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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 us 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.

15. SUBJECT TERMS
Neurofibromatosis type I, Dystrophic scoliosis, Radiographic characteristics

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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 intervertebal 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.

<u>Dystrophic classification ('dys')</u>: 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 <u>non</u>-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 'Yes'	Dystrophic No	*percent*	Dystrophic Yes	*percent*	Total
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	10	6	1	0	2	39
	51.28	25.64	15.38	2.56	0.00	5.13	
Y	11	12	16	14	10	20	83
	13.25	14.46	19.28	16.87	12.05	24.10	
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	24	5	2	1	2	39
	12.82	61.54	12.82	5.13	2.56	5.13	
Y	4	22	24	16	9	8	83
	4.82	26.51	28.92	19.28	10.84	9.64	
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	15	3	3	2	0	39
	41.03	38.46	7.69	7.69	5.13	0.00	
Y	9	16	29	15	7	7	83
	10.84	19.28	34.94	18.07	8.43	8.43	
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	7	0	2	0	0	39
	76.92	17.95	0.00	5.13	0.00	0.00	
Y	28	18	18	9	8	2	83
	33.73	21.69	21.69	10.84	9.64	2.41	
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	4	2	1	0	1	39
	79.49	10.26	5.13	2.56	0.00	2.56	
Y	52	8	10	7	3	3	83
	62.65	9.64	12.05	8.43	3.61	3.61	
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 wedgeing (‘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

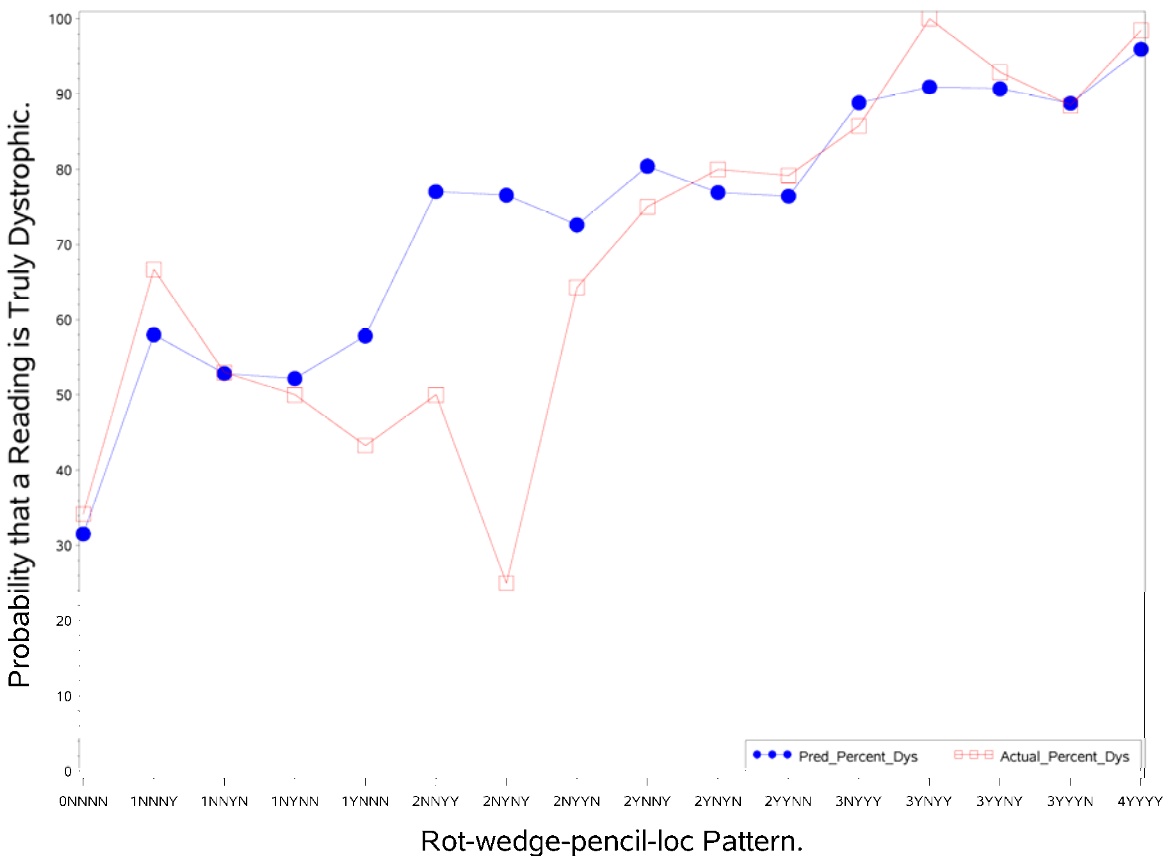
When forward selection was used, the results were identical with the results for backward selection (table below); this gives increased confidence that the chosen four characteristics are likely the ones that really matter. Stepwise selection was also tried, with identical results.

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 2YNYN.

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	2NYNY	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	3YNYY	90.9022	100.000
14	3YYNY	90.6772	92.857
15	3YYYN	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	Minimum Acceptable 95% Lower Confidence Limit Sample size						
0.85	0.50	0.55	0.60	0.65	0.70	0.75	0.80
	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.

    - To date we have a total of 14 potential subjects to be enrolled in phase 2 of this study.
    - We are in the process of obtaining informed consent for these subjects.

b. Once informed consent is obtained participants will be referred to Axial Biotech. Axial Biotech will send the participants a buccal swab kits with a self addressed stamped envelope.

c. Participants will be asked to swab the inside of their cheeks and to collect DNA sample and mail them back to Axial Biotech for genetic testing. They will be guided by written instructions telephone instructions and/or internet video instruction.

The rest of the tasks have yet to be started:

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

Task 4: Preparation of reports, analysis of data and preparation of manuscript (year 3.)

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 wedgeing ('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, a draft abstract shown below will be submitted to future national and international medical society meetings for podium or poster presentations.

Abstract

BACKGROUND: Dystrophic scoliosis is prevalent in patients with NF type 1. It is a significant cause of morbidity and its treatment is extremely challenging. Early detection and subsequent intervention may provide better outcomes. Certain radiographic characteristics have been shown to be strongly associated with dystrophic scoliosis and can be used as predictors.

OBJECTIVE: This multicenter study aims to validate these radiographic characteristics of dystrophic modulation through an interobserver reliability and logistic regression analysis.

METHODS: Full body scoliosis radiographs in NF1 patients from multiple institutions across the United States were graded either as dystrophic or non-dystrophic scoliosis by 5 spine surgeons . Each set of radiographs were also assessed by the presence or absence of 8 radiographic characteristics namely: vertebral wedging; vertebral rotation; short sharp angular curve; rib penciling; vertebral scalloping; widened interpedicular distance; atypical location; and spindling of transverse processes. Interoberver reliability analysis was performed to assess agreement and logistic regression was done to determine which radiographic characteristics were significant predictors of dystrophic scoliosis.

RESULTS: A set of 122 cases of scoliosis radiographs in NF1 patients from multiple institutions across the United States were read by 5 spine surgeons graded using 8 radiographic dystrophic characteristics. Of the 122 cases 68% (83/122) were classified by the contributing institution as dystrophic and 32% (39/122) were classified as non-dystrophic. For 415 readings (5 surgeons) on the 83 radiographs that were truly dystrophic, the overall sensitivity was 74.7%. Similarly, for the 195 readings on radiographs 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. Logistic regression modeling results indicate that four characteristics: rib penciling, vertebral rotation, vertebral wedge and atypical location, were strongly associated with true dystrophic status. If all four characteristics were present at once, the odds of that radiograph being truly dystrophic are 51 times higher than if none were present. To put it another way, the model predicts that the probability of a radiograph being truly dystrophic is about 31% if none of these four characteristics were present. The probability rises to about 52-58% if one of the four characteristic were present, 72-80% if two were present, 88-91% if three were present, and to about 96% if all four were present.

CONCLUSION: The 8 radiographic characteristics were significantly associated with dystrophic modulation in NF1 patients with scoliosis. Presence of rib penciling, vertebral rotation, vertebral wedging and atypical location were found to be most significant predictors of dystrophic scoliosis.

CONCLUSION:

No conclusions yet.

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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.									
	Xray ID#	Dystrophic Deformity	Sharp angular curve	Rib Penciling	Vertebral Rotation	Vertebral scalloping	Vertebral Wedging	Spindling of transverse processes	Widened interpedicular distance	Atypical location
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
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SUPPORTING DATA:

Please see body.