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RESEARCH

Possible association of single nucleotide polymorphisms in the 3' untranslated region of HOXB9 with acetabular overcoverage

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Objectives

Excessive acetabular coverage is the most common cause of pincer-type femoroacetabular impingement. To date, an association between acetabular over-coverage and genetic variations has not been studied. In this study we investigated the association between single nucleotide polymorphisms (SNPs) of paralogous Homeobox (HOX)9 genes and acetabular coverage in Japanese individuals to identify a possible genetic variation associated with acetabular over-coverage.

Methods

We investigated 19 total SNPs in the four HOX9 paralogs, then focused in detail on seven of those located in the 3' untranslated region of *HOXB9* (rs8844, rs3826541, rs3826540, rs7405887, rs2303485, rs2303486, rs79931349) using a case-control association study. The seven *HOXB9* SNPs were genotyped in 316 subjects who had all undergone radiological examination. The association study was performed by both single-locus and haplotype-based analyses.

Results

The genotype and allele frequencies of the five *HOXB9* SNPs showed significant association with acetabular over-coverage compared with controls (rs7405887 OR = 3.16, p = 5.29E-6, 95% CI 1.91 to 5.25). A significant difference was also detected when haplotypes were evaluated (OR = 2.59, p = 2.61E-5, 95% CI 1.65 to 4.08). The two *HOXB9* SNPs (rs2303485, rs2303486) were associated with decreased acetabular coverage (rs2303485 OR = 0.524, p = 0.0091, 95% CI 0.322 to 0.855; rs2303486 OR = 0.519, p = 0.011, 95% CI 0.312 to 0.865).

Conclusions

The five *HOXB9* SNPs (rs8844, rs3826541, rs3826540, rs7405887, rs79931349) were associated with acetabular over-coverage. On the other hand, the two SNPs (rs2303485 and rs2303486) were associated with the lower acetabular coverage. The association of rs2303486 would be consistent with the previous study. Therefore, the *HOXB9* SNPs might be involved in the morphogenesis of acetabular coverage, and could be an independent risk factor for developing pincer-type femoroacetabular impingement.

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Keywords: Acetabular overcoverage; HOXB9; SNP; Pincer-type FAI; Case-control study

Article focus

- To date, an association between acetabular over-coverage and genetic variations has not been studied. We investigated the association between single nucleotide polymorphisms (SNPs) of paralogous HOX9 genes and acetabular over-coverage.

Key messages

- The *HOXB9* SNPs might be involved in the morphogenesis of acetabular coverage, and could be an independent risk factor for developing pincer-type femoroacetabular impingement.

Strengths and limitations

- **Strengths:** This is the first report on the association of genetic variations in acetabular over-coverage. The *HOXB9* SNPs showed significant association with acetabular over-coverage compared with controls.

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Table I. Characteristics of AO cases and controls

Group	Subjects (n)	Age (yrs)	Gender (%, female)	Sharp angle[20]	AR (%)
AO	130	29.3 (SD 10.5)	*96.2*	38.9° (SD 2.1)*	*32.3*†
Control	186	42.1 (SD 16.1)	*94.6*	46.4° (SD 3.4)	*6.5*

* p < 0.001 *versus* controls, Mann–Whitney U test
† Odds ratio = 6.92, p = 1.87E-9, 95% CI 3.47 to 13.8, chi-squared test
AO, acetabular overcoverage; AR, acetabular retroversion; SD, standard deviation

- **Limitations:** More studies should be conducted with larger sample numbers and using different ethnic groups.

Introduction

The hip is a ball-and-socket joint. The socket is formed by the acetabulum, which is part of the large bone of the pelvis. The degree of acetabular coverage is correlated with several hip diseases, such as acetabular dysplasia (AD), femoroacetabular impingement (FAI), labral tears, cartilage damage, and hip osteoarthritis (OA).[1-3] AD is a major and common cause of secondary hip OA, particularly in women.[1] It is characterised by a shallow acetabulum and decreased coverage of the femoral head. This condition appears to be related to a number of different factors.[2] Several studies have investigated the association between genetic variations and AD.[4-10] On the other hand, the morphogenesis of acetabular over-coverage (AO) remains poorly understood. Excessive acetabular coverage has been reported to be related to FAI (pincer morphological features).[3,11,12]

FAI is characterised by contact arising from abnormal morphological features of the hip joint, and has been associated with early OA of the hip. Patients with FAI are young, usually in their 20s to 40s. The estimated prevalence of FAI is 10% to 15%.[13] The morphological mechanism of FAI is well established.[3] However, the genetic predisposition in the pathogenesis of FAI remains unknown. Two main types of FAI have been described in relation to morphological variations of the acetabulum and femoral head, namely: pincer-type and cam-type impingement. Pincer-type impingement occurs on the excessive acetabular coverage, and is more common in women.[11] AO is correlated with the radiologic depth of the acetabular fossa. To date, an association between AO and genetic variations has not been studied. Considering the involvement of genetic variations in several examples of AD studies, it is worth investigating whether any genetic variation is associated with susceptibility to AO.

Homeobox (HOX) genes encode a family of transcription factors of fundamental importance for body patterning along the anteroposterior (AP) axis during skeletal development.[14,15] Particularly, paralogous Hox9 genes were shown to have an essential role in the development of the limb buds.[16-19] In the present study, to identify a possible genetic variation associated with AO, we investigated the association between single nucleotide polymorphisms (SNPs) of paralogous HOX9 genes and AO.

Patients and Methods

Study subjects and radiographs. The study examined 316 unrelated Japanese subjects who came to our hospital and underwent radiological examination. To exclude some diseases, all subjects completed a standard questionnaire regarding their medical history. All cases were sporadic in nature. Some individuals were under medical treatment and/or had received operative therapy. The evaluation of acetabular coverage was based on radiological findings. Radiographs were taken with a tube-to-film distance of 100 cm. The pelvises were positioned supine, with their frontal anatomical plane parallel to the film plate. The central beam was directed to the midpoint between the pubic symphysis and a horizontal line connecting both anterior and superior iliac spines. All the radiographs fulfilled the criteria for correct pelvic positioning with regard to both the axial and the transverse pelvic rotation.[3] The angle formed by a line from the inferior pelvic teardrop to the superior lateral edge of the acetabulum, and a line horizontal from the inferior pelvic teardrop constituted the Sharp angle.[20] In this study, AO was defined as a Sharp angle < 42° on at least one side. Two authors (TS and EC) independently measured the Sharp angle twice.

The mean age of the 130 subjects (125 females and five males) with AO was 29.3 years (SD 10.5; 20 to 88). The mean Sharp angle on the AO side was 38.9° (SD 2.1; 32.2° to 41.9°). The mean age of the 186 control subjects (with a Sharp angle ≥ 42° on both sides; 176 females and ten males) was 42.1 years (SD 16.1; 17 to 72). The mean Sharp angle on the side with more coverage was 46.4° (SD 3.4; 42.1° to 60.0°) (Table I). DNA was extracted from peripheral blood leukocytes. All participants gave written informed consent for the genetic analysis. The Medical Ethics Committee of the University of Miyazaki approved the study.

Genotyping. Following extraction of genomic DNA, genotyping of the polymorphisms was performed by PCR amplification of 19 total SNPs in four HOX9 paralogs (*HOXA9*, *HOXB9*, *HOXC9*, *HOXD9*) using forward and reverse primers 1, 2 and 3. PCR products were directly sequenced using an ABI Prism 3130 sequencer (Applied Biosystems, Foster City, California). Genotype and allele frequencies of the SNPs were determined by direct counting. The pair-wise linkage disequilibrium (LD) patterns and block partition of the SNPs identified in the

Table II. Polymorphisms of the paralogous HOX9 genes investigated in this study

						Japanese HapMap*	
Gene	**dbSNP name**	**Region**	**Alleles**	**Position**	**Primer**	**Allele**	**MAF**
HOXA9	rs3801776	promoter	G/A	26500955	for 1/ rev 1	A	0.257
	rs3839805	promoter	A/C	26501106	for 1/ rev 1		
	rs886340	promoter	A/G	26503174	for 2/ rev 2	G	0.140
	rs2237336	promoter	T/C	26503691	for 2/ rev 2		
	rs11978680	exon1	C/A	26500538	for 3/ rev 3		
	rs11975265	exon1	A/C	26500666	for 3/ rev 3		
HOXB9	rs8844	3' UTR	C/T	5351988	for 1/ rev 1	T	0.155
	rs3826541	3' UTR	C/A	5352234	for 1/ rev 1		
	rs3826540	3' UTR	T/C	5352296	for 1/ rev 1		
	rs7405887	3' UTR	G/C	5352794	for 2/ rev 2		
	rs2303485	3' UTR	G/A	5352841	for 2/ rev 2		
	rs2303486	3' UTR	A/T	5352947	for 2/ rev 2	T	0.409
	rs79931349	3' UTR	G/A	5353011	for 2/ rev 2		
HOXC9	rs11829948	exon1	T/C	16537643	for 1/ rev 1		
	rs7137528	exon1	G/C	16537773	for 1/ rev 1		
	rs2241820	exon1	T/C	16537803	for 1/ rev 1	C	0.252
HOXD9	rs3731794	promoter	C/A	27195846	for 1/ rev 1		
	rs711821	exon1	A/C	27197432	for 2/ rev 2		
	rs10665265	exon1	C/A	27197710	for 2/ rev 2		

SNP, single nucleotide polymorphism; UTR, untranslated region; MAF, minor allele frequency; for, forward; rev, reverse
* International HapMap Project[22,23] Data Release #28, August 10

3' untranslated region (UTR) of *HOXB9* gene were analysed using Haploview version 4.2.[21] Japanese HapMap genotyping data of the HOX9 SNPs were retrieved from the International HapMap Project[22,23] website by querying the HapMap Data Release #28. In this study, we selected seven SNPs in the 3' UTR of *HOXB9*. This is because in previous studies, Rouault et al[24] and Hao et al[10] selected two SNPs in the 3'UTR of *HOXB9* (rs8844, rs2303486), which were included in these seven SNPs, to investigate an association with congenital dislocation of the hip (CDH) using French and Chinese-based populations, respectively, and in the HapMap Data Release #28, the Japanese genotype and allele frequency data of *HOXB9* SNPs were shown mainly in the 3' UTR region (rs8844, rs2303486 and rs4239158).

Statistical analysis. All numerical values are expressed as the mean with standard deviation. The difference in the Sharp angle between cases and controls was analysed using a Mann–Whitney U test. The frequency of acetabular retroversion (AR) was assessed by the chi-squared test. The Hardy–Weinberg equilibrium for each group and the comparisons of genotype and allele frequencies between groups were performed using the chi-squared test with SNPAlyze (ver.7 DYNACOM Co., Ltd., Chiba, Japan). Values of $p < 0.05$ were considered statistically significant. A statistical analysis for power was performed using G*Power3 version 3.1.9[25-28] under the following conditions: Power (1-beta); 80%, Test family; chi-squared tests, Effect size; 0.158, Type I error rate; 0.05, Df; 1.

Corrected p-values were used, derived from the Bonferroni correction for multiple comparisons, where appropriate.

Results

In 316 subjects, 130 were AO-positive cases and 186 were controls. As shown in Table I, 32.3% of AO cases had acetabular retroversion (AR OR = 6.92, p = 1.87E-9, 95% CI 3.47 to 13.8). We performed genotyping of 19 SNPs in the four HOX9 paralogs (Table II) by PCR amplification using forward and reverse primers. We found a novel SNP located in the 3' UTR of *HOXB9*, c1486G>A (rs79931349). In this study, we focused on seven specific SNPs located in the 3' UTR of *HOXB9* that were significantly associated with AO (Tables III and IV). The minor allele frequencies for these SNPs were all > 0.05. The statistical power was 80% at the effect size 0.158.[25-28] The remaining 12 SNPs in the HOX9 paralogs did not show a significant association with AO (data not shown).

In the single-locus association analysis, distributions of genotypes in AO cases and controls were all in accordance with the Hardy–Weinberg equilibrium (Table III). The genotype and allele frequencies of the five *HOXB9* SNPs (rs8844, rs3826541, rs3826540, rs7405887, rs79931349) showed statistically significant differences between AO cases and controls (rs7405887 OR = 3.16, p = 5.29E-6, 95% CI 1.91 to 5.25). On the other hand, the two SNPs (rs2303485, rs2303486) were associated with the decreased acetabular coverage (rs2303485 OR = 0.524, p = 0.0091, 95% CI 0.322 to 0.855; rs2303486 OR = 0.519, p = 0.011, 95% CI 0.312 to 0.865) (Table IV). Subsequently, we carried out a haplotype-based association study using Haploview,[21] which was used to visualise the structure of pair-wise LD between the SNPs, and to define haplotype blocks. A total of five SNP pairs showed high LD values, and constituted a

Table III. Genotype and allele frequencies of the HOXB9 SNPs in the 130 AO cases and 186 controls

dbSNP	Genotypes (%)			Alleles (%)		MAF	HWE exact
Groups							p-value
rs8844	CC	CT	TT	C	T		
AO	76 (58.5)	49 (37.7)	5 (3.8)	201 (77.3)	59 (22.7)		
Control	151 (81.2)	33 (17.7)	2 (1.1)	335 (90.1)	37 (9.9)	0.152	0.696
rs3826541	CC	CA	AA	C	A		
AO	76 (58.5)	49 (37.7)	5 (3.8)	201 (77.3)	59 (22.7)		
Control	151 (81.2)	33 (17.7)	2 (1.1)	335 (90.1)	37 (9.9)	0.152	0.696
rs3826540	TT	TC	CC	T	C		
AO	77 (59.2)	48 (36.9)	5 (3.8)	202 (77.7)	58 (22.3)		
Control	151 (81.2)	33 (17.7)	2 (1.1)	335 (90.1)	37 (9.9)	0.150	0.696
rs7405887	CC	CG	GG	C	G		
AO	75 (57.7)	50 (38.5)	5 (3.8)	200 (76.9)	60 (23.1)		
Control	151 (81.2)	32 (17.2)	3 (1.6)	334 (89.8)	38 (10.2)	0.155	0.411
rs2303485	GG	GA	AA	G	A		
AO	96 (73.8)	29 (22.3)	5 (3.8)	221 (85.0)	39 (15.0)		
Control	111 (59.7)	67 (36.0)	8 (4.3)	289 (77.7)	83 (22.3)	0.193	0.677
rs2303486	AA	AT	TT	A	T		
AO	43 (33.1)	56 (43.1)	31 (23.8)	142 (54.6)	118 (45.4)		
Control	38 (20.4)	101 (54.3)	47 (25.3)	177 (47.6)	195 (52.4)	0.495	0.303
rs79931349	GG	GA	AA	G	A		
AO	76 (58.5)	50 (38.5)	4 (3.1)	202 (77.7)	58 (22.3)		
Control	151 (81.2)	33 (17.7)	2 (1.1)	335 (90.1)	37 (9.9)	0.150	0.696

AO, acetabular overcoverage; MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium

Table IV. Association of the HOXB9 SNPs and haplotypes in the AO cases and controls

	Genotypes				Alleles			
dbSNP		OR	p-value	95% CI		OR	p-value	95% CI
rs8844	CC *vs* CT+TT	3.07	9.93E-6	1.85 to 5.09	T *vs* C	2.66	1.12E-5	1.70 to 4.15
rs3826541	CC *vs* CA+AA	3.07	9.93E-6	1.85 to 5.09	A *vs* C	2.66	1.12E-5	1.70 to 4.15
rs3826540	TT *vs* TC+CC	2.97	1.84E-5	1.79 to 4.93	C *vs* T	2.60	1.88E-5	1.66 to 4.07
rs7405887	CC *vs* CG+GG	3.16	5.29E-6	1.91 to 5.25	G *vs* C	2.64	1.10E-5	1.69 to 4.11
rs2303485	GG *vs* GA+AA	0.524	0.00912	0.322 to 0.855	A *vs* G	0.614	0.0219	0.404 to 0.934
rs2303486	AA *vs* AT+TT	0.519	0.01128	0.312 to 0.865	T *vs* A	0.754	0.0818	0.549 to 1.036
rs79931349	GG *vs* GA+AA	3.07	9.93E-6	1.85 to 5.09	A *vs* G	2.60	1.88E-5	1.66 to 4.07
	Haplotypes	**AO (%)**	**Control (%)**	**OR**	**p-value**	**95% CI**		
	C-C-T-C-o-●-G	200 (76.9)	333 (89.5)					
	T-A-C-G-o-●-A	56 (20.0)	33 (9.7)	2.59	2.61E-5	1.65 to 4.08		

SNP, single nucleotide polymorphism; AO, acetabular overcoverage; OR, odds ratio for recessive model; CI, confidence interval; ○,G/A; ●,A/T. The remaining five haplotypes (C-C-T-G-o-●-A, C-C-T-G-o-●-G, T-A-C-G-o-●-G, T-A-C-C-o-●-A, T-A-T-G-o-●-A) showed frequencies less than 0.05

single haplotype block in the 3' UTR of *HOXB9* (Fig. 1). Results of the haplotype analysis for *HOXB9* are presented in Table IV. A total of seven possible haplotypes were obtained, five of which had a frequency < 5%; therefore, we analysed the two most prevalent haplotypes (C-C-T-C-G/A-A/T-G and T-A-C-G-G/A-A/T-A). The haplotype with minor alleles of five SNPs (T-A-C-G-G/A-A/T-A) showed a significant association with AO (OR = 2.59, p = 2.61E-5, 95% CI 1.65 to 4.08).

Discussion

Excessive acetabular coverage is thought to be the major cause of pincer-type FAI.[3,11,12] While AD has been reported to be associated with several genetic variants,[4-10] the gene variants of AO remain unclear. Nevertheless, prior to our study, no other studies have investigated the association between genetic variations and AO. To the best of our knowledge, this is the first report on the association of genetic variations of HOX9 in AO.

The mammalian Hox complex contains 39 genes organised into four different clusters, Hox A, B, C, and D. Based on DNA sequence and the position of the genes on their respective chromosomes, individual members of the four clusters have been classified into 13 paralogous families. Members of a paralogous family often share similar gene expression patterns. The chromosomal order of the HOX genes is collinear to the relative position of their expression domains along the AP axis of the developing embryo, such that the more 3' the gene is located, the more anterior its expression boundary.[14,15,29]

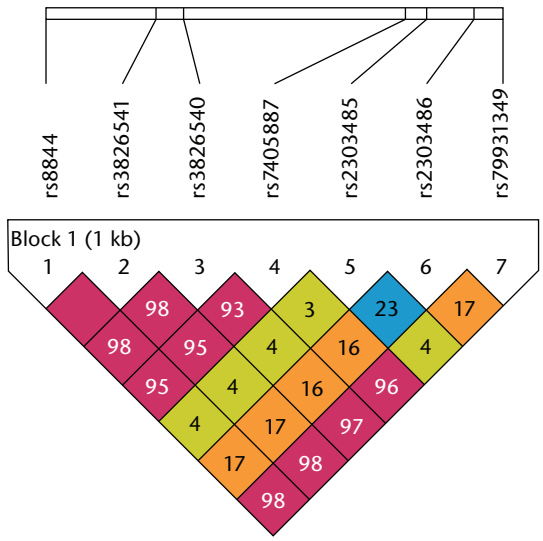

Fig. 1

LD plot of seven single nucleotide polymorphisms (SNPs) in the 3' UTR of HOX*B9*. r^2 values that correspond to SNP pairs are expressed as percentages and shown within the respective squares. Higher r^2 values are indicated in red. These five SNPs constitute a haplotype block that spans the 3' UTR of the HOX*B9* gene.

Using gene targeting, abdominal B-related genes, which are paralogous to Hox9-13 genes, were shown to have an essential role in the development of the limb buds.[16,30,31] In humans, Muragaki et al[32] demonstrated that synpolydactyly, an inherited abnormality of the hands and feet, is caused by expansion of a polyalanine stretch in the amino–terminal region of *HOXD13*. Mortlock and Innis[33] reported the identification of a *HOXA13* nonsense mutation in a family with hand–foot–genital syndrome. Paralogous HOX9 genes are located in the most 3' regions of the abdominal B-related gene regions in HOX complexes. Moreover, HOX9 genes are expressed specifically in the anterior portion of the developing limb buds.[17] Therefore, HOX9 paralogs might be involved in the development of the hip region. In 2009, Rouault et al[24] investigated the association of the CDH with two *HOXB9* SNPs (rs8844 and rs2303486) in a sample population from France. The case-control study revealed no significant association between CDH and *HOXB9* SNPs.[24] On the other hand, quite recently, Hao et al[10] reported that rs2303486 was associated with CDH in a Chinese population. However, these studies did not evaluate a potential associated with AO. Human *HOXB9* is located on the chromosome 17q21.3. *HOXB9* cDNA is 2711 bp long with a 180 bp sequence element called the homeobox in exon2. *HOXB9* encodes a 250 amino acid protein (NCBI Gene ID: 3219). Based upon genetic predisposition and the possible important function of *HOX* genes in the pathogenesis of skeletal disorders, it appears worthwhile to investigate whether *HOXB9* gene polymorphisms are associated with susceptibility to AO.

In this case-control study, we identified the five SNPs (rs8844, rs3826541, rs3826540, rs7405887, rs79931349) located in the 3' UTR of the *HOXB9* gene that are associated with AO (rs 7405887 OR = 3.16, p = 5.29E-6, 95% CI 1.91 to 5.25; Table IV), and may be an independent risk factor for future development of AO. Interestingly, the two *HOXB9* SNPs indicated OR < 1 respectively (rs2303485 OR = 0.524, p = 0.0091, 95% CI 0.322 to 0.855; rs2303486 OR = 0.519, p = 0.011, 95% CI 0.312 to 0.865) (Table IV). These two SNPs could be associated with decreased acetabular coverage. Lower acetabular coverage is a major and common cause of CDH, which is a contrasting disease to pincer-type FAI. The association of rs2303486 would be consistent with Hao et al's report.[10] Therefore, *HOXB9* might be relevant to acetabular morphogenesis. However, the morphological mechanism of *HOXB9* is not obvious, although HOX9 paralogs are critical during organogenesis, limb development and growth of tumour.[18,19,34,35] Further studies are needed to clarify the mechanism in detail that links the *HOXB9* SNPs with the acetabular morphology. As shown in Table I, 32.3% of AO cases had AR (OR = 6.92, p = 1.87E-9, 95% CI 3.47 to 13.8). AR appears to be related to AO. AR is defined as a 'crossover' sign,[36,37] and is also thought to be the common cause of pincer-type FAI.[3,11,12] The *HOXB9* SNPs may be associated with the pathogenesis of AR in the same way as AO.

This study had several limitations, such as a small sample size and a lack of replication study. Therefore, more studies should be conducted with larger sample numbers and using different ethnic groups. These future studies on *HOXB9* function and its relevance to AO will contribute not only to the elucidation of the pathogenic mechanism of pincer-type FAI, but also to the identification of a novel therapeutic target for the disease. The *HOXB9* SNPs could be a predictive tool to identify AO, helping to delay or prevent the onset of pincer-type FAI.

Supplementary material

A table showing the primers used in this study, as well as a figure of direct sequences of genomic DNA from Japanese individuals, showing the five *HOXB9* SNPs (red boxes), are available alongside the online version of this article at www.bjr.boneandjoint.org.uk

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- T. Sekimoto: Study design, Data collection and analysis, Writing the paper
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ICMJE Conflict of Interest:

- None declared