

## *Invited Comment*

# Recent Milestones in Achondroplasia Research

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Achondroplasia is by far the most common of the human chondrodysplasias. Its name was coined over a century ago to distinguish individuals with disproportionate short stature from individuals with proportionate short stature. The term was subsequently used to differentiate short limb from short trunk dwarfism. The fundamental clinical, genetic, radiographic, and histologic features of achondroplasia were delineated in the 1960s and early 1970s [Maroteaux and Lamy, 1964; Zellweger and Taylor, 1965; Langer et al., 1967, 1968; Silverman, 1968; Rimoin et al., 1970; Scott, 1976].

By the late 1970s, attention began to focus on complications that occurred over time and on the natural history of achondroplasia in general. For example, growth curves for height, body segments, and head circumference were developed that allowed growth of achondroplastic children to be compared to other achondroplastic children [Horton et al., 1978]. Many papers were published on the orthopedic [Kopits, 1976], neurologic [Lutter and Langer, 1977; Galanski et al., 1978; Morgan and Young, 1980; Hecht et al., 1984], obstetric [Lattanzi and Harger, 1982], respiratory [Stokes et al., 1983], and social [Scott, 1977] complications of achondroplasia. There was no stronger advocate for natural history studies than Judith G. Hall. In the aggregate, they made it possible to provide anticipatory care for achondroplasia. In fact, these and other studies led to publication in 1995 of a policy statement from the American Academy of Pediatrics entitled “Health Supervision for Children with Achondroplasia”; the principal consultant was Judith G. Hall [1995]. The recommendations from this statement still serve as the basis for managing achondroplasia.

Interest in the pathogenesis of achondroplasia grew in the 1980s. A number of groups studied the morphology, ultrastructure, and biochemistry of the achondroplasia growth plate [Stanescu et al., 1977; Sillence et al., 1979; Maynard et al., 1981; Pedrini-Mille and Pedrini, 1982; Horton et al., 1988]. While these studies identified many subtle differences between the achondroplastic and normal growth

plates, they did not yield the basic defect in achondroplasia. In fact, in comparison to similar studies on the spondyloepiphyseal dysplasia family of human chondrodysplasias, in which such studies had been very informative [Lee et al., 1989; Murray et al., 1989; Tiller et al., 1995], these studies were not very revealing.

Similarly, several animal models including known mouse mutants were described as models of achondroplasia [Maroteaux and Lamy, 1964; Sande and Bingel, 1983; Bonucci and Nicoletti, 1988; Sannasgala and Johnson, 1990]. While these animal models shared many features with human achondroplasia, none corresponded precisely.

The major obstacle faced by researchers investigating human and animal model skeletal tissues in search of an underlying defect was that the “achondroplasia gene” was not known. Despite much progress with the Human Genome Project generating markers that could be used for linkage analysis in the early 1990s, achondroplasia eluded attempts at linkage. This was presumably because informative families were small in both number and size since most cases of achondroplasia result from new mutations. Finally, the achondroplasia locus was mapped to the distal short arm of chromosome 4 in 1994 [Francomano et al., 1994; Velinov et al., 1994]. Within a few months, mutations of the gene encoding fibroblast growth factor receptor 3 (*FGFR3*) were discovered in achondroplasia [Rousseau et al., 1994; Shiang et al., 1994; Bellus et al., 1995a]. Unexpectedly, almost all patients with clinical features of typical achondroplasia had the same, recurrent G480R mutation, which substitutes an arginine for a glycine residue in the transmembrane domain of the tyrosine kinase-coupled transmembrane receptor that is expressed in the growth plate.

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Within a few months, *FGFR3* mutations were also identified in thanatophoric dysplasia types I and II and in hypochondroplasia [Tavormina et al., 1995; Bellus et al., 1995b].

With the gene and mutations identified, attention turned to how the mutations disturb linear bone growth. Biochemical studies of the receptor combined with knockout experiments in mice revealed that *FGFR3* is a negative regulator of chondrocyte proliferation and differentiation in the growth plate and that the mutations in achondroplasia and related disorders activated the receptor [Colvin et al., 1996; Deng et al., 1996; Naski et al., 1996]. Thus, they can be viewed as gain-of-function mutations. Achondroplasia has now been genetically modeled in mice through both transgenic and gene targeting (knockin) approaches [Naski et al., 1998; Chen et al., 1999; Garofalo et al., 1999; Wang et al., 1999; Iwata et al., 2000].

Studies addressing the mechanism responsible for the gain of receptor function suggest that the mutation stabilizes the dimerized receptor [Webster and Donoghue, 1996]. Briefly as depicted in Figure 1, the product of the *FGFR3* gene is an *FGFR3* monomer. Binding of FGF ligands to *FGFR3* cause these monomers to dimerize (1). This alters the conformation of the receptor and activates its tyrosine kinase activity. This activation leads to phosphorylation of selected tyrosine residues in the cytoplasmic domain of the receptor, which when phosphorylated, serve as docking sites for signaling molecules that are recruited to the receptor (2) and subsequently initiate the propagation of signals through pathways that eventually impact proliferation and differentiation.

Most evidence to date suggests that signals propagated through the STAT and MAP kinase signaling pathways (3) are most relevant to inhibition of chondrocyte proliferation and differentiation (4) [Webster and Donoghue, 1996; Murakami et al., 2004]. Although the precise mechanism is not known, the stabilization of *FGFR3* dimerization by mutation is believed to enhance the receptor's kinase activity [Webster and Donoghue, 1996]. We have also shown that the increased kinase activity disturbs the normal turnover of the activated receptor allowing it to accumulate within cells leading to amplification of *FGFR3* signals [Cho et al., 2004].

With the molecular pathogenesis of achondroplasia emerging, interest has begun to shift to therapy intended to counter the effects of the overactive receptor. Three strategies have been considered to date (Fig. 1). The first two involve inhibiting, or more precisely downregulating, the tyrosine kinase activity of *FGFR3* [Aviezer et al., 2003]. The first involves chemical inhibitors selective for the *FGFR3* tyrosine kinase. This approach has been used successfully to treat cancers, most notably chronic lymphocytic leukemia with the Bcr-Abl tyrosine kinase inhibitor—Gleevec [Bennasroune et al., 2004].

The second therapeutic strategy relies on blocking antibodies to interfere with binding of FGF ligands to *FGFR3* [Aviezer et al., 2003]. This blocking strategy using the Herceptin monoclonal antibody against the epidermal growth factor receptor (EGFR) has been employed to treat breast cancer [Bennasroune et al., 2004]. It should be noted that the molecular mechanism responsible for achondroplasia is essentially the same as that of many cancers—gain of

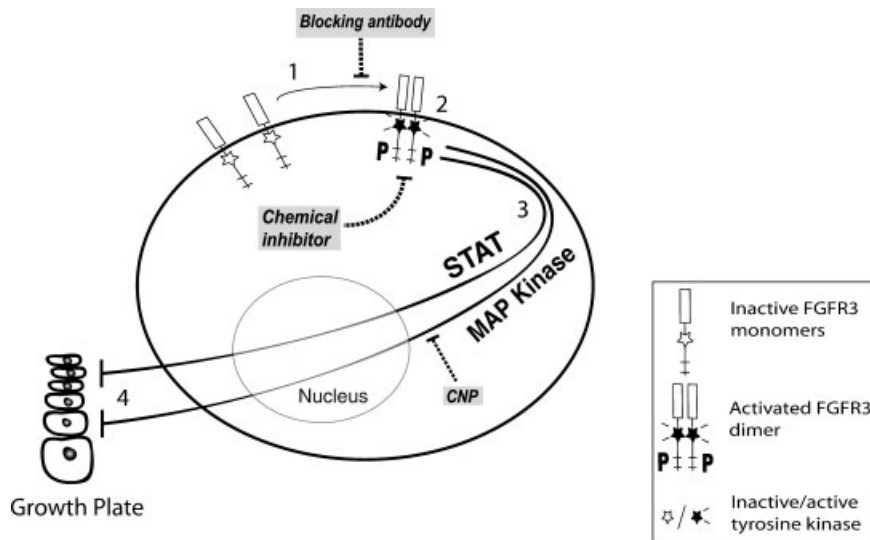


FIG. 1. Schematic depiction of *FGFR3* activation. Binding of FGF ligands to receptor induces dimerization (1), which in turn leads to phosphorylation of key tyrosine residues in the cytoplasmic domain of the receptor (2). The phosphorylated tyrosine residues function as docking sites for recruitment of signaling molecules that initiate the propagation of *FGFR3* signals. The STAT and MAP kinase pathways (3) appear to be most relevant in regulating proliferation and differentiation of growth plate chondrocytes (4). These pathways exert their inhibitory effects largely through influencing transcription of other genes in the nucleus whose products directly influence cell behaviors in the growth plate. Therapeutic approaches under consideration include chemical kinase inhibitors, blocking antibodies, and CNP as shown against a gray background.

function of a tyrosine kinase-coupled receptor or signaling molecule, which explains why the same therapeutic strategies are being explored.

The third possibility involves C-type natriuretic peptide (CNP), which because of its effects on fluid and electrolyte balance and vascular tone is being considered as a therapeutic agent for certain cardiovascular diseases [Scotland et al., 2005]. Since CNP has recently been shown to downregulate FGF-induced activation of MAP kinase signaling pathways in growth plate chondrocytes and to counteract the effects of the achondroplasia mutation in mice, it has been suggested as a possible treatment for achondroplasia [Yasoda et al., 2004].

All of these therapeutic approaches are in the early stages of development. Nevertheless, they offer promise that definitive, FGFR3-directed therapies for achondroplasia will be in the foreseeable future. If and when they reach the clinical trial phase of development, it will be essential to utilize the extensive definitions of clinical manifestations and well documented natural history of achondroplasia delineated in the pre-FGFR3 years to determine if they work and, if so, to monitor their effects.

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