Review article

Osteogenesis imperfecta: translation of mutation to phenotype

Peter H Byers, Gillian A Wallis, Marcia C Willing

By the end of the recently completed Fourth International Conference on Osteogenesis Imperfecta (Pavia, Italy, 9-12 September 1990) more than 70 mutations in the two genes that encode the chains of type I collagen, the major protein of bone, had been identified as the molecular cause of different forms of osteogenesis imperfecta (OI). Although by no means complete, the set of mutations in hand provides a rough guide to how to predict the phenotypic effects of mutations in type I collagen genes, predicts that certain classes of mutations will give rise to very mild phenotypes that will blend with common disorders, such as osteoporosis, and clarifies the genetic aspects of the widely used clinical classification of OI.¹

Clinical classification of OI

For almost a century clinicians have attempted to provide a rationale by which to understand the readily apparent clinical heterogeneity in OI. The early distinction of 'congenita' forms (those with fractures at birth) from 'tarda' forms (those who develop fractures at a later period),² followed by acknowledgment of further phenotypic variation,³ provided early guides. About a decade ago, these early classifications were largely supplanted, at least for geneticists, by the scheme developed by Sillence *et al*¹ that incorporated genetic, radiographic, and phenotypic criteria. Biochemical and genetic studies have further amended and refined this classification (table). The value of the classification is often questioned because of the difficulty in deciding where

the borders of the types of OI lie and how early a subject can be 'classified'. It is clear that there are molecular reasons for the clinical uncertainties and that they reflect the heterogeneous nature of the causative mutations.

Two classes of mutations, those that reduce the amount of type I collagen synthesised by tissues, and those that alter the structure of the molecules made, are responsible for almost all forms of OI (fig 1). In general, the defective synthesis mutations produce the mildest phenotypes. Mutations that alter the structure of the chains of type I procollagen and lead to the formation of abnormal molecules produce a very wide range of phenotypes, from extremely mild to lethal. Because the severity of the phenotype depends on the chain in which the mutation occurs, as well as the nature and location of the mutation, there is considerable overlap of the phenotypic entities. Molecular studies are now designed, in part, to determine how these mutations produce disease and why OI phenotypes occur in about 1 in 5000 to 1 in 10 000 subjects.

Relevant collagen biosynthesis

To understand how mutations in collagen genes result in osteogenesis imperfecta, it helps to describe the genes and their products, and the manner in which the protein is synthesised and processed by the cell (see references 4 and 5 for more complete reviews of collagen biosynthesis and gene structure). Two genes, COL1A1 located on chromosome 17 and COL1A2 located on chromosome 7, encode the pro α 1(I) and pro α 2(I) chains of type I procollagen, respectively. These genes each contain more than 50 exons that contain the coding sequences for proteins of about 1400 amino acids. The gene structure reflects the domain structure of the protein. The first five exons encode the signal sequence and an amino-terminal propeptide (the function of which is unclear). The sixth exon encodes a protease cleavage site. The next

Departments of Pathology, Medicine, and Pediatrics,
University of Washington, Seattle, WA 98195, USA.
P H Byers, G A Wallis, M C Willing

Correspondence to Dr Byers, Department of Pathology,
SM-30, University of Washington, Seattle, WA 98195,
USA.

Molecular basis of osteogenesis imperfecta.

OI type	Clinical features	Inheritance*	Biochemical and genetic abnormalities
I	Normal stature, little or no deformity, blue sclerae, hearing loss in 50%, dentinogenesis imperfecta is rare and may distinguish a subset	AD	<i>Common</i> 'Non-functional' COL1A1 allele <i>Rare</i> Substitution for glycine residue in carboxyl-terminal telopeptide of $\alpha 1(I)$. Substitution of glycine at position 94 of the triple helix in pro $\alpha 1(I)$. Exon deletion in pro $\alpha 2(I)$ triple helical domain
II	Lethal in the perinatal period, minimal calvarial mineralisation, beaded ribs, compressed femurs, marked long bone deformity, platyspondyly	AD (new) AR (rare)	<i>Common</i> Substitutions for glycyl residues in the triple helical domain of the $\alpha 1(I)$ chain and $\alpha 2(I)$ chain. <i>Rare</i> Rearrangement in the COL1A1 and COL1A2 genes. Exon deletions in triple helical domain of COL1A1 and COL1A2. Substitutions and small deletions in the non-triple helical carboxyl-terminal propeptide. Tripeptide deletion in pro $\alpha 1(I)$ triple helix Small deletion in $\alpha 2(I)$ on the background of null allele
III	Progressively deforming bones, usually with moderate deformity at birth. Sclerae variable in colour, often lighten with age. Dentinogenesis imperfecta common, hearing loss common. Stature very short	AD AR (uncommon)	Point mutations in the COL1A1 and COL1A2 gene Frameshift (4 bp deletion) in COL1A2 that prevents incorporation of pro $\alpha 2(I)$ chains into molecules
IV	Normal sclerae, mild to moderate bone deformity and variable short stature, dentinogenesis imperfecta is common and hearing loss occurs in some	AD	Point mutations in COL1A1 and COL1A2 gene. Exon skipping mutations in COL1A2

*AD, autosomal dominant; AR, autosomal recessive.

43 exons encode the core 1014 amino acid triple helical domain of the chain, that consists of repeating tripeptide units with the sequence of Gly-X-Y in which X and Y exclude cysteine and tryptophan. The final four exons encode an additional protease cleavage site and the globular carboxyl-terminal domain of the protein that is involved in molecular assembly.

The genes are coordinately transcribed, the mRNAs are processed and transported to the cytoplasm where they are translated on membrane bound polysomes. The precursor proteins are inserted through the membrane of the rough endoplasmic reticulum and the signal sequences are cleaved. During chain elongation, all the prolyl residues (about 100 in each chain) that precede glycine in the triple helix and some of the lysines (less than 10 in each chain) are hydroxylated by the enzymes prolyl 4-hydroxylase and lysyl hydroxylase, respectively. Some hydroxylysine residues are glycosylated and a single asparagine linked oligosaccharide is added to each chain in the carboxyl-terminal propeptide.

Each procollagen molecule contains two pro $\alpha 1(I)$ chains and a single pro $\alpha 2(I)$ chain. The correct assembly of molecules is directed by interaction of the pro α chains through their carboxyl-terminal globular domains. After aggregation in the 2:1 ratio the chains fold the triple helix from the carboxyl-terminal end

towards the amino-terminus. The triple helix requires a glycine in every third position to fold correctly. Although the modification by the hydroxylating and glycosylating enzymes is initiated during chain elongation, it ceases only with the formation of a stable triple helix, at which point the molecule is no longer a substrate. The completed molecule is transported out of the cell through the Golgi apparatus and deposited in the pericellular environment where the two propeptides are cleaved from the molecule and the collagen molecules spontaneously aggregate to form fibril structures. The formation of fibrils depends on the uniformity of subunits and abnormal molecules have highly deleterious effects on fibril structure.

Point mutations are common

Despite the repetitive nature of much of the collagen gene (which might be suspected to lead to recombinational events), point mutations are the most common events that lead to OI phenotypes. These mutations may result in substitutions for glycine residues within the triple helix, may produce exon skipping events if they occur in the consensus splice donor or acceptor sites, or may alter the ability of chains to aggregate into molecules if they occur within

Figure 1 Two classes of mutations in type I collagen genes have different effects on collagen molecules. The first (top) decrease the synthesis of type I collagen by cells but the structure of the molecules synthesised is normal. The second (bottom) alter the structure of the molecules and generally have more deleterious phenotypic consequences. (Reprinted from Wenstrup *et al.*,⁷⁴ with permission.)

the domains that encode the carboxyl-terminal pro-peptide.

Point mutations in the triple helix: genotype-phenotype relationship

As a rule, substitutions for glycine residues in the triple helix of the $\alpha 1(I)$ chain have the most severe phenotypic consequences when they occur towards the carboxyl-terminal end of the molecule and are mildest towards the amino-terminal end. However, there is a marked influence of the nature of the substituting amino acid and there may be important effects of local domain structure (fig 2).

Cysteine for glycine substitutions have been the most readily characterised because the presence of cysteine in the triple helical domain of $\alpha 1(I)$, from which it normally is excluded, is detected at the protein level by the formation of a disulphide bonded

dimer of $\alpha 1(I)$ chains. Substitutions of cysteine for glycine at positions 988,⁶ 904,⁷ 748,⁸ 718,⁹ and 691¹⁰ are lethal, those at 526⁹ and 415¹¹ produce an OI type III phenotype, those at 382, 223, 205 (Pruchno *et al.*, unpublished data), 178,¹² and 175¹³ produce an OI type IV phenotype, and those at 94,⁹ 46 (Starman *et al.*, unpublished data), 43,¹⁴ and 19¹⁵ produce even milder phenotypes. Of the two major aberrations from this general picture, a mild phenotype in a woman with a substitution at 904,⁷ and a lethal phenotype with substitution at residue 244,¹⁶ only the former is explained by somatic mosaicism for the mutation which was lethal in her child. The lethal outcome in a child with a cysteine for glycine substitution at 244 is unexpected, given the fairly smooth 'phenotypic gradient', but might be explained by an unidentified structural feature of the molecule in that region, or by death from causes unrelated to OI.

With three exceptions, substitutions of serine for glycine carboxyl-terminal to residue 550 of the triple helix are lethal¹⁷⁻²¹; a substitution at 460 (Willing and Byers, unpublished data) produces an OI type III/IV phenotype. Two mutations, one at 832²² and the other at 844,²³ produce OI type III/IV phenotypes and are flanked by mutations that result in lethal phenotypes. Mosaicism has not, apparently, been excluded in either instance but the possibility of a tolerant domain in the molecule has also been suggested. A very severe but non-lethal phenotype that results from a substitution at 1009¹⁹ may reflect unusual effects of mutations very near the end of the triple helix.

Two mutations that result in substitution of arginine for glycine (at residue 154²¹ in unrelated subjects) are not lethal while the others that produce the same substitution in different domains²⁴⁻²⁸ (Willing and Byers, unpublished data) and all those identified so far that substitute valine²⁸⁻³⁰ or aspartic acid^{31,32} (Starman *et al.*, unpublished data) for glycine are lethal. The paucity of mutations that result in substitutions of alanine²⁸ for glycine suggests that in many regions of the gene such an event would produce a mild phenotypic effect. Thus, because of the bias toward studying mutations in the lethal phenotype, the substitutions that result in mild phenotypes have not been identified.

In contrast to substitutions for glycine within the triple helix of $\alpha 1(I)$, for the far smaller number of similar mutations within $\alpha 2(I)$ there is no semblance of an orderly relationship between phenotype and position or nature of the substituting residue. For example, cysteine for glycine substitutions which produce a mild phenotype (at residue 646)³³ in dominant families may be flanked by those which are more severe or lethal in effect.³³⁻³⁵ Perhaps the nature of the surrounding sequence or of a special domain within the chain explains this finding. Most

Figure 2 Map of point mutations in COL1A1 (top) and COL1A2 (bottom) that result in substitutions for glycine within the triple helical domain of the $\alpha 1(I)$ and $\alpha 2(I)$ chains, respectively. For each locus, the location of each point mutation is represented along the chain. The substituting residue is listed on the left and the residue number of the substituted glycine is listed along the line. The triple helix contains 1014 amino acids and the first glycine is represented as residue 1. The phenotype of each mutation is denoted below the mutation.

other point mutations in the COL1A2 gene have proved to be lethal³⁶⁻³⁹ although a serine for glycine at 661,⁴⁰ a valine for glycine at 586,²⁸ and an arginine for glycine at 1012⁴¹ are notable exceptions. Because linkage between mild dominant forms of OI and polymorphic loci in COL1A2 has been shown,⁴²⁻⁴⁵ point mutations in the COL1A2 gene may prove to be common once the mutations in these families have been identified. It should be noted, however, that a significant number of the mutations in the COL1A2 gene from mildly affected subjects and their families result in exon skipping or in insertion of intron material (see below).

Exon skipping mutations within the triple helix

A consequence either of point mutations or of small genomic deletions, exon skipping mutations in the type I collagen genes are the second most common cause of OI characterised (fig 3). For the COL1A1 gene and the pro $\alpha 1(I)$ chain, the majority of mutations that give rise to exon skipping events are either very severe or lethal^{46, 47} (Wallis *et al*, unpublished data). Mild effects can occur, however, if the mutation involves exons near the 5' end of the gene (for example, exon 8, encoding amino acids 19 to 36 of the triple helix)⁴⁸ or if the proportion of chains synthesised by the mutant mRNA is small (for example, exon 17

Figure 3 Map of the location of exon skipping mutations in the *COL1A1* (top) and *COL1A2* (bottom) genes. The maps of the two genes are drawn roughly to scale but the sizes of the introns are more accurately denoted for *COL1A1*. Note that in *COL1A1* exons 33 and 34 are fused to form a single 108 bp exon and that the *COL1A2* gene is about twice the size of the *COL1A1* gene. In each case, except for exon 14 in *COL1A1*, the affected subject is heterozygous for the mutation. The splicing scheme indicates that one allele is processed normally but the other allele results in an mRNA from which the exon indicated has been skipped. There is inefficient splicing of exon 14 in both the *COL1A1* alleles in the subject with OI type II that results in about half the mRNA from each allele having the deleted structure.

skipping with a low proportion of the abnormal product).⁴⁹

For the *COL1A2* gene, exon skipping mutations display an orderly gradient of phenotypic expression from lethal to quite mild as they progress from the 3' to the 5' end of the helix coding domain.^{14 50–54} The transition from lethal to non-lethal appears to occur between exons 28 and 21, but the extent to which instability of the abnormal mRNA contributes to the phenotype is unclear. This trend predicts that mutations even closer to the 5' end than exon 9 may give surprisingly mild phenotypes. In both *COL1A1* and *COL1A2*, skipping of exon 6, which contains the amino-terminal procollagen protease site, produces the Ehlers-Danlos syndrome type VII phenotype,^{55 56} a dramatic shift from OI.

Point mutations in the non-triple helical carboxyl-terminal domains

A small number of mutations has been identified outside the triple helical domain in the *COL1A1* gene. Substitution of cysteine for the glycine three residues removed from the carboxyl-terminal end of the triple

helix has a mild effect.⁵⁷ Similarly, substitution for a single cysteine in the carboxyl-terminal propeptide by tryptophan has a mild effect, perhaps because the chain is excluded from molecules¹⁷ (see below). In contrast, substitution of arginine for leucine⁴⁷ in a conserved region of the carboxyl-terminal domain results in a lethal phenotype, apparently because the abnormal chain interferes with secretion of the procollagen molecules into which it is incorporated.

Multiexon rearrangements are lethal when expressed in proteins but shorter deletions may be milder

Despite the repetitive nature of the collagen genes, rearrangements within *COL1A1* and *COL1A2* are rare causes of osteogenesis imperfecta and, when they have occurred, have been lethal. A three exon deletion from within the triple helix coding domain of the *COL1A1* gene^{58 59} and a seven exon deletion from within *COL1A2*⁶⁰ have been identified, although the mechanisms by which they occurred are uncertain. A more complex partial duplication within *COL1A1* was also lethal and presumably arose by recombination.⁶¹

In contrast, shorter deletions have different effects depending on the mutation. For example, two independent mutations which each remove one Gly-Ala-Pro triplet from the three-fold repeat between 868 and 876 of the $\alpha 1(I)$ chain have been lethal.^{20 62} A three amino acid (Gly-Pro-Pro) deletion near the carboxyl-terminal end of the triple helix of the $\alpha 2(I)$ chain (residues 1009 to 1011) is, however, mild (Wallis *et al*, unpublished data). Deletion of the three nucleotides that encode the valine at position 255 of the $\alpha 2(I)$ chain produces the OI type III phenotype.⁶³

Frameshift mutations have differing effects that depend on their location

Frameshift mutations within the triple helical coding domain have proved difficult to identify and none has yet been characterised. In contrast, a frameshift mutation in COL1A2 that alters the coding sequence by insertion of intron sequence in the triple helix,⁶⁴ and a frameshift mutation in the carboxyl-terminal domain of COL1A2,⁶⁵ have, surprisingly, resulted in mild phenotypes. The mild phenotype of the six amino acid insertion between residues 585 and 586 of the triple helix may result from the intracellular retention of molecules that contain the abnormal chain, or from instability of the abnormal mRNA, or both.

Frameshift mutations in the carboxyl-terminal domains of the two genes differ in their phenotypic effect. One mutation, a single base insertion in a COL1A1 allele,⁶⁶ has a lethal effect, apparently because the molecules that contain the mutant allele are not secreted efficiently, leading to a paucity of normal molecules in the matrix. In contrast, one mutation in the COL1A1 gene⁶⁷ and another in the COL1A2 gene⁶⁵ have mild phenotypic effects in the heterozygous state because they prohibit incorporation of the abnormal chains into molecules. The COL1A2 frameshift does, however, produce a moderately severe phenotype in the homozygote,⁶⁸ the consequence of synthesising type I procollagen molecules that contain only pro $\alpha 1(I)$ chains.

Translation of mutation to phenotype

The phenotypic consequence of mutations in type I collagen genes reflects the gene in which the mutation occurred, the nature of the mutation, the location of the mutation, and the effect of the mutation on the behaviour of the abnormal chain and on the behaviour of molecules formed by the chain.

Excluded allelic products generally produce mild phenotypes

In general, mutations that prohibit incorporation of an abnormal chain into a molecule, or that simply reduce the amount of the chain available, would be expected to have mild clinical consequences⁶⁹ and are

found most often among subjects with OI type I (fig 1).^{67 70 71} Very few such mutations have yet been identified, probably because of the difficulty in detecting mutations that lower the expression of the abnormal allele. These mutations may have to be identified at the genomic level, a formidable task with genes that encompass 18 kb and 38 kb and have more than 50 exons apiece.

Included but abnormal products vary in severity

If abnormal chains are included in molecules and those molecules are at least moderately stable, the phenotypic effects are more deleterious than if an abnormal chain does not aggregate into a molecule. If, however, an abnormal chain leads to very rapid intracellular degradation of molecules that incorporate the chain, the clinical consequences should differ depending on the gene in which the mutation occurs. Mutations in the COL1A1 gene may be highly deleterious and even lethal because they destroy three-quarters of all the type I procollagen molecules synthesised (half of all molecules contain one abnormal pro $\alpha 1(I)$ chain and a quarter contain two abnormal pro $\alpha 1(I)$ chains) (fig 1).⁶⁶ A similar mutation in the COL1A2 gene could result in loss of only half the molecules made and so might be similar in effect to a null COL1A1 allele (that is, only half the normal amount of type I procollagen molecules are completed in either case) but only if none of the abnormal molecules leaked from the cell.

When an abnormal chain is included in a molecule, the translation of mutation to phenotype depends on several factors. First, almost all molecules that contain chains with mutations in the triple helical domains are less stable than their normal counterparts and are secreted less efficiently. Second, the mutation alters the structure of the mature molecule so that during processing in the rough endoplasmic reticulum the chains remain accessible to the modifying enzymes and undergo additional hydroxylation of lysyl residues in the triple helix and additional hydroxylysyl glycosylation. These molecules may have an extremely long period of residence in the rough endoplasmic reticulum (thus altering cellular architecture and perhaps other functions of the cell). Third, the abnormal molecules that leave the cell are not proteolytically processed as efficiently as the normal molecules and interfere with the normal process of fibril nucleation and growth. Fourth, abnormal fibrils are probably inefficient substrates for mineralisation. Finally, the requirements of bone for molecular structure may be greater than those of skin and other soft tissues to explain, in part, the relative tissue specificity of these mutations.

Point mutations and deletions (large or small) affect the intracellular processing of molecules in much the same way, probably because both introduce phase

shifts in chain registration that reduce stability.^{67 72} That is, chains in these molecules become over-modified and the molecules are secreted inefficiently. As a result, the phenotypic consequences of diverse mutations are similar. There is not yet a good explanation for the effects of specific substitutions for glycyl residues in the triple helix, but the size of the substituting residue and interactions with nearby residues probably play a role in determining the phenotypic effect.

Genetics of osteogenesis imperfecta

As a result of biochemical, molecular genetic, and linkage studies there is now little question that the vast majority of subjects with OI are heterozygous for mutations in one of the genes that encode the chains of type I procollagen.^{45 73 74} There are rare instances of homozygosity^{46 65} or compound heterozygosity⁷⁵ for mutations in these genes. There are also uncommon forms of OI that appear to result from mutations in non-collagen genes and are probably inherited in an autosomal recessive fashion.⁷⁶

There has been controversy about the mode of inheritance of the lethal forms of OI and of some of the deforming varieties. Some clinical studies appeared to provide evidence for autosomal recessive inheritance of lethal OI¹ and of recessive inheritance of the progressive deforming varieties of OI.⁷⁷ More recent clinical studies⁷⁸ and biochemical and molecular evidence⁷³ have now made it clear that the lethal form of OI almost always results from heterozygosity for mutations in one of the genes of type I collagen (uniparental disomy for an exon skipping mutation provides a remarkable exception, especially as the mutation results in inefficient exon removal⁴⁶). Biochemical studies, and some molecular studies, provide the same conclusion for deforming varieties of OI.²¹⁻²³ Despite the molecular evidence of heterozygosity for dominant mutations, recurrence of these phenotypes among sibs born to phenotypically normal parents is not rare. Indeed, for OI type II, the sib recurrence rate is about 6%.⁷³

The modest recurrence risk for OI type II is explained by parental gonadal, and usually somatic, mosaicism in the vast majority of such families.³² The low recurrence risk for OI type III is probably explained by two different mechanisms: parental mosaicism and rare instances of autosomal recessive inheritance, possibly involving non-collagen genes.

The rate of parental mosaicism for collagen gene mutations seems high when compared to other disorders, but comparable studies in other genes have not been completed.

Mutation mechanisms

Collagen genes are probably good reporters of mutations. First, there is a high density of invariant

and required glycine residues in the triple helical domain (one-third of all amino acids). Substitution for either of the first two nucleotides of the glycine codon (GGN) changes the amino acid. Thus 22% (two out of nine) of all nucleotides encoding the triple helical domain will probably give rise to a phenotypic change in the heterozygote. Second, the large exon number and sensitivity to exon loss in the protein provides more than 100 additional mutation sensitive sites in each gene. Third, the need to maintain structure in the globular carboxyl-terminal domain to allow for interactions that generate a triple chain molecule provides an unknown number of additional targets. Finally, because type I collagen forms fibrils from identical subunits, appearance of any abnormal molecules interferes with the production of normal structure.

The majority of mutations in collagen genes that produce recognisable forms of OI are point mutations, most of which change a glycine codon to that for another amino acid. Only two of the more than 70 mutations catalogued here have occurred independently in unrelated subjects.²¹ Both recurrent mutations appeared at CpG dinucleotides and are consistent with deamination of a methyl cytosine to produce thymidine. Because there is considerable bias in the manner in which mutations have been recognised and analysed, the mechanistic basis of mutations is uncertain. It appears, however, that mutations at CpG dinucleotides are not overrepresented, although those sites may be sequences where recurrent mutations are more likely to appear.²¹

It is surprising to us, given the repetitive structure of the type I collagen genes and the ready identification of large deletions within the type III collagen gene,⁷⁹ that large deletions within either gene of type I collagen are uncommon. The multiexon deletions that have been identified occurred as results of intron-intron events and only a single instance of recombination through exon exchange has been recognised. Although it may be that the structure of the gene itself is sufficient to limit recombination, it is possible that other explanations may be necessary to explain the paucity of such events. For example, the presence of other essential genes imbedded within introns or transcripts read from the 'collagen' non-coding strand could limit viability of even heterozygotes for large deletions.

Conclusion

Osteogenesis imperfecta presents a remarkable clinical spectrum, from virtually undetectable to lethal, as a consequence of mutations in the two genes that encode the chains of type I collagen. The phenotypic expression of these mutations reflects the gene in which the mutations occur, the nature of the mutation, the effect of the mutation on the behaviour

of the gene and abnormal protein, and the consequences of the changes in protein behaviour for assembly of a normal extracellular matrix. The analysis of more than 70 mutations provides insight into the relation of mutation to disease but it is clear that we are still a long way from understanding the grammar by which phenotype is translated from genotype. We estimate that it will take the results of more than 200 mutations to begin to saturate the mutation map and, even then, the finding of unexpected domains of either greater or lesser severity may require additional study.

Collagen genes have proved to be a remarkably robust biological system in which to study mutations because a great deal is known about protein structure, because the protein lives a dual existence in the intracellular and extracellular environments, and because the large collagen gene family means that findings in one system have broad implications. Few investigators had anticipated that substitution of single glycine residues within the triple helix could have such profound effects on folding, modification, secretion, and phenotype. Studies of OI defects have revitalised interest in the intracellular life style of collagen molecules, in the molecules that are involved in their maturation, and on how mutations alter structure and function. At the same time, it has become clear that a more complete understanding is necessary of the structure of bone, the manner in which the protein matrix of bone is built, and how alterations in the amount or structure of the constituent collagens produce aberrant fibrils and, thus, tissues.

The consequences of somatic and germline mosaicism for mutations have been most completely explored in collagen genes but have implications for other genes. Somatic mosaicism at high levels is a result of early embryonic mutation. It is not clear whether collagen genes are at greater risk than other genes for mutation during early embryogenesis, whether entry into the germ cell lineage offers protection from mutation, or whether there is mutation limited to the gene derived from only one parent. It is clear, however, that these studies raise further questions about the timing of new dominant mutations, oblige us to reconsider how to provide genetic counselling in dominantly inherited disorders, and force us to stop taking dogma for granted.

While it is likely that the models developed for understanding the behaviour of type I procollagen molecules will serve for all the fibrillar collagens,⁸⁰ we may need to modify our expectations when dealing with collagens that differ in structure. Nevertheless, the methods for identifying and characterising mutations in collagens are now well established and progress in understanding the consequences of mutations in other collagen genes should be rapid.⁸¹

Finally, we should not underestimate the contri-

bution that heritable mutations in important matrix genes, like collagens, can play in a wide range of late onset, degenerative disorders, and how the study of disorders like OI can point the way to identification of candidate mutations (see references 5, 80, 82, and the last paragraphs of many grant applications for more detailed discussion of these points).

The original work reported here was supported in part by grants from the National Institutes of Health (AR 21557), the March of Dimes Birth Defects Foundation (6-298), the Arthritis Foundation, and by the Michael Geisman Memorial Fellowship from the Osteogenesis Imperfecta Foundation.

- 1 Silience DO, Senn A, Danks DM. Genetic heterogeneity in osteogenesis imperfecta. *J Med Genet* 1979;16:101-16.
- 2 Looser E. Zur kenntnis der osteogenesis imperfecta congenita et tarda. *Mitt Grenzgeb Med Chir* 1906;15:161.
- 3 Seedorf KS. *Osteogenesis imperfecta. A study of clinical features and heredity based on 55 Danish families comprising 180 affected persons*. Copenhagen: Ejnar Munksgaard, 1949.
- 4 Byers PH. Disorders of collagen biosynthesis and structure. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic basis of inherited disease*. 6th ed. New York: McGraw-Hill, 1989:2805-44.
- 5 Prockop DJ, Constantinou CD, Dombrowski KE, et al. Type I procollagen: the gene-protein system that harbors most of the mutations causing osteogenesis imperfecta and probably more common heritable disorders of connective tissue. *Am J Med Genet* 1989;34:60-7.
- 6 Cohn DH, Byers PH, Steinmann B, Gelinas RE. Lethal osteogenesis imperfecta resulting from a single nucleotide change in one human pro α 1(I) collagen allele. *Proc Natl Acad Sci USA* 1986;83:6045-7.
- 7 Constantinou D, Nielson KB, Prockop DJ. A lethal variant of osteogenesis imperfecta has a single base mutation that substitutes cysteine for glycine 904 of the α 1(I) chain of type I procollagen. The asymptomatic mother has an unidentified mutation producing an overmodified and unstable type I procollagen. *J Clin Invest* 1989;83:574-84.
- 8 Vogel BE, Minor RR, Freund M, Prockop DJ. A point mutation in a type I procollagen gene converts glycine 748 of the α 1 chain to cysteine and destabilizes the triple helix in a lethal variant of osteogenesis imperfecta. *J Biol Chem* 1987;262:14737-44.
- 9 Starman BJ, Eyre D, Charbonneau H, et al. Osteogenesis imperfecta. The position of substitution for glycine by cysteine in the triple helical domain of the pro α 1(I) chains of type I collagen determines the clinical phenotype. *J Clin Invest* 1989;84:1206-14.
- 10 Prockop DJ. Mutations in collagen genes. The lessons of rare diseases applied to common diseases. *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:50.
- 11 Nicholls AC, Oliver J, Renouf D, Pope FM. Type I collagen mutation in osteogenesis imperfecta and inherited osteoporosis. *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:48.
- 12 Cetta G, Tenni R, Rossi A, Antoniazzi F, Vitellaro-Zuccarello L, Valli M. The substitution of cys for gly 178 in α 1(I) triple helical domain: mutual influence of the mutation with neighboring amino acids. *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:8.
- 13 Pretorius PJ, Wirtz MK, Rao VH, et al. Glycine to cysteine substitution at position 175 in an alpha 1 chain of type I collagen produces a clinically heterogeneous form of osteogenesis imperfecta. *Third International Conference on the Molecular Biology and Pathology of Matrix, Philadelphia, 13-16 June 1990*. Abstract III-40.
- 14 Rowe DW, Stover ML, McKinstry M, et al. Molecular mechanisms (real and imagined) for osteogenic bone disease. *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:57.

- 15 Nicholls AC, Oliver J, Renouf D, Pope FM. Type I collagen mutation in osteogenesis imperfecta and inherited osteoporosis. *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:48.
- 16 Fertala A, Westerhausen A, Morris GM, Rooney JE, Prockop DJ. Two cysteine substitutions in the type I procollagen genes (COL1A1 and COL1A2) that cause lethal osteogenesis imperfecta. The location of glycine substitutions does not in any simple way predict their effects on protein function or phenotype. *Am J Hum Genet* 1990;47:216 (abstract 848).
- 17 Bateman JF, Hannagan M, Lamande S, Moeller I, Chan D, Cole WG. Collagen I mutation in perinatal lethal osteogenesis imperfecta. *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:2.
- 18 Westerhausen A, Kishi J, Prockop DJ. One mutation that substitutes serine for glycine $\alpha 1$ -598 and another mutation that substitutes serine for glycine $\alpha 1$ -631 of type I procollagen (COL1A1) both cause lethal osteogenesis imperfecta but have different effects on the thermal stability of the protein. *Am J Hum Genet* 1989;45:229 (abstract 902).
- 19 Cohn DH, Wallis G, Zhang X, Byers PH. Serine for glycine substitutions in the $\alpha 1(I)$ chain of type I collagen: biological plasticity in the Gly-Pro-Hyp clamp at the carboxyl-terminal end of the triple helical domain. *Third International Conference on the Molecular Biology and Pathology of Matrix, Philadelphia, 13-16 June 1990*. Abstract III-1.
- 20 Wallis GA, Starman BJ, Byers PH. Clinical heterogeneity explained by molecular heterogeneity and somatic mosaicism. *Am J Hum Genet* 1989;45:228 (abstract 895).
- 21 Pruchno CJ, Cohn DH, Wallis GA, et al. Osteogenesis imperfecta due to recurrent point mutations at CpG dinucleotides in the COL1A1 gene of type I collagen. *Hum Genet* (in press).
- 22 Marini JC, Grange DK, Gottesman GS, Lewis MB, Koepf DA. Osteogenesis imperfecta type IV: detection of a point mutation in one $\alpha 1(I)$ collagen allele (COL1A1) by RNA/RNA hybrid analysis. *J Biol Chem* 1989;264:9694-9.
- 23 Pack M, Constantinou CD, Kalia K, Nielsen KB, Prockop DJ. Substitution of serine for $\alpha 1(I)$ -glycine 844 in a severe variant of osteogenesis imperfecta minimally destabilizes the triple helix of type I procollagen. The effects of glycine substitutions on thermal stability are either position or amino acid specific. *J Biol Chem* 1989;264:19694-9.
- 24 Bateman JF, Chan D, Walker ID, Rogers JG, Cole WG. Lethal osteogenesis imperfecta due to the substitution of arginine for glycine at residue 391 of the $\alpha 1(I)$ chain of type I collagen. *J Biol Chem* 1987;262:7021-7.
- 25 Wallis GA, Starman BJ, Zinn AB, Byers PH. Variable expression of osteogenesis imperfecta in a nuclear family is explained by somatic mosaicism for a lethal point mutation in the $\alpha 1(I)$ gene (COL1A1) of type I collagen in a parent. *Am J Hum Genet* 1990;46:1034-40.
- 26 Bateman JF, Lamande SR, Dahl HHM, Chan D, Cole WG. Substitution of arginine for glycine 664 in the collagen $\alpha 1(I)$ chain in lethal perinatal OI. *J Biol Chem* 1988;263:11627-30.
- 27 Wallis GA, Starman BJ, Schwartz MF, Byers PH. Substitution of arginine for glycine at position 847 in the triple helical domain of the $\alpha 1(I)$ chain of type I collagen produces lethal osteogenesis imperfecta. Molecules that contain one or two abnormal chains differ in stability and secretion. *J Biol Chem* 1990;265:18628-33.
- 28 Lamande SR, Dahl HHM, Cole WG, Bateman JF. Characterization of point mutations in the collagen COL1A1 and COL1A2 genes causing lethal perinatal osteogenesis imperfecta. *J Biol Chem* 1989;264:15809-12.
- 29 Patterson E, Smiley E, Bonadio J. RNA sequence analysis of a perinatal lethal osteogenesis imperfecta mutation. *J Biol Chem* 1989;264:10083-7.
- 30 Westerhausen A, Constantinou CD, Prockop DJ. A mutation that substitutes valine for glycine $\alpha 1$ -637 in a type I procollagen gene (COL1A1) and causes lethal osteogenesis imperfecta. Evidence for a cooperative block of micro-unfolding between amino acid positions 637 and 775. *Am J Hum Genet* 1990;47:242 (abstract 951).
- 31 Zhuang J, Constantinou CD, Ganguly A, Prockop DJ. A single base mutation in type I procollagen that converts glycine $\alpha 1$ -541 to aspartate in a lethal variant of osteogenesis imperfecta. Detection of the mutation with carbodiimide reaction. *Third International Conference on the Molecular Biology and Pathology of Matrix, Philadelphia, 13-16 June 1990*. Abstract III-54.
- 32 Cohn DH, Starman BJ, Blumberg B, Byers PH. Recurrence of lethal osteogenesis imperfecta due to parental mosaicism for a dominant mutation in a human type I collagen gene (COL1A1). *Am J Hum Genet* 1990;46:591-601.
- 33 Wenstrup RJ, Shrago-Howe AW, Lever LW, Phillips CL, Byers PH, Cohn DH. The effects of different cysteine for glycine substitutions within $\alpha 2(I)$ chains: evidence of distinct structural domains within the type I collagen triple helix. *J Biol Chem* (in press).
- 34 Edwards MJ, Byers PH, Cohn DH. Mild osteogenesis imperfecta produced by somatic mosaicism for a lethal mutation in a type I collagen gene. *Am J Hum Genet* 1990;47:215 (abstract 846).
- 35 Fertala A, Rooney J, Westerhausen A, Prockop DJ. Single base mutation that converts the codon for glycine $\alpha 2$ -787 to cysteine in the type I procollagen gene (COL1A1) causes a lethal variant of osteogenesis imperfecta. *Third International Conference on the Molecular Biology and Pathology of Matrix, Philadelphia, 13-16 June 1990*. Abstract III-23.
- 36 Bateman JF, Hannagan M, Lamande S, Moeller I, Chan D, Cole WG. Collagen I mutation in perinatal lethal osteogenesis imperfecta. *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:2.
- 37 Tsuneyoshi T, Constantinou CD, Mikkelsen M, Prockop DJ. A substitution of arginine for glycine $\alpha 2$ -694 in a gene for type I procollagen (COL1A2) that causes lethal osteogenesis imperfecta. Further definition of a cooperative block for micro-unfolding of the triple helix between residues 637 and 775. *Am J Hum Genet* 1990;47:240 (abstract 943).
- 38 Marini JC, Grange DK, Lewis MB. Detection of a mutation in $\alpha 2(I)$ mRNA in a proband with lethal type II osteogenesis imperfecta. *Am J Hum Genet* 1989;46:205 (abstract 805).
- 39 Baldwin CT, Constantinou CD, Dumars KW, Prockop DJ. A single base mutation that converts glycine 907 of the $\alpha 2(I)$ chain of type I procollagen to aspartate in a lethal variant of osteogenesis imperfecta. The single amino acid substitution near the carboxyl terminus destabilizes the whole triple helix. *J Biol Chem* 1989;264:3002-6.
- 40 Spotila LD, Constantinou CD, Sereda L, Riggs BL, Prockop DJ. Substitution of serine for gly $\alpha 2$ -661 in the gene for type I procollagen (COL1A2) as a cause of post-menopausal osteoporosis. *Am J Hum Genet* 1990;47:237 (abstract 931).
- 41 Wenstrup RJ, Cohn DH, Cohen T, Byers PH. Arginine for glycine substitution in the triple-helical domain of the products of one $\alpha 2(I)$ allele (COL1A2) produces the osteogenesis imperfecta type IV phenotype. *J Biol Chem* 1988;263:7734-40.
- 42 Tsipouras P, Myers JC, Ramirez F, Prockop DJ. Restriction fragment length polymorphism associated with the pro- $\alpha 2(I)$ gene of human type I procollagen. Application to a family with an autosomal dominant form of osteogenesis imperfecta. *J Clin Invest* 1983;72:1262-7.
- 43 Wallis G, Beighton P, Boyd C, Mathew CG. Mutations associated with the pro- $\alpha 2(I)$ collagen gene are responsible for several cases of osteogenesis imperfecta type I. *J Med Genet* 1986;23:411-6.
- 44 Sykes BC, Ogilvie DJ, Wordsworth BP, Anderson DJ, Jones N. Osteogenesis imperfecta is linked to both type I collagen structural genes. *Lancet* 1986;ii:69-72.
- 45 Sykes B, Ogilvie D, Wordsworth P, et al. Consistent linkage of dominantly inherited osteogenesis imperfecta to the type I collagen loci: COL1A1 and COL1A2. *Am J Hum Genet* 1990;46:293-307.
- 46 Bonadio J, Ramirez F, Barr M. An intron mutation in the human $\alpha 1(I)$ collagen gene alters the efficiency of pre-mRNA splicing and is associated with osteogenesis imperfecta type II. *J Biol Chem* 1990;265:2262-8.
- 47 Wallis GA, Starman BJ, Chessler SD, Willing MC, Byers PH. Mutations in the CB6 and carboxyl-terminal regions of COL1A1 causing lethal OI have different effects on the stability and secretion of the type I collagen molecule. *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:55.
- 48 Bateman JF, Hannagan M, Lamande S, Moeller I, Chan D, Cole WG. Collagen I mutations in perinatal lethal osteogenesis imperfecta. *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:2.
- 49 Pruchno CJ, Willing MC, Starman BJ, Aylsworth A, Byers PH. Inefficient splicing and production of both a normal and shortened mRNA from the same COL1A1 allele of type I collagen in a father and son with osteogenesis imperfecta (OI). *Am J Hum Genet* 1989;46:213 (abstract 837).
- 50 Ganguly A, Baldwin CT, Conway D, Prockop DJ. Use of a carbodiimide reaction to locate single-base mutations. Detection of a single-base mutation in an intron of the type I procollagen

- gene (COL1A2) that causes abnormal RNA processing in a proband with lethal osteogenesis imperfecta (type II). *Am J Hum Genet* 1990;47:217 (abstract 852).
- 51 Tromp G, Prockop DJ. Single base mutation in the pro α 2(I) gene of type I procollagen that causes efficient splicing of RNA from exon 27 to exon 29 and synthesis of a shortened but in-frame pro α 2(I) chain. *Proc Natl Acad Sci USA* 1988;85:5254-8.
 - 52 Superti-Furga A, Raghunath M, Pistone FM, Comano C, Steinmann B. Deletion of nucleotides +2 to +40 in intron 21 of COL1A2 leading to skipping of exon 21 observed in a boy with short stature, osteoporosis, dentinogenesis imperfecta, but no fractures (OI type IV). *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:37.
 - 53 Zhuang J, Constantinou CD, Prockop DJ. Heterozygous defect that deletes the codons of exon 13 from the mRNA of the α 2 chain of type I procollagen in a patient with mild osteogenesis imperfecta. *Third International Conference on the Molecular Biology and Pathology of Matrix, Philadelphia, 13-16 June 1990*. Abstract III-55.
 - 54 Kuivaniemi H, Sabol C, Tromp G, Sippola-Thiele M, Prockop DJ. A 19-base pair deletion in the pro α 2(I) gene of type I procollagen that causes in-frame RNA splicing from exon 10 to exon 12 in a proband with atypical osteogenesis imperfecta and in his asymptomatic mother. *J Biol Chem* 1988;263:11407-13.
 - 55 Weil D, Bernard M, Combates N, et al. Identification of a mutation that causes exon skipping during collagen pre-mRNA splicing in an Ehlers-Danlos syndrome variant. *J Biol Chem* 1988;263:8561-4.
 - 56 Weil D, D'Allesio M, Ramirez F, et al. A base substitution in the exon of a collagen gene causes alternative splicing and generates a structurally abnormal polypeptide in a patient with Ehlers-Danlos syndrome type VII. *EMBO J* 1989;8:1705-10.
 - 57 Cohn DH, Apone S, Eyre DR, et al. Substitution of cysteine for glycine within the carboxyl-terminal telopeptide of the α 1(I) chain of type I collagen produces mild osteogenesis imperfecta. *J Biol Chem* 1988;263:14605-7.
 - 58 Chu ML, Gargiulo V, Williams CJ, Ramirez F. Multiexon deletion in an osteogenesis imperfecta variant with increased type III collagen mRNA. *J Biol Chem* 1985;260:691-4.
 - 59 Barsh GS, Roush CL, Bonadio J, Byers PG, Gelinas RE. Intron-mediated recombination may cause a deletion in an α 1 type I collagen chain in a lethal form of osteogenesis imperfecta. *Proc Natl Acad Sci USA* 1985;82:2870-4.
 - 60 Willing MC, Cohn DH, Starman BJ, Holbrook KA, Greenberg CR, Byers PH. Heterozygosity for a large deletion in the α 2(I) collagen gene has a dramatic effect on type I collagen secretion and produces perinatal lethal osteogenesis imperfecta. *J Biol Chem* 1988;263:8398-404.
 - 61 Cohn DH, Pruchno CJ, Zhang X, Byers PH. Homology-mediated recombination between type I collagen gene (COL1A1) exons results in an intragenic tandem duplication and lethal osteogenesis imperfecta. *Am J Hum Genet* 1990;47:110 (abstract 427).
 - 62 Hawkins JR, Superti-Furga A, Steinmann B, Dalglish R. Analysis of mutation in type III osteogenesis imperfecta by RNase A protection mapping. *Molecular Biology of the Extracellular Matrix. British Connective Tissue Society Meeting, Leicester, England, 26-27 September 1989*.
 - 63 Molyneux K, Byers PH, Dalglish R. A new mutation in the α 2(I) gene of a patient with non-lethal osteogenesis imperfecta. *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:19.
 - 64 Wenstrup RJ, Schrago-Howe A, Lever L, Phillips C. Mutations in COL1A2 that result in nonlethal OI phenotypes. *Fourth International Conference on Osteogenesis Imperfecta, Pavia, Italy, 9-12 September 1990*. Abstracts, 1990:58.
 - 65 Pihlajaniemi T, Dickson LA, Pope FM, et al. Osteogenesis imperfecta: cloning of a pro α 2(I) collagen gene with a frameshift mutation. *J Biol Chem* 1984;259:12941-4.
 - 66 Bateman JF, Lamande SR, Dahl HHM, Chan D, Mascara T, Cole WG. A frameshift mutation results in a truncated nonfunctional carboxyl-terminal pro α 1(I) propeptide of type I collagen in osteogenesis imperfecta. *J Biol Chem* 1989;264:10960-4.
 - 67 Willing MC, Cohn DH, Byers PH. Frameshift mutation near the 3' end of the COL1A1 gene predicts an elongated pro α 1(I) chain and results in osteogenesis imperfecta type I. *J Clin Invest* 1990;85:282-90.
 - 68 Nicholls AC, Osse G, Schloos HG, et al. The clinical features of homozygous α 2(I) collagen deficient osteogenesis imperfecta. *J Med Genet* 1984;21:257-62.
 - 69 Sykes B. The molecular genetics of collagen. *BioEssays* 1983;3:112-7.
 - 70 Barsh GS, David KE, Byers PH. Type I osteogenesis imperfecta: a nonfunctional allele for pro α 1(I) chains of type I procollagen. *Proc Natl Acad Sci USA* 1982;79:3838-42.
 - 71 Genovese C, Rowe D. Analysis of cytoplasmic and nuclear messenger RNA in fibroblasts from patients with type I osteogenesis imperfecta. *Methods Enzymol* 1987;145:223-34.
 - 72 Vogel BE, Doelz R, Kadler KE, Hojima Y, Engel J, Prockop DJ. A substitution of cysteine for glycine 748 of the α 1 chain produces a kink at this site in the procollagen I molecule and an altered N-proteinase cleavage site over 225 nm away. *J Biol Chem* 1988;263:19249-55.
 - 73 Byers PH, Tsiouras P, Bonadio JF, Starman B, Schwartz RC. Perinatal lethal osteogenesis imperfecta (OI type II): a biochemically heterogeneous disorder usually due to new mutations in the genes for type I collagen. *Am J Hum Genet* 1988;42:237-48.
 - 74 Wenstrup RJ, Willing MC, Starman BJ, Byers PH. Distinct biochemical phenotypes predict clinical severity in nonlethal variants of osteogenesis imperfecta. *Am J Hum Genet* 1990;46:975-82.
 - 75 de Wet WJ, Pihlajaniemi T, Myers J, Kelly TE, Prockop DJ. Synthesis of a shortened pro α 2(I) chain and decreased synthesis of pro α 2(I) chains in a proband with osteogenesis imperfecta. *J Biol Chem* 1983;258:7721-7.
 - 76 Aitchison K, Ogilvie DJ, Honeyman M, Thompson E, Sykes BC. Homozygous osteogenesis imperfecta unlinked to collagen I genes. *Hum Genet* 1988;78:233-6.
 - 77 Sillence DO, Barlow KK, Cole WG, Dietrich S, Garber AP, Rimoin DL. Osteogenesis imperfecta type III. Delineation of the phenotype with reference to genetic heterogeneity. *Am J Med Genet* 1986;23:821-32.
 - 78 Thompson EM, Young ID, Hall CM, Pembrey ME. Osteogenesis imperfecta type IIA. Evidence for dominant inheritance. *J Med Genet* 1987;24:386-9.
 - 79 Superti-Furga A, Steinmann B, Ramirez F, Byers PH. Molecular defects of type III procollagen in Ehlers-Danlos syndrome type IV. *Hum Genet* 1989;82:104-8.
 - 80 Kuivaniemi H, Tromp G, Prockop DJ. Mutations in collagen genes. Causes of rare and some common diseases in man. *FASEB J* (in press).
 - 81 Barker DF, Hostikka SL, Zhou J, et al. Identification of mutations in the COL4A5 collagen gene in Alport syndrome. *Science* 1990;248:1224-7.
 - 82 Shapiro JR, Rowe DW. Collagen genes and brittle bones. *Ann Intern Med* 1983;99:700-4.