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**Genetic Evaluation for the Scoliosis Gene(s) in Patients with
Neurofibromatosis 1 and Scoliosis**

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14. ABSTRACT Dystrophic or non-dystrophic scoliosis is one of most common skeletal manifestations of Neurofibromatosis type 1. Dystrophic scoliosis has a more progressive and debilitating course than non-dystrophic scoliosis thus requiring in most cases surgical intervention. Experts have recommended early intervention for better outcomes but tools for early detection of dystrophic scoliosis have not been developed. The goal of this study is to develop validated radiographic and genetic tools for early detection of dystrophic or non-dystrophic scoliosis. Early detection will allow physicians to provide more timely interventions and consequently improve outcomes and overall clinical management in patients with Neurofibromatosis type 1. Early detection may also lessen the number of imaging modalities such as radiographs and MRIs, thereby lowering cost of medical management. Work to date has focused on radiographic criteria for dystrophic modulation and validation of this radiographic scoring system. Initial patient recruitment for genetic marker testing has begun.				
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INTRODUCTION

Neurofibromatosis type 1 (NF1) is a common autosomal dominant genetic disorder occurring in 1:4000 worldwide. Scoliosis is perhaps the most common skeletal problem in patients with NF1 with a prevalence of 10-69%. There are two types: dystrophic and non dystrophic scoliosis. Dystrophic scoliosis appears to have a poorer prognosis. Dystrophic changes develop over time and may not necessarily appear at initial presentation. Therefore the development and validation of a radiographic scheme to classify dystrophic scoliosis is needed to aide in distinguishing dystrophic from non dystrophic scoliosis and allow early detection and intervention and is our first objection. The second objective rests on the fact that NF1 has marked variability of clinical expression. There is evidence that other genes may play a role in NF1 expression. Current research has identified candidate genetic SNP markers that can predict progressive and non-progressive curves in Adolescent Idiopathic Scoliosis (AIS) with a high degree of reliability. If the same genetic markers are present in non-dystrophic scoliosis then this will allow earlier, more accurate prognostication, and perhaps improve treatment. Thus our hypothesis is that NF1 patients with non-dystrophic or dystrophic scoliosis have the same genetic markers as patients with AIS.

Table: NINE RADIOGRAPHIC CHARACTERISTICS OF DYSTROPHIC DEFORMITY IN NF1.

Characteristics	% incidence
Rib penciling	62
Vertebral rotation	51
Posterior vertebral scalloping	31
Vertebral wedging	36
Spindling of transverse processes	31
Anterior vertebral scalloping	31
Widened intervertebral foramina	29
Enlarged intervertebral foramina	25
Lateral vertebral scalloping	13

From Durrani AA, Crawford AH, Choudry SN, et al.

Body

NF 1 patients with scoliosis can present as either non dystrophic or dystrophic scoliosis. Non dystrophic scoliosis behave and evolve similarly to that of AIS patients. Therefore, we hypothesize that:

Neurofibromatosis type 1 patients with non-dystrophic scoliosis have a similar curve progression risk profile markers as patients with Adolescent Idiopathic Scoliosis. Dystrophic scoliosis patients will not have the same curve progression risk profile as AIS.

To test this hypothesis this study was divided into two main phases. Phase 1 involves the development and validation of a radiographic scheme to classify radiographic dystrophic changes in patients with NF1 scoliosis. In phase 2 of the study, this validation scheme will be used to distinguish dystrophic vs. non dystrophic scoliosis patients and correlate that with genetic marker testing.

Phase 1:

The aim of the first phase is to development and validation of a scheme to classify dystrophic changes

in patients with NF 1 scoliosis with the goal of creating a validated clinical radiographic grading scheme for the diagnosis dystrophic scoliosis in NF1 patients.

Hypothesis: Radiographic characteristics of dystrophic deformity described by Crawford and Durrani et. al. will distinguish dystrophic scoliosis from non-dystrophic scoliosis.

A checklist of radiographic findings indicating dystrophic curves has been developed. However this has not been validated to date.^[8] Our team has experience in developing and validating spinal radiographic measures with particular expertise in validation of reliability of scoliosis measurements.^[4,7,11,12,13,18,19,20,21,22,27,28,29,30,31] From these radiographs (and from other example images available from participating surgeons' files) the spectrum of severity of these findings will be selected. For each category a severity scale will be developed. Intra- and inter-observer reliability will then be tested and reported.

Analysis Methods

The general objective of this study is to evaluate the operating characteristics of diagnostic procedures, based on radiographs, for dystrophic scoliosis. We are interested in (1) estimating the reliability of between-observer evaluations, and (2) estimating the sensitivity and specificity of radiography based classification relative to the 'gold standard' of a definitive clinical diagnosis.

Reliability

The primary outcome variable of interest is whether a patient's radiograph indicates dystrophic scoliosis. This is a binary outcome. We will quantify the intra-observer reliability for each assessor, using the agreement between each assessor's first and second readings of a given patient radiography. We will also quantify the inter-observer reliability for both the agreement among experts and the agreement between experts and non-experts, using the kappa measure of agreement.

The sample size for the inter-observer reliability assessment was estimated for two situations of interest:

In the first, we are interested in the level of agreement between two experts. We assume that the proportion of agreement will be approximately 70%, and wish to define the level of agreement within a 95% confidence level margin of error of 10%. That is, if the observed proportion of agreement is 70%, we would want the 95% confidence interval for the true proportion of agreement to be (60%, 80%). This will require a sample size of **81 patient radiographs**.

In the second, we are interested in the level of agreement between an expert and a non-expert. We assume that the proportion of agreement will be approximately 50%, and wish to define the level of agreement within a 95% confidence level margin of error of 10%. This necessitates a sample size of **97 patient radiographs**.

Predictive Ability: Sensitivity and Specificity:

First, we will determine how well each of the nine radiographic characteristics alone predicts dystrophic scoliosis using standard diagnostic test criteria of sensitivity and specificity.

Second, we will assess which combinations of the nine characteristics most accurately and precisely predict dystrophic scoliosis using multiple logistic regression, with the known dystrophic status as the binary outcome and the nine radiographic characteristics as binary predictors. From this we will obtain a composite variable which is predictive of dystrophic scoliosis. We will estimate the sensitivity and specificity of this composite logistic predictor, again using the established clinical diagnosis as the gold standard.

The sample size for assessing the sensitivity and specificity of the composite predictor was estimated

assuming that the test sensitivity and specificity will both be 90% and that we would like the 95% exact binomial confidence intervals for each to be (80%, 98%). This will require a sample size of 75 dystrophic patient radiographs and 75 non-dystrophic patient radiographs.

Phase 1 Tasks:

The estimated time to completion of aim 1 is 1.5 years from the official start of this project (August 1, 2010).

To accomplish aim 1 the following tasks and their status are enumerated below:

- a. Preoperative radiographs of patients with dystrophic and non dystrophic scoliosis will be evaluated. All radiographs in film format will be scanned and converted to digital format. Dr. Ledonio and Dr. Polly will collect and initially evaluate the radiographs.
 - Letters to solicit de-identified whole spine radiographs of NF1 patients with scoliosis were sent to 10 spine surgeons who are members of the SDSG. To date a total of 252 radiographs from 123 cases of dystrophic or non dystrophic scoliosis were screened and evaluated by first Dr. Ledonio then by Dr. Polly. One case was excluded for a total of 122 cases. Of which 83 (68%) were dystrophic and 39 (32%) were non dystrophic scoliosis cases.
- b. A grading scheme for severity of each dystrophic factor will be developed by Dr. Crawford and Dr. Polly (see minutes in appendix).
 - On April 21-22, 2011 experts from Texas Scottish Rite, Cincinnati Children's Hospital and Axial Biotech gathered at the Department of Orthopaedic Surgery, University of Minnesota's special grand rounds event to lecture on their experiences on the treatment Neurofibromatosis type 1 patients with scoliosis. This was followed by a study group meeting to discuss and clarify the definitions for the radiographic characteristics of dystrophic scoliosis. The radiographic characteristics agreed upon were as follows:
 1. Short sharp angular curve
 2. Rib Penciling
 3. Vertebral rotation
 4. Vertebral scalloping
 5. Vertebral Wedging
 6. Spindling of transverse processes
 7. Widened interpedicular distance
 8. Atypical location
- c. This grading scheme was reviewed by Drs. Polly, Crawford, Sucato, and Larson for initial face validity.
 - The following day a sample set of the radiographic cases were graded (as present or not present) using each of the above characteristics followed by a determination of either dystrophic or non dystrophic.
- d. A set of images was sent to several scoliosis surgeons for intra- and inter-observer reliability testing to determine generalized reliability.
 - 122 sets of scoliosis radiographs were sent to 5 spine surgeons for grading.
 - Data were then screened, cleaned and entered into a database (appendix) and sent to the statistician for analysis as described previously. The results are as follows:

Statistical Report

Data Set {Program: Ledonio analysis 2011-06-14.sas.}

Spinal x-rays from 122 patients were evaluated independently by 5 orthopedic surgeons ('readers') on the presence or absence of 8 characteristics (e.g. 'rib penciling') and on whether they would diagnose the patient as dystrophic or not. The five surgeons were not aware of the clinical diagnosis for the patients. The resulting dataset contained 5 observations for each of the 122 x-rays or 610 total observations on 9 variables. {File: Radiographic grading database 6-13-11.xls, received in corrected form from Dr. Ledonio on 6-15-11.}

The 'gold standard' clinical diagnosis for each x-ray, made by the patient's surgeon based on clinical data, physical examination, MRI and CT scans, surgical observations and results, as well as the x-ray data, were provided in a separate file. {File: Key NF1 Scoliosis Films.xls, received from Dr. Ledonio on 6-14-11.}

All statistical analysis was carried out using SAS 9.2.

Results

Proportion Dystrophic

Overall, 363 of the 610 readings (59.5%) were deemed dystrophic ('dys'). For a given reader, the proportion deemed dystrophic ranged from 45.1% to 67.2% as shown in the table below. The differences among readers are statistically significant (Pearson's chi-square test, p-value = 0.0060). If the reader with the lowest proportion (Sucato) is excluded, the differences among readers are no longer significant (p-value = 0.7201).

Reader	Frequency No- dystrophic (percent)	Frequency Yes- dystrophic (percent)	Total
Carreon	47 (38.52)	75 (61.48)	122
Crawford	45 (36.89)	77 (63.11)	122
Larson	40 (32.79)	82 (67.21)	122
Polly	48 (39.34)	74 (60.66)	122
Sucato	67 (54.92)	55 (45.08)	122
Total	247 (40.49)	363 (59.51)	610

The *actual* diagnosis was dystrophic for 83 of the 122 x-rays, or 68%. All of the readers underestimated the proportions that were dystrophic.

Accuracy (Sensitivity and Specificity)

A comparison of the actual diagnosis ('dys_true') to the reader's diagnosis ('dys') for the 610 readings is shown in the table below. For the $83 * 5 = 415$ readings on the 83 x-rays that were truly dystrophic, the readers overall were correct only 74.7% of the time, i.e. their overall sensitivity was 74.7%. Similarly, for the 195 readings on x-rays that were truly non-dystrophic, the readers overall were correct only 72.8% of the time, i.e. their overall specificity was 72.8%. The agreement between the true diagnosis and the overall readers' diagnoses, as assessed using the kappa statistic, is 0.44 or 'fair'.

Note that with a sample size of 122 x-rays, the margin of error for both the sensitivity and specificity is about 8%, which is well within the desired precision of 10% used in the original sample size estimate.

Actual diagnosis ↓ (‘dys_true’)	Readers		Total
	No-dystrophic	Yes-dystrophic	
No-dystrophic	142 (72.82%)	53 (27.18%)	195
Yes-dystrophic	105 (25.30%)	310 (74.70%)	415
Total:	247	363	610

Byrt (in *Epidemiology* 1996: 7: 561) proposed these guidelines for interpreting kappa statistics:

0.93 – 1.00	Excellent agreement
0.81 – 0.92	Very good agreement
0.61 – 0.80	Good agreement
0.41 – 0.60	Fair agreement
0.21 – 0.40	Slight agreement
0.01 – 0.20	Poor agreement
≤ 0.00	No agreement

The sensitivity, specificity and agreement with the true diagnosis for each reader is shown in the table below. The agreement with the true diagnosis is ‘fair’ for all readers.

Reader	Sensitivity	Specificity	Agreement with true diagnosis (kappa)
OVERALL	74.7 %	72.8 %	0.44
Carreon	77.1	71.8	0.46
Crawford	77.1	66.7	0.42
Larson	83.1	66.7	0.49
Polly	74.7	69.2	0.41
Sucato	61.5	89.7	0.43

Inter-Observer Reliability

The inter-observer reliability was assessed using Fleiss’ kappa measure of agreement, using the MAGREE macro in SAS and double-checked using the `kappam.fleiss` function in the `irr` package in R. The kappa values for the 8 x-ray characteristics, as well as for the dystrophic diagnosis, for the 122 x-rays read by 5 readers, are shown in the table below. The degree of agreement ranges from ‘poor’ for Vertebral scalloping and Widened interpedicular distance to (just barely) ‘good’ for Vertebral wedging.

Characteristic	Variable name	Fleiss’ kappa
Dystrophic diagnosis	Dys	0.612
Vertebral wedging	Wedge	0.619 - max
Vertebral rotation	Rot	0.589
Sharp angular curve	Curve	0.602
Rib penciling	Pencil	0.414
Vertebral scalloping	Scall	0.140 - min
Widened interpedicular distance	Wide	0.182
Atypical location	Loc	0.276
Spindling of transverse processes	Spind	0.424

The rate at which each characteristic was observed in x-rays deemed dystrophic by a given reader and in x-rays deemed non-dystrophic by a given reader is shown in the table below. The association between each characteristic

and dystrophic diagnosis is highly significant (chi-square test, p-value < 0.0001) for all eight characteristics. The characteristics most often observed in x-rays deemed dystrophic were wedge, rot and curve.

Variable Name	Rate observed in all 610 readings	Rate observed in x-rays deemed dystrophic by a given reader	Rate observed in x-rays deemed non-dystrophic by a given reader
Wedge	61.5 %	90.6 %	18.6 %
Rot	61.2	89.3	19.8
Curve	52.5	84.3	5.7
Pencil	42.8	63.1	13.0
Scall	40.7	57.9	15.4
Wide	36.1	54.8	8.5
Loc	22.3	35.0	3.6
Spind	15.1	23.4	2.8

The rates observed in x-rays that truly were dystrophic vs. non-dystrophic are shown in the second table below. The association between each characteristic and true dystrophic diagnosis is highly significant (chi-square test, p-value < 0.0001) for seven of the eight characteristics, and slightly less significant (p-value = 0.0011) for the eighth (spind).

Variable Name	Rate observed in all 610 readings	Rate observed in truly dystrophic x-rays (sensitivity)	Rate observed in truly non-dystrophic x-rays (1 - specificity)
Wedge	61.5 %	75.9 %	30.8 %
Rot	61.2	76.1	29.2
Curve	52.5	65.3	25.1
Pencil	42.8	54.4	18.0
Scall	40.7	46.8	27.7
Wide	36.1	43.9	19.5
Loc	22.3	29.6	6.7
Spind	15.1	18.3	8.2

The inter-observer reliability was investigated further by counting the number of times a given characteristic was said to be present by the five readers. This count ('sum_dys', 'sum_wedge', etc.) varied from 5 if all 5 readers said the characteristic was present, to 0 if all 5 readers said it was not present. The raw data for agreement on each of the 8 characteristics plus the dystrophic classification are given in the Appendix. The summary tables are shown below.

Dystrophic classification ('dys'): Of the 83 truly dystrophic x-rays, 42 (50.6%) were correctly classified as dystrophic by all five readers. Eight (9.6%) were incorrectly classified non-dystrophic by all five readers. There was some degree of disagreement for the remaining 33 (39.8%) dystrophic x-rays. Similarly, of the 39 non-dystrophic x-rays, 22 (56.4%) were classified correctly by all five readers, four (10.3%) were classified incorrectly by all five readers, and there was some disagreement about the remaining 13 (33.3%).

Number of readers saying			Dystrophic		Total
'Yes'	Dystrophic No	percent	Yes	percent	
0	22	56.41%	8	9.64%	30
1	2	5.13	4	4.82	6
2	5	12.82	6	7.23	11
3	3	7.69	8	9.64	11
4	3	7.69	15	18.07	18
5	4	10.26	42	50.60	46
Total	39	100.00%	83	100.00%	122

Ignoring the true diagnosis, the sum of yes answers for dystrophic diagnosis ranged from 0 (24.6% of readings) to 5 (37.7%) for the 122 x-rays, as shown below.

'dys' sum_yes	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	30	24.59%	30	24.59%
1	6	4.92	36	29.51
2	11	9.02	47	38.52
3	11	9.02	58	47.54
4	18	14.75	76	62.30
5	46	37.70	122	100.00

Vertebral wedging ('wedge'):

dys_true	sum_wedge						
Frequency, Row Pct	0	1	2	3	4	5	Total
N	18	7	3	2	4	5	39
	46.15	17.95	7.69	5.13	10.26	12.82	
Y	9	1	8	7	13	45	83
	10.84	1.20	9.64	8.43	15.66	54.22	
Total	27	8	11	9	17	50	122

'wedge' sum_yes	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	27	22.13	27	22.13
1	8	6.56	35	28.69
2	11	9.02	46	37.70
3	9	7.38	55	45.08
4	17	13.93	72	59.02
5	50	40.98	122	100.00

Vertebral rotation ('rot'):

dys_true	sum_rot						
Frequency, Row Pct	0	1	2	3	4	5	Total
N	18	6	3	5	5	2	39
	46.15	15.38	7.69	12.82	12.82	5.13	
Y	10	2	2	7	21	41	83
	12.05	2.41	2.41	8.43	25.30	49.40	
Total	28	8	5	12	26	43	122

'rot' sum_yes	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	28	22.95	28	22.95
1	8	6.56	36	29.51
2	5	4.10	41	33.61
3	12	9.84	53	43.44
4	26	21.31	79	64.75
5	43	35.25	122	100.00

Sharp angular curve ('curve'):

dys_true	sum_curve						
Frequency, Row Pct	0	1	2	3	4	5	Total
N	24	2	2	3	6	2	39
	61.54	5.13	5.13	7.69	15.38	5.13	
Y	16	1	7	11	17	31	83
	19.28	1.20	8.43	13.25	20.48	37.35	
Total	40	3	9	14	23	33	122

	'curve' sum_yes	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	0	40	32.79	40	32.79
	1	3	2.46	43	35.25
	2	9	7.38	52	42.62
	3	14	11.48	66	54.10
	4	23	18.85	89	72.95
	5	33	27.05	122	100.00

Rib penciling ('pencil'):

	dys_true	sum_pencil					
	Frequency, Row Pct	0,	1,	2,	3,	4,	5, Total
N	, 20 , , 51.28 ,	10 , , 25.64 ,	6 , , 15.38 ,	1 , , 2.56 ,	0 , , 0.00 ,	2 , , 5.13 ,	39
Y	, 11 , , 13.25 ,	12 , , 14.46 ,	16 , , 19.28 ,	14 , , 16.87 ,	10 , , 12.05 ,	20 , , 24.10 ,	83
Total	31	22	22	15	10	22	122

	'pencil' sum_yes	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	0	31	25.41	31	25.41
	1	22	18.03	53	43.44
	2	22	18.03	75	61.48
	3	15	12.30	90	73.77
	4	10	8.20	100	81.97
	5	22	18.03	122	100.00

Vertebral scalloping ('scall'):

	dys_true	sum_scall					
	Frequency, Row Pct	0,	1,	2,	3,	4,	5, Total
N	, 5 , , 12.82 ,	24 , , 61.54 ,	5 , , 12.82 ,	2 , , 5.13 ,	1 , , 2.56 ,	2 , , 5.13 ,	39
Y	, 4 , , 4.82 ,	22 , , 26.51 ,	24 , , 28.92 ,	16 , , 19.28 ,	9 , , 10.84 ,	8 , , 9.64 ,	83
Total	9	46	29	18	10	10	122

	'scall' sum_yes	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	0	9	7.38	9	7.38
	1	46	37.70	55	45.08
	2	29	23.77	84	68.85
	3	18	14.75	102	83.61
	4	10	8.20	112	91.80
	5	10	8.20	122	100.00

Widened interpedicular distance ('wide'):

dys_true	sum_wide						
Frequency, Row Pct	0,	1,	2,	3,	4,	5,	Total
N	16 , 41.03	15 , 38.46	3 , 7.69	3 , 7.69	2 , 5.13	0 , 0.00	39
Y	9 , 10.84	16 , 19.28	29 , 34.94	15 , 18.07	7 , 8.43	7 , 8.43	83
Total	25	31	32	18	9	7	122

'wide' sum_yes	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	25	20.49	25	20.49
1	31	25.41	56	45.90
2	32	26.23	88	72.13
3	18	14.75	106	86.89
4	9	7.38	115	94.26
5	7	5.74	122	100.00

Atypical location ('loc'):

dys_true	sum_loc						
Frequency, Row Pct	0,	1,	2,	3,	4,	5,	Total
N	30 , 76.92	7 , 17.95	0 , 0.00	2 , 5.13	0 , 0.00	0 , 0.00	39
Y	28 , 33.73	18 , 21.69	18 , 21.69	9 , 10.84	8 , 9.64	2 , 2.41	83
Total	58	25	18	11	8	2	122

'loc' sum_yes	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	58	47.54	58	47.54
1	25	20.49	83	68.03
2	18	14.75	101	82.79
3	11	9.02	112	91.80
4	8	6.56	120	98.36
5	2	1.64	122	100.00

Spindling of transverse processes ('spind'):

dys_true	sum_spind						
Frequency, Row Pct	0,	1,	2,	3,	4,	5,	Total
N	31 , 79.49	4 , 10.26	2 , 5.13	1 , 2.56	0 , 0.00	1 , 2.56	39
Y	52 , 62.65	8 , 9.64	10 , 12.05	7 , 8.43	3 , 3.61	3 , 3.61	83
Total	83	12	12	8	3	4	122

'spind' sum_yes	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	83	68.03	83	68.03
1	12	9.84	95	77.87
2	12	9.84	107	87.70
3	8	6.56	115	94.26
4	3	2.46	118	96.72
5	4	3.28	122	100.00

Logistic regression

Logistic regression was carried out in order to determine which combination of x-ray characteristics was best able (despite the lack of agreement among readers) to predict true dystrophic status for the N=610 readings. The log odds of an x-ray being truly dystrophic were modeled as a function of the eight x-ray characteristics listed above (coded as 1 if present and -1 if not). No higher order terms or interaction terms were considered.

When backward elimination was used to determine which characteristics were most predictive of true dystrophic status, four characteristics (spind, curve, wide and scall) were eliminated since they were not significant at the alpha = 0.05 level (table below).

Summary of Backward Elimination

Step	Effect Removed	DF	Number In	Wald Chi-Square	Pr > ChiSq
1	spind	1	7	0.0360	0.8495
2	curve	1	6	0.0631	0.8016
3	wide	1	5	0.3541	0.5518
4	scall	1	4	0.6924	0.4053

The modeling results indicate that four characteristics, pencil, rot, wedge and loc, are strongly associated with true dystrophic status. The odds of an x-ray being truly dystrophic are 2.43 times higher when the reader saw rib penciling ('pencil') than when the reader did not. Similarly the odds of an x-ray being truly dystrophic are 2.97 times higher if the reader saw vertebral rotation ('rot'), 2.37 times higher if he saw vertebral wedging ('wedge') and 3.00 times high if he saw atypical location ('loc'). If the reader saw all four of these characteristics at once, the odds of that x-ray being truly dystrophic are 51 times higher than if he saw none of the four characteristics.

Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	1.1940	0.1708	48.8548	<.0001
pencil Y	1	0.4445	0.1216	13.3687	0.0003
rot Y	1	0.5455	0.1212	20.2577	<.0001
wedge Y	1	0.4310	0.1218	12.5297	0.0004
loc Y	1	0.5488	0.1650	11.0591	0.0009

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits
pencil Y vs N	2.432	1.510 3.917
rot Y vs N	2.977	1.851 4.788
wedge Y vs N	2.368	1.469 3.816
loc Y vs N	2.997	1.569 5.722

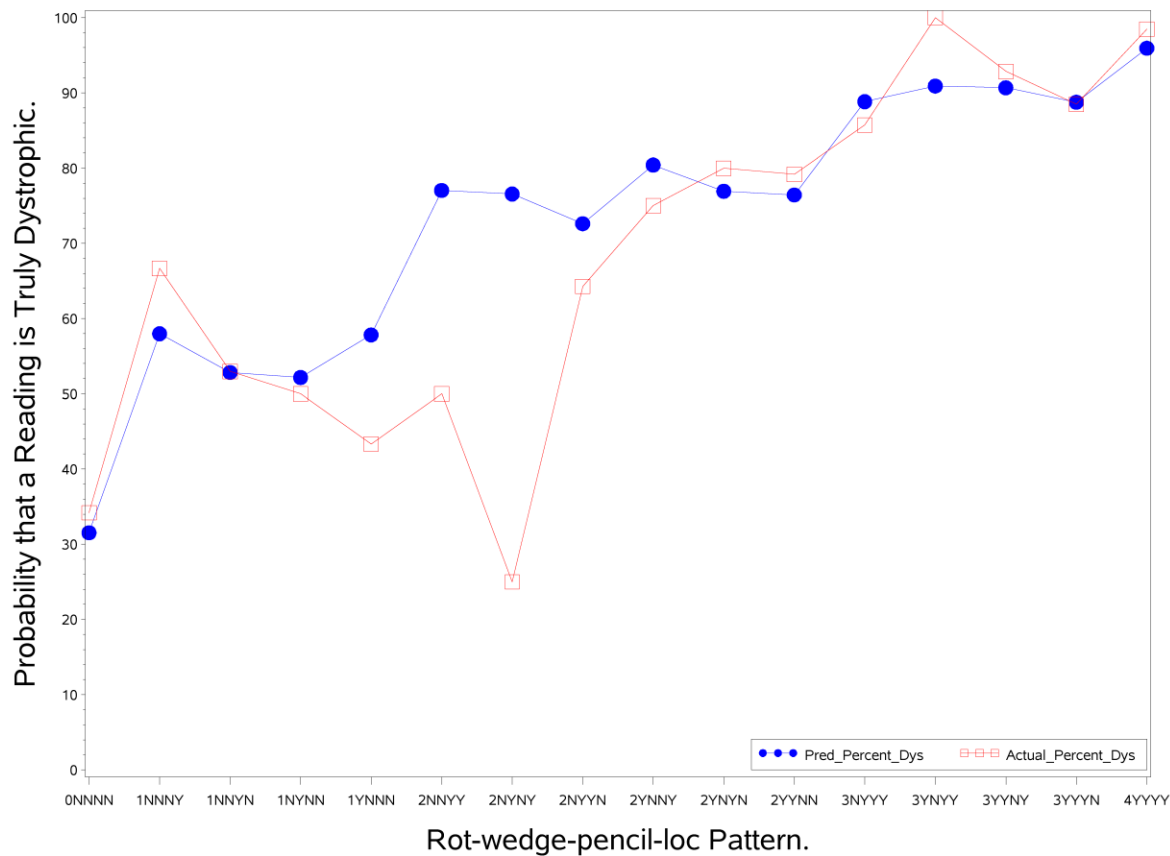
When forward selection was used, the results were identical with the results for backward selection (table below); this gives increased confidence that the chosen four characteristics are likely the ones that really matter. Stepwise selection was also tried, with identical results.

Summary of Forward Selection

Step	Effect Entered	DF	Number In	Score Chi-Square	Pr > ChiSq
1	rot	1	1	122.9014	<.0001
2	wedge	1	2	28.5889	<.0001
3	pencil	1	3	14.1359	0.0002
4	loc	1	4	11.8334	0.0006

The model-predicted probability of being dystrophic (blue dots) and the actual probability of being dystrophic (red squares) are given in the table and figure below, as a function of a created variable called 'sum4_pattern4'. The first digit of this variable gives the number of the four characteristics in the model which were observed in a given reading. The remaining four digits of this variable are NNNN if all four characteristics (rot, wedge, pencil and loc, in that order) were not observed by the reader, YNNN if the reader observed only rot and not the other three characteristics, and so on. So if a reader saw rot and pencil, the pattern variable would be 2YNNY.

Obs	sum4_ pattern4	Pred_ Percent_ Dys	Actual_ Percent_ Dys
1	0NNNN	31.5248	34.194
2	1NNNY	57.9768	66.667
3	1NNYN	52.8273	52.941
4	1NYNN	52.1564	50.000
5	1YNNN	57.8183	43.333
6	2NNYY	77.0428	50.000
7	2NYYN	76.5635	25.000
8	2NYYN	72.6159	64.286
9	2YNNY	80.4213	75.000
10	2YNYN	76.9276	80.000
11	2YYNN	76.4467	79.167
12	3NYYY	88.8225	85.714
13	3YNNY	90.9022	100.000
14	3YYNY	90.6772	92.857
15	3YYYY	88.7578	88.489
16	4YYYY	95.9447	98.462



Keep in mind that since each x-ray was read five times, and the five readings did not always agree, a given x-ray may contribute to as many as five different patterns.

The model predictions are reasonably close to the actual values. The model predicts that the probability of an x-ray being truly dystrophic is about 31% if the reader saw none of these four characteristics. The probability rises to about 52-58% if the reader saw one of the four characteristics, to about 72-80% if he saw two of them, to about 88-91% if he saw three of them, and to about 96% if he saw all four of them.

Phase 2

The aim of phase 2 of this study is to perform genetic testing on patients with NF 1 who have had clinical treatment for scoliosis.

Hypothesis: The curve progression risk profile for AIS is also found in non-dystrophic but not in dystrophic scoliosis.

The samples in Aim #1 would be the same samples with non-dystrophic scoliosis with a known outcome at skeletal maturity. These samples will be collected retrospectively according to inclusion and exclusion criteria and final outcome. The statistical analysis would be a simple comparison to see whether the sensitivity of the genetic panel in NF1 patients with scoliosis is similar to the AIS study (85%). The study will test NF1 patients, in both dystrophic and non dystrophic categories, that have been treated with fusion surgery.

Genotyping:

Genetic testing will be done at Axial Biotech. DNA collection and genotyping of the sample cohorts with 53 single-nucleotide polymorphism (SNP) markers associated with progression to a surgical curve in AIS patients (Table 5). The results of the SNP marker analysis are represented as a numerical score and as high, intermediate or low risk genetic profile for curve progression. The validated scheme in Aim 1 will be used to classify the scoliosis as dystrophic or non dystrophic.

Specifically, two millimeters of saliva is collected in an DNA Genotek (Ottawa, Canada), Oragene OG-300 sample collection kit. DNA samples are extracted from the saliva using MagNA Pure Compact magnetic bead extraction protocols (Roche Applied Sciences, Indianapolis, IN). Genotypes are determined using 53 Taqman™ assays (Applied Biosystems, Inc., Foster City, CA) designed to detect the each SNP. The Taqman assay is an allele discrimination assay using PCR amplification and a pair of fluorescent dye detectors that target each SNP. One fluorescent dye is attached to the detector that is a perfect match to the first allele (e.g. an “A” nucleotide) and a different fluorescent dye is attached to the detector that is a perfect match to the second allele (e.g. a “C” nucleotide). During PCR, the polymerase will release the fluorescent probe into solution where it is detected using endpoint analysis in an Applied Biosystems 7900HT Real-Time instrument. Genotypes are determined using Applied Biosystems automated Taqman genotyping software, SDS v2.3. After genotypes are determined the risk progression score is determined for each patient using a logistic regression algorithm determined during the discovery and validation phases of the original research. All samples and scores are tracked in a Laboratory Information Management System. Testing is done in Axial Biotech’s CLIA/CAP accredited laboratory.

Analysis Methods and Assessment of Data:

The objective of Aim 2 is to evaluate the clinical utility of a set of genetic markers in NF1 patients that have been treated clinically. These genetic markers have previously been validated as markers associated with the development of surgical curves (> 40 degree Cobb angle in a growing spine) in adolescent idiopathic scoliosis patients. This study will attempt to confirm, in NF1 surgical patients with non-dystrophic scoliosis, the 85% sensitivity observed in surgical adolescent scoliosis patients.

Sample Size Determination:

Two cohorts will be collected, NF1 patients with dystrophic scoliosis that have been treated clinically and NF1 patients with non-dystrophic scoliosis that have been treated clinically. A sample size of at least 100 patients is required to evaluate the sensitivity (lower 95% CI = between 0.70 to 0.75). In anticipation of enrollment drop outs we are approved to recruit 140 subjects to meet sample size requirement of 100 patients.

Sample Size Determination

Expected Sensitivity	<u>Minimum Acceptable 95% Lower Confidence Limit</u> Sample size						
	0.50	0.55	0.60	0.65	0.70	0.75	0.80
0.85	18	26	33	52	85	176	624

Phase 2 tasks:

The estimated time to completion of aim 2 is 1.5 years after the end of phase 1.

To accomplish aim 2 the following tasks and their status are enumerated below:

Task 2: Identification, recruitment and informed consent acquisition of 200 NF1 patients with scoliosis from SDSG and NF support groups.

- a. Once identified, letters of invitation to participate in this study together with informed consent form was

sent by Dr. Polly and his staff. The research coordinator at the University of Minnesota will keep track of study participants. Dr. Christopher Moertel was a resource for patient recruitment along with the Spinal Deformity Study Group and Children's Tumor Foundation. Also included was Cincinnati Children's Hospital with Dr. Alvin Crawford as the site-PI.

- During the course of the study approximately 1200 letters were sent to patients diagnosed with NF type 1. Of these 54 qualify for the study. 10 were excluded because they did not meet inclusion criteria. In addition, upon IRB approval we utilized several different social media venues by advertising our study on ClinicalTrials.gov, Children's Tumor Foundation, and The Littlest Tumor Foundation. Midwest Society. Expanding our efforts in this manner allowed us to recruit 11 additional individuals and additional 30 expressing interest. Currently we are awaiting consent letters and samples of 10 additional individuals. Additionally, our collaboration with University of Utah was also used to enroll 19 additional individuals in our study. We have plans to expand our recruitment efforts to other organizations and create presence at events organized by different NF1 foundations. As our study was approved for no cost extension, we will utilize new recruitment methods and increase our efforts in order to reach a proposed 100 study participants.
 - At this point a total of 47 subjects have consented and were enrolled in phase 2 of this study. Their samples are processed by Affiliated Genetics, a company formerly known as Axial Biotech (name change occurred recently).
- b. Once informed consent is obtained participants are referred to Affiliated Genetics (formerly Axial Biotech). Affiliated Genetics sends the participants a buccal swab kits with a self-addressed stamped envelope. Some participants would receive buccal swab kits along with their informed consent in order to expedite the process and decrease a burden on patient by decreasing the amount of involvement necessary to participate in the study. This action has allowed increasing recruitment efforts.
- c. Participants will be asked to swab the inside of their cheeks and to collect DNA sample and mail them back to Affiliated Genetics for genetic testing. They will be guided by written instructions telephone instructions and/or internet video instruction.

Task 3: Perform genetic testing on patients with NF 1 who have had clinical treatment for scoliosis at Affiliated Genetics with Drs. Ogilvie and Ward. (2nd – 3rd years).

- Results of the first 17 swab samples have been reported. Additionally, 30 samples are pending processing by Affiliated Genetics.

Task 4: Preparation of reports, analysis of data and preparation of manuscript (year 4.)

KEY RESEARCH ACCOMPLISHMENTS:

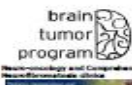
- Collection of a large sample size of de-identified scoliosis radiographs of patients with NF 1 from a multiple centers across the United States.
- Creation of database of radiographic grading for dystrophic scoliosis for 122 sets of scoliosis radiographs 68% of which are dystrophic and 32% are non-dystrophic.
- For 415 readings on the 83 x-rays that were truly dystrophic, the overall sensitivity was 74.7%. Similarly, for the 195 readings on x-rays that were truly non-dystrophic, the overall specificity was 72.8%. The agreement between the true diagnosis and the overall readers' diagnoses, as assessed using the kappa statistic, is 0.44 or 'fair'.
- The degree of agreement for the 8 radiographic characteristics for dystrophic scoliosis ranges from 'poor' for Vertebral scalloping and Widened interpedicular distance to 'good' for Vertebral wedging.

- The association between each characteristic and dystrophic diagnosis is highly significant (chi-square test, p -value < 0.0001) for all eight characteristics. The characteristics most often observed in x-rays deemed dystrophic were vertebral wedging, vertebral rotation and sharp angular curve.
- The modeling results indicate that four characteristics, pencil, rot, wedge and loc, are strongly associated with true dystrophic status. The odds of an x-ray being truly dystrophic are 2.43 times higher when the reader saw rib penciling ('pencil') than when the reader did not. Similarly the odds of an x-ray being truly dystrophic are 2.97 times higher if the reader saw vertebral rotation ('rot'), 2.37 times higher if he saw vertebral wedging ('wedge') and 3.00 times high if he saw atypical location ('loc'). If the reader saw all four of these characteristics at once, the odds of that x-ray being truly dystrophic are 51 times higher than if he saw none of the four characteristics. To put it another way, the model predicts that the probability of an x-ray being truly dystrophic is about 31% if the reader saw none of these four characteristics. The probability rises to about 52-58% if the reader saw one of the four characteristics, to about 72-80% if he saw two of them, to about 88-91% if he saw three of them, and to about 96% if he saw all four of them.

REPORTABLE OUTCOMES:

As a result of phase 1 efforts, four abstracts were accepted as poster presentations at the IMAST and CTF annual meetings. (See appendix)

Poster for CTF annual meeting 2012:



Neurofibromatosis Type I and Scoliosis: A Multicenter Study to Determine Radiographic Predictors of Dystrophic Scoliosis

Moertel, Christopher L.; Loder, Charles Gerald T.; Poffy Jr., David W.; Bearer, Ann M.; Crawford, Alvin H.; Sucato, Daniel J.; Carroon, Leah Y.; Larson, A. Noelle; Stevenson, David A.; Vitale, Michael G.

1. University of Minnesota, Minneapolis, MN; 2. Cincinnati Children's Hospital, Cincinnati, OH; 3. Texas Scottish Rite Hospital for Children, Dallas, TX; 4. Norton Leatherman Spine Center, Louisville, KY; 5. Mayo Clinic, Rochester, MN; 6. University of Utah, Salt Lake City, UT; 7. Columbia University Medical Center, New York, NY

This study was funded by a research grant from: DOD Neurofibromatosis Investigation-Related Research Award (#W81XWH-05-1-0488)

Neurofibromatosis Type I and Scoliosis: A Multicenter Study to Determine Radiographic Predictors of Dystrophic Scoliosis

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This study was funded by a research grant from: DOD Neurofibromatosis Investigation-Related Research Award (#W81XWH-05-1-0488)

INTRODUCTION

Scoliosis in Neurofibromatosis type I: Dystrophic or non-dystrophic?

- Nondystrophic and dystrophic
- Most common osseous defect
- 2% of pts with scoliosis will have NF-1
- 30% of patients with NF-1 have spine disorders
- Dystrophic scoliosis features (Crawford 2004, 2007)

Natural History

- Carver et al. JUS 87 1989
 - Treated (n=4) and untreated (n=22) w/ NF-1 scoliosis
 - 75% untreated group had kyphoscoliosis
 - Severe anterior scalloping - progressed 22°/yr
 - All others 7°/yr progression and 8°/yr of kyphosis
- Miles et al. Spine 1994
 - Vertebral subluxation, disc wedging and peripheral vascular dysplasia
 - prognostic factors that predict progression after resection

Certain radiographic characteristics have been reported to predict dystrophic scoliosis, but their predictive value is not well described. It is unclear which set of radiographic features are most predictive of dystrophic scoliosis and will stand up in a robust statistical model. This study aims to determine which combination of x-ray characteristics was best able to predict true dystrophic status.

EXAMPLES

Sharp angular curve



Dural ectasia; vertebral scalloping



RESULTS

Logistic regression analysis modeling: backward, forward and stepwise elimination

Modeling indicates that rib penciling; vertebral rotation, vertebral wedging and atypical location are strongly associated with dystrophic status (p -values < 0.001). The other four characteristics were not significantly associated with dystrophic status, given the presence of the first four characteristics in the model (p -values > 0.4).

- Spinning of transverse processes
- Short sharp angular curve
- Widened interpedicular space
- Vertebral scalloping
- $p > 0.05$

Strong predictors of dystrophic scoliosis:

- Rib penciling
- Vertebral rotation
- Vertebral wedging
- Atypical location
- $p < 0.05$

The odds of an x-ray being dystrophic were 2.43 times higher when rib penciling was present; vertebral rotation - 2.97, vertebral wedging - 2.37, & atypical location 3.00

If all 4 characteristics patterns were present there would be a 51 times higher risk of dystrophic curve pattern.

Model summary

- The model predicts that the probability of an x-ray being truly dystrophic is about 31% if the reader saw none of these four characteristics.
- The probability rises to about 52-58% if the reader saw one of the four characteristics, to about 72-80% if he saw two of them, to about 88-91% if he saw three of them, and to about 96% if he saw all four of them.

METHODOLOGY

Study Design: Scoliosis radiographs of 122 NF1 patients from multiple institutions were graded by NF-experienced spine surgeons as dystrophic or non-dystrophic based on eight radiographic characteristics: vertebral wedging, vertebral rotation, sharp angular curve, rib penciling, vertebral scalloping, widened interpedicular distance, atypical location, and spinning of transverse processes. Of the 122 cases, 83 (68%) were classified by the contributing institution as dystrophic and 39 (32%) were classified as non-dystrophic. Logistic regression was used to model the odds of an x-ray being dystrophic as a function of the 8 radiographic characteristics. Backward elimination, forward elimination, and stepwise selection were used to determine which characteristics were most predictive of dystrophic status.

Eight Radiographic characteristics of Dystrophic scoliosis

- Vertebral wedging
- Vertebral rotation
- Sharp angular curve
- Rib penciling
- Vertebral scalloping
- Widened interpedicular distance
- Atypical location
- Spinning of transverse processes

The 'gold standard' clinical diagnosis for each x-ray, made by the patient's surgeon based on clinical data

Combination of Hx, PE, MRI and CT scans, surgical observations and results.

RESULTS

The actual diagnosis was dystrophic for 83 of the 122 x-rays, or 68%, and 39(32%) were non-dystrophic.

Readers underestimated the proportion that were dystrophic.

For a given reader, the proportion deemed dystrophic ranged from 45.1% to 87.2% as shown in the table below. The differences among readers are statistically significant (Pearson's chi-square test, p -value = 0.0006). If the reader with the lowest proportion (37%) is excluded, the difference among readers are no longer significant (p -value = 0.7291).

Reader	Frequency Non-dystrophic (n=39)	Frequency Dystrophic (n=83)
1	47	25
2	43	27
3	42	42
4	42	42
5	46	34
6	46	36
7	37	45
8	37	45
Total	327	405

All 8 characteristics are strongly associated with dystrophic scoliosis ($p < 0.002$).

The association is strongest for atypical location (OR=4.45) and weakest (not significant) for scalloping (OR=1.59).

Characteristic	Sensitivity	Specificity	Relative Risk* (95% CI)
Vertebral rotation	91.1%	70.8%	2.46 (1.68 - 3.58)
Vertebral wedging	79.2%	69.2%	2.47 (1.69 - 3.63)
Sharp angular curve	89.3%	78.5%	2.66 (1.82 - 3.94)
Rib penciling	84.4%	62.0%	1.65 (1.12 - 2.35)
Vertebral scalloping	86.3%	70.3%	1.68 (1.12 - 2.52)
Widened interpedicular distance	41.5%	30.5%	2.25 (1.48 - 3.43)
Atypical location	81.6%	66.8%	4.45 (3.14 - 6.15)
Spinning of transverse processes	88.0%	58.6%	2.22 (1.34 - 3.74)

*Risk of disease: using the traditional method in determining x-rays vs. non-dystrophic x-rays.

Discussion: Dystrophic Modulation

- Curran et al. Spine 2000
 - Modulation occurred 65% of patients
 - Modulation occurred in 51% of patients scoliosis presented before 7 years and 20% after 7 years
 - Rib penciling only factor influenced progression
 - Progression rate: scoliosis 12° and kyphosis 8°
- Dystrophic modulation may explain underestimation of dystrophic diagnosis by 5 readers.

CONCLUSION

Only four of the 8 classic radiographic findings of dystrophic scoliosis are most predictive. Further research to predict dystrophic curve patterns should focus on these radiographic markers.

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University of Minnesota
Division of the Center

Abstract #1

TITLE: Neurofibromatosis type I with Dystrophic Scoliosis: A Multicenter Inter-observer Reliability Study of Radiographic Characteristics

AUTHORS (LAST NAME, FIRST NAME):

Ledonio, Charles Gerald T.1; Polly, David W.1; Brearley, Ann M.1; Crawford, Alvin H.2; Sucato, Daniel J.3; Carreon, Leah Y.4; Larson, A. Noelle5; Stevenson, David6; Vitale, Michael G.7; Moertel, Christopher L.1

INSTITUTIONS (ALL):

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ABSTRACT BODY:

Summary (80 words max): This multicenter radiographic assessment study has shown that there is good reliability to detect dystrophic scoliosis in NF1 patients by assessing radiographic characteristics of dystrophic modulation.

Introduction: Scoliosis in patients with Neurofibromatosis type I (NF1) can manifest as dystrophic or non-dystrophic. In contrast to nondystrophic, dystrophic scoliosis is rapidly progressive making treatment challenging. 8 radiographic characteristics have been reported to predict dystrophic scoliosis, but the inter-observer reliability is not well described. Rating systems should have high inter-rater reliability to be generalizable. Careful validation of these predictive factors may facilitate early detection and timely treatment intervention to improve outcomes. The purpose of this study is to assess the inter-observer reliability of 8 radiographic characteristics of dystrophic modulation in NF1.

Methods: Scoliosis xrays of 122 NF1 patients from multiple institutions across the United States were graded by 5 spine surgeons as dystrophic or non-dystrophic, based on 8 radiographic characteristics of dystrophic modulation: wedging, rotation, sharp angular curve, rib penciling, scalloping, widened interpedicular distance, atypical location, and spindling transverse processes. The curves were classified by each submitting institution as dystrophic or non-dystrophic. Inter-observer reliability analysis was performed using Fleiss' kappa.

Results: Of the 122 cases, 83(68%) were classified by the contributing institution as dystrophic and 39(32%) were classified as non-dystrophic. The agreement beyond chance among the 5 readers for the overall dystrophic diagnosis was 0.61(good). The agreement beyond chance for each radiographic characteristic ranges from 0.62 for wedging to 0.14 (poor) for scalloping(Table 1). For dystrophic diagnosis, all 5 readers agreed that a case was dystrophic in 46 of 122 cases, and non-dystrophic in 30 of 122 cases, but there was some disagreement in 46 cases. For wedging, where the agreement was 'good', the readers completely agreed more than half of the time. In contrast, where the agreement was 'poor', the readers disagreed in nearly all the cases.

Conclusion: Overall dystrophic diagnosis can be reliably assessed by radiographic characteristics. Some radiographic characteristics, such as wedging, can be reliably assessed with good agreement. The agreement on other characteristics, such as scalloping, is poor.

Table 1. Kappa statistics

Characteristic	kappa
Dystrophic diagnosis	0.612
Vertebral wedging	0.619
Sharp angular curve	0.602
Vertebral rotation	0.589
Spindling of transverse processes	0.424
Rib penciling	0.414
Atypical location	0.276
Widened interpedicular distance	0.182
Vertebral scalloping	0.140

Abstract #2

TITLE: Neurofibromatosis type 1 and Dystrophic Scoliosis: A Multicenter Study of Accuracy of Surgeons' Radiographic Assessment

AUTHORS (LAST NAME, FIRST NAME):

Ledonio, Charles Gerald T.1; Polly, David W.1; Brearley, Ann M.1; Larson, A. Noelle5; Sucato, Daniel J.3; Carreon, Leah Y.4; Crawford, Alvin H.2; Stevenson, David6; Vitale, Michael G.7; Moertel, Christopher L.1

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ABSTRACT BODY:

Summary (80 words max): Experienced spine surgeons reviewed 122 scoliosis radiographs of NF1 patients and to establish the predictive value of 8 factors classically associated with a dystrophic scoliosis. All 8 factors were significantly associated with dystrophism, some more sensitive or more specific than others.

Introduction: Scoliosis in NF1 patients can manifest as dystrophic or non-dystrophic. Early detection and subsequent intervention may provide better outcomes. Certain radiographic characteristics are associated with dystrophism but their predictive value has not been well-described. This study aims to determine the accuracy of radiographic assessment of dystrophic modulation in NF1 patients with scoliosis.

Methods: Scoliosis radiographs of 122 NF1 patients from multiple institutions were graded by 5 spine surgeons as dystrophic or non-dystrophic based on 8 radiographic characteristics: wedging, rotation, short sharp angular curve, rib penciling, scalloping, wide interpedicular distance, atypical location, and transverse processes spindling. Of 122 cases, 83(68%) were classified by contributing institution as dystrophic and 39(32%) as non-dystrophic(used as reference standard). Sensitivity and specificity were calculated for the overall assessment and for each characteristic. The association between each characteristic and dystrophic scoliosis was tested using chi-square and quantified as a relative risk (RR).

Results: For the overall assessment, the readers concurred with the assessment of dystrophic scoliosis with a sensitivity of 75% (310/415reads). Similarly, the readers correctly assessed non-dystrophic scoliosis for specificity of 73%(142/195). Positive predictive value 85% and negative predictive value was 57%. Among readers, the sensitivity ranged from 61% to 83% and the specificity from 67% to 90%. For the 8 radiographic characteristics individually, sensitivity ranges from 18% for spindling to 76% for rotation, and the specificity ranges from 69% for wedging to 93% for atypical location. All 8 characteristics are strongly associated with dystrophic scoliosis ($p<0.002$). The association is strongest for atypical location ($RR=4.45$) and weakest, (still significant) for scalloping ($RR=1.9$).

Conclusion: 8 radiographic characteristics were significantly associated with dystrophic modulation in NF1 patients with scoliosis. Wedging and rotation were most sensitive, atypical location and transverse processes spindling were most specific. On balance, atypical location and rib penciling had the strongest association with dystrophic scoliosis.

Table 1

Characteristic	Sensitivity	Specificity	Relative Risk* (95% CI)
Vertebral rotation	76.1 %	70.8 %	2.60 (2.08 – 3.26)
Vertebral wedging	75.9	69.2	2.47 (1.98 – 3.07)
Sharp angular curve	65.3	74.9	2.60 (2.02 – 3.34)
Rib penciling	54.4	82.0	3.03 (2.22 – 4.15)
Vertebral scalloping	46.8	72.3	1.69 (1.32 – 2.17)
Widened interpedicular distance	43.9	80.5	2.25 (1.66 – 3.05)
Atypical location	29.6	93.3	4.45 (2.58 – 7.67)
Spindling of transverse processes	18.3	91.8	2.23 (1.34 – 3.72)

*Risk of a rater seeing the indicated characteristic in dystrophic x-rays vs. in non-dystrophic x-rays.

Abstract #3

TITLE: Neurofibromatosis Type I and Scoliosis: A Multicenter Study to Determine Radiographic Predictors of Dystrophic Scoliosis

AUTHORS (LAST NAME, FIRST NAME):

Ledonio, Charles Gerald T.1; Polly, David W.1; Brearley, Ann M.1; Larson, A. Noelle3; Sucato, Daniel J.2; Crawford, Alvin H.4; Carreon, Leah Y.5; Stevenson, David6; Vitale, Michael G.7; Moertel, Christopher L.1

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ABSTRACT BODY:

Summary (80 words max): Dystrophic scoliosis in NF1 patients can be best predicted by the following radiographic findings – vertebral wedging, rotation, rib penciling, and atypical curve location. If all four factors are present, there is a 51 times increased risk of a dystrophic curve.

Introduction: Scoliosis in Neurofibromatosis type I (NF1) can manifest as non-dystrophic or dystrophic, which can cause rapid progressive deformity. It is unclear which set of radiographic features are most predictive of dystrophic scoliosis and will stand up in a robust statistical model.

Methods: Scoliosis radiographs of 122 NF1 patients from multiple institutions were graded by five fellowship trained spine surgeons as dystrophic or non-dystrophic based on eight radiographic characteristics: vertebral wedging, vertebral rotation, sharp angular curve, rib penciling, vertebral scalloping, widened interpedicular distance, atypical location, and spindling of transverse processes. Of the 122 cases, 83 (68%) were classified by the contributing institution as dystrophic and 39 (32%) were classified as non-dystrophic. Logistic regression was used to model the odds of an x-ray being dystrophic as a function of the 8 radiographic characteristics. No other predictors, higher order terms or interactions were considered. Backward elimination, forward elimination, and stepwise selection were used to determine which characteristics were most predictive of dystrophic status.

Results: Modeling indicates that rib penciling, vertebral rotation, vertebral wedging and atypical location are strongly associated with dystrophic status (p-values < 0.001). The other four characteristics were not significantly associated with dystrophic status, given the presence of the first four characteristics in the model (p-values > 0.4). The odds of an x-ray being dystrophic were 2.43 times higher when rib penciling was present (Table 1). Similarly, the odds ratio for dystrophic curves were: vertebral rotation – 2.98, vertebral wedging – 2.37, atypical location 3.00. If all 4 characteristics patterns were present there would be a 51 times higher risk of dystrophic curve pattern.

Conclusion: Only four of the 8 classic radiographic findings of dystrophic scoliosis are most predictive. Further research to predict dystrophic curve patterns should focus on these radiographic markers.

Table 1. Odds ration of radiographic characteristics

Characteristic	Odds Ratio (95% CI)
Vertebral rotation	2.98 (1.85 – 4.79)
Vertebral wedging	2.37 (1.47 – 3.82)
Rib penciling	2.43 (1.51 – 3.92)
Atypical location	3.00 (1.57 – 5.72)

Abstract Publications and Presentations:

1. Charles Gerald T. Ledonio, MD; David W. Polly, MD; Ann M. Brearley, PhD; Alvin H. Crawford, MD; Daniel J. Sucato, MD, MS; Leah Y. Carreon, MD,MSc; A. Noelle Larson, MD; David Stevenson; Michael G. Vitale, MD, MPH; Christopher L. Moertel, MD. Neurofibromatosis Type I with Dystrophic Scoliosis: A Multicenter Inter-Observer Reliability Study of Radiographic Characteristics [abstract]. In: 19th International Meeting on Advanced Spine Techniques (IMAST); 2012, July 18-21; Istanbul, TURKEY: IMAST; 2012. Final Program, Abstract nr 534. E-poster
2. Charles Gerald T. Ledonio, MD; David W. Polly, MD; Ann M. Brearley, PhD; A. Noelle Larson, MD; Daniel J. Sucato, MD, MS; Alvin H. Crawford, MD; Leah Y. Carreon, MD,MSc; David Stevenson; Michael G. Vitale, MD, MPH; Christopher L. Moertel, MD. Neurofibromatosis Type I and Scoliosis: A Multicenter Study to Determine Radiographic Predictors of Dystrophic Scoliosis [abstract]. In: 19th International Meeting on Advanced Spine Techniques (IMAST); 2012, July 18-21; Istanbul, TURKEY: IMAST; 2012. Final Program, Abstract nr 545. E-poster
3. Charles Gerald T. Ledonio, MD; David W. Polly, MD; Ann M. Brearley, PhD; A. Noelle Larson, MD; Daniel J. Sucato, MD, MS; Leah Y. Carreon, MD, MSc; Alvin H. Crawford, MD; David Stevenson; Michael G. Vitale, MD, MPH; Christopher L. Moertel, MD. Neurofibromatosis Type 1 and Dystrophic Scoliosis: A Multicenter Study of Accuracy of Surgeons' Radiographic [abstract]. In: 19th International Meeting on Advanced Spine Techniques (IMAST); 2012, July 18-21; Istanbul, TURKEY: IMAST; 2012. Final Program, Abstract nr 549. E-poster
4. Charles Gerald T. Ledonio, MD; David W. Polly, MD; Ann M. Brearley, PhD; A. Noelle Larson, MD; Daniel J. Sucato, MD, MS; Leah Y. Carreon, MD, MSc; Alvin H. Crawford, MD; David Stevenson; Michael G. Vitale, MD, MPH; Christopher L. Moertel, MD. Neurofibromatosis Type 1 and Dystrophic Scoliosis: A Multicenter Study of Accuracy of Surgeons' Radiographic [abstract]. In: 19th International Meeting on Advanced Spine Techniques (IMAST); 2012, July 18-21; Istanbul, TURKEY: IMAST; 2012. Final Program, Abstract nr 549. E-poster
5. David Polly, M.D., Charles Ledonio, M.D., Christopher Moertel, MD Neurofibromatosis Type I and Scoliosis: A Multicenter Study to Determine Radiographic Predictors of Dystrophic Scoliosis [Podium presentation]. In: International Congress on Early Onset Scoliosis and Growing Spine (ICEOS); 2012, November 15-16; Dublin, Ireland.
6. Christopher Moertel, MD; David Polly, M.D; Charles Ledonio, M.D., *Radiographic Assessment Reliability of Dystrophic Modulation in NF1 Patients with Scoliosis* [Podium Presentation]. In: Children's Tumor Foundation, UMN Symposium; Minneapolis, Minnesota (MN), May 16 2012.
7. Ledonio, Charles Gerald T.; Polly, David W.; Brearley, Ann M.; Crawford, Alvin H.; Sucato, Daniel J.; Carreon, Leah Y.; Larson, A. Noelle; Stevenson, David; Vitale, Michael G.; Moertel, Christopher L.

Neurofibromatosis type I with Dystrophic Scoliosis: A Multicenter Inter-observer Reliability Study of Radiographic Characteristics. (Poster# 298) Global Spine Congress, April 4-6, 2013. Hong Kong.

8. Ledonio, Charles Gerald T.; Polly, David W.; Brearley, Ann M.; Crawford, Alvin H.; Sucato, Daniel J.; Carreon, Leah Y.; Larson, A.Noelle; Stevenson, David; Vitale, Michael G.; Moertel, Christopher L. Neurofibromatosis type 1 and Dystrophic Scoliosis: A Multicenter Study of Accuracy of Surgeons' Radiographic Assessment. (Poster# P299) Global Spine Congress, April 4-6, 2013. Hong Kong.
9. Ledonio, Charles Gerald T.; Polly, David W.; Brearley, Ann M.; Crawford, Alvin H.; Sucato, Daniel J.; Carreon, Leah Y.; Larson, A. Noelle; Stevenson, David; Vitale, Michael G.; Moertel, Christopher L. Neurofibromatosis type 1 and Dystrophic Scoliosis: A Multicenter Study of Accuracy of Surgeons' Radiographic Assessment. Poster, NASS Value Award nominee. *North American Spine Society (NASS)* 28th Annual Meeting. October 9-12, 2013. New Orleans, Louisiana.

CONCLUSION:

No conclusions have been made at this juncture. We are approved for no cost extension as we would like to reach our recruitment goal of 100 individuals. Letter of approval is included with this document in the attachments.

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APPENDICES

Grading sheet

Name:				Date:							
Instructions: 1) Enter the ID of each radiograph. 2) Write a check mark or "Y" for each characteristic that is present for each radiograph.											
	<u>Xray ID#</u>	<u>Dystrophic Deformity</u>	<u>Sharp angular curve</u>	<u>Rib Penciling</u>	<u>Vertebral Rotation</u>	<u>Vertebral scalloping</u>	<u>Vertebral Wedging</u>	<u>Spindling of transverse processes</u>	<u>Widened interpedicular distance</u>	<u>Atypical location</u>	
1											
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E-posters Abstract #1

Neurofibromatosis type 1 with Dystrophic Scoliiosis: A Multicenter Inter-observer Reliability Study of Radiographic Characteristics

Lindholm, Charles David M¹, Pelly, David M¹, Benavise, Amy M¹, Choudhry, Anil M¹, Sussler, David J¹, Carlson, Leah T¹, Lissner, A. Todd², Shewman, David A³, Vignar, William J¹, Marder, Christopher L¹

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Abstract: The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

E-Poster #54: NF1 & Dystrophic scoliosis: A Multicenter Inter-observer

Presented by: Charles Lindholm, MD, PhD, University of Colorado, Aurora, CO, United States

Abstract: The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

Scoliosis in Neurofibromatosis type1: Dystrophic or non-dystrophic

Abstract: The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

Natural History

Abstract: The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

Radiographic characteristics of dystrophic scoliosis

Abstract: The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

Objective

The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

Materials and Methods

Abstract: The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

Inter-observer

Abstract: The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

Results

Abstract: The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

Actual Dystrophic diagnosis

Variable Name	Rate observed in 122 cases (n=122)	Rate observed in 30 cases (n=30)	Rate observed in 45 cases (n=45)
Neural wedging	81.2%	70.0%	80.0%
Neural rotation	81.2%	70.0%	80.0%
Sharp angular curve	81.2%	70.0%	80.0%
Rib penciling	42.8%	30.0%	40.0%
Neural interpedicular distance	42.8%	30.0%	40.0%
Apical location	36.1%	30.0%	40.0%
Spinal location	22.9%	30.0%	40.0%
Spinal location	18.1%	30.0%	40.0%

Discussion: Dystrophic Modulation

Abstract: The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

Summary

Abstract: The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

Thank you

Abstract: The purpose of this study is to assess the inter-observer reliability of a radiographic characteristics of dystrophic modulation in NF1.

Abstract #2

[illegible][illegible]

Scoliosis in Neurofibromatosis type1:
Dystrophic or non-dystrophic

- Nondystrophic and dystrophic
- Most common osseous defect
- 2% of pts with scoliosis will have NF-1
- 30% of patients with NF-1 have spine disorders
- Dystrophic more severe

Classified OGN 2007

Natural History

- **Calder et al. 2012, 2013**
 - **Transcribed and sequenced 1002 of 1007 individuals**
 - This is a **large group** of **hybrids**
 - **Genetic analysis** suggesting **hybridization** 20% to
 - All **show 7% hybridization** and **6% of hybrids**
- **Wolfe et al. 2014**
 - **Genetic analysis** the **weight and growth** of **hybrids** **program** **before the hybrid**
 - **program** **the hybrids**



Hybrid percentage 5 to 10 degrees

University of Minnesota

[illegible]

Objective

This study aims to determine the accuracy of radiographic assessment of dystrophic modulation in NF1 patients with scoliosis.

[illegible]

Results

- The actual diagnosis was dyspeptic for 63 of the 122 (51%), or 60% and 39(32%) were non-dyspeptic
- Readers underestimated the proportions that were dyspeptic.

Reader	Frequency Dyspeptic (percent)	Frequency Dyspeptic (percent)
1	46 (38%)	51 (41%)
2	46 (38%)	77 (63%)
3	46 (38%)	42 (34%)
4	46 (38%)	76 (62%)
5	47 (39%)	66 (54%)
Total	247 (20%)	362 (29%)

Results			
	Reader	Sensitivity	Specificity
• Dystrophic scoliosis: Sensitivity of 75% (310/415 reads)	OVERALL	74.7 %	72.0 %
• Non-dystrophic: Specificity of 79% (142/195 reads).	1	77.1 %	71.8
• Positive predictive value = 85%	2	77.1 %	66.7
• Negative predictive value = 57%.	3	83.3 %	66.7
	4	74.7 %	69.2
	5	61.5 %	66.7

Results

- All fit characteristics are strongly associated with *log10*(scale) ($p < 0.0005$).
- The association is strongest for atypical locations (30–45) and weakest, (but significant) for scaling (30–2.50).

Characteristic	Specificity	Sensitivity	Relative AUC ^a (95%CI)
Location: center	75.2	72.0	2.49 (2.00–3.09)
Location: edge	77.6	69.2	2.47 (2.00–3.05)
Scale: upper 10%	80.3	76.9	2.50 (2.02–3.04)
Scale: lower 10%	80.3	76.9	2.50 (2.02–3.04)
Scaling: typical	44.0	73.2	1.39 (1.14–1.71)
Scaling: atypical	44.0	73.2	1.39 (1.14–1.71)
Location: intermediate distance	45.9	85.5	2.24 (1.84–2.75)
Aggregation:	70.3	76.9	2.44 (2.02–2.97)
Scaling: homomorphism	38.1	76.9	2.22 (1.84–2.70)

Note: a score being less than 1 indicates that a specific scale is associated with a specific location.

Discussion: Dystrophic Modulation

- *Dumortier et al, Spine 2000*
 - Modulation occurred 65% of patients
 - Modulation occurred in 51% of patients scoliosis presented before 7 years and 25% after 7 years
 - Rib penciling: only factor influenced progression
 - Progression rate: scoliosis 12° and kyphosis 8°
- Dystrophic modulation may explain underestimation of dystrophic diagnosis by S raters.

Summary

- The 8 radiographic characteristics were significantly associated with dystrophic modulation in NF1 patients with scoliosis.
- Wedging and rotation were most sensitive, atypical location and transverse processes spindling were most specific.
- On balance, atypical location and rib perching had the strongest association with dystrophic scoliosis.

[illegible]

Neurofibromatosis Type I and Scoliosis: A Multicenter Study to Determine Radiographic Predictors of Dysmorphic Scoliosis

Jefferys, Charles David M^{1,2}, Dally, David M¹, Brackley, Ann M¹, Jackson, A. Noel³, Rogers, David A⁴, Connor, Lisa T⁴, Connors, Anne M⁴, Stevenson, David A⁴, Vlastakis, William J⁵, Marder, Christopher L⁶

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E-Poster # 545: NF1 & Scoliosis: Multicenter Radiographic Predictors

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 4. **Co-Presenters:** Anne M. Cholewicki, MD
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 8. **Co-Presenters:** Christopher J. Leung, MD

9. **Co-Presenters:** William H. Phipps, MD, PhD
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 12. **Co-Presenters:** William H. Phipps, MD, PhD
 13. **Co-Presenters:** William H. Phipps, MD, PhD
 14. **Co-Presenters:** William H. Phipps, MD, PhD
 15. **Co-Presenters:** William H. Phipps, MD, PhD
 16. **Co-Presenters:** William H. Phipps, MD, PhD
 17. **Co-Presenters:** William H. Phipps, MD, PhD
 18. **Co-Presenters:** William H. Phipps, MD, PhD
 19. **Co-Presenters:** William H. Phipps, MD, PhD
 20. **Co-Presenters:** William H. Phipps, MD, PhD

Abstract: The purpose of this study was to determine the radiographic predictors of scoliosis in patients with NF1. A multicenter study was conducted involving 10 centers. The study included 100 patients with NF1 and 100 controls. The patients were divided into two groups: those with scoliosis and those without. The controls were divided into two groups: those with scoliosis and those without. The radiographic predictors of scoliosis were determined by comparing the two groups. The results of the study are as follows:

- 1. Scoliosis was present in 50% of the patients with NF1 and 20% of the controls.
- 2. The radiographic predictors of scoliosis in patients with NF1 were:

Scoliosis in Neurofibromatosis type1: Dystrophic or non-dystrophic			
	Lise A. Fenderson et al (1)		
	Number	Range	Age (yr)
• Nondystrophic and dystrophic			
• Most common osseous defect			
• 2% of pts with scoliosis will have NF-1			
• 30% of patients with NF-1 have spine disorders			
• Dystrophic more severe			

Natural History

- Colwell et al., JGIM 1992
 - Treated (JGIM) and untreated (JGIM) at 10% incidence
 - 70% untreated group had high cholesterol
 - Serum uric acid: untreated - 5.0 mg/dl; treated 3.7 mg/dl
 - 32.6% in progression and 0% in regression
- Wilson et al., Spine 1994
 - Vertebral fractures: the average incidence among patients with asymptomatic progressive degenerative changes before the onset of symptoms after vertebroplasty



Spinal progression 5 to 10 degrees

**University of Minnesota
Division of Endocrinology**

Radiographic characteristics of dysphagic scoliosis

- Certain radiographic characteristics have been reported to predict dysphagic scoliosis, but their predictive value is not well described.
- It is unclear which set of radiographic features are most predictive of dysphagic scoliosis and will stand up in a robust statistical model.

Table 1. 100 DISPHAGIC CHARACTERISTICS OF DYSPHAGIC PATIENTS (N=10)

Scoliosis	Scoliosis
Asymmetry	Asymmetry
Asymmetry	Asymmetry
Asymmetry	Asymmetry
Asymmetry	Asymmetry
Asymmetry	Asymmetry
Asymmetry	Asymmetry
Asymmetry	Asymmetry
Asymmetry	Asymmetry
Asymmetry	Asymmetry
Asymmetry	Asymmetry



Objective

This study aims to determine which combination of x-ray characteristics was best able to predict true dystrophic status.

```

graph TD
    A[1. Denaturation: heating to 94°C to separate DNA strands] --> B[2. Annealing: binding of primers to single DNA strands]
    B --> C[3. Extension: synthesis of new DNA strands by DNA polymerase]
    C --> D[4. Repeating the cycle for 25-30 cycles]
    D --> E[5. Final extension: a final cycle of extension to ensure all DNA strands are fully extended]
    E --> F[6. Purification: removal of primers and other components]
    F --> G[7. Analysis: visualization of the amplified DNA fragments]
    
```

Materials and Methods

1. **Denaturation:** heating to 94°C to separate DNA strands
2. **Annealing:** binding of primers to single DNA strands
3. **Extension:** synthesis of new DNA strands by DNA polymerase
4. **Repeating the cycle for 25-30 cycles**
5. **Final extension:** a final cycle of extension to ensure all DNA strands are fully extended
6. **Purification:** removal of primers and other components
7. **Analysis:** visualization of the amplified DNA fragments

The final amplified DNA fragments are used for various applications, such as:

- **Cloning:** insertion into a plasmid vector for transformation into bacteria
- **Sequencing:** determination of the DNA sequence
- **Restriction digestion:** digestion with restriction enzymes for analysis
- **Electrophoresis:** separation of DNA fragments by size using agarose gels
- **Visualization:** staining of DNA bands with ethidium bromide or other fluorescent dyes

Results

- The actual diagnosis was dystrophic for 83 of the 122 x-rays, or 68% and 39(32%) were non-dystrophic
- Readers underestimated the proportions that were dystrophic.

Reader	Presumed Dystrophic (n=83)	Presumed Non-dystrophic (n=39)
1	65 (78%)	15 (38%)
2	61 (73%)	17 (43%)
3	62 (75%)	16 (41%)
4	68 (82%)	7 (18%)
5	67 (81%)	10 (26%)
Total	343 (41%)	383 (95%)

Logistic regression analysis: modeling backward, forward and stepwise elimination

Strong predictor of dysplastic features:

- Spindling of transverse process
- Short sharp angular curve
- Widened interpedicular space
- Vertebral scalloping
- $p = 0.05$

Strong predictor of dysplastic features:

- Rib penolting
- Vertebral rotation
- Vertebral wedging
- Atypical location
- $p < 0.05$

Results

- The odds of an x-ray being dystrophic were 2.43 times higher when rib penciling was present: vertebral rotation = 2.50, vertebral wedging = 2.37, a apical location 3.00
- If all 4 characteristics pattern were present there would be a 51 times higher risk of dystrophic curve pattern.

Characteristic	Odds Ratio (95% CI)
Vertebral rotation	2.50 (1.25 - 4.75)
Vertebral wedging	2.37 (1.17 - 4.75)
Rib penciling	2.43 (1.25 - 4.52)
Apical location	3.00 (1.52 - 5.72)

Model summary

- The model predicts that the probability of an x-ray being truly dystrophic is about 31% if the reader saw none of these four characteristics.
- The probability rises to about 52-58% if the reader saw one of the four characteristics, to about 73-80% if he saw two of them, to about 85-91% if he saw three of them, and to about 96% if he saw all four of them.

Conclusion

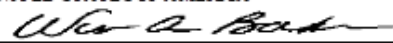
- Only four of the 8 classic radiographic findings of dystrophic scoliosis are most predictive
 - Rib penciling
 - Vertebral rotation
 - Vertebral wedging
 - Atypical curve location
- Further research to predict dystrophic curve patterns should focus on these radiographic markers.

[illegible]

SUPPORTING DATA:

Please see body.

NO COST EXTENSION APPROVAL DOCUMENTS:

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT				1. CONTRACT ID CODE	PAGE OF PAGES	
				S	1	9
2. AMENDMENT/MODIFICATION NO. P00001		3. EFFECTIVE DATE 31-Jul-2013		4. REQUISITION PURCHASE REQ. NO. W81XWH-10-1-0489		5. PROJECT NO. (If applicable)
6. ISSUED BY US ARMY MEDICAL RESEARCH ACQUISITION ACT DIRECTOR 820 CHANDLER STREET FORT DETRICK MD 21702-5014		CODE W81XWH		7. ADMINISTERED BY (If other than item 6) US ARMY MEDICAL RESEARCH ACQUISITION ACT ATTN: CHASEN DEBNER 301-819-8585 CHASEN.DEBNER.CIV@MAIL.MIL FORT DETRICK MD 21702		CODE W81XWH
8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code) REGENTS OF THE UNIVERSITY OF MINNESOTA OFFICE OF SPONSORED PROJECTS 200 OAK STREET SE MINNEAPOLIS MN 55455-2009				9A. AMENDMENT OF SOLICITATION NO.		
				9B. DATED (SEE ITEM 11)		
				X 10A. MOD. OF CONTRACT/ORDER NO. W81XWH-10-1-0489		
				X 10B. DATED (SEE ITEM 13) 01-Aug-2010		
CODE 0DH95		FACILITY CODE				
11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS						
☐ The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of offer ☐ is extended, ☐ is not extended. Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods: (a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.						
12. ACCOUNTING AND APPROPRIATION DATA (If required)						
13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.						
A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.						
B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).						
X C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF: Federal Demonstration Partnership, Phase V, dated July 1, 2008						
D. OTHER (Specify type of modification and authority)						
E. IMPORTANT: Contractor ☒ is not, ☐ is required to sign this document and return _____ copies to the issuing office.						
14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.) Modification Control Number: cdeener135321 This modification extends the period of performance of the grant by 12 months at no additional cost to the agreement per the recipient's request dated 25 July 2013. An annual technical reports shall be submitted no later than 31 August 2013 and the final report is due by 31 August 2014. Quarterly submissions of the SF 425 shall continue during the no-cost extension period. The next quarterly SF 425 shall be submitted no later than 31 October 2013. Administrative changes have been made as well. See the Summary of Changes. Pt David Polly Title: Genetic Evaluation for the Scoliosis Gene(s) in Patients with Neurofibromatosis 1 and Scoliosis Period of Performance: 01 August 2010- 31 August 2014 (research ends 31 July 2014) Award Amount: \$705,183 Obligated Amount: \$705,183						
Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.						
15A. NAME AND TITLE OF SIGNER (Type or print)				16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print) WENDY A. BAKER / CONTRACTING OFFICER TEL: 301-819-2034 EMAIL: wendy.baker.civ@mail.mil		
15B. CONTRACTOR/OFFEROR (Signature of person authorized to sign)		15C. DATE SIGNED		16B. UNITED STATES OF AMERICA BY  (Signature of Contracting Officer)		16C. DATE SIGNED 31-Jul-2013
EXCEPTION TO SF 30 APPROVED BY OIRM 11-84				30-105-04		STANDARD FORM 30 (Rev. 10-83) Prescribed by GSA FAR (48 CFR) 53.243

SECTION SF 30 BLOCK 14 CONTINUATION PAGE

SUMMARY OF CHANGES

SECTION 00010 - SOLICITATION CONTRACT FORM

The discount terms has changed from net 7 days to Net 7 Days.

The 'administered by' organization has changed from

USA MED RESEARCH ACQ ACTIVITY

ATTN: JASON KUHNS

301-619-1861

JASON.KUHNS1@US.ARMY.MIL

FORT DETRICK MD 21702

to

US ARMY MEDICAL RESEARCH ACQUISITION ACT

ATTN: CHASEN DEENER

301-619-8585

CHASEN.N.DEENER.CIV@MAIL.MIL

FORT DETRICK MD 21702

CLIN 0001

The CLIN extended description has changed from Period of Performance: 01 August 2010- 31 August 2013 (research ends 31 July 2013) to Period of Performance: 01 August 2010- 31 August 2014 (research ends 31 July 2014).

DELIVERIES AND PERFORMANCE

The following Delivery Schedule item for CLIN 0001 has been changed from:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	UIC
---------------	----------	-----------------	-----

POP 01-AUG-2010 TO

31-AUG-2013 N/A USA MED RESEARCH MAT CMD

1077 PATCHEL STREET

BLDG 1056

FORT DETRICK MD 21702

FOB: Destination W91ZSQ

To:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	UIC
---------------	----------	-----------------	-----

POP 01-AUG-2010 TO

31-AUG-2014 N/A USA MED RESEARCH MAT CMD

1077 PATCHEL STREET

BLDG 1056

FORT DETRICK MD 21702
FOB: Destination W91ZSQ

SECTION 00800 - SPECIAL CONTRACT REQUIREMENTS

The following have been modified:

A. This award is made under the authority of 31 U.S.C. 6304 and 10 U.S.C. 2358. The recipient's statement of work and the revised budget, dated 15 July 2010, for this proposal submitted in response to the Fiscal Year 2009 (FY09) Department of Defense Neurofibromatosis Research Program Investigator Initiated Research Award Announcement, which closed 14 April 2009, are incorporated herein by reference. The Catalog of Federal Domestic Assistance Number relative to this award is CFDA 12.420.

B. ACCEPTANCE OF AWARD. The recipient is not required to countersign this assistance award. In case of disagreement, the recipient shall notify the Grants Officer and not assess the award any costs until such disagreement(s) is resolved.

C. MAXIMUM OBLIGATION (SEP 2006) (USAMRAA)

The maximum obligation for support of the project will not exceed the amount specified in the award, as amended. USAMRAA does not amend assistance agreements to provide additional funds for such purposes as reimbursement for unrecovered indirect costs resulting from the establishment of final negotiated rates or for increases in salaries, fringe benefits and other costs.

D. TERMS AND CONDITIONS: The recipient agrees to the General Terms and Conditions of the Federal Demonstration Partnership, Phase V, dated July 1, 2008 and Department of Army – Agency Specific Requirements. Modifications to the General Terms and Conditions dated July 1, 2008 are modified as indicated below.

1. PATENTS AND INVENTIONS (DEC 2001) (USAMRAA)

a. The recipient shall use the Interagency Edison through the National Institutes of Health Commons (<http://www.iedison.gov/>) for filing of Patent Application and Invention Disclosure. Negative reports are required and shall be submitted on a DD Form 882 to the Grants Officer. (DD Form 882 can be located on web site <http://www.usamraa.army.mil>).

b. Invention reports are due annually and at the end of the period of the award. Annual reports are due 30 days after the anniversary date of the award and final reports are due 30 days after the expiration of the award. The award will NOT be closed out until all invention reporting requirements are met.

2. TECHNICAL REPORTING REQUIREMENTS (DEC 2008) (USAMRAA)

Format Requirements for Annual/Final Reports

a. Annual reports must provide a complete summary of the research accomplishments to date with respect to the approved Statement of Work. Journal articles can be substituted for detailed descriptions of specific aspects of the research, but the original articles must be attached to the report as an appendix and appropriately

referenced in the text. The importance of the report to decisions relating to continued support of the research can not be over-emphasized. An annual report shall be submitted within 30 calendar days of the anniversary date of the award for the preceding 12 month period. If the award period of performance is extended by the Grants Officer, then an annual report must still be submitted within 30 days of the anniversary date of the award. A final report will be due upon completion of the extended performance date that describes the entire research effort.

b. A final report summarizing the entire research effort, citing data in the annual reports and appended publications shall be submitted at the end of the award performance period. The final report will provide a complete reporting of the research findings. Journal publications can be substituted for detailed descriptions of specific aspects of the research, but an original copy of each publication must be attached as an appendix and appropriately referenced in the text. All final reports must include a bibliography of all publications and meeting abstracts and a list of personnel (not salaries) receiving pay from the research effort.

Although there is no page limitation for the reports, each report shall be of sufficient length to provide a thorough description of the accomplishments with respect to the approved Statement of Work. Submission of the report in electronic format (PDF or Word file only), shall be submitted to <https://ers.amedd.army.mil>.

All reports shall have the following elements in this order

FRONT COVER: Sample front cover provided at https://mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting. The Accession Document (AD) Number should remain blank.

STANDARD FORM 298: Sample SF 298 provided at https://mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting. The abstract in Block 13 must state the purpose, scope, major findings and be an up-to-date report of the progress in terms of results and significance. Subject terms are keywords that may have previously assigned to the proposal abstract or are keywords that may be significant to the research. The number of pages shall include all pages that have printed data (including the front cover, SF 298, table of contents, and all appendices). Please count pages carefully to ensure legibility and that there are no missing pages as this delays processing of reports. Page numbers should be typed: please do not hand number pages.

TABLE OF CONTENTS: Sample table of contents provided at https://mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting.

INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

BODY: This section of the report shall describe the research accomplishments associated with each task outlined in the approved Statement of Work. Data presentation shall be comprehensive in providing a complete record of the research findings for the period of the report. Provide data explaining the relationship of the most recent findings with that of previously reported findings. Appended publications and/or presentations may be substituted for detailed descriptions of methodology but must be referenced in the body of the report. If applicable, for each task outlined in the Statement of Work, reference appended publications and/or presentations for details of result findings and tables and/or figures. The report shall include negative as well as positive findings. Include problems in accomplishing any of the tasks. Statistical tests of significance shall be applied to all data whenever possible. Figures and graphs referenced in the text may be embedded in the text or appended. Figures and graphs can also be referenced in the text and appended to a publication. Recommended changes or future work to better address the research topic may also be included, although changes to the original Statement of Work must be approved by the Army Grants Officer's Representative. This approval must be obtained prior to initiating any change to the original Statement of Work.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

manuscripts, abstracts, presentations; patents and licenses applied for and/or issued; degrees obtained that are supported by this award; development of cell lines, tissue or serum repositories; informatics such as databases and animal models, etc.; funding applied for based on work supported by this award; employment or research opportunities applied for and/or received based on experience/training supported by this award.

CONCLUSION: Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

REFERENCES: List all references pertinent to the report using a standard journal format (i.e. format used in Science, Military Medicine, etc.).

APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES.**

Mark all pages of the report which contain proprietary or unpublished data that should be protected by the U.S. Government. **REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE.** It is the responsibility of the Principal Investigator to advise the U.S. Army Medical Research and Materiel Command when restricted limitation assigned to a document can be downgraded to Approved for Public Release. **DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS.**

Manuscripts/Reprints, Abstracts

A copy of manuscripts or subsequent reprints resulting from the research shall be submitted to the USAMRMC. An extended abstract suitable for publication in the proceedings of the applicable research program is required in relation to a DOD meeting planned during the term of this award. The extended abstract shall (1) identify the accomplishments since award and (2) follow instructions to be prepared by the USAMRMC and promulgated at a later date. The extended abstract style will be dependent on the discipline.

3. PAYMENTS

ADVANCE PAYMENTS AND FULL FUNDING (DEC 2008) (USAMRAA)

a. Payments. Advance payments will be made to the recipient. Questions relative to payment issues involving Defense Finance and Accounting Service shall be directed to usarmy.detrack.medcom-usamraa.mbx.aa3@mail.mil.

b. Electronic Funds Transfer. All advance payments to the recipient will be made by electronic funds transfer (EFT). The recipient shall contact the Defense Finance and Accounting System (DFAS) named on the face page of this award to make arrangements for EFT. Failure to do so may result in nonpayment.

c. If the recipient fails to perform, the Grants Officer shall notify DFAS in writing to withhold payments.

d. Advance Payment Schedule

Year One \$240,988

Amount	On or About
\$60,247	01 August 2010
\$60,247	01 October 2010
\$60,247	01 January 2011
\$60,247	01 April 2011

Year Two \$239,385

Amount	On or About
\$59,846	01 July 2011
\$59,846	01 October 2011
\$59,846	01 January 2012
\$59,847	01 April 2012

Year Three \$224,810

Amount	On or About
\$56,202	01 July 2012
\$56,203	01 October 2012
\$56,202	01 January 2013
\$56,203	01 April 2013

e. Financial Reporting Requirements:

Federal Financial Report (SF 425): Quarterly and Final Reports (For reporting individual assistance agreements)

Reporting period end dates fall on the end of the calendar quarter for quarterly reports (3/31, 6/30, 9/30, 12/31) and the end date of the assistance agreement period of performance for the final report. Reports are due 30 days after the reporting period end date for quarterly reports and 90 days after the end date of the assistance agreement for the final report.

The SF425 and instructions for completion can be obtained from <https://usamraa.army.mil>. All SF425's shall be submitted electronically to usarmy.detrack.medcom-usamraa.mbx.sf425@mail.mil. The award number assigned

by USAMRAA, which looks similar to W81XWH-XX-X-XXXX shall be included in the subject line of the electronic submission.

NOTE: The SF425 is a single form that consolidates and replaces the Federal Cash Transaction Report (SF272.SF272A) and the Financial Status Report (SF269/SF269A)

f. Interest Bearing Account. Unless exempted by applicable Treasury-State agreements in accordance with the Cash Management Improvement Act (CMIA) (31 U.S.C. 3335), the recipient shall deposit all advance payments in an interest bearing account. Interest over the amount of \$250 per year shall be remitted annually to the Department of Health and Human Services, Payment Management System, P.O. Box 6021, Rockville, MD 20852. A copy of the transmittal letter stating the amount of interest remitted shall be sent to the U.S. Army Medical Research Acquisition Activity, ATTN: MCMR-AAA-AC, 820 Chandler Street, Fort Detrick, MD 21702-5014.

4. PROHIBITION OF USE OF HUMAN RESEARCH (JAN 2007) (USAMRAA)

**** PROHIBITION – READ FURTHER FOR DETAILS ****

Research under this award involving the use of human subjects, to include the use of human anatomical substances and/or human data, may not begin until the U.S. Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protections Office (HRPO) approves the protocol. Written approval to begin research or subcontract for the use of human subjects under the applicable protocol proposed for this award will be issued from the US Army Medical Research and Materiel Command, HRPO, under separate letter to the recipient. A copy of this approval will be provided to the US Army Medical Research Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in withholding of funds and or the termination of the award.

5. PROHIBITION OF USE OF LABORATORY ANIMALS (JAN 2007) (USAMRAA)

**** PROHIBITION – READ FURTHER FOR DETAILS ****

Notwithstanding any other provisions contained in this award or incorporated by reference herein, the recipient is expressly forbidden to use or subcontract for the use of laboratory animals in any manner whatsoever without the express written approval of the US Army Medical Research and Materiel Command, Animal Care and Use Office (ACURO). The recipient will receive written approval to begin research under the applicable protocol proposed for this award from the US Army Medical Research and Materiel Command, ACURO, under separate letter. A copy of this approval will be provided to the US Army Medical Research and Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in the termination of the award.

6. PROHIBITION OF USE OF HUMAN CADAVERS (JAN 2007) (USAMRAA)

**** PROHIBITION – READ FURTHER FOR DETAILS****

Research under this award using human cadavers may not begin until the U.S. Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protections Office (HRPO) approves the protocol. Written approval to begin research or subcontract for the use of human cadavers under the applicable protocol proposed for this award will be issued from the US Army Medical Research and Materiel Command, HRPO, under separate letter to the recipient. A copy of this approval will be provided to the US Army Medical Research Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in withholding of funds and or the termination of the award.

7. SUPPORTING INFORMATION (APR 2008) (USAMRAA)

Information such as subawards, consultant agreements, vendor quotes, and personnel work agreements may be required in order to support proposed costs or to determine the employment status of personnel under the assistance agreement. The Government's receipt of this information does not constitute approval or acceptance of any term or condition included therein. The terms and conditions of the assistance agreement take precedence over any term or condition included in supporting information.

8. TRAFFICKING VICTIMS PROTECTION ACT (May 2008) (USAMRAA)

Trafficking in persons.

a. Provisions applicable to a recipient that is a private entity.

1. You as the recipient, your employees, subrecipients under this award, and subrecipients' employees may not--

i. Engage in severe forms of trafficking in persons during the period of time that the award is in effect;

ii. Procure a commercial sex act during the period of time that award is in effect; or

iii. Use forced labor in the performance of the award or subawards under the award.

2. We as the Federal awarding agency may unilaterally terminate this award, without penalty, if you or a subrecipient that is a private entity--

i. Is determined to have violated a prohibition in paragraph a.1 of this award term; or

ii. Has an employee who is determined by the agency official authorized to terminate the award to have violated a prohibition in paragraph a.1 of this award term through conduct that is either--

A. Associated with performance under this award; or

B. Imputed to you or the subrecipient using the standards and due process for imputing the conduct of an individual to an organization that are provided in 2 CFR 180, "OMB Guidelines to Agencies on Governmentwide Debarment and Suspension (Nonprocurement)," as implemented by our agency at 2 CFR part 1125.

b. Provision applicable to a recipient other than a private entity. We as the Federal awarding agency may unilaterally terminate this award, without penalty, if a subrecipient that is a private entity--

1. Is determined to have violated an applicable prohibition in paragraph a.1 of this award term; or

2. Has an employee who is determined by the agency official authorized to terminate the award to have violated an applicable prohibition in paragraph a.1 of this award term through conduct that is either--

i. Associated with performance under this award;

ii. Imputed to the subrecipient using the standards and due process for imputing the conduct of an individual to an organization that are provided in 2 CFR part 180, "OMB Guidelines to Agencies on Governmentwide Debarment and Suspension (Nonprocurement)," as implemented by our agency at 2 CFR part 1125.

c. Provision applicable to any recipient.

1. You must inform us immediately of any information you receive from any source alleging a violation of a prohibition in paragraph a.1 of the award term.

2. Our right to terminate unilaterally that is described in paragraph a.2. or b. of this section:

i. Implements section 106(g) of the Trafficking Victims Protection Act of 2000 (TVPA), as amended (22 U.S.C. 7104(g)), and

ii. Is in addition to all other remedies for noncompliance that are available to us under this award.

3. You must include the requirements of paragraph a.1 of this award term in any subaward you make to a private entity.

d. Definitions. For the purpose of this award term:

1. "Employee" means either:

i. An individual employed by you or a subrecipient who is engaged in the performance of the project or program under this award; or

ii. Another person engaged in the performance of the project or program under this award and not compensated by you including, but not limited to, a volunteer or individual whose services are contributed by a third party as an in-kind contribution toward cost sharing or matching requirements.

2. "Forced labor" means labor obtained by any of the following methods: the recruitment, harboring, transportation, provision, or obtaining of a person for labor or services, through the use of force, fraud, or coercion for the purpose of subjection to involuntary servitude, peonage, debt bondage, or slavery.

3. "Private entity":

i. Means any entity other than a State, local government, Indian Tribe, or foreign public entity, as those terms are defined in 2 CFR 175.25.

ii. Includes:

A. A nonprofit organization, including any nonprofit institution of higher education, hospital, or tribal organization other than one included in the definition of Indian Tribe at 2 CFR 175.25(b).

B. A for-profit organization.

4. "Severe forms of trafficking in persons," "commercial sex act," and "coercion" have the meanings given at section 103 of the TVPA, as amended (22 U.S.C. 7102).

(End of Summary of Changes)