Exclusion of COL2A1 and VDR as Developmental Dysplasia of the Hip Genes

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Abstract Developmental dysplasia of the hip (DDH) is a spectrum of disorders affecting the proximal femur and/or acetabulum leading to an abnormal formation of the hip. Genetic factors are involved in the etiology of DDH. Early recognition of DDH affords the best results from treatment and a better knowledge of the genetics of DDH could enhance early diagnosis. Variants in the Type II collagen (COL2A1) and vitamin D receptor (VDR) genes have been associated with patients with osteoarthritis of the hip secondary to DDH, suggesting these genes could contribute to DDH. To see whether there was linkage between the COL2A1/VDR locus and nonsyndromic DDH, we conducted a linkage study on 11 families with multiple cases of DDH. We demonstrated no evidence of linkage between the COL2A1/VDR locus and nonsyndromic DDH (LOD score < -2), suggesting, although variants in these genes could play a role in osteoarthritis in patients with DDH, they do not contribute to nonsyndromic DDH. The search for causal gene variants should proceed with other candidates.

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Introduction

Developmental dysplasia of the hip (DDH) is a spectrum of disorders affecting the proximal femur and/or acetabulum leading to an abnormal formation of the hip. DDH is observed with variable degrees of severity from mild lateral instability of the femoral head in a shallow acetabulum with slight capsular laxity to complete dislocation of the joint [4, 18]. It is one of the most common skeletal congenital conditions [19, 20]; it is more frequently observed in females than in males with a ratio of approximately 5:1 and the left hip is more affected than the right. There is evidence of a different incidence among populations with higher rates among whites (one per 1000 live births), whereas it is very rare in China and in the United States among blacks [17, 18]. DDH incidence is reportedly 10 times higher in Italy than in the rest of Europe [2], with the highest rate observed in Ferrara, where the incidence is 18.4 new cases per 1000 live births [15].

DDH can be observed as an isolated anomaly or as part of a Mendelian or chromosomal syndrome [21]. The etiology of nonsyndromic DDH is not known, but wide evidence of familial aggregation [19, 20], high concordance between monozygotic twins (41%) compared with dizygotic twins (2.8%), and high risk of recurrence (5%) for subsequently born siblings [19] strongly suggest a crucial role for genetic factors. Oligohydramnios and, in general, factors limiting fetal motility have also been associated with an increased risk for nonsyndromic DDH [19]. Therefore, this skeletal condition could be considered a multifactorial disease in which both environmental and genetic factors play a role. Carter and Wilkinson [3] suggested a two-gene model on the basis of which

Wynne-Davies [20] hypothesized two etiologically distinct groups of patients with nonsyndromic DDH, one group with acetabular dysplasia and the other group with joint laxity. The nature of nonsyndromic DDH inheritance has been studied by complex segregation analysis in 171 families of probands treated in Ferrara [15]. In that study, we proposed a two-locus model in which recessive transmission at both loci was favored [15].

Little is known about the nature of susceptibility genes for DDH. Cilliers and Beighton [5] described a wide South African family with recurrence of a form of DDH evolving to osteoarthritis in adulthood (Beukes familial hip dysplasia). This clinical phenotype was inherited as an autosomal-dominant trait, as in early-onset osteoarthritis, but association with the Type II collagen gene (COL2A1) was excluded [1]. Linkage analysis of a Beukes familial hip dysplasia family pointed to a locus in 4q35, but no specific gene has been identified so far [11]. In an Italian cohort of patients with osteoarthritis of the hip, Granchi et al. [10] reported an association with single-nucleotide polymorphisms in the COL2A1 and vitamin D receptor (VDR) genes when the degenerative disease was secondary to DDH, whereas no association was evident in patients with idiopathic osteoarthritis. COL2A1 and VDR are adjacent genes located in 12q13 that play a role in cartilage formation and in bone mineralization, respectively. The evidence of Granchi et al. [10] could be interpreted as suggesting an association between the COL2A1/VDR locus and DDH and suggesting variants in the COL2A1 and/or VDR genes might play a role in the etiology of this skeletal disorder.

We asked whether the COL2A1/VDR locus played a role in the etiology of nonsyndromic DDH, assuming a dominant or a recessive model or using a model-free nonparametric linkage (NPL) analysis.

Materials and Methods

We recruited multiplex DDH families through the Center for the Study of DDH of the St Anna Hospital of Ferrara (Italy). Pediatricians, orthopaedists, and geneticists collaborate in this center, and the neonatal screening of DDH is performed by clinical and sonographic detection. We included the families from the same pool studied in the complex segregation analysis of Sollazzo et al. [15]. To admit a proband to the study, we used the following criteria: (1) nonsyndromic DDH diagnosis; (2) clinical and echographic neonatal diagnosis performed in all cases by the same clinician (GA); and (3) evaluation of DDH severity according to the sonographic classification of the anatomic-pathologic alterations of the hip by Graf and Wilson [9]. Only families including probands with Graf Grade 3 and 4 were included in the study. To limit the underlying genetic heterogeneity of the trait, only families of Italian origin were included. Eleven families were enrolled in the study, including 41 affected and 73 unaffected individuals (Table 1). We performed a complex segregation analysis to assess the model of inheritance of DDH using a sample of 171 families [15]. The analysis favored a two-locus recessive-recessive model. Using this model, we performed a power calculation analysis with the SLINK computer program [13]. A set of 11 pedigrees was selected because it gave a power of 84% (significant at greater than 80%). Those families include probands with Graf Grade 3 and 4 because, the higher the severity grade is, the better is the fit on the segregation model previously identified [15]. The ratio of females to males affected was 5.83:1. A sample of peripheral blood or a buccal swab was obtained from each participating subject. The study was approved by the relevant local ethics committee. Participation in the study was dependent on obtaining informed consent.

Table 1. Descriptions of the families studied

Family number	Males	Females	Unaffected	Affected	Males affected	Females affected	Total family members
1	5	4	6	3	2	1	9
2	3	3	3	3	0	3	6
3	3	10	8	5	0	5	13
4	9	8	13	4	0	4	17
5	3	4	5	2	1	1	7
6	4	8	7	5	0	5	12
7	4	8	7	5	0	5	12
8	4	4	4	4	1	3	8
9	2	4	3	3	0	3	6
10	2	7	5	4	2	2	9
11	8	7	12	3	0	3	15
Total	47	67	73	41	6	35	114

Fig. 1 The position of the COL2A1 and VDR genes is relative to flanking markers in the 9.6-cM interval between D12S1663 and D12S368. Physical distances (in Mb) are indicated below the horizontal line.

We isolated genomic DNA from whole blood using the NucleonTM BACC1 DNA Extraction Kit (Amersham Biosciences, Piscataway, NJ) or from buccal swabs using the QIAamp[®] DNA Mini Kit (QIAGEN[®], Valencia, CA).

Linkage studies were performed by analysis of short tandem repeat polymorphic markers. A panel of three short tandem repeat polymorphic markers (D12S1663, D12S85, D12S368) that span approximately 9.6 cM on chromosome 12q, including the candidate COL2A1/VDR locus, were chosen (Fig. 1).

Genotyping was carried out using Applied Biosystems (Foster City, CA) primer sets. Polymerase chain reactions were performed in a 20- μ L volume using reagent concentrations and temperature profiles as recommended by the reagent manufacturer (Applied Biosystems). Fluorescent-labeled polymerase chain reaction product electrophoresis was performed by separation on 4.25% polyacrylamide gels run using an ABI PRISM[®] 377 Sequencer (Applied Biosystems). Lane tracking and allele calling were performed using GeneScan[®] and Genotyper[®] software (Applied Biosystems). Individuals were genotyped once. Two investigators (MR, AC) independently scored all the chromatograms and were blinded to the identity of the individuals. Only in the case of different interpretation was the genotyping of the family repeated.

Linkage analysis was assessed using Genehunter 2.1 software (Whitehead Institute for Biomedical Research, Cambridge, MA). Autosomal-dominant and autosomal-recessive models with 25%, 50%, or 75% penetrance were applied, as well as a model-free NPL analysis. Multipoint LOD score and NPL analyses were performed across the 9.6-cM interval between D12S1663 and D12S368 on chromosome region 12q12–q13, including the adjacent candidate genes VDR and COL2A1.

Results

Our data demonstrate the COL2A1 and VDR genes do not play an important role in DDH etiology. Assuming an autosomal-dominant model (Fig. 2) and considering high

Fig. 2 Multipoint LOD scores across the D12S1663 and D12S368 interval on 12q were calculated using Genehunter 2.1 software (Whitehead Institute for Biomedical Research, Cambridge, MA). Distances are shown in cM. When an autosomal-dominant model with penetrance values of 75% and 50% was used, the LOD scores were less than -2 , thus excluding linkage. When a penetrance value of 25% was used, the LOD score was widely negative.

penetrance values (50% or 75%), the observed LOD score at the COL2A1/VDR locus was less than -2 , thus supporting an exclusion of these candidate genes. Assuming a lower penetrance value (25%), an LOD score of -1.4 resulted. When an autosomal-recessive model (Fig. 3) was assumed, the LOD scores at the COL2A1/VDR locus were widely negative with all three penetrance levels used (25%, 50%, 75%). This strongly rules out COL2A1 and VDR as DDH genes under an autosomal-recessive model. Model-free NPL analysis (Fig. 4) resulted in a z value of -0.7 ($p = 0.75$) at the COL2A1/VDR locus. This evidence, although not supporting an exclusion of COL2A1 or VDR, makes improbable a contribution of variants in these genes in the etiology of DDH.

Fig. 3 Multipoint LOD scores across the D12S1663 and D12S368 interval on 12q were calculated using Genehunter. Distances are shown in cM. When an autosomal-recessive model with penetrance values of 75%, 50%, or 25% was used, the LOD scores were less than -2 , thus excluding linkage.

Fig. 4 Nonparametric LOD scores across the D12S1663 and D12S368 interval on 12q were calculated using Genehunter. Distances are shown in cM. A z value of -0.7 was found at the COL2A1/VDR locus, making it improbable there is a contribution of the COL2A1 and VDR genes in the etiology of DDH.

Discussion

Genetic factors play an important role in the etiology of DDH but presently no gene is linked with the abnormality. The COL2A1 and VDR genes were suggested as possibly

involved in DDH determination [10]. The aim of this study was to investigate the genetics of DDH. We asked whether the COL2A1 and VDR genes were in linkage with non-syndromic DDH. We performed a linkage analysis on a sample of 11 pedigrees chosen from a pool of families previously studied in a complex segregation analysis work [15]. The candidate genes used were the COL2A1/VDR locus. They were considered first because of their possible involvement in DDH. The data from this analysis support the exclusion of the COL2A1 and VDR genes in DDH etiology.

Although the number of families enrolled in the study is small, the sample size has enough power to support the analysis. Moreover, although our data are not suggestive of linkage for the COL2A1 and VDR genes, which are, respectively, determinants of cartilage and bone metabolism, this is the first molecular approach to the genetics of DDH, thus suggesting other genes for growth and ligament laxity should be evaluated.

Nonsyndromic DDH is widely considered a multifactorial polygenic disorder, but the nature of its genetic component is still unknown [14–16]. Carter and Wilkinson [3] hypothesized two different genetic systems could be responsible for the etiology of DDH, one related to the dysplasia of the acetabulum and another controlling the capsule around the hip. Wynne-Davies [20] supported this hypothesis and suggested nonsyndromic DDH cases could be sorted into two etiologic sets, a first group with acetabular dysplasia inherited as a polygenic trait and mostly related to patients with late diagnosis and a second group with joint laxity, including most neonatal patients in which, besides genetic predisposition, the action of environmental factors was relevant [17]. A complex segregation analysis in 171 DDH families [15] is consistent with the model of Wynne-Davies and provided evidence for recessive transmission of the two major genetic factors.

The polygenic component of nonsyndromic DDH etiology is likely to include common gene variants, but the identity of the specific genetic susceptibility factors producing hip dysplasia is still completely unknown. Strategies to track down these gene variants may include linkage or association analyses and can be set up starting from candidate genes or using a genome-wide approach. So far, no linkage analyses have been performed to identify nonsyndromic DDH genes, and the only report of an association analysis leading to possible DDH susceptibility genes is the evidence of Granchi et al. [10] of an association between two restriction enzyme fragment polymorphisms (RFLP) in the COL2A1 (PvuII RFLP) and VDR (BsmI RFLP) genes in patients with osteoarthritis secondary to DDH. Using a case-control approach, these authors reported an association between the PvuII RFLP and osteoarthritis of the hip secondary to DDH, whereas no differences were

observed when comparing the patients with idiopathic osteoarthritis with the control subjects. Among patients with osteoarthritis, the frequency of homozygotes for the PvuII RFLP was decreased in subjects with former DDH compared with idiopathic patients. The results reported by Granchi et al. [10] could be explained by hypothesizing that variants in the COL2A1 and VDR genes either act as genetic risk factors for nonsyndromic DDH or are associated with only the degenerative aspects of osteoarthritis and do not directly contribute to the etiology of DDH.

We asked whether the segregation of the COL2A1/VDR locus is associated with nonsyndromic DDH in families with recurrence of cases. The families we studied were chosen from the pool of families considered for the previously performed complex segregation analysis work [15], and the probands admitted in the study showed a Graf Grade 3 or 4 sonographic aspect. We included the more severely affected probands because of the evidence that the severity of the disease contributes to the determination of the model of genetic transmission [15]. In the 11 multiplex families included in the study, no evidence of linkage between the COL2A1/VDR locus and nonsyndromic DDH was observed assuming either a dominant or a recessive model. Using penetrance values ranging from 75% to 25%, the obtained LOD scores were all negative and exclusion of the COL2A1/VDR locus could be considered for penetrance values as low as 50% under the dominant model and 25% under the recessive model. This evidence indicates mutations in COL2A1 or VDR genes are not major risk factors for nonsyndromic DDH. Although not main players in the etiology of DDH, gene variants in one of the two candidate genes could in theory still act as minor risk factors. In our study, the NPL score at the COL2A1/VDR locus is clearly negative ($z = -0.7$), and although the observed z value does not completely exclude a role, it also does not support a role of COL2A1 or VDR in nonsyndromic DDH.

Our data refute the hypothesis of an etiologic role of COL2A1 or VDR in nonsyndromic DDH proposed by Granchi et al. [10] based on evidence of an association in patients with osteoarthritis secondary to DDH. The outcome of our model-free NPL analysis suggests variants in COL2A1 or VDR should rather be considered as possible determinants of degenerative disease in patients with DDH. According to this model, common variants in one of these two genes could act as low-penetrance genetic factors that increase the chance of developing osteoarthritis in patients who have previously developed hip dysplasia.

Because haplotypes in the COL2A1 gene have been associated with generalized radiographic osteoarthritis [12], we can hypothesize a common variant in this gene could cause osteoarthritis in patients who experienced hip

dysplasia while causing no particular harm in patients who have had normal development of the hip.

Earlier diagnosis is required to obtain substantial progress in treating DDH [6–8]. The knowledge of the genetics of DDH is crucial for its prevention and early diagnosis and could possibly offer the basis for new strategies of clinical and surgical intervention. Our data indicate neither COL2A1 nor VDR is involved in the etiology of nonsyndromic DDH and therefore contribute only in excluding these two genes from the list of candidates. Because the genetic component of DDH remains to be explored, the search for causal gene variants will proceed using other candidates or using a genomewide approach.

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