Fibroblast Growth Factor Receptor and Related Skeletal Disorders

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Abstract

Osteochondrodysplasias are a heterogeneous group of disorders. To date, more than 450 skeletal conditions have been characterized. Many of the skeletal dysplasias arise during the prenatal period and are able to be diagnosed by ultrasonography. Fibroblast growth factor (FGF) signaling, including the ligands and their receptors, plays an important role in the function of chondrocytes and osteocytes that contribute to bone patterning. Two of the most common types of skeletal dysplasias are achondroplasia and thanatophoric dysplasia, emphasizing the importance of FGF signaling in skeletal development. This chapter focuses on the many distinct skeletal disorders arising from mutations in the FGF receptor (FGFR) family of genes that are responsible for forms of syndromic and non-syndromic craniosynostosis and chondrodysplasias.

Keywords

Osteochondrodysplasias • Skeletal dysplasia • Fibroblast growth factor • FGF • Fibroblast growth factor receptor • FGFR • Achondroplasia • Thanatophoric dysplasia • Craniosynostosis • Chondrodysplasias

Introduction

Osteochondrodysplasias are a heterogeneous group of disorders. To date, greater than 450 skeletal conditions have been characterized [1–3]. Many of the skeletal dysplasias arise during the prenatal period and can be clinically diagnosed by ultrasonography. The perinatal prevalence of skeletal dysplasias is conservatively suggested to be 2.3 in 10,000 with estimates as high as 1 in 4,000–5,000 births [4, 5]. Fibroblast growth factor (FGF) signaling, including the ligands and their receptors, play an important role in the function of chondrocytes and osteocytes that contribute to bone patterning. Two of the most common types of skeletal dysplasias are achondroplasia and thanatophoric dysplasia, emphasizing the importance of FGF signaling in skeletal development [6]. Since mutations found in the FGF receptor (FGFR) genes result in some of the most common types of skeletal dysplasias, genetic testing is an effective diagnostic tool for determination of the type of skeletal dysplasia and establishing recurrence risks. This chapter focuses on the many distinct skeletal disorders arising from mutations in the FGFR family of genes that are responsible for forms of syndromic and non-syndromic craniosynostosis and chondrodysplasias.

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Fibroblast Growth Factor Signaling

The FGF signaling pathway plays a prominent role in the growth and shaping of the skeletal system through regulation

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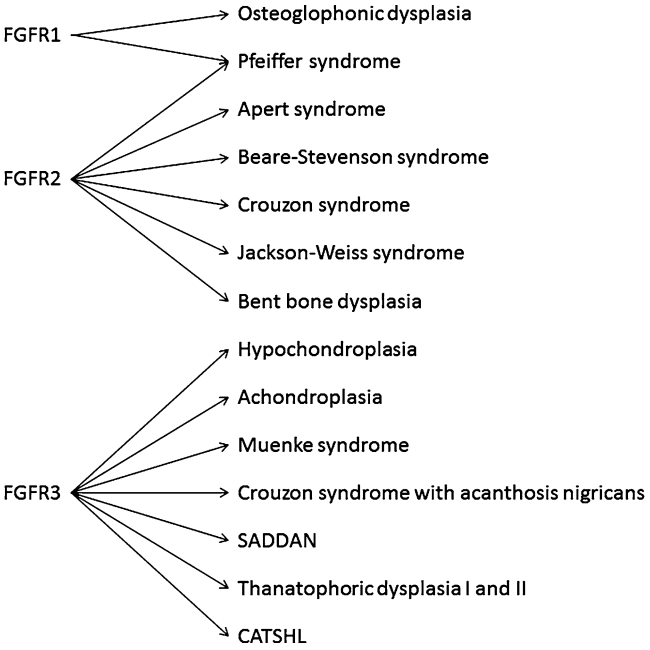

Figure 12.1 FGFR-associated syndromic conditions

Table 12.1 *FGFR1*, *-2*, and *-3* genes

Gene	OMIM	Chromosomal location	Gene organization
FGFR1	*136350	8p11.23-p11.22	18 exons, 17 coding
FGFR2	*176943	10q26.13	18 exons, 17 coding
FGFR3	*134934	4p16.3	18 exons, 17 coding

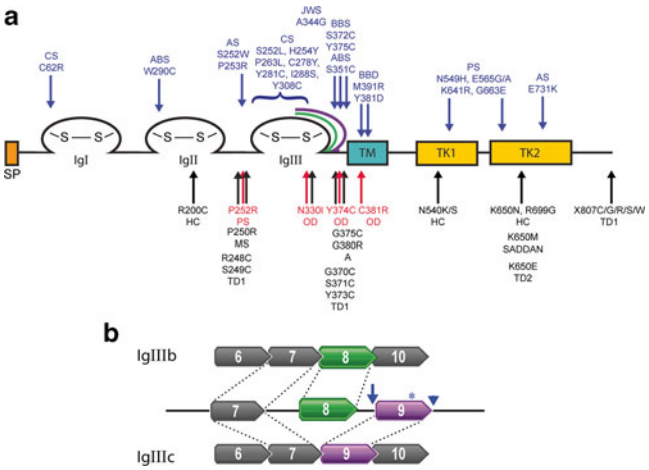

Figure 12.2 FGFR protein structure. (**a**) Common FGFR protein structure showing the signal peptide domain (SP, *orange*), the three immunoglobulin loops (IgI, IgII, and IgIII) with cysteine–cysteine disulfide bonds (S–S), the transmembrane domain (TM, *blue*), and the bifurcated tyrosine kinase domain (TK1 and TK2, *yellow*). The alternatively spliced carboxy-region of IgIII is illustrated as *green* and *purple lines*. Locations of selected mutations in *FGFR1* (*red*), *FGFR2* (*blue*), and *FGFR3* (*black*) are indicated by *arrows*, with the associated disease above or below the mutation. *A* achondrodysplasia, *ABS* Antley-Bixler syndrome, *AS* Apert syndrome, *BBD* bent bone dysplasia, *BSS* Beare-Stevenson syndrome, *CS* Crouzon syndrome, *HC* hypochondrodysplasia, *JWS* Jackson-Weiss syndrome, *MS* Muenke syndrome, *OD* osteoglophonic dysplasia, *PS* Pfeiffer syndrome, *TD1* and *TD2* thanatophoric dysplasia type 1 and 2, respectively. (**b**) Alternative splicing of exons 8 and 9 that code for the IgIII carboxy-terminal region. Isoform FGFR-IIIb (*top*) includes exon 8 (*green*). Isoform FGFR-IIIc (*bottom*) includes exon 9 (*purple*). Examples of *FGFR2* splicing mutations are shown: c.940-2A>G (*arrow*), c.1032G>A (A344A) (*asterisk*); and c.1084+3A>G (*arrowhead*)

of chondro- and osteoblastogenesis [7]. Twenty-three known FGF family members act as ligands to the FGFRs to influence cellular proliferation and differentiation. The FGF ligands interact with four high-affinity receptors (*FGFR1* through -4). Signaling is initiated by a FGFR monomer that binds to a FGF ligand, which requires heparin sulfate proteoglycan to facilitate the interaction [9]. FGF ligands bind to the FGFRs with different affinities and specificities [8]. Ligand binding induces homodimerization of the receptors and autophosphorylation of the tyrosine residues located in the cytoplasmic domain to propagate the intracellular signal [10, 11].

FGF Receptors

FGFRs are membrane-bound receptor tyrosine kinases. Of the four receptors, only mutations in *FGFR1*, *-2*, and *-3* (OMIM *136350, *176943, *134934) result in skeletal disorders (Fig. 12.1). The structures of the FGFR paralogs are very similar (Table 12.1). The FGFR paralogs share the same organization consisting of three immunoglobulin-like extracellular domains denoted as IgI, IgII, and IgIII, a membrane traversing hydrophobic region, and a bifurcated intracellular tyrosine kinase domain that propagates the signal to downstream pathways (Fig. 12.2a). The three immunoglobulin-like regions are stabilized by cysteine-cysteine disulfide bonds. The *FGFR1-3* genes produce alternative splicing of the IgIII domain, which generates two isoforms that exhibit tissue-specific expression. The amino aspect of the IgIII region is referred to as IgIIIa. The carboxy half of the IgIII loop contains one of two alternate exons (Fig. 12.2b) denoted as IgIIIb and IgIIIc that code for the C-terminal region of the IgIII domain [12, 13]. The two isoforms preferentially bind to particular FGF ligands and are differentially expressed in epithelial and mesenchymal tissues during development [14]. Studies indicate that it is the FGFR2c and FGFR3c isoforms, expressed in the mesenchyme, that are particularly involved in proper bone patterning [15].

Molecular Basis of Disease

Mutations in the *FGFR1*, *-2*, and *-3* genes account for approximately 15–20 % of all craniosynostosis and chondrodysplasias. FGFR-related skeletal anomalies are a result of gain-of-function variants that constitutively activate the receptor function [16–18]. Activated FGFRs receptors cause increased cellular proliferation and premature osteoblast differentiation [19–21]. The FGFR constitutive activation is by either a FGF ligand-dependent or -independent mechanism. Ligand-dependent mechanisms arise due to mutations that improve binding of FGF ligands and dimerization of the receptors prolonging signaling activity [22]. Alternatively, ligand-independent mechanisms include the following: (1) enhancement of receptor dimerization due to an immunoglobulin domain structural change such as a gain or loss of a cysteine residue within the loops; (2) augmentation of dimerization due to intramembrane domain changes in amino acid charge that increases hydrogen bonding; and (3) alterations in the kinase domain causing constitutively active phosphorylation [23–25].

Mutations causing FGFR-related craniosynostosis and/or chondrodysplasia tend to cluster in specific domains of the receptors (Fig. 12.2a). These clusters illustrate the importance of the domains in receptor function. Analogous mutations found in each of the different FGFRs tend to mirror phenotypic effects between the receptors. However, distinctions between analogous mutations in the different receptors and their phenotypes reveal their independent roles during normal skeletogenesis.

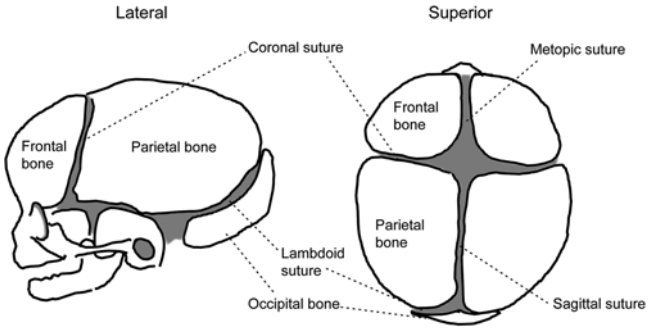

Figure 12.3 Skull sutures of a newborn. (*left*) Lateral view. (*right*) Superior view

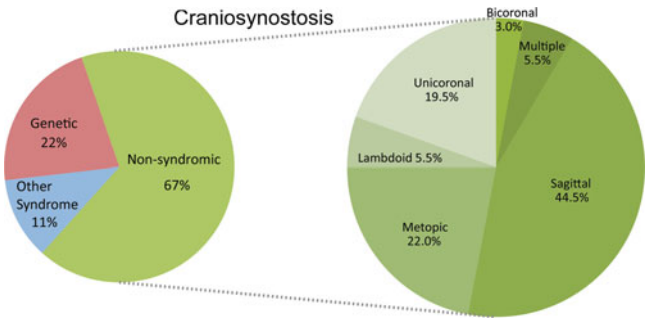

Figure 12.4 The prevalence of different forms of craniosynostosis derived from analysis of a 215-patient cohort affected with syndromic and nonsyndromic forms of craniosynostosis (*left*). Phenotypic categorization of nonsyndromic cases based upon affected sutures (*right*) (adapted with permission from Wilkie et al. 2010 [32])

Craniosynostosis and Chondrodysplasia

Craniosynostosis

Craniosynostosis arises from the premature fusion of one or more of the cranial sutures resulting in a dysmorphic skull. Distortions of the skull arise from uneven growth patterns between the sutures and depend on the location and timing of the fusion events [26]. The cranial sutures are the leading edges of the growing intramembranous bones that comprise the skull vault or calvaria. The calvaria consists of the left and right frontal, squamous occipital, squamous temporal, and the left and right parietal bones that will form the neurocranium (Fig. 12.3). At birth, the frontal bones are separated by the metopic suture. The coronal suture separates the frontal bones from the left and right parietal bones. The lambdoid suture separates the parietals from the occipital bone. The suture leading edges function as ossification centers. The calvaria of the neurocranium that forms the desmocranium ossifies as intramembranous bone forming the vault of the neurocranium. Normally, the metopic suture begins to close after the first year and closure is completed by the seventh year, generating the frontal bone [27]. The sagittal, coronal, and lambdoidal sutures generally complete fusion between 20 and 40 years of age. *De novo* or autosomal dominantly inherited mutations in the *FGFR1–3* genes that enhance signaling result in the inhibition of the osteogenic proliferation program, thereby causing premature fusion of cranial sutures [28]. Mutations associated with isolated nonsyndromic forms of craniosynostosis have been found in each of the *FGFR1–3* genes [29–31].

Nonsyndromic or isolated single-suture forms of craniosynostosis are more common than syndromic and number 1 in 2,100–3,000 live births [17]. Sagittal synostosis is the most common, followed by coronal, metopic, and then lambdoid suture synostosis (Fig. 12.4) [32, 33]. Nonsyndromic craniosynostosis is heterogeneous and many of the causes remain unknown. Often full sequencing of the *FGFR* genes may be requested to rule out the receptors and potentially detect *de novo* mutations.

Chondrodysplasias

In addition to influencing the growth of the neurocranium, FGF signaling also participates in the development of the skeletal system exemplified by its role in the growth of the long bones of the appendicular skeleton. Dominantly inherited and *de novo* gain-of-function mutations result in varying degrees of dwarfism as illustrated by particular *FGFR3* mutations that cause hypochondroplasia and achondroplasia. Observed in both craniosynostosis and chondrodysplasia cases, germline mutations due to advanced paternal age are a significant contributor to these disorders [34, 35].

Clinical Utility of Testing

Syndromic forms of *FGFR*-related craniosynostosis and chondrodysplasia are diagnosed clinically, based on their well-described phenotypes. Three-dimensional ultrasonography is able to detect early skeletal anomalies, including short bones, premature skull fusion, and other characteristic syndromic features [6, 36]. Molecular testing is performed to confirm the diagnosis and provide recurrence risks in pregnancies with either suspected germline mosaic transmission in families with a history of a previously affected fetus, or in families with an affected parent and a 50 % probability of passing on the deleterious allele. In such cases of inheritance, examination of the contributing parent may reveal a mild phenotype. For severe cases, *de novo* mutations occurring in the male germline may be responsible. Advanced paternal age is known to increase the risk of having affected offspring. Generally, targeted mutation analysis is sufficient to confirm a well-characterized phenotype and is the first tier of testing. Sequencing of all the exons may be performed if no mutation is found initially.

Available Assays

Different methods have been developed to identify mutations within the *FGFR* genes. The vast majority of mutations are missense and nonsense, with splicing and in-frame small insertions and deletions being much rarer. Detection methods include targeted mutation analysis, scanning of specific exons, and sequencing of all the coding regions as well as their intron/exon boundaries. Targeted analysis is based on testing the patient's DNA for previously described mutations. Using restriction fragment length polymorphism (RFLP) methods, regions of interest within the coding sequences can be amplified by polymerase chain reaction (PCR) and digested with restriction endonucleases that would characteristically identify specific mutations based on altered banding patterns observed by gel electrophoresis and staining (Fig. 12.5). Incomplete digestion of a restriction site may lead to aberrant results and an incorrect heterozygosity interpretation; therefore, both positive and negative digestion controls are essential for RFLP analysis. Alternatively, allele-specific oligonucleotide PCR with primers targeted to wild-type alleles and previously characterized mutations may be performed, resulting in identifiable amplicon patterns. The drawback to targeted analysis is that mutations outside the scope of the assay will be missed.

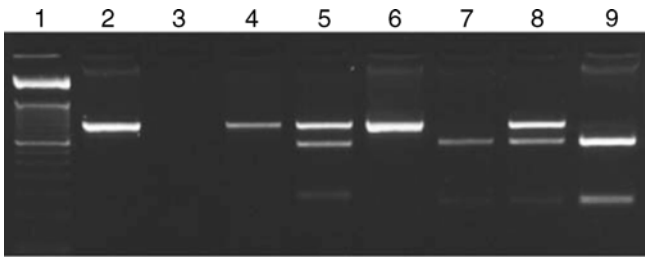

Figure 12.5 Thanatophoric dysplasia type 2 restriction fragment length polymorphism (RFLP) assay. Amplification of the *FGFR3* exon 15 and digestion of patient and control DNA give characteristic banding patterns diagnostic for the p.Lys650Glu mutation. *Lane 1*, 50 base pair (bp) size marker; *lane 2*, undigested 450 bp amplicon; *lane 3*, no DNA template PCR control; *lanes 4–6*, restriction digest with *Bsm*AI; *lanes 7–9*, digest with *Bbs*I. *Lanes 4, 6, 7,* and *9*, control DNA. *Lanes 5* and *8*, patient DNA. *Lanes 4–6* show a gain of a *Bsm*AI restriction site in the patient DNA (*lane 5*). *Lanes 7–9* demonstrate loss of a *Bbs*I digest site in the patient's DNA (*lane 8*)

Mutation scanning by denaturing high-performance liquid chromatography (DHPLC) has been employed to screen the exons of the FGFR genes whereby patient's profiles suggest potential mutations in comparison to unaffected and affected controls. Issues arise in scanning techniques such as DHPLC as well as high-temperature melting profiles and single-stranded conformational polymorphism in their inability to identify specific mutations or distinguish between known mutations and rare polymorphisms. Unique profiles identifying potential mutations must then be directly sequenced. Sequence analysis of the coding exons is the most comprehensive of the methods ensuring that any point mutation and small deletions and duplications will be identified within the amplified regions. Massively parallel sequencing, using next-generation sequencing platforms, also offers the option to perform sequence analysis on many samples concurrently through barcoding of individual patient's DNA and using bioinformatics to separate the reads by patient and analyze the sequence data.

Deletion/duplication analysis may be performed by multiplex ligation-dependent probe amplification or exon level array-based comparative genomic hybridization and then confirmed by quantitative PCR. These methods measure the relative amounts of each exonic region where probes are placed, comparing the quantitative results to internal controls, usually housekeeping gene(s) found on other chromosomes.

Interpretation of Test Results

The majority of *FGFR* mutations causing skeletal dysplasia are point mutations. Interpretation of novel missense variants is becoming easier as the knowledge base grows. To date, there are approximately 155 known mutations within the *FGFR1-2-3* that cause skeletal dysplasias. Current lists of mutations are available in the Human Gene Mutation Database (www.biobase-international.com/product/hgmd). Databases that include polymorphisms, such as dbSNP (www.ncbi.nlm.nih.gov/projects/SNP/), the 1000 Genomes Project (www.1000genomes.org/), and exome sequencing projects (Exome Aggregation Consortium [ExAC], exac.broadinstitute.org; Exome Variant Server, http://evs.gs.washington.edu/EVS/), provide information on the genetic variants and their frequencies in the general population [37, 38]. Variants of unknown significance can be evaluated using predictive algorithms that may provide insight into whether they are benign or pathogenic. These predictions take into consideration the biochemical properties of the amino acid change, including size, charge, polarity, steric constraints, and evolutionary conservation of the residue within the protein domain. Internet-based predictive tools, including PolyPhen2, SIFT, and Mutation Taster, provide analysis of the probable functional consequences of missense variants [39–41]. The results of these prediction tools have to be considered as one piece of evidence.

Rare splicing mutations causing skeletal dysplasia have been documented. Mutations, such as the synonymous change p.Ala344Ala (c.1032G>A) in the immunoglobulin IIIc (IgIIIc) domain of *FGFR2*, activate a cryptic splice donor site (Fig. 12.2b) [42]. Conversely, weakening the endogenous donor site (c.1084+3A>G and c.1084+1G>A) induces exon skipping and preferential splicing of the IgIIIb isoform [43–45]. Similarly, *FGFR2* mutations causing Pfeiffer syndrome affect the IgIIIc acceptor site (c.940-2A>G or A>T) [46]. Unknown variants found in the intronic regions of the gene can be analyzed through splicing algorithms, such as the Human Splice Finder (www.umd.be/HSF/) [47] and Netgene2 (www.cbs.dtu.dk/services/NetGene2/) [48], to predict their influence on the precursor mRNA.

Laboratory Issues

Commercial test kits for analyzing the *FGFR* genes are not available, so testing is performed using laboratory developed tests. Test development and validation may be achieved using cell lines obtained from the Coriell Cell Repository (http://ccr.coriell.org). Proficiency testing is available through the College of American Pathologists Molecular Genetics Laboratory sequencing surveys. Additionally, interlaboratory sample exchanges and internal repeat testing of blinded samples can be used to meet the proficiency testing requirements.

FGFR-Related Skeletal Syndromes

Apert Syndrome

Apert syndrome (OMIM#101200), also known as acrocephalosyndactyly, is characterized by craniosynostosis, facial hypoplasia, broad thumbs and great toes, and digit fusion of the hands and feet (syndactyly) described as a "mitten-hand" malformation. As the most common of the craniosynostosis syndromes, Apert syndrome accounts for approximately 4.5 % of all cases with an occurrence estimated as 1 in 65,000 to 80,000 [49, 50]. The majority of cases result from *de novo* mutations attributed to increasing paternal age [51].

The majority of cases (99 %) are caused by one of two point mutations in the *FGFR2* gene [52]. The common mutations are p.Ser252Trp, which accounts for approximately two-thirds of the cases, and p.Pro253Arg accounting for one third of cases. These mutations, located at the IgII-IgIII linker region, enhance ligand binding affinity [9]. Rarer mutations have been observed surrounding these two mutations in the linker region. Diagnostic testing can be performed by targeted Sanger sequencing.

Apert syndrome is clinically diagnosed. Identification of the specific mutation helps in determining the potential risk for further affected pregnancies due to germline mosaicism. With an affected parent, there is a 50 % probability that a pregnancy will result in an affected child.

Pfeiffer Syndrome

Patients presenting with Pfeiffer Syndrome (OMIM#101600) exhibit brachycephaly, hypertelorism, ocular proptosis, a flat midface, broad thumbs, and medially deviated great toes, and occasionally with hearing loss and variable cutaneous syndactyly [17]. Pfeiffer syndrome is a genetically heterogeneous disorder. Mutations have been found in both *FGFR1* and *FGFR2*. *FGFR2* mutations account for 95 % of patients' mutations. One activating mutation, p.Pro252Arg in the *FGFR1* gene, accounts for approximately 5 % of the diagnosed patients. Mutations in *FGFR2* are found in the IgIIIa and IgIIIc regions as well as the tyrosine kinase domain, and tend to be phenotypically more severe than the *FGFR1*-derived phenotype.

Crouzon Syndrome With or Without Acanthosis Nigricans

Crouzon syndrome (OMIM#123500) patients have multiple suture fusions or coronal fusions causing brachycephaly, trigonocephaly, and rare reports of cloverleaf skull malformation also known as kleeblattschädel [53, 54]. Attributes

typically include hypertelorism, a small midface, beaked nose and protrusion of the eyes. Hands and feet are generally normal. Heterozygous mutations in the *FGFR2* gene show high penetrance and variability of the phenotype within families. Approximately half of the cases are inherited and the other half arise from *de novo* mutations. Increasing paternal age is a contributing factor for *de novo* mutations [35]. The prevalence is estimated as 1 in 65,000 live births [55].

Patients with Crouzon syndrome with acanthosis nigricans (OMIM#612247) are typically female, display hyperpigmentation of the skin, hyperkeratosis, and other skin findings. A specific *FGFR3* heterozygous mutation, p.Ala391Glu, has been identified and is located in the transmembrane domain [56].

Muenke Syndrome

Muenke syndrome (OMIM#602849) displays incomplete penetrance and a variable phenotype even within families [57]. Prevalence in the population is estimated to be 1 in 30,000 live births. Sporadic and familial cases have been reported. Characteristics include bi- or unicoronal synostosis, midfacial hypoplasia, ptosis, and downslanting palpebral fissures. Some affected individuals have additional features that may include sensorineural hearing loss, developmental delay, brachydactyly, and coned epiphyses in the hands and feet. Muenke craniosynostosis is a result of a specific heterozygous mutation, p.Pro250Arg, found in the linker region between domains IgII and IgIII of *FGFR3* [58–61]. Targeted testing may be performed by sequencing the seventh exon of *FGFR3* or by RFLP analysis using the *Msp*I endonuclease.

Beare-Stevenson Syndrome

Beare-Stevenson (OMIM#123790), also known as cutis gyrate syndrome of Beare and Stevenson, is a rare and severe disorder. Patients characteristically have body-wide skin furrows (cutis gyrate), acanthosis nigricans, skin tags, bifid scrotum, and anogenital anomalies. The craniosynostosis may be severe and present as a cloverleaf skull with hypertelorism, a broad nasal bridge, cleft palate, and hypodontia. Two heterozygous point mutations in the *FGFR2* gene, p.Ser372Cys and p.Tyr375Cys, account for 50–60 % of cases, suggesting locus heterogeneity. The resulting cysteine residues are thought to increase ligand-independent dimerization. These *de novo* mutations are analogous to the mutations in *FGFR3* causing thanatophoric dysplasia. An intragenic deletion, c.1506del63, has recently been described and is proposed to alter gene splicing in favor of the IgIIIb isoform of *FGFR2*. Loss of the 21 amino acids encoded by exons 8 and 9 is suggested to cause aberrant expression of *FGFR2b* [62].

Jackson-Weiss Syndrome

Jackson-Weiss Syndrome (OMIM#123150), inherited in an autosomal dominant manner, has been most prominently described in an extended Amish family with a p.Ala344Gly mutation in the IgIIIc domain of *FGFR2* [63–66]. Fully penetrant with variable severity, the characteristics of the syndrome include craniosynostosis with facial anomalies, broad great toes, and webbing of the second and third toes [67]. A few reports suggest mutations in *FGFR2* and *FGFR1* exhibit phenotypic traits similar to Jackson-Weiss syndrome indicating that Crouzon, Jackson-Weiss, and Pfeiffer syndromes may represent a spectrum of craniosynostotic and digit malformations [68, 69].

Antley-Bixler Syndrome Type 2

Antley-Bixler syndrome (ABS; trapezoidocephaly-synostosis syndrome) is a rare and severe heterogeneous disorder with mutations found in both the *FGFR2* gene (autosomal dominant; type 2; OMIM#207410) and the cytochrome P450 oxidoreductase (*POR*) gene (autosomal recessive; type 1; OMIM#201750). ABS type 2 is characterized by craniosynostosis of the coronal and lambdoid sutures, midfacial hypoplasia, radiohumeral and digit fusions, exophthalmos, and arachnodactyly [70, 71]. The mutations associated with ABS include p.Trp290Cys and p.Ser351Cys, both found in the IgIII domain of the *FGFR2* gene [72]. Mutations at these positions also have been associated with the milder phenotype of Crouzon syndrome [73].

Osteoglophonic Dysplasia

Osteoglophonic dysplasia (Fairbank-Keats syndrome; OMIM#166250), a very rare disorder, is typified by variable craniosynostosis and rhizomelic dwarfism with a "hollowed-out" appearance of the tubular bones on radiographs, depression of the nasal bridge, unerupted teeth, frontal bossing, and prognathism similar to achondroplasia. Mutations in *FGFR1* are found in the conserved amino acids clustered in the C-terminal region of the IgIII immunoglobulin domain, the linker region, and the transmembrane domain. The *FGFR1* p.Tyr372Cys mutation is analogous to both the *FGFR2* p.Tyr375Cys mutation that causes Beare-Stevenson syndrome and the p.Tyr373Cys *FGFR3* mutation that causes thanatophoric dwarfism type 1, indicating the importance of that amino acid position in the functional role of the receptors [74].

Achondroplasia

Achondroplasia (OMIM#100800) arises from mutations in the *FGFR3* gene that inhibit chondrocyte proliferation within the endochondral growth plate resulting in the shortening of long bones. Achondroplasia is the most common form of FGFR-related short-limbed dwarfism [75, 76], with an occurrence of 1 in 10,000 to 30,000 live births [4, 5]. Two common variants, c.1138G>A (~98 %) and c.1138G>C (1–2 %), result in a p.Gly380Arg mutation in the transmembrane domain of *FGFR3* [77]. The achondroplasia mutation is the most common *de novo* disease-causing mutation known. There is a strong paternal origin for the mutation, mostly in fathers over the age of 35 years [34]. A second mutation, described in several published accounts, suggests that p.Gly375Cys also causes achondroplasia [78–80].

Testing for the c.1138G>A mutation may be performed by RFLP digestion of exon 10 with the *Sfc*I restriction enzyme. It has been noted, however, that complete digestion is not consistently observed for the assay and other molecular methods may be required to differentiate between the G>A and the G>C mutations, the heterozygous form, and the lethal homozygous form [81–83].

Severe Achondroplasia, Developmental Delay, and Acanthosis Nigricans (SADDAN)

Severe achondroplasia, developmental delay and acanthosis nigricans (SADDAN; OMIM#187600), is caused by a c.1949A>T mutation (p.Lys650Met) in *FGFR3*. The substitution of a methionine residue at position 650 differentiates SADDAN from type 2 thanatophoric dysplasia, which arises from a glutamic acid substitution at the same position (c.1948A>G; Lys650Glu). The SADDAN amino acid change induces constitutive kinase activity that is threefold greater than normal [84].

Hypochondroplasia

Hypochondroplasia (OMIM#146000) is clinically diagnosed as a mild form of skeletal dysplasia. Clinical diagnosis is usually by short limbs detected on ultrasonography, which prompts diagnostic testing. The *FGFR3* c.1138G>A mutation that causes achondroplasia has been found in about 5 % of hypochondroplasia cases. *FGFR3* mutations account for only 50–70 % of cases, suggesting genetic heterogeneity. Of those mutations in *FGFR3*, 70 % are a recurrent p.Asn540Lys amino acid change located in the tyrosine kinase 1 domain (TK1), while others are rarer. Testing may be performed by RFLP analysis of an exon 13 PCR product, which will detect the two c.1659C>A/G, p.N540K mutations. A *Bsp*MI restriction site is abolished by the c.1620C>A mutation, and the c.1620C>G mutation creates a novel *Alu*I restriction site [85]. The other known mutations may be detected by sequencing exons 10, 13, and 15 of *FGFR3*.

Thanatophoric Dysplasia

Thanatophoric dysplasia (TD) is the most common lethal condition of short-limbed skeletal dysplasia with a distorted head and has an estimated incidence of 1 in 20,000 to 50,000 live births. Two types of TD are clinically diagnosed based on ultrasound and radiographic findings [86–88]. Type 1 (OMIM#187600) patients have prominently curved femurs, while type 2 (OMIM#187601) patients typically have a severe form of craniosynostosis often referred to as a cloverleaf skull and a small chest [89].

Several different gain-of-function mutations in *FGFR3* cause TD type 1. Mutations p.Arg248Cys, p.Ser249Cys, p.Ser371Cys, and p.Tyr373Cys create novel cysteine residues in the extracellular and intramembranous domains, while other mutations causing TD type 1, such as p.Ter807Arg, p.Ter807Cys, p.Ter807Gly, p.Ter807Ser, and p.Ter807Trp, obliterate the stop codon resulting in extension of the intracellular domain by an additional 141 amino acids [90, 91].

TD type 2 is caused by the *FGFR3* transition mutation c.1948A>G, coding for p.Lys650Glu [92, 93]. The mutation causes multiple cranial sutures to fuse prematurely resulting in a cloverleaf skull malformation. The importance of the lysine 650 codon, situated in the tyrosine kinase-domain activation loop of FGFR3, is emphasized by the wide range of clinical phenotypes observed based, on the different amino acid substitutions and their ability to influence kinase activity. Similar to the previously mentioned SADDAN p.Lys650Met mutation, substitution of the lysine 650 for a glutamine or asparagine residue is associated with a milder hypochondrodysplasia phenotype [94].

Bent Bone Dysplasia: FGFR2 Type

Bent bone dysplasia-FGFR2 type (OMIM#614592) has recently been attributed to two heterozygous mutations in the FGFR2 transmembrane domain, c.1172T>G (p.Met391Arg) and c.1141T>G (p.Tyr381Asp) that reduce its localization to the plasma membrane [95]. A phenotype of perinatal lethality with hypertelorism, midface hypoplasia, micrognathia, prematurely erupted prenatal teeth, low-set posteriorly rotated ears, and clitoromegaly in females. Distinct radiological findings include coronal craniosynostosis with poorly mineralized calvaria, curved appendicular skeletal defects, and clavicle hypoplasia. The nuances of the genotype-

phenotype correlations are observed in the mutation of the tyrosine 381 residue to asparagine (p.Tyr381Asn, c.1141T>A) that causes Crouzon syndrome [96]. These studies suggest a spectrum of severity for altered FGFR2 activity.

Other *FGFR*-Associated Disorders

In addition to the activating mutations resulting in craniosynostosis and chondrodysplasia, loss-of-function mutations in the *FGFR* genes cause a variety of different syndromes.

CATSHL Syndrome

Dominantly inherited, camptodactyly, tall stature, scoliosis, and hearing loss (CATSHL; OMIM#610474) is caused by a *FGFR3* p.Arg621His heterozygous missense mutation residing within the tyrosine kinase domain generating a loss-of-function that promotes endochondral bone growth [97]. Recently, a novel homozygous *FGFR3* c.1167C>A p.Thr546Lys, mutation has been described as also causing skeletal overgrowth [98].

Kallmann Syndrome

Hypogonadotropic hypogonadism, also known as Kallmann syndrome (OMIM#308700), is a heterogenic disorder with mutations found most commonly in the *KAL* gene (KAL1, X-linked; OMIM *300836) as well as *FGFR1* (KAL2, OMIM#147950). Other genes that account for 5 % or less of cases include *PROKR2*, *PROK2*, *CHD7*, and *FGF8*, while an additional five genes are known to account for the autosomal recessive form. Sensitivity of testing for clinically diagnosed Kallmann syndrome is approximately 30 % for the aforementioned genes [99]. Loss-of-function mutations in *FGFR1* account for approximately 10 % of cases of type 2 Kallmann Syndrome with deletions being rare contributors to the disorder. Kallmann syndrome exhibits a 5:1 male to female ratio with an incidence of approximately 1 in 8,000 to 10,000 in males and 1 in 40,000 to 50,000 in females [100, 101]. Patients characteristically have olfactory bulb dysgenesis (anosmia) and hypogonadotropic hypogonadism, with boys also having micropenis and cryptorchidism. Mutations in the *FGFR1* gene also may result in cleft lip and/or palate, agenesis of the teeth, and digital malformations [102].

LADD Syndrome

Lacrimo-auriculo-dento-digital (LADD) syndrome (OMIM#149730; Levy-Hollister syndrome) is a dominant, heterogeneous disorder with mutations found in the *FGF10* gene as well as the tyrosine kinase domains of *FGFR2* (p.Ala648Thr, p.Ala628Thr) and *FGFR3* (p.Asp513Asn) [103–105]. Variants in the receptor kinase domain associated with LADD syndrome reduce phosphorylation activity [106]. Affected individuals typically exhibit hypoplasia/aplasia of the tear and salivary ducts, malformed ears and deafness, hypodontia, and digital anomalies mostly affecting the thumbs [107–109].

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