PERIPHERAL MUSCLE WEAKNESS IN RASOPATHIES

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ABSTRACT: *Introduction*: RASopathies are a group of genetic conditions due to alterations of the Ras/MAPK pathway. Neurocutaneous findings are hallmark features of the RASopathies, but musculoskeletal abnormalities are also frequent. The objective was to evaluate handgrip strength in the RASopathies. *Methods:* Individuals with RASopathies (e.g., Noonan syndrome, Costello syndrome, cardio-facio-cutaneous [CFC] syndrome, and neurofibromatosis type 1 [NF1]) and healthy controls were evaluated. Two methods of handgrip strength were tested: GRIP-D Takei Hand Grip Dynamometer and the Martin vigorimeter. A general linear model was fitted to compare average strength among the groups, controlling for confounders such as age, gender, height, and weight. *Results:* Takei dynamometer: handgrip strength was decreased in each of the syndromes compared with controls. Decreased handgrip strength compared with sibling controls was also seen with the Martin vigorimeter ($P < 0.0001$). *Conclusions:* Handgrip strength is decreased in the RASopathies. The etiology of the reduced muscle force is unknown, but likely multifactorial.
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The RASopathies consist of distinct syndromes which are due to mutations in genes involved in regulation of the Ras/mitogen-activated protein kinase (MAPK) pathway.[1] Examples of these causative genes include *NF1*, *HRAS*, *BRAF*, *MEK1/2*, *PTPN11*, *KRAS*, *RAF1*, *SOS1*, and *SPRED1*. Multiple organ systems are affected in the RASopathies, and there is variable phenotypic overlap. Some of these syndromes include neurofibromatosis type 1 (NF1), Noonan syndrome (NS), cardio-facio-cutaneous syndrome (CFC), Costello syndrome (CS), Legius syndrome, capillary malformation-arteriovenous malformation syndrome, and multiple lentigines syndrome. The RASopathies consist of a range of common (e.g., NF1, NS) to very rare genetic disorders (e.g., CS, CFC). As a group, most primary care physicians will likely follow patients with RASopathies in their clinical practices.

Although neurocutaneous findings are frequently mentioned as hallmark manifestations of RASopathies, the musculoskeletal system is also affected frequently. Some of the musculoskeletal problems are specific for individual syndromes (e.g., long bone bowing/pseudarthrosis and sphenoid wing dysplasia in NF1), but the phenotypic overlap is significant for many of the musculoskeletal problems. Frequent musculoskeletal findings in the RASopathies include scoliosis, pectus anomalies, pes planus, hip dysplasia, low bone mineral density, bone cysts, and hypotonia (reviewed by Stevenson and Yang, 2011).[2]

Decreased motor proficiency, decreased strength, and muscle abnormalities have been reported in NF1.[3–6] There have been reports of muscle abnormalities (e.g., myopathy, weakness) in isolated cases or small cohorts of other RASopathies besides NF1.[7–12] Some data from a transgenic mouse model has further supported an abnormality of muscle in the clinical setting.[13] Significant muscle abnormalities including muscle fibrosis, reduced muscle fibers, and reduced muscle force were shown in mice lacking *Nf1* in the early limb bud mesenchyme.[13] In addition, fewer myosin heavy chain expressing cells and decreased myotube numbers were reported in C2C12 mouse myoblasts transfected with plasmids harboring *HRAS*, *BRAF*, and *MEK1* mutations, which are causative genes for CS and CFC.[7] In an effort to provide objective data to support our clinical observation of weakness, our goal was to assess muscle function by measuring isometric hand and forearm strength in individuals who have a RASopathy.

MATERIALS AND METHODS

Participants. Clinical data from cohorts of individuals with NF1, NS, CS and CFC were obtained through a physical examination and medical history collection. Individuals were recruited at parent support group meetings and through referral to the University of Utah Clinical Genetics Research Program. For classification of a specific diagnosis, individuals were examined by a medical geneticist (D.S. or K.A.R.). The diagnosis was based on the clinical phenotype with supportive genotypes when

Abbreviations: NF1, neurofibromatosis type 1; CFC, cardio-facio-cutaneous syndrome; CS, Costello syndrome; NS, Noonan syndrome; kgf, kilogram-force; kPa, kilopascal; LSM, least square mean

Key words: cardiofaciocutaneous syndrome, Costello syndrome, muscle force, neurofibromatosis, Noonan syndrome, RASopathy

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Table 1. Handgrip analysis using Takei dynamometer in cohort #1.

Group	*N*	Mean height -cm- (range)	Mean weight -kg- (range)	Mean age -years- (range)	Handgrip (kgf) LSM	95% Confidence limits	*P*-value*
CS	20	134 (99–152)	43 (18–68)	15.7 (6–31)	9.12	(6.76, 11.48)	<0.0001
CFC	15	134 (95–158)	31 (17–66)	12.6 (5–27)	11.00	(8.39, 13.61)	<0.0001
NS	15	146 (109–163)	48 (12–105)	21 (6–47)	13.56	(11.28, 15.85)	=0.0006
NF1	59	132 (104-172)	32 (16–80)	10.4 (5–22)	14.20	(13.04, 15.37)	<0.0001
Controls	53	156 (101–188)	52 (18–84)	13.2 (5–23)	18.22	(16.86, 19.58)	Overall <0.0001

**Comparison of handgrip strength (kgf) between syndromic groups and controls adjusted for possible confounders. P-values are not adjusted for multiple comparisons. kgf, kilogram-force; LSM, least square mean; CS, Costello syndrome; CFC, cardiofaciocutaneous syndrome; NS, Noonan syndrome; NF1, neurofibromatosis type 1.*

available. Individuals with NF1 were only included if they fulfilled the NIH clinical diagnostic criteria.[14] Control subjects were healthy individuals or siblings of participants without a known syndrome or orthopedic condition. Individuals who could not follow the commands of the measurement protocol due to cognitive impairment or age were not included.

Written informed consent was obtained, and the study was approved by the Institutional Review Board at the University of Utah and the University of California at San Francisco.

Measurements. Handgrip strength was initially obtained using the GRIP-D Takei Hand Grip Dynamometer instrument per protocol instructions for the Takei Physical Fitness Test (GRIP-D item# 5101, Takei Scientific Instruments Co., LTD, Tokyo, Japan) in a cohort of individuals with NF1, CS, CFC, NS, and healthy controls (Cohort #1). In brief, individuals were in an upright position and held the dynamometer in 1 hand with the grip range adjusted so that the second joint of the forefinger is bent through 90 degrees. The instrument was then held down at the participant's side without letting the arm touch the body with the arm fully extended. Individuals were then asked to exert full force with the hand for approximately 3 seconds to obtain the maximum kilogram-force (kgf), during which verbal encouragement was provided. A total of 4 measurements were obtained, alternating from the right hand, to the left hand, to the right hand, and back again to the left hand with approximately 30 seconds rest between each measurement. The average of the 4 measurements for each individual was used. The reported measurement range of the GRIP-D Takei dynamometer instrument is 5.0 to 100 kgf with a minimum unit of 0.1 kgf. The reported measuring accuracy of the instrument is ± 2 kgf in 50 kgf.

Because the Takei dynamometer requires the participant to generate a minimum of 5 kgf, we conducted a study on an additional cohort (Cohort #2) of CS, CFC, and NS participants and normal sibling controls less than 18 years of age using the Martin vigorimeter.[15] NF1 individuals were not available during the measurements with the Martin vigorimeter. There was an overlap of 5 individuals with Costello syndrome in the cohort using the Takei dynamometer and the cohort using the Martin vigorimeter, but there was no overlap in the individuals with NS, CFC, or controls. These data from the Martin vigorimeter were collected 2 years after collection of data from the Takei dynamometer from the initial cohort. The medium bulb was used for all studies, and measurements were all performed consistently (B.A.T. or K.A.R.). Each participant was placed with the forearm on the table with the wrist in the neutral position. Three measurements were obtained in succession from the self identified dominant hand. Participants were asked to "squeeze the bulb as hard as you can" and were further encouraged during the task. There were approximately 10 seconds of rest between each reading. Height and weight were not available in this cohort.

Statistical Analysis. For data generated from the Takei dynamometer, a general linear model was fitted to compare average strength score among the 5 groups, controlled for possible confounders such as age, gender, height, and weight. Tukey-Kramer multiple-comparison adjusted *P*-values were obtained. The statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, North Carolina). For data generated from the Martin vigorimeter a similar comparison was performed controlling for possible confounders of age and gender.

RESULTS

For measurements of grip strength with the Takei dynamometer, a total of 162 individuals participated (mean age 13.2 years; 73 females, 89 males). Demographics of each group were as follows: NF1 ($n = 59$; mean age 10.4 years; range, 4.5–21.5 years; 31 males, 28 females), controls ($n = 53$; mean age, 13.2 years; range, 4.9–22.7 years; 28 males, 25 females), CS ($n = 20$; mean age, 15.7 years; range, 6.2–30.7 years; 11 males, 9 females),

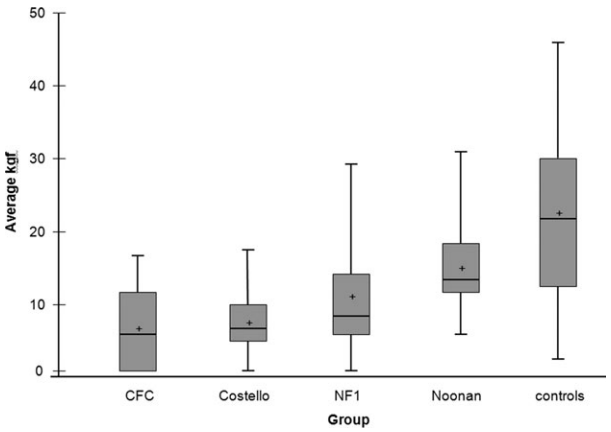

FIGURE 1. Box plots of raw unadjusted handgrip strength scores (kgf) using the Takei dynamometer in syndromes of the Ras/MAPK pathway and healthy controls from cohort #1. (CFC, cardiofaciocutaneous syndrome; NF1, neurofibromatosis type 1; kgf, kilogram-force).

CFC ($n = 15$; mean age, 12.6 years; range, 4.9–27.1 years; 9 males, 6 females), NS ($n = 15$; mean age, 21 years; range, 6.4–47.4 years; 10 males, 5 females) (Table 1).

Raw average grip strength values were as follows: NF1 (mean, 10.4 kgf; range, 0–29.5 kgf), Controls (mean, 22.2 kgf; range, 1.3–45.5 kgf), CS (mean, 7.2 kgf; range, 0–17.4 kgf), CFC (mean, 6.4 kgf; range, 0–16.6 kgf), NS (mean, 15.1 kgf; range, 5.5–30.3 kgf) (boxplots of raw means/medians in Fig. 1).

A general linear model was fitted to compare average forearm strength among the 5 groups with controls for possible confounders such as age, gender, height, and weight. All syndromic groups had significantly lower least square mean strength scores than the control group (see Table 1). Differences were still significant after Tukey-Kramer multiple-comparison adjusted *P*-values: CFC ($P < 0.0001$), CS ($P < 0.0001$), NF1 ($P = 0.0005$), and NS ($P = 0.0053$). Other notable group differences, after Tukey-Kramer adjustment, showed decreased strength in CS compared with NF1 ($P = 0.0021$) and CS compared with NS ($P = 0.0591$). The model also showed that height ($P < 0.0001$) and weight ($P = 0.0005$) were significantly associated with average strength scores, but age and gender were not significant (although age was significant in a univariate analysis, but not after controlling for height and weight, which is consistent with older subjects being bigger).

To address the fact that a few individuals were unable to generate enough force on the Takei dynamometer, we used the Martin vigorimeter to measure handgrip pressure in children 18 years and younger on an additional group of CS, CFC, and NS participants (Cohort #2). All raw averages handgrip strength values measured in kilopascals (kPa) for each syndrome were lower than the average score for the normal sibling controls (Fig. 2). Differences were significant after Tukey-Kramer multiple-comparison adjusted *P*-values: CFC ($P < 0.0001$), CS ($P < 0.0001$), and NS ($P < 0.0001$) (Table 2). The model also showed a significant interaction between age and groups (which indicates handgrip strength increases by age, but much faster in the controls than all other syndromic groups), but gender was not statistically significant, and height and weight were not available for inclusion in the model.

DISCUSSION

All of the Ras/MAPK syndromes that we tested had decreased generation of muscle force using both the handgrip Takei dynamometer and the Martin vigorimeter. This is in concordance with clinical observations of weakness, hypotonia, impaired motor proficiency, delayed motor milestones, and decreased muscle mass in many of the RASopathies.[2,7] This study provides objective data to suggest that disturbance of Ras signaling leads to skeletal muscle weakness, which likely contributes to the various clinical musculoskeletal findings broadly observed in the RASopathies. The etiology of the reduced muscle force is not known, but it is likely multifactorial.

Animal studies and *in vitro* cellular studies have provided some information on the role of the Ras/MAPK pathway on skeletal development and muscle function. One conditional *Nf1* mouse model shows dramatic abnormalities of muscle, including reduced muscle fibers, reduced muscle force, and defects in myogenesis,[13] providing evidence that increased signaling

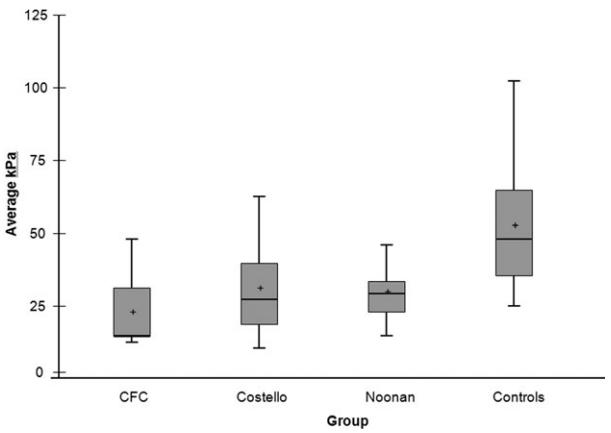

FIGURE 2. Box plots of raw unadjusted handgrip strength scores (kPa) using the Martin vigorimeter in selected syndromes of the Ras/MAPK pathway and healthy sibling controls from cohort #2. (CFC, cardiofaciocutaneous syndrome; kPa, kilopascal).

Table 2. Handgrip analysis using Martin vigorimeter in cohort #2.

Group	*N*	Gender (F/M)	Mean age -years- (Range)	Handgrip (kPa) LSM	95% Confidence limits	*P*-value*
CS	13	8/5	13.4 (6 – 18)	25.7	(18.6, 32.8)	<0.0001
CFC	6	5/1	9.3 (4 – 15)	25.2	(16.1, 34.4)	<0.0001
NS	10	5/5	10.0 (5 – 18)	29.1	(22.2, 36.0)	<0.0001
Controls	17	8/9	9.3 (6 – 14)	63.2	(47.3, 69.2)	Overall <0.0001

**Comparison of handgrip (kPa) between syndromic groups and controls, based on a general linear model with an interaction between age and Group (gender was not statistically significant), with Tukey-Kramer multiple comparison adjustment. kPa, kilopascal; F, female; M, male; LSM, least square mean; CS, Costello syndrome; CFC, cardiofaciocutaneous syndrome; NS, Noonan syndrome; NF1, neurofibromatosis type 1.*

through the Ras/MAPK plays a role in skeletal muscle development. With *in vitro* studies, Tidyman et al. showed that mutations that cause CS and CFC inhibit myoblast differentiation.[7] Also, an *Nf1* mutant fly model showed a reduced locomotive index in response to stress.[16] These animal models and *in vitro* studies support further investigation in the clinical realm.

Myopathies result in weakness, and there have been reports of individuals with CS with weakness and an increased density of muscle spindles on muscle biopsy suggesting an underlying myopathy.[10–12,17] Tidyman et al. evaluated the clinical muscle biopsies of several individuals with CS and CFC showing abnormal skeletal muscle including excessive variability of muscle fiber size and type II predominance.[7]

Mitochondrial dysfunction has also been reported in humans and animal models of selected RASopathies.[7,16,18,19] Lee et al. showed lower mitochondrial membrane potential and ATP content using mouse fibroblasts and human lymphoblast cell lines from 2 Noonan syndrome patients.[18] Kleefstra et al. reported a cohort of 5 children with mitochondrial encephalomyopathy with *de novo* RAS/MAPK gene mutations, but they did not see significant metabolic features of oxidative phosphorylation dysfunction on a prospectively screened cohort of 18 patients with a RASopathy.[19] In addition, of the muscle biopsies reviewed by Tidyman et al., 6 individuals with CS or CFC did not show abnormal mitochondrial structure by light and electron microscopy, but all had abnormal respiratory chain enzyme analysis.[7] The handgrip strength test performed was an isometric measure of only 2 maximal forces per muscle groups and hence was unlikely to lead to significant lactic acidosis and impaired function directly from the test. However, mitochondrial dysfunction may result in chronic fatigue and impaired metabolic efficiency with subsequent muscle atrophy and weakness over time, which in turn would attenuate the typical age-related increase in strength over time.

Souza et al. speculated that vitamin D and calcium concentrations and other metabolic differences potentially contribute to decreased muscular forces in NF1.[5] Vitamin D deficiency has been associated with decreased muscle function in the general population,[20] and 25-hydroxyvitamin D deficiency has been reported in some individuals with NF1,[21] but this has not been systematically assessed in the other RASopathies.

It is also possible that joint laxity, hypotonia, and hand anomalies potentially could have interfered with the functional generation of maximal force with the dynamometer that was selected which requires a self generated voluntary task. In addition, cognitive deficits may have contributed to the ability to accurately follow commands during the handgrip strength measurement protocol leading to suboptimal muscle force production. The range of cognitive function varies between and within the syndromic groups and makes it difficult to determine if subjects' cognitive function impacted the performance of the task. Cognitive deficits in NF1 and Noonan syndrome that would impair the performance of a simple task would be rare but are not as unlikely in CFC and CS, which may explain the decreases in CS compared with NF1 and NS. However, we excluded participants who were unable to follow commands to perform the handgrip measurement. A few individuals were unable to generate enough force to reach the minimum measurement range of 5.0 kgf for the dynamometer used, resulting in a recorded value of 0 kgf. It is likely that some force was produced, although it did not reach the minimum measurement range and could potentially bias the overall group values. However, eliminating individuals with a score of 0 kgf would also provide bias. We elected to include individuals who followed commands to complete the procedure, although no force was recorded on the Takei dynamometer.

To determine if an alternate handgrip strength device would confirm our findings and potentially overcome potential limitations of the Takei

dynamometer for children with cognitive delays and clinical weakness, hand grip strength was measured on additional groups of CS, CFC, and NS children using the Martin vigorimeter. The Martin vigorimeter measures spherical grip pressure and is deemed a reliable device for use in children[15] and the elderly as it is less dependent on hand anthropometry.[22] It has been compared and validated against other dynamometers.[22–24] Using the Martin vigorimeter, we measured handgrip strength on cognitively impaired children as young as 4 years of age and confirmed decreased strength in a separate cohort. All individuals evaluated were able to generate some measurable force with the Martin vigorimeter. There was a small overlap of participants in both cohorts for individuals with CS but no overlap in the other groups. In addition, the measurements of the 5 overlapping CS individuals in which the Martin vigorimeter was used were performed 2 years after the initial measurement with the Takei dynamometer.

Decreased physical activity levels based on questionnaires have been reported in some of the RASopathies.[25] Whether or not an inherent muscle abnormality leads to decreased physical activity, or decreased physical activity due to other issues results in subsequent weakness, is difficult to determine. Increased bone resorption markers have also been reported in NF1, CS, CFC, and NS,[25] which potentially could be a secondary effect of abnormal muscle function and subsequent decrease in load bearing activities. Therefore muscle weakness could be a contributing factor to the osteopenia and osteoporosis observed in several of the RASopathies.

This study provides objective evidence of decreased strength using a handgrip dynamometer in the RASopathies. Based on the results of decreased strength in addition to the known musculoskeletal problems in the RASopathies, we think that physical therapy and/or occupational therapy assessments should be considered for patients. It is likely that increasing strength will result in improved ability to participate in physical activities with subsequent improvement in quality of life. In addition, we theorize that the frequency and onset of many of the musculoskeletal abnormalities associated with the RASopathies, including late-onset hip dysplasia, scoliosis, and contractures, might be improved if muscle strength and tone could be normalized.

Future studies including of longitudinal assessment of strength as patients age, studies to assess efficacy of strength training, and studies on associations of co-morbidities (e.g., cognitive impairment, contractures, cardiomyopathy) will be informative. Better delineation of the pathophysiology of reduced muscle force will help to determine what therapies will be most efficacious.

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