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PRINCIPAL INVESTIGATOR: Dr. David Stevenson

CONTRACTING ORGANIZATION: University of Utah
SALT LAKE CITY, UT 84112-9023

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David Stevenson, MD

E-Mail: david.stevenson@hsc.utah.edu

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13. SUPPLEMENTARY NOTES  

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 without 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.

15. SUBJECT TERMS  
Neurofibromatosis type 1, bone, pseudarthrosis

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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. Given that we have asked for a year no-cost extension we have not performed all of the tasks for the final data analyses as described below and we will perform this in our final report.

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. Funds from the no-cost extension will be utilized in part to retain the clinical coordinator in order to continue to complete the project.
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. We have continued to send out recruitment flyers to NF support groups and orthopedic agencies. In addition, Dr. Stevenson has spoken at several NF support group functions and attempted to provide wider knowledge of the study.

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 previously identified these individuals who are potential study participants for future contact. We have focused on those with tibial bowing primarily.

During this time period we recruited individuals 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 previously 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 was 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 19 individuals with NF1 with tibial bowing. We are continuing to recruit additional individuals. We have enrolled 87 individuals with NF1 without tibial bowing to function as controls. To help in recruitment primarily of NF1 individuals with bowing, Dr. Stevenson previously traveled to the Children's Tumor Foundation (CTF) NF Forum for advertisement to the US clinic coordinators and chapter leaders and the study was highlighted by
CTF on their website. In addition the study was advertised through the CTF Registry.

Examples of the radiographs of a few of the NFI 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 our 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, 3 individuals have sustained a tibial fracture and one individual underwent elective surgery prior to fracture. One individual (Participant #1) sustained a fracture of the tibia and had intramedullary rod placed. The second individual (Participant #9) sustained a fracture of the tibia and had external fixator placed. The third individual (participant #14) sustained a tibial fracture and had intramedullary rod placed. Another individual (Participant
#12) elected to undergo surgical procedures to correct the bowing prior to fracture (See Fig. 2).

![Fig. 2. Radiograph of Participant #12 who had surgical intervention to try and decrease the degree of bowing.]

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 16/19 participants (see results in Table 1), and in 2 of the 3 individuals with +z-score differences we think that these individuals had physiologic bowing and not pathologic tibial dysplasia (see Figures 3,4).

Since only a few 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. However, two of the three individuals who fractured had the largest negative difference of z-score between the bowed and non-bowed tibia (z-scores of -8.0 and -5.0. 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

<table>
<thead>
<tr>
<th>Participant #</th>
<th>Tibia Affected</th>
<th>Z-score Right Tibia</th>
<th>Z-score Left Tibia</th>
<th>Difference between bowed and non-bowed tibia</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>Left</td>
<td>-0.7</td>
<td>-1.0</td>
<td>-0.3</td>
</tr>
<tr>
<td>#2</td>
<td>Left</td>
<td>-3.3</td>
<td>-2.4</td>
<td>+0.9</td>
</tr>
<tr>
<td>#3</td>
<td>Left</td>
<td>+1.3</td>
<td>-1.0</td>
<td>-2.3</td>
</tr>
<tr>
<td>#4</td>
<td>Right</td>
<td>-3.7</td>
<td>-0.7</td>
<td>-3.0</td>
</tr>
<tr>
<td>#5</td>
<td>Right</td>
<td>-0.5</td>
<td>+0.3</td>
<td>-0.7</td>
</tr>
<tr>
<td>#6</td>
<td>Right</td>
<td>-4.2</td>
<td>-1.7</td>
<td>-2.5</td>
</tr>
<tr>
<td>#7</td>
<td>Left</td>
<td>-0.3</td>
<td>-1.0</td>
<td>-0.7</td>
</tr>
<tr>
<td>#8</td>
<td>Left</td>
<td>-0.3</td>
<td>-3.9</td>
<td>-3.6</td>
</tr>
<tr>
<td>#9</td>
<td>Right</td>
<td>-7.5</td>
<td>+0.5</td>
<td>-8.0</td>
</tr>
<tr>
<td>#10</td>
<td>Right</td>
<td>-4.5</td>
<td>-0.7</td>
<td>-3.2</td>
</tr>
<tr>
<td>#11</td>
<td>Right</td>
<td>-3.2</td>
<td>-2.4</td>
<td>-0.8</td>
</tr>
<tr>
<td>#12</td>
<td>Left</td>
<td>+3.2</td>
<td>-5.2</td>
<td>-8.4</td>
</tr>
<tr>
<td>*#13</td>
<td>Left</td>
<td>0</td>
<td>+2.6</td>
<td>+2.6*</td>
</tr>
<tr>
<td>#14</td>
<td>Right</td>
<td>-5.2</td>
<td>-0.2</td>
<td>-5.0</td>
</tr>
<tr>
<td>*#15</td>
<td>Right</td>
<td>-0.1</td>
<td>-0.8</td>
<td>+0.7*</td>
</tr>
<tr>
<td>#16</td>
<td>Left</td>
<td>-2.3</td>
<td>-3.9</td>
<td>-1.6</td>
</tr>
<tr>
<td>#17</td>
<td>Right</td>
<td>-2.2</td>
<td>-0.5</td>
<td>-1.7</td>
</tr>
<tr>
<td>#18</td>
<td>Left</td>
<td>+0.7</td>
<td>-2.8</td>
<td>-3.5</td>
</tr>
<tr>
<td>#19</td>
<td>Left</td>
<td>0.9</td>
<td>-2.8</td>
<td>-3.7</td>
</tr>
</tbody>
</table>

*These two individuals had very minimal lateral vs. anterolateral bowing without radiographic findings of tibial dysplasia (i.e., cortical thickening and medullary canal narrowing) – see Figure 3 and 4 and although these individuals were included in the study as they were referred and being treated as tibial dysplasia, we think given the young age when the bowing was noted is most consistent with probably physiologic bowing of infancy and hence an indicator that bone ultrasound is helpful in differentiating pathologic tibial bowing vs. physiologic bowing. Longitudinal follow-up will help clarify this question.
Fig. 3. Radiograph of Participant #13 with minimal anterior bowing on left (B,C) compared to right (A), and no significant lateral bowing without cortical thickening or medullary canal narrowing. This was very difficult to discern on examination (D).

Fig. 4. (A) Radiograph of Participant #15 with history per parent of tibial bowing that was thought to be bilateral with possible increase on the right. Bracing was used bilaterally for 6 months and then stopped. (B) Upon our examination we could not see any anterior bowing and only mild bilateral bowing which we thought was likely within normal limits and most consistent with physiologic bowing.

Examples of photos of the bowed tibia of each NF1 individual with tibial bowing that have been enrolled are shown below in Fig. 5.
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 the urine was frozen prior to being sent for analysis. All urine samples have been sent to Dr. Pasquali’s laboratory for pyridinium crosslink analysis. Pyridinium crosslinks are currently being run in a stepwise approach in batches. We have results of the first 13 NF1 individuals with tibial bowing (Table 2) and the rest are still undergoing analysis. The average DPD/PYD ratio was 0.31 which is well above the mean that we have previously reported in children without NF1 (i.e. approximately 0.22) (Stevenson et al. 2010). We will perform statistical analysis during the next year from the pyridinium crosslinks of the NF1 individuals without bowing for comparison.

<table>
<thead>
<tr>
<th>NF1 individuals with Tibial Bowing</th>
<th>Pyridinoline (PYD) umol/mol creatinine</th>
<th>Deoxy-pyridinoline (DPD) umol/mol creatinine</th>
<th>DPD/PYD Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1-07</td>
<td>408</td>
<td>141</td>
<td>0.35</td>
</tr>
<tr>
<td>NF1-211</td>
<td>524</td>
<td>150</td>
<td>0.29</td>
</tr>
<tr>
<td>NF1-217</td>
<td>127</td>
<td>33</td>
<td>0.26</td>
</tr>
<tr>
<td>NF1-223</td>
<td>250</td>
<td>65</td>
<td>0.26</td>
</tr>
<tr>
<td>NF1-225</td>
<td>211</td>
<td>54</td>
<td>0.26</td>
</tr>
<tr>
<td>NF1-229</td>
<td>508</td>
<td>128</td>
<td>0.25</td>
</tr>
<tr>
<td>NF1-231</td>
<td>290</td>
<td>93</td>
<td>0.32</td>
</tr>
<tr>
<td>NF1-232</td>
<td>290</td>
<td>90</td>
<td>0.32</td>
</tr>
<tr>
<td>NF1-243</td>
<td>412</td>
<td>147</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>-----</td>
<td>---</td>
</tr>
<tr>
<td>NF1-246</td>
<td>357</td>
<td>107</td>
<td>0.30</td>
</tr>
<tr>
<td>NF1-254</td>
<td>358</td>
<td>147</td>
<td>0.41</td>
</tr>
<tr>
<td>NF1-257</td>
<td>507</td>
<td>174</td>
<td>0.35</td>
</tr>
<tr>
<td>NF1-260</td>
<td>654</td>
<td>201</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*Measurements are average of two consecutive first morning voids.

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

-Blood samples for pit resorption assays have been obtained on individuals with tibial bowing and NF1 individuals without tibial bowing. Samples are shipped via FedEx to Dr. Yang at Indiana University.

In **Figure 6** we show data that osteoclast activity is increased in NF1 compared to controls as we have previously described (Stevenson et al., 2011), and that pit resorption in individuals with NF1 with bowing compared to NF1 individuals without bowing is increased.

**Figure 6.** **Top panel:** Example of resorption area (outlined in dotted yellow line) of an individual without NF1, NF1 individual without tibial bowing, and an individual with NF1 with tibia bowing. **Bottom panel:** Percent of pit resorption area per low power field is increased in the NF1 individuals with bowing compared to NF1 individuals without bowing and controls.
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 collected discarded tissue from surgical procedures of two of the individuals with tibial bowing proceeded to fracture in March 2014, and we are currently processing the sample (example of tissue shown Fig. 7).

![Tissue sample from surgery of two different participants (A and B) who developed fracture of the bowed tibia.](image)

Figure 7. Tissue sample from surgery of two different participants (A and B) who developed fracture of the bowed tibia.

We have extracted DNA from peripheral blood of all individuals with tibial bowing and will continue to monitor them for development of fracture. For the individuals who have fractured in which we have tissue (see Fig. 7), we are currently extracting DNA from bone for exome sequencing. Whole genome amplification has been performed on the DNA extracted from several other individuals with stored pseudarthrosis tissue. Given the advances in next generation sequencing we are utilizing exome sequencing for our analyses. As mentioned in the task above these analyses will take place in a prioritized fashion given the cost of next generation sequencing which is even more important now that we are utilizing exome sequencing.

DNA extraction from bone and whole genome amplification (WGA): DNA extraction from bone is challenging. There are three key facts which affect the yield of the DNA extraction; 1) There are low cell numbers (e.g. osteocytes) in bone, 2) release of DNA from osteocytes nucleus is difficult, 3) calcium precipitation. A Qiagene kit was used for DNA extraction from bone tissue. Figure 8 is gel picture for the DNA extractions from four different pieces of the bone from tibial dysplasia. 10 ug of bone has been used for each extraction. The yield of DNA is between 50ng and 1000ng/ per sample. Due to the low yield of DNA whole genome amplification was pursued to meet the requirement for exome sequencing (200 ug of DNA required from Illumina TrueSeq Exome capture, 1.5ug of DNA for Nimblegen Exome and 3ug of
DNA for Agilent Exome capture). Whole genome amplification has been performed on some of the samples with low yield of DNA using GenomePlex® (Sigma-Aldrich Co.). Please see the gel pic show below showing post amplification. The fist WGA yielded 981.3 ng/ul in 100 ul of DNA and the second WGA (lane 2) yielded 983.7 ng/ul in 100 ul of DNA, see Figure 9.

Figure 8. DNA extractions from four pieces of bone tissue from a patient. Lane 1,2,5,6 are the molecular weight markers. Lane 3,4,7,8 are DNA from different extractions.

Figure 9. Lane 1 and 2 are whole genome amplifications from the same sample. Lane 3 is high molecular weight marker, Lane 4 is 50bp molecular weight marker.

Exome Sequencing: Our new approach is to use exome sequencing to examine the entire coding region of the genome. A significant finding is that we have been able to identify a second hit in 4/5 individuals, and in the one
individual without a confirmed somatic NF1 mutation there is still the question of a possible second hit (IVS+1,ex15) (Table 3). This dramatically shifts the thought that mutations in other genes are the causative factor for tibial dysplasia in NF1. In communication with collaborators from a different institution, this is further confirmed and hence we think that double inactivation of NF1 is necessary for development of tibial dysplasia in NF1 (manuscript submitted).

Table 3. NF1 Mutations in Pseudarthrosis Tissue vs. Germline

<table>
<thead>
<tr>
<th>Age at Diagnosis/Presentation</th>
<th>NF1 Inherited</th>
<th>NF1 Somatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth (bowing); fracture 18 month</td>
<td>p.R1849Q</td>
<td>17q LOH</td>
</tr>
<tr>
<td>Bowing noticed at 2 months; 7 years of age sx for PA</td>
<td>IVS-2, ex15</td>
<td>p.N45fs</td>
</tr>
<tr>
<td>Pseudarthrosis at 10 years (amputation at 18 years)</td>
<td>p.V7155fs</td>
<td>? IVS+1,ex15</td>
</tr>
<tr>
<td>Bowing noted at birth; fracture at 3 mo</td>
<td>IVS-2,ex2</td>
<td>P.E1192X</td>
</tr>
<tr>
<td>Fracture at 2 weeks</td>
<td>n.d.</td>
<td>p.R1276X</td>
</tr>
</tbody>
</table>

It is still unknown what cell type harbors the specific NF1 second hit. Based on our results of the samples analyzed in Table 3, there is low level mosaicism providing evidence of a mixed cellular population. This may be the reason for the ambiguity of whether or not there is a somatic mutation in the single case in Table 3. We are now proceeding with analysis of separate sections from tissue blocks of the above individuals to determine the specific tissue in which the second hit occurred (i.e. cortical bone, periosteum, etc.) (see Figure 10).
Figure 10. Comparison of the somatic mutation in an individual with neurofibromatosis type 1 (NF1) and tibial pseudarthrosis. The top panel show sequence from DNA extracted from blood. The bottom 3 panels show sequence from DNA extracted from different sections of the surgical sample. The somatic mutation is not shown in DNA extracted from the sample that was purely bone and lower levels of the mutation seen in the mixed bone and pseudoarthrosis sample suggesting that the somatic mutation arises from the hypercellular proliferating tissue in between the bone segments likely from the periosteum.

Even though our data are quite convincing that the salient factor for tibial dysplasia development is double inactivation of \textit{NF1}, there is still a possibility of modifier genes impacting the subsequent natural history of tibial bowing, as not all individuals with tibial bowing fracture and there is variable expressivity. For example, in one sample we identified a variant in PTPN11 which is involved in the RAS/MAPK signal transduction pathway (see Figure 11). However, this variant is not a somatic event, although it could still provide a modifying event and the child had severe bowing and pseudoarthrosis that required amputation.
Figure 11. Comparison of Sanger sequencing of variant identified in PTPN11 from exome sequencing showing PTPN11 variant in both blood and bone tissue.

We are using a tier analysis to first analyze the 16 genes in RAS/MAPK Pathway as initially proposed, although exome sequencing will now allow for a much broader approach to look at a multitude of other genes that can be identified on exome. Using a variant analysis for the whole blood sample compared to bone tissue, the mutations in only bone tissue are considered as secondary events and are more likely to play a role as genetic modifiers of pseudarthrosis.

h. Interim analyses and manuscripts.
- Interim analyses are described above respectively for each aspect of the study. A manuscript describing the genetic analyses has been written and has been submitted to Human Molecular Genetics and is currently under review.

i. Annual reports will be written.
- This has been performed herein.

Task 3. Data Analysis (Months 42-48): Given that we have asked for a year no-cost extension we have not performed all of the tasks for the final data analyses as described below and will perform this in our final report. Portions of the tasks below have been performed and are described above.

a. Review the data entered and updates fracture and surgical history from biannual phone interviews.
- This has been performed as described above.

b. Perform statistical analysis of data from QUS, urine crosslinks, and osteoclast pit resorption assays.
- This is described above in part and will be performed in whole upon the final report.

c. Summarize the results of genetic and histologic analyses of osseous tissue.
- This is described above.

d. A final report will be written.
- This will be done at the end of the no-cost extension.
Key Research Accomplishments

- Enrollment of 106 individuals with NF1.
- Nineteen 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 16/19 individuals, and in 2 of the 3 individuals with positive z-score differences we think that the individuals have physiologic bowing rather than tibial dysplasia. This is a key finding as it will allow for future surrogate marker for clinical trials focused on therapeutics to improve bone quality prior to fracture in a non-invasive and age specific manner.
- DNA extraction from peripheral blood for somatic mutation comparison in tibial tissue.
- Successful whole genome amplification of pseudarthrosis tissue.
- Documentation of double inactivation in NF1 in tissue samples as the primary event leading to tibial dysplasia.
- Confirmation of increased bone resorption in NF1.
Reportable Outcomes

Given that this proposal is primarily prospective in which we are following NF1 individuals with tibial bowing over time to see who will fracture, reportable outcomes and research accomplishments will be limited within the timeframe of the grant. However the following manuscript reporting our data on the genetic analyses of the pseudarthrosis tissue has been submitted and is currently under review in JBMR:


The following abstracts and presentations were given in which aspects of the current study supported some of the rationale for discussion:


*Bone Health in NF1*. Invited speaker at the Children’s Tumor Foundation NF Forum. New Orleans, Louisiana (June, 2012)


*Comparison of the Musculoskeletal Findings in RASopathies*. Invited speaker at the Bone Series, Vanderbilt University, Nashville, TN, April, 2013.

*Muscle in NF1*. Invited speaker at the Children’s Tumor Foundation International NF Conference, Monterrey, CA, June, 2013.


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
Appendices
none