CLINICAL RESEARCH

Lethal osteogenesis imperfecta congenita and a 300 base pair gene deletion for an $\alpha 1(I)$ -like collagen

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Abstract

Broad boned lethal osteogenesis imperfecta is a severely crippling disease of unknown cause. By means of recombinant DNA technology a 300 base pair deletion in an $\alpha 1(I)$ -like collagen gene was detected in six patients and four complete parent-child groups including patients with this disease. One from each set of the patients' clinically unaffected parents also carried the deletion, implying that affected patients were genetic compounds.

The study suggests that prenatal diagnosis should be possible with 100% accuracy in subjects without the deletion and with 50% accuracy in those who possess it (who would be either heterozygous—normal, or affected with the disease).

Introduction

Osteogenesis imperfecta is a heterogeneous connective tissue disease in which fragile osteoporotic bones are a major feature. Other tissues such as skin, tendons, sclerae, and blood vessels are also abnormal.¹ Sillence and Rimoin classified the disease into four major clinicogenetic groups.² Two of these are relatively mild autosomal dominant diseases, while the inheritance of the

others is less clear and they produce severely disabling diseases. The most severe of these is Sillence type II osteogenesis imperfecta, also called osteogenesis imperfecta congenita, lethal perinatal osteogenesis imperfecta, and Vrolik's disease. There are at least two subgroups of patients—those with hardened, widened osteoporotic bones (broad boned variant), and those with relatively normal bones (thin boned type). Broad boned osteogenesis imperfecta is a particularly severe form of the disease, causing severe crippling deformities, early death, and great distress to affected families. Fatal respiratory infections are common in infancy due to the severely deformed and restricted chest, and fetal deaths are apparently associated in some instances with congenital heart disease and various vascular abnormalities.³ The overall prevalence of osteogenesis imperfecta is almost 1/10 000 in live births, and the prevalence of lethal broad boned disease has been estimated at about 1/60 000 in live births.⁴

The inheritance and recurrence rates of lethal broad boned osteogenesis imperfecta have been estimated at 3% on the basis that most patients are the result of new autosomal dominant mutations. Population studies tend to support this view,⁵ although other explanations for the low prevalence within families include a high incidence of miscarriages or a natural reluctance of families with one affected child to risk another. Although theoretically the disease may be diagnosed in utero using ultrasound localisation of the fetus at about the 20th week of pregnancy, early diagnosis and accurate genetic counselling are desirable.

We present data from four affected families showing that their children affected with the disease carried a deletion of 300 base pairs for an $\alpha 1(I)$ -like gene. This was similar to and possibly identical with a similar deletion that we have found in a father and son affected with mild Ehlers-Danlos syndrome type II. Every patient with the broad boned lethal form of the disease known to us also carried this deletion. Furthermore, in each affected family one of the two unaffected parents of each child carried an identical gene defect. Both parents in family 1 showed mild clinical features such as loose jointedness, blue sclerae, and joint extensibility, which are reminiscent of the mildest forms of the Ehlers-Danlos syndrome. This suggests that some forms of lethal osteogenesis imperfecta congenita are genetic compounds and implies that intrauterine diagnosis by amniocentesis or trophoblastic biopsy should now be possible.

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Patients and methods

Patients with severe osteogenesis imperfecta were referred to Northwick Park Hospital by various interested colleagues. Each patient and parents were personally examined, relevant x ray pictures and clinical photographs obtained, and skin fibroblast cultures established from 4 mm punch biopsy of the lateral aspect of the chest (in affected children) or the medial aspect of the upper arm (in unaffected parents). Blood samples (2-5 ml from children and 20-30 ml from adults) were obtained and chromosomal DNA prepared from them.⁶ This was then digested with the restriction endonucleases Eco RI, Bam HI, or both. The 40 kilobase (kb) DNA coding for a collagen $\alpha 1(I)$ -like gene cosH col 1⁷ was used as a probe in the blotting experiments of Southern.⁸ In some instances the 3' terminal 4.3 kb Eco RI fragment of the clone was used to probe the Southern blots. Blotting and hybridisation procedures were as described,⁶ denatured human placental DNA being used to compete for repetitive sequences.⁹ In each instance single and double digestion using Bam HI, Eco RI, or both were carried out at 37°C using standard conditions.

Skin fibroblast cultures were established and maintained in Dulbecco's MEM medium containing 10% fetal calf serum and ascorbic acid 28 μ mol (50 mg)/l.¹⁰ After radiolabelling for 24 hours with ¹⁴C-proline in the same medium procollagens were isolated in the presence of phenylmethylsulphonylfluoride 280 μ mol (50 mg)/l, parahydroxymercuribenzoate 140 μ mol (50 mg)/l, and 0.01M edetic acid pH 7.4 to prevent proteolytic cleavage.¹¹ The proteins were separated by sodium dodecyl sulphate gel electrophoresis on 5% polyacrylamide slab gels.¹² In other experiments samples were treated with pepsin for six hours at 15°C to remove the procollagen extension peptides and the collagens analysed by slab gel electrophoresis and visualised by photofluorography.¹³ In a third set of experiments post-translational hydroxylation of procollagens was inhibited by adding α , α' -dipyridyl to the culture medium.¹⁴

FAMILY HISTORIES

Family 1—The patient was the first child of first cousin Sri Lankan parents. The child was born post-term and severe osteogenesis imperfecta was diagnosed at birth. Death occurred at 5 weeks from a respiratory tract infection. Postmortem examination showed grossly distorted, widened, osteoporotic bones. Subsequently the parents elected to have another pregnancy and a normal daughter was born.

Family 2—A first child of unrelated Ugandan Asian parents was born at the 37th week of pregnancy, weighing 2500 g. Osteogenesis imperfecta with severe short limbed dwarfism was diagnosed at birth, and the child died at 48 hours. Postmortem examination showed grossly distorted osteoporotic bones.

FIG 1—Posteroanterior (left) and lateral (right) whole body radiographs of fetus with lethal osteogenesis imperfecta. Note broadened, widened ribs and long bones with poorly calcified skull.

Family 3—This child was the second of unrelated Indian parents. Osteogenesis imperfecta was diagnosed at birth, and death occurred on the fifth day of life.

Family 4—A first child of unrelated Anglo-Saxon parents had survived to the age of 21 months. Grossly deforming short limbed dwarfism had been noted at birth, and he was small and extremely fragile. He had survived several respiratory tract infections and was completely cot bound and needed constant care and attention.

FIG 2—Eco RI digested DNA from three sets of parents and one affected patient hybridised with the 40 Kb ³²P nick translated cosH col 1. Track 7 shows abnormal pattern from one affected patient, and tracks 1-6 show patterns from three sets of parents of such children. One of each set (tracks 1, 3, and 5) also shows abnormal gene, while tracks 2, 4, and 6 are normal and show only normal 4.3 kb fragment.

Results

The x ray changes were characteristic of the disease and showed broadened, osteoporotic limbs; shortened, widened ribs; and a poorly calcified skull (fig 1). Postmortem examination of two of the children showed characteristic changes with broadened, widened collagen deficient bones (paper in preparation).

Southern blots of DNA from affected patients and four of their eight parents showed clear differences from normal controls when digested with either Eco RI, Bam HI, or double digestions of these enzymes. Eco RI digestion showed heterogeneity of the 3' fragment, which migrated as a doublet of 4.3 and 4.0 kb in affected subjects instead of the normal 4.3 kb single fragment (fig 2). In Bam HI digestions the normal 5.2 kb 3' fragment migrated as a smaller 4.9 kb band in affected children and their carrier parents (not shown). This abnormality was very clearly shown with a double digestion with Eco RI and Bam HI (fig 3). Normally the 4.3 kb Eco fragment is

cleaved into two pieces of 3.2 and 1.1 kb while clinically normal heterozygous parents and affected patients have an extra band of 2.9 kb (fig 3). Equally convincing results were obtained whether the whole cosmid (not shown) or the 3' Eco RI fragments were used as probes. There were clear differences between the patterns of carrier parents and those of their spouses. Four of the eight parents (one from each pair of parents of the four affected children) showed a restriction pattern similar to that of the affected child. Four affected children and their parents therefore showed the characteristic Eco/Bam doublets (fig 3). The restriction pattern of the second parent of each pair was invariably normal in this respect (figs 2 and 3).

FIG 3—DNA double digested with Eco RI and Bam HI and probed with ^{32}P nick translated 3. Eco RI 4.3 kb fragment of cosH col 1. Tracks 1-6 show patterns from parents of patients with broad boned lethal osteogenesis imperfecta congenita shown in tracks 7-9. One track from each set of parents shows normal 3.2 and 1.1 kb fragment (tracks 2, 4, and 6), while tracks 1, 3, and 5 have extra 2.9 kb band which is also present in affected patients.

Despite numerous experiments with other collagen probes such as cDNA clones to $\alpha 1(\text{I})$ and $\alpha 2(\text{I})$ and genomic probes to $\alpha 2(\text{I})$ we were unable to find any other consistent differences which identified a second abnormality in the other parents or the affected children. We also analysed the collagens and procollagens secreted into the medium by cultured skin fibroblasts from our four patients. The collagens showed a characteristic delay in the migration of the collagen $\alpha 1(\text{I})$ chains (fig 4). This change was abolished in the presence of α , α' -dipyridyl and appeared to be caused by post-translational over-hydroxylation of certain lysine residues.

Discussion

This paper describes a specific 300 base pair deletion in an $\alpha 1(\text{I})$ -like collagen gene in four children with lethal broad boned osteogenesis imperfecta and four of their eight parents. We have not observed similar changes in any other form of the disease, nor in over 400 samples from normal controls; we have, however,

identified a closely similar gene deletion in two patients with Ehlers-Danlos syndrome type II. This is consistent with a carrier frequency of less than 1/1000 based on Silience's estimate of 1/60 000 for all patients with type II osteogenesis imperfecta, since only some of these would have the broad boned lethal form of the disease. Because the defect is detectable with more than one restriction enzyme, probably it is not caused by a restriction site polymorphism, and the deletion itself is unlikely to be polymorphic in light of its rarity and its specificity for this disease. In any case the deletion served as a useful marker of this disease in these particular families.

Intrauterine diagnosis would be accurate in the 50% of pregnancies which do not carry the Eco/Bam doublet, and carriers of the gene either would have lethal osteogenesis imperfecta or be clinically normal heterozygotes. Either the whole cosmid or the 3' 4.3 kb Eco RI fragment could be used as probes. The patterns obtained with the latter are simple and easy to interpret, especially when double digestions with Eco RI and Bam HI are used. This would therefore be the probe of choice with small quantities of DNA such as those from trophoblastic biopsy or amniocentesis. Pregnancies carrying the marker gene might then be terminated immediately or followed up to full

FIG 4—Photofluorogram of radiolabelled collagens produced in culture. Conditions arranged so that $\alpha 1(\text{III})$ separated from $\alpha 1(\text{I})$ and $\alpha 2(\text{I})$ chains. Tracks 1 and 5 show patterns from normal controls, and tracks 2-4 are from patients with lethal osteogenesis imperfecta showing delayed migration of $\alpha 1(\text{I})$ chains.

term if ultrasound of the fetus confidently excluded the disease. Each of our patients with lethal osteogenesis imperfecta carried a gene deletion which caused minor clinical abnormalities in their carrier parents and which has been implicated in the causation of Ehlers-Danlos syndrome type II. Although we were surprised that the first cousin parents in family 1 did not carry the same gene defect, this may be explained statistically, since such people share only one eighth of their genes. Possibly patients with

lethal osteogenesis imperfecta are genetic compounds inheriting from one parent the 300 base pair deletion described here and from the other a so far unidentified collagen abnormality. There are already precedents at the protein level to support this—for example, in the patient with osteogenesis imperfecta and both pro $\alpha 1(I)$ and pro $\alpha 2(I)$ defects described by Byers *et al.*¹⁵ It is noteworthy in this respect that each of our four patients had a delayed $\alpha 1(I)$ migration, suggesting that a subtle defect in $\alpha 1(I)$ collagen might have been the second defect. If this is confirmed then the inheritance of lethal osteogenesis imperfecta in our patients would have been effectively autosomal recessive (doubly heterozygous) and the recurrence risk 25% in the families. Ours would be the first example of the familial transmission of a collagen gene deletion in lethal osteogenesis imperfecta. A 500 base pair deletion in a collagen pro $\alpha 1(I)$ gene has already been identified in this disease¹⁶ and may be an example of the second type of abnormality which we suspect.

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Chronic headache: the role of deformity of the nasal septum

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Abstract

To ascertain a possible causal relation between chronic headache and nasal stenosis arising from deformity of the nasal septum 79 people suffering from both symptoms were observed over one year. On entry to the study they were randomly allocated to either an operative or a control group. The patients in the operative group underwent nasal reconstructive surgery, and the control patients were observed without treatment. Comparison of the two groups showed a considerable decrease in headache after operation.

These findings support the suggestion of a causal relation between chronic headache and nasal stenosis and indicate that a nasal origin should be borne in mind in cases of otherwise unexplained chronic headache.

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Introduction

Many textbooks on otorhinolaryngology state that nasal septal deformity with blocking of the nose can cause chronic headache. The question of a causal relation between the two symptoms has been studied. Hansen and Sherman reported cases in which headache improved after nasal surgery, and others have also reported their experience. We are unaware of any reports of prospective controlled trials on this subject. We therefore carried out a controlled clinical trial to assess the effect of nasal reconstructive surgery on chronic headache in patients with nasal airway obstruction.

Patients and methods

The study was carried out from April 1980 to November 1982. Eighty three patients referred to the ear, nose, and throat department for nasal septal deformities met the criteria for inclusion in the study—namely, a history of chronic headache for at least two years; nasal obstruction due to septal deformity; and a lower age limit of 15. Patients with facial pain of purely neuralgic character or with sinusitis were not included.

Patients on the waiting list for nasal surgery were allocated to one of two groups. Those at the top of the waiting list were assigned to undergo operation; those on the waiting list who were not due to be admitted for operation for a year (the waiting time then being about two years) served as controls. Thus the patients in the two groups entered the trial at the same time and consecutively from two separate places on the waiting list without any selection.