

Original Article

Variants rs3732378 and rs3732379 of gene CX3CR1 are not associated with developmental dysplasia of the hip in Han Chinese population

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Received October 13, 2016; Accepted October 25, 2016; Epub February 1, 2017; Published February 15, 2017

Abstract: Introduction: Developmental dysplasia of the hip (DDH) is one of the most common inborn disabilities of the hip joint. This study intended to investigate and validate the association between gene Chemokine (C-X3-C motif) receptor 1 (CX3CR1) and DDH in Han Chinese population. Materials and methods: We enrolled 409 children radiology confirmed complete dislocation of the femoral head sporadic DDH patients and 351 healthy controls (set A) to conduct a case-control association study. Another set of nine families with DDH history were enrolled, which consist of 19 DDH patients and 15 healthy families (set B). All samples were genotyped by Taqman assay on ABI Viia 7 real-time polymerase chain reaction (PCR) instrument. Results: No significant difference was detected in genotype distributions or allelic frequencies both in set A and set B, even after patients were stratified by sex (all $P > 0.05$). In addition, two DDH patients in same family (family I) carried different variations of CX3CR1. Conclusion: Our study demonstrates that the association between variants of gene CX3CR1, rs3732378 and rs3732379, and DDH in Han Chinese population is negative, which was discordant with previous study. Then, the significance of CX3CR1 in DDH should be further evaluated carefully.

Keywords: Developmental dysplasia of the hip, genetics, CX3CR1, SNP rs3732378, SNP rs3732379

Introduction

Developmental dysplasia of the hip (DDH, OMIM #142700) is one common skeletal disorder, presenting with shallow acetabulum and decreased coverage of the femoral head [1]. Incidence of DDH varies from 0.1% to 1.84% in different populations [2]. DDH is a multifactorial disease with both environmental and genetic risk factors [3]. Though Mechanical factors are suggested, it is accepted that genetic components are a crucial part in the etiology of DDH. Several DDH susceptibility genes were discovered by association study in Chinese and Caucasian populations [4-7]. What's more, the risk of DDH in first-degree relatives of those affected by the disorder have 12-fold increased [8]. However, no unequivocal genetic factors have been detected.

Chemokine (C-X3-C motif) receptor 1 (CX3CR1), a receptor of chemokine, functions as a

receptor for the ligand fractalkine, which mediates cellular adhesive and migratory functions and is known to be expressed in mesenchymal stem cells destined to become chondrocytes [9]. In a recent study by Feldman et al [10], a missense mutation in CX3CR1, rs3732378, was identified in all affected DDH members in a large multi-generation family. The Thr280-Met280 transition caused by rs3732378 could enhance cellular adhesive and migratory functions [11]. And Li et al [12] reported a case-control study about rs3732378 and rs3732379 of CX3CR1 can increase the risk of DDH. Though these study identified a significant genetic risk factor for DDH in their crowd, it is not known how prevalent this variant will be in the overall DDH patient population or whether this variant is associated with DDH in other population.

Based on the knowledge above, we conducted a genetic study to investigate the prevalence of rs3732378 and rs3732379 of CX3CR1 in Han

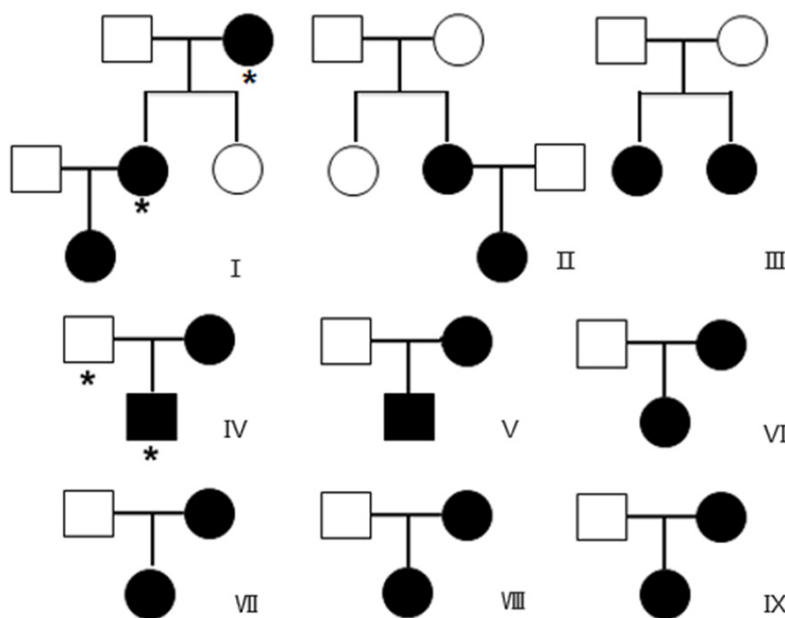


Figure 1. Pedigree of 19 DDH members in 9 families. I-IX are the number of families. Filled in symbols denote DDH patients. The asterisk indicates individuals with heterozygote AG in rs3732378 and TC in rs3732379.

Methods

DNA was extracted from all the subjects from peripheral blood using the NucleoSpin Blood QuickPure Kit (Macherey-Nagel GmbH & Co. KG, Düren, German) according to the manufacturer's protocol. The samples were genotyped by Taqman assay on ABI Viia 7 real-time polymerase chain reaction (PCR) instrument (Applied Biosystems, ABI, Foster City, CA, USA) and laboratory personnel blinded to case status performed genotyping. 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. First of all, two-sided chi-squared tests were performed to determine the significance of differences in allelic frequencies and $P < 0.05$ was considered statistically significant. Hardy-Weinberg equilibrium was calculated by chi-squared test in both control and case groups.

Results

Distributions of genotypes of rs3732378 and rs3732379 in both sporadic cases and controls were conformed to Hardy-Weinberg equilibrium in set A and set B (all $P > 0.05$). Genotyping of rs3732378 in set A showed the minor allele A with a frequency of 2.44% and allele G with a frequency of 97.56% in sporadic DDH cases, was not associated with DDH ($P = 0.15$) (**Table 1**). Genotyping of rs3732379 in set A showed the minor allele T with a frequency of 4.52% and allele C with a frequency of 95.48% in sporadic DDH cases, was not associated with DDH ($P = 0.16$) (**Table 2**). And genotyping of

Chinese DDH population and validate the association between CX3CR1 and DDH in Han Chinese population.

Materials and methods

Patients

We enrolled 409 children radiology confirmed complete dislocation of the femoral head sporadic DDH patients and 351 healthy controls (set A) to conduct a case-control association study. Another set of nine families (**Figure 1**) with DDH history were enrolled, with a total of 19 DDH patients and 15 healthy first degree relatives (set B). DDH patients were also consecutively recruited from the Center of Diagnosis and Treatment for Development dysplasia of hip, Kang'ai Hospital. Controls were enrolled at the Physical Examination Center, Drum Tower Hospital, affiliated to the Medical School of Nanjing University. The diagnosis of DDH was made on the basis of clinical criteria and radiographic evidence by experts. All controls had no symptom or history of DDH. All the subjects were Han Chinese living in or around Nanjing. The study was approved by the ethical committee of the Nanjing University and the ethical committee of Nanjing Drum Tower Hospital, and informed consent was obtained from all patients and controls.

Association between CX3CR1 and DDH

Table 1. Association between SNP rs3732378 of the CX3CR1 gene with DDH in Han Chinese population

Population	Genotype	Case				Control				Allele G frequency		Genotype GG frequency					
		Allele G frequency	Genotype GG frequency	Genotype	Sum	Allele G frequency	Genotype GG frequency	Genotype	Sum	P value	OR (95% CI)	P value	OR (95% CI)				
		AA	AG	GG	Sum	AA	AG	GG	Sum								
Set A	Total	1	18	390	409	97.56%	95.35%	0	26	325	351	96.30%	92.59%	0.15	1.54 (0.85-2.78)	0.11	1.64 (0.89-3.02)
	Female	1	12	355	368	98.10%	96.47%	0	10	123	133	96.24%	92.48%	0.09	2.02 (0.88-4.60)	0.06	2.22 (0.95-5.19)
	Male	0	6	35	41	92.68%	85.37%	0	16	202	218	96.33%	92.66%	0.23	0.48 (0.18-1.27)	0.22	0.46 (0.17-1.26)
Set B	Total	0	3	16	19	92.11%	84.21%	0	1	14	15	96.67%	93.33%	0.78	0.40 (0.04-4.01)	0.78	0.38 (0.04-4.09)
	Female	0	2	15	17	94.12%	88.24%	0	0	4	4	100%	100%	-	-	-	-
	Male	0	1	1	2	75.00%	50.00%	0	1	10	11	95.45%	90.90%	0.29	0.14 (0.00-2.94)	0.30	0.10 (0.00-3.15)

CX3CR1 = the gene Chemokine (C-X3-C motif) receptor 1; SNP = single nucleotide polymorphism; DDH = developmental dysplasia of the hip; OR = odds ratio; CI = confidence interval.

Table 2. Association between SNP rs3732379 of the CX3CR1 gene with DDH in Han Chinese population

Population	Genotype	Case				Control				Allele C frequency		Genotype CC frequency					
		Allele C frequency	Genotype CC frequency	Genotype	Sum	Allele C frequency	Genotype CC frequency	Genotype	Sum	P value	OR (95% CI)	P value	OR (95% CI)				
		TT	TC	CC	Sum	TT	TC	CC	Sum								
Set A	Total	1	35	373	409	95.48%	91.20%	0	22	329	351	96.87%	93.73%	0.16	0.68 (0.40-1.17)	0.19	0.69 (0.40-1.20)
	Female	1	29	338	368	95.79%	91.85%	0	6	127	133	97.74%	95.49%	0.15	0.53 (0.22-1.27)	0.16	0.53 (0.22-1.31)
	Male	0	6	35	41	92.68%	85.37%	0	16	202	218	96.33%	92.66%	0.23	0.48 (0.18-1.27)	0.22	0.46 (0.17-1.26)
Set B	Total	0	3	16	19	92.11%	84.21%	0	1	14	15	96.67%	93.33%	0.78	0.40 (0.04-4.01)	0.78	0.38 (0.04-4.09)
	Female	0	2	15	17	94.12%	88.24%	0	0	4	4	100%	100%	-	-	-	-
	Male	0	1	1	2	75.00%	50.00%	0	1	10	11	95.45%	90.90%	0.29	0.14 (0.00-2.94)	0.30	0.10 (0.00-3.15)

CX3CR1 = the gene Chemokine (C-X3-C motif) receptor 1; SNP = single nucleotide polymorphism; DDH = developmental dysplasia of the hip; OR = odds ratio; CI = confidence interval.

Association between CX3CR1 and DDH

rs3732378 and rs3732379 in another set consisting of familial cases and controls (set B) produced another negative result ($P=0.43$ and 0.43) (Tables 1 and 2). Furthermore, no significant difference was found even patients were stratified by sex. Detailed genotype of DDH members in families was illustrated in Figure 1. Genotyping of rs3732378 and rs3732379 exist mutations of heterozygote in same 4 members. And the majority of DDH members have nothing with CX3CR1 mutations.

Discussion

This study denied the association between rs3732378 and rs3732379 of CX3CR1 and DDH in Han Chinese population. The prevalence of allele in sporadic DDH patients is similar to that in healthy controls. Though CX3CR1 is not associated with DDH in Han Chinese sporadic cases, familial DDH patients showed an insignificantly lower prevalence of allele G in rs3732378 and allele C in rs3732379. However, this insignificance is possibly caused by our limited familial DDH number.

Infection, inflammatory monocytes, amyotrophic lateral sclerosis, macular degeneration, etc have been verified in associated with gene CX3CR1 [13-16]. So, healthy individuals should be enrolled as controls. In study of Feldman et al [10] the controls in a large multi-generation family still had a high percent variant of CX3CR1. In recent study by Li et al [12], they excluded patients with syndromic hip dislocation which were typical DDH and enrolled in our study. And in their study the best association is $P=0.001$ which was not adjusted by gender and less persuasive. In addition, selective comparisons maybe a reason for discordance between results, after all the mutations of CX3CR1 in different crowds are different [16, 17]. Furthermore environmental risk factors for DDH including breech presentation, oligohydramnios (deficiency of amniotic fluid), primiparity (first-born) and inappropriate nursing positions can't be neglected [8, 18]. So, to verify the association between CX3CR1 and DDH in Han Chinese population, nine families with DDH history were further investigated.

DDH members in families are shown in Figure 1 and the result was still negative. One patient without inheriting genetic mutation but still suffered from DDH in the affected family I. What's more, another patient inherited CX3CR1 mutation from his healthy father but his DDH mother

has no association with CX3CR1 mutation (family IV). And other familial DDH members haven't mutation either. Then, our results showed that the rs3732378 and rs3732379 of CX3CR1 may have no association with DDH in Han Chinese population.

Denial of association between CX3CR1 and DDH in Han Chinese population is premature. Further genetic studies with more familial DDH patients rather than sporadic cases would be a better approach to understand the development of DDH. CX3CL1-CX3CR1 axis participates in the early stage of osteoblast differentiation, CX3CR1-deficient pre-osteoclasts showed impaired differentiation [19]. CX3CL1-CX3CR1 axis also promotes osteoclast recruitment and subsequent bone resorption [20]. Yet there is no reports regarding CX3CL1-CX3CR1 in pelvic development or hip joint formation, which necessitate relevant studies with genetically modified animal models to get further insight.

Several possible reasons may contribute to the negative results of the current study. Firstly, we only identified 1 homozygote AA of rs3732378 and 1 homozygote TT of rs3732379 phenotype carrier (0.2%) in a sporadic DDH patient. Due to prevalence of DDH and the rarity of this homozygote, it is unlikely to conclude the association between the AA or TT phenotype and DDH in the whole population. Secondly, statistical power and sample size to detect the association between CX3CR1 and DDH may be limited, and further study was necessary.

Conclusions

The variants of gene CX3CR1, rs3732378 and rs3732379, are not associated with DDH in Han Chinese population. Future large sample-size based studies with diverse populations are necessary and CX3CL1-CX3CR1 in pelvic development or hip joint formation should be given priority to.

Acknowledgements

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

Disclosure of conflict of interest

None.

Association between CX3CR1 and DDH

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