Distinct Biochemical Phenotypes Predict Clinical Severity in Nonlethal Variants of Osteogenesis Imperfecta

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Summary

We reviewed clinical and biochemical findings from 132 probands with nonlethal forms of osteogenesis imperfecta (OI) whose fibroblasts were sent to the University of Washington for diagnostic studies in the years 1981–87. In cells from 86% of probands with nonlethal OI we identified biochemical alterations compatible with heterozygosity for a mutation that affected expression or structure of α chains of type I procollagen. We observed two major biochemical phenotypes. Cells from 40 probands (group A) secreted about half the normal amount of normal type I procollagen and no identifiable abnormal molecules; these patients were generally of normal stature, rarely had bone deformity or dentinogenesis imperfecta, and had blue sclerae. Cells from 74 probands (group B) produced and secreted normal and abnormal type I procollagen molecules; these patients were usually short and had bone deformity and dentinogenesis imperfecta, and many had grey or blue-grey sclerae. In cells from an additional 18 probands (group C) we were unable to identify altered type I procollagen synthesis or structure. Detection of these abnormalities has value in the determination of mode of inheritance and in the prediction of clinical severity.

Introduction

Osteogenesis imperfecta (OI) is a generalized connective-tissue disorder characterized principally by osseous fragility. The clinical spectrum in OI ranges from death in the perinatal period to mild osseous fragility in adulthood. About 10 years ago Sillence et al. (1979) proposed a classification of OI on the basis of clinical, radiographic, and genetic features that distinguished four types: OI type I (mild, dominantly inherited OI with bone fragility and blue sclerae), OI type II (perinatal lethal), OI type III (progressive deforming), and OI type IV (dominant with normal sclerae and mild deformity). Recent linkage studies using polymorphic loci in the genes that encode the chains of type I collagen (Tsipouras et al. 1983, 1984; Sykes et al. 1986, 1990; Wallis et al. 1986), and biochemical and molecular

genetic studies (reviewed in Byers 1989) have shown that most individuals with dominantly inherited forms of OI have mutations in either the COL1A1 or COL1A2 genes, which encode the proα1(I) and proα2(I) chains of type I procollagen, respectively. It has been suggested that the clinical heterogeneity observed in OI might be explained by the nature and location of the mutations in the COL1A1 and COL1A2 genes and by the effects of these mutations on the structure and synthesis of type I collagen molecules (Byers 1989). Analyses of the type I procollagen molecules synthesized by dermal fibroblasts cultured from affected individuals with OI have identified two broad biochemical groups: (1) those who synthesize and efficiently secrete about half the expected amount of structurally normal type I procollagen (Barsh et al. 1981; Genovese and Rowe 1987; Willing et al. 1990) and (2) those who produce both normal and abnormal populations of molecules which are then secreted (Bonadio et al. 1985; Bonadio and Byers 1986; Wenstrup et al. 1986).

To determine whether biochemical studies correlate with phenotype, we reviewed the clinical features of 224 individuals with nonlethal OI on whom we per-

formed analyses of collagens synthesized by cultured dermal fibroblasts during the 7 years of 1981–87. We can identify alterations in the amount or structure of type I procollagen synthesized in cells from more than 86% of the 132 probands for whom we have adequate clinical descriptions. A decrease in the amount of normal type I procollagen synthesized generally resulted in the mildest OI phenotype, while synthesis of some normal and some abnormal type I procollagen molecules resulted in phenotypes that varied from mildly deforming bone disease with normal or slightly short stature to severely deforming bone disease with moderate to marked short stature.

Methods

Assessment of Clinical Information

Clinical information was requested from referring physicians who sent cultured cells for type I collagen analysis. Thirty-five of the 132 probands included in the study were examined by one or more of the authors (R.J.W., M.C.W., or P.H.B.).

Age, height or description of stature, presence of bone deformity, presence or absence of dentinogenesis imperfecta, assessment of hearing status, family history of OI, and the referring diagnosis of OI type according to the Sillence classification were recorded for each individual. Fracture number was often not included in clinical descriptions and for some patients could only be estimated and thus was not included in the data collected.

An individual was considered short if his or her height was at or below the third percentile for age or if the referring clinician stated that the patient had short stature. Bowing of extremities was based on X-ray reports of the femurs, tibia, and humerus. Wormian bones were not considered as bone deformities. Bone deformities due to misalignment of specific fractures were also discounted. Dentinogenesis imperfecta was considered present if the teeth were opalescent, discolored, or fragile. Hearing was assessed by audiometric studies. Because many patients were children or young adults who were below the age at which hearing loss might be expected, we did not include hearing status in our analyses. The presence of other affected family members was determined by family history, physical examination, and, in some instances, by biochemical analysis of collagen synthesized by fibroblasts from other family members. Scleral hue was determined by recording the reported or apparent scleral hues and by inspection. Color cards were not used.

We included patients in the analysis if we had information on height and bone deformity and on either dentinogenesis imperfecta or scleral color or both. Of the 92 nonlethal OI probands excluded for lack of clinical information, in 41 there were no data on height, long-bone deformity, scleral color and dentinogenesis imperfecta; in 48 there was no information on height and long-bone deformity; and in three for whom there was information on height and deformity there was no information on scleral hue and dentinogenesis imperfecta.

Analysis of Procollagens and Collagens Synthesized by Dermal Fibroblasts Cultured from OI Probands

Thirty-five of the 132 cell strains included in the present study were from biopsies obtained by one of the authors. The remaining 97 cell strains were sent from other institutions and were adapted to our culture conditions prior to being labeled with radioactive amino acids. Most biopsies were obtained from skin of the upper arm. The cultures were maintained in Dulbecco-Vogt modified Eagle medium containing 10% FCS (GIBCO), 100 U penicillin/ml, 100 µg streptomycin/ml, and 2.5 mM glutamine in a humidified atmosphere of 9% CO_2/air at 37°C. Control cell strains were obtained from individuals who showed no evidence of connective-tissue disease. All samples were obtained with appropriate consent.

[^{3}H]proline-labeled procollagens were prepared, harvested, and separated in SDS–polyacrylamide gels according to a method described elsewhere (Bonadio et al. 1985). The collagen α chains were prepared by partial proteolysis of [^{3}H]proline-labeled procollagen with pepsin. Proα and α chains were detected after electrophoresis by radioautofluorography using EN^{3}HANCE (Dupont–New England Nuclear) as the fluor.

Results

In the years 1981–87, we analyzed fibroblasts from 420 individuals with OI (table 1). Of the affected individuals, 351 were probands and 69 were affected family members identified as a result of family studies. One hundred twenty-seven of the probands had the perinatal lethal form of OI; clinical, genetic, and biochemical findings on many of these have been reported elsewhere (Byers et al. 1988). Two hundred twenty-four probands had nonlethal forms of OI. We had sufficient clinical information to determine the severity of the phenotype in 132 individuals who form the study population in the present report.

Table I

OI Cell Strains Analyzed at the University of Washington 1981–87

Category	N
All OI cell strains	420
OI probands[a]	351
Nonlethal OI probands[b]	224
Nonlethal OI probands with clinical information (study population)[c]	132

[a] If more than one family member was affected, only the proband in a family was included.

[b] Clinical and genetic analyses of many lethal OI probands analyzed in this period have been previously reported.

[c] Chosen on the basis of availability of comprehensive clinical information.

We identified two major types of alterations in the synthesis or structure of type I procollagen among these cell strains. Forty of 132 cell strains (group A) secreted about half the normal amount of structurally normal type I procollagen, as evidenced by the decreased ratio of type I to type III procollagen secreted into the culture medium (fig. 1). No abnormal type I collagen molecules were secreted. Cells from 74 (group B) of the 132 patients produced normal and abnormal populations of proα1(I) and proα2(I) chains of type I procollagen. The abnormal proα chains and the α chains produced from them by limited proteolytic digestion with pepsin had electrophoretic mobilities that were slower than both (a) those of the normal molecules produced by the same cells and (b) those produced by control cells (fig. 1). The apparent increase in molecular weight was due to increased posttranslation modification (especially lysyl hydroxylation and glycosylation of hydroxylysine) in the triple-helical domain of molecules that incorporate an abnormal chain (Byers 1989). Five of the 74 cell strains in group B produced type I procollagen molecules in which specific abnormalities could be identified directly from the screening analysis (fig. 1). Eighteen cell strains (group C) synthesized type I procollagen molecules that we could not distinguish from those synthesized by control cell strains.

Most of the individuals in group A had very mild OI: only 12% had short stature, 8% had long-bone deformity, 12% had dentinogenesis imperfecta, and all had blue sclerae (fig. 2). In contrast, 92% of those in group B were short statured, 85% had long-bone deformity, and 76% had dentinogenesis imperfecta; 85% also had blue or grey sclerae. In the remaining 13% of individuals (group C) we were unable to identify either alterations in the amount of type I procollagen molecules made or the presence of a structurally abnormal population of molecules. Sixty-seven percent of individuals in group C had short stature, 70% had long-bone deformity, and 30% had dentinogenesis imperfecta (fig. 2). Of the 92 probands excluded for lack of sufficient clinical information, 30 had biochemical findings characteristic of group A, 52 had findings characteristic of group B, and 10 had findings indistinguishable from those in the controls (group C).

All but one of the group A patients had been referred with the diagnosis of OI type I (table 2). Among those in biochemical group B, 11% were referred with OI type I, 42% with OI type IV, 33% with OI type III, 8% with OI type III/IV, and 6% with OI of uncertain type. Those in group C were thought to have OI type I (17%), OI type IV (34%), OI type III (29%), or unclassifiable OI (22%). It is surprising that 25 of 31 patients with the clinical diagnosis of OI type III had overmodified pro-α and α chains; OI type III has been considered an autosomal recessive disorder (Sillence et al. 1979), yet these biochemical findings have been associated with heterozygosity for a mutation altering the structure of a type I collagen chain in both lethal and nonlethal OI cell (Byers 1989).

Discussion

Most patients with nonlethal OI have one of two biochemical phenotypes: (1) secretion of approximately half the normal amount of apparently normal type I procollagen or (2) synthesis and secretion of some normal type I procollagen and some molecules in which the constituent chains have undergone increased posttranslational modification along some or all of the triple-helical domain. Individuals in the former group were generally of normal stature, rarely had bone deformity or dentinogenesis imperfecta, and all had blue sclerae. In contrast, individuals in the latter group were usually short statured and had long-bone deformities and dentinogenesis imperfecta. A small group of patients with clinically apparent OI but without clear evidence of type I collagen abnormalities (group D) were usually short and had long-bone deformities and blue sclerae, but only a minority had dentinogenesis imperfecta.

Thus, for the vast majority of individuals with OI, analysis of both the amount of type I procollagen synthesized by cultured fibroblasts and the electrophoretic mobility of the constituent chains yields diagnostic and prognostic information and, even in the absence of family history, establishes the mode of inheritance of the

A Medium · Cells — lanes 1 2 3 4. Labels: FN, proα1(III), proα1(I), proα2(I). Bottom labels: C OI C OI.

B Medium · Cells — lanes 1 2 3 4 5 6. Labels: [α1(III)]₃, α1(I), α2(I). Bottom labels: C OI C OI C OI.
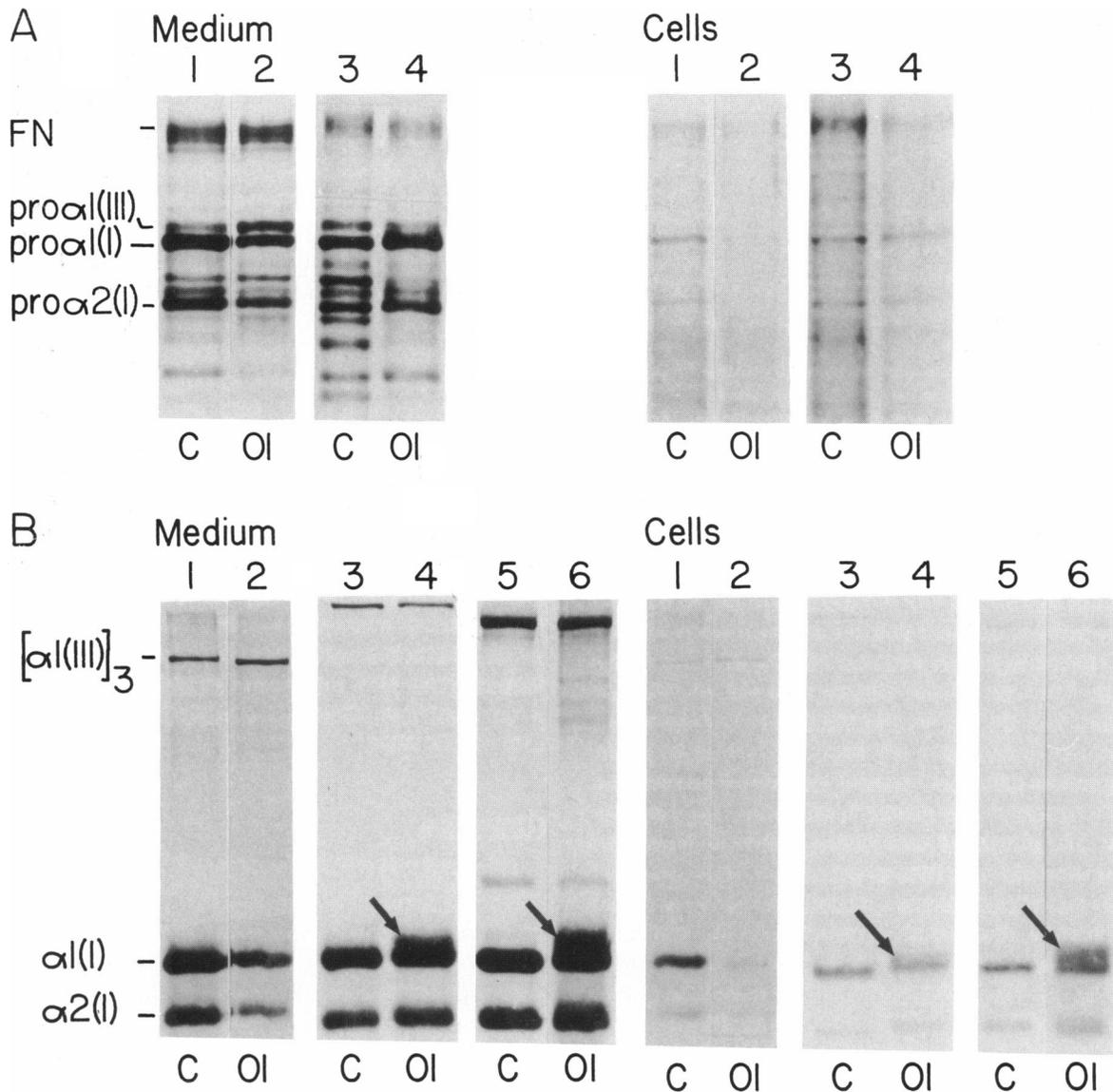
condition. In the small group in which we were unable to find type I collagen abnormalities, we could not exclude the possibility of a mutation in a type I collagen gene.

The majority of individuals we evaluated did not have a family history of OI; of the 86 individuals for whom we had pedigree information, 41% had a positive family history (60% in group A, 30% in group B, and 33% in group C). While this illustrates that new mutations account for some individuals in all groups of OI, we cannot, because of ascertainment bias, estimate accurately the mutation rate. That is, we think we were more likely to be asked to evaluate individuals who were the only affected members of their families than to be asked to evaluate those who were in large families with one or more previously diagnosed individuals. In the latter group, the diagnosis does not usually come into question, and biochemical or genetic analysis may only become an issue either if there is marked variability in the clinical phenotype in members of a single family or if prenatal diagnosis is a concern.

Among individuals with OI who have mild phenotypes and whose cells synthesize about half the normal amounts of type I procollagen, there is relatively little

C

Medium Cells

1 2 3 4   1 2 3 4

FN —

proα1(III)
proα1(I)
proα2(I) —

C OI OI OI   C OI OI OI

D

Medium Cells

1 2 3 4   1 2 3 4

$[\alpha I(III)]_3$ —

αl(I) —
α2(I) —

C OI OI OI   C OI OI OI
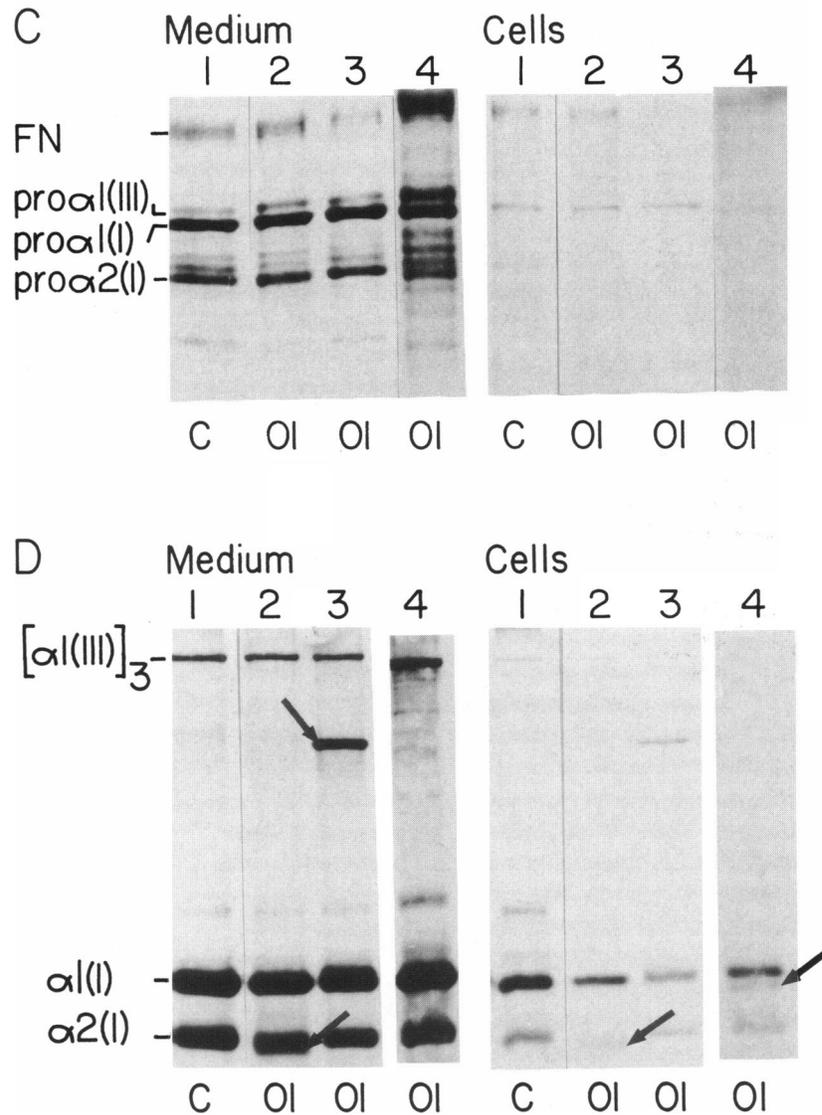
Figure I   Autoradiofluorogram of proα and α chains of collagens synthesized by cells cultured from patients with different forms of OI. *A,* Proα chains in the medium and cell layer of two controls (C) and two patients with OI (OI). Lanes 2 contain proα chains secreted into the medium and retained within the cell layer from a patient with the clinical diagnosis of OI type I and a group A biochemical phenotype. Lanes 4 contain proα chains secreted into the medium or retained within the cell layer from a patient with the clinical diagnosis of OI type IV and a group B biochemical phenotype. Bands below proα2(I) are proteolytic conversion products from proα chains to α chains. *B,* Pepsin-treated proα chains (α chains) of type I collagen and type III collagen from the same patients as in *A.* Lanes 5 and 6, in both medium and cell layer, contain molecules synthesized by cells from a patient with the clinical diagnosis of OI type III and a group B biochemical phenotype. In OI type I, cells synthesize and secrete about half the normal amount of type I procollagen. In OI type IV and OI type III, cells synthesize normal type I procollagen and some molecules which undergo increased posttranslational modification which results in delayed electrophoretic mobility (see arrows in *B,* lanes 4 and 6 of medium and cells). *C* and *D,* proα and α chains, respectively, synthesized by cells from patients with forms of OI in which the molecular lesions are directly identifiable. Cells from the patient with OI type IV (Lanes 2) synthesize a population of proα2(I) chains from which a small region in the triple-helical domain between residues 6 and 327 was deleted (arrows indicate chains with faster than normal mobility). The position of the deletion was determined by analysis of cyanogen bromide peptides of α2(I) chains (data not shown). Cells from the patient with OI type IV (Lanes 3) synthesized some proα1(I) chains that contained a cysteine residue in the triple-helical domain and resulted in formation of a disulfide-bonded dimer (see arrow) when the α chains were run under nonreducing conditions. Cells from the patient with OI type I (lanes 4) synthesized two populations of proα1(I) chains, one of which was normal and one of which had deleted 33 amino acids in the triple-helical domain encoded by a single exon.
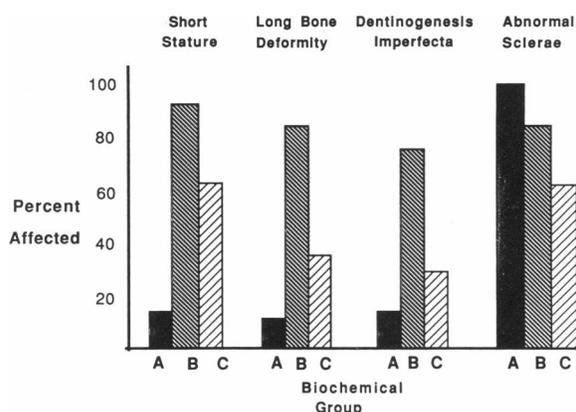
Figure 2 Prevalence of major complications of OI in each major biochemical phenotypic group. Group A shows decreased production of normal type I collagen molecules; group B shows production of both normal and overmodified populations of type I collagen molecules; and group C shows no apparent abnormality.

variation in the degree of clinical severity, and the biochemical phenotype also tends to be uniform. The decrease in production of type I procollagen by cells from individuals with OI type I may result from one of several mechanisms: loss of COL1A1 allele that encodes the proα(I) chains of type I procollagen, mutations that affect transcription or processing of the COL1A1 transcript (Genovese and Rowe 1987), or failure to incorporate an abnormal proα1(I) chain into a type I procollagen molecule (Willing et al. 1990). In all of these cases, a decreased pool of proα1(I) chains limits the rate of type I procollagen assembly and production (fig. 3). The secretion of half of the normal amount of type I procollagen is generally accompanied by continued synthesis of normal amounts of other matrix proteins, so that the aberrant matrix presumably reflects altered ratios of components.

Among individuals whose fibroblasts synthesize an abnormal population of type I procollagen, there is a wide range of clinical phenotypes. Cells both from individuals who have only mild short stature and mild deformity and from those who have severely deforming OI—even perinatal lethal OI—may be difficult or impossible to distinguish by SDS-PAGE of proα and α chains of type I collagen.

In contrast to cell strains from group A, those in group B secrete less than the normal amount of structurally normal type I procollagen molecules and, in addition, synthesize and secrete structurally abnormal molecules. The production of abnormal type I procollagen by cells from individuals with deforming varieties of OI has been demonstrated to result from heterozygosity for mutations in COL1A1 or COL1A2 alleles (fig. 3), most commonly those which result in substitutions for single glycine residues in the triple-helical domain. Several substitutions for glycine residues have been demonstrated in cells from patients with the perinatal lethal form of OI (OI type II) (Cohn et al. 1986; Bateman et al. 1987, 1988; Vogel et al. 1987; Baldwin et al. 1988; Constantinou et al. 1989), and evidence of similar mutations in COL1A1 and COL1A2 that result in nonlethal deforming OI (Wenstrup et al. 1988, and in press; Starman et al. 1989; C. J. Pruchno, G. A. Wallis, D. H. Cohn, M. C. Willing, B. J. Starman, and P. H. Byers, unpublished data) is emerging. Propagation of a normal triple helix during assembly of type I procollagen molecules depends on the presence of glycine in every third position (reviewed in Byers 1989). Substitution for any glycine residue either delays triple-helix propagation or alters the triple-helical structure so that additional posttranslational lysyl hydroxylation and hydroxylysyl glycosylation (which normally cease when a triple helix is formed) can continue. The increased modification occurs largely amino-terminal to the mutation site within the triple-helical domain of all chains in molecules that contain one or more abnormal chains, and it may contribute to altered rates of secretion and

Table 2

Clinical Presentation and Initial Clinical Diagnosis of Nonlethal OI Probands

	OI Type[a]					
BIOCHEMICAL FINDINGS	I	IV	III/IV[b]	III	U[c]	Total
Group A: decreased type I/type III ratio	39			1		40
Group B: overmodified proα and α chains	8	31	6	25	4	74
Group C: normal findings	3	6		5	4	18

[a] Classification is that of Sillence et al. (1979).
[b] Phenotype of severity intermediate between OI type III and OI type IV.
[c] No OI subtype could be applied.

Normal

8 pro α1(I)
4 pro α2(I)   ➡   4 procollagen

Mild OI (Group A)

4 pro α1(I)   2 procollagen
4 pro α2(I)   ➡   +
                                             2 pro α2(I)
                                                (degraded)

Deforming OI (Group B)

8 pro α1(I)
2 pro α2(I)   ➡   2 procollagen
2 pro α2(I)*   2 procollagen*

OR

4 pro α1(I)   1 procollagen
4 pro α1(I)*   ➡   2 procollagen*
4 pro α2(I)
                                             1 procollagen*
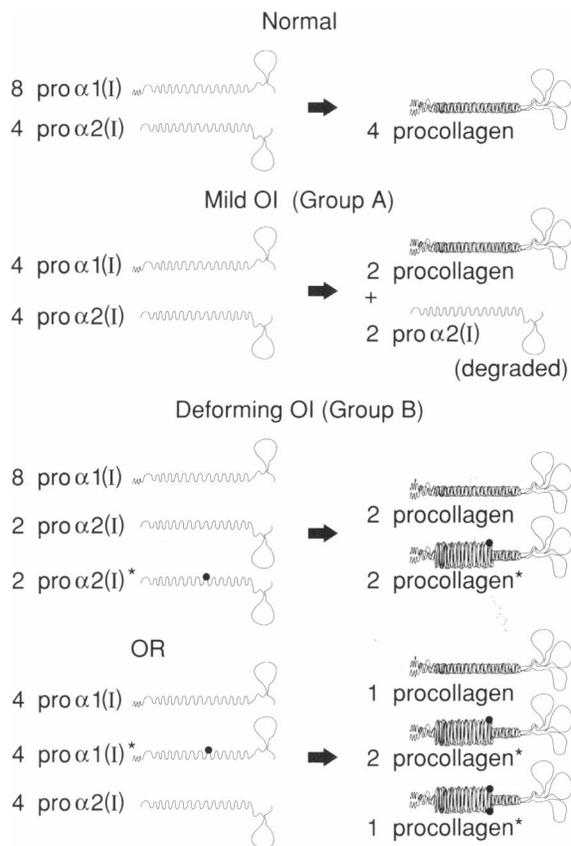
Figure 3   Stoichiometric relationship of normal and abnormal α chains of type I collagen resulting from dominant mutations which produce the observed biochemical phenotypes in OI. Group A corresponds best to OI type I, and group B is represented by OI types III and IV. Molecules which contain mutant chains (the site of mutation is indicated by the black dot in the triple-helical domain) undergo increased posttranslational modification amino-terminal to the site of mutation, may be inefficiently secreted with increased degradation within cells, and result in defective collagen fibrillogenesis. The broadened area of the triple helix depicts "overmodification."

abnormal collagen fibrillogenesis that would alter bone mineralization.

The severity of the clinical phenotype resulting from substitutions for single glycine residues in the triple-helical domain of either proα1(I) or proα2(I) chains probably is determined by the chain in which the substitution occurs, the position of the substitution in the chain, and the nature of the substituting residue. Substitutions for glycines may be less deleterious in α2(I) than in the same region of α1(I) (Vogel et al. 1987; Byers 1989; Wenstrup et al., in press). In addition, identical substitutions for glycine in proα1(I) chains are clinically more severe when they are near the carboxyl-terminus of the triple helix than when they are near the amino-

terminal end (Starman et al. 1989). This latter effect probably reflects the extent of posttranslational modification, the efficiency of secretion of the molecules, and the rate at which the abnormal molecules are assimilated into the matrix. Because mutations near the amino-terminal end of the triple-helical domain may have relatively little effect on posttranslational modification or secretion efficiency, they may be difficult to detect and probably account for some of the individuals in group C. While we do not yet have enough data on the nature and location of point mutations that result in the deforming varieties of OI to predict the precise phenotype, it is clear that such mutations generally result in more severe phenotypes than do mutations that only affect the amount of type I procollagen produced. Some of the difficulty in classification of OI reflects the complex relationship between mutations and phenotype.

In terms of practical benefits to the patients and their families, investigation of the biochemical basis of OI in sporadically affected individuals can help to define the mode of inheritance, the expected recurrence risk among siblings and children of the affected individual, and, to some extent, the natural history. Because the sensitivity of the assay is high, it can also be useful in the context of an evaluation for suspected child abuse when fractures have been detected. We believe that the biochemical findings in group A or group B show virtually 100% specificity for OI. Furthermore, because chorionic villus cells cultured from the placentas of fetuses affected with different forms of OI synthesize the same abnormal collagens as do their skin cells, such studies can also facilitate prenatal diagnosis for individuals where linkage analysis is not feasible (P. H. Byers and B. J. Starman, unpublished data). Finally, these studies suggest that strategies aimed at treatment of OI should take into consideration the nature of the biochemical defect and should be targeted appropriately for each biochemical phenotype.

Acknowledgments

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