University of Cincinnati

Date: 5/7/2012

I, Meron Y Azage B.S., hereby submit this original work as part of the requirements for the degree of Master of Science in Genetic Counseling.

It is entitled:
Fracture Rates in Adults with Neurofibromatosis Type 1

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This work and its defense approved by:

Committee chair: Elizabeth Schorry, MD

Committee member: Robert Hopkin, MD

Committee member: Lisa Martin, PhD
Fracture Rates in Adults with Neurofibromatosis Type 1

A thesis submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of

Master of Science in the Department of Pediatrics of the College of Medicine 2012 by

Meron Azage

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Abstract

About 30-50% of patients with Neurofibromatosis Type 1 (NF1) have disease involving the skeletal system. Osteoporosis and low bone density are common findings; however increased rate of fractures in patients with NF1 has not yet been established in a well-designed clinical study. The purpose of this study is to compare the prevalence of fractures between adults with NF1 and unaffected controls including unaffected siblings/spouses of these patients. We hypothesized that the NF1 cohort will have fracture rates that are higher than unaffected controls. A retrospective questionnaire was administered to 38 adults with NF1 and 36 unaffected adults. Participants were asked to report fractures that occurred during their lifetime as well as in the past 10 years. The number of fractures, the bone location, age of participant when the fracture occurred, cause of fracture (such as trauma), time for healing, treatment and complications were assessed. We stratified fracture rates in cases and controls for age and sex, and statistically adjusted for other risk factors such as ethnicity, body mass index (BMI), tobacco use, menopause status, level of physical activity and dietary calcium intake. While adults with NF1 had higher 10 year fracture rate than controls, the difference did not reach statistical significant. Given the relatively small sample size in this study, collection of additional data is warranted. Improving our understanding of skeletal phenotype of NF1 may change clinical management, improve standard of care of patients and inform future drug trials for bone disease.
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List of Symbols

1. Body mass index (BMI)
2. Bone mineral content (BMC)
3. Bone mineral density (BMD)
4. Confidence Interval (CI)
5. Deoxypyridinoline (Dpd)
6. Dual-energy X-ray absorptiometry (DEXA)
7. Neurofibromatosis Type 1 (NF1)
8. Pyridinoline (Pyd)
9. Quantitative ultrasonometry (QUS)
10. Standard Deviation (SD)
Background

Neurofibromatosis Type 1 (NF1) is a common neurocutaneous disorder with a prevalence of about 1/3000. The \textit{NF1} gene encodes the protein neurofibromin, a tumor suppressor that functions by regulating Ras. The mechanism by which Neurofibromin functions to alter bone remodeling is not well understood; however mouse models of NF1 have shown defects in function of osteoblasts and osteoclasts (Kolanczyk et al., 2007; Stevenson et al., 2011; Yu et al., 2005). At least 10-50% of patients with NF1 have disease involving the skeletal system (Illes et al., 2001). Presentation of musculoskeletal findings is variable, and can include short stature, dysplastic scoliosis, pectus excavatum, tibial dysplasia/pseudarthrosis and sphenoid wing dysplasia. It has been reported that patients with NF1 have decreased bone mineral density and content because of pathological bone remodeling (Kolanczyk et al., 2007). Osseous abnormalities such as low bone mineral density (BMD), osteoporosis and osteopenia are well recognized as a phenotype of NF1; however fracture rates in the adult NF1 patient population are not well documented.

Despite a now well-documented decrease in BMD in patients with NF1, few studies have investigated whether this translates into an increased risk of fracture. Our group recently performed a study of over 200 children and adolescents (ages 5-20 years) with NF1 and found no difference in lifetime fracture rate compared to a control group (George-Abraham et al., 2012). Tucker et al conducted an exploratory study of BMD in adult NF1 patients using a fracture history questionnaire. In their study, NF1 patients reported a 4 fold increase in lifetime fracture events compared to their unaffected siblings/spouses (Tucker et al., 2009); however, data was collected differently for subjects versus controls. The objective of the current study was to describe fractures in adult patients with NF1 and to determine if fracture rates in adults between the ages of 40-70 with NF1 are different than in unaffected adults. The primary outcome measure to be evaluated was fracture rate over the previous 10 year period. This study aims to confirm the findings of the study done by Tucker et al by performing direct data collection from cases and controls.
This study attempts to add to current literature and improve our understanding of skeletal phenotype of NF1.

**Methods**

This study is a comparative, cross-sectional study that collected quantitative data on fracture history using a questionnaire. This study received IRB approval from Cincinnati Children's Hospital Medical Center (CCHMC) and University of Cincinnati. Participants in this study were adults with NF1 and unaffected controls, between the ages of 40-70, who met inclusionary criteria. Cases met the NIH diagnostic criteria (Ferner et al., 2007) for NF1 if two or more features listed below were present.

1. Six or more café au lait macules over 15 mm in greatest diameter.
2. Two or more neurofibromas of any type or one plexiform neurofibroma.
3. Freckling in the axillary or inguinal regions.
4. Optic nerve glioma.
5. Two or more Lisch nodules (iris harmatomas).
6. Sphenoid dysplasia/tibial dysplasia with or without pseudoarthrosis.
7. A first-degree relative (parent, sibling, or offspring) with NF1 who meets the NIH diagnostic criteria for NF1.

**Inclusion Criteria for Cases**

- Age: 40-70 years.
- Meet NIH diagnostic criteria for NF1.

**Exclusion Criteria for Cases**

- Diagnosis of NF2 or sub-type of NF such as segmental NF.
- Age younger than 40 and older than 70 years.
- Diagnosis of chronic illness/ genetic condition affecting bone health.
- Steroid use.
- Nonambulatory.

All study participants who completed the study questionnaire were offered a $10 gift card as compensation. Recruitment of participants was performed in person, by mail and on the internet.

Participants were recruited in 1 of 4 following different ways

1. The NF family conference: held on July 30, 2011, is an annual one day event organized by CCHMC and the Children’s Tumor Foundation. Attendees who agreed to participate in our study were consented and provided with the questionnaire. We mailed questionnaires to siblings/spouses of adults with NF1 who did not attend the conference.

2. Participants were recruited at the adult neurofibromatosis clinic in the Department of Internal Medicine and Pediatrics of the University of Cincinnati. We also recruited parents or grandparents of children with NF1 who were seen at the pediatric neurofibromatosis clinic at CCHMC.

3. Questionnaires were mailed to patients with NF1 previously seen in the NF Clinic and their spouses/siblings. The study questionnaire was mailed to nonresponders a second time one month after the initial mailing.

4. We submitted this research study to be listed on the websites of the Children's Tumor Foundation (www.ctf.org) and Neurofibromatosis, Inc. (www.nfnetwork.org). Both organizations maintain Facebook pages for individuals with NF and their families. The Children's Tumor Foundation has affiliates in nearly all states and the website features links to 47 different states with a Facebook page. We contacted the site administrators (if listed) to obtain permission to post on their respective Facebook walls a link to the study poster.
Study Questionnaire

In addition to low BMD, there are several significant risk factors that contribute to fractures in adults (Kanis et al., 2009). The study questionnaire assessed additional information which may be associated with NF1 or fracture rates including age, sex, height, weight, ethnicity, dietary calcium intake, calcium supplement use, level of physical activity and medication use. The questionnaire utilized in this study was adapted from the following 4 different previously published questionnaires.

1. Center for Pediatric Nutrition Research, University of Utah Health Sciences Center personal health history questionnaire which was originally developed and validated to assess nutritional data in youth/adolescents (Rockett et al., 1997).

2. A questionnaire from a German study (translated into English) on fracture history in patient with NF1 (Tucker et al., 2009).

3. The Short Calcium Questionnaire (version SCQ-2002) is a food frequency questionnaire validated in adults to estimate calcium (Sebring et al., 2007).

4. The Canadian Multicenter Osteoporosis Study Questionnaire (CaMoS), which was developed to assesses the burden of osteoporosis and fracture in Canadian women and men (Kreiger et al., 1999).

The self-administered questionnaire used in this study was pretested for face validity in 4 adults. Pretesters were healthcare providers familiar with the NF1 patient population, and support staff of the Department of Human Genetics at CCHMC. The readability level of the validated survey, which takes 15-20 minutes to complete, was found to be at the 8th grade level. This was critical because approximately 50% of patients with NF1 are expected to have varying degrees of a learning disability. A study staff was available to answer questions for participants who were recruited in person. Questionnaires were assessed for completeness and if unclear responses were given, participants were contacted by phone to clarify responses.
Data Analysis

The purpose of this study was to compare the frequency of fractures between adults with NF1 and same-age unaffected adults. Our sample size calculation, using fracture incidence as reported by Tucker et al, indicated that with a conservative sample size of 60 per group, we would have more than 80% power to detect a difference in frequency of fractures with an error rate of 0.05. This sample size would enable us to detect a true probability of exposure among cases of .199. To ensure comparability of the groups, we first tested for differences in age, sex, ethnicity, BMD, tobacco use, menopause status, level of physical activity and dietary calcium intake using t-tests and chi-square goodness of fit, as appropriate. Then, we measured history of fractures in the past 10 years as a primary outcome variable and compared percentage of participants in our cases and controls who report at least one fracture. A chi-square analysis was used to test our hypothesis that adults with NF1 would report fractures at higher rates than unaffected controls. The Type I error of 0.05 was considered significant. Data analysis was done using JMP, Version 7.

Results

We recruited 40 adults with NF1 and 37 healthy controls. We excluded 2 participants who did not meet the age requirement and 1 duplicate questionnaire, resulting in a total of 38 adults with NF1 and 36 controls. Of the 74 individuals who met our inclusion criteria, 36 (48%) respondents were recruited from various clinic sites, 24 (32%) were recruited from various websites, 6 (8%) were recruited by mail and 8 (19%) were recruited at the NF family conference. Demographic characteristics of respondents are represented in Table 1. The mean age of cases and controls was 51.8 years (95% CI 49.0-54.6) and 51.1 years (95% CI 48.2-54.1) respectively. Overall, our NF patients and controls were similar with respect to ethnicity, age of menopause, exercise habits, dietary calcium intake and calcium supplement use. History of cancer diagnosis was similar in the two groups: there were 5 cases and 2 controls who reported a history of cancer. However, there were differences between our cases and controls with respect to sex
and BMI. Cases were more likely to be female (71%) compared to our controls (47%) (p= 0.0369). Additionally, controls had a higher mean calculated BMI than cases (p = 0.0125).

We compared the proportion of individuals who had at least one fracture in the past 10 years by NF status. There were a total of 16 fracture events in the last 10 years reported; 12 total fractures in the NF group and 4 in the control group. There were a total of 14 (19%) respondents who have had at least 1 fracture event; 10 (26%) individuals were cases and 4 (11%) were controls. However, this difference did not reach statistical significance (p=0.0951). As sex and BMI exhibited differences between case status, we tested whether each of these variables were associated with 10 year fracture rate. As there was no difference in males and females in fracture rate (p=0.8446) or in BMI and fracture rate (p=0.9800), no additional adjustment was warranted.

We then looked at other outcome variables such as the frequency of lifetime fractures and the location of fractures. There were 2 respondents who were not sure about their lifetime fracture history and 45 individuals who have never had a fracture. Amongst adults with NF1, there were 15 (42%) individuals who reported at least one fracture event in their lifetime and 8 (53%) individuals who reported more than one fracture event in their lifetime. Amongst controls, 12 (33%) individuals reported at least one fracture event in their lifetime and 6 (50%) individuals reported having had more than one fracture event in their lifetime. There were 47 total lifetime fracture events reported amongst cases and controls. Individuals with NF1 reported 27 (57%) of those events while controls reported the remaining 20 (43%) fracture events. There was no statistical difference between the two groups in reporting of lifetime fractures (p=0.071). Lifetime fracture history was not affected by confound variables such as age, sex, history of cancer diagnosis and BMI. Fractures reported involved the fingers/ hands, wrist, sternum, ribs, humerus, fibula and toes/foot. Fracture location data was collapsed into those involving the lower extremity (leg/foot), the upper extremity (arm/hand) and other (all other fractures). There was no statistical
difference in the location of fractures amongst those who have NF1 and those who were healthy controls (p=0.1570).

**Discussion**

This study compares the prevalence of fractures between adults with NF1 and same-age unaffected adults. Although we found a greater number of fractures in the past 10 years in NF1 adults compared to controls, this difference was not statistically significant. Although the pathogenesis of bone disease in NF1 is still not fully understood; multiple studies have elucidated basic differences in NF1 bone. Heterozygous Nf1+/− and cre-recombinant mice have been used as animal models to study the role of Neurofibromin on bone development (Yu *et al.*, 2005). Kolanczyk *et al* demonstrated that the mouse model had reduced growth because of reduced differentiation and proliferation of osteoblasts and chondrocytes. Reduced mobility of the joints caused by congenital fusion of the hip joint was also noted. Histological examination of cortical bone of mutants revealed increased porosity, decreased tissue calcium content, large non-mineralized areas and abnormal vasculature within the bone (Kolanczyk *et al.*, 2007). Cre-recombinant mouse models, which knocked out both NF1 alleles in specific tissues, have documented that in addition to abnormal differentiation of osteoblasts, increased proliferation and size of osteoclasts, the bone-resorbing cells, occur in NF1.

Human bone densitometry, biochemical and histomorphometric studies conducted thus far indicate that generalized bone disease is significant in NF1. NF1 patients had smaller periosteal circumferences, smaller cortical area and decreased bone mineral content resulting in a narrower bone at the tibial diaphyseal site (Stevenson *et al.*, 2009). An early study used dual-energy X-ray absorptiometry (DEXA) and found that 11 of 12 patients with NF1 had BMD measuring 1 SD below the mean, which implies an increased risk of fracture (Illes *et al.*, 2001). Since BMD is not drastically reduced in this condition, it is reasonable to assume that the risk of fracture may only be increased marginally in adults with NF1.
Several other larger studies have been conducted on BMD in NF1 patients. A cross-sectional study using quantitative ultrasonometry (QUS) of 104 adults (ages 20-80) found that patients with NF1 had lower BMD than a reference population (Lammert et al., 2005). This finding was consistent in a smaller study of BMD and bone mineral content (BMC) in 34 study subjects (Kuorilehto et al., 2005). Complete skeletal densitometry of 84 individuals with NF1 (5-18 years of age) showed generalized decrease of BMD in the hip, femoral neck and lumbar spine (Stevenson et al., 2007). The spine was the most affected with mean BMD Z-scores of -1.38 ± 1.05 (95% CI -1.62; -1.13), which is significantly below the normal range (Brunetti-Pierri et al., 2008).

In addition to low BMD, patients with NF1 have been shown to have low serum 25-hydroxyvitamin D concentration which may predispose them to fractures (Lammert et al., 2006). Vitamin D is important for maintaining bone health and it has been suggested that reduced serum 25-hydroxyvitamin D concentrations contribute to the bone defects seen in NF1. Supplementation of vitamin D has been shown to improve BMD in patients with NF1 (Seitz et al., 2010). Stevenson et al conducted a study on Pyridinoline (Pyd) and deoxypyridinoline (Dpd), which provided additional evidence of poor bone health in patients with NF1. Pyd and Dpd, which are crosslinks in collagen, contribute to bone strength. In a study of 59 NF1 children (ages 5-19), 19% of NF1 patients without skeletal dysplasia and 38% of those with skeletal dysplasia had Pyd/Dpd ratio that was elevated indicating high bone turnover (Stevenson et al., 2008).

Our study does not support the findings of the one prior published study of fracture rates in adults with NF1. Tucker et al found lifetime fracture rates in adult NF1 patients were significantly higher than in unaffected siblings/spouses. In their study, methodology included a fracture history questionnaire completed on site by NF1 subjects; fracture history of spouses/ siblings was assessed by a follow-up phone call to the NF1 patients. They found a total of 41 lifetime fractures in 72 adults with NF1 (ages 18-
72 years). The control group, which consisted of 73 unaffected siblings and 22 spouses, reported a total of 6 lifetime fractures. Patients with NF1 reported significantly more fractures (33% lifetime fracture for adults with NF1 versus 8% in controls); including vertebral and hip fractures at relatively young ages than controls in this study (Tucker et al., 2009).

While the findings of the Tucker et al study regarding the increased rates of lifetime fracture in NF1 patients was statistically significant (p<0.001), the data collection methods used was a source of bias because NF1 patients were asked to report fracture history for their spouses/siblings. The lifetime fracture incidence (8%) in their control group is much lower than has been documented in many populations around the world, where lifetime fracture risk by age 50 ranges between 20% - 55% (Ahmed et al., 2009; Nguyen et al., 2007; van Staa et al., 2001). We suspected the number of lifetime fractures reported in controls in the Tucker et al study were a serious underestimation, and therefore calls their conclusions into question. Our study, although not yet statistically significant due to small sample size, suggests that there is an increase in 10 year fracture rate in adults with NF1, but that increase is not as great as the 4-fold increase suggestion by the Tucker et al study.

Limitations

There are several limitations to this study, the most significant being low response rate and small sample size. While we attempted to achieve the sample size of 60 participants in each group by utilizing various recruitment methods, low response rate was a draw back in our data collection. We believe power analysis of our primary outcome, fracture history in the last 10 years, can reach statistical significance with additional data. Post-hoc power calculation, assuming the probability of fracture in the two groups is accurate in our data, showed we need a sample size of 204 to be able to reject the null hypothesis with probability of 0.8. We found differences in the sex ratio between our cases and controls and this was a likely source of bias. The use of a questionnaire that has not been validated is another notable limitation.
About 50% of individuals with NF1 have variable degrees of learning/ cognitive disability. It was necessary for the study staff member, administering the questionnaire in a face-to-face encounter, to assist some individuals with explaining some questions on the survey. Participants who received a mailed in questionnaire may have experienced difficulty completing this survey. It is therefore possible that a bias was introduced by utilizing 2 different (mailed questionnaire vs. completing a questionnaire with study staff) data collection methods.

Fracture history collected was not verified by medical records or radiographic imaging to confirm fractures reported by participants. Accuracy of self-reports of fracture history is affected by various factors and previous studies have indicated overreporting as well as underreporting (Chen et al., 2004; Joakimsen et al., 2001). A study has reported a 71% reporting accuracy of single-site fractures in postmenopausal women (Chen et al., 2004). We do not expect accuracy of reports of fracture history to differ between cases and controls; however studies that gather fracture history supported by clinical data may help us get a better understanding of fracture risk in patients with NF1. Several factors affecting bone health such as BMD and use of hormone replacement therapy were not assessed.

**Conclusion**

This study attempts to contribute to the body of published literature on the osseous phenotype of NF1, specifically the prevalence of fractures in adults with this condition. The prevalence of having had at least one fracture in the past 10 years was 26% and 11% in our cases and controls respectively. Previously reported differences between adults with NF1 and healthy controls with respect to fracture rate are not supported by data in our study. We were not able to detect a statistically significant difference in prevalence rates as our study was underpowered. If the true probability of having a fracture in adults with NF1 is 33%, as reported by Tucker et al, we should have been able to identify this difference in our cohort. Future studies that are adequately powered are needed to look at clinically significant fracture
risk in NF1. It might be reasonable to conclude that the prevalence of fractures in adults with NF1 is only modestly higher than in healthy controls.
Bibliography


### Tables

**Table I. Descriptive Characteristics of Study Participants**

<table>
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<th>Comparison P Value</th>
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<td>NF1 (n = 38)</td>
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<th>NF1</th>
<th>NF1 vs. Control</th>
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<tr>
<td>Age (yrs)</td>
<td>51.1 ± 8.6</td>
<td>51.8 ± 8.6</td>
<td>0.73</td>
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<tr>
<td>Male, n (%)</td>
<td>19 (53)</td>
<td>11 (29)</td>
<td>0.0369*</td>
</tr>
<tr>
<td>Caucasian Race, n (%)</td>
<td>33 (92)</td>
<td>32 (84)</td>
<td>0.33</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>28.5 ± 5</td>
<td>25.7 ± 4.1</td>
<td>0.0125*</td>
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<tr>
<td>Age of Menopause</td>
<td>47 ± 3.9</td>
<td>48 ± 7.2</td>
<td>0.71</td>
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<td>Calcium intake (mg/d)</td>
<td>1317.8 ± 536</td>
<td>1170.7 ± 487</td>
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<tr>
<td>Intense Exercise (1 hour/ wk), n (%)</td>
<td>24 (67)</td>
<td>22 (58)</td>
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<tr>
<td>Fracture Events</td>
<td>Group</td>
<td>Comparison P Value</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------</td>
<td>--------------------</td>
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<tr>
<td></td>
<td>Control</td>
<td>NF1</td>
<td>NF1 vs. Control</td>
</tr>
<tr>
<td></td>
<td>(n = 36)</td>
<td>(n = 38)</td>
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</tr>
<tr>
<td>None</td>
<td>32 (89%)</td>
<td>28 (74%)</td>
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<tr>
<td>Past 10 years</td>
<td>4 (11%)</td>
<td>10 (26%)</td>
<td>0.0951</td>
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<tr>
<td>Lifetime</td>
<td>12 (33%)</td>
<td>15 (42%)</td>
<td>0.7891</td>
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Table III. Number of fractures in the past 10 years by skeletal site and group (n = total number of fractures)

<table>
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<th>Fracture site</th>
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<th>NF1 (n = 12)</th>
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<td>Ribs</td>
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<td>Wrist</td>
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<td>Sternum</td>
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