Skeletal muscle and motor deficits in Neurofibromatosis Type 1

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Abstract

Neurofibromatosis Type 1 (NF1) is a genetic neurocutaneous disorder with multisystem manifestations, including a predisposition to tumor formation and bone dysplasias. Studies over the last decade have shown that NF1 can also be associated with significant motor deficits, such as poor coordination, low muscle tone, and easy fatigability. These have traditionally been ascribed to developmental central nervous system and cognitive deficits. However, recent preclinical studies have also illustrated a primary role for the \textit{NF1} gene product in muscle growth and metabolism; these findings are consistent with clinical studies demonstrating reduced muscle size and muscle weakness in individuals with NF1. Currently there is no evidence-based intervention for NF1 muscle and motor deficiencies; this review identifies key research areas where improved mechanistic understanding could unlock new therapeutic options.

Keywords: Neurofibromatosis Type 1, NF1, Muscle, Weakness, Neuropathy

Introduction

Neurofibromatosis Type 1 (NF1) is an autosomal dominant genetic disorder that affects approximately 1 in 3000 births\textsuperscript{1,2}. Individuals with NF1 are predisposed to developing abnormalities in a number of body systems; individually and cumulatively they can have a significant impact on quality of life. For example, skeletal deformities such as scoliosis and pseudarthroses, are uncommon but can be associated with considerable morbidity\textsuperscript{3}. Benign, malignant, and disfiguring tumors (termed neurofibromas) are characteristic of the condition and can be challenging to manage\textsuperscript{4}. Neuropsychological impairments in NF1, such as executive dysfunction, inattention, specific learning disorder and reduced social competency, can result in reduced social participation and social isolation\textsuperscript{5}.

The clinical diagnosis of NF1 relies on fulfilling at least two of the seven diagnostic criteria; café au lait macules, skinfold freckling, neurofibromas, Lisch nodules, optic pathway tumors, bone dysplasia or a family history\textsuperscript{2}. Notably, some of these features are congenital in origin, while some manifest over time at characteristic developmental stages\textsuperscript{6}.

Skeletal muscle and motor deficits, such as reduced muscle size\textsuperscript{7}, muscle weakness\textsuperscript{8,9}, and poor co-ordination\textsuperscript{9} are increasingly recognized as common manifestations of NF1. Historically these deficits have been attributed to central nervous system dysfunction\textsuperscript{11}. However, recent preclinical and clinical studies have indicated a primary role for the \textit{NF1} gene product, neurofibromin, in muscle growth and metabolism. Neurofibromin has been characterized as a GTPase activating protein that is a negative regulator of the Ras GTPase\textsuperscript{12}. Loss of functional neurofibromin leads to a dose-dependent increase in Ras signaling, which can profoundly affect cell proliferation, differentiation, and function [reviewed in \textsuperscript{12}].

In this review we will discuss the evidence for neurological and cognitive deficits contributing to the motor/muscle phenotype. This review also refocuses discussion on previous studies that describe a vital role for \textit{NF1} in skeletal muscle de-
## Table 1. Motor impairment in NF1.

| AUTHOR & YEAR | DESIGN                      | SUBJECTS                                      | GENDER, AGE | OUTCOME MEASURES                                      | RESULTS                                      |
|---------------|-----------------------------|-----------------------------------------------|-------------|-------------------------------------------------------|----------------------------------------------|
| Eldridge et al. 1989 | Case-control study | NF1 n=13 Unaffected sibling controls n=13 | NF1 6-27 yrs n=3F n=10M Controls n=9F n=4M | PANESS test of gross and fine motor function | Gross and fine motor function ↓ (p=<0.0001) |
| Hofman et al. 1994 | Case-control study | NF1 n=12 Unaffected sibling controls N=12 | NF1 6-14 yrs n=10M n=2F Controls 7-16 yrs n=6M n=4F | PANESS test of gross and fine motor function | Gross and fine motor function ↓ (p=<0.01) |
| North et al. 1995 | Case-control study | NF1 n=51                                      | 8-16 yrs    | Berry Test of visual-motor integration (VMI)           | VMI ↓ (Norm mean 100, NF1 mean 92.4)          |
|                |                             |                                               |             | Henderson Test of motor impairment                     | Coordination deficits                        |
|                |                             |                                               |             |                                                       | Mild n=11                                    |
|                |                             |                                               |             |                                                       | Moderate n=7                                 |
|                |                             |                                               |             |                                                       | Definite n=13                                |
|                |                             |                                               |             |                                                       | Specific problems with:                     |
|                |                             |                                               |             |                                                       | Manual dexterity                            |
|                |                             |                                               |             |                                                       | Balance                                      |
|                |                             |                                               |             |                                                       | Ball skills                                  |
| Dilts et al. 1996 | Case-control study | NF1 n=20 Unaffected sibling controls n=20 | NF1 6-16 yrs Controls 6-17 yrs | Developmental test of visual-motor integration (VMI) | VMI ↓ (p=<0.05) |
| Hyman et al. 2003 | Prospective longitudinal study (1992-2000) | NF1 n=32                                      | N/A         | Berry Test of visual-motor integration (VMI)           | VMI ↓ (p=<0.002) |
| Billingsley et al. 2003 | Case-control study | NF1 n=38 Healthy Controls n=38 | NF1 n=16M n=22F Controls n=21M n=17F | Finger tapping test of fine motor speed | Fine motor speed ↓ (p=0.05) |
| Feldmann et al. 2003 | Case-control study | NF1 n=100 Healthy controls n=100 | NF1 6-37 yrs n=57F n=43M Controls 6-39 yrs n=51F n=49M | Motorische Leistungs-Serie (MLS) computer based-motor performance task | Steadiness (no. of contacts) ↓ (p=<0.05) |
|                |                             |                                               |             |                                                       | Steadiness (time of contacts, s) ↑ (p=<0.01) |
|                |                             |                                               |             |                                                       | Tapping test (no. of contacts) ↓ (p=<0.01)   |
| Hyman et al. 2005 | Case-control study | NF1 n=81 Unaffected sibling controls n=49 | NF1 8-16 yrs 47%F 53%M Controls 8-16 yrs 59%F 41%M | Grooved peg board test of fine motor coordination TOVA test of motor speed | Fine motor coordination ↓ (p=0.003) Motor speed ↓ (p=0.007) |
| Johnson et al. 2010 | Case-control study | NF1 n=26                                      | NF1 4-15 yrs n=13M n=13F | Bruininks Oseretsky Test of Motor Proficiency | Total motor composite ↓ (p=<0.05) |
development, as well as the emerging pre-clinical models that suggest a novel regulatory role for \( NF1 \) in muscle metabolism.

**Neurological and cognitive deficits may contribute to impaired motor skill**

Children with NF1 have been shown to have a generalized decrease in intellectual function (mean IQ in the low 90’s) as well as a higher rate of specific cognitive deficits (i.e. attention, visuoperceptual skills, language and executive function)\(^{13,14} \). Neurocognitive studies have also frequently reported impairment in motor abilities in individuals with NF1 (Table 1), including mild impairment in gross and fine motor tasks\(^{14,16} \) and impairment on the Beery test of visuomotor integration, which requires subjects to draw increasingly difficult shapes and figures\(^{17-19} \). Individuals with NF1 also present with deficits in a range of functional tasks that likely have significant impact on quality of life. For example, significant impairment in balance, muscle strength, and upper limb co-ordination in NF1 has been observed using the BOT-2 test of motor proficiency\(^{10,20} \). Mild to moderate deficits in manual dexterity, balance, and ball handling skills\(^{19} \), deficient motor timing and reaction time\(^{21} \), reduced fine motor speed\(^{22} \), and impaired handwriting in NF1 children has also been reported\(^{23} \).

A recent study has highlighted a correlation between measures of cognition and gait\(^{10} \). Gait assessment was performed in 46 children and adolescents with NF1 (ages 7-17yrs) using the GAITRite electronic walkway, and results were compared with a battery of cognitive tests, including the Wechsler Intelligence Scale for children and sub-tests from the Cambridge Automated Neuropsychological Test Battery. The largest correlations were found between deficits in gait width and spatial working memory \((r=0.594, p<0.01)\), and deficits in running speed and agility with impaired strategy generation \((r=0.549, p<0.01)\), supporting speculation of a link between NF1 motor deficits and abnormal central nervous system (CNS) function\(^{11,24} \).

While the mechanisms of interaction between the CNS and peripheral nervous system (PNS) and motor dysfunction are unclear, structural and functional brain abnormalities are a feature of the condition that may contribute to motor impairments. Neurofibromin plays a significant role in the developing brain, with loss of expression resulting in increased cell

### Table 1. (continuous from previous page)

| AUTHOR & YEAR | DESIGN | SUBJECTS | GENDER, AGE | OUTCOME MEASURES | RESULTS |
|---------------|--------|----------|-------------|------------------|---------|
| Knab et al. 2011 | Case-control study | NF1 n=70 | NF1 12.3±2.5 yrs n=36M n=34F Controls 10.7±2.1 yrs n=6M n=13F | Berry Test of visual-motor integration (VMI) | VMI ↓ \((p<0.001)\) |
| Debrabant et al. 2014 | Case-control study | NF1 n=20 | NF1 n=9M n=11F Controls n=11M n=9F | The visual-motor reaction time test (VRT) | VRT variables ↓ \((p<0.05)\) VMI Copy test ↓ \((p<0.001)\) Tracing test ↓ \((p=0.013)\) |
| Champion et al. 2014 | Case-control study | NF1 n=46 | NF1 7-17 yrs n=26M n=20F | Bruininks Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2) Gait assessment using the GAITRite electronic walkway | Balance ↓ \((p<0.001)\) Running speed & agility ↓ \((p<0.001)\) Upper limb coordination ↓ \((p<0.001)\) Impaired gait in NF1: Velocity ↓, Cadence ↓, Stride length ↓, Single support ↓, Base of support ↓, Step time ↑, Double support ↑ |
| Gilboa et al. 2014 | Case-control study | NF1 N=30 | NF1 8-16 yrs n=9M n=21F Controls 8-16 yrs n=9M n=21F | Berry Test of visual-motor integration (VMI) and motor coordination (MC) subtest The Hebrew Handwriting Evaluation (HHE) | VMI & MC ↓ \((p=0.007)\) HHE performance ↓ \((p=0.000)\) |
proliferation and differentiation, ultimately impacting morphology\textsuperscript{25}. A recurrent finding has been an increase in total brain volume, involving both grey and white matter\textsuperscript{26}. Diffusion weighted magnetic resonance imaging (MRI) in individuals with NF1 indicates reduced white matter integrity in a number of regions closely linked to motor function. These include the: (1) corpus callosum, (2) caudate nucleus; a sub-region of the basal ganglia involved in goal-direct behavior and voluntary movement, and (3) thalamus; a sub-cortical nuclear complex that receives and relays cortical and sub-cortical inputs that sub-serve sensory and motor mechanisms, as well as cognitive abilities\textsuperscript{27,28}. Volumetric studies report these structures as abnormally large in NF1 cohorts\textsuperscript{29,30}, suggesting a reduced signal-to-noise ratio\textsuperscript{31,32}. Moore et al have further shown significant associations between increased corpus callosum size and reduced motor performance in children with NF1\textsuperscript{33}. There is also mounting evidence for functional abnormalities within the thalamus and caudate in individuals with NF1. Both adult and pediatric positron emission tomography studies report thalamic hypometabolism\textsuperscript{34,35}, suggesting reduced thalamic signal processing, and a recent event-related functional MRI study identified abnormal right caudate activation during a spatial working memory task\textsuperscript{36}; a cognitive ability significantly associated with impaired gait in NF1\textsuperscript{10}. Individuals with NF1 also commonly show focal areas of high T2 signal intensity on MRI, which have been associated with reduced fine motor skill\textsuperscript{11}.

Mechanistically, one clinical study has examined sensory and motor neuropathy in NF1 as possibly contributory. Electrophysiological measures in 39 individuals with NF1 aged 10-56 revealed motor polyneuropathy was a rare manifestation, and while abnormalities in multimodal evoked potentials (visual, auditory and sensory) were seen commonly, many were associated with tumors or lesions\textsuperscript{37}. Further clinical studies correlating neurological assessments with strength and other motor outcomes are needed to define the influence of the CNS and PNS in the NF1 motor phenotype.

While preclinical mouse models have been used to investigate mechanisms underlying some neurocognitive deficits, a relationship between cognition and motor performance has not yet been defined in these systems. Abnormalities such as the learning and attention deficits have been extensively examined in the \textit{Nf1}\textsuperscript{\textsuperscript{\textsuperscript{-/-}}} mouse\textsuperscript{38-43}. For example, increased GABA release in the hippocampus has been shown to underlie learning deficits, and can be rescued by inhibition of ERK signaling\textsuperscript{38}, while Lovastatin treatment has been shown to rescue deficits in learning and attention\textsuperscript{42}. However, interactions between these deficits and motor function remain unknown. The most significant motor deficit seen in this mouse line was impaired grip strength using a hanging wire test\textsuperscript{44}, and there was no demonstration of a neurocognitive basis for this result. Furthermore, this line failed to show any deficiencies in motor performance tests linked to cerebellar function\textsuperscript{45}.

Decreased dopamine levels in the striatum have also been identified in a further \textit{Nf1}\textsuperscript{\textsuperscript{\textsuperscript{-/-}}} mouse model with bi-allelic \textit{Nf1} inactivation in glia\textsuperscript{46}. These mice exhibited reduced exploratory behaviors as well as selective and non-selective attention abnormalities, which were rescued by pharmacologic intervention to restore dopamine levels. While speculative, abnormal dopamine levels may underlie some of the motor impairments observed in individuals with NF1. Indeed some gait characteristics identified in a pediatric NF1 cohort, including a shorter step length and longer step time, resemble those seen in early Parkinson disease; a disorder associated with reduced dorsal striatal dopamine levels\textsuperscript{47}. Further insight into the interactions between neurocognitive development and motor deficits may come from more advanced mouse models of conditional double inactivation of \textit{Nf1} in neurons\textsuperscript{47}. This remains an important area for future investigation.

### Reduced muscle size and impaired muscle function in NF1

In 2005, Stevenson et al. published data from an analysis of 40 individuals with NF1 using peripheral quantitative computed tomography (pQCT) scanning\textsuperscript{7}. Individuals with NF1 presented with a significant reduction in muscle cross-sectional area compared to age matched controls. Comparative findings were seen in a pediatric NF1 cohort where lean tissue was measured using dual-energy x-ray absorptiometry (DEXA)\textsuperscript{48}. Children with NF1 had a significantly reduced lean tissue mass. While reduced muscle size may imply a reduction in strength\textsuperscript{49,50}, muscle functional outcomes were not assessed in these primarily radiographic studies\textsuperscript{48,51}. However, in a seminal 2009 study, Souza et al performed hand grip dynamometry testing of 21 subjects (age 7-60 yrs) with NF1 compared to gender, aged, and physical activity matched controls, and demonstrated a significant reduction in grip strength in the NF1 cohort\textsuperscript{8}.

Findings of muscle functional impairment in NF1 have been subsequently reported in clinical studies (Table 2). Reduced strength of the hip extensor muscles has been described using hand held dynamometry\textsuperscript{52}. Likewise, a 2013 trial investigating lower body muscle function in NF1 children, found that jumping force (N/kg) and jumping power (W/kg) were both significantly reduced\textsuperscript{53}. Aerobic exercise performance may also be impaired. A cohort of 17 individuals with NF1, along with gender, age, and bodyweight matched control subjects, underwent maximal oxygen consumption (VO\textsubscript{2} max) testing, a measure of maximal aerobic exercise capacity. Individuals with NF1 had a reduced VO\textsubscript{2} max as well as a reduced maximal systolic blood pressure\textsuperscript{54}. While the authors acknowledged that there was some difficulty in recruiting activity matched controls, it remains to be thoroughly investigated whether reduced physical activity accounts for reduced exercise capacity in NF1. Given the mounting evidence for motor and muscular deficits in NF1, continued exercise studies of this kind are warranted, particularly those assessing quality of life outcome measures.

Insight gained from research into NF1 muscle function may be applicable to other related genetic diseases. NF1 belongs to the RASopathy family of diseases, which includes Costello syndrome, Cardiofaciocutaneous syndrome\textsuperscript{55}, and Noonan syndrome.
syndrome. In a 2012 clinical study, hand-grip dynamometry was used to assess muscle strength across these syndromes. Reduced grip strength and muscle weakness was identified as a common feature of them all. It is unclear whether these conditions share a common mechanism for muscle weakness downstream of altered Ras signaling. However, if this is the case, any successful pathway-targeted interventions that improve NF1 muscle performance, may have broad clinical applicability to other RASopathies.

**NF1 is critical for skeletal muscle development**

Early evidence suggesting a critical role for NF1 in skeletal muscle development came from in vitro experiments assessing gene expression during myoblast differentiation. Levels of NF1 mRNA, and neurofibromin were elevated during differentiation, and a concomitant decrease in activated p21-Ras was observed. Subsequent studies have demonstrated that Ras overexpression can inhibit myoblast differentiation.
In mice, double inactivation of Nf1 was found to be embryonically lethal, and Nf1 null embryos showed underdeveloped cardiac and skeletal muscle60. However, it remained unclear whether these effects on muscle were secondary to some other failure in the developmental program, and it wasn’t until recently that a key role for Nf1 in muscle was demonstrated in an animal model. Kolanczyk et al. developed a limb-specific Nf1 knockout mouse (Nf1-/-) using a Prx1-Cre transgene to drive deletion of Nf1 in cells of the mesenchymal lineage51. This mouse strain showed reduced muscle weight, muscle weakness, and muscle fibrosis. Furthermore, consistent with in vitro data, analysis of mutant embryos revealed hyperactive Ras/MAPK signaling, and impaired myoblast differentiation in the developing limbs52.

Nf1 regulation of muscle metabolism

While the Nf1-/- mouse model demonstrated the requirement of Nf1 for normal limb muscle development, Prx1-Cre driven recombination is not restricted to the muscle lineage. Thus, the inactivation of Nf1 in other mesenchymal tissues including adipocytes, connective tissue, and bone, were potential confounders to interpretation. To overcome this, a skeletal muscle specific Nf1 knockout mouse (Nf1Muo-/-) was subsequently generated, by crossing the MyoD-Cre transgenic mouse63 with the Nf1flox line60.

Homozygous Nf1 inactivation in muscle was lethal in the first week of life, and while pups were born at normal bodyweight, they had stunted growth and a high rate of maternal infanticide was observed. Electron microscopy (EM) imaging of Nf1Muo-/- muscle specimens showed no evidence of cytoarchitectural abnormalities, including protein aggregates, myofibril disruption, or Z-line streaming64. While Nf1-/- muscle has been described as dystrophic62, this may be a monomer, as neither mouse model nor patient muscle biopsies have been characterized as having progressive loss of cytoskeletal or membrane protein integrity65.

EM of 3-day old Nf1Muo-/- muscle samples unexpectedly revealed excessive accumulations of intramyocellular lipid, which was subsequently confirmed by Oil Red O staining64. This led to speculation that Nf1 may have a key role in the regulation of muscle lipid metabolism. Analysis of adult Nf1Muo-/- muscle samples revealed similarly elevated triglyceride levels, 10-fold that of controls64.

Increased fatty acid synthesis may underlie these accumulations in the Nf1Muo-/- mice, as a substantive increase in the expression of fatty acid synthase was observed65. Metabolic dysregulation was also seen for a range of mitochondrial enzymes, including succinate dehydrogenase (SDH), β-hydroxyacyl-CoA dehydrogenase (BHAD), and medium-chain acyl-CoA dehydrogenase (MCAD). The expression of mitochondrial fatty acid transport protein carnitine palmitoyl transferase-1 (CPT-1), and membrane transport proteins, CD36, and fatty acid transport protein 4 (FATP4) were also reduced64.

Interpretation of these metabolic and molecular perturbations remains challenging. For example, it is difficult to separate any primary deficits responsible, from any downstream or compensatory metabolic changes in the mature Nf1Muo-/- muscle. Interestingly, analysis of neonatal Nf1Muo-/- muscle showed only the intramyocellular lipid phenotype, suggesting that lipid accumulation may be the initiating factor, and that subsequent molecular and metabolic dysregulation is temporal in nature64. Although the precise mechanisms remain unknown, these mouse data demonstrate a novel metabolic regulatory role for neurofibromin in muscle.

One possibility is that NF1 muscle has commonalities with the lipid storage myopathies (LSMs), which also present with progressive muscle weakness and muscle lipid accumulation66. If parallels are found to exist with the LSMs, this may provide insight into potential interventions for NF1. For example, primary carnitine deficiency (PCD) leads to an impairment of lipid transport into the mitochondria, a resultant accumulation of lipid droplets, and muscle weakness. PCD patients have been successfully treated with high dose L-carnitine supplementation66. While such speculation is attractive to entertain, evidence for lipid accumulation in human NF1 samples has not been firmly established. Identifying lipid accumulation in human NF1 muscle biopsies will be an important goal for researchers in order to demonstrate the relevance of these murine models.

Key questions remain unanswered

A number of historical and recent studies raise important questions regarding the role of the NF1 gene in muscle development and function.

In the 1990s, Gutmann et al identified cardiac and skeletal muscle isoforms of NF167,68. To date, the functional importance of these isoforms remains unclear. It is possible that they have a unique role in the regulation of muscle development and/or metabolism. Isoform-specific knockout models may be able to provide insight into the role of alternatively spliced variants of neurofibromin in muscle. Furthermore, the genetics of NF1 in muscle are yet to be elucidated. Some manifestations of NF1 are associated with heterozygosity (haploinsufficiency) and others with double inactivation. For example, local double inactivation in muscle was lethal in the knockin mouse60.

One intriguing possibility is that NF1 mutations and associated weaknesses may indirectly influence physical activity. Recent data indicates that children with NF1 have reduced participation in formal and informal physical activities71. Finding ways to accommodate this into both studies of the underlying biological weaknesses and strategies for exercise-based intervention may have its own challenges.

One intriguing possibility is that NF1 mutations and asso-
associated muscle weakness may increase the risk or severity of other related or unrelated conditions. For example, spinal complications such as scoliosis can be a major source of morbidity in NF1. Both bone density and paraspinal muscle strength can affect scoliotic progression. Late onset scoliosis has been described in adolescents with NF1 with no underlying abnormalities, and it is possible that weakness or hypotonia may be contributory. In addition, two recent case reports suggest the potential for interactions between NF1 and other muscle-related genetic conditions. One report describes an individual with digeny for mutations in NF1 and ryanodine receptor 1, resulting in myopathy. The other shows a previously unreported mutation in an NF1 locus leading to mitochondrial complex I deficiency, hypotonia, and developmental delay.

Continued identification of clinical presentations of this kind may provide useful insight.

From a therapeutic standpoint, it will be critical to ascertain the capacity of exercise regimes to modify the muscle and motor phenotypes in NF1. The current literature is limited to a single case study that reports improved jumping and throwing performance in children with NF1, following a plyometric training program. However, this study was small (n=3), the children were of variable ages and genders, and the study lacked a control cohort. Larger randomized and controlled exercise intervention studies are greatly needed to answer questions regarding the effects of exercise training on motor control, muscle size and strength, fatigue, and quality of life outcomes in individuals with NF1.

While physical therapies are often favored if they can produce significant benefits, pathway-specific pharmacological interventions remain a potential treatment for those found unresponsive to exercise. The Ras-MEK-ERK pathway is the canonical pathway in NF1, but this signaling cascade is also recognized for its role in muscle. Constitutively active MEK has been shown to directly bind and repress myogenic transcription factors, inhibiting myogenic differentiation in vitro. Furthermore, in a cancer setting, MEK/ERK inhibition has been shown to be anabolic for skeletal muscle in humans and mice. This pathway is likely to be of particular relevance for interventions aiming to improve muscle function in NF1.

Conclusion

Souza et al. can be credited for initiating an explosion of activity in the field of NF1 muscle research in 2009. Since then, a range of clinical studies have confirmed reduced motor performance and/or muscle impairment in individuals with NF1. While there are associations between neurological abnormalities and the NF1 muscle/motor phenotype, the mechanisms underlying these interactions are yet to be elucidated, and remain an area for further research. Mechanistically, recent studies using genetically modified mouse models have provided strong evidence for a metabolic regulatory role for neurofibromin in muscle, likely contributing to the phenotype.

This review has also reflected on a number of historically overlooked or potentially undervalued studies. The key roles for NF1 in muscle development are preceded by studies showing increases in NF1 gene and protein expression during myogenic differentiation. The findings of deficits in muscle strength are similarly preceded by radiographic studies showing decreases in muscle mass. However, it is the studies describing muscle-specific NF1 isoforms that are perhaps the most relevant to revisit, as these isoforms may have as yet undefined roles in the NF1-muscle phenotype.

In summary, there have been significant advances in our understanding due to a co-ordination of basic and clinical research studies. Continued investigation into the underlying biology may translate into new approaches for intervention.

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