The Molecular and Genetic Basis of Fibroblast Growth Factor Receptor 3 Disorders: The Achondroplasia Family of Skeletal Dysplasias, Muenke Craniosynostosis, and Crouzon Syndrome with Acanthosis Nigricans*

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ABSTRACT

Achondroplasia, the most common form of short-limbed dwarfism in humans, occurs between 1 in 15,000 and 40,000 live births. More than 90% of cases are sporadic and there is, on average, an increased paternal age at the time of conception of affected individuals. More then 97% of persons with achondroplasia have a Gly380Arg mutation in the transmembrane domain of the fibroblast growth factor receptor (FGFR) 3 gene. Mutations in the FGFR3 gene also result in hypochondroplasia, the lethal thanatophoric dysplasias, the recently described SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans) dysplasia, and two craniosynostosis disorders: Muenke coronal craniosynostosis and Crouzon syndrome with acanthosis nigricans. Recent evidence suggests that the phenotypic

differences may be due to specific alleles with varying degrees of ligand-independent activation, allowing the receptor to be constitutively active.

Since the Gly380Arg achondroplasia mutation was recognized, similar observations regarding the conserved nature of FGFR mutations and resulting phenotype have been made regarding other skeletal phenotypes, including hypochondroplasia, thanatophoric dysplasia, and Muenke coronal craniosynostosis. These specific genotype-phenotype correlations in the FGFR disorders seem to be unprecedented in the study of human disease. The explanation for this high degree of mutability at specific bases remains an intriguing question. (*Endocrine Reviews* **21**: 23–39, 2000)

I. Introduction

THE FIRST phenotype known to be caused by a mutation in the gene encoding fibroblast growth factor receptor (FGFR) 3 was achondroplasia (Fig. 1), the most common form of human dwarfism (1, 2). The achondroplasia family of skeletal dysplasias, as described by Spranger (3), also includes the mildly severe hypochondroplasia (Fig. 2) and the lethal thanatophoric dysplasia (TD) (Fig. 3). Recently, SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans) dysplasia (Fig. 4), a skeletal dysplasia with features of both achondroplasia and TD, has been

added to this family of disorders (4). These other disorders in the achondroplasia family also result from mutations in the FGFR3 gene (4–12). In individuals with achondroplasia the skeleton is the primary system involved in the phenotype, and all of the disorders in the achondroplasia family of skeletal dysplasias involve some degree of short stature and/or abnormal ossification of bony structures.

Although achondroplasia, hypochondroplasia, and TD have been recognized as genetic disorders for decades, the first reports of their molecular basis were published only very recently (1, 2, 13, 14). Since then, a number of mutations that result in these disorders have been described, and their possible effects on skeletal development postulated. FGFR3 mutations have also been described in two craniosynostosis phenotypes: Muenke coronal craniosynostosis (Fig. 5) (15–17) and Crouzon syndrome with acanthosis nigricans (Fig. 6) (18). In general, the relationship between mutations in the FGFR3 gene and other FGFR genes, and the phenotypes that result from these mutations, have broken new ground in the understanding of human mutations and genetic disorders. In the FGFR genes, more than any other, there is a highly conserved relationship between mutations at particular amino acids and resulting phenotypes (1, 2, 5, 6, 15, 17–20). Moreover, the FGFR3 nucleotides mutated in the majority of cases of achondroplasia and Muenke craniosynostosis are among the most highly mutable nucleotides in the human genome.

The clinical spectrum of the achondroplasia family of dis-

FIG. 1. Typical achondroplasia, seen here in a husband and pregnant wife. Note the disproportionate short stature with rhizomelic (proximal) shortening of the limbs, relative macrocephaly, and midface hypoplasia. Some of the additional manifestations of achondroplasia are lumbar lordosis; mild thoracolumbar kyphosis, with anterior beaking of the first and/or second lumbar vertebra; short tubular bones; short trident hand; and incomplete elbow extension. [Reproducted with permission.]

orders ranges from mildly affected hypochondroplasia to inevitably lethal TD (21, 22). This article reviews the molecular and genetic basis and clinical features of these skeletal dysplasias and the craniosynostosis phenotypes that result from mutations in the FGFR3 gene. Although there are significant exceptions to this generalization, dominant mutations in the human FGFR3 gene recognized to date predominantly affect bones that develop by endochondral ossification, while dominant mutations involving FGFR1 and FGFR2, such as Pfeiffer syndrome, Crouzon syndrome, Apert syndrome, Beare-Stevenson cutis gyrata syndrome, and Jackson-Weiss syndrome (19, 20, 23–40), principally cause syndromes that involve bones arising by membranous ossification. In this review we discuss the structure and function of the normal and mutant FGFR3 gene. Finally, we
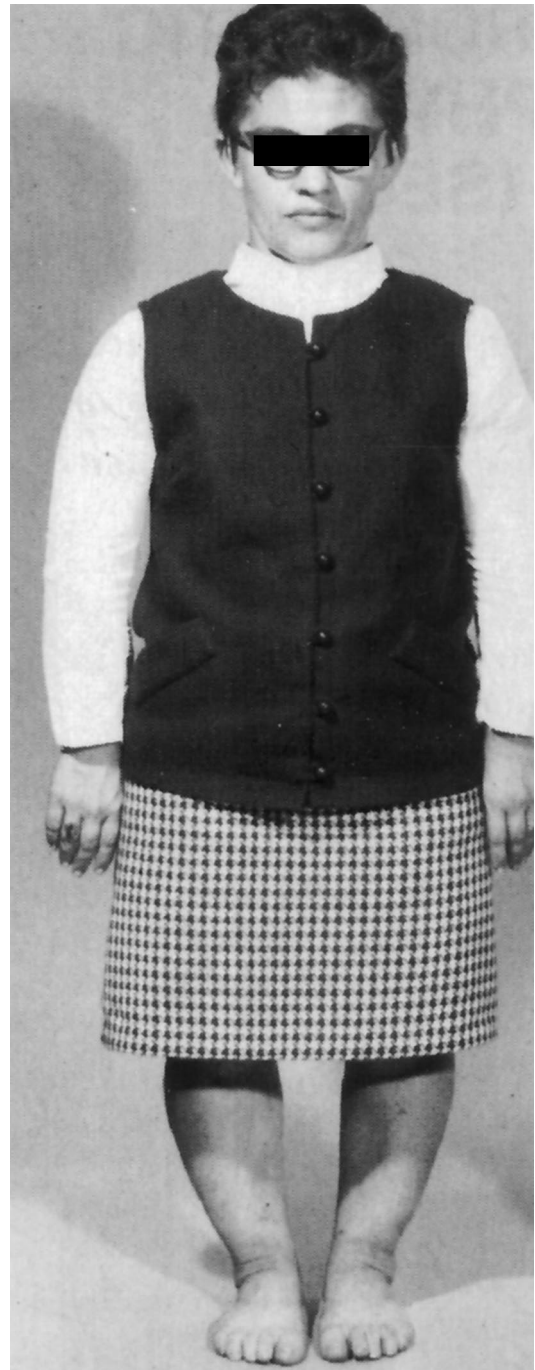

FIG. 2. Typical hypochondroplasia. Notice small stature, especially in the bowed lower limbs, and stubby hands and feet. In hypochondroplasia, limbs are usually short, without rhizomelia, mesomelia, or acromelia, but may have mild metaphyseal flaring. Brachydactyly and mild limitation in elbow extension can be evident. Spinal manifestations may include anteroposterior shortening of lumbar pedicles. The spinal canal may be narrowed or unchanged caudally. Lumbar lordosis may be evident. [Reprinted with permission from Beals RK 1969 Hypochondroplasia: a report of five kindreds. Journal of Bone & Joint Surgery (Am) 51:728–736.]

summarize the implications of the molecular basis of these disorders and potential for GH therapy in patients with achondroplasia and hypochondroplasia.
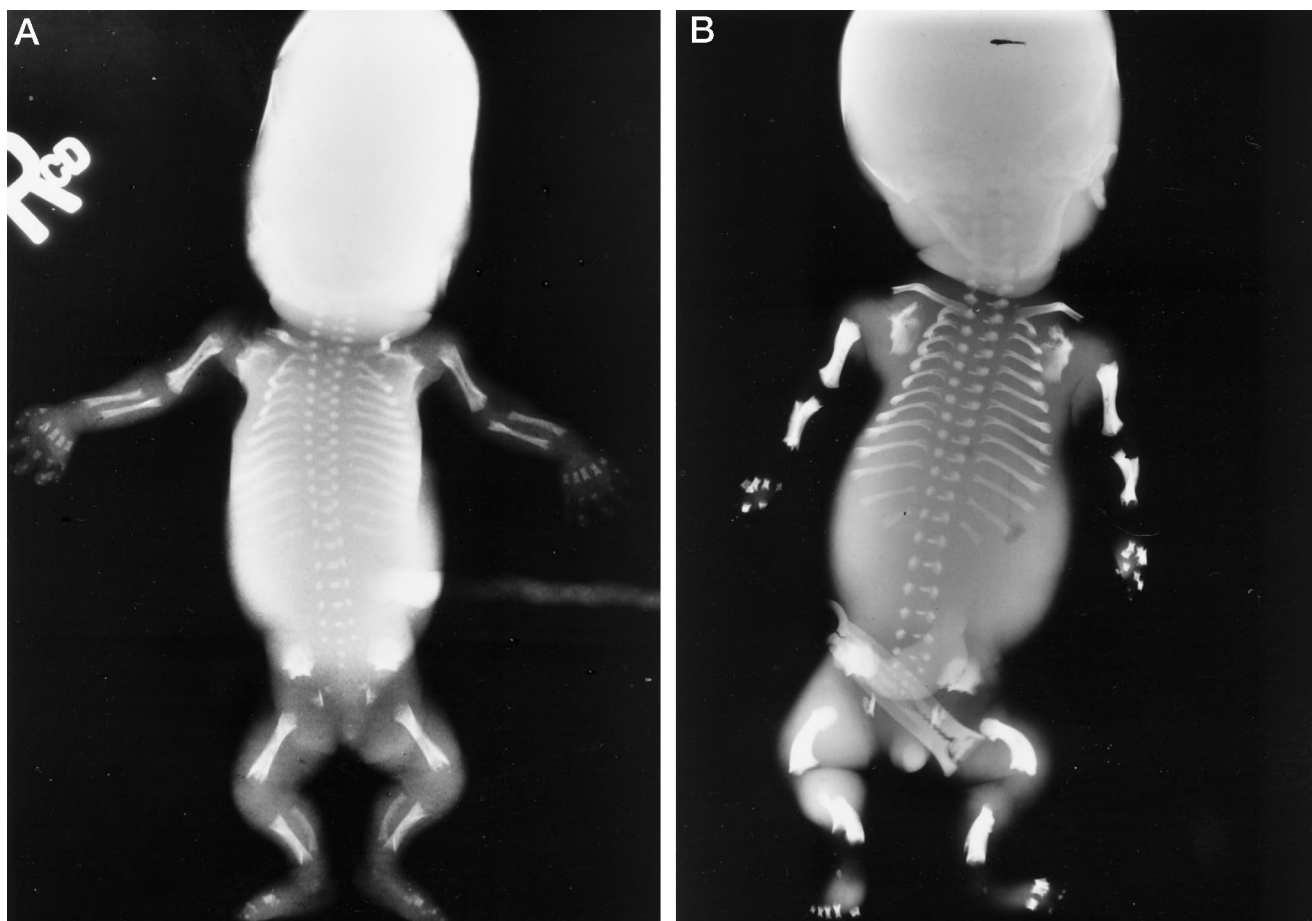
Fig. 3. Thanatophoric dysplasia. The features of TD include micromelic shortening of the limbs, macrocephaly, platyspondyly, and reduced thoracic cavity with short ribs. *A*, TD type II. Note the straight femurs. Cloverleaf skull may also be a feature of TD II. *B*, TD type I. Note the curved femurs. [Figures courtesy of Dr. Ralph Lachman.]

II. Fibroblast Growth Factor Receptor 3 (FGFR3)

In humans, the FGFRs represent a family of four tyrosine kinase receptors (FGFR 1–4) that bind fibroblast growth factors (FGFs) with variable affinity (41). The FGF family of proteins consist of at least 18 structurally related, heparan-binding polypeptides that play a key role in the growth and differentiation of various cells of mesenchymal and neuroectodermal origin (42–45). FGFs are also implicated in chemotaxis, angiogenesis, apoptosis, and spatial patterning (46, 47). The FGFs share many structural features. Distinction between these ligands is determined by different expression patterns during and after development, as well as different affinities for specific FGFRs. FGF 1, 2, 4, 8, and 9 have been shown to bind with high affinity or to activate FGFR3 (48–52).

The FGFR3 gene maps to human chromosome 4p16.3 (53). The cDNA was originally isolated in the search for the Huntington disease gene on chromosome 4 (54, 55). The 4.4-kb cDNA contains an open reading frame of 2,520 nucleotides, encoding an 840-residue protein. The human and mouse FGFR3 genes have recently been characterized (56–58) and span approximately 16.5 kb and 15 kb, respectively. Both genes consist of 19 exons and 18 introns. In both genes, the translation initiation and termination sites are located in

exons 2 and 19, respectively. The 5′-flanking regions lack typical TATA and CAAT boxes. However several putative *cis*-acting elements are present in the promoter region, which is contained within a CpG island (57, 58). The promoter regions of both the human and mouse FGFR3 genes are very similar, with several conserved putative transcription factor-binding sites, suggesting an important role for these elements and their corresponding transcription factors in the transcriptional regulation of FGFR3 (58). It has been demonstrated that the 100 bp of FGFR3 sequence 5′ to the initiation site are sufficient to confer a 20- to 40-fold increase in transcriptional activity (59). FGFR3 sequences between −220 and +609 are sufficient to promote tissue-specific expression (59).

Proteins in the family of fibroblast growth factor receptors (FGFRs) have a highly conserved structure (Fig. 7). The mature FGFR3 protein, like all of the FGFRs, is a membrane-spanning tyrosine kinase receptor with an extracellular ligand-binding domain consisting of three immunoglobulin subdomains, a transmembrane domain, and a split intracellular tyrosine kinase domain (Fig. 7) (60). Ligand binding requires dimerization of two monomeric FGFRs and includes a heparin-binding step. Promiscuous dimerization is observed; for example, in addition to dimerizing with itself,

Fɪɢ. 4. SADDAN dysplasia is characterized by extreme short stature, severe tibial bowing, profound developmental delay, and acanthosis nigricans. A, Young girl. Notice the moderate bowing of the femurs with reverse bowing of the tibia and fibula. B, Man in early twenties. Notice the extreme short stature and severe acanthosis nigricans. Individuals with SADDAN dysplasia also have had seizures and hydrocephalus during infancy with severe limitation of motor and intellectual development. [Reprinted with permission from G. A. Bellus *et al*.: *Am J Med Genet* 85:53–65, 1999 (106). © Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

FGFR1 may dimerize with FGFR2, FGFR3, or FGFR4. Similar dimerization combinations of other FGFR monomers are also possible. Differing combinations of dimers are observed in different tissues and different stages of development, and this diversity of dimers probably plays an important role in skeletal differentiation (60).

A further element of complexity is introduced by the presence of alternative splice sites in the FGFR genes. These are

FIG. 5. Muenke coronal craniosynostosis. Facial findings of affected individuals from 18 families. *Black circles* (●) denote postoperative photographs. Clinical manifestations consist of bicoronal synostosis, unicoronal synostosis, macrocephaly, and abnormal skull shape. A high arched palate, sensorineural hearing loss, and developmental delay can also be evident. [Reprinted with permission from M. Muenke *et al.*: *Am J Hum Genet* 60:555–564, 1997 (17). © The University of Chicago.]

FIG. 6. Crouzon syndrome with acanthosis nigricans. Female with brachycephaly, ocular protosis, and hypertelorism (*left*). Also evident are manifestations of hyperpigmentation, hyperkeratosis, and melanocytic nevi (*right*). [Reprinted with permission from Jameson JL (ed): *Principles of Molecular Medicine*, 1998 (149). © Humana Press.]

found in the third immunoglobulin domain (closest to the membrane) and typically splice in an alternative exon for this domain. The Ig domain 3 is encoded by two separate exons: exon IIIa encodes the N-terminal part of the domain, and the C-terminal half is encoded by either exon IIIb or IIIc (48, 61). The splice forms differ in their ligand affinity and preferential ligand binding, as well as tissue-specific expression. FGFR3 with exon IIIb has a high ligand specificity for FGF-1 (also known as acidic FGF) (48) and is expressed in mouse embryo, skin, and epidermal keratinocytes (61). The splice form containing exon IIIc was detected in the developing mouse brain and in the spinal cord and in all other bony structures (62, 63). Developmental expression of FGFR3 suggests this protein plays a significant role in skeletal development. Outside the nervous system, the highest levels of FGFR3 are observed in cartilage rudiments of developing bone (64). In the mouse, FGFR3 has an unique pattern of expression during organogenesis. FGFR3 is expressed in the germinal epithelium of the neural tube. At one day postpartum and in the adult mouse and rat brain, FGFR3 is expressed diffusely (64, 65). In the chick, FGFR3 is ubiquitously expressed in the mesoderm of limb and feather buds (66). Understanding the developmental expression patterns of FGFR3 has aided in the understanding of the human phenotypes that result from mutations in this gene. These phenotypes, including the achondroplasia family of skeletal dysplasias, Muenke coronal craniosynostosis, and Crouzon syndrome with acanthosis nigricans, are discussed below.

III. Clinical and Molecular Studies

A. The achondroplasia family of skeletal dysplasias

Dr. Jurgen Spranger (3) was far ahead of his time when he first described families of skeletal dysplasias. Before the first mutation in COL2A1, the gene that encodes type II collagen, was identified, he recognized that achondrogenesis, hypochondrogenesis, spondyloepiphyseal dysplasia, and Stickler syndrome were members of the same family of skeletal dysplasias. Similarly, he classified achondroplasia, hypochondroplasia, and TD in the same family, based on similarities in their skeletal and histological phenotypes. He grouped these disorders into families, despite the wide variation in their severity. Time, together with the vast progress in molecular and genetic studies of the skeletal dysplasias, has confirmed Dr. Spranger's clinical observations.

The achondroplasia family, as described by Spranger (3), is characterized by a continuum of severity ranging from mild (hypochondroplasia) and more severe forms (achondroplasia) to lethal neonatal dwarfism (TD). The identification of FGFR3 mutations in each of the disorders in the "achondroplasia family" of skeletal dysplasias, as well as COL2A1 mutations in the "type II collagenopathies" (67), fortified Dr. Spranger's remarkable power of clinical observation.

Achondroplasia and TD type II (see below) both appear to be genetically homogeneous (and, most of the time, homoallelic) conditions in that they are caused by a single nucleotide substitution in more than 95% of cases (1, 2, 5, 7, 10). Interestingly, the opposite situation was observed in association with mutations with other FGFR-related defects. In the craniosynostosis syndromes caused by mutations in FGFR1, FGFR2, or FGFR3, similar mutations, but in different receptors, have been found to cause distinct phenotypes: FGFR1 Pro252Arg results in Pfeiffer syndrome; FGFR2 Pro253Arg results in Apert syndrome; and FGFR3 Pro250Arg causes Muenke craniosynostosis (15). FGFR2 mutations are also associated with Crouzon, Pfeiffer, and Jackson-Weiss syn-
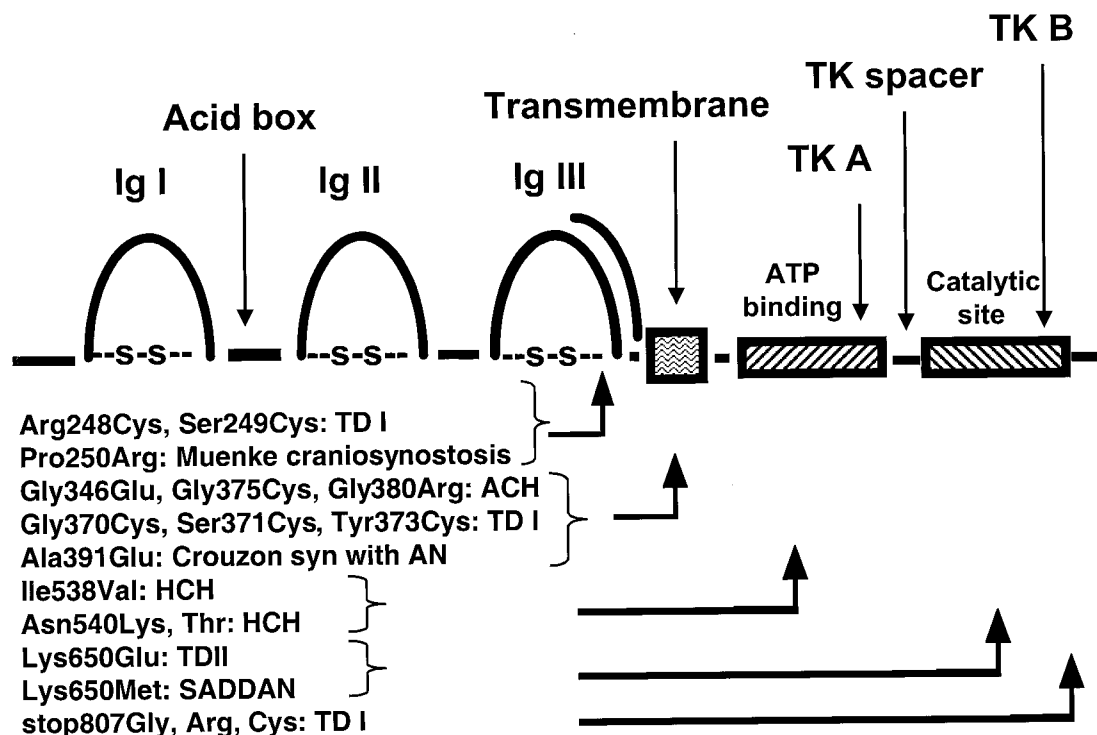
FIG. 7. Schematic diagram of a prototypical FGFR protein. Three Ig-like domains (IgI–IgIII) are indicated by loops, closed with disulfide bridges. These Ig-like domains are extracellular and responsible for ligand binding. Alternative splicing in the C-terminal half of the third Ig-like loop is indicated by an extra "half" loop. The acid box is a stretch of acidic amino acids found in all FGFRs between IgI and IgII. The tyrosine kinase (TK) domains are found intracellularly. The tyrosine kinase (TK) A domain contains the ATP binding site. The tyrosine kinase (TK) B domain contains the catalytic site. Also shown are the FGFR3 mutations and their approximate corresponding locations within the protein. ACH, Achondroplasia; AN, acanthosis nigricans; HCH, hypochondroplasia.

dromes (19, 20, 68); interestingly, all three phenotypes can be caused by a FGFR2 Cys342Arg mutation.

1. Achondroplasia. Achondroplasia, the most common cause of dwarfism in man, occurs in approximately between 1 in 15,000 and 1 in 40,000 live births. It is an autosomal dominant disorder with complete penetrance, characterized by short-limbed dwarfism, macrocephaly, depressed nasal bridge, frontal bossing, and trident hands (Fig. 1) (69, 70). X-rays show a shortening of long bones with squared-off iliac wings, a narrow sacrosciatic notch, and distal reduction of the vertebral interpedicular distance (Fig. 8) (69, 70). Physical and radiographic findings of the disorder are remarkably consistent. Histopathology demonstrates a defect in the maturation of the cartilage growth plate of long bones. More than 90% of the cases are sporadic, and there is an increased paternal age at the time of conception of the affected individuals, suggesting that the *de novo* mutations are of paternal origin. Affected individuals are fertile and achondroplasia is transmitted as a fully penetrant autosomal dominant trait (21, 71). In contrast, homozygous achondroplasia is usually lethal in the neonatal period and affects 25% of the offspring of matings between two parents with heterozygous achondroplasia (72).

In 1994, the gene responsible for achondroplasia was mapped to a region of 2.5 mb of DNA at the telomeric end of the short arm of chromosome 4 (4p16.3) (13, 14, 73). Significantly, it mapped very close to another elusive disease gene locus, that of Huntington disease. Only a few months

later, the candidate region for achondroplasia was recognized to contain the gene encoding FGFR3 (1, 2). Mapping of the achondroplasia locus allowed Dr. John Wasmuth and associates (1) at the University of California, Irvine, the laboratory that had identified the FGFR3 cDNA in the search for the Huntington disease gene, to quickly screen this gene for mutations in achondroplasia probands; mutations in FGFR3 were quickly identified. Concurrently, Rousseau *et al.* (2) also identified the same FGFR3 mutations as the cause of achondroplasia. FGFR3 mutations that result in TD were identified soon thereafter, confirming the allelic nature of the disorders (see below) (5). The identification by Bellus *et al.* (6) of a conserved FGFR3 mutation that causes hypochondroplasia completed, at the time, the allelicism of the achondroplasia family of skeletal dysplasias.

The first reports of mutations in FGFR3 causing achondroplasia (1, 2) indicated that 37 of 39 mutations studied were exactly the same, a G-to-A transition at nucleotide 1138 (G1138A). The remaining two mutations were a G-to-C transversion at the same nucleotide (G1138C). Both mutations result in the substitution of arginine for the glycine residue at position 380 (Gly380Arg) in the transmembrane domain of the protein (Figs. 7 and 9). Most analyses were performed on heterozygous achondroplasia patients, but the Gly380Arg mutation was also detected in several cases of homozygous achondroplasia, in which both parents of the proband had achondroplasia. In 1995, Bellus *et al.* (74) confirmed the remarkable degree of genetic homogeneity of the disorder by
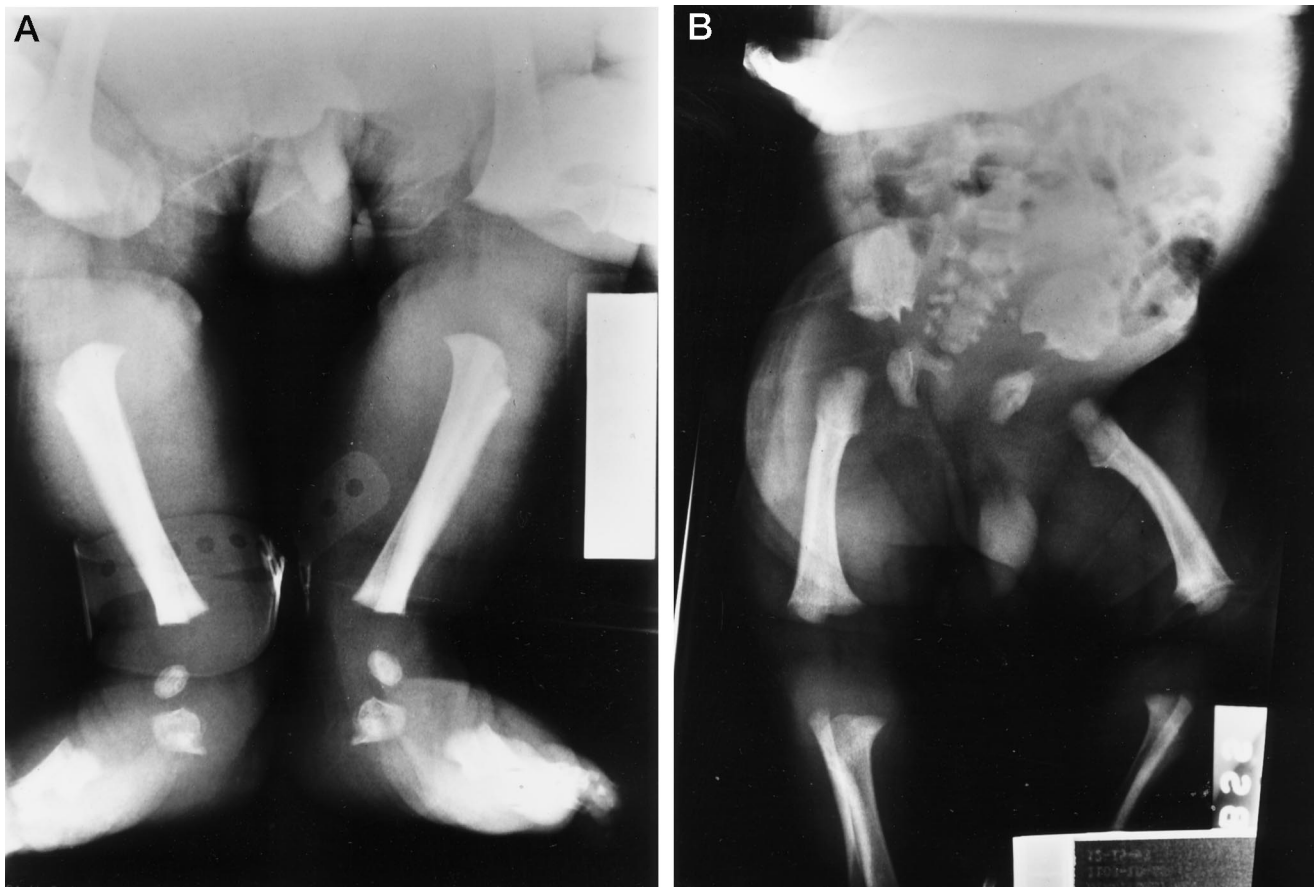
FIG. 8. Radiographic features of achondroplasia. Lower limbs in a young child. Note widened metaphyses, "chevron seat" epiphyses, and short long bones. Radiographically, manifestations can also include lumbar lordosis and mild thoracolumbar kyphosis, with anterior beaking of the first and/or second lumbar vertebra; small cuboid-shaped vertebral bodies with short pedicles and progressive narrowing of the lumbar interpedicular distance; small iliac wings with narrow greater sciatic notch; short tubular bones; metaphyseal flaring; short trident hand with short proximal midphalanges; and short femoral neck. [Figures courtesy of Dr. Ralph Lachman.]

finding the Gly380Arg mutation in 153 of 154 achondroplastic alleles. In this series, the G-to-A transition accounted for 150 alleles, while the G-to-C transversion was found in 3. [The last patient was later rediagnosed as having SADDAN dysplasia, based on phenotypic findings much more severe than those found in typical achondroplasia (see below). Therefore Bellus *et al.* (74) found FGFR3 mutations in 100% of their cohort, with the two achondroplasia mutations ob-
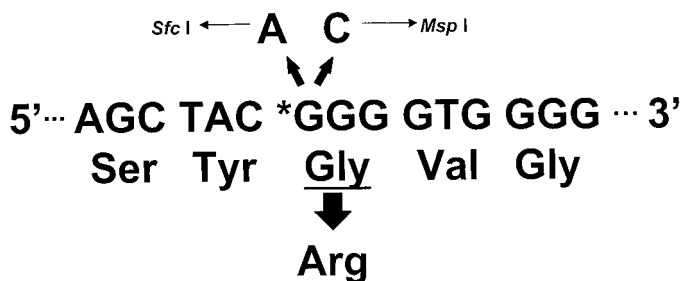

FIG. 9. The common FGFR3 mutations causing achondroplasia both result in Gly380Arg amino acid substitutions. Shown is the FGFR3 sequence surrounding the site of the common mutation. A G1138A mutation creates a novel *Sfc*I site; a G1138C mutation creates a *Msp*I site. The nucleotide changed in the common mutation (G1138) is depicted by an (*). The glycine residue (Gly380) is *underlined*.

served in all 153 of their patients with true achondroplasia.] Thus, the vast majority of cases of achondroplasia are caused by the same Gly380Arg mutation. Exceptions include two cases, reported by Superti-Furga *et al.* (75) and Nishimura *et al.* (76), in which a Gly375Cys mutation was detected five amino acids away from the common codon 380 mutation, and an achondroplasia patient with a novel Gly346Glu mutation identified by Prinos *et al.* (77).

Very recently, studies from various countries (Sweden, Japan, and China) showed the Gly380Arg mutation in all achondroplasia patients studied, confirming the remarkable genetic homogeneity of achondroplasia (78–83). This observation and the relatively high incidence of achondroplasia suggest that nucleotide 1138 of the FGFR3 gene is the most mutable nucleotide described so far in the human genome. The homogeneity of mutations in achondroplasia is unprecedented for an autosomal dominant disorder and may explain the relatively moderate variability in the phenotype of the disease (74). We have recently demonstrated that, as previously expected, FGFR3 mutations in sporadic cases of achondroplasia occur exclusively on the paternally derived chromosome, suggesting an advanced paternal age effect and that factors influencing DNA replication or repair during

spermatogenesis may predispose to the occurrence of the achondroplasia mutation (84).

2. Hypochondroplasia. The findings in patients with achondroplasia prompted the search for FGFR3 mutations in other disorders considered related to achondroplasia. Hypochondroplasia (Fig. 2) is an autosomal dominant condition characterized by short stature, micromelia, and lumbar lordosis. Clinical symptoms, radiological features, and histopathological aspects are similar to, but milder than those seen in achondroplasia (85, 86). Many cases are first referred for endocrinological evaluation of short stature.

McKusick *et al.* (87) first proposed that achondroplasia and hypochondroplasia are allelic, based on the similarities in phenotype between the two disorders and the identification of a severely dwarfed patient whose father had achondroplasia and whose mother had hypochondroplasia. More than two decades later, molecular linkage studies supported allelism of achondroplasia and hypochondroplasia (14, 88). Subsequently, heterozygous FGFR3 mutations were detected in DNA from persons with hypochondroplasia: C-to-A or C-to-G transitions at nucleotide 1620 (C1620A, C1620G), resulting in an Asn540Lys substitution in the proximal tyrosine kinase domain (6). These observations have since been confirmed in several laboratories (8, 89–91). In 1996, Prinster *et al.* (92) also found the C-to-A and C-to-G changes at nucleotide 1620 in Italian hypochondroplasia patients, and a novel FGFR3 Ile538Val that results in hypochondroplasia was also identified (93). However, studies of other families with hypochondroplasia have shown the phenotype to be unlinked to chromosome 4p16.3 (94, 95). In three familial cases not linked to chromosome 4, Rousseau *et al.* (89) reported that the phenotype was milder, macrocephaly and shortening of the bones were less obvious, the hands were normal, and no metaphyseal flaring was noted, as compared with hypochondroplasia probands, due to the FGFR3 Asn540Lys mutation. Prinster *et al.* (96) also described nine cases of hypochondroplasia not due to the FGFR3 Asn540Lys mutation. The authors stated that although they could not identify firm genotype-phenotype correlations, in their study the Asn540Lys mutation was most often associated with disproportionate short stature, macrocephaly, and with radiological findings of unchanged or narrow interpedicular distance and fibula longer than the tibia (96). This observation supports the view that unlike achondroplasia, hypochondroplasia is a clinically and genetically heterogeneous condition (85, 86, 95–97).

3. TD. TD (Fig. 3) is one of the more common sporadic lethal skeletal dysplasia, affecting approximately 1 of 60,000 births. The features include micromelic shortening of the limbs, macrocephaly, platyspondyly, and reduced thoracic cavity (98, 99). In the most common subtype (type I, TD I), femurs are curved, while in type II (TD II), straight femurs are present and cloverleaf skull may also be a feature of the phenotype. Interestingly, mutational studies have confirmed the classification of TD into these two subtypes (5). Affected individuals usually die in the neonatal period. However, a limited number of cases with prolonged survival have been reported (100, 101).

Mutations in the FGFR3 gene have been identified in both types of TD (5, 7, 9). Indeed, heterozygous mutations were found to cluster mainly to two different locations in the FGFR3 gene, depending on the phenotype. While TD II was accounted for by a single recurrent mutation in the tyrosine kinase 2 domain (Lys650Glu), TD I results from either a stop codon mutation or missense mutations in the extracellular domain of the gene (11). Interestingly, all missense mutations found so far created cysteine residues (9, 12).

In the first report of FGFR3 mutations in TD, Tavormina *et al.* (5) demonstrated a sporadic mutation causing a Lys650Glu change in the tyrosine kinase domain in 16 of 16 TD II patients. In the same study, the authors also report a mutation causing an Arg248Cys change in 22 of 39 TD I patients and a Ser371Cys mutation was found in one additional infant with TD I. Interestingly, the first 15 TD patients tested for the Lys650Glu mutation were not separated based on TD subtype. Of those 15, nine had the mutation. It was not until after the molecular analysis that the radiographs of the TD probands were reexamined and separated into subgroups based on straight or curved femurs. Nine patients had straight femurs, consistent with TD II. Those nine patients all had the Lys650Glu mutation. The remaining six had curved femurs, consistent with a TD I phenotype (5).

Subsequently, Rousseau *et al.* (7) reported mutations in the stop codon (stop807Gly, stop807Arg, and stop807Cys) in five additional patients with TD I. The latter mutations removed the normal translation stop signal and are predicted to result in a protein 141 amino acids longer than normal if translation continues to the next in-frame stop codon (7, 10). In 1996, Rousseau *et al.* (11) reported two novel missense mutations (Tyr373Cys and Gly370Cys), creating cysteine residues in the extracellular domain of the receptor in 9 of 26 TD I patients, giving further support to the view that newly created cysteine residues in the extracellular domain of the protein appear to play a key role in the severity of the disease (5, 7, 10, 11). Pokharel *et al.* (102), in late 1996, found the mutation most commonly reported in previous European and North American studies, the Arg248Cys substitution, in five of five Japanese TD I patients. The reported patients included cases with the usual presentation, and also, a case with a 9-yr follow up, representing an unusually mild clinical course for TD. This may suggest that, similarly to achondroplasia, TD I is a genetically homogenous condition (10, 102).

Histopathologically, cases with the Lys650Glu substitution demonstrated relatively more preservation of the physeal chondrocyte columns with identifiable proliferative and hypertrophic zones. The fibrous band was present only adjacent to the periosteum. In contrast, the fibrous band was more extensive and the column preservation poorer in cases with the Arg248Cys substitution (103). In this study, 91 cases of TD were examined for clinical, radiographic, and histological findings. Every case of TD examined had an identifiable FGFR3 mutation (103). Interestingly, radiographically, all of the cases with the Lys650Glu substitution demonstrated straight femurs with craniosynostosis and, frequently, a cloverleaf skull. In all other cases, the femurs were curved (103).

The platyspondylic lethal skeletal dysplasias (PLSDs) are a heterogeneous group of short-limb dwarfing conditions,

with TD the most common form. Three other types of PLSD, or TD variants (San Diego, Torrance, and Luton), have been distinguished from TD. The most notable difference between TD and the variants is the presence of large rough endoplasmic reticulum inclusion bodies within chondrocytes of the variants. Brodie *et al.* (104) examined 22 cases of TD variants for the presence of missense mutations in the FGFR3 gene. All 17 cases examined of the San Diego type (PLSD-SD) were heterozygous for some of the same FGFR3 mutations that cause TD I. Of the 17 FGFR3 mutations identified, 7 were Arg248Cys mutations, 2 were Ser249Cys mutations, 6 were Tyr373Cys mutations, and 2 were stop codon mutations. No mutations were identified in the Torrance and Luton types. Large inclusion bodies were found in 14 cases of PLSD-SD, with the material retained within the rough endoplasmic reticulum staining with antibody to the FGFR3 protein. The authors speculate that the radiographic and morphological differences between TD and PLSD-SD may be due to other genetic factors (104).

4. SADDAN dysplasia. SADDAN dysplasia (Fig. 4) is a recently described phenotype also belonging to the achondroplasia family of skeletal dysplasias. SADDAN dysplasia was originally named SSB dysplasia, for skeletal, skin, and brain dysplasia, as these are the three systems predominantly affected in this condition (4, 105, 106). SADDAN dysplasia is characterized by extreme short stature, severe tibial bowing, profound developmental delay, and acanthosis nigricans (4, 104). A novel mutation in the FGFR3 gene, A1949T (Lys650Met), has been reported in three unrelated patients with SADDAN dysplasia (4, 107). These three patients have all survived past infancy, with two patients now young adults, without the need for prolonged ventilatory assistance. Individuals with the Lys650Met mutation have skeletal findings distinct from both TD I and TD II. These findings included absence of craniosynostosis or cloverleaf skull anomaly and moderate bowing of the femurs with reverse bowing of the tibia and fibula. Survival past infancy has led to the observation of phenotypic manifestations that may not occur in surviving children with TD, including development of acanthosis nigricans in the cervical and flexural areas. Individuals with SADDAN dysplasia also had seizures and hydrocephalus during infancy with severe limitation of motor and intellectual development. The Lys650Met mutation has also been identified in two patients with TD type I, (107, 108). Interestingly, substitution of the identical amino acid residue by glutamic acid (Lys650Glu) results in TD II.

B. Craniosynostosis disorders

FGFR3 mutations have also been identified in individuals with disorders not in the achondroplasia family of skeletal dysplasias. These include nonsyndromic craniosynostosis, recently referred to as Muenke coronal craniosynostosis, and Crouzon syndrome with acanthosis nigricans.

1. Muenke coronal craniosynostosis. Recently Bellus *et al.* (15) identified a FGFR3 Pro250Arg amino acid substitution caused by a C749G transversion in 10 unrelated patients with autosomal dominant or sporadic cases of craniosynostosis (Fig. 5). This mutation is in the region of the gene that encodes the extracellular domain of the FGFR3 protein. The FGFR3 residue mutated in these individuals, FGFR3 Pro250, corresponds to the exact residue in two other FGFR genes in which mutations cause craniosynostosis syndromes (23–40). FGFR1 Pro252Arg and FGFR2 Pro253Arg amino acid substitutions result in Pfeiffer and Apert syndromes, respectively (23–40).

Muenke *et al.* (17) provided extensive information on a series of 61 individuals from 20 unrelated families in which coronal craniosynostosis is due to the FGFR3 Pro250Arg mutation, defining a new clinical syndrome that might be referred to as Muenke coronal craniosynostosis (16). Considerable phenotypic variability is observed in individuals with this mutation. In addition to the craniosynostosis, some patients had radiographic abnormalities of their hands and feet, including thimble-like middle phalanges, coned epiphyses, and carpal and tarsal fusions. Brachydactyly was observed in some patients, as was sensorineural hearing loss. Developmental delay was observed in a minority of the patients. Reardon *et al.* (109) discussed the clinical manifestations in nine individuals with this mutation. Four of these individuals had mental retardation. Reardon *et al.* (109) suggested that there was a significant overlap between Saethre-Chotzen syndrome and the phenotype produced by this mutation. Saethre-Chotzen is caused by mutations in the TWIST gene (110), and patients originally diagnosed with Saethre-Chotzen in which an FGFR2 or FGFR3 mutation has been identified should be reclassified. Golla *et al.* (111) described a large German family with the Pro250Arg mutation in which there was also considerable phenotypic variability among individuals.

2. Crouzon syndrome with acanthosis nigricans. Crouzon syndrome is characterized by cranial synostosis, hypertelorism, exophthalmos and external strabismus, parrot-beaked nose, short upper lip, hypoplastic maxilla, and a relative mandibular prognathism, and is caused predominantly by mutations in the gene for FGFR2 (Fig. 6) (19, 23, 24, 26–28, 112). Recently, a FGFR3 Ala391Glu (G-to-A transition at nucleotide 1172) substitution was identified in individuals with a phenotype of Crouzon craniosynostosis in association with acanthosis nigricans (18, 113). Meyers *et al.* (18) identified this mutation in a mother and daughter and two sporadic cases with this condition. This mutation is in the FGFR3 transmembrane domain, situated close to the recurrent achondroplasia mutation. The patients had a typical Crouzon syndrome phenotype. Skeletal survey showed no evidence for the skeletal manifestations of achondroplasia, TD, or hypochondroplasia, although they did have hydrocephalus, possibly caused by stenosis of the jugular foramen (114), and some of the cases had interpediculate narrowing (18).

The acanthosis nigricans in the patients with the FGFR3 Ala391Glu mutation was characterized by verrucous hyperplasia and hypertrophy of the skin with hyperpigmentation and accentuation of skin markings, distributed in a distinctive fashion including not only the axillae and neck, but also the chest, abdomen, breasts, perioral, and periorbital areas, and nasolabial folds (18). Meyers *et al.* (18) noted multiple melanocytic nevi over the face, trunk, and extremities of all four of their patients.

One of the patients with Crouzon syndrome with acanthosis nigricans due to the FGFR3 Ala391Glu mutation reported by Meyers *et al.* (18) has a second cousin with Crouzon syndrome. This individual does not have acanthosis nigricans. The phenotype in this patient is due to the FGFR2 Ser347Cys mutation (19).

IV. Biochemical Analysis of FGFR3 Mutations

Binding of the FGF ligand to the FGFR leads to dimerization of the receptor, which, in turn, initiates autophosphorylation of several tyrosine residues in the cytoplasmic domain (Fig. 10). Cell surface-bound heparan sulfate proteoglycans are required to help the ligand-receptor complex to form (115). Phosphorylation of the FGFR tyrosine residues stimulates tyrosine kinase activity, possibly by stabilizing the activation loop of the kinase in a conformation that allows substrates and ATP to access the catalytic site (116, 117). Furthermore, the phosphorylated tyrosine residues act as binding sites for substrates containing Src homology or phosphotyrosine binding domains, providing a means to recruit and phosphorylate other molecules, furthering the FGFR signal transduction pathway.

Recent evidence suggests that the phenotypic differences among the individual diseases that comprise the achondroplasia family of disorders may be due to specific alleles with varying degrees of ligand-independent activation. These alleles can be generated by missense mutations occurring at different domains within FGFR3 (118). Mutations allow the receptor to be constitutively active. Mutations in different domains may have differing effects on the signal transduction pathways initiated by the receptor.

Targeted disruption of the FGFR3 gene causes enhanced bone growth of long bones and vertebrae in mice, suggesting that FGFR3 negatively regulates bone growth (118, 119). Thus, FGFR3 mutations in the achondroplasia family of skeletal dysplasias can probably be interpreted as gain-of-function mutations that activate the fundamentally negative growth control exerted by the FGFR3 pathway (118, 120). The fact that the recessive loss-of-function mutation produces a phenotype in mice, which appears to be the opposite of those seen in achondroplasia, hypochondroplasia, or TD in humans, suggests that the human phenotype may result from a constitutive, or ligand-independent activation of the receptor (118).

Based on the current knowledge about signal transduction by the FGF pathway, activation of FGFRs normally occurs only after ligand binding (121). After studying *Xenopus* FGFRs, Neilson and Friesel (122) also found that different point mutations may activate FGFRs by distinct mechanisms, and that ligand-independent FGFR activation may be a feature skeletal dysplasias have in common.

Additional evidence for the gain-of-function hypothesis was provided by Webster *et al.* (123), who recently demonstrated profound constitutive activation of the FGFR3 tyrosine kinase (~100-fold above the wild type) associated with the Lys650Glu mutation, which is known to cause TD type II. The authors demonstrated a specificity for position in FGFR3, as well as charge, in terms of amino acid changes that result in altered kinase activation. The authors speculated that the TD type II mutation in the FGFR3 activation loop mimicked the conformational changes that activate the tyrosine kinase domain (123). This activation is normally initiated by ligand binding and autophosphorylation of the receptor. Using immunoprecipitation followed by an *in vitro* kinase assay, Webster and Donoghue (124) also found that the mutation in TD increased autophosphorylation activity

FIG. 10. A putative model for FGFR3 signaling. The receptor is shown with both a extracellular and intracellular domain. Binding of the ligand (FGF) to the receptor in the presence of heparan sulfate proteoglycans, results in receptor dimerization and autophosphorylation of several FGFR3 tyrosine residues in the cytoplasmic domain, which stimulates tyrosine kinase activity. These phosphorylated tyrosine residues provide a means to recruit and phosphorylate other molecules, furthering the FGFR3 signal transduction pathway. Recent studies have shown that mutations in the FGFR3 gene can allow constitutive, ligand-independent activation of the receptor. For the common achondroplasia and TD mutations, this leads to the activation of Stat1 and cell cycle inhibitors, eventually leading to cell growth arrest.
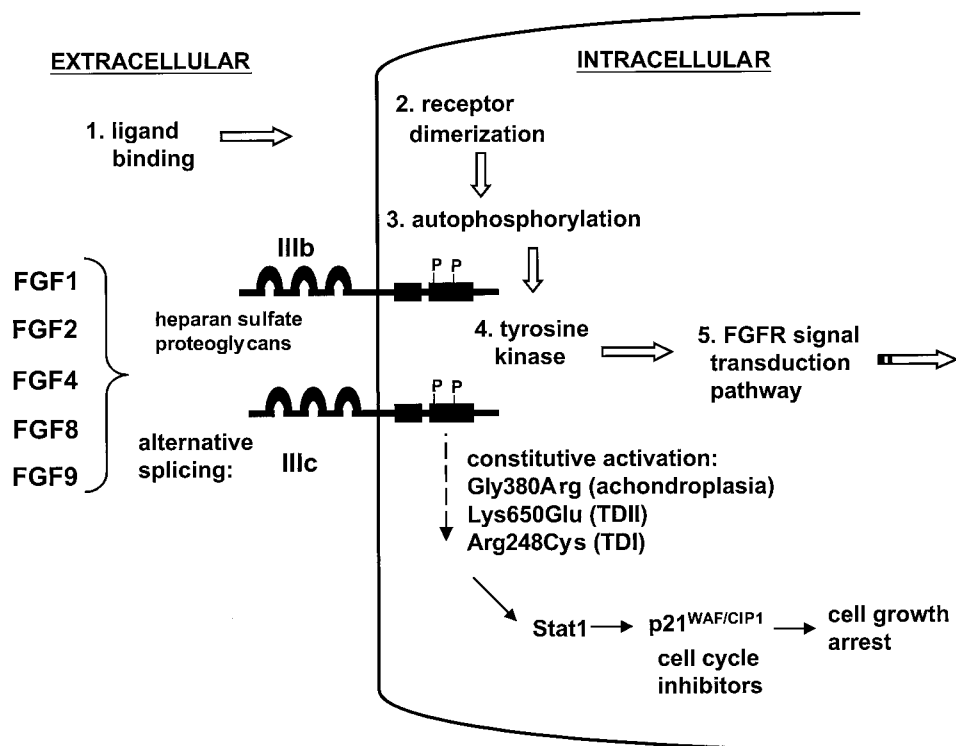
of the FGFR3 relative to the wild-type or achondroplasia mutant receptor.

Subsequently, Webster and Donoghue (124, 125) found similar constitutive FGFR3 activation associated with the Gly380Arg mutation, known to result in achondroplasia. Moreover, Naski *et al.* (126) demonstrated that the Gly380Arg, the Lys650Glu (TD II), and the Arg248Cys (TD I) mutations constitutively activate the receptor, as evidenced by ligand-independent receptor tyrosine phosphorylation and cell proliferation. Interestingly, but perhaps not surprisingly, the mutations that are responsible for TD activated the FGFR3 receptor more strongly than the mutations causing achondroplasia. It has further been demonstrated that the constitutive tyrosine kinase activity of FGFR3 containing the TD II mutation specifically activates the transcription factor Stat1 (signal transducer and activator of transcription) (127, 128). This mutant receptor also induced nuclear translocation of Stat1, induced expression of the cell-cycle inhibitor p21(WAF/CIP1), and resulted in growth arrest of the cell. Stat1 activation and increased p21(WAF/CIP1) expression was found in chondrocytes from a TD II fetus, but not in cells from a non-TD fetus. The authors suggest that in TD, Stat1 may be used as a mediator of growth retardation in bone development, and that abnormal STAT activation and p21(WAF/CIP1) expression due to the mutant FGFR3 receptor may be responsible for the resulting phenotype (127).

Naski *et al.* (129) examined the effects of an activated FGFR3 specifically targeted to growth plate cartilage in mice. The resulting mice were dwarfed, with axial, appendicular, and craniofacial skeletal hypoplasia (129). FGFR3 inhibited endochondral bone growth by disrupting chondrocyte proliferation and differentiation. The Indian hedgehog signaling pathway and bone morphogenic protein (Bmp) 4 expression were also down-regulated in growth plate chondrocytes from these mice, suggesting that FGFR3 is an upstream negative regulator of the hedgehog signaling pathway and that FGFR3 may coordinate the growth and differentiation of chondrocytes with the growth and differentiation of osteoprogenitor cells (129).

Wang *et al.* (130) and Li *et al.* (131) developed mouse models for achondroplasia. The mice are significant for their small size, including shortening of the long bones, especially the femur (130, 131). Also evident was a short craniofacial area, midface hypoplasia with protruding incisors, distorted skull with anteriorly shifted foramen magnum, and kyphosis (130, 131). Histological examination revealed narrowed and distorted growth plates in the long bones, vertebrae, and ribs of these mice, demonstrating that achondroplasia results from a gain of FGFR3 function, leading to inhibition of chondrocyte proliferation (130). Stat1, Stat5a, and Stat5b were activated by expression of the mutant receptor, and p16, p18, and p19 cell cycle inhibitors were up-regulated, also leading to inhibition of chondrocyte proliferation (131). Fewer maturing and hypertrophic chondrocytes were generated in the growth plates of these mutant mice, resulting in a "less-active" growth plate (131).

Thompson *et al.* (132) demonstrated that a chimera containing the transmembrane and intracellular domain of FGFR3 with the achondroplasia mutation fused to the extracellular domain of platelet-derived growth factor (PDGF), induces ligand-dependent differentiation of PC-12 cells. When stably transfected into PC12 cells, which contain no endogenous PDGF receptor, this chimera can be specifically activated by PDGF to signal through the altered FGFR3 intracellular domain. These chimeras induce ligand-dependent autophosphorylation of the chimera receptor and stimulated strong phosphorylation of mitogen-activated protein (MAP) kinase and phospholipase C. Compared with cells transfected with a chimera with normal FGFR3 sequences, cells transfected with the chimera with the FGFR3 achondroplasia mutation were more responsive to ligand, with less sustained MAP kinase activation, indicative of a primed or constitutively-on condition. This observation is consistent with the hypothesis that these mutations weaken ligand control of the FGFR3 receptor, and may provide a biochemical explanation for the observation that the TD phenotype is more severe than that of achondroplasia (132). Subsequently, using similar chimeras, this same group analyzed the effects of six FGFR3 mutations that result in skeletal dysplasias (133). The three tyrosine kinase domain mutations (Lys650Glu, Lys650Met, and Asn540Lys) all resulted in strong ligand-independent tyrosine phosphorylation, especially the Lys650Glu TD type II (133). Lys650Met (TD type I) and Lys650Glu mutations resulted in autoactivation of the receptor sufficient to produce partial differentiation of the PC-12 cells (133). Chimeras containing mutations in the transmembrane domain of FGFR3 (achondroplasia mutations Gly375Cys and Gly380Arg, and Crouzon syndrome mutation Ala391Glu) displayed normal expression and activation, but did exhibit a greater response to lower concentrations of ligand.

Similar autonomous receptor activation has been observed before with mutations in other tyrosine kinase receptors, such as FGFR2, epidermal growth factor, colony stimulating factor 1, and the RET oncogene (134–139). Additional studies will need to be done before the cellular and biochemical consequences of these mutations are fully understood. It will be important to understand the transcriptional differences caused by FGFR3-mediated signal transduction in both normal and disease states.

V. GH Treatment

GH therapy has been proposed as a possible treatment for the short stature of achondroplasia. It was thought that children with chondrodysplasias will not grow in response to GH therapy because of an inability of the abnormal growth cartilage to respond. However, studies have shown that there is an increase in growth velocity, especially during the first year of treatment, which may be beneficial. A number of studies have been done that suggest that a gain in growth rate is possible during 1–2 yr of treatment (140–144), but the usefulness of GH treatment in achondroplasia will be known only when a study of final height is completed. Although it is unlikely that long-term GH therapy will significantly increase height in achondroplasia, long-term prospective, controlled studies are still needed before a conclusion can be developed.

Growth has increased during the early phases of GH therapy in both patients with achondroplasia and hypochondroplasia: 34 patients with achondroplasia or hypochondroplasia in the National Cooperative Growth Study have been treated with an average dose of GH of 0.317 mg/kg per week for an average of 2.6 yr and have gained an average of 0.7 sd in height. These data suggest that the abnormal growth cartilage in patients with chondrodysplasia responds to GH therapy (144). Weber *et al.* (143) studied the effects of recombinant human GH treatment in six prepubertal children with achondroplasia, ranging in age from 2 to 8 yr. During the year of treatment the growth velocity increased from 1.1 to 2.6 cm/year in three patients, while in the others no variation was detected, confirming the individual variability in the response to GH treatment.

To clarify the effectiveness of GH treatment of short stature in achondroplasia, a long-term treatment study with a large number of patients was performed (140): 42 children (16 males and 26 females, age 3–14 yr) with achondroplasia were examined. After the evaluation, the children were treated with GH for more than 2 yr, and then posttreatment growth velocity and body proportion parameters were determined. The annual height gain during GH therapy was significantly greater than before therapy (3.9 ± 1.0 cm/yr before treatment *vs.* 6.5 ± 1.8 cm/yr for the first year, and 4.6 ± 1.6 cm/yr for the second year of treatment), and body disproportion was not aggravated during the treatment period. The authors concluded that GH might be beneficial in the treatment of short stature in children with achondroplasia in the first 2 yr of treatment (140).

In another study, 15 children with achondroplasia, 7 boys (4.8–12.2 yr of age) and 12 girls (5.7–2.2 yr of age), were treated daily with human GH at a dosage of 1 IU/kg/week (141). Auxological assessments were performed 6 months before, at initiation of, and at 6, 12, and 24 months after initiation of GH therapy. During the first semester of GH treatment, a significant increase in height velocity, from 3.2 to 8.3 cm/yr, was observed in all children. However, during the second semester, a relative decrease in growth rate was observed. By the end of the first year, height velocity had increased from 3.2 to 6.9 cm/yr (mean, 3.7 cm/yr; range, 1.1–8 cm/yr) in 13 children and remained unchanged in 2 children. Height velocity declined during the next 12 months and, by the end of the second year of treatment, had increased in only 7 of the 9 children who had completed 2 yr of therapy (mean increase, 3.1 cm/yr); 2 children did not respond to GH therapy. These studies demonstrate that GH treatment resulted in an increased growth rate in some children with achondroplasia; however, the amount of increase declined during the second year of treatment, and the final heights of these individuals is not yet known.

VI. Implications

The identification of FGFR3 mutations in each of the disorders in the "achondroplasia family" of skeletal dysplasias has had a tremendous impact on our understanding of human genetics. Nonetheless, these remarkable molecular findings have only raised many additional intriguing questions.

Why are particular nucleotides of the FGFR3 gene so highly mutable? In studies aimed at determining the mutation rates of CpG dinucleotides in the human factor IX gene, calculated mutation rates at these "highly" mutable sites are 2–3 orders of magnitude lower than those calculated for the FGFR3 mutations causing achondroplasia and Muenke craniosynostosis (145).

Moreover, the high degree of phenotypic specificity associated with FGFR3 mutations is highly unusual in the study of human genetics and disease. That more than 97% of persons with achondroplasia have exactly the same amino acid substitution at nucleotide 1138 was a first in the study of human mutations and genetic disorders. Furthermore, the common Pro250Arg amino acid substitution, which causes Muenke coronal craniosynostosis, adds to the uniqueness of genotype-phenotype correlations in the FGFR disorders. The explanation for this high degree of mutability remains an intriguing question. Since the G1138A achondroplasia mutation was recognized, similar observations have been made in FGFR3 and other human FGFR genes regarding other skeletal phenotypes, including hypochondroplasia and TD, and Pfeiffer and Apert syndromes. Furthermore, it seems that particular nucleotides in FGFR genes are more highly susceptible to mutation than other nucleotides. There is a high degree of correlation in the locations of observed mutations from one FGFR to another. Again, this conservation of mutations at particular sites in the FGFR genes is a very intriguing biological phenomenon. It is possible that FGFR mutations in the same locations have been identified because mutations at these sites are capable of conferring constitutive activation of the receptor, while mutations at other sites do occur, but do not lead to severe phenotypic changes and, thus, have not yet been identified. However the different degrees of constitutive activation cannot explain all the differences in the resulting phenotypes. Furthermore, why do some FGFR3 mutations result in a relatively small amount of skeletal changes, such as in Muenke craniosynostosis and Crouzon syndrome with acanthosis nigricans? These questions remain to be answered.

The prenatal diagnosis of many skeletal dysplasias is difficult to make. A certain sonographic diagnosis of a *de novo* case is rarely possible. In fact, achondroplasia is almost never detected on prenatal ultrasound before the third trimester. In face of uncertainty, physicians sometimes elect to emphasize the most severe alternative diagnoses. In a recent retrospective study, 25% of achondroplasia patients were given an incorrect prenatal diagnosis of a lethal or very severe disorder (146). By identifying mutations responsible for skeletal dysplasias, mutational analysis can be offered when a short-limb disorder is detected by ultrasound; however, indiscriminate use of FGFR3 molecular testing cannot be recommended. Thus, the prenatal diagnosis becomes more effective, making it possible to reduce the amount of incorrect and potentially harmful information provided to the parents (146, 147), thereby helping to avoid unnecessary terminations. Therefore, the high degree of specificity of the FGFR3 G1138A mutation for the achondroplasia phenotype has profound implications for persons with achondroplasia, their families, and their physicians. Because the achondroplasia mutations are easily detectable by molecular means,

the molecular diagnosis is one that can now be performed in many molecular diagnostic laboratories. One very positive outcome of the ability for molecular diagnosis is to provide couples at risk for children with homozygous achondroplasia with reliable prenatal diagnosis for the inevitably lethal condition. Individuals providing genetic counseling should keep in mind that there are other disorders with mild degrees of limb shortening that will not be diagnosed by FGFR3 molecular analysis, and that most cases diagnosed in the second trimester with short limbs and a small chest will have a lethal form of dwarfism, but, most likely, not TD or homozygous achondroplasia. These cases clearly do not have achondroplasia and there are many forms of lethal skeletal dysplasias other than TD; therefore, molecular testing for the common FGFR3 mutations cannot be recommended. The precise diagnosis in these cases is best made after birth or by radiographs and histology.

Additionally, as has been found with many genetic disorders in the past, understanding the physiology behind the achondroplasia family of disease, and other skeletal dysplasias, has the potential to help us understand the normal mechanisms of skeletal growth and development. As we gain a greater understanding of why a particular phenotype results from a particular, but specific, mutation in the FGFR3 gene, we should gain insight into the molecular mechanisms that distinquish one bone from another.

Acknowledgments

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