

# Correspondence

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## Single base pair alterations as the predominant category of mutation in type I osteogenesis imperfecta

SIR,

The molecular pathology of type I osteogenesis imperfecta (OI) is beginning to be understood. Genetic linkage studies in a number of families have shown the type I collagen gene (*COL1A1*) to be the usual site of mutation in this dominantly inherited disorder.<sup>1</sup> A deficiency in the level of *COL1A1* mRNA or pro $\alpha$ 1(I) collagen is also frequently observed.<sup>2,3</sup> In a limited number of subjects a pro $\alpha$ 1(I) collagen defect has been defined and for some of these cases the genetic lesion is also known.<sup>4</sup> The DNA mutations include single base pair alterations as well as large deletion and insertion defects. However, because these cases were all initially identified as protein defects the observed DNA abnormalities do not necessarily represent the true spectrum of type I OI mutations. The present challenge in extending our understanding of this disorder is therefore to overcome the technical difficulties involved in studying the frequently encountered cases in which no protein defect is apparent or a *COL1A1* null allele exists.

In an attempt to meet this challenge we directly examined the *COL1A1* gene in genomic DNA samples from a series of nine independent type I OI patients. For five of these subjects previous linkage studies had shown *COL1A1* to be the defective loci<sup>1</sup> (data not shown). In the other four cases a *COL1A1* null allele had been indicated by protein and RNA studies (P Byers, 1988, personal communication).

The technique used for this study involved the S1 nuclease directed cleavage of heteroduplex DNA molecules formed between genomic material and cloned sequences. This approach to genomic DNA analysis was first described by Chebloune *et al.*<sup>5</sup> The methodology used for our study (a modified version of that described in the initial report) was known to permit the detection of short length variations of the order of 4 bp in heterozygous subjects but not single base pair alterations.<sup>6</sup>

In all nine subjects the complete 18 kb *COL1A1* gene as well as 2 kb of 5' flanking sequence was examined as 11 contiguous domains of 1.4 to 2.5 kb. No mutation was found. A positive control, consisting of a 4 bp length mismatch, showed all experiments to be qualitatively successful.

These results indicate that in the nine cases examined, and hence probably in the majority of type I OI subjects, the genetic defect is likely to be a single base pair alteration. This being so it is necessary that the nature of all normal sequence variations in and around the *COL1A1* gene be determined so that small mutations such as these can be identified and the molecular pathology of type I OI more completely defined.

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## Lethal osteogenesis imperfecta

SIR,

Two of us recently reported an infant with lethal osteogenesis imperfecta associated with a 46,XY,inv(7)(p13q22) karyotype in the *Journal of Medical Genetics*.<sup>1</sup> The parents are phenotypically normal; the mother has a 46,XX,inv(7)(p13q22) karyotype, and the father has a 46,XY karyotype. Because the gene for the pro $\alpha$ 2(I) chain of type I procollagen (*COL1A2*), a component of the principal collagen in bone, is assigned to the long arm of chromosome 7 at a site between bands q21 and q22,

we speculated that the karyotypic abnormality could be implicated in the infant's disease.

Analyses of type I procollagen gene products synthesised by fibroblastic cells (#GM 09324, mother, and 09325, father; National Institute of General Medical Sciences Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ, USA) cultured from biopsies of parental skin have now been completed. The techniques used are described elsewhere.<sup>2</sup> The synthesis of pro $\alpha$ 1(I) and pro $\alpha$ 2(I) chains, the electrophoretic mobility of the chains, and the efficiency of secretion of the intact molecules were all normal. It seems unlikely that the karyotypic abnormality present in the mother and infant was responsible for the infant's disease.

De novo mutations in type I collagen genes are the most frequent cause of lethal osteogenesis imperfecta.<sup>2</sup> While it is possible that the rearrangement involving 7p13q22 could predispose to mutations which alter *COL1A2*, we have no direct evidence that supports this hypothesis. The product of this couple's third pregnancy was normal by sonography at 18 weeks' gestational age and the infant was normal at birth.

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#### Genetic heterogeneity in Waardenburg's syndrome

STR,

Since the original description, many associations<sup>1</sup> and heterogeneity<sup>2</sup> have been described in Waardenburg's syndrome (WS). Here we report a study of three sibs of consanguineous parents (uncle-niece) with features suggestive of WS associated with obstructive ileal lesions, inherited as autosomal recessive trait, which we believe may be a variant of WS.

A female neonate was noted at birth to have a white forelock, bilateral blue irides, white eyelashes, a malformed right pinna, and multiple hypopigmented patches of varying sizes on the face (fig 1), both upper arms, and forearms. The inner canthal distance was 22 mm, interpupillary distance 44 mm, and outer canthal distance 70 mm, all within normal limits. Over the next 12 hours, the baby

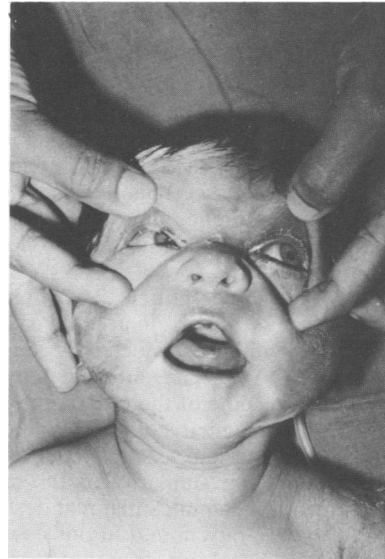


FIG 1 White forelock, light coloured irides, and white eyelashes.



FIG 2 Atretic ileal segments.