The Cognitive Dysfunction and Skeletal Dysplasias of NF1: Are We Ready for Clinical Trials?

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Genetics and Wiley Bicentenary: A Brief Look Backwards, or the Origin of Mendelism from Morphology, and Present and Future Perspectives

“...increasing role in the application of new treatment regimes for...the development of clinical trials.”

Opitz and Carey, 2007. AJMG

Neurofibromatosis Type 1

- Autosomal Dominant Condition
- Approximately 1/3500 people worldwide
- VonRecklinghausen Disease
- Peripheral NF
- Defining Features
  - Cafe-au-lait spots
  - Neurofibromas
  - Lisch nodules
- Distinct from Neurofibromatosis Type 2
The Neurofibromatoses

Neurofibromatosis type 1 (NF1)
- Whole-gene-deletion phenotype (NF1 microdeletion)
- Alternate forms of NF1 (conditions with incomplete/atypical features)
  - Mixed NF
  - Localized NF
    - Segmental NF
    - Gastrointestinal NF
    - Familial spinal NF
    - Familial café-au-lait spots
- Related forms of NF1 (conditions with additional features)
  - NF/Noonan syndrome
  - Watson syndrome

Brems et al., Nat Gen 2007

The Neurofibromatoses

Neurofibromatosis type 1 “like” condition
- CAL spots, freckling - meets NF1 criteria
- Germ line loss of function mutations of SPRED1 (SPROUTY family)

The Neurofibromatoses

Neurofibromatosis type 2 (NF2)
- Alternate form of NF2
  - Schwannomatosis
- Related forms of NF2
  - Schwannomas/vaginal leiomyomas

AJMG Seminars 1999
Diagnostic Criteria for NF1
NIH, 1987: Two of 7
• Multiple café-au-lait spots
• Crowe’s sign: intertriginous freckling pattern
• Lisch nodules of the irides
• Neurofibromas
• Optic pathway tumors
• Skeletal dysplasias
• Family history - first-degree relative

Multiple Café-au-lait Spots
• Most often the first sign
• 80% have >5 by age 1
• No different than “birthmarks”
• Typical pattern of distribution over the flanks and thorax

Crowe’s Sign
• Distinctive freckling
  - Axillae
  - Groin
  - Neck
  - Second sign in ~3/4
• Tend to arise by age 8
• Background lentigines
Optic Pathway Tumor
- Approximately 15%
- Half are symptomatic
- Less aggressive if associated with NF1
- Ages 1 to 4 years
- Astrocyte overgrowth

Neurofibromas
- Dermal (2 or more)
- Arise in early puberty
- Flanks and thorax
- Progressive with age
- Multicellular

Plexiform Neurofibromas
**Lisch Nodules**
- Iris hamartoma
- Not iris freckles, nevus
- Usually bilateral
- Age-dependent
  - Over 90% of cases
  - Present by age 6

**NF1 Gene Discovery**
- Genetically mapped by linkage to 17q11.2
- Balanced translocations identified (1p, 22q)
- Cloned and physically mapped
- Mutations identified
- Full-length cDNA sequenced
- Genomic structure determined
- Alternative splice forms characterized

**NF1 Gene Structure**
- Spans approximately 350-kb of genome
- 60 exons with 3 alternatively spliced exons
- cDNA is 11-13 kb with a 3.5-kb 3’-UTR
- Encodes a peptide of 2818 amino acids
- Housekeeping gene promoter sequence
- 3 expressed genes embedded in intron 27a
- Alu, L1 and AK3 pseudogene insertions
Signposts of the NF1 gene

- GAP-Related Domain
  - Stimulates the intrinsic GTPase of RAS
  - Encoded by exons 21 to 27a
  - 30% homology to p120GAP catalytic domain
  - N-terminus associates with microtubules
  - Known function of neurofibromin

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Cardio-facio-cutaneous syndrome (CFC)
- ERAS, SHP2, MAPK (1/2)
Neurofibromatosis type 1 (NF1)
- [Neurofibromatosis]
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Pleiotropy in NF1

- NF1: a syndrome
- Multiple organ system involvement
- Constitutional growth abnormalities
  - Short stature, macrocephaly
- Propensity for hamatomas, dysplasia, cancer
- Cognitive dysfunction
- Distinctive skeletal manifestations

Clinical Trials: NF1

- Tibial dysplasia
- Learning disabilities/cognitive defect/ADHD

Are We Ready?

Distinctive Osseous Manifestations of NF1

- Scoliosis
  - Dystrophic
  - Typical
- Long bone dysplasias, including pseudarthrosis
- Sphenoid wing dysplasia
- Bone cyst
**Tibial Dysplasia in NF1**

- A localized dysplasia
- One of items of NIH criteria
- Occurs in 2 - 5% of patients with NF1
- Pseudarthrosis end of spectrum: 2%
- Biologically intriguing/medically - surgically challenging, limited knowledge of natural history

**Tibial Dysplasia in NF1**

- Terminology/definition: 
  Tibial dysplasia: disorder of tibia, usually lower, unilateral (patchy); comprises anterolateral bowing (occasionally congenital), fracture and non-union (pseudarthrosis)

**Tibial Dysplasia (TD): General**

- Onset infancy/early childhood (10-20% congenital)
- Prevalence: 1/150,000 - 1/250,000 children
- Primary tibial; rarely bilateral, rarely familial (even in NF1)
Recent Landmarks In The Study of TD In NF1

- Establishment of NNFF Database - 1993
- Stevenson et al., study, 1999
- EPOS multicenter study - 2000, *J Ped Orthoped*
- NNFF Task Force on Orthopedic Manifestations of NF1- Jan 2000, Salt Lake City

Long Bone Dysplasia

- Presents with anterolateral bowing leading to fracture and non-union (pseudarthrosis)
- Unilateral presentation suggests random molecular event

NNFF DB Natural History Study of TD

N = 85
- Male predominance (54 M; 31 F)
- 66% recognized by 1 year
- Median age of fx 2 years (range 0 - 28yr)
- Median # of surgeries 2 (range 0 - 13)
- 16% with amputation
- 43% with fibular dysplasia
Long Bone Dysplasia Natural History

European Pediatric Orthopedic Study (EPOS) - 2000
Multicenter Study of Pseudarthrosis

- Six papers published (340 patients; f/u on 172)
- 54.7% with NF1
- Obtaining union lowest in children under 3 years
- Union achieved in 75%

Classification Schemes of Tibial Dysplasia

Most widely used in past: Andersen, Boyd

Andersen:
- I Congenital
- Dysplastic
- Cystic
- Late
- Club foot

Boyd:
- II Dysplastic / hourglass constrict
- III Cystic
- IV Sclerotic
- V With dysplastic fibula
- VI With introsseous neurofibroma

Crawford Classification

![Image of Crawford Classification]
Multicenter Study of Natural History of TD/NF: Shriners

- Case-control study of TD/NF
- Adult, fully mature patients, TD/NF
- Registry of NF1/TD / TD alone
- Neurofibromatosis Orthopedic Core Facility (NOCF) - tissue samples

Double Inactivation of NF1 in PA Tissue Study

Patient #1
- 42 yr old man with NF1
- Anterolateral bowing right leg at birth
- Tibia fx (at 1 yr); tibia-fibula syndesmosis - union achieved
- Re-fracture at 41 yr (pseudarthrosis 8 mo later)
- Pseudarthrosis resected (partial healing 6 mo after surgery)

Patient #2

*Stevenson et al., 2006 Am J Hum Genet*
**Patient #1**

- Cellular “fibromatosis-like” tissue between bone ends of fracture gaps
- Lacked bony callus seen in normal fracture healing
- Very minimal endochondral ossification
- No distinctive appearance

**Patient #2**

- Double Inactivation of *NF1* in Pseudarthrosis Tissue
  - Constitutional mutation: nonsense mutation in exon 45 (c.7846C>T; p.R2616X)
  - Mutant allele retained in pseudarthrosis tissue—double inactivation of *NF1*
  - LOH in tibial pseudarthrosis tissue

**Radial and Ulnar Dysplasia**
Do we need more Natural History Studies for TD in NF1?

Are we ready for clinical trials?

Lines of Evidence Suggesting An Intrinsic Skeletal Dysplasia of NF1

- Distinctive osseous manifestations
  TD, sharp angle dystrophic scoliosis

- Decreased bone density - three recent studies in children
  Stevenson et al., 2007

- Signs of vitamin D/calcium dysregulation

- Increased excretion of markers of bone resorption - crosslinks
  Stevenson et al., 2008

- Distinctive osteoblasts of NF1

- Animal models
**NF1 Mouse Models and Skeletal Development**

- Australian group – Schindeler/Little
  - NF1 +/- mice treatment with BMP-2/bisphosphonates
- Indiana group – Yang/Clapp
  - NF1 +/- mice with novel osteoclasto phenotype
- Berlin group – Kolanczyk/Mundlos
  - continued inactivate model recipitulate TD

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**Potential Variables Affecting Outcome in TD**

- Presence of NF1
- Male gender
- Age of onset
- Number of surgeries
- Neurofibroma at site
- Fibular involvement
- Crawford type
- Individual surgeon
- Type of procedure
- Amputation
- Age at last surgery
TD/NF: Outcome Measures

- Number of surgical procedures
- Union/no reunion – PA
- Range of movement at ankle
- Objective gait analyses
- Valgus foot deformity - presence/absence
- Leg length discrepancy - size in cm
- Use of permanent braces/other devices
- Pain – absence/presence
- Limitation of walking distance
- Health-related QOL, PODCI, FAQ
- Amputation

PODCI

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Possible Trials of TD/NF1

- Bracing trial at diagnosis
- Administration of bisphosphonates after surgery – Little protocol
- Randomized trial of uses of BMPs at surgery
Cognitive Dysfunction and Academic Impairment in NF1

- Occur in 40 - 60% of children
- Associated with behavioral problems, including ADHD
- Deficits in IQ, executive function, attention and motor skills
- No specific profile - altered JOL test
  - Ozinoff, AJMG 1999
  - Hyman, Dev Med Child Neurol 2006
  - Acosta, Curr Neurol Neurosci 2006

Animal models:
- NF1 mouse displays learning deficits - Costa et al., 2002
- Silva's group: Lovastatin inhibits of ras and rescues deficits in mouse

Hypothesis: Persons with NF1 will have improved memory and learning on statins (lovastatin)

NF1 - Lovastatin Trials

- DC Children's - adults - Phase I  N=14
- UCLA Phase I - adults - recruiting
- Proposed trial NF Consortium - Dept. of Defense - 9 centers  N=128
  Double-blind placebo controlled
  Ages 10 - 17 yrs., IQ > 80

ClinicalTrials.org
Clinical Trials: NF1

- Learning disabilities/cognitive defect/ADHD
- Tibial dysplasia

Are We Ready?

Clinical Trials: NF1

- LD/NF1 - Animal models
  - Trials - Recruiting
- TD/NF1 - Three models of bone dysplasia
  - No trials
  - Little preliminary data