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14. ABSTRACT Anterolateral tibial bowing is a morbid skeletal manifestation observed in 5% of children with neurofibromatosis type 1 (NF1), typically identified in infancy. The majority of NF1 individuals with tibial bowing will sustain a fracture that will not heal (i.e. pseudarthrosis) resulting in multiple surgeries, poor limb function, and amputation. Some NF1 individuals with tibial bowing, however, do not fracture and the bowing improves over time. Clinical predictors to help drive management are lacking, and the pathophysiology of tibial bowing and pseudarthrosis is not well understood. Our objective is to identify clinical predictors of tibial pseudarthrosis and better understand its pathophysiology. We have begun recruitment and assessed many individuals with NF1 with and with tibial bowing. QUS measurements and osteolytic activities have been assessed in the individuals recruited to date, but numbers are small and hence statistically significant conclusions cannot be made. However, 7/8 individuals with tibial bowing had decreases in speed-of-sound z-scores in the bowed tibia compared to the affected tibia. In addition, we were able to confirm that bone resorption is increased in NF1.					
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Tibial Bowing and Pseudarthrosis in Neurofibromatosis Type 1

(PI: David Stevenson, MD)

Introduction

Anterolateral tibial bowing is a morbid skeletal manifestation observed in 5% of children with neurofibromatosis type 1 (NF1), typically identified in infancy (Friedman and Birch, 1997). The majority of NF1 individuals with tibial bowing will sustain a fracture that will not heal (i.e. pseudarthrosis) resulting in multiple surgeries, poor limb function, and amputation. Some NF1 individuals with tibial bowing, however, will not fracture and the bowing improves over time (Stevenson et al., 2009). Clinical predictors to help drive management are lacking, and the pathophysiology of tibial bowing and pseudarthrosis is not well understood. Our objective is to identify clinical predictors of tibial pseudarthrosis and better understand its pathophysiology. Our integrative proposal will gain novel information about the pathophysiology of tibial bowing and pseudarthrosis using techniques innovative in their application to NF1 tibial dysplasia. As part of the study we will validate use of an imaging modality for tibial bowing for clinical trials and clinical practice. We will also help in the understanding of osteoclast function in tibial bowing. Additionally we will provide novel information on genetic modifiers and pathophysiology of the skeletal phenotype of NF1. Ultimately, our proposal will help in the development of personalized treatment protocols based on an NF1 individual's quantitative ultrasound measurements, osteolytic activity, and somatic mutation profile.

Body

The following section will describe the research accomplishments associated with each task outlined in the approved Statement of Work in which the time frame of this first annual report pertains. Tasks in the approved Statement of Work that fall outside the time of the first 12 months are not included. The individual tasks are underlined below followed by a description of accomplishments related to the task.

Task 1. Plan Development, Patient Recruitment, and Institutional Review (Months 0-6):

- a. Train a clinical coordinator to identify potential subjects and contact appropriate providers to offer enrollment.

-We have trained Heather Hanson as a clinical coordinator on the project. Heather has met personally with the investigators to discuss the project and critical areas including timing of shipment of blood, consenting, number of participants needed and enrollment criteria.

The clinical coordinator has investigated and made contact with organizations that are likely to have contact with individuals who have neurofibromatosis type 1 and tibial bowing prior to fracture. With her help we have sent out recruitment flyers to NF support groups and orthopedic agencies.

- b. Review current research registries to identify and prioritize individuals for recruitment with primary focus in first 6 months on recruitment of individuals with tibial bowing.

-We have an NF Registry in which individuals with NF1 have been recruited and consented to be contacted for future studies. As part of the NF Registry clinical information is contained in our database. We have searched our NF registries for individuals with long bone bowing and NF1 individuals who could serve as controls who have agreed to be contacted for future research. We have identified these individuals who are potential study participants for future contact. We have focused on those with tibial bowing primarily.

At this point in time we have also focused recruitment on those who are coming to the University of Utah for clinical or research purposes for convenience to the family.

- c. Arrange requests, procedures and transfer of prospectively acquired tissue from the NF1 Orthopedic Core Facility (NOCF) for analysis for Specific Aim 3.

-We have received samples from the NOCF that were located at the Shrine Hospital in SLC and the samples are now at the University of Utah.

- d. Assure compliance with USAMRMC and home institutional guidelines on research involving human subjects.

-This has been done.

Task 2. Data Collection, QUS Imaging, and Molecular Analysis (Months 6-42):

- a. Continue to recruit subjects for all specific aims. A projected 150 individuals with NF1 will be recruited over the course of the 3-year period (35 individuals for Specific Aim 1).

-We have enrolled 8 individuals with NF1 with tibial bowing and we have enrolled 28 individuals with NF1 without tibial bowing.

Examples of the radiographs of a few of the NF1 individuals with tibia bowing who have enrolled are shown in **Fig.1** and document the various anterolateral bowing that is typically seen in individuals with NF1. However, the radiographs also show that there is variability in the radiographic features of each individual with NF1 in terms of the tibial structure. This suggests that not all individuals with NF1 who have tibial dysplasia will have the same bone architecture and may result in varying clinical outcomes.



Figure 1. Examples of radiographs (A-D) of the bowed leg of different individuals with NF1 with tibial dysplasia who have enrolled.

- b. Document findings from physical examinations and medical histories on NF1 exam forms for data entry upon enrollment.

-All individuals were personally examined by Dr. Stevenson and the subjects filled out releases of information to obtain radiographs and medical reports to ensure appropriate diagnosis and categorization. Findings were documented through standardized exam forms and entered into spreadsheets by the research coordinator.

c. Biannual phone interviews with individuals with tibial bowing enrolled in Specific Aim 1.

-We have performed biannual phone interviews for individuals with tibial bowing who have reached their required time for phone interviews. In addition we have informed all subjects and their families to contact us for any fracture or surgical intervention.

To date, none of the individuals have sustained a tibial fracture.

d. Obtain QUS at baseline on all NF1 individuals with anterolateral tibial bowing (Specific Aim 1; N=35).

-We have obtained quantitative ultrasound measurements on both legs of individuals with tibial bowing of those who have enrolled. Decreased z-scores for speed of sound as measured by the quantitative ultrasound machine were observed in the affected tibia in 7/8 participants (see results in **Table 1**).

Since none of the individuals have yet fractured, we are unable to determine if the degree of difference in the speed of sound z-scores as measured by quantitative ultrasound between the bowed and non-bowed tibia can help predict who will fracture. We will continue to follow these individuals with our biannual phone interviews to document clinical progression to fracture or continuation of an intact tibia.

Table 1. Mean Z-scores of Speed of Sound from Quantitative Ultrasound of Bowed and Non-bowed Tibia in NF1 Individuals

	Tibia Affected	Z-score Right Tibia	Z-score Left Tibia	Difference between bowed and non-bowed tibia
Participant #1	Left	-0.7	-1.0	-0.3
Participant #2	Left	-3.3	-2.4	+0.9
Participant #3	Left	+1.3	-1.0	-2.3
Participant #4	Right	-3.7	-0.7	-3.0
Participant #5	Right	-0.5	+0.3	-0.7
Participant #6	Right	-4.2	-1.7	-2.5
Participant #7	Left	-0.3	-1.0	-0.7
Participant #8	Left	-0.3	-3.9	-3.6

Examples of photos of the bowed tibia of each NF1 individual with tibial bowing that have been enrolled are shown below in **Fig.2**.



Figure 2. Photographs of the bowed leg of the 8 individuals Participants #1-8 with NF1 with tibial dysplasia.

- e. Obtain urine samples for urinary crosslink measurements to be performed at the University of Utah (Specific Aim 2; N=150).

-We have obtained urine samples from NF1 individuals who have enrolled and have been frozen to be analyzed. We have met with the co-investigators in the Department of Pathology who will be running the assays and have set up the procedures to send samples in batches.

- f. Obtain blood samples for pit resorption assays to be shipped and performed at Indiana University (Specific Aim 2; N=150).

-A subset of the enrolled individuals have had blood samples obtained for pit resorption assays to be performed at Indiana University. However, a few months into the study our collaborators at Indiana University informed us during the past year that there was a shortage in the dentine slides used for the pit resorption assays which resulted in limited supplies for an extended period of time. Therefore for many months the pit resorption assays could not be performed on large numbers of individuals. Our collaborators had a few remaining dentine slides which we saved for individuals with tibial bowing who were scheduled to travel to see us. Therefore, many of the NF1 control individuals without tibial bowing who enrolled did not have blood drawn during this period and will be performed in the upcoming years now that we have been able to obtain the required supplies for the pit resorption assays. In addition, we held off on enrollment of the NF1 control individuals waiting to enroll when we were able to obtain additional dentine slides. We have now addressed the supply shortage and have now been able to get additional dentine slides for the pit resorption assay and have begun to resume active recruitment of the NF1 control individuals.

In **Figure 3** we show data on one NF1 individual documenting increased pit resorption further confirming that osteoclast activity in vitro is increased as we have previously reported. (Stevenson et al., 2011).

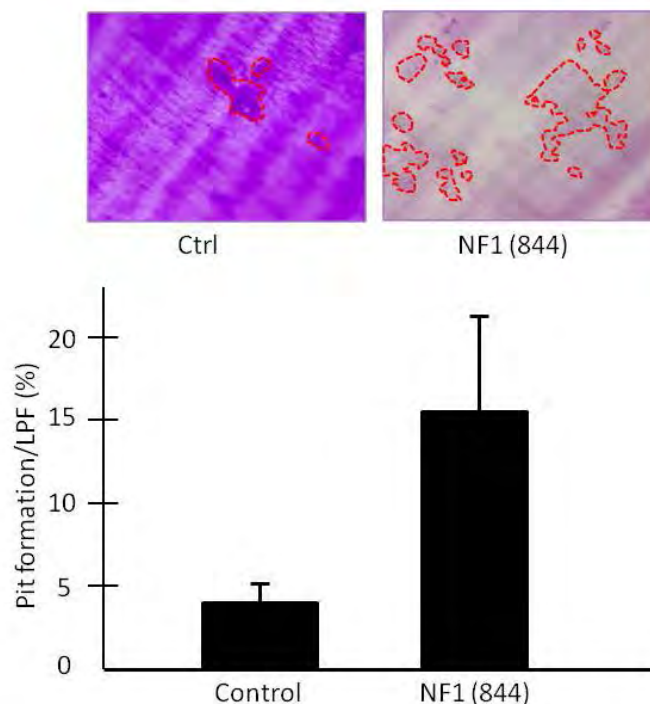


Figure 3: Percent of pit resorption area per low power field of an individual with and without NF1 showing increased osteoclast activity in the NF1 individual. The upper panels show representative slides of pit resorption outlined in dotted red line.

- g. Perform genetic analyses (next-generation sequencing and confirmation Sanger sequencing) and histologic evaluations on osseous tissue specimens obtained at the University of Utah. These analyses will take place with prioritized fashion with first analyses on prospectively acquired tissue and subsequently analyze archived tissues beginning in the second year of the proposal (Specific Aim 3).

-We have continued to collect tissue samples from individuals with tibial pseudarthrosis. We have extracted DNA from peripheral blood of all individuals with tibial bowing and also those in whom we have collected bone tissue through our tissue core. Whole genome amplification has been performed on the DNA extracted from the pseudarthrosis tissue in one individual. As mentioned in the task above these analyses will take place in a prioritized fashion given the cost of next generation sequencing and will be performed primarily in the second and third year.

- h. Interim analyses and manuscripts.

-Interim analyses are described above respectively for each aspect of the study. To date we do not have enough data to generate a manuscript.

- i. Annual reports will be written.

-This has been performed herein.

Key Research Accomplishments

- Enrollment of 36 individuals with NF1.
- Eight individuals with NF1 with tibial bowing without fracture have been recruited and medical histories and examinations documented and followed prospectively.
- Quantitative ultrasound measurements show decreases in the speed of sound of the affected leg compared to the unaffected leg in all but one individual.
- DNA extraction from peripheral blood for somatic mutation comparison in tibial tissue.
- Whole genome amplification of pseudarthrosis tissue
- Confirmation of increased bone resorption in NF1.

Reportable Outcomes

Given that this proposal is primarily a prospective nature of following NF1 individuals with tibial bowing over time to see who will fracture, reportable outcomes and research accomplishments will be limited in the first two years of the study. Hence in this first annual report, outcomes are minimal.

The following abstract was presented at the Western Society for Pediatric Research in which aspects of the study supported some of the rationale for discussion:

Stevenson DA, Allen S, Tidymen WE, Carey JC, Viskochil DH, Stevens A, Hanson H, Sheng X, Thompson GA, Okumura M, Reinker K, Johnson B, Rauhen KA. Peripheral muscle weakness in RASopathies. Oral presentation at the Western Society for Pediatric Research, Carmel, California, January, 2012.

Conclusion

Our integrative proposal will gain novel information about the pathophysiology of tibial bowing and pseudarthrosis. At this point in time we are currently still in the phase of collecting data on individuals with tibial bowing and following them over the course of the study to see if quantitative ultrasound measurements, and osteolytic activity from cultured osteoclasts and urine crosslinks can be used as a predictor of fracture. Our data to date suggest that the bowed tibia has increased porosity based on the decrease in speed of sound z-scores in the affected limb. Ultimately, our proposal will help in the development of personalized treatment protocols based on an NF1 individual's QUS measurements, osteolytic activity, and somatic mutation profile.

References

1. Friedman JM, Birch PH. Type 1 Neurofibromatosis: A descriptive analysis of the disorder in 1728 patients. *Am J Med Genet* 1997;70:138-143.
2. Stevenson DA, Carey JC, Viskochil DH, Moyer-Mileur LJ, Slater H, Murray MA, D'Astous JL, Murray KA. Analysis of radiographic characteristics of anterolateral bowing of the lower leg prior to fracture in neurofibromatosis type 1. *J Pediatr Orthop* 2009;29:385-92.
3. Stevenson DA, Yan J, He Y, Li H, Liu Y, Jing Y, Guo Z, Zhang Q, Zhang W, Yang D, Wu X, Hanson H, Li X, Staser K, Viskochil DH, Carey JC, Chen S, Miller L, Roberson K, Moyer-Mileur L, Yang FC. Multiple increased osteoclast functions in individuals with neurofibromatosis type 1. *Am J Med Genet A* 2011;155:1050-9.

Appendices

none