A Novel Mutation in *FGFR3* Causes Camptodactyly, Tall Stature, and Hearing Loss (CATSHL) Syndrome

Reha M. Toydemir, Anna E. Brassington, Pınar Bayrak-Toydemir, Patrycja A. Krakowiak, Lynn B. Jorde, Frank G. Whitby, Nicola Longo, David H. Viskochil, John C. Carey, and Michael J. Bamshad

Activating mutations of FGFR3, a negative regulator of bone growth, are well known to cause a variety of short-limbed bone dysplasias and craniosynostosis syndromes. We mapped the locus causing a novel disorder characterized by camptodactyly, tall stature, scoliosis, and hearing loss (CATSHL syndrome) to chromosome 4p. Because this syndrome recapitulated the phenotype of the *Fgfr3* knockout mouse, we screened *FGFR3* and subsequently identified a heterozygous missense mutation that is predicted to cause a p.R621H substitution in the tyrosine kinase domain and partial loss of FGFR3 function. These findings indicate that abnormal FGFR3 signaling can cause human anomalies by promoting as well as inhibiting endochondral bone growth.

Fibroblast growth factor receptor 3 (FGFR3) is one of five distinct membrane-spanning tyrosine kinases that participate in a variety of developmental processes. Mutations in *FGFR3* cause at least half a dozen different disorders, including achondroplasia (ACH [MIM 100800]), hypochondroplasia (HCH [MIM 146000]), thanatophoric dysplasia I and II (MIM 187600), Muenke syndrome (MIM 602849), Crouzon syndrome with acanthosis nigricans (MIM 187600), severe ACH with developmental delay and acanthosis nigricans (SADDAN) syndrome,[1] and lacrimo-auriculo-dental-digital (LADD [MIM 149730]) syndrome.[2,3] FGFR3 is a negative regulator of bone growth, and all mutations characterized to date cause constitutive FGFR3 activation and impair endochondral bone growth.[3]

We evaluated a large Utah pedigree in which 27 living affected family members spanning four generations (from a total of 35 affected individuals in seven generations; see fig. 1) were affected with dominantly inherited camptodactyly, tall stature, and hearing loss or CATSHL (pronounced "cat-shul") syndrome (fig. 2). Phenotypic information and DNA were available from 20 of 27 affected individuals. Adult height in males was >97th percentile in 5 of 5 men, with a mean height of 77 inches, and adult height in females was >75th percentile in 9 of 9 and >97th percentile in 8 of 9 women, with a mean height of 70 inches. Camptodactyly of the hands and/or feet (fig. 2) was present in 18 (90%) of 20 individuals, and 17 (85%) of 20 had hearing loss (14 of 20 were documented as having hearing loss, and 3 of 20 acknowledged having hearing loss but refused formal testing). Of 20 individuals, 12 (60%) had developmental delay and/or mental retardation, and several of these had microcephaly (head circumference <2nd percentile). Several had scoliosis and/or a pectus excavatum (fig. 2), although the frequency of occurrence might be underestimated because many family members elected not to undergo chest and/or spine examination. No individual had characteristics of LADD syndrome or craniosynostosis syndromes caused by mutations in *FGFR3*. Marfan syndrome was considered a possible diagnosis, but no affected individuals who were examined had severe myopia, lens dislocation, or aortic-root abnormalities. Therefore, the diagnosis of Marfan syndrome was excluded.

Radiographic findings included tall vertebral bodies with irregular borders and broad femoral metaphyses with long tubular shafts (data not shown). Several affected individuals had a single osteochondroma of the femur, the tibia, or a phalanx; pectus abnormalities; and/or severe thoracolumbar kyphoscoliosis (fig. 2). On audiological exam, each tested individual had bilateral sensorineural hearing loss and absent otoacoustic emissions (fig. 3). By report, the hearing loss was congenital or developed in early infancy, progressed variably in early childhood, and ranged from mild to severe. Computed tomography and magnetic resonance imaging revealed that the brain, middle ear, and inner ear were structurally normal.

To identify the locus for CATSHL syndrome, we performed a genomewide linkage scan, on 20 affected individuals, that revealed a significantly positive LOD score of 3.76 (recombination fraction [θ] 0.001) with marker *D4S412* (table 1), located on the tip of chromosome 4p. A multipoint LOD score estimated from markers saturating this region was 5.1 and reached its maximum at *D4S43* (table 2). No other region of the genome harbored markers with a significantly positive LOD score. Haplotype analysis delimited a critical interval of ~7 Mb (fig. 1) that contained

From the Departments of Human Genetics (R.M.T.; A.E.B.; L.B.J.), Pathology (P.B.T.), Biochemistry (F.G.W.), and Pediatrics (N.L.; D.H.V.; J.C.C.), University of Utah, Salt Lake City; Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock (P.A.K.); and Departments of Pediatrics and Genome Sciences, University of Washington (M.J.B.), and Children's Hospital and Regional Medical Center (M.J.B.), Seattle

Received June 14, 2006; accepted for publication August 10, 2006; electronically published September 26, 2006.

Address for correspondence and reprints: Dr. Michael J. Bamshad, Department of Pediatrics, Division of Genetics and Developmental Medicine, University of Washington School of Medicine, 1959 NE Pacific Street, HSB RR349, Seattle, WA 98195. E-mail: mbamshad@u.washington.edu

Am. J. Hum. Genet. 2006;79:935–941. 0002-9297/2006/7905-0015$15.00

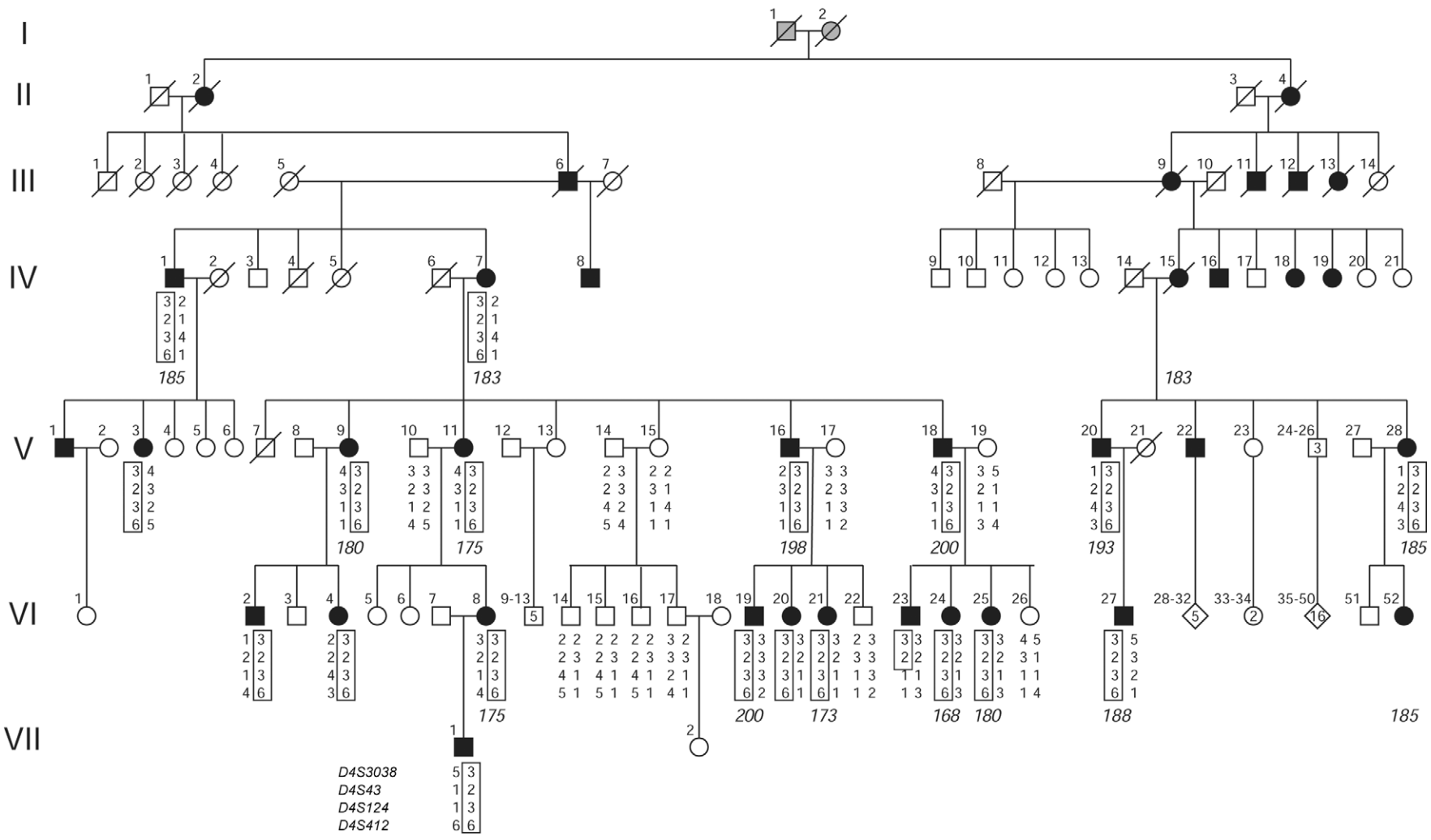

Figure 1. Pedigree of the family with CATSHL syndrome. Filled symbols indicate either affected individuals (*black*) or individuals of unknown status (*gray*), and open symbols indicate unaffected individuals. Genotypes for *D4S3038, D4S43, D4S127,* and *D4S412* are listed, and the disease haplotype segregating with each affected individual is boxed. The height (in centimeters) of each affected adult is indicated in italics.

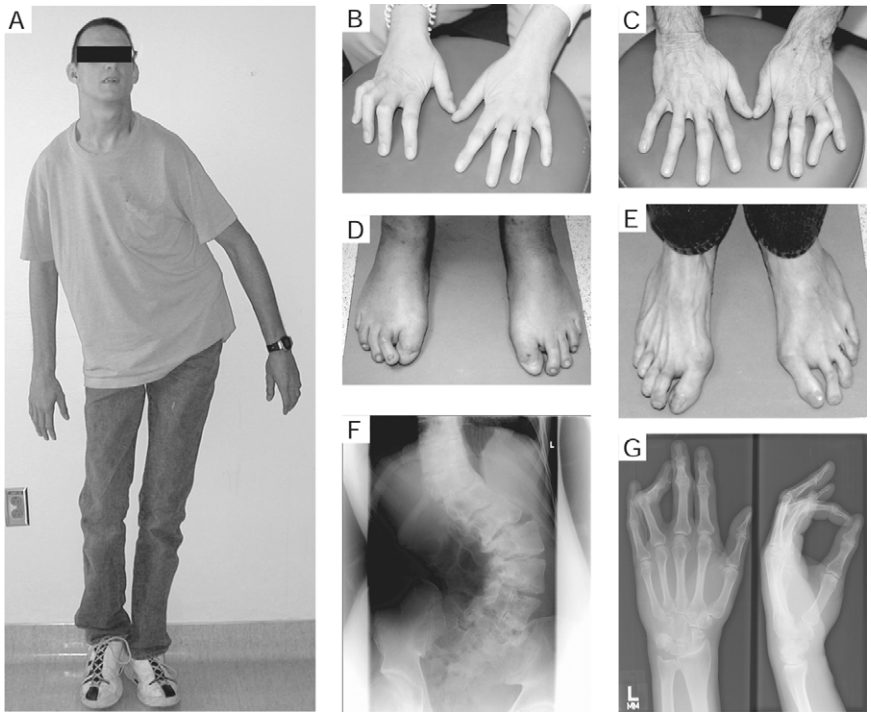

Figure 2. Clinical characteristics of CATSHL syndrome. *A,* Tall stature, pectus excavatum, and scoliotic deformity of the spine. Camptodactyly of the hands (*B* and *C*) and feet (*D* and *E*). *F,* Anterior-posterior radiograph of the thoracolumbar spine, showing ~80° lateral curvature of the lumbar spine. *G,* Radiograph of the hand of an individual with camptodactyly.

~30 genes, including *FGFR3* (Genbank accession number NM_000142). Because the features of CATSHL syndrome overlapped with those of mice homozygous for a *Fgfr3* null allele,[4,5] we screened affected individuals for *FGFR3* mutations by direct DNA sequencing.

In all affected family members tested ($n = 20$), we discovered a G→A missense mutation at nucleotide position +1862 (c.1862G→A) that creates a novel *Dra*III restriction site (fig. 4) and a histidine→arginine substitution (p.R621H). R621 is located in the catalytic loop of the tyrosine kinase domain of FGFR3, and it is invariant in the tyrosine kinase superfamily (fig. 4*c*). No unaffected family members had this variant, nor was it found in 500 chromosomes from individuals matched for geographic ancestry (Western Europe).

The catalytic loop plays a critical role in the transfer of a phosphate ion to its target sites. On the basis of homology modeling done using the crystal structure of FGFR1, the homologous amino acid residue (i.e., R627) is predicted to be critical for the transfer of phosphate.[6] The

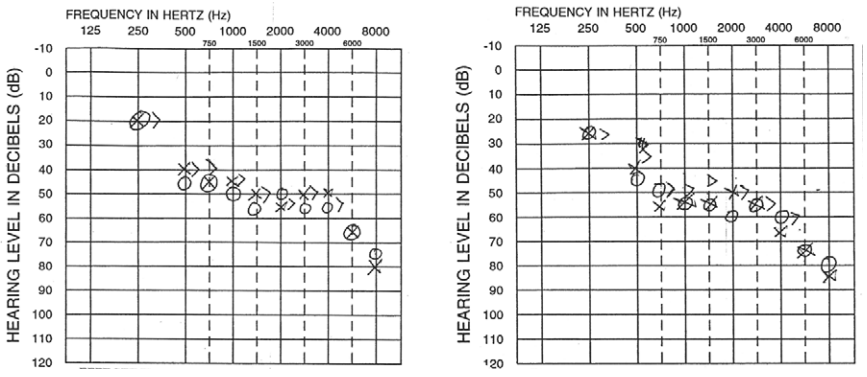

Figure 3. Representative audiograms of two individuals with CATSHL syndrome that demonstrate sensorineural hearing loss. Pure-tone response in the left ear is indicated by a cross (×) and response in the right ear by an open circle (○). Responses in the 500–8,000 Hz range were obtained in the mild sloping to severe hearing loss range, bilaterally.

p.R621H substitution may therefore interfere with the ability of FGFR3 to transfer phosphate to its peptide substrate, resulting in loss of function (fig. 5). This prediction is supported by experiments in which site-directed mutagenesis of the homologous amino acid residue in the kinase domain of the insulin receptor (i.e., R1136) and the C-terminal Src Kinase virtually inactivates the receptor.[7,8]

The anomalies observed in humans with p.R621H recapitulate the defects identified in *Fgfr3*$^{-/-}$ mice.[4,5] The skeletal phenotype of *Fgfr3*$^{-/-}$ mice is characterized by elongated long bones (particularly the femur) and long vertebral bodies that predispose the animals to thoracic kyphoscoliosis and tail kinks. Like the *Fgfr3*$^{-/-}$ mice, only bones formed by endochondral ossification are affected in CATSHL syndrome, and the bones most notably affected are the long bones and vertebral bodies. *Fgfr3*$^{-/-}$ mice also exhibit profound sensorineural deafness that is caused by cochlear defects, including absence of inner and outer pillar cells in the organ of Corti and reduced innervation of the outer hair cells.[4,5] However, the middle

Table 1. Two-Point Linkage Data for All Chromosomes

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

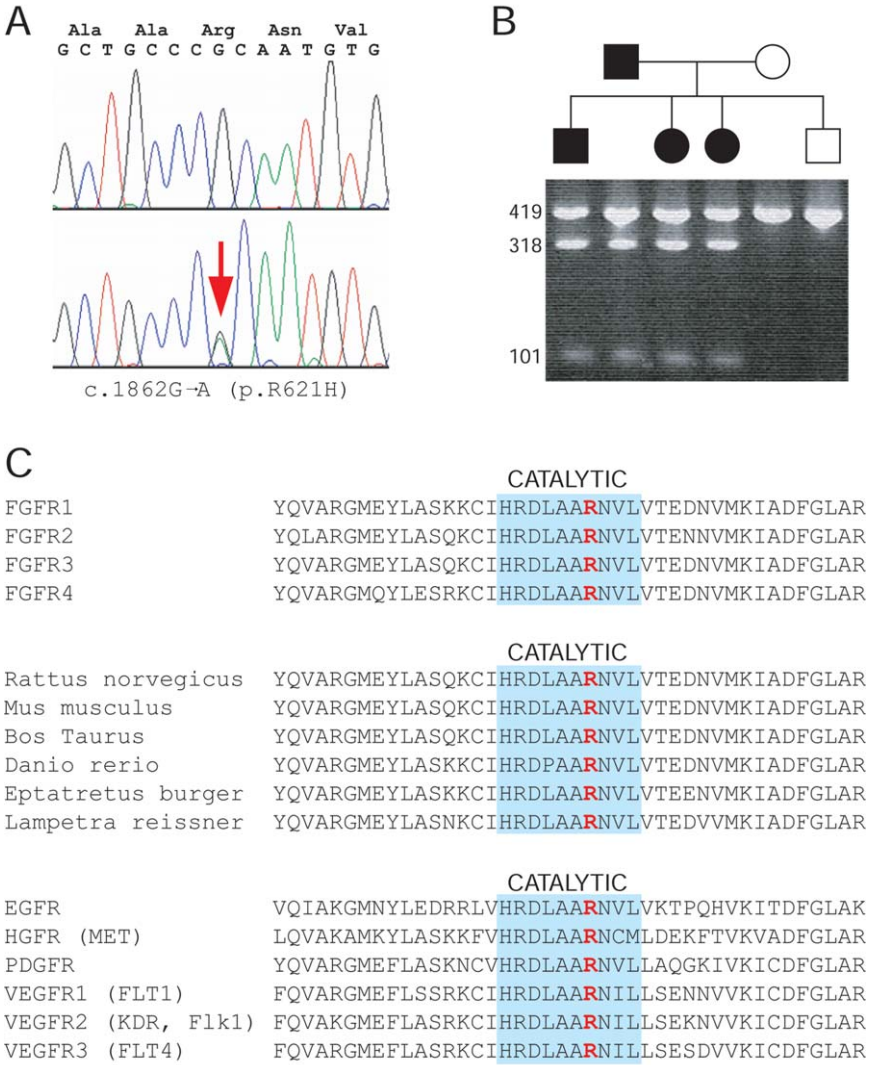

Figure 4. Identification of loss-of-function mutation in *FGFR3* that causes CATSHL syndrome. *A,* A heterozygous G→A *FGFR3* mutation creates a novel *Dra*III restriction site. *B,* Restriction digest with *Dra*III that confirmed homozygosity for the uncut wild-type *FGFR3* allele (419 bp) in unaffected individuals (*open symbols*), whereas affected individuals (*filled symbols*) were heterozygous for a wild-type allele (419 bp) and a mutant allele that cut into two fragments (318 and 101 bp). *C,* Amino acid alignment of different FGFRs. Arginine at codon 621 of the activation domain is conserved among human FGFR1, -2, -3, and -4 (*top*), in all vertebrate FGFR3s characterized to date (*middle*), and in other receptor tyrosine kinases (*bottom*).

ear ossicles and the gross structure of the inner ear of *Fgfr3*$^{-/-}$ mice are normal. Likewise, individuals with p.R621H had sensorineural hearing loss, normal conductive hearing, and no gross abnormalities of the middle or inner ear. In contrast to the static deafness observed in *Fgfr3*$^{-/-}$ mice, the hearing loss in individuals with the p.R621H substitution was progressive. This difference may be a result of the residual activity of the wild-type copy of *FGFR3* in individuals with CATSHL syndrome. It also suggests that the support cells of the organ of Corti might require FGFR3 for maintenance as well as formation, an inference consistent with the expression of Fgfr3 in pillar cells of the adult rat.[9] This requirement may be dose-sensitive, because some individuals with constitutively activating mutations in *FGFR3* also develop sensorineural hearing loss.[10]

Table 2. Results of the Multipoint Linkage Analysis

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

The skeletal phenotypes of both *Fgfr3*$^{-/-}$ mice and individuals with CATSHL syndrome also are similar to those of sheep with a naturally occurring condition called "ovine hereditary chondrodysplasia" or "spider lamb syndrome" (SLS).[11,12] SLS is a codominant condition characterized by modestly increased long-bone length in heterozygotes and elongated "spider-like" legs, a "humped and twisted spine," flexion contractures of the legs, and deformed ribs and sternebra in homozygotes.[12] SLS is caused by a substitution of glutamic acid for valine at

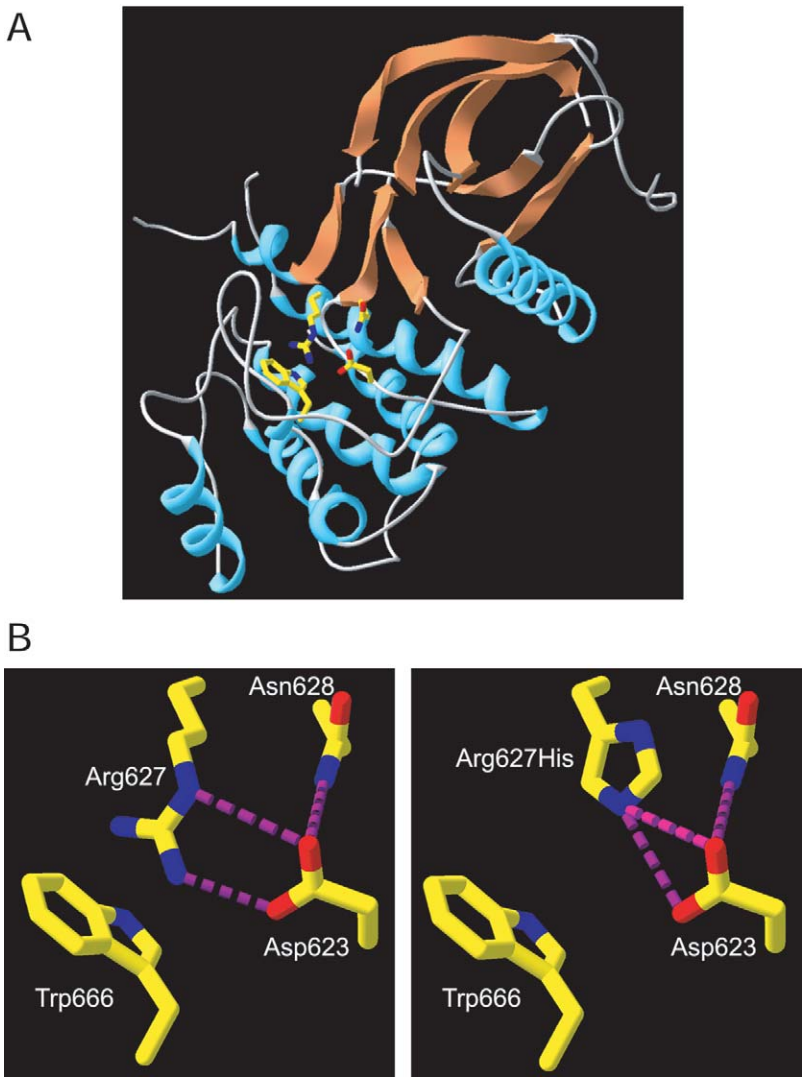

Figure 5. *A,* Ball-and-stick model of the active-site region of the catalytic domain of FGFR1. The model is based on the 0.2-nm crystal structure of the tyrosine kinase domain of the human FGFR1 (RSCB Protein Data Bank entry 1FGK). R627 of FGFR1 is homologous to R621 of FGFR3. *B,* Hypothetical model of FGFR3, showing position of histidine side chain when substituted for R621.

amino acid position 700 (p.V700E) in the tyrosine kinase of Fgfr3, where it is predicted to cause a loss of FGFR3 function.[12] Therefore, both p.R621H and p.V700E cause a dominantly inherited loss of FGFR3 function and similar skeletal anomalies.

For several reasons, it is unlikely that the loss of function caused by p.R621H results from haploinsufficiency. First, mice heterozygous for an *Fgfr3* null allele are phenotypically normal.[4,5] Second, deletion of *FGFR3,* which occurs in most patients with Wolf-Hirschhorn syndrome (WHS [MIM 194190]), is not associated with any of the skeletal defects observed in the individuals with p.R621H.[13] However, it is possible that other genes that are typically deleted in patients with WHS mask the effect of *FGFR3* hemizygosity. Third, the fibroblasts of individuals affected with CATSHL syndrome express both wild-type and mutant (i.e., p.R621H-containing) *FGFR3* RNA in nearly equal proportions, and the expression levels of all five FGFRs in patients are similar to those of normal individuals. Furthermore, both mutant and wild-type FGFR3 localizes to its normal position in the cell membrane (data not shown). These observations suggest that p.R621H might, instead, cause loss of FGFR3 function by a dominant negative mechanism.

Proper FGF signaling requires dimerization of FGFR molecules on the cell surface. Dimerization subsequently promotes the intracellular autophosphorylation of critical tyrosine residues in the activation loop of the receptor.[6] This stabilizes the tyrosine kinase domain in the active conformation, leading to phosphorylation of other tyrosine residues in the activation domain and binding of target proteins. p.R621H-FGFR3 might form a heterodimer with wild-type FGFR3 that reduces or abolishes kinase activity. This mechanism has been shown to underlie the dominant negative effect of several amino acid substitutions in the activation domain of the insulin receptor (MIM 147670), another tyrosine kinase receptor, that cause dominantly inherited insulin resistance.[14,15]

It has been speculated that polymorphisms in *FGFR3* might influence adult height.[16] This hypothesis is supported by the observation that several *FGFR3* mutations cause such mild forms of HCH that the height of affected individuals falls within the normal spectrum.[16] On the other hand, p.V700E is positively correlated with long-bone length in sheep, and the height of p.R621H heterozygotes overlaps with individuals on the taller end of the normal height spectrum. Analogous to the positive association between the level of FGFR3 activation and bone-growth inhibition (i.e., higher levels of *FGFR3* activation cause more-severe limb shortening), our results indicate that increases in long-bone length are associated with FGFR3 impairment. This observation suggests that human stature might be influenced by *FGFR3* activity in a dose-dependent fashion.

Acknowledgments

We thank the individuals and family with this condition for their participation. We thank H. Coon, M. Page, A. Rutherford, R. Sanders, and R. Wiggins, for resources and technical assistance and G. Bellus, S. Mansour, A. Moon, and T. Wright, for comments and suggestions on the manuscript. This work was supported by National Institutes of Health grants HD-048895 and RR-00064, Centers for Disease Control grant U50/CCU822097-02, the Clinical Genetic Research Program at the University of Utah, the Primary Children's Medical Center Foundation, and the Children's Hospital and Regional Medical Center. R.M.T. is a recipient of the University of Utah Graduate Research Fellowship.

Web Resources

Accession numbers and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *FGFR3* cDNA [accession number NM_000142])
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for ACH, HCH, thanatophoric dysplasia I and II, Muenke syndrome, Crouzon syndrome with acanthosis nigricans, LADD syndrome, WHS, and insulin receptor)
RSCB Protein Data Bank, http://www.rcsb.org/pdb/Welcome.do (for human FGFR1 [entry 1FGK])

References

1. Wilkie AOM, Patey SJ, Kan SH, van den Ouweland AMW, Hamel BCJ (2002) FGFs, their receptors, and human limb malformations: clinical and molecular correlations. Am J Med Genet 112:266–278
2. Rohmann E, Brunner HG, Kayserili H, Uyguner O, Nurnberg G, Lew ED, Dobbie A, Eswarakumar VP, Üzümcü A, Ulubil-Emeroğlu M, Leroy JG, Li Y, Becker C, Lehnerdt K, Cremers CW, Yüksel-Apak M, Nurnberg P, Kubisch C, Schlessinger J, van Bokhoven H, Wollnik B (2006) Mutations in different components of FGF signaling in LADD syndrome. Nat Genet 38:414–417
3. Tavormina PL, Bellus GA, Webster MK, Bamshad MJ, Fraley AE, McIntosh I, Szabo J, Jiang W, Jabs EW, Wilcox WR, Wasmuth JJ, Donoghue DJ, Thompson LM, Francomano CA (1999) A novel skeletal dysplasia with developmental delay and acanthosis nigricans is caused by a Lys650Met mutation in the fibroblast growth factor receptor 3 gene. Am J Hum Genet 64:722–731
4. Deng C, Wynshaw-Boris A, Zhou F, Kuo A, Leder P (1996) Fibroblast growth factor receptor 3 is a negative regulator of bone growth. Cell 84:911–921
5. Colvin JS, Bohne BA, Harding GW, McEwen DG, Ornitz DM (1996) Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. Nat Genet 12:390–397
6. Mohammadi M, Schlessinger J, Hubbard SR (1996) Structure of the FGF receptor tyrosine kinase domain reveals a novel autoinhibitory mechanism. Cell 86:577–587
7. Ablooğlu AJ, Frankel M, Rusinova E, Alexander Ross JB, Kohanski RA (2001) Multiple activation loop conformations and their regulatory properties in the insulin receptor's kinase domain. J Biol Chem 276:46933–46940
8. Williams DM, Wang D, Cole PA (2000) Chemical rescue of a

mutant protein-tyrosine kinase. J Biol Chem 275:38127–38130
9. Pirvola U, Cao Y, Oellig C, Suoqiang Z, Pettersson RF, Ylikoski J (1995) The site of action of neuronal acidic fibroblast growth factor is the organ of Corti of the rat cochlea. Proc Natl Acad Sci USA 92:9269–9273
10. Muenke M, Gripp KW, McDonald-McGinn DM, Gaudenz K, Whitaker LA, Bartlett SP, Markowitz RI, et al. (1997) A unique point mutation in the fibroblast growth factor receptor 3 gene (*FGFR3*) defines a new craniosynostosis syndrome. Am J Hum Genet 60:555–564
11. Beever JE, Smit MA, Meyers SN, Hadfield TS, Bottema C, Albretsen J, Cockett NE (2006) A single-base change in the tyrosine kinase II domain of ovine *FGFR3* causes hereditary chondrodysplasia in sheep. Anim Genet 37:66–71
12. Vanek JA, Walter PA, Alstad AD (1989) Radiographic diagnosis of hereditary chondrodysplasia in newborn lambs. J Am Vet Med Assoc 194:244–248
13. Wright TJ, Ricke DO, Denison K, Abmayr S, Cotter PD, Hirschhorn K, Keinanen M, McDonald-McGinn D, Somer M, Spinner N, Yang-Feng T, Zackai E, Altherr MR (1997) A transcript map of the newly defined 165 kb Wolf-Hirschhorn syndrome critical region. Hum Mol Genet 6:317–324
14. Chang PY, Benecke H, Le Marchand-Brustel Y, Lawitts J, Moller DE (1994) Expression of a dominant-negative mutant human insulin receptor in the muscle of transgenic mice. J Biol Chem 269:16034–16040
15. Rau H, Kocova M, O'Rahilly S, Whitehead JP (2000) Naturally occurring amino acid substitutions at Arg1174 in the human insulin receptor result in differential effects on receptor biosynthesis and hybrid formation, leading to discordant clinical phenotypes. Diabetes 49:1264–1268
16. Bellus GA, Spector EB, Speiser PW, Weaver CA, Garber AT, Bryke CR, Israel J, Rosengren SS, Webster MK, Donoghue DJ, Francomano CA (2000) Distinct missense mutations of the FGFR3 Lys650 codon modulate receptor kinase activation and the severity of the skeletal dysplasia phenotype. Am J Hum Genet 67:1411–1421