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The Otto Aufranc Award Identification of a 4 Mb Region on Chromosome 17q21 Linked to Developmental Dysplasia of the Hip in One 18-member, Multigeneration Family

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1 **The Otto Aufranc Award**

2 **Identification of a 4 Mb Region on Chromosome 17q21 Linked to Developmental Dysplasia**
3 **of the Hip in One 18-member, Multigeneration Family**

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31 **Abstract**

32 Developmental dysplasia of the hip (DDH) is a disabling condition of the hip that,
33 depending on geography, can afflict between 20-80% of patients with end-stage arthritis of the
34 hip. Despite its prevalence, the etiology of this disease remains unknown. DDH is a complex
35 disorder with both environmental and genetic causes.. Based on the literature, the candidate genes
36 for the disease are *HOXB9*, collagen type I α 1, and *DLX 3*. The purpose of our study was to map
37 and characterize the gene or genes responsible for this disorder by family linkage analysis. We
38 recruited one 18-member, multigeneration affected family to provide cheek swabs and blood
39 samples for isolation of DNA. Amplified DNA underwent a total genome scan using GeneChip
40 Mapping 250 K Assay (Affymetrix, Santa Clara, CA). We observed only one region with a LOD
41 score greater than 1.5: a 4Mb region on chromosome 17q21.32, yielding a LOD score of 1.82.
42 While a LOD score of 1.82 does not meet the accepted standard for linkage we interpret these
43 data as suggesting the responsible gene could be linked to this region, which includes a cluster of
44 homeobox regulatory system providing cells with specific positional identities along the
45 developing joint and spine. Discovering the genetic basis of the disease would be an important
46 step in understanding the etiology of the disabling condition.

47 **Introduction**

48 Developmental dysplasia of the hip, previously known as congenital dislocation of the hip,
49 is a frequently disabling condition characterized by incomplete formation of the acetabulum
50 and/or femur leading to frequent subluxation or dislocation of the hip, suboptimal joint function,
51 and accelerated wear of the articular cartilage resulting in arthritis. This condition affects one in
52 1000 newborn infants in the United States [34]. The prevalence of the disease is even higher in
53 some parts of the world such as Japan, Italy and other Mediterranean countries [28]. Because of

54 its high prevalence and the undesirable consequences, a screening program is in place in nearly
55 every country that involves clinical examination of the hip of the newborn [29]. Although
56 relatively accurate for detecting gross dislocation or dislocatable hips, the examination cannot
57 detect milder degrees of hip dysplasia [35]. In fact, the dynamic nature of the condition prompted
58 experts to change its name from the traditional “congenital dislocation of the hip” to
59 “developmental dysplasia of the hip.” The reason behind this change two decades ago was two-
60 fold. First, the pathology of the disorder varies per patient and does not consistently result in
61 complete dislocation, but instead in subluxation or dysplasia. Second, should any form of the
62 condition occur, it often happens postnatally and therefore cannot be termed truly congenital [16].
63 Moreover, many patients affected by the condition do not discover their diagnosis until later years
64 of life when arthritis of the hip is instigated [23]. Over the past 20 years, “developmental
65 dysplasia” has become more widely preferred than the misleading “congenital dislocation,”
66 encompassing a wider spectrum of form of the condition than earlier [2, 16]. Developmental
67 dysplasia of the hip (DDH) is therefore a complex disorder with both environmental [29] and
68 genetic causes.

69 Although DDH presents with varying intensity in affected individuals, as determined by
70 the degree of femoral head undercoverage, it also may often affect members of a single family
71 [31]. Based on its pattern of presentation in families, the condition is believed to have a strong
72 genetic basis [35]. Genome-wide scans of large affected families have yielded linkage to
73 chromosomes 4q35, 13q22, and 16p, but no gene mutations in these regions have been found [17,
74 19]. Linkage to chromosome 12q13, where the collagen type 2- α 1 and vitamin D receptor are
75 located, was recently excluded as were two polymorphic sites within the *COL1A1* gene [28]. A
76 recent genetic association study of 335 affected and 622 control individuals found an association

77 of a GDF5 single nucleotide polymorphism (SNP) with DDH [5] (Table 1). Developmental
78 dysplasia of the hip is also seen among dogs, affecting some pedigrees much more commonly.
79 Based on linkage studies in large canine pedigree studies, veterinary researchers have proposed a
80 genetic basis for the disease and have made substantial progress in mapping this disorder [21, 30].
81 Although no mutations in canine genes have been definitely linked to DDH various genetic loci in
82 large canine pedigrees have been linked to DDH.

83 The familial inheritance patterns and a higher concordance rate between identical twins
84 (41.4%) compared with that between fraternal twins (2.8%) strongly support genetic transmission
85 among humans [34, 35]. There is another reason to believe that a single-gene or multiple-gene
86 mutation may be implicated in this condition. Developmental dysplasia of the hip can be
87 associated with other conditions such as clubfoot, renal malformations, and cardiac anomalies
88 [13]. In fact, DDH is not uncommonly part of a syndrome affecting multiple systems in the body
89 [13]. A majority of the patients with DDH, however, have an isolated condition with unilateral or
90 bilateral hip disease.

91 Based on the strong evidence of a genetic contribution to the etiology of DDH, we
92 wondered, without making any assumptions about where potentially causative mutations reside,
93 whether we could identify a genetic haplotype that was uniquely shared by all affected members
94 and not present in unaffected members of the family in this study, elucidating the etiology of their
95 disorder.

96

97 **Patients and Methods**

98 Our institutional prospective database on joint arthroplasty was used to identify patients
99 with DDH. Initially, our electronic database of over 22,000 patients undergoing hip arthroplasty

100 was scanned to identify patients with DDH (International Classification of Diseases, 9th Revision
101 [ICD-9] codes 745.30 and 755.63). We identified 234 patients. We believed some patients with
102 DDH undergoing THA might have been coded under end-stage arthritis (ICD-9 code 715.15).
103 Relying on the notion that most patients with DDH require THA at a younger age, as the next
104 step, we identified all patients younger than age 45 in the database institution. This resulted in the
105 identification of an additional 2450 patients. The radiographs of all 2684 patients were then
106 reviewed and appropriate measurements made to confirm or refute the diagnosis of DDH. After
107 radiographic review, 435 of the approximately 22,000 patients (or about 2%) were believed to
108 have DDH. All of these patients were contacted and asked to return for followup to obtain blood
109 samples and/or buccal swabs for analysis. To date, 130 patients from 28 families have returned
110 and donated DNA samples. At the time of this writing, the analysis had been completed in one
111 18-member, multigeneration family whose proband is severely affected with DDH. All 18 family
112 members consented to study participation and were evaluated both clinically and radiographically
113 by the senior author (JP). Before the initiation of this study, Institutional Review Board approval
114 was obtained.

115 All consenting individuals had an anteroposterior and a cross-leg lateral radiograph of the
116 hip performed as a routine. Other radiographs such as the faux profile to assess anterior coverage
117 or abduction views to assess joint congruity were also performed in patients with DDH
118 contemplating joint preservation surgery. Radiographs for each subject were measured by one of
119 four experienced orthopaedic surgeons with the senior author (JP) confirming measurements for
120 each subject. Analog radiographs were measured manually using an electronic goniometer, and
121 digital radiographs were measured using software with an incorporated angle measurement
122 system. Radiographic diagnosis of dysplasia was made based on standard definition [8, 24, 25].

123 Individuals were deemed to have DDH if one or more of the following was present: (1) Tönnis
124 angle greater than 10° and/or (2) center-edge angle less than 20° [6]. Other measurements
125 included the extrusion index, extent of lateral and superior migration of the femoral head, femoral
126 neck angle, and posterior and anterior acetabular coverage (Table 2) [26]. The diagnosis of DDH
127 was based on these parameters. Patient 001 was the proband in this study. This patient had a hip
128 replaced because of DDH. The preoperative radiographs demonstrated severe arthritis that
129 prevented us from making some of the measurements accurately. Individuals with acetabular
130 retroversion and abnormal extrusion indices that differed from the norm by one standard deviation
131 (Table 3) were categorized as “possible” dysplasia for the purpose of statistical analysis. No
132 family members had been diagnosed with DDH at birth, but rather were diagnosed later in life.

133 Cheek swab or serum DNA gathered from family members was isolated using the
134 QIAamp DNA Blood Mini Kit and amplified using the REPLI-g Mini Kit (Qiagen, Valencia, CA)
135 according to protocol. Amplified DNA was then processed according to the GeneChip Mapping
136 250 K Assay Kit as recommended by the manufacturer (Affymetrix, Santa Clara, CA). Briefly,
137 after Nsp I digestion, fragmented DNA was ligated to adaptor followed by PCR amplification.
138 The PCR product was hybridized to the GeneChip Mapping 250 K Nsp Array and processed with
139 the Fluidics Station and the GeneChip Scanner 3000 (Affymetrix). To eliminate uninformative
140 markers and spurious calls, the following filters were applied to the data set. SNPs were selected
141 for analysis that (1) generated calls for each family member; (2) had confidence scores less than
142 0.3 (confidence scores are inversely related to reliability); (3) were polymorphic with a minor
143 allele frequency of 0.2 or more; and (4) did not generate inheritance errors. This filtering process
144 brought the original 262,314 SNPs down to 6840.

145 Model-based and model-free multipoint LOD score analysis was performed using the
146 software MERLIN Version 1.1.2 (www.sph.umich.edu/csg/abecasis/Merlin/). In the model-based
147 analysis, to account for the diagnostic uncertainty in DDH, we divided individuals into two
148 liability classes with different genetic penetrances (Table 4). Multipoint model-based LOD score
149 analysis over the entire human genome, excluding the sex chromosomes, was performed.

150 Penetrance is the probability of a given individual to be affected given that he or she
151 carries zero, one, or two copies of the disease allele. Liability Class 1 contains individuals with
152 three or more radiographic signs of DDH (1, 9, and 11). We have assigned a 0% probability of
153 affection to individuals in this liability class if they have two normal alleles, and conservatively,
154 we have assigned an 80% penetrance to carriers of one or two mutated alleles. Liability Class 2
155 contains individuals with one of two radiographic signs of DDH (4, 5, 6, 8, 13, and 14).
156 Individuals in this class who happen to be carriers of the mutated allele (either homozygotes or
157 heterozygotes) have the same probability of being affected of 0.8. If an individual has no mutated
158 allele, we assume a probability of affection equal to the incidence of the disorder in the overall
159 population, one per 1000 live births, or 0.001.

160 The analysis conducted assumes autosomal-dominant transmission of the affected allele.
161 We reanalyzed the family using model-free analysis that makes no assumptions about the mode of
162 transmission of this disorder. Individuals with any sign of DDH were included in this analysis (1,
163 4, 5, 6, 8, 9, 11, 13, and 14). Results of this analysis produced a linked region on chromosome 17
164 identical to that found by the model-based analysis using two liability classes as mentioned but
165 with a lower maximum LOD score (data not shown).

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170 **Results**

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172 Based on the data in one family of 18 members in three generations, the DDH phenotype
173 had an autosomal-dominant transmission (Fig. 1). X-linked inheritance was ruled out based on
174 male-to-male transmission of the disease in this pedigree. The maximum multipoint LOD score
175 (1.82) occurred at SNP rs16949053, located 45,844,439 base pairs from the p-term of
176 chromosome 17, with a score of 1.82 (Fig.2A). The region of positive LOD score extended from
177 rs2597165 to rs996379 for approximately 4 Mb.

178 The second largest LOD score, on chromosome 16, was 1.38 at rs11866385 at position
179 10,016,159. However, the two flanking markers both had negative LOD scores, thus limiting the
180 positive LOD score region to a very small interval of approximately 470 Kb. No other genomic
181 region yielded LOD scores greater than 0.5.

182

183 **Discussion**

184 The focus of our study is to map and characterize the gene or genes responsible for this
185 disorder by family linkage analysis. We therefore asked whether we could find a genetic
186 haplotype that was uniquely shared by all affected members and not present in unaffected
187 members of the family in this study, elucidating the etiology of their disorder.

188 Important limitations to this study include both the relatively small family size as well as
189 the diagnostic uncertainty, both of which limit statistical power. A LOD score of 1.82 does not
190 meet the accepted standard for linkage. Therefore, these findings should be viewed conditionally.
191 As elaborated subsequently, the value of our study is that it provides supportive evidence for the
192 biologic importance of this candidate region found to be significant in canine studies and in
193 studies by other investigators studying DDH-affected families in China.

194 The linkage analysis of one multigeneration affected family in this study has demonstrated
195 that DDH does indeed show genetic inheritance with an autosomal-dominant mode of
196 transmission. Furthermore, the mutated gene for the condition appears linked to a region 4Mb in
197 size on chromosome 17q21. The fact that a genome scan of all human chromosomes produces just
198 one plausible candidate region adds credence to the notion that a DDH-causing number of
199 attractive candidate genes in this region (Fig. 2B). These genes were chosen among all genes
200 (approximately 70 genes; see Fig. 2B) within the chromosome 17q21 region defined by our data
201 on the basis of whether their known function might explain the biologic origins of DDH. The
202 entire *HOXB* cluster of homeobox genes is contained in the proximal end of this region. The
203 *HOXB* cluster encodes genes that are part of the developmental regulatory system that provides
204 cells with specific positional identities along the developing spine. One gene in particular,
205 *HOXB9*, is expressed in human embryo from 5 to 9 weeks, a period that coincides with primordial
206 temporal expression, it is expressed in the lumbar, sacral, and caudal regions of the forming
207 provertebra during this time [15]. *PAX1*, another transcription factor responsible for the
208 development of the hip girdle in the chicken, is known to interact with several members of the
209 *HOX* gene family [20].

210 Other attractive candidates in this region include collagen type I- α 1 (*COL1A1*) and *DLX3*
211 (Distal-less homeobox 3). A polymorphism in the collagen type I- α 1 gene might influence hip
212 development [11]. Bone fragility and in its more severe forms, blue sclera and short stature,
213 characterize various forms of osteogenesis imperfecta that are the result of mutations in the major
214 component of connective tissue [3]. Other mutations in *COL1A1* result in Ehlers-Danlos
215 syndrome characterized by laxity of the skin and joints. Still other polymorphisms in the first
216 intron of *COL1A1* are correlated to bone mass and osteoporotic fracture [32].

217 Although polymorphisms in the *PCOL2* and *Sp1* transcription factor binding sites of the
218 *COL1A1* gene do not appear associated with DDH in the Chinese population, *COL1A1* mRNA
219 and protein expression in the hip capsule of DDH-affected children was reportedly decreased
220 compared with age- and gender-matched controls [11, 33]. These observations suggest that
221 polymorphisms other than *PCOL2* and *Sp1* may be influencing *COL1A1* expression in these
222 affected individuals. *DLX3* is a member of a family of transcription factors, including *Runx2*, that
223 regulate the expression of osteocalcin during fetal mouse development [9].

224 Our findings help confirm those of Jiang et al. In 2003 who found after analyzing 101
225 members of Chinese families, that the DDH locus was associated with a marker (D17S1820) that
226 is included in our chromosome 17 candidate region [12]. Additional support for the biologic
227 importance of this region in the etiology of DDH comes from recent studies of Marschall and
228 Distl, in which a quantitative trait locus for hip dysplasia on canine chromosome 9 is syntenic to
229 chromosome 17q21 on the human genome where our candidate gene locus resides [21]. In
230 addition to analyzing the results of a second multigenerational family, we are in the process of
231 sequencing one of a number of these candidate genes.

232 In a small pilot genetic association study, Rouault and coinvestigators recently found no
233 association of DDH with *HOXB9* and *COL1A1* in a small regional French population in which the
234 incidence of this disorder was elevated [27]. Although an intriguing preliminary observation, the
235 limited geographic sampling of this study does not preclude either of the genes playing a
236 causative role in other populations. It is highly probable that DDH is genetically heterogeneous
237 because hip formation involves the activation of numerous genes during development. Indeed,
238 similar contradictory results have surfaced in the past that can be explained by population
239 differences or small sample size and limited statistical power to detect genetic association.

240 Recently, a polymorphism in the Calmodulin (*CALM*) promoter is associated with hip
241 osteoarthritis in the Japanese population and decreases transcription of the gene in vitro and in
242 vivo [22]. In a subsequent study, the *CALM* promoter polymorphism was excluded in a UK white
243 population [18].

244 Our data suggest the presence of a disease-associated mutation/polymorphism in a region
245 of chromosome 17q21 in the affected members of the family in this study. Because our finding,
246 that of Jiang et al. [12] in the Chinese population, and those of the investigators studying the
247 canine hip dysplasia [21] coincide, it is possible to hypothesize that mutations in candidate genes
248 in this region could play a more prevalent role in causing susceptibility to this disorder.

249 We have chosen to compensate for the diagnostic uncertainty of DDH in this study by
250 using reduced penetrance and creating liability classes. Penetrance is the proportion of individuals
251 with a mutation causing a particular disorder who exhibit clinical symptoms of that disorder. The
252 use of liability classes in this study allows for the retention of the statistical power generated by
253 our severely affected family members [1, 9, 11] but still accounts for the diagnostic uncertainty of
254 other possibly affected members.

255 We identified a single genetic locus linked to DDH in one multigeneration North
256 American family. Our evidence suggests this locus is on human chromosome 17q21. We have
257 identified a few potential candidate genes and are actively searching for mutations in a number of
258 them by automated DNA sequence analysis. Identification of mutations or susceptibility-inducing
259 polymorphisms in asymptomatic individuals with DDH might allow implementation of
260 appropriate care in the future.

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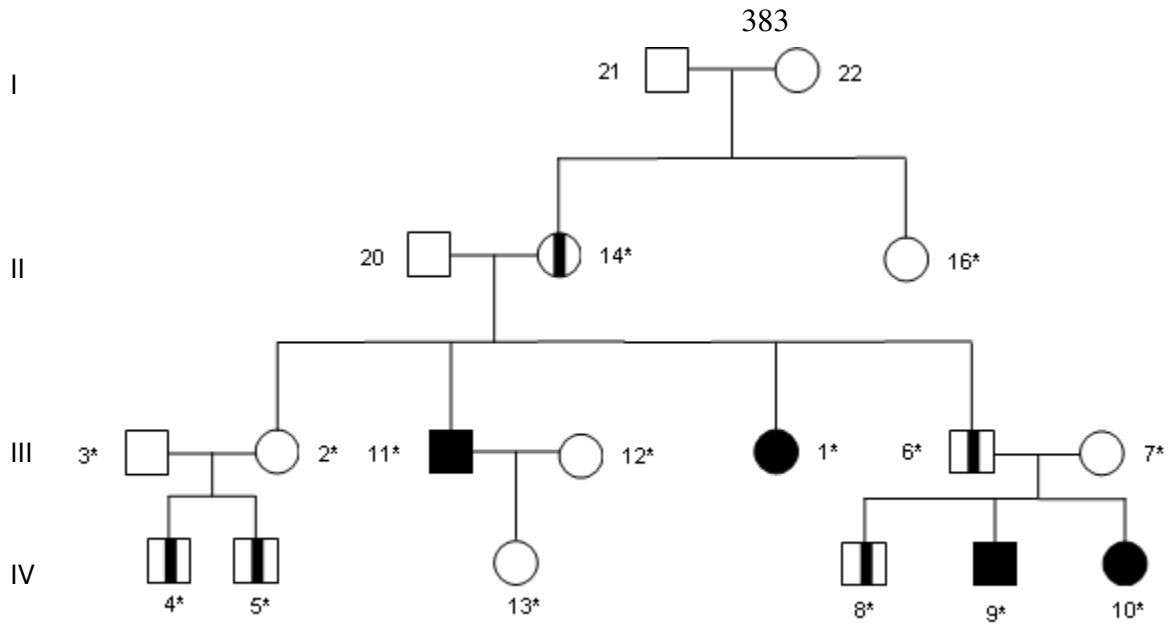
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379 **Figure 1:** This pedigree illustrates one large family showing transmission of the disease through
 380 three generations. Symbols are explained in figure key.

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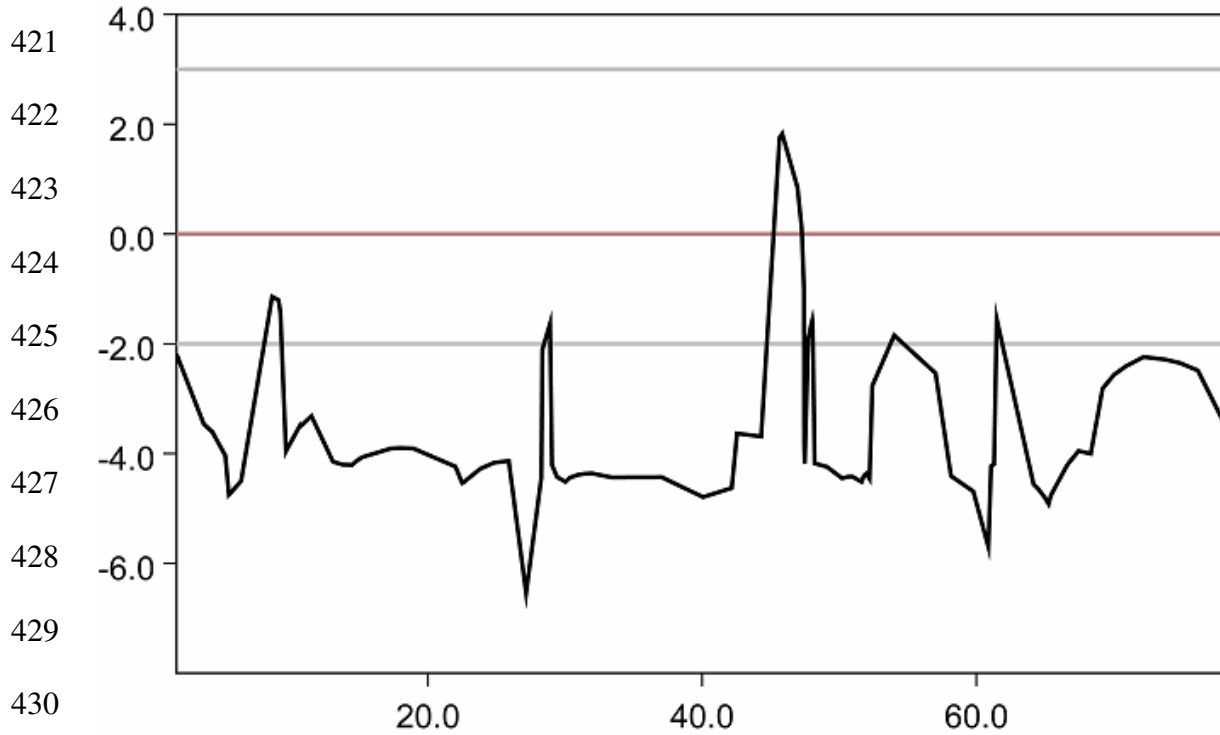
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- * = DNA samples retrieved
- = unaffected female
- = unaffected male
- ◐ = female partially affected, 1-2 radiographic signs
- ◑ = male partially affected, 1-2 radiographic signs
- = affected female, >2 radiographic signs
- = affected male, >2 radiographic signs

417 **Figure 2A:** This graph shows multipoint parametric analysis of the human chromosome 17. The
418 maximum LOD score is 1.8221 at SNP rs16949053 located 45,844,439 base pairs from p-term of
419 chromosome 17.

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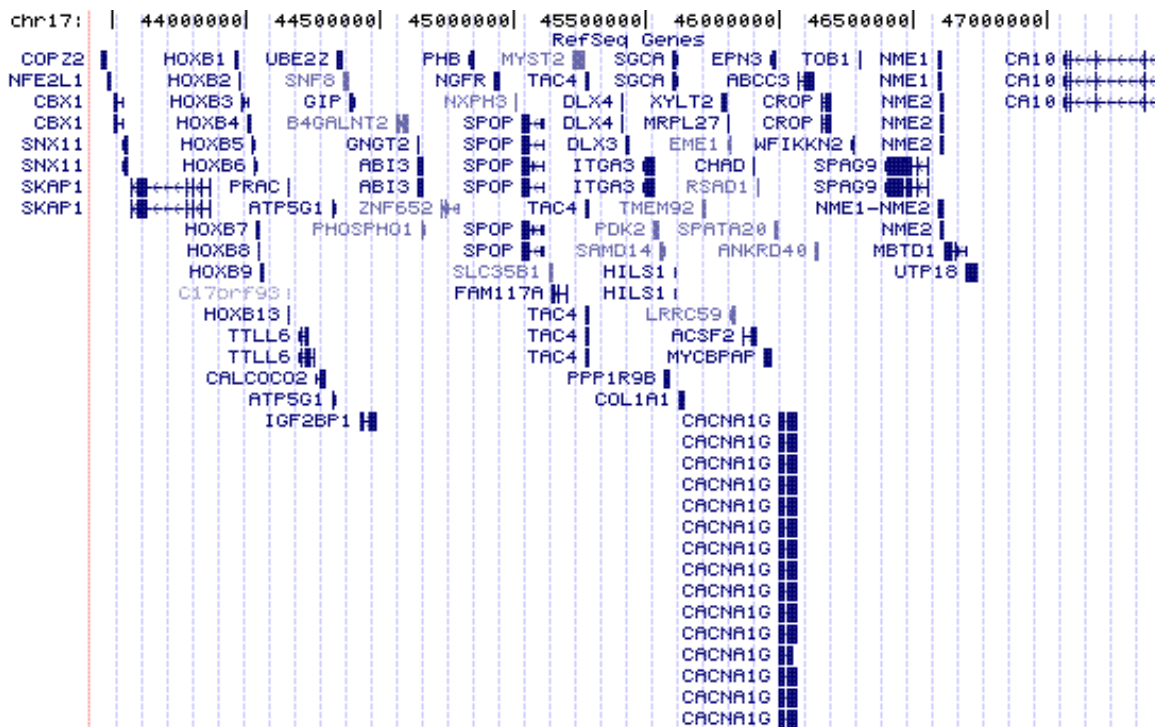
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440 **Figure 2B:** This figure shows an ideogram of chromosome 17 with the box depicting the
441 approximate location of the candidate region. Below this is an enlarged view of the 4.0-MB
442 candidate region showing the position of the 73 transcripts contained in the region [14]. Reprinted
443 with permission from <http://genome.ucsc.edu/cite.html>.

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Table 1:

Table 1. Summary of findings of genetic studies of nonsyndromic DDH and early-onset hip osteoarthritis

Author (year)	Type of study	Conclusion
Rouault et al. (2009) [27]	Pilot candidate locus association study	HOXB9 and COL1A1 association with DDH not supported in study of small regional French population
Dai et al. (2008) [5]	Candidate locus association study of 335 CDH subjects, 622 control subjects with candidate region	Significant association of GDF5 SNP with CDH
Rubini et al. (2008) [28]	Linkage exclusion	COL2A1 and vitamin D receptor excluded from linkage to DDH
Mabuchi and Nakamura (2006) [19]	Whole genome linkage	DDH in one large Japanese family linked to chromosome 13q
Loughlin et al. (2006) [18]	Candidate locus association study	Calmodulin promoter polymorphism not associated with DDH in UK white population
Jiang et al. (2005) [11]	Linkage exclusion	Two polymorphic sites within the COL1A1 gene found not to be linked to DDH in 81 small Chinese families
Mototani et al. (2005) [22]	Candidate locus association study	A functional polymorphic SNP in promoter of the Calmodulin gene found to be associated with hip osteoarthritis in Japanese population
Jiang et al. (2003) [12]	Candidate locus linkage study	Association of one allele of one microsatellite marker (D17S1820) on chromosome 17q21 with CDH phenotype in 101 Chinese families
Granchi et al. (2002) [7]	Candidate locus association study	Pilot study suggests Type II collagen and vitamin D receptor polymorphism might be associated with risk of osteoarthritis in patients with DDH
Ingvarsson et al. (2001) [10]	Whole genome linkage study of large Icelandic family	Locus on chromosome 16p linked to early osteoarthritis of the hip
Cilliers and Beighton (1990) [4]	Whole genome linkage of large African family	Premature (Beukes) degenerative osteoarthritis of the hip mapped to 11 cM region on chromosome 4q35

DDH = developmental dysplasia of the hip; CDH = congenital dislocation of the hip; SNP = single nucleotide polymorphism.

454 **Table 2:** This table shows details of radiographic evaluation that was performed on
 455 members of the affected family. The diagnosis of developmental dysplasia of the hip was
 456 made based on these measurements. Affected members of this family are highlighted.
 457 Abbreviations: SL = Shenton's line, CEA= center edge angle, T = Tonnis, EI = extrusion
 458 index, AC = anterior acetabular coverage, PC = posterior acetabular coverage, FNA =
 459 femoral neck angle, D = disrupted, ND = non-disrupted.
 460

Family member	Age (years)	Shenton's Line (SL)		Center Edge Angle (CEA)		Tonnis (T) (degrees)		Extrusion Index (EI) (mm)		Anterior Acetabular Coverage (AC)		Posterior Acetabular Coverage (PC)		Femoral Neck Angle (FNA)	
		Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
001	39														
009	11	D*	D	12°	18°	24°	24°	16	16	n/a	n/a	n/a	n/a	140°	150°
011	42	D	ND**	3°	6°	20°	24°	28	28	15%	10%	40%	20%	142°	146°
004	6	ND	ND	25°	25°	8°	12°	14	15	20%	30%	70%	50%	134°	132°
005	18	ND	ND	25°	26°	12°	12°	16	16	n/a	n/a	n/a	n/a	136°	136°
006	41	ND	ND	32°	28°	12°	12°	18	18	30%	30%	60%	70%	130°	126°
008	13	ND	ND	28°	32°	10°	6°	14	14	n/a	n/a	n/a	n/a	154°	148°
014	64	ND	ND	18°	22°	0°	0°	14	18	20%	n/a	80%	60%	133°	134°
007	28	ND	ND	24°	22°	12°	10°	14	16	20%	30%	70%	50%	144°	144°
008	13	ND	ND	28°	32°	10°	6°	14	14	n/a	n/a	n/a	n/a	154°	148°
010	n/a	ND	ND	26°	22°	5°	5°	16	18	n/a	n/a	n/a	n/a	155°	165°
002	45	ND	ND	26°	26°	8°	16°	10	10	20%	20%	80%	80%	138°	134°
012	49	ND	ND	17°	24°	0°	0°	3	3	15%	20%	70%	80%	132°	130°
013	n/a	ND	ND	22°	22°	22°	16°	5	6	n/a	n/a	n/a	n/a	132°	130°
003	49	ND	ND	22°	20°	14°	16°	10	10	30%	20%	70%	70%	124°	124°
016	61	n/a	ND	32°	30°	0°	2°	6	6	30%	30%	70%	70%	126°	130°
020	66	ND	ND	32°	32°	12°	9°	12	12	20%	20%	60%	60%	138°	142°

*Disrupted
 **Non-disrupted

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465 **Table 3:** This table demonstrates data on mean, standard deviation and range of normal
466 values of radiographic measurements.

	Mean	SD	Range
Center edge angle (CEA)	23.5°	7.8°	0° - 44°
Tonnis (T) (degrees)	10.8°	7.6°	-7°-32°
Extrusion index (EI) (mm)	8.4	2.6	2-15
Femoral neck angle (FNA)	137.3°	9.0°	110°-154°

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468 **Table 4:** This table shows genetic penetrance classes used to analyze affected family.

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Liability class	Individuals in each class	Homozygous for normal allele	Heterozygous	Homozygous for mutated allele
1	1,9,11	0	0.8	0.8
2	4,5,6,8,13,14	0.001	0.8	0.8

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