISSN:1936-2625/IJCEP0061582

Original Article

A novel missense variant in *TXNDC3* is associated with developmental dysplasia of the hip in Han Chinese population

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Received July 16, 2017; Accepted September 18, 2017; Epub October 1, 2017; Published October 15, 2017

Abstract: Developmental dysplasia of the hip (DDH) is one of the most common inborn disabilities of the hip joint and a common disease with a genetic component for its etiology. However, genetic basis of Developmental dysplasia of the hip (DDH) remains largely unknown. Previous study has identified that *TXNDC3* is significant associated with osteoarthritis and the development of chondrocytes and bone. In this study, we carried out a case-control study to investigate for the association between *TXNDC3* and DDH, to find whether acetabular cartilage and bone formation in hip developmental progress is regulated by *TXNDC3*. We totally enrolled 984 radiology confirmed DDH children and 2043 healthy controls to conduct a case-control association study by genotyping SNP rs10250905 on *TXNDC3*. The rs10250905 variant is further detected in 7 DDH pedigrees which comprise total 15 familial DDH patients. The SNP was significantly associated with DDH, $P = 1.53*10^{-5}$ with the odds ratio of 0.786 (0.705-0.877) for allele T; P = 0.0075 with the odds ratio of 0.761 (0.622-0.930) for genotype TT. Furthermore, the significant difference was also detected in samples when stratified by gender. In case-control study, the allele T frequency in cases (0.397) was lower than in controls (0.456). In addition, the allele T frequency in cases of DDH families was 0.300 and in controls was 0.433. In conclusion, our study demonstrates a novel missense variant of *TXNDC3*, rs10250905, is strongly associated with DDH in Han Chinese population and it shows protective allele T.

Keywords: Developmental dysplasia of the hip, genetics, *TXNDC3*, SNP rs10250905

Introduction

Developmental dysplasia of the hip (DDH; MIM 142700) is one common skeletal disorder characterized by incomplete formation of the acetabulum and/or the proximal femur leading to dysplasia, subluxation or dislocation of the hip, which may induce chronic pain, severe hip dysfunction, and increase the risk of hip osteoarthritis [1, 2]. Incidence of DDH varies from 1 to 18.4 per 1,000 live births in Caucasian population, and 1 to 5 per 1,000 in Chinese population [3]. It is well known that both environmental and genetic factors contribute to the occurrence of DDH [4-6]. Genetic components have been suggested playing a more crucial role in the etiology of DDH than mechanical factors (e.g. breech delivery, high birth weight, primiparity and oligoamnios) [5, 7]. A twin study and several family studies showed that genetic factors play an important role in the etiology of DDH [8-10]. And the risk of DDH in first-degree relatives of those affected by the disorder increases by 12 times [11]. In addition, several DDH susceptibility genes were discovered by association study in Chinese population [12-15]. But the exact etiology of DDH is still unclear.

Thioredoxin domain-containing protein 3 *(TXNDC3)* encodes a thioredoxin protein which is composed of an N-terminal thioredoxin domain and three C-terminal nucleoside diphosphate (NDP) kinase domains. NDP kinases, responsible for the synthesis of nucleoside triphosphates (NTPs), are involved in numerous regulatory processes associated with proliferation, development, and differentiation. They are vital

Table 1. Allele and genotype for the *TXNDC3* SNP rs10250905 in patients and controls in Han Chinese population

Group	Number of subject	Genotype (frequency)			Allele (frequency)		Hardy-Weinberg equilibrium *P* value
		TT	TC	CC	T	C	
All patients	984	160 (0.163)	461 (0.468)	363 (0.369)	781 (0.397)	1187 (0.603)	0.50
All controls	2043	415 (0.203)	1032 (0.505)	596 (0.292)	1862 (0.456)	2224 (0.544)	0.41
Female patients	857	139 (0.162)	409 (0.477)	309 (0.361)	687 (0.401)	1027 (0.599)	0.85
Female controls	854	170 (0.199)	415 (0.486)	269 (0.315)	755 (0.442)	953 (0.558)	0.66
Male patients	127	21 (0.165)	52 (0.409)	54 (0.425)	94 (0.370)	160 (0.630)	0.17
Male controls	1189	245 (0.206)	617 (0.519)	327 (0.275)	1107 (0.466)	1271 (0.534)	0.14

TXNDC3 = Thioredoxin domain-containing protein 3, SNP = single nucleotide polymorphism.

for DNA/RNA synthesis, cell division, macromolecular metabolism and growth. *TXNDC3* has been described to be expressed in testis and regulate oxidative stress in human spermatozoa [16-18]. It was also reported to underlie primary ciliary dyskinesia (PCD), which is characterized by chronic respiratory tract infections and male infertility [19]. Moreover, a 5' single nucleotide polymorphism (SNP) in *TXNDC3* was reported to be associated with osteoarthritis and its specific transcript lacking exon 2 was demonstrated in chondrocytes [20, 21]. Previous studies also indicated association between *TXNDC3* gene and bone mineral density and fracture risk [22]. In a word all these findings have confirmed that *TXNDC3* plays a key role in the development of bone, chondrocytes.

Hereby, we hypothesized that *TXNDC3* might also play a pivotal role in the etiology and pathogenesis of DDH, as acetabular cartilage and bone formation may be regulated by *TXNDC3*. Therefore, we investigated a case-control association study to explore the association between rs10250905 on *TXNDC3* and DDH patients.

Methods and materials

Patients

This is a retrospective case-control study with Level III of evidence. A total of 984 sporadic DDH children patients and 2043 healthy controls were enrolled. We firstly enrolled 386 DDH patients and 558 healthy controls to conduct a case-control association study. We found the association between the missense mutation rs10250905 in *TXNDC3* and DDH. To further validate the result, an independent set of up to 599 cases and 1485 controls and additional 7 DDH pedigrees comprising a total of 15 familial DDH patients and 15 healthy first degree relatives were also enrolled.

Sporadic DDH patients and all members of DDH pedigrees were radiology confirmed and consecutively recruited from the Center of Diagnosis and they were all under treatment of Development dysplasia of hip, in Kang'ai Hospital. Control groups were recruited at the same period of time from the Physical Examination Center, Drum Tower Hospital affiliated to the Medical School of Nanjing University. The diagnosis of DDH patients who all suffered from unilateral or bilateral complete dislocation of the femoral head was made on the basis of clinical criteria and radiographic evidence by experts. All control groups had no symptom or history of skeletal diseases. Subjects with any systemic syndrome were excluded. All the subjects were Han Chinese living in or around Nanjing. The study was approved by the ethical committee of the participating institutions, and informed consent was obtained from patients and controls.

Methods

DNA was extracted from all the subjects either from peripheral blood using the NucleoSpin Blood QuickPure Kit (Macherey-Nagel GmbH & Co. KG, Düren, German) or buccal swabs using the DNA IQ System (Promega, Madison, WI, USA) according to the manufacture's protocol. The SNP rs10250905 was genotyped using a Taqman 50 allelic discrimination assay on an ABI 7300 real-time polymerase chain reaction (PCR) instrument (Applied Biosystems 7300, ABI, Foster City, CA, USA). The samples were genotyped by laboratory personnel blinded to

Table 2. Association of rs10250905 of the *TXNDC3* gene with DDH

	Case						Control						Allele T frequency		Genotype TT frequency	
	Genotype				Allele T frequency	Genotype TT frequency	Genotype				Allele T frequency	Genotype TT frequency	*P* value	OR (95% CI)	*P* value	OR (95% CI)
	TT	TC	CC	Sum			TT	CT	CC	Sum						
Total	160	461	363	984	0.397	0.163	415	1032	596	2043	0.456	0.203	$1.53*10^{-5}$	0.786 (0.705-0.877)	0.0075	0.761 (0.622-0.930)
Female	139	409	309	857	0.401	0.162	170	415	269	854	0.442	0.199	0.015	0.844 (0.737-0.967)	0.047	0.779 (0.608-0.998)
Male	21	52	54	127	0.370	0.165	245	617	327	1189	0.466	0.206	0.004	0.675 (0.516-0.881)	0.278	0.763 (0.468-1.245)

TXNDC3 = Thioredoxin domain-containing protein 3, DDH = developmental dysplasia of the hip; OR = odds ratio; CI = confidence interval.

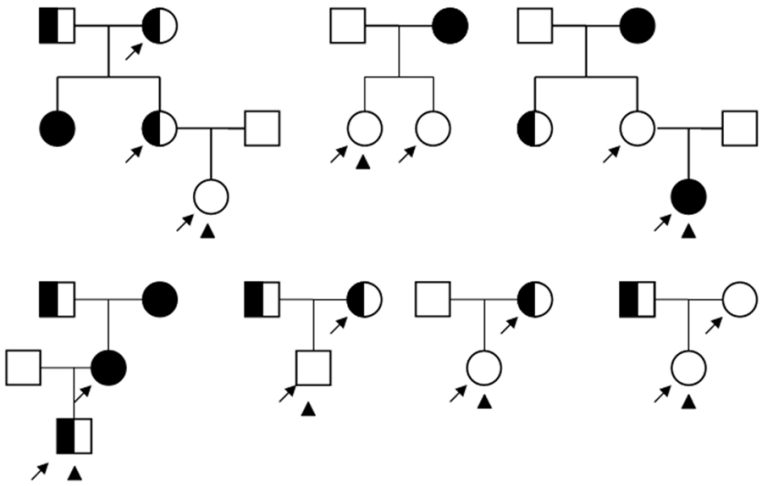

Figure 1. Pedigree of 15 DDH members in 7 families. Symbols filled with black denote TT homozygotes while symbols filled with white-black denote TC heterozygote, and symbols filled with white denote CC homozygotes. The square stands for a male while circles stand for females. Arrows denote DDH members and triangles denote probands.

case status. Genotyping, data entry and statistical analyses results were reviewed by two authors independently. Five percent samples were randomly selected to duplicate and yielded a 100% concordance.

Statistics

SPSS 19.0 system software (SPSS Inc., Chicago, Illinois, USA) was used to test the association between DDH patients and control subjects. The statistical method is as follows. First of all, Hardy-Weinberg equilibrium was calculated by chi-squared test in both control and case groups. Then, two-sided chi-squared tests were performed to determine the significance of differences in genotype and allele distributions frequencies and $P<0.05$ was considered statistically significant. The associations between rs10250905 variants and DDH risk were estimated by computing the odds ratios (ORs) and 95% confidence intervals (CIs).

Results

The ages of DDH patients (mean ± SD) were 22.6 ± 12.1 months (range, 2 to 85 months) and control groups were 55.3 ± 13.4 years (range, 32 to 85 years). Distributions of genotypes of rs10250905 in both case and control groups were conformed to Hardy-Weinberg equilibrium (all $P>0.05$) (**Table 1**). Genotyping of rs10250905 showed that the minor allele T was a significantly protective allele ($P = 1.53*10^{-5}$) and the odds ratio of 0.786 (0.705-0.877) for allele T. Genotype frequency of TT was also significantly different with *p* value of 0.0075 and the odds ratio of 0.761 (0.622-0.930). Furthermore, when subjects were stratified by gender, allele frequencies still showed difference (**Table 2**).

Detection of rs10250905 in 7 DDH pedigrees is shown in **Figure 1**. In all the 7 pedigrees, there were 8 CC homozygotes DDH, 5 TC heterozygotes DDH and 2 TT homozygotes DDH, giving a 0.300 (9/30) frequency of T allele and lower than 0.433 (13/30) in healthy controls. There are 7 probands in DDH families. In the probands stud, 2 probands were presented TC heterozygote and TT homozygote genotype respectively, while other 5 probands were presented CC homozygotes, which demonstrated a 0.214 (3/14) frequency of T allele.

Discussion

Our study indicated, for the first time, the association between DDH and polymorphisms of *TXNDC3* and demonstrated that the missense mutation rs10250905 (Cys208Arg) was associated with DDH in Han Chinese population. Results in sporadic cases indicated that the T allele was a protective allele with significantly lower frequency in sporadic DDH patients. The DDH pedigrees study showed an even lower frequency for T allele in the probands (0.21) than in all familial DDH (0.30), further verifying the protective role of T allele. Previous studies have indicated the association between *TXNDC3* gene and bone mineral density, chondrocyte and osteoarthritis [20-23]. Rs10250905 locates in exon 11 of gene *TXNDC3*, participating in transcription of one NDP domain of *TXNDC3* protein. NDP kinases, responsible for the synthesis of nucleoside triphosphates (NTPs), are involved in numerous regulatory processes associated with proliferation, development, and differentiation. They are vital for

DNA/RNA synthesis, cell division, macromolecular metabolism and growth. However, the function of the Cys208Arg mutation is still unclear. The minor allele frequency of rs10250905 of gene *TXNDC3* in our study was a bit lower than that in Japanese population, but significantly higher than that in any other population according to the data submitted to Hapmap. This frequency variability between different geographic locations calls for replication studies in different ethnicities.

However, limitation of our study should be point out. On the one hand, the number of male subjects in our study was relatively limited due to low prevalence of DDH in males. So the the difference of genotype TT frequency in male wasn't detected in our study. It is necessary to collect a larger number of subjects to confirm this findings.

On other hand , further study towards protein function of *TXNDC3*, though rs10250905 polymorphism leads to a residue change (Cys-208Arg), needs to be done in the *TXNDC3* protein and we have not demonstrated its effect on *TXNDC3* protein function. Association studies in different ethnic populations and functional studies of this susceptibility SNP should be performed to clarify the significance of TXNDC3 as a DDH candidate gene.

In conclusion, our study demonstrates, for the first time, a missense variant of *TXNDC3*, rs10250905, is associated with DDH in Han Chinese population. And, further studies should be conducted with larger sample numbers in different ethnic groups to confirm or refute our findings.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81420108021 and 81101338) and the Natural Science Foundation of Jiangsu Province (Grants No. BK20141088). We thank the patients and their families who donated their blood samples for this study.

Disclosure of conflict of interest

None.

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