

FGFR3 SIGNALING IN ACHONDROPLASIA: A REVIEW

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Abstract

Achondroplasia and related chondrodysplasias are caused by heterozygous mutations of fibroblast growth factor receptor 3 (FGFR3). Virtually all patients with achondroplasia have the same mutation, and all of the FGFR3 mutations activate the FGFR3 signal transduction pathways. There is remarkable correlation between specific mutations and the severity of clinical phenotypes manifestations. The mutations activate the FGFR3 transmembrane receptor by promoting or stabilizing receptor dimerization or by activating kinase activity, in the absence of FGF ligand binding. FGFR3 signals are transmitted through several pathways; the best defined involves activation of STAT1 and induction of the antimitotic protein, p21, in the growth plate. Attempts are underway to experimentally model achondroplasia in transgenic mice by expressing mutant FGFR3 receptors in cartilage.

Key Words: Achondroplasia, chondrodysplasia, fibroblast growth factor, fibroblast growth factor receptor 3 (FGFR3), signal transduction, p21, bone growth, growth plate.

Introduction

Achondroplasia is the most common human chondrodysplasia and the prototype of these disorders [10]. It is a member of a graded series of disorders that ranges from the more severe thanatophoric dysplasia (TD) to the less severe hypochondroplasia. All share a common qualitative clinical phenotype characterized by a long trunk, short extremities, especially proximally, and a large head. TD is lethal in the perinatal period, whereas hypochondroplasia is usually not apparent clinically until early childhood and may be mild enough to escape detection.

Genetics

Achondroplasia was genetically mapped to the short arm of chromosome 4 in 1994 [8, 12, 33]. Heterozygous mutations in patients with achondroplasia of fibroblast growth factor receptor 3 (FGFR3), which maps to this region, were identified within a few months [3, 18, 26, 29]. FGFR3 mutations were soon discovered in TD [19, 30] and subsequently, in hypochondroplasia [4, 17].

Most remarkable about these FGFR3 mutations is their high degree of homogeneity (Fig. 1). Almost everyone with typical achondroplasia has a same glycine 380 to arginine substitution (Gly380Arg) [1, 3, 18, 20, 21, 26, 31, 34]. Similarly, most patients with hypochondroplasia have an asparagine to lysine substitution at residue 540 (Asn540Lys) [4, 17, 22].

Mutations associated with TD, which has been divided clinically into TDI and TDII subtypes, are more dispersed. TDII mutations map to lysine 650 (Lys650Glu), while the majority of TDI mutations substitute cysteine residues for other amino acids at positions 248, 249, 370, 371 and 373 (Arg248Cys, Ser249Cys, Gly370Cys, Ser371Cys, Tyr373Cys) in the extracellular domain [19, 23, 30].

Mutations of FGFR3 have also been found in patients with craniosynostosis associated with acanthosis nigricans involving residues 250 and 391 (Pro250Arg, Ala391Glu).

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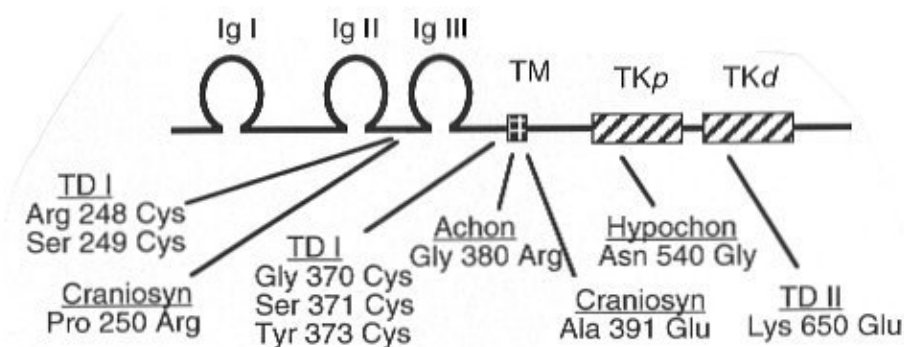
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Figure 1. Schematic showing domain structure of FGFR3 and sites of common human mutations according to clinical phenotypes. Note the tendency for mutations to cluster to specific sites, i.e., neighboring codons. Ig = immunoglobulin, TM = transmembrane, TK_{p/d} = tyrosine kinase proximal/distal, TD = thanatophoric dysplasia, Craniosyn = craniosynostosis, Achon = achondroplasia, Hypochon = hypochondroplasia.



Physiology of FGFR3 Signaling - Normal

FGFR3 is one of four closely related high affinity FGF transmembrane receptors (FGFR1-4). It contains an extracellular ligand-binding domain, a transmembrane domain and a cytoplasmic domain containing a split tyrosine kinase (sub)domain as shown in Figure 1 [2, 32]. FGF receptors are activated by FGF ligands; the most relevant to FGFR3 are FGFs 1, 2, 4, 8 and 9 [5, 9, 11, 16, 25].

The binding of ligand to receptor leads to receptor dimerization and autophosphorylation of tyrosine residues in the cytoplasmic domain. This stimulates intrinsic tyrosine kinase activity. The phosphorylated tyrosines serve as binding sites for cellular substrates [13, 14, 32]. The combination provides a mechanism to both recruit and (trans)phosphorylate molecules that transmit FGFR3 signals. The pathways downstream of the receptor are not well defined. However, the MAP (mitogen-activated protein) kinase, STAT1 (member of a family of proteins that carry signals from activated receptors to the transcription machinery of a cell, i.e., Signal Transduction and Activation of Transcription proteins) and PLC γ (phospholipase C) cascades have been implicated. The ultimate effect of FGFR3 signaling on bone growth is inhibitory. The strongest evidence comes from FGFR3 knockout mice generated by two independent groups which showed that bones grow longer in mice who lacked the receptor [6, 7]. Thus, the mutations observed in achondroplasia and related disorders are gain-of-function mutations which activate the receptor.

Consequences of Mutations

Three mechanisms have been proposed to explain the constitutive activation of FGFR3 by achondroplasia mutations. Ligand-independent stabilization of dimers has been suggested for the Gly380Arg achondroplasia

(transmembrane) mutation [36]. Ligand-independent dimerization resulting from disulfide bond formation between free cysteines in the extracellular domain, which are introduced by mutations, is the most likely explanation for the TDI mutations. There is precedent for this in other receptors that signal through tyrosine kinase, i.e., cysteine mutations in the EGF (Epidermal growth factor) gene and in the RET gene product, in MEN-2A (Multiple Endocrine Neoplasia type 2A) syndrome [24, 27].

The TDII mutation appears to activate the kinase domain directly by altering its baseline conformation from an inhibitory one to a non-inhibitory one [14, 35]. Normally, the latter conformation is induced by (ligand-dependent) autophosphorylation. The mutation acts to mimic this change in the absence of ligand. A similar mechanism may operate for the hypochondroplasia (Asn540Lys) mutation.

There is evidence that PLC γ -mediated increase in calcium may play a role in the aberrant signaling [15]. When the calcium signaling response, i.e., transient increase in intracellular calcium, to FGF2 was measured in fibroblasts from patients and controls, substantially reduced responses were observed for homozygous achondroplasia and TDI, although normal responses were seen for heterozygous achondroplasia and TDII.

There is also evidence that induction of the cyclin-dependent kinase inhibitor, p21, is enhanced by at least the TDII mutation. As mentioned above, STATs are activated by FGFRs. Moreover, Su *et al.* demonstrated that STAT1 is activated in cells transfected with the TDII FGFR3 mutation [28]. They further showed enhanced activation of STAT1 and enhanced induction of p21 expression in the growth plate from TDII patients. This observation could explain the decrease in cell proliferation in the growth plate that would provide the basis for reduced endochondral bone growth in achondroplasia.

Experimental Modeling of Achondroplasia

To experimentally model achondroplasia in mice, Garofalo *et al.* (work in progress) generated transgenic mice in which expression of mutant (Gly380Arg) FGFR3 was targeted to cartilage using the type II collagen promoter. The mutant mice exhibited moderately severe growth deficiency of postnatal onset similar to that seen in humans with achondroplasia. These mice may prove to be valuable models to study this disorder.

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Discussion with Reviewers

O. Jacenko: It would be of interest to provide a more detailed description of the disease phenotypes, particularly since there is a tremendous phenotypic spectrum with respect to severity, as well as the tissues predominantly affected (e.g., skeletal and skull abnormalities are not always coincident). Likewise, it would be relevant to include a description of the histological defects that are seen in both humans and mice with altered FGFR3 (e.g., which cell types appear to be affected in the endochondral skeleton as well as in the cranium).

Authors: At the severe end of the spectrum, TD, there is profound involvement of all bones that grow by endochondral ossification often accompanied by the cloverleaf head deformity, which is thought to result from in utero hydrocephalus. The long bones are extremely short, as are the vertebral bodies. At the other end of the spectrum, hypochondroplasia, there is mild shortening of long bones minimal if any shortening of the vertebral bodies and minimal if any involvement of skull bones. The intermediate clinical phenotype, achondroplasia, is characterized by moderate shortening of long bones, mild shortening of the spine and shortening of bones at the base of the skull. There are usually no non-skeletal primary abnormalities in these disorders.

The growth plate in TD, especially TDI, is highly abnormal. There is a reduction in the number of proliferating and hypertrophic chondrocytes, and the normal columnar architecture is disturbed. However, the extent of these changes usually varies from location to location within even a single tissue section. Areas of relatively normally appearing growth plate may be found occasionally in some cases. Growth plate structure in achondroplasia and in hypochondroplasia is essentially normal, although there may be quantitative shortening in the former condition. Thus, one could speculate that if there is a graded increase in FGFR3 activation as proposed by several authors, there must be some type of threshold above which growth plate structure becomes severely disturbed.

O. Jacenko: The authors might consider comparing the mutation "hot spots" in FGFR3 and the resultant phenotype severities to analogous regions in the other FGFR genes (e.g., FGFR1 and FGFR2 mutations associated with different

forms of craniosynostosis), as well as in other tyrosine kinase receptors (e.g., TIE2).

Authors: As more becomes known about “hot spots” within the human *FGFR* genes and DNA sequences that flank these areas, it may be possible to identify features common to the hot spots in all of these receptor genes. This knowledge may have implications for mutagenesis in general.

O. Jacenko: Based on both the human and murine disease phenotypes, FGFR3 likely is a negative regulator of intrinsic growth rates in the endochondral skeleton; however, in the intramembranously-derived cranium, it appears to have an opposite role in accelerating proliferation/maturation of cells in sutures, leading to premature suture fusion and craniosynostosis. Could the authors comment on these apparently contradictory effects resulting from the same mutations in FGFR3?

Authors: There is no obvious explanation. In some instances, the changes in the skull may be secondary to defective growth of base of the skull, which does grow by endochondral ossification. Potentially, increased mechanical pressure (hydrocephalus) resulting from occlusion of veins that normally carry blood from the brain through the base of the skull, may promote growth at sutures, which is not directly related to FGFR3 expression.

O. Jacenko: The disorders including TDI, TDII, achondroplasia and hypochondroplasia are a result of missense mutations in the FGFR3 gene, and display a graded spectrum of phenotypic severity. Can this spectrum of severity and phenotypic heterogeneity be accounted for solely by the graded activation of the FGFR3 receptor? Of particular interest are the Asn540Gly and Lys650Gly mutations, which affect the tyrosine kinase domains, but result in TDII or hypochondroplasia.

Authors: It is likely that there are other factors that influence phenotypic severity. One possibility is that the mutations, especially those affecting the cytoplasmic domain, activate downstream signaling pathways that are not normally activated by FGFR3.

O. Jacenko: A great degree of phenotypic heterogeneity/variable expressivity has been associated with certain specific mutations (e.g., individuals with hypochondroplasia resulting from Asn540Gly mutations show variable phenotypes involving macrocrania and shortening of limbs; similar variations are seen in the Pro250Arg, where the primary defect involves the skull, with occasional involvement of the appendicular skeleton; mutations in other FGFRs show dissimilar effects on the skull and digits). What might contribute to these individual differences? Has a polymorphism or a modifier gene coincident with the

mutation ever been considered?

Authors: There certainly could be modifier genes involved. However, one must note that humans have genetic backgrounds that are quite different from one another, which makes finding such genes very difficult.

O. Jacenko: The four FGFRs are activated by a number of FGF ligands. If one of the ligands specific for FGFR3 were to be overexpressed, would a phenotype similar to that resulting from FGFR3 mutations be expected?

Authors: If there were a tight ligand-receptor specificity, this would be expected. However, since these specificities are not well characterized *in vivo*, and *in vitro* tends to be relative rather than absolute, it would be difficult to prove that a phenotype resulting from overexpression of a particular ligand was due completely to activation of a single receptor.

O. Jacenko: The authors have suggested that the mutant TDII FGFR3 has constitutive tyrosine kinase activity, which can specifically activate the transcription factor STAT1, and subsequently induce the expression of the cell-cycle inhibitor p21. In general, dysregulation of STAT-responsive genes has been implicated in inflammatory disease and lymphatic abnormalities. Specifically, STAT1 inactivation has demonstrated a critical role for this factor in mediating interferon (IFN)-dependant biological responses (Meraz *et al.*, 1996). Have any immune deficiencies or increased susceptibilities to infections been documented in humans (or in mice) with FGFR3 mutations?

Authors: Patients with achondroplasia and hypochondroplasia are not known to have immune problems. This is in contrast to some human chondrodysplasias in which such problems occur, i.e., cartilage hair hypoplasia. It is conceivable that immune defects exist in patients at the severe end of the spectrum, TD, but are masked by the early death of these infants.

Additional Reference

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