Prenatal prediction of osteogenesis imperfecta (OI type IV): exclusion of inheritance using a collagen gene probe

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SUMMARY Autosomal dominant osteogenesis imperfecta is caused by mutations in the COL1A2 and COL1A1 genes of type I collagen. In a family with OI type IV genetically linked to the COL1A2 gene, we attempted prenatal diagnosis in a pregnancy at risk by genotyping the DNA of the fetus for a COL1A2 gene associated RFLP. Our results showed that the fetus inherited the normal COL1A2 allele from her affected parent. Linkage analysis can thus be used in the prenatal diagnosis of dominantly inherited osteogenesis imperfecta.

Genetic linkage studies with DNA markers have been extensively used in the prenatal diagnosis of various hereditary disorders.¹⁻³ Osteogenesis imperfecta (OI) is a group of genetic disorders of the connective tissue. Owing to its heterogeneity at least four clinical groups have been identified.⁴ OI type IV is transmitted as an autosomal dominant trait and it is characterised by postnatal onset of fractures, mild to moderate skeletal deformity, joint laxity, and in some families dentinogenesis imperfecta. Tsiopoulos *et al*^{5,6} used COL1A2 gene associated RFLPs as markers to show genetic linkage of OI type IV to mutations in the COL1A2 gene. We recently had the opportunity to monitor a pregnancy at risk for OI type IV. Genetic linkage of the OI phenotype to the COL1A2 RFLPs had been previously established in the family.^{6,7} Genotypic analysis of DNA from the fetus showed that she had received the normal allele from her affected parent. Furthermore the segregation of the COL1A1 was discordant to the inheritance of the OI phenotype in the family. This study represents the first example of prenatal prediction using genetic linkage studies in a heritable connective tissue disorder. It should be noted that although postnatal diagnosis is not yet

confirmed, to date the child at 10 months has sustained no fractures, suggesting successful prenatal diagnosis.

Methods

SUBJECTS

Affected and unaffected subjects from a family with autosomal dominant OI were studied with one of the several RFLPs associated with the human COL1A2 genes.

CHORIONIC VILLUS SAMPLING

Chorionic villus sampling was performed by trans cervical aspiration under ultrasound guidance using a 26 gauge Portex (Portex Inc, Wilmington, Massachusetts) catheter. The sample was immediately examined under a low power dissecting microscope to determine its adequacy. Any adherent maternal decidua were dissected free from the sample and the remaining chorionic villi were used for analysis and plated for cell culture. Informed consent for the procedure and the genotypic analysis of the sample was obtained according to institutional guidelines.

RESTRICTION ENDONUCLEASE ANALYSIS OF GENOMIC DNA

Nuclear DNA was isolated from the fetal chorion

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villus tissue and from the leucocytes contained in 10 to 15 ml of EDTA anticoagulated blood according to standard procedures.^{7,8} DNA (10 to 15 µg) was digested to completion under conditions recommended by the commercial supplier. Digested DNA and DNA size markers were separated by electrophoresis in 0.6% or 1% (w/v) agarose gels. The DNA fragments were transferred to nitrocellulose filters⁹ and hybridised with the human COL1A2 and COL1A1 probes for 24 to 48 hours as described. The filters were then washed for 10 minutes at 68°C with each of the following solutions: $2 \times \text{SSC}$, $1 \times \text{SSC}$, $0.5 \times \text{SSC}$, $0.25 \times \text{SSC}$, and $0.1 \times \text{SSC}$ (SSC, buffer containing 0.15 mol/l NaCl in 0.015 mol/l sodium citrate, pH 6). The probes used in these experiments were labelled to a specific activity of 2 to 5×10^8 cpm/µg by nick translation.

DNA PROBES FOR THE HUMAN COL1A2 AND COL1A1 GENES

The cDNA COL1A2 probe used in these experiments has been previously described.¹⁰ This 2.2 kb cDNA contains sequences complementary to the coding regions of the 3' half of the human COL1A2 gene including 1443 nucleotides coding for the C-propeptide region and part of the non-coding region of the corresponding mRNA. The genomic DNA COL1A1 probe is a 2.6 kb fragment towards the 5' end of the gene.¹¹

LINKAGE ANALYSIS

The computer programme LINKAGE¹² was used for calculation of lod scores.

Results

CASE REPORT

The consultand II.2 (figure) is a 31 year old G2 P1 Abl female with osteogenesis imperfecta. During her life time she sustained approximately 40 frac-

tures, with the first occurring at the age of three. Her height was 141 cm (<3rd centile), weight 40.3 kg (<3rd centile), and head circumference 55 cm (50th centile). She presented no deformities of the upper or lower extremities. Other physical findings included triangular facies, mild pectus carinatum, and small joint laxity. Her sclerae were grey. No clinical or radiological signs of dentinogenesis imperfecta were observed. Her first pregnancy resulted in miscarriage at approximately eight weeks. During her second pregnancy chorionic villus sampling was performed at eight weeks and genotype analysis showed the fetus to have received the normal COL1A2 allele from her affected parent. Ultrasound examinations performed at 16, 28, and 32 weeks' of gestation showed the fetal skeleton to be normal. The second pregnancy was complicated by premature labour at 28 weeks' gestation, controlled by intravenous ritodrine followed by oral ritodrine until 32 weeks' gestation. At that time the patient experienced recurrence of uterine contractions requiring both intravenous ritodrine and magnesium sulphate for tocolysis. At 33.5 weeks of gestation an amniocentesis was performed which revealed fetal pulmonary maturity. The proband underwent a primary caesarean section under spinal anaesthesia without complications. A 2100 g (50th centile) female infant, with Apgar scores of 8 and 9 at one and five minutes respectively, was delivered. The infant had no clinical evidence of skeletal deformities or fractures and a gestational age of 32 to 34 weeks by Dubowitz evaluation. The head circumference at birth was 32 cm (50th centile) and the length was 43 cm (10th to 59th centile). At four months her length was 63 cm (50th centile), weight 5.9 kg (25th centile), and head circumference 42.5 cm (75th centile). Her psychomotor development was appropriate for age.

LINKAGE ANALYSIS

The genotypic analysis of the family with an *EcoRI* COL1A2 associated RFLP has been previously reported.^{6,7} The analysis was suggestive of linkage of the OI phenotype to the COL1A2 gene RFLP. In our present studies we genotyped the family with a new *RsaI* COL1A2 associated RFLP.¹³ Affected subjects II.2 and II.4 (figure) were heterozygous for the marker (+/-) while their normal sibs II.1 and II.5 were homozygous (+/+). Since I.1 was also heterozygous while his normal spouse was homozygous, it is obvious that subjects II.2 and II.4 inherited the - allele from their father as well as osteogenesis imperfecta. No recombinants were detected in four informative meioses ($\hat{Z} = 0.90$ at $\theta = 0.00$). The normal spouse of II.2 was also homozygous for the RFLP, so the genotyping of

FIGURE Family with OI type IV. The upper + and - refer to the *RsaI* COL1A2 RFLP. The lower + and - refer to the *RsaI* COL1A1 RFLP. Shaded symbols indicate affected with OI.

their offspring will always be informative. Subject III.1 is homozygous (+/+) as shown both in DNA extracted from chorionic villus tissue and cord blood leucocytes. Since the OI phenotype in this family cosegregated with the - allele we concluded that III.3 had inherited the + allele from her mother and not the one cosegregating with OI. The subsequent genotypic analysis of the family with an *RsaI* COL1A1 associated RFLP¹¹ showed discordant segregation of the marker and the OI phenotype. All four sibs (II.1, II.2, II.4, and II.5) inherited the + allele from their affected parent (I.1) but only II.2 and II.4 also inherited OI (figure).

Discussion

The knowledge of the genetic distance between two points on the human genome has been exploited, both to define the heterogeneity of hereditary disorders and to offer prenatal diagnosis in some.¹⁻³ Genetic distances between various disease and marker loci have been established in almost all 22 autosomes and the X chromosome.¹⁴ Lately, a new category of markers has been used in genetic linkage studies, the restriction fragment length polymorphisms associated with known genes or anonymous DNA fragments.¹⁴

Osteogenesis imperfecta is a heterogeneous group of genetic disorders of the connective tissues, in which defects in type I procollagen have been shown.¹⁵⁻¹⁷ Using RFLPs associated with the COL1A2 gene, located on chromosome 7, we showed that a particular phenotypic subgroup of dominantly inherited OI (OI type IV) is genetically linked to the COL1A2 gene.⁵⁻⁷ Biochemical studies in cultured skin fibroblasts from affected subjects in one of the families genetically linked to the COL1A2 gene showed a defect in the pro $\alpha 2(I)$ chain,¹⁸ a finding corroborated by the study of other mutants.¹⁹ Sykes *et al*¹¹ also showed that autosomal dominant OI (OI types I and IV) is genetically linked to both the COL1A1 and COL1A2 genes. Establishing concordance to one of the two type I collagen structural gene loci and discordance to the other provides a unique approach for prenatal diagnosis in pregnancies at risk. In the case we reported here, the cosegregation of a COL1A2 gene associated RFLP and the OI phenotype was established before the pregnancy. Since the RFLP genotypes of the two parents would always yield information about the affection status of the fetus, we attempted the genotyping of the DNA of the fetus. The analysis of our results showed that the fetus received the normal COL1A2 allele from her affected parent, a result confirmed postnatally.

Since the RFLP used in our studies is within the COL1A2 gene, the possibility of recombination between the marker and the mutation site is highly unlikely. Our assumption about genetic linkage of the OI phenotype to the COL1A2 gene in this particular family was based on the following: (1) the previous demonstration of genetic linkage of OI type IV to the COL1A2 gene,^{5-7, 11} and (2) the exclusion of a major third locus as the site of mutations resulting in OI, strongly suggested by the findings of Sykes *et al*.¹¹ A larger sample of families with dominantly inherited OI, genotyped for the type I collagen genes, is needed in order to establish at a 95% confidence limit the absence of a third locus associated with OI.^{11, 20}

The phenotype of dominantly inherited OI (OI types I and IV) is not always expressed at birth.⁴ In the case we report here, the proband had not sustained any fractures up to the age of 10 months, and, most importantly, her linear growth has been consistently normal (R Dreifuss, personal communication, 1987), an indirect sign of not being affected with OI.⁴

Prenatal diagnosis of osteogenesis imperfecta has been previously performed in the lethal perinatal type of this disorder (OI type II) by ultrasound scanning and biochemical studies.^{21, 22} The sensitivity of either of the two previously mentioned methods is insufficient and therefore unacceptable in the prenatal diagnosis of the mild dominantly inherited OI. Since only 7 to 28% of subjects with this type of OI are born with fractures,^{4, 23} frequently resulting from trauma at birth, ultrasound scanning will give a high number of false negative results. Furthermore the biochemical characterisation of individual OI mutations is attainable in only a small fraction of families with mild dominantly inherited OI, thus making this type of prenatal diagnosis untenable. Genetic linkage studies therefore offer a valid alternative for prenatal detection in this group of genetic disorders of the connective tissue in informative families.

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