

Original Article

Lack of association of a single nucleotide polymorphism in SMOC1 with developmental dysplasia of the hip: a case-control study

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Abstract: Introduction: Developmental dysplasia of the hip (DDH) is one of the most common inborn disabilities of the hip joint. DDH greatly contributes to the occurrence of premature hip osteoarthritis in later life. It has long been supposed that environmental and genetic factors are both the reasons of DDH. However, no unequivocal genetic factors have been detected. Recently, The gene SPARC (secreted protein acidic and rich in cysteine)-related modular calcium binding 1 (SMOC1) has been indicated to be essential for ocular and limb development in human and mice. SMOC1 regulates Bone Morphogenic Protein (BMP) signaling which is important in the skeletal development. Maybe, it is involved in the etiology and pathogenesis of DDH. Materials and methods: We genotyped the SMOC1 SNP rs3742912 using a Taqman 50 allelic discrimination assay on an ABI 7300 real-time polymerase chain reaction (PCR) instrument. 170 children who suffered from the most severe DDH characterized by complete dislocation of the femoral head and 454 control subjects were involved in our study. The genotype distributions and allele frequencies were compared between DDH and healthy control. Results: No significant difference was detected in genotype distributions or allelic frequencies between patients and controls. There was also no significant difference even after patients were stratified by sex (all $P > 0.05$). Conclusion: Our results show that there might be no association between SMOC1 SNP rs3742912 with DDH etiology in Chinese Han population. We hypothesized that it is ligament laxity, not cartilage development which directly related to DDH. Future association work should give priority to genes that for ligament laxity.

Keywords: DDH, genetic factors, SMOC1 gene, BMP signaling, SNP rs3742912

Introduction

Developmental dysplasia of the hip (DDH) has replaced the previous term congenital dislocation of the hip (CDH), as the CDH has limits to reflect all the features of the inborn deformity of the hip [1]. DDH is one of the most common teratogenic diseases in pediatrics. It has a series of abnormalities such as shallow acetabulum, lax capsule, abnormal size or shape of the femoral head, and so on [2-4]. Severe dysplasia can cause serious symptoms even in early childhood [5]. While, mild dysplasia may not affect the normal life of the infants with DDH, but it can result in chronic pain or premature arthritis in adult life [6, 7]. Although the exact etiology of DDH is still unclear, it is well

known that both environmental and genetic factors contribute to the occurrence of DDH [8-10]. Recently, more and more researches hold the idea that genetic factors account mainly position in all the factors to DDH [11]. A twin study and several family studies showed that genetic factors play an important role in the etiology of DDH [11-13]. It also had been reported that a single nucleotide polymorphism in growth differentiate factor 5 (GDF5) had significant association with DDH [14]. Recently, Shi et al's work showed an obvious association between the D repeat polymorphism of ASPN and DDH [15].

SMOC1 is a member of the SPARC matricellular protein family that encodes cell-matrix interaction by binding to extracellular matrix, growth

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Table 1. Allele and genotype for the SMOC1 SNP rs3742912 in patients and controls in Han Chinese population

Group	Number of subject	Genotype (frequency)			Allele (frequency)		Hardy-Weinberg equilibrium
		GG	GA	AA	G	A	P value
All patients	170	62 (0.365)	82 (0.482)	26 (0.153)	206 (0.606)	134 (0.394)	0.90
All controls	454	177 (0.390)	198 (0.436)	79 (0.174)	552 (0.608)	356 (0.392)	0.07
Female patients	153	56 (0.366)	74 (0.484)	23 (0.150)	186 (0.608)	120 (0.392)	0.86
Female controls	144	57 (0.396)	63 (0.438)	24 (0.167)	177 (0.615)	111 (0.385)	0.36
Male patients	17	6 (0.353)	8 (0.471)	3 (0.176)	20 (0.588)	14 (0.412)	0.91
Male controls	310	120 (0.387)	135 (0.435)	55 (0.177)	375 (0.605)	245 (0.395)	0.12

SMOC1 = the gene SPARC (secreted protein acidic and rich in cysteine)-related modular calcium binding 1; SNP = single nucleotide polymorphism.

factors, cell-surface receptors, and cytokines [16, 17]. It has been found that Smoc1 is expressed in the developing optic stalk, ventral optic cup, and limbs of embryos. Mutations in SMOC1 can lead to Waardenburg Anophthalmia syndrome which has a set of features such as anophthalmia, hypoplastic fibula and bowed tibia, and oligodactyly, polydactyly, syndactyly in limbs [18, 19]. It is generally considered that the secreted signaling molecules such as BMP2 plays an important role in the development of the limbs [20]. BMP signaling affects the process of cartilage formation and regulates the skeletal development. SMOC1 functions as a BMP antagonist to balance agonistic and antagonist effects on BMP signaling [19]. Then, SMOC1 has an important effect on the development of limbs by modulating BMP signaling. In addition, SMOC1 null mice recapitulated Waardenburg Anophthalmia syndrome phenotypes [19]. Recently, three families with Waardenburg Anophthalmia syndrome were confirmed mutation in SMOC1. All these findings confirmed that SMOC1 plays a key role in the development of the limbs. We hypothesized that SMOC1 might also play a pivotal role in the etiology and pathogenesis of DDH, as acetabular cartilage formation may be regulated by SMOC1. In this case-control study, we investigated if the SNP (rs3742912) in SMOC1 were associated with DDH in the Chinese Han population.

Materials and methods

Subjects

The study was approved by the ethical committee of the participating institutions, and informed consent was obtained from patients and controls. A total of 624 subjects were

enrolled in this study. All subjects included in the study were Han Chinese origin living in or around Nanjing. No subjects dropped out during the process of the study. One hundred and seventy DDH patients (153 females and 17 males) were enrolled consecutively at the Center of Diagnosis and Treatment for developmental dysplasia of hip, Kang'ai Hospital, China; Four hundred and fifty four healthy controls (144 females and 310 males) were enrolled the same period of time at the Physical Examination Center, Drum Tower Hospital, affiliated to the Medical School of Nanjing University. Patients were diagnosed by expert medical examination with radiographic evidence, and they all suffered from unilateral or bilateral complete dislocation of the femoral head. Patients with systemic syndrome were excluded in the study. The controls never have any symptoms or histories of DDH. Control subjects were identified by detailed inquiry of history and physical examination.

Genotyping

DNA was obtained from all the subjects from peripheral blood using the Chelex-100 method [21] according to the manufacturer's instructions. The SNP rs3742912 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). Laboratory personnel blinded to case status performed genotyping. Two authors independently reviewed the genotyping results and performed statistical analysis.

Statistics

We used chi-square test to determine difference in genotype and allele distributions

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Table 2. Association of rs3742912 polymorphism in SMOC1 with DDH when stratified by gender

Groups compared	GG vs. other combined			AA vs. other combined			G allele vs. A allele			All genotype
	OR	P value	95% CI	OR	P value	95% CI	OR	P value	95% CI	P value
All patients (n = 170) vs. all controls (n = 454)	1.11	0.56	0.77 to 1.60	0.86	0.53	0.53 to 1.39	1.01	0.95	0.78 to 1.30	0.57
Female patients (n = 291) vs. female controls (n = 316)	1.13	0.60	0.71 to 1.81	0.88	0.70	0.47 to 1.65	1.03	0.87	0.74 to 1.43	0.73
Male patients (n = 47) vs. male controls (n = 306)	1.16	0.78	0.42 to 3.21	0.99	0.99	0.28 to 3.58	1.07	0.85	0.53 to 2.16	0.95

DDH = developmental dysplasia of the hip; CI = confidence interval; OR = odds ratio.

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between patients with DDH and the controls. $P < 0.05$ was considered significant difference. The associations between SMOC1 variants and DDH risk were estimated by computing the odds ratios (ORs) and 95% confidence intervals (CIs) with stratified by gender. These tests were performed using SPSS 19.0 system software (SPSS Inc., Chicago, Illinois, USA).

Results

The ages of patients with DDH and controls (mean \pm SD) were 27.5 ± 12.9 months (range 4 to 96 months) and 55.2 ± 13.2 years (range 40 to 87 years), respectively. The data have also been stratified by gender; the ratio of female to male was about nine to one in patients with DDH. Respectively, the distributions of the alleles and genotypes for the SNP rs3742912 are presented in **Table 1**. Distributions of genotypes in the DDH group and control group were both conformed to Hardy-Weinberg equilibrium ($P = 0.90$ and 0.07 , respectively). No significant differences in allele frequency and genotype frequency were detected between DDH and control groups ($P = 0.57$) (**Table 2**). We stratify subjects by sex and compared the genotype frequencies and the allele frequencies. We also found none of positive results (**Table 2**).

Discussion

Recently, more and more researches believed that genetic factors play a more crucial role in the development of DDH. Wynne-Davis proposed two different gene mechanisms to be responsible for the occurrence of DDH, one affecting joint laxity and the other affecting the shape of acetabulum [22]. Carter and Wilkinson confirmed Wynne-Davis's hypothesize, they reported increased incidence of joint laxity with DDH in 1964 [3]. Genes polymorphism which affects capsule laxity and development of acetabular cartilage may be associated with DDH. For the SNP rs3742912 in this study, no significant difference was detected in genotype or allele distribution between DDH and control groups. Also, no significant difference was found when patients were stratified by sex. Then, our results showed that the SNP (rs3742912) in SMOC1 had no association with DDH in Chinese Han population.

Several possible reasons may contribute to the negative results of the current study. Firstly,

statistical power to detect the association between SNP (rs3742912) and DDH may be limited, as our sample size was not big enough, especially for male patients. Secondly, only one SNP (rs3742912) in SMOC1 gene was tested in the present study. Other SNPs within SMOC1 should be tested in the future study. Thirdly, among the genetic factors, we speculated that the root cause of DDH is ligamentous laxity, not acetabulum cartilage development which may be secondary to the former.

Several case-control studies had been carried out to find out the susceptibility genes for DDH, and most of them declared negative results. The reported susceptibility genes all have a same feature, they at least affect ligament development of hip joint [15] or both joint laxity and chondrocytes development of acetabulum [14, 23, 24]. However, there are no reports about any association between the non-susceptibility genes for DDH and ligamentous laxity, although these genes may regulate cartilage development [25, 26]. In the present study, SMOC1 gene had not been declared to regulate tendon or capsule development of the hip joint. Our results were consistent with previous studies.

The severity of DDH is degraded as isolated acetabular dysplasia, subluxation and dislocation of the femoral head. In our study, to best value the association between SMOC1 gene and DDH, only the patients who suffered from the most serious degree of DDH which is characterized as unilateral or bilateral complete dislocation of the femoral head were involved. 170 patients of 624 total subjects were enrolled in our study. The sample size is small, which is the weakness of our study. The strength of our work is that only fully defined DDH patients were enrolled in our study. And, all the patients in our study were infants, which may have less environmental influence than adult DDH patients.

Although failed to suggest the linkage between SMOC1 gene with DDH, our work helped to hypothesize that ligamentous laxity may be directly related to the cause of DDH, and acetabular dysplasia may be secondary to ligamentous laxity as lack of the stimulation of the femoral head and biomechanical function. Future genetic-based studies in DDH are required. Other genes for ligament laxity should be given

priority to. Large sample-size based studies with diverse populations are needed to confirm or refute our findings in future.

Conclusions

The present study did not find any significant association between the SMOC1 polymorphisms (rs3742912) and susceptibility to DDH in the Chinese Han population. We hypothesized it is ligament laxity, not cartilage development directly related to DDH, future association work should give priority to genes that for ligament laxity.

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Disclosure of conflict of interest

None.

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