ORIGINAL ARTICLES

Prenatal diagnosis of diastrophic dysplasia with polymorphic DNA markers

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Abstract

Ultrasonography is a non-invasive method for prenatal detection of diastrophic dysplasia (DTD) in the second trimester of pregnancy. As there is a need for genetic counselling as early as possible we wished to develop a method based on molecular analysis. Five fetuses in families with a previous history of DTD were studied by typing them and their relevant family members for DNA markers closely linked to the DTD gene. The DNA analyses predicted that three of the fetuses were unaffected and two affected. These results were concordant with those obtained by ultrasonography, and the phenotype of the fetus was correctly predicted in all cases. DNA analysis provides a reliable means of prenatal diagnosis in the first trimester of pregnancy.

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Diastrophic dysplasia (DTD) is a severe autosomal recessive chondrodysplasia characterised by disproportionate short stature, generalised joint dysplasia, and deformities of the spine and extremities.[1] Tracheomalacia and kyphosis of the cervical spine cause a significant risk of fatal respiratory and neurological complications. Most patients undergo repeated orthopaedic surgery during childhood, often with unsatisfactory results.[2] The diagnosis is based on clinical and radiographic findings which can be readily distinguished from those of other chondrodysplasias. DTD occurs at low prevalence in most populations, but is exceptionally common among Finns with a carrier frequency of about 1 in 70. The biochemical defect and pathogenesis of DTD are unknown.

Owing to the severe physical handicap caused by DTD most families with an affected child request prenatal diagnosis in subsequent pregnancies. This can be provided by ultrasonography which, however, is feasible only after the first trimester.[3–6] Thus, the diagnosis can only be attained relatively late extending the period of anxiety of the families, and delaying the eventual abortion to midpregnancy. The delay is critical regarding the method by which the termination of pregnancy is performed.

The DTD gene has been localised to distal 5q by linkage analysis.[7,8] The immediate region surrounding DTD is well covered with polymorphic DNA markers several of which do not recombine with the disease gene. Recently strong linkage disequilibrium was detected between the colony stimulating factor 1 receptor locus (*CSF1R*) and *DTD* (table 1) indicating that *CSF1R* is the closest known locus to *DTD*.[9] The aim of this study was to establish prenatal diagnosis that would allow genetic counselling during the first trimester.

Materials and methods

FAMILIES

Pedigrees of the five families studied are shown in fig 1. In families 1 to 3 prenatal diagnosis by DNA analysis was done in parallel with ultrasonography. In families 4 and 5 the actual diagnosis was made by ultrasonography and the fetuses were found to be affected. DNA analysis was performed retrospectively in these two families.

DNA SAMPLES

The source of DNA was blood leucocytes for parents and sibs, cultured amniotic cells from the fetus of family 1, chorion villus samples from the fetuses in families 2 and 3, and skin samples obtained at necropsy from the fetuses in families 4 and 5. Amniocentesis was performed in the 17th gestational week and the placental biopsies in the 12th and 16th gestational weeks. DNA was extracted by standard methods.

DNA MARKERS

The polymorphisms used in this study[9–14] are described in table 2A and the two point linkage data in table 2B. The most likely order of marker loci and their location in relation to the DTD gene on the sex averaged map as derived from linkage analysis is *GRL*-8·2 cM-*DTD*-0·7 cM-*CSF1R*-4·4 cM-*SPARC* (unpublished results).

Table 1 Frequencies of the four StyI-EcoRI haplotypes at CSF1R determined in 152 Finnish DTD chromosomes and 123 control chromosomes.[9]

Haplotype	DTD chromosomes (%)	Normal chromosomes (%)
A=1-1	95	3
B=1-2	1	23
C=2-1	0	6
D=2-2	5	68

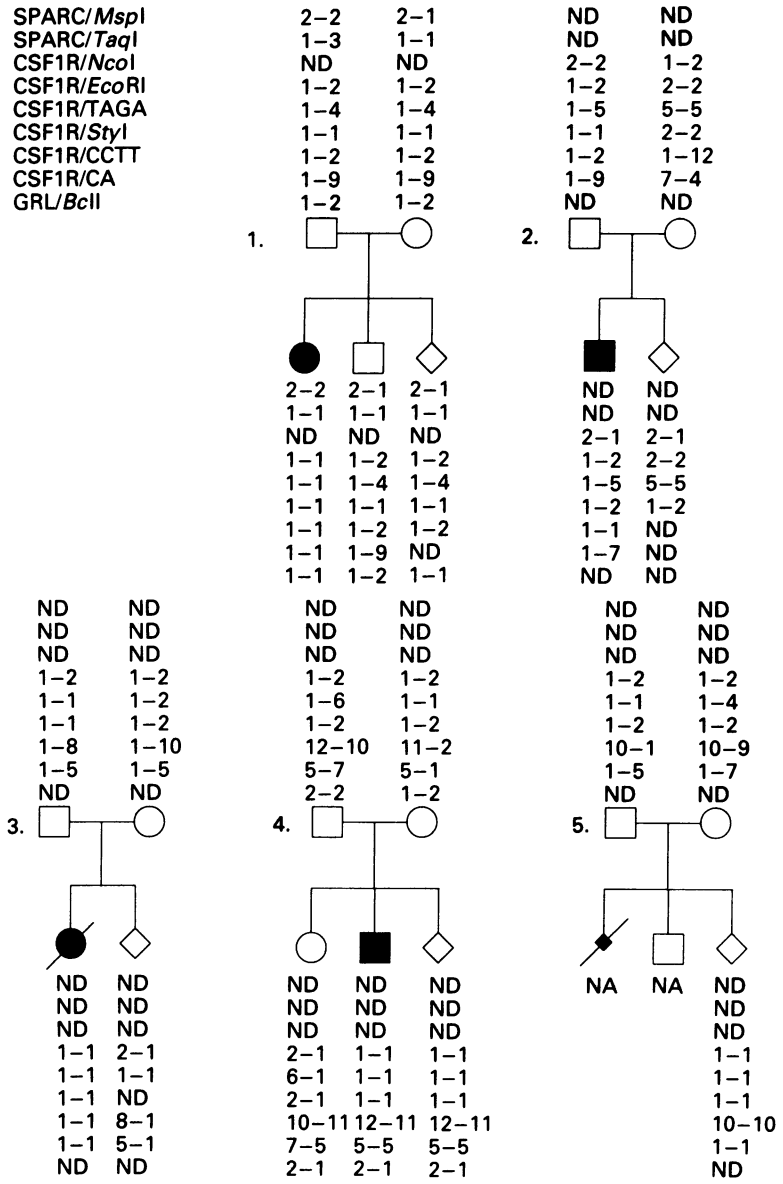

Figure 1 Pedigrees and the genotype data of the families. ND = not determined. NA = sample not available.

Table 2A The polymorphisms used in this study.

Locus	Polymorphism	Probe or PCR primers	Reference
CSF1R	*Eco*RI RFLP	pcfms-104	10
	*Sty*I RFLP	pcfms-104	9
	*Nco*I RFLP	pcfms-104	11
	CA repeat	TGTGTCCAGCCTTAGTGTGCA (F) TCATCACTTCCAGAATGTGC (R)	12
	CTTT/CCTT repeat*	ACAGAGCAAGACTCCATCTCCTT (F) CCAGCATGATAAAACCCTGTCT (R1) GATCCATGCCATTGCACTC (R2)	9
	TAGA repeat	CAGGTTGCTAACCACCCTGT (F) GTGCACACTTGGACAGCATT (R)	9
SPARC	*Taq*I RFLP	pHVON-9-2	13
	*Msp*I RFLP	pHVON-9-2	
GRL	*Bcl*I RFLP	phGR1.2	14

*This repeat was typed by heminested PCR, by first doing 25 cycles with primers F and R1 followed by 25 cycles with F and radiolabelled primer R2

Table 2B Maximum two point lod scores for DTD versus marker loci based on data from 24 Finnish DTD families.

Locus	Zmax	θmax	95% confidence limits
*CSF1R**	16·10	0·009	0·00035–0·044
SPARC†	8·25	0·04	0·01–0·10
GRL	1·37	0·14	-

*Up to six polymorphisms at the *CSF1R* locus were haplotyped for linkage calculations. One crossover within *CSF1R* was observed causing part of *CSF1R* to recombine with *DTD*.[9] In the linkage calculations this was treated as a regular recombination between the two loci.
†Haplotypes constructed from two polymorphisms at the locus were used in linkage calculations.

DNA METHODS

Southern analyses were performed using standard protocols.[15] Polymerase chain reaction (PCR) amplifications were done in a total volume of 10 µl with 20 ng of genomic DNA, 1 pmol of each primer one of which was kinase labelled with γ-^{32}P-ATP, in 100 µmol/l dNTPs, 50 mmol/l KCl, 10 mmol/l Tris-HCl, pH 8·3, 1·5 mmol/l $MgCl_2$, 0·001% gelatin, with 0·25 U *Taq* polymerase. The PCR products were fractionated on 6% polyacrylamide gels containing 7 mol/l urea followed by autoradiography for two to 12 hours.

RISK CALCULATIONS

The probability of the fetus being affected, an unaffected carrier, or non-carrier was calculated using the risk calculation option of the program MLINK of the LINKAGE package.[16] In family 1 the calculation was originally based on data of the flanking marker loci *GRL* and *SPARC*. At that time the close linkage between *DTD* and *CSF1R* had not yet been detected, which also caused the distances between *DTD* and the flanking markers used in the calculations to be slightly different from the ones on the current map. In the remaining four cases calculations were based solely on data on *CSF1R* polymorphisms. For family 5 linkage disequilibrium data were used for estimating the phase of *CSF1R* polymorphisms and *DTD*. Up to six polymorphisms at *CSF1R* were tested, haplotyped, and considered a single locus. The fetus of family 1 was also tested retrospectively for *CSF1R* polymorphisms and a new risk calculation performed.

Results

The structure of four of the five families allowed direct risk calculations based on linkage analysis. At a probability level of more than 95% three fetuses were predicted to be unaffected carriers while one was predicted to be affected (table 3). The results of DNA analysis were concordant with those of ultrasonography, and with the phenotypes of the babies delivered and the aborted fetus examined at necropsy.

The structure of family 5 did not allow standard risk calculations (fig 1). There was no DNA available from the first affected fetus (diagnosed at necropsy after termination of pregnancy based on ultrasonographic findings) nor from the healthy brother, but only from the parents and the fetus under study. This rendered the family formally uninformative for linkage analysis. In this case, however, the strength of the linkage disequilibrium observed in the Finnish population[9] (table 1) could be exploited to establish the phase of *DTD* and the polymorphisms at *CSF1R*, which in turn allowed calculation of the risk by linkage analysis. The probability (P) of one parent carrying *DTD* on a chromosome with haplotype A (table 1) is:

$$\frac{P(A_{DTD}D_{norm})}{P(A_{DTD}D_{norm}) + P(B_{DTD}C_{norm}) + P(C_{DTD}B_{norm}) + P(D_{DTD}A_{norm})}$$

Table 3 The risks of fetuses being affected, unaffected carriers, or non-carriers based on results from polymorphisms at CSF1R. The figures in parentheses for family 1 are the results of the initial risk calculation based on markers GRL and SPARC flanking DTD.

	Family 1	Family 2	Family 3	Family 4	Family 5
Affected	1·3% (19·3%)	1·7%	1·7%	97·3%	97·6%
Unaffected carrier	97·3% (79·7%)	96·5%	96·5%	2·7%	2·4%
Non-carrier	1·3% (1%)	1·7%	1·7%	0%	0%

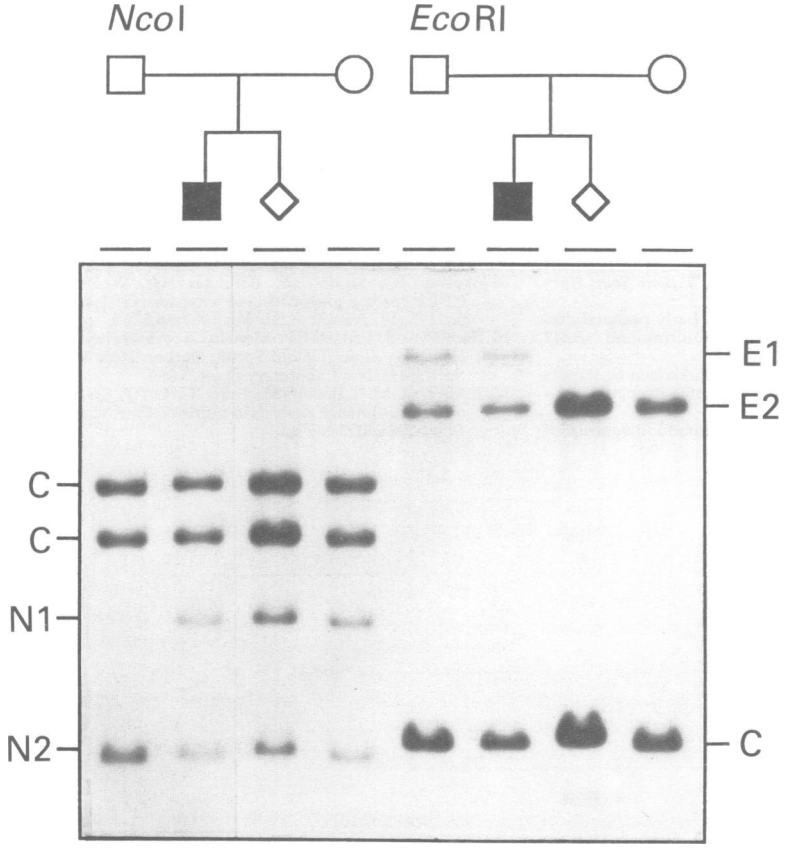

Figure 2 A Southern blot analysis from family 2. Restriction enzyme digestions were done with enzymes NcoI and EcoRI, electrophoresed, blotted, and hybridised with the cDNA clone pcfms-104 from CSF1R. The polymorphic fragments shown by NcoI digestion are N1 (4·1 kb) and N2 (2·9 kb) and the ones shown by EcoRI digestion E1 (29 kb) and E2 (16 kb). C stands for constant bands. The fetus inherited the same allele from the mother as the affected brother, but a different allele from the father and therefore was predicted to be an unaffected DTD carrier.

The figures are $=$

$$\frac{(0{\cdot}95 \times 0{\cdot}68)}{(0{\cdot}95 \times 0{\cdot}68) + (0{\cdot}01 \times 0{\cdot}06) \times (0 \times 0{\cdot}23) + (0{\cdot}05 \times 0{\cdot}03)}$$

$$= 0{\cdot}997.$$

By using the above assumption in the risk calculation the likelihood of the fetus of family 5 being affected was 0·98. This was concordant with the results of fetal ultrasound and the phenotype of the fetus at necropsy after the termination of pregnancy.

Discussion

Ultrasonography is an established and reliable method of prenatal diagnosis in diastrophic dysplasia (DTD). In our experience, out of 18 Finnish at risk pregnancies studied by ultrasonography at 16 to 18 gestational weeks, neither false positive nor false negative diagnoses were made. Five of 18 fetuses were predicted to be affected and each was confirmed by fetal necropsy after the termination of pregnancy. Thirteen fetuses were unaffected. A major handicap of the method is that the time of diagnosis occurs so late that a possible termination can only be done in the second trimester of pregnancy. This study was undertaken to determine if earlier, DNA based diagnosis for DTD might be feasible.

Five pregnancies at risk for DTD were studied by typing the families for DNA polymorphisms closely linked to *DTD*. With the exception of the initial diagnosis in family 1, the DNA diagnoses were based on the information derived from several polymorphisms at a single locus, *CSF1R*. This method was chosen because, despite one recombination having been observed between *CSF1R* and *DTD*, or strictly between *DTD* and part of *CSF1R*,[9] both the linkage disequilibrium and genetic mapping data strongly indicate that *CSF1R* is the closest available marker to *DTD*. It contains at least six polymorphisms: three two allele RFLPs and three highly polymorphic simple sequence length polymorphisms (SSLPs). In the course of our studies on Finnish DTD families, we have typed 123 DTD parents and not found a single person who is not informative for at least one of these polymorphisms.[9] The SSLPs are typed by PCR which allows results to be rapidly obtained from minimal amounts of DNA. The limited availability of DNA from fetuses is a common problem in diagnosis based on Southern blot hybridisation; therefore the existence of useful PCRable markers is an advantage. However, an issue regarding the choice of method might be raised in that some SSLPs have high mutation rates[9] which might conceivably lead to diagnostic errors. Ideally both types of polymorphism should be used, subject to sample availability. Further experience with SSLPs will eventually determine the empirical risk of problems arising from these circumstances.

The risk calculations correctly predicted the phenotype of the fetus in all families. The strong linkage disequilibrium between *CSF1R* and *DTD* allowed phase estimation and risk calculation in a family with a history of DTD but no sample available from the first affected fetus. However, even in Finland linkage disequilibrium cannot be exploited in population screening. Despite the strength of this linkage disequilibrium four out of five Finnish chromosomes 5 carrying the high risk haplotype 'A' are non-DTD chromosomes.

Based on this study we consider the prenatal diagnosis of DTD based on the polymorphisms at *CSF1R* to be reliable enough in the Finnish population to be used as an alternative to ultrasonography in families with an affected child. The diagnosis can be established at least a month earlier than by ultrasonography if chorion villus sampling is performed at 10 weeks' gestation. With the PCR based polymorphisms results can be obtained in a few days. Earlier diagnosis reduces the psychological burden of the families caused by the period of waiting. The time that can be saved by using DNA analysis is critical regarding the

method of termination of the pregnancy if indicated and chosen.

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1 Walker BA, Scott CI, Hall JG, Murdoch JL, McKusick VA. Diastrophic dwarfism. *Medicine (Baltimore)* 1972; **51**:41–59.
2 Ryöppy S, Merikanto J, Marttinen E, Kaitila I. Foot deformities in diastrophic dysplasia. *J Bone Joint Surg (Br)* 1992;**74**:441–4.
3 O'Brien GD, Rodeck C, Queenan JT. Early prenatal diagnosis of diastrophic dwarfism by ultrasound. *BMJ* 1980;**280**:1300.
4 Kaitila I, Ämmälä P, Karjalainen O, Liukkonen S, Rapola J. Early prenatal detection of diastrophic dysplasia. *Prenat Diagn* 1983;**3**:237–44.
5 Gollop TR, Eigier A. Prenatal ultrasound diagnosis of diastrophic dysplasia at 16 weeks. *Am J Med Genet* 1987; **27**:321–4.
6 Gembruch U, Niesen M, Kehrberg H, Hansmann M. Diastrophic dysplasia: a specific prenatal diagnosis by ultrasound. *Prenat Diagn* 1988;**8**:539–45.
7 Hästbacka J, Kaitila I, Sistonen P, de la Chapelle A. Diastrophic dysplasia gene maps to the distal long arm of chromosome 5. *Proc Natl Acad Sci USA* 1990;**87**:8056–9.
8 Hästbacka J, Sistonen P, Kaitila I, Weiffenbach B, Kidd KK, de la Chapelle A. A linkage map spanning the locus for diastrophic dysplasia (DTD). *Genomics* 1991;**11**:968–73.
9 Hästbacka J, de la Chapelle A, Kaitila I, Sistonen P, Weaver A, Lander E. Linkage disequilibrium mapping in isolated founder populations: diastrophic dysplasia in Finland. *Nature Genet* 1992;**2**:204–11.
10 Xu DQX, Guilholt S, Galibert F. Restriction fragment length polymorphism of the human c-fms gene. *Proc Natl Acad Sci USA* 1985;**82**:2862–5.
11 Angert E, Nagarajan L, Huebner K. Previously unreported NcoI RFLP for human CSF1R. *Nucleic Acids Res* 1989;**17**:2153.
12 Polymeropoulos MH, Xiao H, Rath DS, Merril CR. Dinucleotide repeat polymorphism at the human proto-oncogene CSF-1 receptor (CSF1R). *Nucleic Acids Res* 1991;**19**:1160.
13 Naylor SL, Helin-Davies D, Charoenworawat P, Villarreal XC, Long GL. The human osteonectin gene (OSN) has TaqI and MspI polymorphisms. *Nucleic Acids Res* 1989;**17**:6573.
14 Murray JC, Smith RF, Ardinger HA, Weinberger C. RFLP for the glucocorticoid receptor (GRL) located at 5q11-5q13. *Nucleic Acids Res* 1987;**16**:6765.
15 Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning. A laboratory manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 1989.
16 Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;**81**:3443–6.