ment. This PCR product was subcloned into the pBluescript vector (Stratagene) and sequenced. It was found to represent RNA molecules in which the normal exon IIIc 3′ splice site had been used, but not the normal 5′ splice site (Fig. 1C). A new 5′ splice site is used, defining an intron whose 5′ end maps 2 nt downstream from the Crouzon mutation studied.

We interpret these data as follows. In the normal gene, the IIIc exon uses two alternative 5′ splice sites (Fig. 1C), with only very minor use being made of the upstream site. We cannot call the upstream site a cryptic site, as minor use of the upstream site is also made in splicing of pre-mRNA from the endogenous FGFR2 gene in HeLa cells (unpublished work) and has also been described in chicken lung and brain (8). Analogous use of a corresponding internal 5′ splice site has also been described in the IIIc exon of the mouse FGFR1 gene (2). The Crouzon G to A transition makes the upstream site correspond more closely to the 5′ splice site consensus sequence, and the splicing apparatus switches to use of the upstream 5′ splice site. Our data show that the G to A transition in exon IIIc alone is sufficient to provoke the change of 5′ splice sites: no additional mutations in the flanking exons are needed.

The extracellular domain of the normal FGFR2 is composed of 3 Ig domains (5). The third domain, encoded in part by the IIIc exon, is involved in ligand binding (5, 6). As a result of the change in 5′ splice sites, the FGFR2 mRNA produced in Crouzon syndrome now codes for a receptor lacking 17 amino acids of this domain. With this information in hand, it is possible to test the effect of FGFR2 signal transduction of the Crouzon syndrome mutation. This should in turn add to our understanding of the disease.

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Bone morphogenetic protein-4 (BMP-4) is a member of the transforming growth factor-beta (TGF-β) superfamily and is involved in morphogenesis and bone cell differentiation (2). Recombinant BMP-4 can induce ectopic cartilage and bone formation when implanted subcutaneously or intramuscularly in rodents. This ectopic bone formation process resembles the process of bone formation during embryogenesis and fracture healing (10). A cosmide clone containing the complete human bone morphogenetic protein-4 gene (BMP4) was isolated (details to be published elsewhere) and used as a probe to determine the precise chromosomal localization of the human BMP4 gene. This cosmide clone was labeled with biotin-14-dATP and hybridized in situ to chromosomal preparations of metaphase cells as described previously (6). In 20 metaphase preparations, an intense and specific fluorescence signal (FITC) was detected on the q arm of chromosome 14. The DAPI-counterstained chromosomes were computer-

Fine Mapping of the Human Bone Morphogenetic Protein-4 Gene (BMP4) to Chromosome 14q22–q23 by in Situ Hybridization

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were used, which did not allow a more detailed chromosomal sublocalization. The present fine localization of BMP4 to 14q22–q23 makes BMP-4 a possible candidate gene for Holt–Oram syndrome (HOS). HOS is a heritable disorder of skeletal and cardiac development (5). The HOS phenotype is probably determined early in embryogenesis. This heterogeneous disorder (8) may be caused by several types of gene mutations or deletions on either chromosome 12q or 14q. Although one HOS locus has been localized to chromosome 12, other forms of HOS have also been described (1), which are not linked to chromosome 12 (6). In one case, a direct association has been established between HOS and a deletion of the 14q23–q24.2 region (9). The BMP4 localization to 14q22–q23 shown here in combination with the putative role of BMP-4 in limb development (3) and its distribution during development (4) suggests that the disturbed development of skeleton and heart in some HOS patients may be due to a disturbed BMP4 expression or an altered gene product. However, such an association of BMP4 with HOS still must be confirmed by genetic linkage and mutation analyses.

REFERENCES


FIG. 1. Fluorescence in situ hybridization of the BMP4-containing cosmide to region q22–q23 of human chromosome 14. (A) Positively imaged chromosomes showing hybridization signal (arrowhead) and (B) chromosome 14: morbid anatomy with localization of BMP4.