Do HOXB9 and COL1A1 genes play a role in congenital dislocation of the hip? Study in a Caucasian population

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Summary

Objective: Congenital dislocation of the hip (CDH), which is one of the most common congenital skeletal disorders, corresponds to an abnormal seating of the femoral head in the acetabulum. It is commonly admitted that CDH presents a genetic component. However, little is known about the genetic factors involved. This study aimed to determine the role of two potential candidate genes on chromosome 17 in CDH: HOXB9 (involved in limb embryonic development) and COL1A1 (involved in joint laxity).

Method: We set up a case-control association study (239 cases and 239 controls) in western Brittany (France) where CDH is particularly frequent. The set of informative single nucleotide polymorphisms (SNPs) in each gene was selected using Tagger and genotyped using the SNaPshot™ method (n = 2 and n = 10, respectively). The association was tested both through single-locus and haplotype-based analyses, using SAs and Haploview softwares. In addition, we carried out the transmission disequilibrium test (TDT) with the same polymorphisms from a sample of 81 trios (i.e., 81 patients included in the case-control study and their both parents).

Results: The case-control study revealed no significant association between CDH and the tagSNPs selected in both HOXB9 and COL1A1. Moreover, the TDT did not reveal distortion in allelic and haplotype transmission of the studied markers.

Conclusion: Our study did not support an association between HOXB9 and COL1A1 and CDH in our population. These negative findings were obtained by population- and family-based designs. Analysis of the genetic component of CDH should focus on other candidate genes.

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Key words: Congenital dislocation of the hip, Association study, Case-control study, Transmission disequilibrium test, Candidate genes, COL1A1, HOXB9.

Introduction

Congenital dislocation of the hip (CDH – OMIM #142700) is one of the most common congenital skeletal disorders. It results from an abnormality of the seating of the femoral head in the acetabulum1-3. The condition, which affects mainly females (sex-ratio: 1:8), may induce severe functional handicap if not treated early, and increases risk to develop hip osteoarthritis4,5.

CDH is particularly frequent in Caucasian populations, with an incidence about 1–10 per 1000 live births. Clusters of CDH have been observed in some ethnic groups such as the Navajo Indians (67/1000)6 and the Lapps (50/1000)7, but this condition is also very common in our area (Finistère, western Brittany, France). An epidemiological study performed in the 1960s showed that the incidence among girls reached 38/1000 in a specific area called “Pays Bigouden”8,9.

Besides a mechanical component linked to the pregnancy and delivery conditions, it is commonly admitted that CDH presents a genetic component. The main factors that argue in favor of a genetic determination for the disease are the ethnic predisposition and the familial aggregation observed. Higher recurrence risks of CDH have been reported in first-degree relatives of patients10,11, as well as higher concordance in monozygotic than in dizygotic twins (40% vs 3%)12. The hereditary transmission has also been highlighted in a large segregation analysis performed on 171 pedigrees13. In addition, the availability of animal models supports the genetic hypothesis. Hip dysplasia occurs with a high frequency in some breed canine varieties, such as German Shepherd dogs and Labrador...
CDH can be considered as a multifactorial disease, however little is known about the genetic factors involved. It has been suggested that the genetic predisposition to CDH could involve two genetic systems: the first one linked to acatabular dysplasia (corresponding to a shallow acetabulum at birth) and the second one linked to capsular joint laxity (corresponding to a weak mechanical resistance of the capsule that could result from a defect of the connective tissue)18–20.

The genetic factors involved in CDH may therefore affect acatabular morphology or capsular laxity. Two genes located on chromosome 17 at position 17q21.3 appear as interesting candidate genes. The first one – HOXB9 – is included in a cluster of Homeobox genes and the encoded protein functions as a sequence-specific transcription factor, which is involved in cell proliferation and differentiation. The HOX genes are essential for limb development, where they participate in both growth and organization of the structures21,22. The second gene – COL1A1 – belongs to the superfamily of genes encoding collagen; it encodes the α1 chain of collagen type I. Such genes are of particular interest because of their involvement in resistance and elasticity of the tissues. They have been associated with a large spectrum of diseases such as Ehlers–Danlos syndrome, osteogenesis imperfecta, chondrodysplasia and low bone mineral density23–25. Moreover, biochemical studies have shown that CDH is associated with alterations in the metabolism of collagen, which could explain the joint laxity observed26. The involvement of these two genes in CDH has been suggested by a Chinese study which, later on, refuted the implication of COL1A125,26.

In view of these ambiguous findings, we proposed to study the association between HOXB9 and COL1A1 genes and CDH in our area (western Brittany, France) where this condition is particularly frequent.14–17

Four centres involved in the screening and treatment of CDH in this area participated in the study (Hospital of Brest, Pont L’Abbé, Quimper and Roscoff) (Fig. 1).

**Method**

**STUDY DESIGN**

In order to determine whether HOXB9 and COL1A1 may play a role in CDH, we set up a genetic-based association study in the area of Finistère (western France) where that condition is particularly frequent.8–9

Four centres involved in the screening and treatment of CDH in this area participated in the study (Hospital of Brest, Pont L’Abbé, Quimper and Roscoff) (Fig. 1).
regression models were used to estimate the strength of the associations through calculation of odds-ratio (OR) with their 95% confidence interval (95% CI). The alternative patterns of inheritance (dominant, recessive and multiplicative) were tested for each SNP by using a specific coding of the “genotype” variable. To account for multiple testing, the Bonferroni correction was applied. This consists in dividing the usual significance level of the test of likelihood ratio (LRT) and by calculating OR with their 95% CIs.

As our questionnaire gives information on the main mechanical factors known to be involved in CDH, only breech presentation was significantly more frequent in cases than in controls (12.5% vs 1.4%, OR = 10.1 [3.0–34.5], P < 0.0001). Joint hyperlaxity was present in 11.0% of them and was also significantly more frequent in cases than in controls (11.0% vs 1.8%, OR = 6.7 [2.3–19.7], P < 0.0001).

RESULTS OF THE CASE-CONTROL STUDY

The case-control study was based on 239 patients and 239 controls. Two SNPs were genotyped in the HOXB9 gene, and 10 in the COL1A1 gene. Genotyping was successful for all the markers in the whole sample. The accuracy of genotyping was validated in the way that the sequencing results of the retested markers did not differ from those obtained initially.

Single-locus association study

No significant association was observed between the two polymorphisms selected in HOXB9 and CDH. In contrast, a weak association tended to be observed for two of the SNPs selected in COL1A1, this before applying the Bonferroni correction. The concerned markers were rs2857396 (CC vs CT + TT: OR = 0.26 [0.07–0.96], P = 0.043) and rs1107946 (AA + AC vs CC: OR = 0.63 [0.41–0.98], P = 0.038), for which study power was respectively assessed to 90% and 67%. However, the significance of these results did not survive correction for multiple testing. Moreover, the complementary analyses performed in the subgroup of patients without breech presentation and in the subgroup without family history revealed no significant association (data available upon request).

Haplotype-based association study

LD and haplotype blocks observed for the two genes revealed a single haplotype block in HOXB9 and three blocks in COL1A1 (Fig. 3). Results of the haplotype analysis for each gene are presented in Tables IIa and b. For HOXB9, haplotype frequencies did not differ between cases and controls. In contrast, for COL1A1, a weak association tended to birthweight (≥4 kg) in 15.1% of them. CDH affected the first sibling in 42.7% of cases and three patients were part of multiple pregnancies. The proportion of postmature babies (≥42 weeks of gestation) was 5.9%. A postural anomaly was observed in 11.4% of the CDH patients. Among these mechanical factors, only breech presentation was significantly more frequent in cases than in controls (12.5% vs 1.4%, OR = 10.1 [3.0–34.5], P < 0.0001). Joint hyperlaxity was present in 11.0% of them and was also significantly more frequent in cases than in controls (11.0% vs 1.8%, OR = 6.7 [2.3–19.7], P < 0.0001).
be observed for the third haplotype block (LRT = 7.2, \(P = 0.0280\)) which included the markers rs207555 and rs1107946, the latter being associated with CDH in the single-locus analysis. More precisely, frequency of the CC haplotype was estimated at 89.7% in cases and 85.3% in controls (\(P = 0.0396\)), whereas frequency of the CA haplotype was estimated at 0.6% in cases and 2.5% in controls (\(P = 0.0193\)). Thus, by considering the most common haplotype (CC) as reference, the OR associated with the CA haplotype was 0.23 (95% CI = [0.06-0.84], \(P = 0.026\)). Again, the significance of all these findings disappeared after correction for multiple testing.

RESULTS OF THE TDT

Analysis of the family data set revealed similar negative findings. Table III shows, for each of the SNPs selected in the HOXB9 and COL1A1 genes, the ratio of transmissions to nontransmissions of the possibly overtransmitted allele from heterozygous parents to their affected offspring. This analysis did not reveal preferential transmission of any alleles of the selected markers. The haplotype-based analysis found similar results. In the same way, no transmission disequilibrium was observed for any of the 16 alleles of the microsatellite marker D17S1820.

Discussion

The present study examines the involvement of two genes on chromosome 17 (HOXB9 and COL1A1) in CDH in a Caucasian population where the incidence of that condition is high. These genes may play a role in acetabular dysplasia or joint laxity, and are therefore interesting candidate genes.

Our findings do not support the role of these two genes in CDH in our population. They are consistent with the results observed in a Chinese population for the COL1A1 gene\(^{26}\).
Our negative findings were obtained by two different approaches (case-control and family-based studies) that rely both on single-locus and haplotype-based analyses. Moreover, our study used selection methods of informative markers based on LD information, contrary to previous studies which usually consisted in genotyping only one or two genetic markers per gene. Our sample is also one of the largest used to date in genetic studies carried out on CDH (even if it remains of modest size for such kind of studies). It should nevertheless be noted that family history of CDH appears particularly frequent in our study. This could be the result of several phenomena (high incidence of CDH in our region, lack of accuracy of the variable based on patients’ responses) and may have induced a selection bias. Nevertheless, the results remain unchanged in the subgroup of patients without family history.

It is commonly admitted that CDH involves genetic and mechanical factors. The mechanical component is linked to the conditions of pregnancy and delivery. It mainly corresponds to factors that reduce the mobility of the fetus in the womb and induce excessive pressure on the flexed thigh-bone. Among them, we note breech presentation, oligoamnios, primiparity, high birthweight, postmaturity and presence of postural anomalies.

Besides this mechanical component, the observation of clusters of CDH and the familial nature of the condition suggest the existence of a genetic component for CDH. Familial studies have reported higher risks of recurrence for first-degree relatives and showed that these risks increased if several children or two generations were affected. More-
team. This case-control study, based on 338 patients and 622 controls, found a significant association between a functional SNP (rs143383) located in the 5’UTR of the GDF5 (growth differentiation factor 5) gene which plays a crucial role in joint morphogenesis. This SNP was already found to be associated with osteoarthritis susceptibility in both Asian and European populations.

In conclusion, to date, only one gene involved in the etiology of CDH has been reported (GDF5). The other studies have excluded the role of several genes in CDH (COL1A1 in a Chinese population) or in DDH (COL2A1, ESRI and VDR in Caucasian populations). Our study do not support the involvement of HOXB9 and COL1A1 in a Caucasian population.

Analysis of the genetic component of CDH needs further investigations and should focus on other candidate genes. Identification of the genetic factors involved in CDH should advance understanding of the respective roles of genetic and environmental factors in that complex disease. It would furthermore avoid late diagnosis and treatment, and promote earlier identification of patients at risk of developing hip osteoarthritis.

Conflict of interest

The authors declare that they have no conflict of interest.

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