

Mapping of developmental dysplasia of the hip to two novel regions at 8q23-q24 and 12p12

LIXIN ZHANG*, XIAOWEN XU*, YUFAN CHEN, LIANYONG LI, LIJUN ZHANG and QIWEI LI

Department of Pediatric Orthopaedics, Shengjing Hospital of China Medical University,
Shenyang, Liaoning 110004, P.R. China

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Abstract. Developmental dysplasia of the hip (DDH), previously known as congenital hip dislocation, is a frequently disabling condition characterized by premature arthritis later in life. Genetic factors play a key role in the aetiology of DDH. In the present study, a genome-wide linkage scan with the Affymetrix 10K GeneChip was performed on a four-generation Chinese family, which included 19 healthy members and 5 patients. Parametric and non-parametric multipoint linkage analyses were carried out with Genespring GT v.2.0 software, and the logarithm of odds (LOD) score and nonparametric linkage (NPL) score were calculated. Parametric linkage analysis was performed, assuming an autosomal recessive trait with full penetrance and Affymetrix 'Asian' allele frequencies. The strongest evidence for linkage was found on chromosome 8q23-24, with a peak LOD score of 2.658 ($\theta=0$), covering 2.377 Mb from single nucleotide polymorphisms (SNPs) rs724717 to rs720132. This interval included nine additional successive SNPs: rs1566071, rs1902121, rs756404, rs702768, rs777813, rs2033995, rs147959, rs2884367 and rs1898287. The same region also yielded the highest NPL score of 2.883 ($P=0.0156$) from the non-parametric multipoint linkage analysis. Additionally, the second highest NPL score of 2.727 ($P=0.0156$) and LOD score of 2.528 ($\theta=0$) were obtained on chromosome 12p12 for three consecutive markers (rs1919980, rs763853 and rs725124). This region overlapped a narrow distance of 0.642 Mb. Notably, in addition to these two regions; no significant linkage was identified for other chromosomal regions (with LOD and NPL scores >2.0). For the first time, at least for this pedigree, the evidence in the present

study showed that DDH is mapped to two novel regions at 8q23-q24 and 12p12.

Introduction

Developmental dysplasia of the hip (DDH), previously known as congenital hip dislocation, is a frequently disabling condition characterized by premature arthritis later in life (1). The term encompasses a spectrum of diseases ranging from minor acetabular dysplasia to irreducible dislocation, which affects 25-50 in 1,000 live births among Lapps and Native Americans but is very rare among southern Chinese and African populations (1). A number of factors, including genetic mutations and intrauterine and postnatal environmental factors, are thought to contribute to the disease (2).

Genetic influence on DDH has been long known but is still poorly understood. Although most clinical cases appear to be sporadic, 12-33% of patients have a family history of DDH (3,4). One study reported a 10-fold increase in the incidence of DDH among the parents of index patients and a seven-fold increase among the siblings compared with the general population (3), which suggested a complex aetiology involving multiple genes interacting with environmental factors. Several genes, such as collagen alpha-1 (I) chain, homeobox protein Hox-B, basement membrane-specific heparan sulfate proteoglycan core protein, plasma membrane calcium-transporting ATPase 4, pregnancy-associated plasma protein-A2 (PAPPA2), teneurin-3 (TENM3), growth/differentiation factor 5 (GDF5) and transforming growth factor beta-1 proprotein have been investigated in various populations as candidates (5-11). However, to the best of our knowledge, few results have been replicated in other populations, suggesting that geographic and ethnic factors probably also play a key role in disease aetiology. Therefore, locating and subsequently identifying the causative genes for DDH are essential. Two identified genes have been found based on the genetic analysis of large segregating DDH families after mapping of the causative regions. A variant in the CX3C chemokine receptor 1 (CX3CR1) gene was found in all DDH-affected members of a 72-member, four-generation affected family (12). Watson *et al* (13) identified a mutation in the UFM1 specific peptidase 2 (UFSP2) gene in a family with Beukes hip dysplasia. Genetic variations in other diseases were also found after locating causative regions. Shrimpton *et al* (14) found

Correspondence to: Professor Lianying Li, Department of Pediatric Orthopaedics, Shengjing Hospital of China Medical University, 36 Sanhao Street, Heping, Shenyang, Liaoning 110004, P.R. China
E-mail: loyo_ldy@163.com

*Contributed equally

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that chromosome 2q31 was associated with congenital vertical talus, which led to the detection of a mutation in the homeobox protein *Hox-D10* gene using whole-genome linkage analysis. Additionally, the vitamin D receptor gene was associated with late-onset Alzheimer's disease after mapping susceptibility loci to chromosome 12q13 (15).

In the present study, a genome-wide linkage scan with the Affymetrix 10K GeneChip was performed on a four-generation Chinese family, which included 19 healthy members and 5 patients affected with DDH. For the first time, at least for this pedigree, the evidence showed that the disease is mapped to two novel regions at 8q23-q24 and 12p12.

Materials and methods

Patients. The proband (III-6), a 28-year-old female, appeared at the Department of Paediatric Orthopaedics, Shengjing Hospital of China Medical University for consultation for her two-year-old son (IV-3), as she had a family history of DDH. The pedigree of the Chinese family is shown in Fig. 1. The four-generation Chinese family, including 24 individuals, resides in a village in northeast China. Family members were invited to participate in the study, and those aged ≥ 18 years gave informed consent. Approval was obtained from the Human Research Ethics Committee of Shengjing Hospital of China Medical University. The status of the 24 individuals was established on the basis of clinical and radiological examinations. Five individuals (aged 23-72 years) had DDH, including two males (III-9 and III-10) and three females (I-1, III-6 and III-7). Anterior-posterior radiography of the pelvis revealed that all affected members had similar bilateral hip dislocation, which was classified as grade IV according to the Tönnis classification system (Fig. 2) (16). These individuals did not have any other system abnormalities associated with Down syndrome, Marfan syndrome, Ehlers-Danlos syndrome or dyschondroplasia and were therefore diagnosed with isolated DDH. All other members of the pedigree had normal appearance and height (163-175 cm).

Genome-wide scan and linkage analysis. Blood samples were obtained from all members of the family except for two individuals (III-3 and III-5). DNA was extracted using QIAamp DNA Blood mini kit (Qiagen GmbH) through a standard procedure. A genome-wide scan was performed on the 22 individuals using Affymetrix 10K single nucleotide polymorphism (SNP) arrays at GeneTech Biotechnology Co., Ltd. Parametric and nonparametric multipoint linkage analyses were performed using Genespring GT v.2.0 software (Agilent Technologies, Inc.), and the logarithm of odds (LOD) and NPL scores were calculated (Fig. 3). Parametric linkage analysis was performed, assuming an autosomal recessive trait with full penetrance and Affymetrix 'Asian' allele frequencies.

Results

The strongest evidence for linkage was found on chromosome 8q23-24, with a peak LOD score of 2.658 ($\theta=0$), covering 2.377 Mb from SNP rs724717 to rs720132. This interval included nine additional successive SNPs: rs1566071, rs1902121, rs756404, rs702768, rs777813, rs2033995, rs147959, rs2884367

and rs1898287. The same region also yielded the highest NPL score of 2.883 ($P=0.0156$) from non-parametric multipoint linkage analysis. Additionally, the second highest NPL score of 2.727 ($P=0.0156$) and an LOD score of 2.528 ($\theta=0$) were obtained on chromosome 12p12 for three consecutive markers (rs1919980, rs763853 and rs725124; Table I). This region overlapped a narrow distance of 0.642 Mb. Notably, in addition to the two regions, no significant linkage was identified for other chromosomal regions (with both LOD and NPL scores >2.0).

Discussion

The aetiology of DDH may involve complex interactions between many genes and the environment. Carter and Wilkinson (17) postulated the existence of two genetic systems underlying the disease: The former, polygenic, is related to dysplasia of the acetabulum, and the latter, possibly dominant, controls the capsule around the hip joint. In accordance with the above hypothesis, Wynne-Davies (18) proposed that two aetiological subtypes of DDH may be observed: One with acetabular dysplasia and another with joint laxity. During the past decade, genome-wide scans and association studies of large families have mapped DDH to chromosome regions 3p22 (American), 4q35 (Africans), 16p (Icelandic), 13q22 (Japanese), 20q11 (Chinese) and 15q13 and 19p13 (Saudi). Genetic mutations, such as *CX3CR1*, *UFSP2*, *ubiquinol-cytochrome-c reductase complex*, *PAPPA2*, *GDF5* and *TENM3*, were identified in these regions. The present results and previously identified chromosome regions may be related to the growing development of the hip joint according to the function of genes included in these regions (10,12,13,19-22). DDH was previously associated with the D17S1820 marker from chromosome region 17q21 by analysing 303 individuals from 101 Chinese trios (data not shown). The results were further confirmed by Feldman *et al* (23) through analysis of a multi-generation American family with 18 members by linkage.

The Chinese family, as reported here, features a rare example of non-syndromic DDH suggestive of an uncommon autosomal recessive model, which is different from previously reported autosomal dominant traits (4,12). However, two unaffected members of the family (III-3 and III-5) refused to provide blood samples, which has limited the power of linkage analysis. Nevertheless, this large pedigree may still be useful for genetic linkage studies to locate candidate genes. For the family, genome-wide scanning has provided strong evidence for regions linked with the disease. Although neither LOD nor NPL scores exceeded 3.0, this may provide a basis for the accumulation of more evidence for the linkage.

Maximum LOD and NPL scores (2.658 and 2.883, respectively) were obtained on chromosome 8q23-24. The region spans 2.377 Mb from SNPs rs724717 to rs720132. Four genes are known to reside in this region, including transcriptional repressor GATA binding 1 (*TRPS1*), eukaryotic translation initiation factor 3 subunit H (*EIF3H*), RAD21 cohesin complex component (*RAD21*) and rRNA-binding ribosome biosynthesis protein UTP23 (*UTP23*). Among the four candidates, *UTP23* encodes a small subunit processome component, which is required for pre-18S rRNA maturation (24). The product of *RAD21* is involved in the repair of double-strand DNA breaks and chromatid cohesion during mitosis (25). Eukaryotic

Table I. LOD score of two novel chromosome regions.

Chromosome region	Length (Mb)	Variation	LOD at θ					
			0	0.05	0.1	0.2	0.3	0.4
8q23-q24	2.377	1-9	2.658	2.361	2.058	1.442	0.851	0.349
12p12	0.642	10-12	2.528	2.240	1.946	1.349	0.777	0.297

Numbers (1-9) refer to rs1566071, rs1902121, rs756404, rs702768, rs777813, rs2033995, rs147959, rs2884367 and rs1898287, respectively. Numbers (10-12) refer to rs1919980, rs763853 and rs725124, respectively. LOD, logarithm of odds.

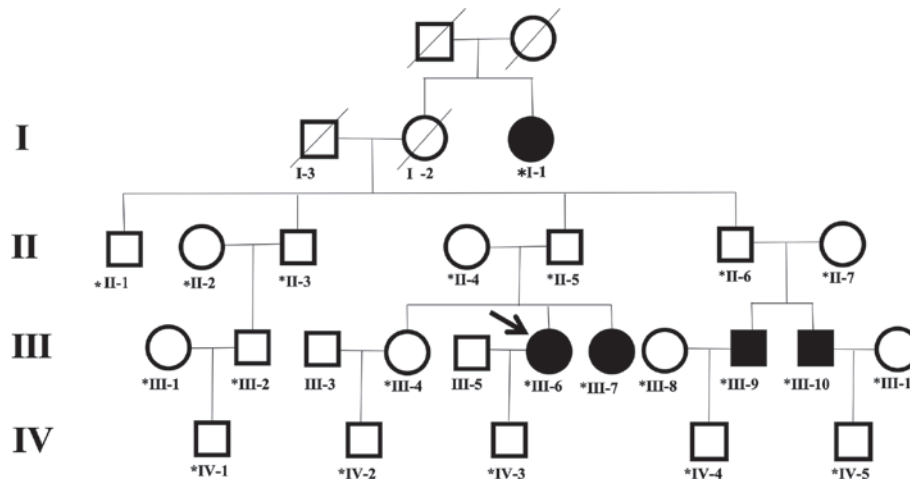


Figure 1. Pedigree of the four-generation Chinese family affected with dysplasia of the hip. The arrow indicates the proband. Asterisks indicate family members whose samples were used for the genome-wide scan.

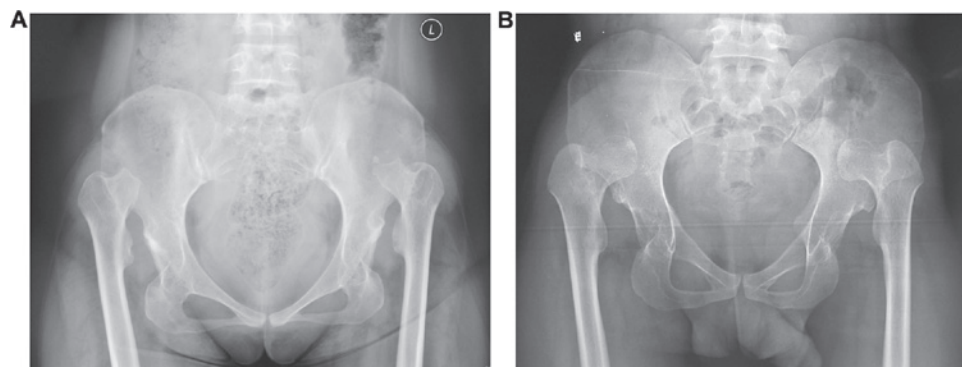


Figure 2. Radiographs showing a Tönnis grade IV bilateral hip dislocation of (A) proband III-6 and (B) proband III-9.

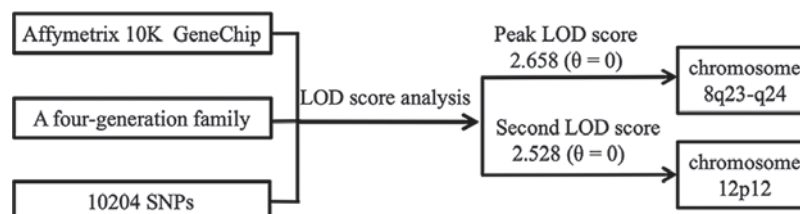


Figure 3. Nucleotide polymorphism array analysis workflow chart. SNP, single nucleotide polymorphism; LOD, logarithm of odds.

translation initiation factor 3 subunit H (*EIF3H*) is a subunit of the eukaryotic translation initiation factor 3 complex. The

intact *EIF3H* protein contributes to efficient translation initiation on 5' leader sequences harbouring multiple upstream open

reading frames, and normal levels of EIF3H are implicated in growth control through protein synthesis (26,27).

TRPS1 seems to be the most attractive candidate from the 8q23-24 region. *TRPS1* binds DNA through a single GATA-type zinc-finger domain that recognizes a consensus DNA sequence. Mutations in the *TRPS1* gene may cause tricho-rhino-phalangeal syndrome (TRPS) (28), an autosomal dominant type craniofacial and skeletal dysplasia. In addition to craniofacial deformities, patients with TRPS generally have skeletal anomalies, including short stature, hip abnormalities (dysplasia, dislocation of the hip or joint laxity), cone-shaped epiphyses and premature closure of growth plates, which have reflected defects in endochondral ossification. Long bones of the vertebrate appendicular skeleton are formed through the process of endochondral ossification when initial cartilaginous anlagen are replaced by bone (29). Various transcription factors and signalling pathways may be involved in the regulation of endochondral bone formation. The *Runx2* transcription factor is the master regulator of osteoblast differentiation and is required for chondrocyte hypertrophy. *TRPS1* physically interacts with *Runx2* and represses *Runx2*-mediated trans-activation. Transgenic mice experiments demonstrated that loss of repression of the *Runx2*-*Ihh*-positive regulatory loop induced by *TRPS1* mutation can result in altered endochondral bone formation, which is characterized by the dysregulation of chondrocyte differentiation and uncoupling of processes of perichondrial mineralization and chondrocyte maturation (30). Thus, *TRPS1* is the most promising gene and should be investigated in future studies.

SOX5 is the only gene known to reside in the 12p12 region. The gene encodes a member of the SOX (SRY-related HMG-box) family of transcription factors involved in the regulation of embryonic development and cell fate determination. *Sox5*, together with *Sox6*, is essential for the establishment of cartilage growth plates and thus the proper and timely development of endochondral bones through regulation of chondrocyte differentiation and proliferation (31). Foetuses of *Sox5*-*Sox6* double null mice may die with severe, generalized chondrodysplasia and fail to form the epiphyseal plates and endochondral bones. Notably, chondrodysplasia can also be observed at the dysplastic acetabulum in DDH, albeit mild and partial. *SOX5* gene knockdown inhibited *MMP-9* gene expression, contributing to fibroblast survival, proliferation, migration and invasion in a rheumatoid arthritis study (32). Therefore, the potential effects of the *SOX5* gene on the development of non-syndrome DDH cannot be excluded.

According to the analysis above, the two novel regions may affect articular cartilage formation, not only through regulating chondrocyte differentiation and proliferation, but also by uncoupling processes of perichondrial mineralization and chondrocyte maturation. They may also be related to joint development by promoting fibroblast growth.

As a complex disease, DDH is genetically heterogeneous due to population differences and the complexity of hip development. It is likely that different candidate genes have contributed to the susceptibility to this disorder in different populations. The present study showed a genome-wide linkage analysis for a Chinese DDH family and the identification of two novel loci at 8q23-24 and 12p12. The present results indicated the presence of a disease-associated

mutation or polymorphism in the mapped regions of the affected members. With a number of candidate genes identified from the two regions (*TRPS1* and *SOX5* in particular), mutations in these genes can be screened through sequence analysis. These studies may lead to the eventual identification of novel mutations or polymorphisms predisposing individuals to the disease, which may enable early diagnosis and optimal treatment.

To conclude, the evidence presented showed that developmental dysplasia of the hip is mapped to two novel regions at 8q23-q24 and 12p12. The present results indicate the presence of a disease-associated mutation or polymorphism in the mapped regions for the affected members.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LL contributed to the conception of the study. YC and QL contributed significantly to data analysis and manuscript preparation. XX and LXZ analysed the data and wrote the manuscript. LJZ helped perform data analysis with constructive discussions. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethics approval was obtained from the Human Research Ethics Committee of Shengjing Hospital of China Medical University. Family members agreed to be included in this research study.

Patient consent for publication

Consent for publication was obtained from participants.

Competing interests

The authors declare that they have no competing interests.

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