STRATEGIES AND OUTCOMES OF PRENATAL DIAGNOSIS FOR OSTEOGENESIS IMPERFECTA: A REVIEW OF BIOCHEMICAL AND MOLECULAR STUDIES COMPLETED IN 129 PREGNANCIES

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SUMMARY

We completed prenatal diagnostic studies from 129 pregnancies at risk for osteogenesis imperfecta (OI). Studies in 107 pregnancies were completed by analysis of collagen synthesized by cells cultured from chorionic villus biopsies and the remaining 22 used direct mutation identification or analysis of polymorphic restriction sites in the COL1A1 gene of type I collagen. The vast majority of studies ($n=113$) were obtained to identify fetuses with OI type II (the perinatal lethal form) and some fetuses affected with OI type III or IV (the deforming varieties). Of the 50 couples who had had one previous affected pregnancy with the lethal form of OI, one had a second affected pregnancy, a rate of 2 per cent. Two of the seven unaffected couples (28 per cent) who had had two previous affected pregnancies with OI type II had a third affected pregnancy; none of the three with two previous pregnancies with OI type III had a third. Pregnancies at risk for OI type I could not be ascertained reliably by biochemical analysis of cultured CVS cells but were identified by direct analysis of the causative mutation or the use of linked markers in families. All prenatal diagnostic studies were undertaken only after earlier diagnostic studies (biochemical or molecular) had been completed on the proband, a necessary strategy for accurate results. In all pregnancies at risk for OI type II, OI type III, and OI type IV studied with biochemical strategies and in pregnancies at risk for OI type I studied with molecular techniques, there were neither false-negative nor false-positive results. Diagnostic information can be obtained within 20–30 days of biopsy using biochemical techniques and within 10–14 days when molecular strategies are used. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

The clinical heterogeneity apparent in osteogenesis imperfecta (OI) (Sillence *et al.*, 1979) is explained largely by different mutations in the two non-syntenic genes, COL1A1 (chromosome 17) and COL1A2 (chromosome 7), which encode the proα1(I) and proα2(I) chains, respectively, of type I procollagen (Byers, 1993, 1995). Although rare autosomal recessive forms of OI due to mutations in these genes (Nicholls *et al.*, 1984; Pihlajaniemi *et al.*, 1984) and possibly in non-collagen genes (Aitchison *et al.*, 1988; Wallis *et al.*, 1993) exist, the vast majority of individuals with OI are heterozygous for dominantly acting mutations. These abnormalities fall into two general classes—one in

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which there is too little type I procollagen synthe-sized but the product is normal (OI type I) and one in which abnormal molecules are made (OI type II, OI type III, and OI type IV) (Wenstrup et al., 1990; Byers, 1993).

Prenatal diagnosis is of concern in families with OI in several situations: (1) where there is a risk because of parental germline mosaicism (Byers et al., 1988) for the birth of a second (or subse-quent) child with osteogenesis imperfecta to be born to normal parents, (2) where there is the risk for the birth of a child in a family affected with an autosomal dominant form of OI, and (3) where there is the risk for the birth of a child affected with an autosomal dominant form of OI where the parent is the only affected individual in the family.

The form of prenatal diagnostic studies used for a particular pregnancy depends on the nature of diagnostic studies performed earlier and the type of abnormality identified. If cultured fibroblasts from an affected parent or child have been studied and an abnormal type I procollagen molecule has been identified, then mesenchymal cells cultured from chorionic vilus biopsies taken at about 10 weeks' gestation, which synthesize collagens comparable to dermal fibroblasts, can be used for diagnostic studies. In large families, linkage studies may identify the gene and allele which contain the mutant sequence (Tsipouras et al., 1983, 1986; Wallis et al., 1986; Sykes et al., 1986, 1990), thus permitting prenatal diagnosis by haplotype analy-sis of fetal DNA. This type of molecular exami-nation has been used in pregnancies at risk for OI type I (Lynch et al., 1991) where the mutant allele results in diminished collagen production without an identifiable abnormal collagen product (Willing et al., 1992). Because OI represents a 'private' mutation in most families, mutational analysis of DNA from CVS or amniotic fluid cells can be used only in pregnancies where the molecular defect has been previously identified. Specific mutations have not been identified in most affected individuals.

Ultrasound determination of fetal limb length and morphology can reliably identify fetuses with OI type II by 14–16 weeks' gestation, even in the absence of a previously affected sib (Shapiro et al., 1982; Dinno et al., 1982; Chervenak et al., 1982; Constantine et al., 1991; Thompson, 1993; Dimaio et al., 1993), and can identify some fetuses with the progressive deforming variety of OI (OI type III) by 18–20 weeks' gestation (Thompson, 1993; Aylsworth et al., 1984; Robinson et al., 1987). In the absence of fracture or significant bowing,

ultrasound cannot be used to identify fetuses with the milder forms of OI, even in the presence of a positive family history.

Over the last 15 years, we have performed prenatal diagnosis for 129 pregnancies using bio-chemical and molecular genetic strategies. Here we describe the efficacy with which the techniques permit prenatal diagnosis, the limitations of strategies for some forms of OI, and findings concerning recurrence risks in families.

METHODS

Cultured chorionic villus (CV) cells arrived in our laboratory, on average, 10–14 days after the CV sampling (CVS) procedure was undertaken (at about 10 weeks' gestation). Results of biochemical studies of cultured CVS cells were completed, on average, 14 days after the cells arrived. If DNA extracted from cultured cells was shipped, results were completed approximately 6 days after arrival (to identify a known mutation) and 14 days after arrival for COL1A1 or COL1A2 marker studies. Samples were received with concerns about recur-rence of OI type I, OI type II, OI type III, and OI type IV. Because in some instances details about the clinical history of the affected proband are incomplete, the nomenclature 'OI type III/IV' is used throughout to represent either OI type III or OI type IV, the deforming varieties of OI.

Biochemical analysis of proteins synthesized by cultured CVS cells

Chorionic villi were aspirated from pregnancies (usually 10–12 weeks' gestation) at risk for differ-ent forms of osteogenesis imperfecta, at the refer-ral sites. The tissues were cleaned of maternal tissues and the cells were dispersed with col-lagenase and then plated in Chang medium that contained 20 per cent fetal bovine serum at the referring centre. Cells were passaged with trypsin, seeded into T-25 flasks, and when the cell density approached confluence, shipped by overnight express to Seattle. A gestational age-matched control CVS cell line generally was shipped simultaneously from the referring centre. The cells were then seeded into culture dishes (250 000 cells per 35 mm dish) and allowed to attach and spread overnight in Chang medium that contained 20 per cent fetal calf serum. Proteins were bio-synthetically labelled with [³H]proline (without serum supplement) using procedures previously
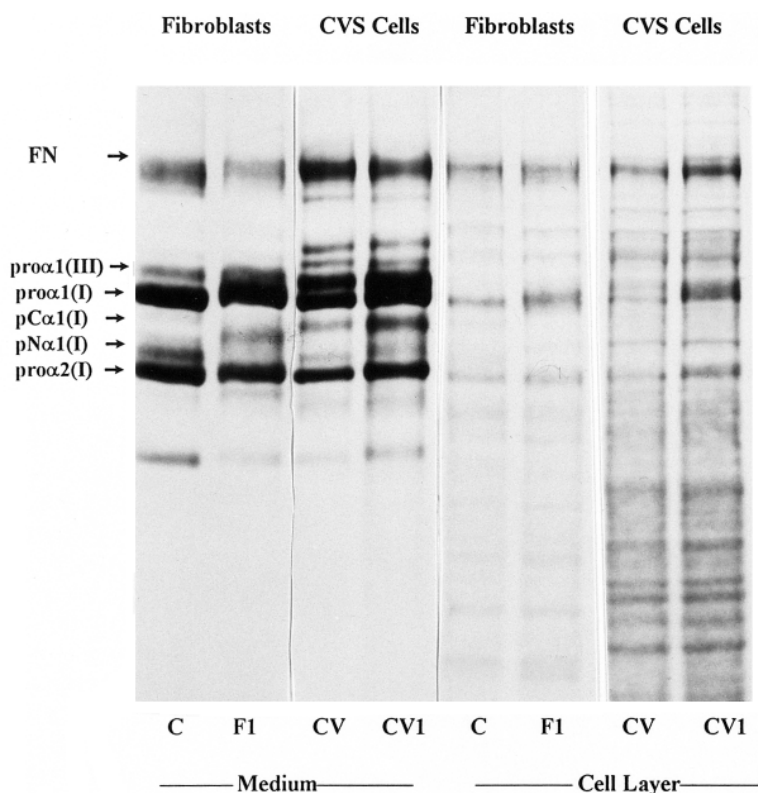
Fig. 1—Autoradiogram of proα chains of type I and type III procollagens synthesized by control fibroblasts (C), cells from the previous affected infant with OI type II in the family (F1), control CVS cells (CV), and CVS cells from a fetus at risk for OI type II (CV1). There is blurring of the distinction between proα1(I) and proα1(III) in cells from the affected infant (F1) and from the pregnancy (CV1), consistent with an affected pregnancy

established for cultured fibroblasts (Bonadio *et al.*, 1985), harvested, and examined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1). With three exceptions, cells from the proband in each family had been studied prior to the analysis of the collagens synthesized by chorionic villus cells.

In preliminary attempts to examine type I procollagen and collagen synthesized by amniotic fluid (AF) cells, AF cells were cultured according to standard procedures, shipped from the referring centre, and then labelled in parallel to fibroblasts and CVS cells. AF cells were examined for collagen synthesis.

Mutation detection or haplotype analysis of DNA extracted from CVS cells or amniocytes

DNA was submitted to our laboratory from referring centres for molecular analysis. For direct mutation analysis, primers that flanked the identified mutation of COL1A1 or COL1A2 in genomic DNA were used to amplify by polymerase chain reaction (PCR) the suspected region (Saiki *et al.*, 1988). If the mutation altered a restriction endonuclease site, the amplified fragment was digested with the enzyme as directed by the manufacturer. Alternatively, the sequence of the amplified region was determined using one of the amplification primers and the dideoxy chain-termination method with Sequenase® (US Biochemicals) as the DNA polymerase.

Three polymorphic restriction endonuclease sites in or near the COL1A1 gene, *Mnl*I in the 3′ untranslated region, an *Rsa*I site in intron 5, and an *Msp*I site upstream of the 5′ end of COL1A1, were amplified using previously published primer sequences (*Mnl*I, Willing *et al.*, 1990; *Rsa*I, D'Alessio *et al.*, 1988; *Rsa*I, Ogilvie *et al.*, 1987). To determine if only one COL1A1 allele was

Table I—Outcome of prenatal diagnostic studies done in pregnancies at risk for different forms of OI

	Total	Biochemical	DNA	Affected
OI type II	57	52	5	3
OI type III or IV	56	48	8	12
OI type I	16	7	9	11
Total	129	107	22	26

expressed in RNA (diagnostic of OI type I), either an allele-specific primer extension assay (Willing et al., 1992) or concurrent analysis of genomic DNA and cDNA genotypes by digestion of amplified products with MnlI was used. Haplotypes were constructed by inspection.

Statistical analysis

T-test assuming equal variance from the Microsoft Excel data analysis program was utilized to compare means. Confidence intervals were calculated from the standard error of the mean and the Student t-test for degrees of freedom.

RESULTS

Biochemical and molecular studies of CVS cells

Biochemical studies of cultured CVS cells were completed in 107 instances (Table I). With three exceptions, collagens synthesized by cells from an affected proband (either sibling or parent) had been examined and previously found to be abnormal and compatible with heterozygosity for a mutation in one of the genes of type I collagen. In 113 pregnancies at risk for OI type II, III or IV, 15 affected pregnancies were identified (Table I). In the pregnancies predicted as affected, the diagnosis was confirmed by ultrasound examination of the fetus, by biochemical studies of cells from the fetus, by autopsy or radiographs after pregnancy termination, or, at birth, by physical examination and/or review of skeletal radiographs. In the unaffected pregnancies, a normal ultrasound and/or examination at birth confirmed the prenatal prediction. There were no false-negative or false-positive test results. With one exception, all 'control' CVS samples provided were normal. In the one instance in which apparently overmodified bands were seen, repeated ultrasound examination of the fetus was normal. This may represent a false positive.

Biochemical study of cultured CVS cells in pregnancies at risk for OI type I was abandoned after an initial assessment of reliability and accuracy. The biochemical marker (diminished type I procollagen) in OI type I is less amenable to identification. Six pregnancies at risk for OI type I were studied biochemically; in four cases, the fetus was predicted to be affected and in the two remaining cases, we were unable to make a determination about the status of the fetus. Three terminations were subsequently undertaken and cells from the fetus sent for biochemical confirmation. In two, the diagnosis was confirmed, but in one of the three cases, the study of collagens synthesized by fibroblasts cultured from the fetus could not substantiate the abnormalities identified in cultured CVS cells. Thus, of six pregnancies studied, one may represent a false positive and in two the findings were not diagnostic.

Biochemical studies of amniocytes

The major cells that grow from the amniotic fluid following diagnostic amniocentesis at 16 weeks are the epithelioid AF cells (Hoehn et al., 1974), which synthesize only one of the chains of type I procollagen, the proα1(I) chain (Crouch and Bornstein, 1978). These chains are extensively overmodified and have an aberrant electrophoretic mobility so that AF cells usually are not useful for prenatal diagnosis. Two samples (one at risk for OI type II and one at risk for OI type I) were submitted for prenatal diagnosis. It was not possible to determine if either pregnancy was affected.

Molecular studies of DNA isolated from CVS cells or amniocytes

Molecular studies of DNA isolated from CVS cells were performed in 22 instances, of which 15 were direct mutational analysis (Table II) and seven relied on examination of COL1A1 markers. Direct mutation analysis identified 11 unaffected and four affected pregnancies (one with OI type I, one with OI type II, and two with OI type III/IV) of which three were confirmed by biochemical or molecular studies after termination or by ultrasound examination prior to termination; no additional studies were done in the fourth (OI type I). All pregnancies predicted as 'normal' were unaffected at birth. Marker studies were

Table II—Prenatal diagnosis of OI by analysis of specific mutations

	CTBP No.	Risk phenotype	Gene	Mutation	Method of detection	Outcome
1	95-327*	OI type I	COL1A1	Loss of mutant allele (intron 48 $G^{+1}{\rightarrow}A$)	Sequencing	Affected
2	92-027†	OI type II	COL1A2	G452C	HphI site eliminated	Affected
3	95-837	OI type III/IV	COL1A1	Exon 48 skip (intron 47 $C^{-3}{\rightarrow}G$)	XhoI site eliminated	Affected
4	96-005	OI type IV	COL1A2	Exon 38 skip (intron 37 $A^{-2}{\rightarrow}G$)	Allele-specific oligonucleotide hybridization	Affected
5	95-786	OI type I	COL1A1	Exon 17 skip (intron 17 $G^{+1}{\rightarrow}G$)	DdeI site created	Normal
6	94-170	OI type II	COL1A1	G448S	AvaII site eliminated	Normal
7	94-501	OI type II	COL1A1	G448C	AvaII site eliminated	Normal
8	93-417‡	OI type II	COL1A1	G451S	NheI site created	Normal
9	94-838‡	OI type II	COL1A1	G451S	NheI site created	Normal
10	94-727	OI type II	COL1A1	Exon 27 skip (intron 26 $A^{-2}{\rightarrow}C$)	MspI site created and PstI site eliminated	Normal
11	93-614	OI type III	COL1A1	G526C	HpaII site eliminated	Normal
12	96-358	OI type III	COL1A1	G868C	BsaJI site eliminated	Normal
13	93-378	OI type IV	COL1A1	Exon 10 skip (intron 9 $G^{-1}{\rightarrow}T$)	AluI site created (cDNA)	Normal
14	95-330	OI type IV	COL1A2	G238S	SacII site eliminated	Normal

*Mutation previously identified by Willing et al. (1994).
†Prenatal diagnosis on molecular basis published by Dimaio et al. (1993) with molecular diagnostic studies performed by Daniel Cohn, PhD, Cedars Sinai Medical Center, Los Angeles. The mutation was reported by Edwards et al. (1992).
‡One of two unaffected pregnancies identified in one family with a single previous pregnancy affected with OI type II.
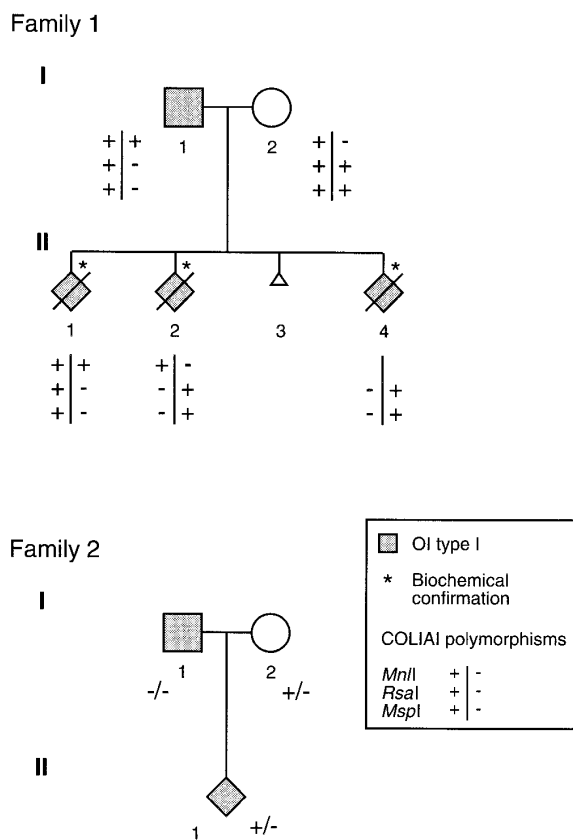
Fig. 2—Pedigrees of two families in which prenatal diagnosis of osteogenesis imperfecta was performed by examining COL1A1-linked polymorphic markers. Family 1 includes an affected father (I-1) with OI type I and three affected pregnancies (II-1, II-2, and II-4) studied by examining COL1A1 markers MnlI, RsaI, and MspI. Family 2 includes an affected father (I-1) and an affected pregnancy with OI type I studied by examining MnlI alleles (genomic DNA in the parents and fetus; mRNA in the fetus)

informative in six or seven pregnancies at risk for OI type I and identified six affected fetuses.

Prenatal diagnosis by use of polymorphic markers in the COL1A1 gene fell into two categories, one in which the linkage phase of the marker and the phenotype could be determined and the nuclear family was informative, and one in which the linkage phase of the marker(s) was not known because the affected parent was the first in the family, or the mating was not completely informative. In OI type I, a further step in diagnostic studies was available because the disorder results, in almost all instances, from reduced amounts of the mRNA products of one COL1A1 allele (Willing et al., 1992). In family 1 (Fig. 2), I-1

was the first affected individual in the family; biochemical studies demonstrated a reduction in the amount of type I collagen synthesis, but the linkage phase of the markers in the COL1A1 could not be determined uniquely (in contrast to those of his wife) and he was homozygous for the only marker (MnlI) expressed in the mRNA. When this became apparent, the family requested that the first pregnancy be terminated because of diagnostic uncertainty. Biochemical studies using cultured fetal dermal cells demonstrated that the fetus was affected and analysis of the genotype permitted the phase to be set in the father and identified the mutant allele (MnlI+, RsaI−, MspI−) (Fig. 2). Analysis of the RsaI and MspI sites permitted the determination that both subsequent pregnancies were also affected and analysis of the MnlI-bearing sequence in the mRNA from cells of the second fetus confirmed that the product of the MnlI(+) allele from the father was in low abundance (Fig. 3). In family 2 (Fig. 2), the affected father was also homozygous (−/−) at the polymorphic MnlI site but the unaffected mother was heterozygous. The fetus was heterozygous at the MnlI site and the paternal MnlI(−) allele was in low abundance, compatible with an affected fetus. The pregnancy is continuing.

Predicted recurrence risk and outcome

We analysed 57 pregnancies at risk for the lethal form of OI and an additional 56 at risk for OI type III or IV (Table III). In the 50 pregnancies at risk for recurrence of OI type II in which there had been one previous affected pregnancy in each family, there was a single recurrence (2 per cent). In the families of seven of the pregnancies at risk for recurrence of OI type II where there were two previous affected pregnancies, two affected pregnancies were identified, a recurrence rate of 28 per cent, significantly greater than in the proportion seen in the 'one previous affected' group ($P<0.01$). There were three pregnancies at risk for OI type III/IV that occurred in a single family with normal parents but two previous affected children; all three were normal. In the 31 pregnancies at risk for recurrence of OI type III or IV as a result of one previous affected child, one affected pregnancy was identified (3 per cent). In 22 pregnancies with a parent affected with OI type III or IV, 11 affected pregnancies were identified (50 per cent). There were 16 pregnancies at risk for OI type I in families with an affected parent; 11 were affected (68 per cent).
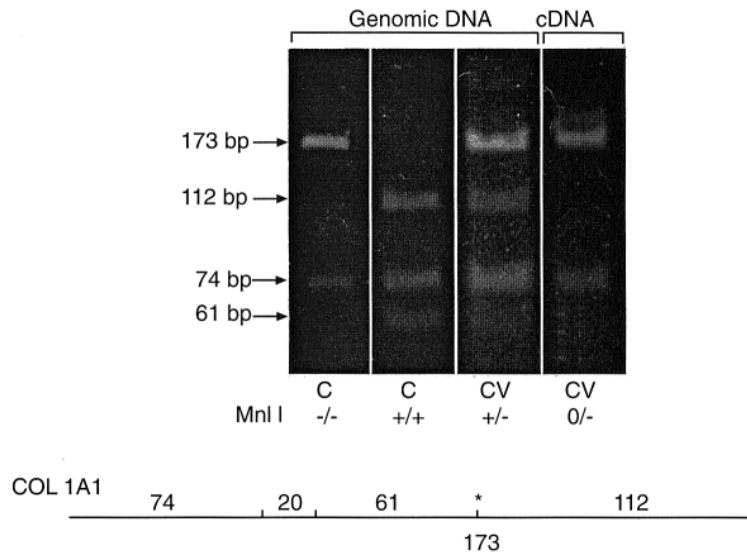
Fig. 3—Results of an *Mnl*I digest of genomic DNA and cDNA from messenger RNA derived from fetal CVS cells in family 1. The cDNA shows significant diminished *Mnl*I+ allele in the fetal mRNA, consistent with a null allele derived from the affected father and an affected pregnancy. The map below indicates the *Mnl*I sites in a 267 bp region of the COL1A1 3′ untranslated region; the polymorphic *Mnl*I site is indicated by the asterisk

Table III—Prenatal diagnosis in different types of OI and measured recurrence risk

OI type	N	Affected	Recurrence rate (%)
OI type II			
Two previous affected	7	2	28
One previous affected	50	1	2
OI type III or IV			
Two previous affected	3	0	—
One previous affected	31	1	3
Affected parent	22	11	50
OI type I			
Affected parent	16	11	68

DISCUSSION

Different OI phenotypes result from different mutations in the genes that encode the chains of type I procollagen (Byers, 1993, 1995). OI type I generally is a consequence of mutations that affect the synthesis of the proα1(I) chains and decrease the synthesis of type I procollagen by half (Barsh *et al.*, 1982; Rowe *et al.*, 1985; Genovese and Rowe, 1987; Wenstrup *et al.*, 1990). Because type I procollagen molecules must have two or more of the three chains as proα1(I) chains, a decrease in synthesis of proα1(I) chains effectively decreases the synthesis of type I procollagen. Mutations in a COL1A1 allele that result in a premature termination-codon, although they would predict the synthesis of truncated proα1(I) chains, in fact produce mRNA products that either are not transported to the cytoplasm (Stover *et al.*, 1993; Redford-Badwal *et al.*, 1996) or are rapidly degraded (Willing *et al.*, 1994). In contrast, OI type II, OI type IV, and most forms of OI type III generally result from mutations in either a COL1A1 or a COL1A2 allele that alter the structure of the chain synthesized so that the type I procollagen molecules into which the abnormal chains are incorporated are abnormal. As a consequence, cells from affected individuals synthesize some normal and some abnormal molecules. The abnormal molecules can generally be detected because the mutations alter the intracellular post-translational modification of all chains in the abnormal molecules and thus shift their electrophoretic mobility. Such mutations include rare instances of gene rearrangement and genomic deletions, splicing defects which remove single exons and, most commonly, point mutations which result in substitution for glycine within the
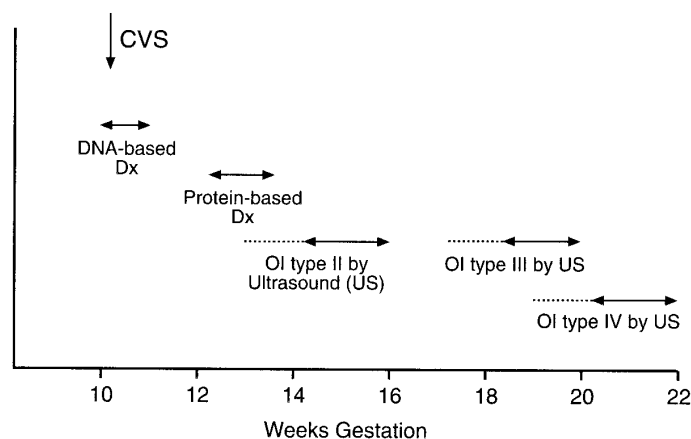
Fig. 4—Timing of prenatal diagnostic studies for different forms of osteogenesis imperfecta by weeks of gestation

triple helical domain of either chain (Byers, 1993, 1995). Because there is only a single copy of the COL1A1 and COL1A2 gene in the haploid human genome, any tissue which expresses these genes coordinately and assembles type I procollagen molecules in a normal fashion could be used to identify alterations in the amount synthesized or in the structure of the synthesized products. Chorionic villus cells do both and clearly can be used for accurate prenatal detection of fetuses with mutations that affect the structure of these genes. These cells are not, however, useful for the accurate identification of variation in the amounts of type I collagen synthesized and thus have limited value in the evaluation of cultured cells in pregnancies at risk for OI type I. This lack of reliability appears to be because as CVS cells age in culture, the relative amounts of type I and type III procollagens synthesized change. Thus, the prenatal diagnosis of OI type I is best accomplished by analysis of linked polymorphic markers in the COL1A1 gene or direct mutational analysis.

The best strategy to employ when considering prenatal diagnosis in a pregnancy at risk for OI depends on the recurrence risk in the family, the information available concerning the family structure and linked markers, whether an affected family member has been studied previously, and whether the precise mutation is known (Fig. 4).

For the perinatal lethal form of OI, an abdominal ultrasound examination can detect an affected fetus by 15–16 weeks' gestation (Raghunath *et al.*, 1994). In a pregnancy known to be at risk, vaginal probe ultrasound may be informative by 13 weeks'

gestation (Dimaio *et al.*, 1993). With current technology in experienced centres, it is unlikely that this diagnosis will be missed by abdominal sonography at 15–16 weeks. The majority of OI type II samples that we received for biochemical diagnostic studies (not prenatal diagnostic studies) during the period from 1982 to 1994 were obtained from fetal samples in which the diagnosis was suspected on routine ultrasound screening, the pregnancy terminated, and samples were sent to confirm the diagnosis. The biochemical diagnosis of OI type II can also be achieved by analysis of cultured CVS cells or by direct analysis of DNA for known mutations. The former usually provides results by 13–14 weeks' gestation (about 8–10 days to grow cells, a day for shipping, and another 7–10 days for the biochemical studies following the procedure for 10 weeks' gestation). The latter can provide the diagnosis within 4–6 days following the biopsy. Thus, for OI type II, if the molecular defect is not known, biochemical analysis of cultured CVS cells provides a diagnosis 1–2 weeks earlier than ultrasound studies. Raghunath *et al.* (1994) have indicated that direct analysis of proteins extracted from CVS cells can provide a diagnosis of OI type II. Their study, however, reports only normal outcomes so that demonstration of abnormal proteins by this method is not yet assured.

The advantages of the use of biochemical or molecular studies becomes clearer for the non-lethal forms of OI. The diagnosis of OI type III usually cannot be made by ultrasound studies until 19–20 weeks' gestation, at about which time limb

length begins to fall below the fifth percentile (Constantine *et al.*, 1991; Thompson, 1993). To be sure that growth is failing, a baseline ultrasound at 16 weeks' gestation and weeky or biweekly monitoring of growth are necessary. The diagnosis of OI type IV is usually not apparent by ultrasound until after 20 weeks' gestation when limb length discrepancy may become apparent or femoral bowing appears. In many instances, the diagnosis of OI type IV cannot be identified by ultrasound during pregnancy. OI type I is very difficult to identify by ultrasound and is only rarely apparent because of femoral bowing or fracture. Limb length is almost always normal. Biochemical examination of cultured CVS cells (OI type III/IV), or direct molecular analysis of a specific mutation, or evaluation of linked markers (all forms of OI) provides diagnostic studies in the same time as the studies in pregnancies at risk for OI type II. For the non-lethal disorders, then, the biochemical or molecular studies provide the most expeditious means of prenatal diagnosis if requested by parents.

The accuracy of prenatal diagnosis of osteogenesis imperfecta is largely dependent on the prior study of an affected individual in the family. Wenstrup *et al.* (1990) previously demonstrated that biochemical studies of collagens synthesized by fibroblasts cultured from skin of individuals with non-lethal forms of OI could detect abnormalities of collagen production or collagen structure in 85–90 per cent of those cell strains. We have identified abnormalities in collagen structure in proteins synthesized by cells cultured from about 98 per cent of infants with OI type II (data not shown). Thus, for the non-lethal forms of OI in which no prior studies have been done, there is a relatively low chance (10–15 per cent) that no evidence of a biochemical abnormality would be present in cells from an affected pregnancy; that risk is significantly lower for the OI type II group. If prenatal diagnostic studies are undertaken in the absence of prior biochemical or molecular studies, a normal result in the context of concern about OI type III or IV may not exclude the diagnosis of OI and often commits the family to additional costly ultrasound studies. Furthermore, because OI is not the only reason for fracture, it is important to know the correct diagnosis prior to doing specific biochemical studies for prenatal diagnosis.

Prenatal diagnostic studies for OI may also be used in the clarification of a diagnosis suggested by sonographic studies. The differential diagnosis of OI type II at 16 weeks' gestation is limited: achondrogenesis or hypochondrogenesis, thanatophoric dysplasia, and autosomal recessive hypophosphatasia being the most common. All are lethal and if the pregnancy is terminated, biochemical studies post-termination can provide the diagnosis, provide realistic recurrence risks, and earlier prenatal diagnosis in subsequent pregnancies. Fetal skin biopsies or placental biopsies provide little value, especially if the pregnancy is to be terminated regardless of outcome. The recognition of femoral (and other limb) bowing later in pregnancy can lead to some uncertainty of diagnosis; for example, different forms of OI, campomelic dysplasia. In these situations, CVS biopsy is less feasible, analysis of collagens synthesized by amniocytes is not useful, and only analysis of collagens synthesized by placental or fetal skin cells is likely to provide a specific diagnosis of a form of OI. In addition, given the time taken for collagen studies to be completed, it would likely be too late for intervention once results were available. These procedures have an increased risk of pregnancy loss which must be considered in the decision of whether to pursue additional studies or defer diagnostic studies until delivery.

Previous estimates for the recurrence risk for OI type II in siblings were 0–25 per cent (Sillence *et al.*, 1979; Thompson *et al.*, 1987; Byers *et al.*, 1988) and for OI type III it was 0 or 25 per cent (Sillence *et al.*, 1986; Thompson *et al.*, 1988) depending on the families studied. In some instances, these were empirical studies and in others, the estimates relied not only on the family studies, but also on assumptions regarding the mode of inheritance (e.g. Sillence *et al.*, 1979; Young *et al.*, 1987). It is now clear that the perinatal lethal form almost always results from new dominant mutations in the genes of type I collagen (Byers, 1993) and that recurrence of the OI type II phenotype in a sibship results from germline or mixed germline and somatic mosaicism in one of the parents (Cohn *et al.*, 1990; Cohen-Salal *et al.*, 1991; Bonaventure *et al.*, 1992; Edwards *et al.*, 1992; Mottes *et al.*, 1993). Similarly, in non-South African families (see Wallis *et al.*, 1993), recurrence of OI type III in sibships with normal parents usually results from parental mosaicism for a dominant mutation (Namikawa *et al.*, 1995; Raghunath *et al.*, 1995; Lund *et al.*, 1996). These risks need to be refined depending on the situation. From population studies, the estimated risk for the birth of a child with OI type II

is about 1:50 000 live births (Connor *et al.*, 1985; Orioli *et al.*, 1986, 1995). The birth of an infant with OI type II reflects a mutation that could have occurred at any of several stages: during the embryonic development of the parent who is a somatic and germline mosaic for the mutation; during the evolution of the germline in the parent; or during the formation of a single egg or sperm. The recurrence risk in each situation is different. In the first, it depends on the number of cells allocated to the germ cell lineage and the proportion of those cells that contain the mutant allele. In no case would the recurrence risk exceed 50 per cent, the risk if all allocated germline cells contained a copy of the mutant allele. This situation would occur only if the number of cells allocated to the germ lineage were small. The recurrence risk if a mutation occurs during expansion of the germ cell lineage depends on how many divisions have taken place prior to the mutational event. Only if the mutation occurs at the time of allocation or within the next few divisions is the risk likely to be significant (Wijsman, 1991). The risk for recurrence if the mutation affects a single sperm or egg is the mutation rate at that locus, again likely to be negligible. Thus, effectively, the empirically measured recurrence risk is shared unequally by different classes of families. Recurrence of the phenotype in sibs identifies a family in which a parent is very likely to be a somatic and germline mosaic for the mutation. Although the number of families with more than one pregnancy with OI type II is small, the recurrence rate in our population was two in seven pregnancies, or 28 per cent. In contrast, when the pregnancies following only a single previous affected pregnancy were monitored, the recurrence rate was 1 in 50 pregnancies, or 2 per cent ($P < 0.01$). Although there were no recurrences among the families at risk for OI type III, a similar stratification of risk is likely. Thus, in counselling families with previous pregnancies affected by a form of OI due to a dominant mutation, it is important to recognize that the recurrent risk cited should be stratified depending on the number of previous affected pregnancies.

In past studies, by identifying OI type II pregnancies, assessing sibship size, and estimating the recurrence risk based on numbers of unaffected siblings, a second affected fetus was identified in 6 per cent of pregnancies (Byers *et al.*, 1988). The 2 per cent recurrence of OI type II observed in our current study is a prospective ascertainment of the risk of couples with one previous affected pregnancy for whom prenatal diagnostic studies were undertaken. In each instance, the sample size was small and the methods used to calculate the recurrence risk differed so that statistical comparison is difficult. In the present study, the risk calculation is derived only from those with a single previous affected pregnancy and suggests that the lower risk of 2 per cent needs to be considered when counselling couples in the equivalent category. In addition, on the basis of our analysis of parent of origin of mutations in families with germinal mosaicism, this risk is probably divided equally between the two parents if they should initiate a pregnancy with a different partner (unpublished data).

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