RESEARCH REVIEW

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Skeletal Abnormalities in Neurofibromatosis Type 1: Approaches to Therapeutic Options

Florent Elefteriou,[1]* Mateusz Kolanczyk,[2,3] Aaron Schindeler,[4,5] David H. Viskochil,[6,7] Janet M. Hock,[8] Elizabeth K. Schorry,[9] Alvin H. Crawford,[10] Jan M. Friedman,[11] David Little,[4,5] Juha Peltonen,[12,13] John C. Carey,[6,7] David Feldman,[14] Xijie Yu,[8] Linlea Armstrong,[11] Patricia Birch,[11] David L. Kendler,[15] Stefan Mundlos,[2,3] Feng-Chun Yang,[16,17] Gina A\giostratidou,[18] Kim Hunter-Schaedle,[18] and David A. Stevenson[6,7]

[1]Department of Medicine, Vanderbilt Center for Bone Biology, Vanderbilt University Medical Center, Nashville, Tennessee

[2]Max Planck Institute for Molecular Genetics, FG Development and Disease, Berlin, Germany

[3]Institute for Medical Genetics, Charité, Universitätsmedizin Berlin, Berlin, Germany

[4]Department of Orthopaedic Research and Biotechnology, The Children's Hospital at Westmead, Sydney, Australia

[5]Discipline of Paediatrics and Child Health, Faculty of Medicine, University of Sydney, Sydney, Australia

[6]Division of Medical Genetics, Department of Pediatrics, University of Utah, Salt Lake City, Utah

[7]Shriners Hospital for Children, Salt Lake City, Utah

[8]Maine Institute for Human Genetics and Health, Brewer, Maine

[9]Human Genetics Division, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

[10]Department of Orthopedics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

[11]Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada

[12]Department of Cell Biology and Anatomy, University of Turku, Turku, Finland

[13]Department of Dermatology, University of Turku, Turku, Finland

[14]Department of Orthopedic Surgery, New York University Hospital for Joint Diseases, New York, New York

[15]Department of Medicine, University of British Columbia, Vancouver, BC, Canada

[16]Department of Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana

[17]Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana

[18]Children's Tumor Foundation, New York, New York

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*Correspondence to:
Florent Elefteriou, Department of Medicine/Clinical Pharmacology, Vanderbilt University Medical Center, Center for Bone Biology, 2215 Garland Avenue, Medical Research Building IV, Room 1225E, Nashville, TN 37232-0575. E-mail: florent.elefteriou@vanderbilt.edu
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The skeleton is frequently affected in individuals with neurofibromatosis type 1, and some of these bone manifestations can result in significant morbidity. The natural history and pathogenesis of the skeletal abnormalities of this disorder are poorly understood and consequently therapeutic options for these manifestations are currently limited. The Children's Tumor Foundation convened an International Neurofibromatosis Type 1 Bone Abnormalities Consortium to address future directions for clinical trials in skeletal abnormalities associated with this disorder. This report reviews the clinical skeletal manifestations and available preclinical mouse models and summarizes key issues that present barriers to optimal clinical management of skeletal abnormalities in neurofibromatosis type 1. These concepts should help advance optimal clinical management of the skeletal abnormalities in this disease and address major difficulties encountered for the design of clinical trials.

Key words: neurofibromatosis; NF1; bone; skeletal dysplasia; osteoblast; osteoclast; tibial dysplasia; pseudarthrosis

INTRODUCTION

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder caused by mutations in the *NF1* gene, which encodes the tumor suppressor neurofibromin [Cawthon et al., 1990; Viskochil et al., 1990; Marchuk et al., 1991]. People with NF1 are constitutionally heterozygous for an *NF1* loss-of-function mutation, that is, they are haploinsufficient for *NF1*. The skeleton is frequently affected in patients with NF1 [Crawford and Schorry, 1999]. Manifestations include focal bony lesions associated with significant morbidity, mild shortness of stature and reduced bone mineral density (BMD). The natural history and pathogenesis of NF1 skeletal abnormalities are poorly understood and currently therapeutic options for these manifestations are limited.

In February 2008, the Children's Tumor Foundation (CTF) convened an International NF1 Bone Abnormalities Consortium to review current approaches to clinical management of NF1 skeletal abnormalities, to discuss available preclinical models of these disorders, and to identify barriers that might be impeding progression to future clinical trials for NF1 skeletal abnormalities. In this research review and report of the Consortium meeting, we summarize the state of knowledge of NF1 skeletal manifestations, discuss potential interventions of each, and review mouse models as the foundation of preclinical trials. This report also highlights priorities for future research.

INSIGHTS ON NF1 SKELETAL MANIFESTATIONS

The skeletal phenotypes of NF1 can be categorized as either generalized or focal manifestations. Generalized skeletal manifestations (osteopenia/osteoporosis and shortness of stature) are common, but usually mild. Focal abnormalities (tibial dysplasia, short angle scoliosis, and sphenoid wing dysplasia) are less common, but cause significant morbidity. The relationship of generalized skeletal manifestations to the occurrence or progression of focal skeletal abnormalities is unknown, and the overall understanding of bone growth, remodeling, and repair in NF1 is critical to therapy development.

Osteoporosis

Decreased BMD in both sexes at an early age has been reported in up to 50% of individuals with NF1 [Illes et al., 2001; Kuorilehto et al., 2004b; Lammert et al., 2005; Dulai et al., 2007; Stevenson et al., 2007; Yilmaz et al., 2007; Brunetti-Pierri et al., 2008; Duman et al., 2008]. This may be related to an inadequate increase in bone remodeling observed by both bone histomorphometry and changes in circulating bone markers [Stevenson et al., 2008; Seitz et al., 2009]. Despite the consensus that children with NF1 present with low BMD, interpretation of these studies is challenging because bone densitometry measures in adult populations cannot be extrapolated to children, for reasons that include confounding of DXA results by growth and differences in fracture epidemiology in children and adolescents [Rauch et al., 2008]. Adding to the complexity of interpreting skeletal changes in the pediatric NF1 population, the criteria for osteoporosis in children under age 19 were recently redefined [Lewiecki et al., 2008; Rauch et al., 2008]. None of the published studies on bone density in NF1 children meet these more stringent new recommendations; few meet the requirement that the sample size of the control reference set consider sex, age, and race/ethnicity. In addition, NF1 datasets are often spread across a large age range with few subjects and based on T-scores that do not enable comparison to age-adjusted sex-specific norms. Therefore, the severity of the osteoporosis in this pediatric population and its implications are still unclear. An increased incidence of fractures in patients with NF1 has been reported, but sample sizes were small [Brunetti-Pierri et al., 2008; Tucker et al., 2008]. Another challenge to interpreting low BMD in patients with NF1 correctly is posed by recent reports of low vitamin D (14–21 ng/ml serum 25-dihydroxy-vitamin D, 25-OH-D) and osteomalacia in a subset of patients with NF1 [Lammert et al., 2005; Seitz et al., 2009]. Controlled studies that are adequately powered, normalize calcium homeostasis and consider age, sex, and race/ethnicity norms, are needed to determine if the apparent high prevalence of low BMD in patients with NF1 predicts increased fracture risk and a need for intervention.

Assessments and interventions. *NF1 children and adolescents.* At present, there are no clinical trials to support the use of osteoporotic drugs in this population, irrespective of whether or not they exhibit NF1. In the absence of treatment guidelines, treatment should be conservative and correct measured deficiencies in hormones (vitamin D, thyroid, estrogen, etc.) that are known to regulate skeletal growth and maturation. A review of bone mass and bone size during skeletal growth concluded changes were dependent on calcium intake and exercise, especially in prepubertal children [Heaney and Weaver, 2005]. Conservative therapy to promote bone health such as treatment with calcium, vitamin D, and weight-bearing exercise therefore forms the first line of therapy in children with NF1 and low bone mass.

Conservative therapy is no longer adequate once fractures have occurred. Non-pharmacologic therapies that use skeletal loading via physical therapy [Chad et al., 1999] or standing on vibration platforms [Ward et al., 2004] have not been tested in subjects with NF1. Osteoporosis drugs are either anabolic or anti-resorptive. Anabolic drugs are limited to two forms of parathyroid hormone and are contraindicated in children because of the increased incidence of osteosarcoma in rats [Tashjian and Gagel, 2006]. Anti-resorptive drugs such as the bisphosphonates and antibodies that target osteoclasts have been used to reduce osteoporotic fractures in adults, but their effect on BMD and fracture risk in children with NF1 is unknown.

NF1 adults. At this time, treatment of osteoporosis in adults with NF1 should not differ from that of people who do not exhibit NF1, until further information is obtained. Because low serum 25-OH-D and osteomalacia has been reported in patients with NF1 and low bone mass, patients should be evaluated for vitamin supplementation. The recent change in reference standards for serum 25-OH-D from 10 ng/ml, considered the "rachitic" value for many years, to 20 ng/ml (50 nmol/L) [Wagner and Greer, 2008; Mimouni and Shamir, 2009], has implications on the need for intervention and effective dosing. A study using 1,000 IU vitamin D to correct serum levels in four patients with NF1 reported increased BMD [Seitz et al., 2009], while a lower dose of 400 IU in another study did not improve BMD [Bruenetti-Pierri et al., 2008]. Sample size in both studies is too small for definitive conclusions on effective dose. Standard of care requires osteoporotic adults over age 50 years to take 1,200 mg calcium and 800–1,000 IU vitamin D per day, and to reduce clinical risk factors by regular weight bearing and muscle strengthening exercises and avoidance of smoking and excessive alcohol. Selection of approved anabolic or anti-resorptive drugs to prevent or treat osteoporotic fractures should follow the standard practice. Clinical trials are needed to determine which of currently available therapies are most effective to treat patients with NF1 and osteoporosis.

Short Stature and Macrocephaly

Individuals with NF1 tend to be shorter than expected for their families [Szudek et al., 2000; Virdis et al., 2003], with 20–30% of adults with NF1 estimated to have a height below the 3rd centile. Growth velocity in these individuals is typically normal or near normal before puberty, then declines. Short stature in patients with NF1 is usually proportional. Scoliosis, growth hormone deficiency, and other NF1-related complications can contribute to short stature, but the cause of short stature in most patients with NF1 is unknown.

Head circumference in patients with NF1 tends to be large, and macrocephaly (head circumference >2 SD above the mean) occurs in about one-fourth of patients [Szudek et al., 2000]. However, much of this is thought to be due to enlargement of the brain [Greenwood et al., 2005]. It is not clear if skull growth contributes to macrocephaly.

Dysplasia of the Tibia and Other Long Bones

Long bone dysplasia is seen in a small percentage (3–4%) [Friedman and Birch, 1997] of patients with NF1 and most commonly involves the tibia, although other long bones can be affected. Affected individuals usually present in infancy with unilateral anterolateral bowing of the lower leg (Fig. 1a), although a child may be born with fracture and/or pseudarthrosis, or develop these shortly after birth (Fig. 1b,c). The anterolateral bowing seen in patients with NF1 is distinct from the bilateral physiological bowing common in children as they begin to walk. Radiographically, tibial bowing in NFL patients prior to fracture usually appears as cortical thickening and medullary canal narrowing at the apex of the convexity, typically near the junction of the middle and distal thirds of the tibia [Stevenson et al., 2007] (Fig. 2). Various radiographic classification systems for tibia bowing have been proposed [Hardinge, 1972; Andersen, 1973; Bassett et al., 1981; Boyd, 1982; Crawford, 1986; Crawford and Bagamery, 1986; Crawford and

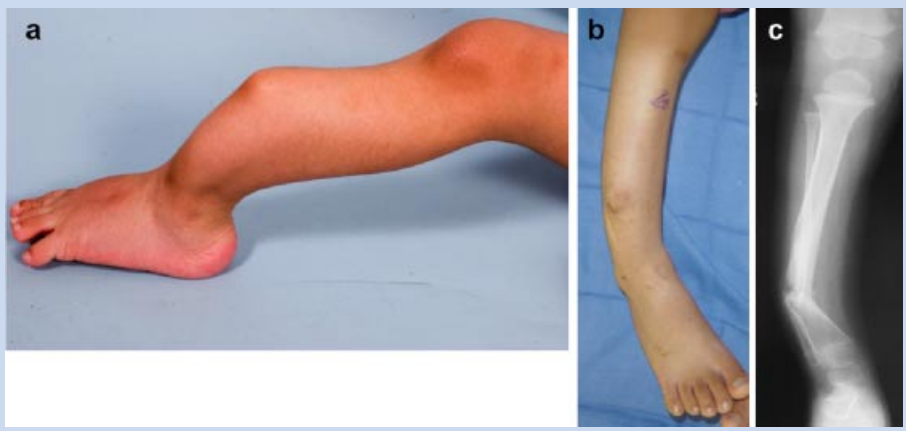

FIG. 1. a: Anterolateral bowing of the lower leg of a child with NF1 prior to fracture. b: Photograph of the leg of a child with NF1 with tibial pseudarthrosis. c: Radiograph of the affected leg pictured in (b).

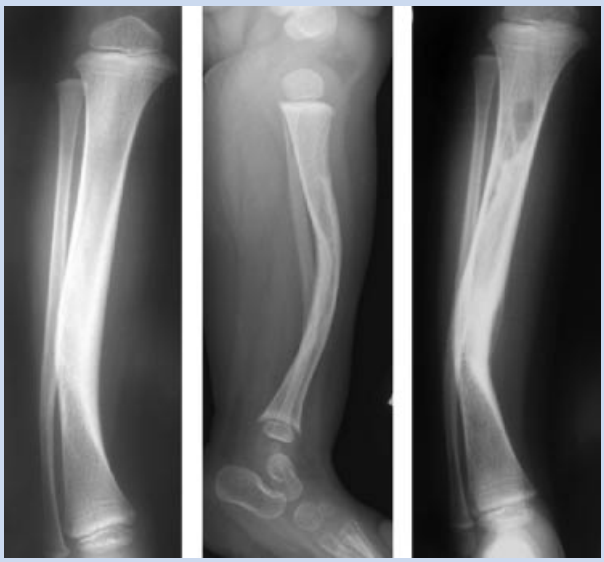

FIG. 2. Radiographs of the lower limb of individuals with NF1 with anterolateral bowing of the leg showing cortical thickening near the apex of the bowing with narrowing of the medullary canals.

Schorry, 1999; Hefti et al., 2000] though several types may represent changes over time.

The bowed long bone frequently sustains fracture (Fig. 3) usually before age 3 years, often with minimal trauma, leading to subsequent non-union or "pseudarthrosis." Histologically, the fibrous pseudarthrosis tissue seen at the fracture site is not a neurofibroma; rather it is a fibrous overgrowth of unspecified cell origin.

Assessments and interventions. The current standard for treatment of long bone bowing in children is bracing to prevent fracture [Bara et al., 2007; Ofluoglu et al., 2008]. Although evaluations of brace type, duration of use, or long-term benefits have not been published, the majority of members of this Consortium advocated early bracing until the child achieves maturity. However, in some cases bracing should continue into adulthood [Crawford and Schorry, 2006]. The Consortium orthopedists recommended routine bracing of the dysplastic long bone upon diagnosis of bowing and agreed that prophylactic surgery should be avoided.

Treatment for long bone pseudarthrosis is often unsatisfactory, requiring multiple surgeries or ultimately amputation [Coleman and Coleman, 1994; Stevenson et al., 1999; Traub et al., 1999; Wientroub and Grill, 2000]. The Consortium members thought that bracing after fracture should continue, delaying surgery until mid-childhood (≈5–8 years of age). Complications of long bone pseudarthrosis include residual angular deformity, ankle stiffness, limb length discrepancy, refracture, and chronic pain. Most surgeons resect the pseudarthrotic region and perform bone bridging and fixation via intramedullary stabilization devices, free vascularized fibular grafting (contralateral or ipsilateral), or external fixation (e.g., Ilizarov technique), either alone or in combination with transankle fixation [Johnson et al., 1990; Friedlaender et al., 2001; Vander Have et al., 2008]. Attempts have been made to promote bone healing with electrical stimulation, varying periods of postoperative immobilization, supplemental bone grafting, and application of bone morphogenetic proteins and stem cells [Johnson et al., 1990; Friedlaender et al., 2001; Kitoh et al., 2004; Vander Have et al., 2008].

Several series of surgical outcomes have been reported [von Satzger and Herbst, 1981; Murray and Lovell, 1982; Anderson et al., 1992; Coleman and Coleman, 1994; Gilbert and Brockman, 1995; Traub et al., 1999; Grill et al., 2000; Romanus et al., 2000; Tudisco et al., 2000; Wientroub and Grill, 2000; Dobbs et al., 2004;

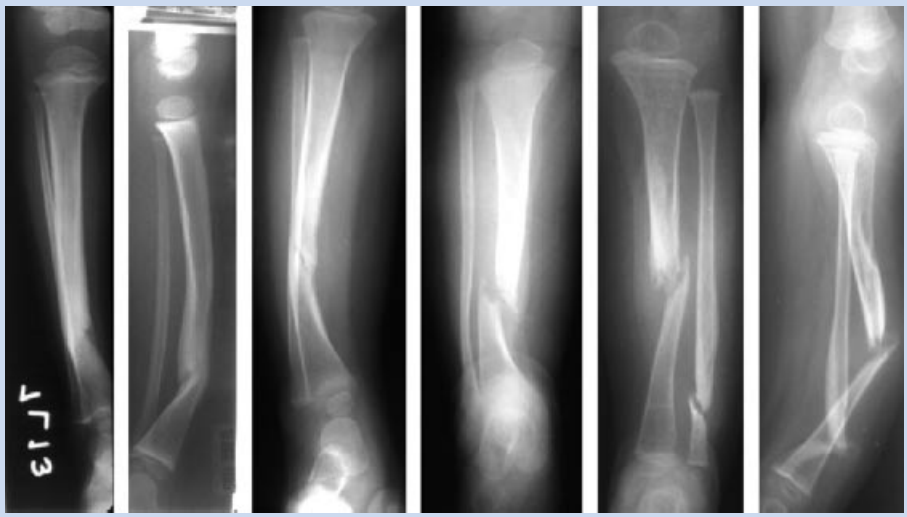

FIG. 3. Radiographic examples showing different stages of fracture and pseudarthrosis of dysplastic long bones in individuals with NF1.

Ohnishi et al., 2005; Bara et al., 2007]; however, the number of patients is usually small and there is no consensus regarding the treatment for pseudarthrosis. Comparison of studies is difficult because of limited follow up, age differences, lack of designation of NF1, severity of the dysplasia, number of surgeries, and use of ancillary treatments.

Overall, tibial dysplasia with pseudarthrosis is a challenging NF1 skeletal manifestation. Our knowledge of its natural history and pathogenesis is limited, and there is a lack of consensus regarding therapeutic approaches. The Consortium participants agreed that clinical trials should assess the outcome of continued bracing after fracture in comparison to early surgery, or the effects of pharmacologic augmentation at the time of surgery on time to healing and potential re-fracture. More data from preclinical drug trials are needed to guide selection of therapeutic agents and drug delivery methods. It is likely a combination of pharmacologic and non-pharmacologic approaches will be advantageous to provide optimal fracture healing in dysplastic tibiae.

Vertebral Defects

Ten to 33% of children with NF1 have vertebral deformity and 2% of children with scoliosis have NF1 [Vitale et al., 2002]. Two types of spinal curvature occur in NF1: dystrophic and non-dystrophic (Fig. 4a,b). The term dystrophic has been utilized in the orthopedic literature to describe a dysplastic type of scoliosis with a rapid course of progression [Durrani et al., 2000]. Although no diagnostic criteria for dystrophic scoliosis exist, Durrani et al. [2000] described nine specific radiographic features associated with dystrophic scoliosis (rib penciling, vertebral rotation, posterior vertebral scalloping, anterior vertebral wedging, lateral vertebral scalloping, vertebral wedging in either the sagittal or coronal plane, spindling of the transverse process, widened interpedicular distance, and enlarged intervertebral foramina). Non-dystrophic scoliosis is more common [Vitale et al., 2002] and resembles idiopathic adolescent scoliosis in the NF1-unaffected population. In rare instances, non-dystrophic scoliosis can progress to the dystrophic form. Dystrophic scoliosis usually presents in the preadolescent child with sharp angulation over a short segment of the spine. It is potentially debilitating and may rapidly progress to neurological impairment. Distinctive radiographic features of dystrophic scoliosis include a short-segment sharply angulated curve (involving four to six vertebrae), scalloping of vertebral margins, vertebral wedging, spinal canal widening, defective pedicles, and rib-penciling [Crawford et al., 2007].

Dystrophic scoliosis is frequently associated with paraspinal or other internal neurofibromas adjacent to the vertebrae [Khong et al., 2003; Ramachandran et al., 2004]. Tumors have been observed via MRI in 69% of patients with NF1 and dystrophic scoliosis (E. Schorry and A. Crawford, unpublished results) with a higher incidence in those with short-segment sharp curves versus non-dystrophic curves (77% vs. 24%). Alwan et al. [2005] hypothesized that vertebral bone in NF1 responds differently to the presence of a paraspinal tumor; however, there are no prospective data regarding the relationship of paraspinal neurofibromas to dystrophic scoliosis. If the adjacent neurofibromas were to contribute to the development and progression of dystrophic scoliosis, then therapies to reduce or stabilize the tumors could provide a promising approach to scoliosis prevention. It would be useful to collect data on vertebral abnormalities and scoliosis in patients enrolled in clinical trials for the treatment of plexiform neurofibromas. These data should include plain radiographs of the spine to assess degree of curvature, vertebral scalloping, vertebral wedging, defective pedicles, rib-penciling, and progression of these features; biochemical markers of bone resorption; and lumbar BMD.

Dural ectasia, defined as widening of the dural sac surrounding the spinal cord, can occur in patients with NF1 and dystrophic scoliosis (Fig. 4d) [Schonauer et al., 2000; Tubbs and Oakes, 2002; Khong et al., 2003]. It is not known if this is a primary malformation or secondary to the vertebral abnormalities. Dural ectasia may be a primary mesodermal dysplasia of the meninges [Casselman and Mandell, 1979] or an expansion of dural tissue into new space created by vertebral body bone loss. The vertebral column can dislocate or erode, causing rib dislocation into the spinal canal, resulting in spinal cord injury. Kyphoscoliosis is another NF1

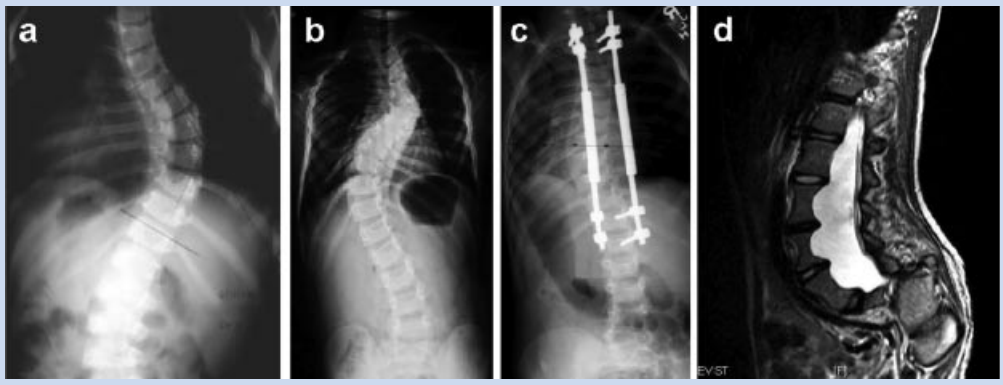

FIG. 4. Imaging of the spine of NF1 individuals with (a) non-dystrophic scoliosis, (b) early onset dystrophic scoliosis, (c) scoliosis treated with growing rods, and (d) dural ectasia and pseudomeningoceles.

FIG. 5. Pectus excavatum in a 6-year-old boy with NF1.

skeletal abnormality, in which vertebral bodies may be so severely deformed as to resemble malformations. Weakening of spinal stabilizers (i.e., facets, pedicles, and ligaments), perhaps by dural ectasia with meningocele formation, may lead to kyphosis.

Assessments and interventions. If a child is skeletally immature and the spine curvature measures ≈25–45°, bracing is typically used, while surgery is commonly employed if the curvature progresses to >45° before maturity or 55° after maturity (Fig. 4c). Skeletally immature patients with dystrophic scoliosis with a curvature >30° typically require a unique management approach due to progression. Imaging should look carefully for paravertebral tumors and dystrophic changes that may be missed on plain radiographs. Presurgical characterization is critical as the lamina may be thin, the canal affected by dural ectasia or intraspinal tumors, and a rib may have displaced into the spinal canal, all of which have an increased risk of poor post-surgical outcome.

Surgical treatment with fusion and growing rods is complex. Variables such as age, gender, associated neurofibromas, location and degree of the curve, and associated radiographic dystrophic features make trial design difficult. Poor understanding of the natural history of dystrophic scoliosis and a lack of animal models additionally hinder progress. We do not yet understand the pathogenesis of NF1 dystrophic scoliosis and there are no clear pharmacologic adjunctive options. If effective therapy for neurofibromas is found, secondary effects may improve dystrophic scoliosis. The occurrence of vertebral pseudarthrosis after surgery [Kim and Weinstein, 1997] suggests intrinsic abnormalities of the vertebral bony matrix in NF1. If focal decreases in BMD of specific vertebrae cause microfractures and vertebral wedging with subsequent development of scoliosis, then pharmacologic agents to increase vertebral strength may be appropriate.

There is no effective treatment for NF1-related dural ectasia and investigation of the natural history, frequency, and pathophysiology is needed.

Chest Wall Deformities

Chest wall deformities in patients with NF1 are incompletely understood but thought to be present in as many as 50% of patients [Riccardi, 1999] (Fig. 5). The relationship of chest wall deformities to scoliosis in patients with NF1 is not clear. The chest wall deformities are principally cosmetic; surgical treatment is rarely required.

Sphenoid Wing Dysplasia

Cranial defects in patients with NF1 usually involve the sphenoid wing (Fig. 6). In a cohort of 256 probands with NF1, 11% had a dysplastic sphenoid wing [Friedman and Birch, 1997]. Most cranial defects are associated with plexiform neurofibromas [Jacquemin et al., 2002, 2003], often with infiltration and decalcification of cranial bones adjacent to tumors. Other lesions associated with sphenoid wing defects include arachnoid cysts, dural ectasia, or buphthalmos. Sphenoid wing dysplasia is thought to be a secondary response of bone to the adjacent soft tissue abnormality. However, two observations suggest the existence of a bone cell-autonomous defect and may help elucidate the etiology of the dysplastic sphenoid wing: NF1 sphenoid wing lesions have been associated with tibial and vertebral dysplasia [Alwan et al., 2007], and formation of this skull structure proceeds through endochondral bone formation, which is defective in NF1 [Kolanczyk et al., 2007]. As a

FIG. 6. Neuroimaging of an individual with NF1 with left sphenoid wing dysplasia. The arrowheads indicate the dysplastic left sphenoid remnant. For comparison, the black arrow marks the normal appearing contralateral sphenoid bone. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

congenital malformation, or as a secondary bony defect sphenoid wing dysplasia is not a primary target for therapeutic prevention. It may be more promising to first apply sensitive imaging techniques to screen patients with sphenoid wing dysplasia for adjacent tumors, which may be amenable to therapy.

Other Skeletal Manifestations of NF1

Other rarer bone abnormalities are observed in patients with NF1. Cystic osseous lesions, often identified incidentally during radiographic knee exam, are occasionally seen in the absence of tumors or long bone dysplasia [Colby and Saul, 2003; Lee and Cho, 2006]. The lesions rarely fracture or show progressive deformity, and biopsy generally shows non-ossifying fibromas. Dental abnormalities, including increased caries and early primary tooth eruption, and periapical cemental dysplasia have been reported in patients with NF1 [Lammert et al., 2007; Tucker et al., 2007; Visnapuu et al., 2007]. Periapical cemental dysplasia may be confused with chronic inflammation on radiographic analysis and precipitate unnecessary dental procedures. More information regarding NF1 dental abnormalities is needed.

MOUSE MODELS AND PATHOPHYSIOLOGY OF NF1

Difficulties in understanding the human pathophysiology of NF1 skeletal defects led to the development of mouse models to determine the role of *Nf1* in bone cells and facilitate preclinical studies. *Nf1* mRNA and neurofibromin are expressed in mouse bone and cartilage during development and adulthood [Kuorilehto et al., 2004a], and more specifically in mesenchymal stem cells, chondrocytes, osteoblasts [Elefteriou et al., 2006; Kolanczyk et al., 2007], and osteoclasts [Yang et al., 2006a]. This pattern of expression suggested that NF1-related skeletal defects stem in part from primary osseous defects caused by bone cellular dysfunctions related to generalized *NF1* heterozygosity, and/or to *NF1* loss of function in specific bone cell types.

The early embryonic lethality of $Nf1^{-/-}$ embryos precluded skeletal analyses [Brannan et al., 1994; Jacks et al., 1994; Lakkis and Epstein, 1998] and the first NF1 mouse bone study used $Nf1^{+/-}$ mice. $Nf1^{+/-}$ osteoprogenitors and osteoblasts had defects in proliferation and differentiation in vitro and constitutive activation of Ras and Erk signaling, as observed in $Nf1^{+/-}$ Schwann cells [Yu et al., 2005]. *Nf1* haploinsufficiency affected the monocytic lineage as well, based on the observation that $Nf1^{+/-}$osteoclast precursors undergo faster differentiation than wild-type (WT) counterparts in vitro, due to activation of a Ras-AKT-PI3K pathway. Rho-GTPaseRac 1 is a crucial Ras-mediated effector in $Nf1^{+/-}$ osteoclasts [Yan et al., 2008] and is a potential therapeutic target.

Despite the cellular in vitro phenotypes of $Nf1^{+/-}$ osteoblasts [Yu et al., 2005] and osteoclasts [Yang et al., 2006b], no bone mass phenotype nor focal bone dysplasia were seen in $Nf1^{+/-}$ mice, which suggests that *NF1* heterozygosity alone is insufficient to explain tibial or vertebral dysplasia in NF1 patients; this is supported by the low incidence and focal nature of bony dysplasias among these patients. An attractive theory is somatic inactivation of the normal *NF1* allele in a finite set of cells from the affected tissues. This is supported by the demonstration of double inactivation of *NF1* in tissue harvested from pseudarthrosis sites in some, but not all, patients with NF1 [Stevenson et al., 2006; Sakamoto et al., 2007]. A bi-allelic deficiency of *Nf1* in committed osteoblasts, generated using transgenic mice expressing Cre-recombinase under the control of the osteoblast-specific 2.3 kb collagen type 1 promoter ($Nf1^{-/-}_{ob}$ mice), produced mice with no patterning or size defects, but with high bone turn-over [Elefteriou et al., 2006]. The bones of $Nf1^{-/-}_{ob}$ mice exhibited increased collagen synthesis and delayed mineralization, causing a prominent increase in osteoid surfaces similar to what is observed in human NF1 bone histological sections [Elefteriou et al., 2006; Brunetti-Pierri et al., 2008; Seitz et al., 2009] (Fig. 7). The bones of $Nf1^{-/-}_{ob}$ mice also exhibited increased osteoclast numbers and bone resorption similar to humans with NF1 [Stevenson et al., 2008]. These in vivo studies indicated that neurofibromin in osteoblasts inhibits collagen synthesis, promotes mineralization, and also regulates osteoclastogenesis by limiting the expression of receptor activator for nuclear factor κ B ligand (RANKL), the major osteoblast-derived cytokine promoting the formation of mature osteoclasts. Constitutive activation of Ras, Erk, Rsk2, and the transcription factor ATF4 was identified as major regulator of increased collagen synthesis and osteoclastogenesis in response to *Nf1* deficiency [Elefteriou et al., 2006] (Fig. 8).

Another mouse model, the $Nf1^{Prx-/-}$ model, deleted both alleles of *Nf1* in mesenchymal cells and their derivatives (osteoblasts, adipocytes, chondrocytes, muscle cells, and endothelial cells of the skullcap and developing limb bud mesenchyme). These mice had growth retardation and grossly abnormal, stunted limbs [Kolanczyk et al., 2007], including bowing of the tibia similar to that seen in NF1 patients. Increased porosity and decreased calcium content was proposed as the cause of bowing. The $Nf1^{Prx-/-}$ mouse is characterized by delayed osteoblast differentiation, which was not observed in the $Nf1^{-/-}_{ob}$ mouse due to the late recombination event

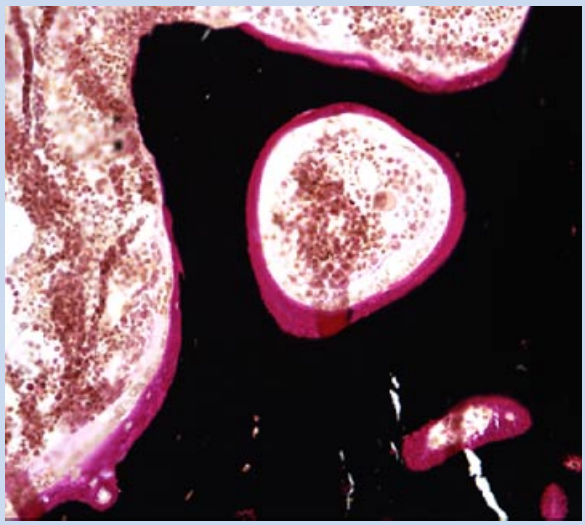

FIG. 7. Presence of non-mineralized collagen (osteoid, pink) at a site of NF1 bone non-union. Undecalcified sections were stained by van Gieson/von Kossa (400×). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

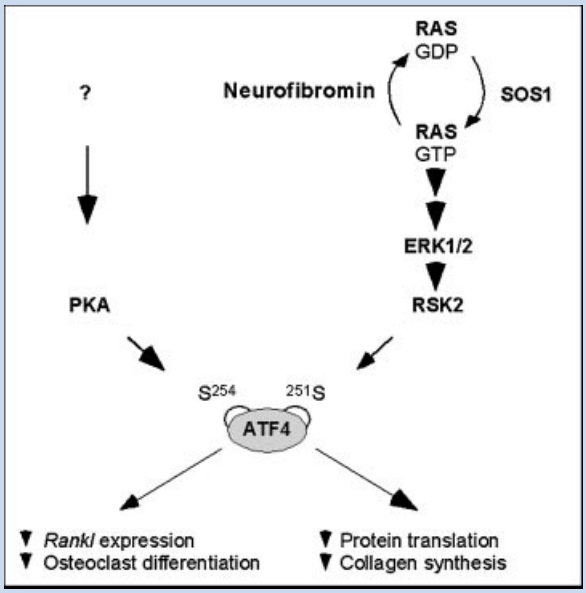

FIG. 8. Signaling pathway engaged by neurofibromin in mature osteoblasts. Neurofibromin negatively regulates collagen synthesis via its control of RAS, ERK1/2, RSK2, and ATF4 activation. It also negatively regulates osteoclastogenesis via a less defined pathway involving PKA, ATF4, and RANKL.

driven by the 2.3 kb collagen type 1 promoter. Other differences with the $Nf1_{ob}^{-/-}$ model are normal *Rankl* expression and an increase in blood vessel invasion of bone cortex. It is not clear if such a blood vessel invasion defect is caused by loss of *Nf1* in endothelial or bone-forming cells. On the other hand, the $Nf1^{Prx-/-}$ mice recapitulate some features of $Nf1_{ob}^{-/-}$ mice, such as an increase in osteoid surfaces and increased number of osteoclasts. These similarities could be explained by the finding that although the differentiation of osteoblasts from $Nf1^{Prx-/-}$ mice is impaired in vitro, $Nf1^{Prx-/-}$ mice generate $Nf1^{-/-}$ mature osteoblasts that function like $Nf1^{-/-}$ osteoblasts from $Nf1_{ob}^{-/-}$ mice. As observed in osteoblasts from $Nf1_{ob}^{-/-}$ and $Nf1^{+/-}$ mice, osteoblasts and osteoprogenitor cells from the $Nf1^{Prx-/-}$ mice display constitutive Ras and Erk activation. In comparing the $Nf1^{Prx-/-}$ and the $Nf1_{ob}^{-/-}$ models, constitutive Erk signaling appears to regulate different functions in osteoblast progenitors (inhibiting differentiation) versus mature osteoblasts (promoting collagen synthesis and delaying mineralization). Thus far, the $Nf1^{Prx-/-}$ mouse model most closely recapitulates human NF1-related bone abnormalities. These mice also had delayed cortical bone injury repair making them a good model for preclinical studies related to bone healing [Kolanczyk et al., 2008]. Table I summarizes the major characteristics of the three published mouse models.

Despite similarities with the human condition, no mouse model fully models the human skeletal manifestations, in part due to the limitations of the genetic manipulations. In the $Nf1_{ob}^{-/-}$ and $Nf1^{Prx-/-}$ mice, virtually all mature osteoblasts lack both copies of *Nf1*, while in NF1 patients, most osteoblasts are heterozygous for *NF1* and only a fraction of osteoblasts and osteoprogenitors, likely limited to the localized dysplastic sites, may have complete inactivation. In the $Nf1_{ob}^{-/-}$ mouse, osteoprogenitors have both normal *Nf1* alleles, and thus this model does not account for the role of *Nf1* in early osteoblast differentiation. In $Nf1^{Prx-/-}$ mice, vertebral abnormalities seen in NF1 patients are not observed because Cre -recombination is limited to the appendicular skeleton. These models also lack the $Nf1^{+/-}$ microenvironment that underlies various NF1 phenotypes. Although these mouse models have advanced our understanding of *Nf1* functions in bone cells, more sophisticated models and methods are needed, not only to trigger *Nf1* loss-of-function in specific bone cell types, but also to affect specific skeletal elements at various developmental stages.

Despite these limitations, *Nf1*-deficient mice are highly valuable in preclinical testing of candidate therapies for NF1 skeletal defects. Initial preclinical studies using these mice reported delayed bone healing in $Nf1^{+/-}$ mice upon distal tibia fracture [Schindeler et al., 2008a]. While $Nf1^{+/-}$ mice do not replicate the bowing and spontaneous fractures seen in patients with NF1 and tibial dysplasia, they indicate that *Nf1* haploinsufficiency is sufficient to model defective NF1 bone repair. This result also suggests that fracture healing might be impaired in non-dysplastic bones in patients with NF1. The cell types and molecular mediators that contribute to poor $Nf1^{+/-}$ healing remain to be fully characterized.

Various studies have been initiated in preclinical mouse models to assess the potential efficacy of selected drugs on bone formation, repair and remodeling. A recent study demonstrated that the combination of bisphosphonates and bone morphogenetic protein 2 (BMP2, a protein which induces bone and cartilage formation) improved net bone production by $Nf1^{+/-}$ cells in an in vivo model of heterotopic bone formation [Schindeler et al., 2008b]. This treatment did not attempt to correct the specific signaling defects associated with $Nf1^{-/-}$ or $^{+/-}$ osteoblasts, but represented an initial approach. Bisphosphonates are currently approved for other applications, so they could transition rapidly to NF1 clinical trials. Kolanczyk et al. [2008] recently reported that the drug lovastatin improves cortical bone injury healing defects observed in the $Nf1^{Prx-/-}$ mice.

SUMMARY

Following the meeting, the NF1 Bone Consortium committed to establishing a clinical and research network to collaborate in the development of preclinical studies and clinical trials in order to identify effective management strategies and therapeutic approaches for NF1 skeletal abnormalities. Key issues from this literature review and discussion from the multidisciplinary NF1 Bone Consortium meeting were identified.

The key issues that present barriers to optimal clinical management of NF1 skeletal abnormalities are the following:

(1) NF1 is a multifaceted, polysystemic disease. A number of skeletal abnormalities are highly morbid with a natural history distinct from that of the general population. Some manifestations, such as dystrophic scoliosis, can lead to clinically significant consequences if neglected.

TABLE I. Major Characteristics of the *Nf1* Mouse Models

	$Nf1^{+/-}$ [Yu et al., 2005]	$Nf1_{ob}^{-/-}$ [Elefteriou et al., 2006]	$Nf1^{Prx-/-}$ [Kolanczyk et al., 2007]
Loss of *Nf1* activity	Whole body	Mature osteoblasts	Limb mesenchymal osteoprogenitors and progeny (chondrocytes, osteoblasts, endothelial cells, adipocytes, muscles)
Genetic setting	+/−	−/−	−/−
Osteoblast differentiation	Decreased	Normal	Decreased
Collagen synthesis	ND	Increased	ND
Mineralization	ND	Delayed	ND
Osteoclast differentiation	Increased (following ovariectomy)	Increased (osteoblast-dependent)	Increased (mechanism ND)
Bone quality	ND	Decreased crystalinity	Increased cortical porosity Decreased calcium content
Mechanical properties	Decreased stiffness	Decreased stiffness	ND
Parallels with human NF1 skeletal phenotype	Increased bone resorption Delayed bone healing	Increased bone resorption Delayed bone healing Increased osteoidosis	Increased bone resorption Tibial bowing Low bone density Delayed bone healing Increased osteoidosis

ND, not determined.

(2) Consensus guidelines for the treatment of the specific orthopedic manifestations in patients with NF1 do not exist and clinical management practices for each NF1 skeletal abnormality are highly variable.
(3) Limited knowledge is available about the pathophysiology of NF1 skeletal abnormalities. This presents considerable limitations in determining how to approach the design and conduct of clinical trials for NF1 skeletal abnormalities. A better understanding of the natural history and the development of new therapies and long-term orthopedic management will be essential to improve patient care.
(4) The cost of clinical trials, limited available funding for orphan disorder trials, lack of animal models recapitulating the skeletal phenotypes, limited data from preclinical studies, lack of availability of candidate therapeutic interventions with few side effects, potential poor patient compliance with protocols due to co-morbidities and lack of and difficulty in identifying available outcome measures and endpoints were identified as current barriers to successful skeletal abnormalities clinical trials in patients with NF1.

The following concepts will direct future research plans to help advance optimal clinical management for NF1 skeletal abnormalities:

(1) In patients with NF1, the morbidity associated with either dystrophic scoliosis or tibial dysplasia is much greater than that of short stature or macrocephaly. This helps define priorities for future trials.
(2) In patients with NF1, findings such as low BMD and short stature reflect a generalized alteration of bone. The relationship of this generalized skeletal condition to the development and progression of characteristic focal skeletal abnormalities like tibial dysplasia and dystrophic scoliosis is not clear.
(3) Prospective studies to determine the relationship of spinal neurofibromas in patients with dystrophic scoliosis are needed to determine if early treatment of spinal neurofibromas could prevent dystrophic scoliosis. It will be beneficial to collect data on bone and scoliosis in clinical trials treating neurofibromas.
(4) Available mouse models recapitulate some, but not all, of the bone abnormalities in patients with NF1. Mouse data helped clarify that *NF1* haploinsufficiency is likely related to the generalized NF1 bone remodeling defects, whereas total loss of *NF1* function is likely related to the focal (dysplastic) defects. Identifying neurofibromin cellular functions, target genes and downstream signaling pathways remains a priority to understand the etiology of the NF1 skeletal manifestations.
(5) Ras/Erk constitutive activation occurs in *Nf1*-deficient osteoblasts (as in Schwann cells), and inhibition of Ras/Erk signaling by lovastatin in an NF1 mouse model improves bone healing defects.

The following are examples of feasible areas to start the development of NF1 skeletal abnormalities clinical trials based on our current understanding of bone disease in NF1:

(1) The prevention of fracture during initial presentation of long bone bowing would likely improve quality of life. Therefore, trials investigating early and prolonged bracing to prevent fracture in patients with long bone bowing, including trials utilizing different types of braces, would be beneficial.
(2) Anecdotally, early surgical intervention in children with NF1 and tibial pseudarthrosis results in poorer outcomes compared to later surgical intervention. Bracing for tibial dysplasia after fracture may be a preferred strategy to delay surgery and optimize the chance of positive outcomes. Bracing studies after fracture are of high priority (e.g., early surgery vs. bracing and later surgery). Agreement on the standardization of bracing protocols among surgeons will be difficult, but is required for future trials focused on tibial dysplasia.
(3) Some patients with NF1 have decreased BMD, and defects in vitamin D metabolism, osteoclastogenesis or bone cell response to systemic signals regulating bone remodeling are likely involved. However, an increased incidence of fractures has not yet been firmly established. Based on human data and healing defects observed in $Nf1^{+/-}$ mice, studies investigating fractures are needed in individuals with NF1 to determine whether fracture healing defects are observed in dysplastic bones only or generally in the NF1 skeleton. Currently, except for adult cases with unequivocal osteoporosis, the consensus among NF1 Bone Consortium participants was not to intervene for mild to moderate decreases in BMD. A clinical trial of the efficacy of standard treatment for osteoporosis in adults with NF1 with documented osteoporosis is recommended.
(4) Use of locally applied biological mediators (e.g., bone morphogenetic protein) at the time of surgery in patients with pseudarthrosis is an attractive option in order to avoid complications of systemic administration of pharmacologic agents. Based on data from the animal models, a variety of cell types and signaling pathways are likely to be involved in NF1 patients with bone manifestations. Therefore, combination therapies, using both anabolic and anti-catabolic medications, will likely give optimal results.

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