Published in final edited form as:
Am J Med Genet A. 2012 September ; 0(9): 2225–2232. doi:10.1002/ajmg.a.35535.

The Learning Disabilities Network (LeaDNet): Using Neurofibromatosis Type 1 (NF1) as a Paradigm for Translational Research

Maria T. Acosta[1,2,*], **Carrie E. Bearden**[3], **Xavier F. Castellanos**[4], **Laurie Cutting**[5], **Ype Elgersma**[6], **Gerard Gioia**[1], **David H. Gutmann**[7], **Yong-Seok Lee**[8], **Eric Legius**[9], **Maximillian Muenke**[2], **Kathryn North**[9], **Luis F. Parada**[10], **Nancy Ratner**[11], **Kim Hunter-Schaedle**[12], and **Alcino J. Silva**[3]

[1]The Gilbert Neurofibromatosis Institute, Children's National Medical Center, Washington District of Columbia [2]National Human Genome Research Institute, National Institute of Health, Bethesda, Maryland [3]University of California, Los Angeles, California [4]New York University, New York, New York [5]Vanderbilt University, Nashville, Tennessee [6]Erasmus University, Rotterdam, The Netherlands [7]Washington University School of Medicine, St. Louis, Missouri [8]University of Leuven, Leuven, Belgium [9]Children's Hospital at Westmead, University of Sydney, Sydney, Australia [10]University of Texas Southwestern, Dallas, Texas [11]Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio [12]Children's Tumor Foundation, New York, New York

Abstract

Learning disabilities and other cognitive disorders represent one of the most important unmet medical needs and a significant source of lifelong disability. To accelerate progress in this area, an international consortium of researchers and clinicians, the Learning Disabilities Network (LeaDNet), was established in 2006. Initially, LeaDNet focused on neurofibromatosis type 1 (NF1), a common single gene disorder with a frequency of 1:3,000. Although NF1 is best recognized as an inherited tumor predisposition syndrome, learning, cognitive, and neurobehavioral deficits account for significant morbidity in this condition and can have a profound impact on the quality of life of affected individuals. Recently, there have been groundbreaking advances in our understanding of the molecular, cellular, and neural systems underpinnings of NF1-associated learning deficits in animal models, which precipitated clinical trials using a molecularly targeted treatment for these deficits. However, much remains to be learned about the spectrum of cognitive, neurological, and psychiatric phenotypes associated with the NF1 clinical syndrome. In addition, there is a pressing need to accelerate the identification of specific clinical targets and treatments for thesephenotypes. The successeswith NF1 have allowed LeaDNet investigators to broaden their initial focus to other genetic disorders characterized by

*Correspondence to: Maria T. Acosta, M.D., Department of Neurology, Children's National Medical Center, 111 Michigan Av. NW, Washington, DC 20010. macosta@childrensnational.org.

How to Cite this Article: Acosta MT, Bearden CE, Castellanos XF, Cutting L, Elgersma Y, Gioia G, Gutmann DH, Lee Y-S, Legius E, Muenke M, North K, Parada LF, Ratner N, Hunter-Schaedle K, Silva AJ. 2012. The Learning Disabilities Network (LeaDNet): Using neurofibromatosis type 1 (NF1) as a paradigm for translational research. Am J Med Genet Part A 158A:2225–2232.

learning disabilities and cognitive deficits including other RASopathies (caused by changes in the Ras signaling pathway). The ultimate mission of LeaDNet is to leverage an international translational consortium of clinicians and neuroscientists to integrate bench-to-bedside knowledge across a broad range of cognitive genetic disorders, with the goal of accelerating the development of rational and biologically based treatments.

Keywords

neurofibromatosis type 1; learning disabilities; RAS/MAPK pathway; neurodevelopmental disorders; Learning Disabilities Network

INTRODUCTION: DEFINING THE NF1 COGNITIVE PHENOTYPE: WHAT WE KNOW AND DON'T KNOW

Our appreciation of the spectrum of the NF1 cognitive phenotype has significantly expanded over the last decade. Common features of the NF1 cognitive phenotype include problems with visuospatial learning and memory, deficits in executive function, including prominent working memory deficits, and difficulties with sustaining and switching of attention [Acosta et al., 2006]. Global cognitive deficits can be reliably identified in children as young as 2 years of age [Lorenzo et al., 2011]. Behavioral problems and academic difficulties that mimic the manifestations present in children with typical attention deficit/hyperactivity disorder (ADHD) are present in up to 60% of patients with diagnosis of NF1 [Acosta et al., 2006; Hyman et al., 2006]. ADHD is thought to contribute to the learning disabilities and poor social skills in NF1 [Barton and North, 2004]. NF1 is associated with low-to-normal IQ scores, with mean Full Scale IQ (FSIQ) in the low 90s. The frequency of intellectual disability in NF1 is ~6%, which is about twice the frequency in the general population. Intellectual function tends to be stable in individuals with NF1 over time [Hyman et al., 2005]. Because of the various behavioral and cognitive problems associated with NF1, up to 75% of affected children have learning problems, and most school-age children with NF1 need additional support in the form of special education or remedial teaching [Krab et al., 2008a].

Despite major recent advances in our understanding of the clinical and neurobiological aspects of the cognitive deficits in NF1, there is a pressing need to advance current knowledge of the behavioral and cognitive deficits associated with this condition. This information will be critical to guide potential clinical and behavioral interventions currently envisioned for NF1. While some areas of behavior and cognition research have advanced, others have lagged behind. For example, the neurocognitive profile of NF1 has now been fairly well characterized. The profile involves a typical pattern of difficulties in attention, visuospatial skills, and working memory [Acosta et al., 2006]. A recent translational study [Shilyansky et al., 2010] revealed hypoactivation of cortical striatal networks and working memory deficits in *Nf1* mice and in NF1 patients. The studies with NF1 patients used parametric working memory tasks and functional neuroimaging (fMRI) to show that the degree of hypoactivation of corticostriatal networks in NF1 was predictive of the degree of impairment in working memory performance [Shilyansky et al., 2010]. However, other areas

(e.g., social cognition) in NF1 have received comparatively little attention. For example, there is extensive evidence that the RAS/mTOR pathway is implicated in the autism spectrum disorders (ASD), and that NF1 affects this signaling pathway. Unfortunately, little is currently known about ASDs and other psychiatric phenotypes in NF1. Interestingly, there is evidence suggesting that the rates of the ASDs are higher in the NF1 population, and that *Nf1* mutations in mice affect social interactions and other behavioral measures associated with ASDs. Moreover, there is growing evidence that social cognition deficits are a key target for intervention in psychiatric disorders, such as schizophrenia, since these deficits are a key predictor of psychosocial outcome. Further investigation of social cognition and other ASD phenotypes in NF1, may be of great importance for the development of targeted interventions that will improve cognitive and psychiatric health of individuals with NF1.

Neuroimaging techniques have provided important, although controversial, insights into specific NF1-related learning problems. However, no systematic approach has been taken to resolve and understand these controversial findings. Some of the neuroimaging findings reported by multiple research groups include potential increases in gray matter volume, increases in the cross-sectional area of the corpus callosum, and the presence of T2 hyperintensities (T2H) in the thalamus [Hyman et al., 2005, 2007; Acosta et al., 2006; Pride et al., 2010]. Unfortunately, the tentative and controversial nature of some of these measures has prevented them from being used clinically. Larger sample sizes and longitudinal studies are necessary to better understand and characterize both possible neuroanatomic changes in NF1 over time and the real impact of these changes in cognitive and behavioral deficits.

The purpose of our review is to provide a comprehensive overview of the current state of knowledge of cognitive disorders in NF1 and their treatment and illustrate how Learning Disabilities Network (LeaDNet) represents a paradigm for related translational research.

THE GENETICS OF NFI-ASSOCIATED COGNITIVE AND BEHAVIORAL DEFICITS

As a single gene disorder, NF1 has enormous potential for elucidating gene–brain–behavior connections. These connections are far more difficult and problematic to establish in multigenic disorders, such as ADHD, autism, and schizophrenia, where both the underlying mechanisms and associated phenotypes are far more diverse and complex [Acosta et al., 2004]. Hopefully, the lessons learned in NF1 may be helpful for designing similar studies in more complex and heterogeneous conditions. Data from animal models and clinical neuroimaging studies have shown that NF1 genetic deficits impact both adult function and early brain development. These deficits ultimately manifest in a wide variety of clinical features, from clinically asymptomatic to severe phenotypes. Insights gained from studies of the genetic basis of NF1 phenotypes have been crucial for understanding and developing treatments for this condition.

The *NF1* gene encodes neurofibromin, a large 2,818 amino acid protein containing a GTPase-activating protein (GAP)-related domain, which serves to accelerate the inactivation of the RAS signaling molecule. Previous studies with Nf1 heterozygous mutant mice (*Nf1*+/−) showed that these mice have learning and memory and other behavioral deficits that

parallel key features of the cognitive phenotype of NF1 patients [Silva et al., 1997; Costa et al., 2002; Cui et al., 2008]. Studies of these mutant mice revealed that their hippocampal learning and memory deficits are caused by impairments in long-term potentiation (LTP) [Costa et al., 2002; Cui et al., 2008], a synaptic mechanism involved in learning and memory [Lee and Silva, 2009]. Further studies of these LTP deficits revealed that neurofibromin, the protein encoded by the *NF1* gene, regulates Ras/MAPK signaling presynaptically in hippocampal GABAergic neurons, and that in *Nf1*+/− mice GABA release is enhanced [Cui et al., 2008]. A number of observations showed that this increase in GABA release is the cause for the LTP and learning deficits described for the *Nf1*+/− mice. These included the finding that doses of a GABA receptor blocker that do not affect LTP or learning in control mice, completely reverse the LTP and learning deficits of the *Nf1*+/− mutant mice [Cui et al., 2008]. More importantly, lovastatin, an FDA-approved drug that decreases the isoprenylation and therefore the activation of RAS, can reverse the GABA release, LTP and learning impairments of the *Nf1*+/− mutant mice [Li et al., 2005].

There are three major alternatively spliced exons of the *NF1* gene: exon 9a, 23a, and 48a. The *NF1* isoform containing exon 48a is highly expressed in adult and embryonic cardiac and muscle tissues, but not in the adult brain [Gutmann et al., 1995]. In contrast, the most commonly expressed alternative isoform NF1-Ex23a (NF1 type II) is expressed in the brain, and is particularly highly expressed in astrocytes. This isoform has decreased RAS-GAP activity, and mice lacking exon 23a show specific hippocampal learning impairments [Costa et al., 2001]. Interestingly, expression of the alternative isoform NF1-exon 9a is exclusively restricted to neurons of the central nervous system [Gutmann et al., 1999]. Exon 9a encodes only 10 amino acids in the amino terminal region of neurofibromin. NF1-exon 9a expression is enriched in the septum, striatum, cortex, and hippocampus beginning in the first week of postnatal life. However, until recently, the importance of this neuronal isoform was unclear. Nf1-9a*/9a*mutant mice, which express neurofibromin at normal levels but lack exon 9a, have spatial learning and hippocampal plasticity deficits that are similar to *Nf1*+/− mice. Like the *Nf1*+/− mice, the *Nf1*-9a*/9a*mutant mice show an increase in GABA-mediated inhibition which leads to LTP deficits. All together, these results indicate that neurofibromin exon 9a is critical for synaptic plasticity and learning in the central nervous system.

In addition to functioning as a negative RAS regulator, studies in Drosophila and mouse homozygous cells indicate that neurofibromin is also a positive regulator of the cAMP/PKA pathway [Dasgupta et al., 2003; Brown et al., 2011, 2012]. In flies, cAMP signaling is critical for learning. *Nf1* null Drosophila mutants showed reduced AC activity as well as deficits in olfactory learning, which can be rescued by expression of rutabaga, a constitutively active AC [Guo et al., 2000]. Interestingly, unlike learning deficits, *Nf1*-regulated RAS activity and its GAP domain are responsible for long-term memory in Drosophila. Thus, neurofibromin regulates learning and memory in Drosophila by two separate signaling mechanisms (cAMP and Ras signaling, respectively) [Medeiros et al., 2007]. Distinct functional domains of neurofibromatosis type 1 (NF1) regulate immediate memory versus long-term memory formation [Ho et al., 2007]. Abnormal RAS/MAPK signaling is also responsible for circadian deficits in *Nf1* mutant Drosophila, a result that

suggests that it may be worth testing the hypothesis that sleeping problems in NF1 could be treated drugs that target this signaling pathway (e.g., statins) [Williams et al., 2001].

LESSONS FROM OTHER COGNITIVE DISORDERS

For the first time [Costa et al., 2002] animal model studies of NF1 showed that it was possible to intervene in adult mice and dramatically rescue a number of behavioral phenotypes, including attention and cognitive deficits [Li et al., 2005; Cui et al., 2008]. Previous dogma maintained that neurodevelopmental disorders, such as NF1, are caused by developmental changes irreversible in adults. The work with NF1 motivated similar studies in a dozen other conditions, including tuberous sclerosis, fragile X, Angelman syndrome, Down syndrome, and Rett syndrome. All together, these studies showed that it is possible to reverse key cognitive deficits associated with neurodevelopmental disorders, even when the treatments are initiated in adult mice [Ehninger et al., 2008b]. Although this effort is still in its infancy, follow-up clinical trials suggest that similar adult interventions may also be successful in patients from these various disorders.

Importantly, research in an array [Arcos-Burgos et al., 2004] of other medical conditions associated with cognitive deficits can also inform our understanding of the NF1 phenotype. Examples include (but are not limited to) ADHD, Williams syndrome, ASD, tuberous sclerosis (TSC), and fragile X syndrome, all conditions with some parallel neurological deficits (and therefore clinical needs) as NF1.

A decade of genetic studies of ADHD—the most common behavioral disorder in children with a prevalence of up to 10% [Acosta et al., 2008]—has provided an invaluable opportunity to study and better understand the connections between genetics, behavior, and biological mechanisms. Genome-wide linkage studies in large multigenerational families have identified chromosomal regions containing putative ADHD genes [Arcos-Burgos et al., 2010; Jain et al., 2011]. Recently, the first ADHD susceptibility genes were identified, and interactions between them have been reported to double the risk for ADHD. Interestingly, detailed clinical studies have shown that interactions between two gene loci may be able to predict both ADHD severity and its long-term outcome [Acosta et al., 2011b]. ADHD is common in individuals affected with the RASopathies.

The RASopathies are a group of clinically overlapping disorders caused by mutations in genes coding for key components of the RAS-MAP-kinase pathway [Rauen et al., 2010]. The *NF1* gene was the first gene identified in a human genetic disorder that coded for a protein of the RAS-MAP-kinase pathway. Clinical studies have shown that the RASopathies are generally associated with cognitive and behavioral problems. In addition to NF1, studies in individuals with Legius, Costello, Noonan, and CFC syndrome (all RASopathies) showed cognitive and behavioral problems of different degrees. For example, learning and attention deficits are frequently reported in children with Legius syndrome caused by mutations in *SPRED1* [Messiaen et al., 2009; Rauen et al., 2010] a negative regulator of the RAS-MAPK pathway at the level of RAS–RAF interaction. Further studies in *Spred1* knockout mice revealed hippocampal synaptic plasticity and learning deficits similar to those previously described in *Nf1*+/− heterozygous mice [Denayer et al., 2008]. A considerable amount of

data from humans and animal models indicate that the RAS-MAP-kinase pathway is an important regulator of learning, memory, and behavior. Pharmacological manipulations of this pathway are obvious choices for clinical trials designed to test treatments for the learning disabilities in these children [Li et al., 2005; Acosta et al., 2011a]. Similarly, studies in animal models of Noonan syndrome also point to deregulations in Ras/MAPK signaling responsible for deficits in plasticity and subsequent learning and memory impairments.

Recent human genetic studies have implicated the PI3 kinase/mTOR pathway in a small subset of autism cases that are related to genetically inherited diseases [Hoeffer and Klann, 2010]. A conditional knockout mouse line with *Pten* deleted in a subset of postmitotic neurons in the cortex and hippocampus had normal life span, but exhibited stereotypic behavioral abnormalities reminiscent of ASDs [Kwon et al., 2006; Zhou et al., 2009]. This led to the development of an inducible Nestin-creERT2; Ptenflox/flox mouse line in which cre gene expression, and therefore *Pten* ablation, can be induced pharmacologically (by tamoxifen) at various developmental stages in the neural stem cell population. Studies using this inducible line addressed two important issues: First, whether targeting Pten ablation to the stem/progenitor cell lineage is sufficient to cause ASD behaviors in this mouse model. Second, whether the previous PI3K pathway mouse model (Nse-cre; Ptenflox/flox) identified a specific anatomic region responsible for certain forms of ASDs. Preliminary results show that *Pten* deletion in hippocampal stem cells results in many phenotypic similarities with a previous mouse model, where *Pten* was deleted in mature neurons of the hippocampus and cortex. This suggests a role for hippocampal stem cells in the development of ASDs. It ispossible that social cognition deficits in NF1 are caused by a similar mechanism since NF1/MAPK signaling may affect common downstream signaling components, including mTOR signaling [Li et al., 2012].

Animal studies with TSC, a genetic condition caused by mutations in a GTPase complex, have also recently pointed to a potential targeted treatment. Just as NF1, TSCis characterized by somatic and CNS phenotypes, and an increase in the incidence of ASDs, cognitive deficits, and seizures. Studies of animal models of TSC revealed changes in synaptic plasticity and learning and memory [Ehninger et al., 2008a; Auerbach et al., 2011]. As with *Nf1*+/− mutant mice, *Tsc2*+/− mice show alterations in hippocampal LTP and deficits in hippocampal learning and memory. Interestingly, important aspects of the phenotypes associated with TSC are only fully revealed when environmental factors (i.e., immune activation during pregnancy) are considered and included [Zhao et al., 2010]. *Tsc2*+/− mutant mice only show social interaction deficits, a hallmark phenotype of the ASDs, when their mothers' immune system is challenged during pregnancy [Zhao et al., 2010]. Importantly, studies in TSC patients found evidence consistent with this hypothesis [Zhao et al., 2010]. This suggests the possibility that important aspects of the phenotypes of neurodevelopmental disorders, including those in *NF1*, may only be revealed, studied, and understood, when environmental factors are taken into consideration.

A combination of clinical observations and neurobiological findings will be critical for advancing the understanding and developing treatments for complex genetic or/and clinical conditions. Some examples of those complex genetic conditions include extreme conditions like holoproscencephaly (HPE). HPE has been used as a model of the potential effects of

complex genetic deficits in normal brain development. HPE is the most common anomaly of the developing human forebrain, seen in 1:250 conceptions, with a high rate of fetal demise and a birth prevalence of 1:8,000 live births [Solomon et al., 2010]. Over a dozen genes, contributing to several signaling pathways have been implicated in the etiology of HPE [Roessler and Muenke, 2010]. In addition, a number of environmental factors including maternal serum cholesterol levels, and teratogens, have also been implicated [Edison and Muenke, 2004; Johnson and Rasmussen, 2010]. Incomplete penetrance and variable expressivity has led to tremendous interfamilial phenotypic variability in HPE. Infants with severe CNS anomalies and severe cognitive disability have been observed to have family members with identical gene mutations but very different phenotypes. These family members with identical mutations appear to be clinically and phenotypically normal, and may even be intellectually gifted with an above average IQ. Partial penetrance is also observed in NF1 phenotypes, and understanding its causes is an important future goal of NF1 research.

The variability in the clinical cognitive profile of NF1 could be caused by a number of factors, including the presence of micro-deletions [Descheemaeker et al., 2004], somatic mosaicism, subtle *NF1* missense mutations, modifying genes, factors governing biallelic *NF1* gene inactivation during brain development, and poorly understood environmental factors [Ehninger et al., 2012]. Future cognitive studies of NF1 will require more extensive genetic and genomic approaches. These studies could be directed at unraveling the complex genomic and environmental factors that can modify NF1 phenotypes (learning deficits, ADHD, etc.) in both animal models and human subjects. To understand and treat this and other disorders of ras signaling, it will be critical to understand the genomic and environmental complexity behind the cluster of phenotypes associated with each of these disorders, since this information most likely will instruct clinical decisions as to the best treatment for any one particular patient with a RASopathy.

ADVANCING THERAPEUTIC INTERVENTIONS FROM MOUSE MODELS TO THE CLINIC

What do we know about brain development and cognitive mechanisms from NF1 animal models, and how can we apply this knowledge to clinical care and development of therapeutic interventions? Progress in animal models, and in the clinical understanding of NF1 cognitive defects, has begun to provide the basis for an initial foray into identifying and testing targeted interventions for NF1 learning disabilities. Tools from cognitive neuroscience have been useful in translational research and in treatment development for cognitive impairments in NF1. Behavioral and electro-physiological studies showed that the working memory deficits of *Nf1*+/− mice are probably caused by abnormally enhanced activity-dependent GABA release in cortico-striatal inhibitory networks. Parallel fMRI studies in NF1 individuals showed that the hypoactivation of dorsal lateral prefrontal circuits correlated with their working memory deficits [Shilyansky et al., 2010]. Together, these integrative mouse and human studies suggest that one function of neurofibromin is to regulate the prefrontal and striatal inhibitory networks critical for working memory performance.

Recent studies using *Nf1* genetically engineered mice have been instructive for elucidating the cellular and molecular defects underlying the cognitive deficits seen in children with NF1. Another *Nf1* mouse model was engineered that used the Cre recombination system and the germline *Nf1* heterozygous null mutation to generate a *Nf1*+/− mutant with a complete loss of the *Nf1* gene in GFAP expressing glial cells (*Nf1* OPG mouse) [Brown et al., 2010b]. Unlike the *Nf1*+/− mice, the *Nf1* OPG mice developed optic gliomas [Bajenaru et al., 2003; Zhu et al., 2005; Brown et al., 2012], a common tumor seen in approximately 15% of children with NF1 [Listernick et al., 1997]. The OPG mouse was used to gain insight into the frequently encountered attention system abnormalities seen in children with NF1. These studies reported novel defects in non-selective and selective attention without an accompanying hyperactivity phenotype. Specifically, the *Nf1* OPG mice exhibit reduced rearing in response to novel objects and environmental stimuli [Brown et al., 2010a]. Similar to children with NF1, the attention system dysfunction in these mice is reversed by treatment with methylphenidate (MPH) suggesting a defect in brain catecholamine homeostasis. Using several methods, including positron emission tomography (PET), these studies demonstrated that the attention system abnormalities in *Nf1* OPG mice are the consequence of reduced dopamine (DA) levels in the striatum, which is normalized following either MPH or L-dopa administration [Brown et al., 2011]. Interestingly, previous studies demonstrated that the attention deficits observed in *Nf1*+/− mice could be reversed with a drug (lovastatin) that reversesþthe increased Ras signaling and abnormally high GABA release deficits described in these mice. This demonstrates that two very different treatments, likely working at different steps in the pathways affected, can be used to treat attention deficits in mice with *NF1* mutations. Similarly, multiple treatments will be useful in NF1 patients to address potential heterogeneity caused by poorly understood genetic and environmental factors: NF1 patients resilient to one treatment may nevertheless benefit from another.

Interestingly, recent studies showed that in addition to deficits in GABA release and LTP caused by hyperactivation of the Ras signaling pathway, the *Nf1*+/− mutation also results in a cell-autonomous defect in neuron growth cones and neurite extension that is caused by deregulation of cAMP signaling [Brown et al., 2010b]. Collectively, these data suggest that CNS phenotypes in NF1 may be treated by agents that normalize abnormalities in either Ras signaling, cAMP signaling, or DA homeostasis.

Studies in mouse models have led to the first targeted therapeutic intervention trials in children with NF1. Studies of these mice showed that abnormal Ras activation is associated with impaired hippocampal-based learning that is reversible with lovastatin, a commonly prescribed medication. The initial results of phase 1 studies, have demonstrated that two commonly used statins that permeate the blood–brain barrier (simvastatin and lovastatin) can be safely prescribed in the NF1 population [Krab et al., 2008b; Acosta et al., 2011a]. In addition, these studies provided preliminary evidence that statin treatment may ameliorate specific cognitive deficits in children with NF1 [Acosta et al., 2011a]. An initial small double-blind trial using simvastatin had limitations in terms of selection sample and maybe in treatment duration time [Krab et al., 2008b]. However, two additional large multicenter double-blind placebo control clinical trials, targeting cognitive deficits in NF1, are currently on-going http://www.clinicaltrials. gov/ct2/show/NCT00352599?term=neurofibromatosis

+type+1&rank=l http://www.clinicaltrials.gov/ct2/show/NCT00853580?term=neurofibromatosis+type+1&rank=7.

Recently, resting-state functional magnetic resonance imaging (rs-fMRI) approaches, which assess the brain's intrinsic functional architecture, have been successfully used to index human brain function. The brain exhibits ultra-low frequency fluctuations (<0.1 Hz), which are easily observable during blood oxygenation level-dependent (BOLD) fMRI runs of as little as 6 min. These spontaneous fluctuations show temporal coherence between anatomically connected brain regions, and are functionally related through co-activity in response to task demands [Bajenaru et al., 2003; Zhu et al., 2005; Raichle, 2010; Brown et al., 2012]. The patterns of positive and negative temporal correlation delineate the entire complement of functional circuitry of any individual. These synchronous patterns are both universal and individually specific in their extent and strength. This methodology has the potential to provide regionally specific information regarding single subjects at baseline, and after potentially therapeutic interventions. A recent small pilot study of seven children with NF1, scanned before and after a 12-week treatment with lovastatin (open label), illustrated the promise and potential of this powerful approach for understanding the neurocognitive underpinnings of the behavioral and cognitive deficits associated with NF1 [Chabernaud et al., 2012].

Other potential interventions that could be tested in the NF1 population include some already available stimulant medications for ADHD. As mentioned above, a recent study with *Nf1* OPG mice suggest that abnormal DA homeostasis is a key contributor to the attention deficits associated with this condition, and that these deficits may be reversed with MPH. These results with the mutant mice provide an incentive for pursuing comprehensive clinical trials of stimulant medication in children with NF1. The availability of preclinical Nf1 mouse models with attention system deficits, which can be rapidly assessed behaviorally and neurochemically (PET imaging) [Brown et al., 2010a, 2011], provide new opportunities for understanding the genomic and environmental variables that could affect the clinical impact of MPH treatments.

Despite the justified optimism concerning the promise of current animal model studies of NF1, a cautionary note is in order: the road from animal model to human behavior is far from straightforward. Several limitations exist, including the differences between species, the complexity of the fundamental neurocognitive mechanisms, our poor understanding of these conditions, and the enormous variability of the clinical manifestations targeted by these potential interventions.

POSSIBLE FUTURE INTERVENTION TRIALS FOR NF1 READING DISABILITIES

As mentioned above, approximately half of children with NF1 have learning disabilities. Of the various types of learning disabilities, reading disabilities are common in this population and often the most debilitating, because of their importance for academic and job success. Treatment of reading disabilities in the general population with no *identified* genetic syndrome (i.e., idiopathic reading disability; IRD) has been well-researched with outcomes

described in terms of reading achievement, using standardized tests, and neurobiological correlates of improved reading skill, using neuroimaging. If those interventions were useful in children with NF1, then reading disabilities could be an important clinical target in this population [Cutting and Levine, 2010]. Previous studies have shown that children with NF1 and reading disabilities performed similarly to children with IRD in phonological, rapid naming, and reading comprehension measurements, but demonstrated more pronounced visual spatial deficits [Cutting and Levine, 2010]. In recent studies, findings suggest that children with NF1 with reading difficulty respond to intervention techniques known to work with IRD, and, furthermore, respond to particular reading intervention approaches better than others. Nevertheless, there is still need to gain a deeper understanding of the cognitive characteristics associated with reading disabilities in children with NF1, and how this treatment may interact with drug therapy, as well as presence of ADHD. In addition, it is necessary to confirm behavioral observations that treatment with a reading intervention results in neurobiological changes (measured using fMRI and DTI neuroimaging) in this population as compared to children with IRD. Ultimately, improvement in the quality of life (e.g., school performance) of children with NF1 may depend on a complex combination of behavioral and drug therapy. Drugs, such as statins and MPH, treat the abnormal biochemistry and physiology of the condition, but they do not address the need to bring these children up to par with their peers in a wide range of social and cognitive skills. Extensive behavioral interventions, in parallel with appropriate pharmacological treatments, may be needed for treating the cognitive and behavioral phenotypes associated with NF1.

THE LEADNET CONSORTIUM: A MODEL FOR ADVANCING NF1 COGNITIVE UNDERSTANDING AND THERAPEUTIC DEVELOPMENT

Cognitive deficits have a long-term impact in the quality of life of the NF1 population. These deficits are complex and it is clear that an organization like LeaDNet, where neuroscientists and clinicians work together, is needed to accelerate and facilitate research and develop effective treatments for these problems. LeaDNet is also critical for clinical studies to test the efficacy of treatments developed in animal models, since these trials need large numbers of patients that are only possible in multicenter collaborative studies. Additionally, the LeaDNet collaborative network will also have a central role in developing simpler cognitive and behavioral measurements that are easily comparable across clinical centers and that facilitate the participation of clinical sites with different levels of expertise. For example, the use of standard measurements that can be obtained from interviews with parents, teachers, and caregivers could be encouraged and promoted with the help of LeaDNet investigators all over the world.

The LeaDNet structure will also be central to continue to tighten the connections and communication between neuroscientists working in animal models, cognitive neuroscientists and clinicians participating in clinical trials, so that advances in the laboratory can reach the clinic efficiently and effectively. LeaDNet's involvement in NF1 has been successful, particularly in this area, as the statin cognitive trials indicate. It is possible that trials targeting indications, such as ASD and ADHD in the NF1 population will follow closely behind; thus, observations in mouse models promise to continue to inform and guide clinical

trials for NF1 patients with these conditions, and LeaDNet will have an important role in catalyzing these collaborations. Beyond studies in animal models, it is also important to introduce new technological innovations into clinical studies. This includes new brain imaging techniques, novel cognitive instruments, and biomarkers to track clinical progression, a process that is tremendously facilitated when neuroscientists with different expertise collaborate with clinicians. The need for biomarkers is especially critical, since improvements in certain cognitive measures may take longer to be evident than the duration of clinical trials. Very likely, improvements in specific cognitive domains may require not only the reversal of underlying biochemical deficits (e.g., increased Ras/MAPK signaling), but also behavioral and cognitive interventions that will need time to improve the quality of life of NF1 patients. The common denominator in all of these important future initiatives isthe need for close collaborations amongst clinicians and neuroscientists working in different universities and centers around the world. The complexity of these challenges can only be met effectively within a collaborative framework like the one provided by LeaDNet.

In summary, studies in NF1 have broken new ground in the understanding and treatment of neurodevelopmental disorders, and similar strategies in other genetic models have already shown that the approaches developed for NF1 may be generally applicable. Similarly, strategies and findings from studies of fragile X, ASD, ADHD, HPE, schizophrenia, TSC, and reading disabilities can also be applied in NF1. For example, cognitive neuroscience strategies that have been widely used in autism and schizophrenia, and that take advantage of a detailed dissection of the cognitive phenotypes of these disorders, are much needed in NF1. We have reason for hope: we are closer than ever to a targeted treatment for cognitive deficits associated with NF1. With LeaDNet we have in place a collaborative network of a highly diverse group of neuroscientists and clinicians that will be critical for translating advances in the understanding of this condition into clinical interventions, and hopefully, measureable improvements in the quality of life of the patients we all serve.

REFERENCES

Acosta MT, Arcos-Burgos M, Muenke M. Attention deficit/hyperactivity disorder (ADHD): Complex phenotype, simple genotype? Genet Med. 2004; 6:1–15. [PubMed: 14726804]

Acosta MT, Gioia GA, Silva AJ. Neurofibromatosis type 1: New insights into neurocognitive issues. Curr Neurol Neurosci Rep. 2006; 6:136–143. [PubMed: 16522267]

Acosta MT, Castellanos FX, Bolton KL, Balog JZ, Eagen P, Nee L, Jones J, Palacio L, Sarampote C, Russell HF, Berg K, Arcos-Burgos M, Muenke M. Latent class subtyping of attention-deficit/hyperactivity disorder and comorbid conditions. J Am Acad Child Adolesc Psychiatry. 2008; 47:797–807. [PubMed: 18520958]

Acosta MT, Kardel PG, Walsh KS, Rosenbaum KN, Gioia GA, Packer RJ. Lovastatin as treatment for neurocognitive deficits in neurofibromatosis type 1: Phase I study. Pediatr Neurol. 2011a; 45:241–245. [PubMed: 21907886]

Acosta MT, Velez JI, Bustamante ML, Arcos-Burgos M, Muenke M. A two-locus genetic interaction between LPHN3 and 11 q predicts ADHD severity and long-term outcome. Transl Psychiatry. 2011b; 1:e17. DOI: 10.1038.14 Published online 5 July 2011. [PubMed: 22832519]

Arcos-Burgos M, Castellanos FX, Pineda D, Lopera F, Palacio JD, Palacio LG, Rapoport JL, Berg K, Bailey-Wilson JE, Muenke M. Attention-deficit/hyperactivity disorder in a population isolate: Linkage to loci at 4q13.2, 5q33.3, 11q22, and 17p11. Am J Hum Genet. 2004; 75:998–1014. [PubMed: 15497111]

Arcos-Burgos M, Jain M, Acosta MT, Shively S, Stanescu H, Wallis D, Domene S, Velez JI, Karkera JD, Balog J, Berg K, Kleta R, Gahl WA, Roessler E, Long R, Lie J, Pineda D, Londono AC, Palacio JD, Arbelaez A, Lopera F, Elia J, Hakonarson H, Johansson S, Knappskog PM, Haavik J, Ribases M, Cormand B, Bayes M, Casas M, Ramos-Quiroga JA, Hervas A, Maher BS, Faraone SV, Seitz C, Freitag CM, Palmason H, Meyer J, Romanos M, Walitza S, Hemminger U, Warnke A, Romanos J, Renner T, Jacob C, Lesch KP, Swanson J, Vortmeyer A, Bailey-Wilson JE, Castellanos FX, Muenke M. A common variant of the latrophilin 3 gene, LPHN3, confers susceptibility to ADHD and predicts effectiveness of stimulant medication. Mol Psychiatry. 2010; 15:1053–1066. [PubMed: 20157310]

Auerbach BD, Osterweil EK, Bear MF. Mutations causing syndromic autism define an axis of synaptic pathophysiology. Nature. 2011; 480:63–68. [PubMed: 22113615]

Bajenaru ML, Hernandez MR, Perry A, Zhu Y, Parada LF, Garbow JR, Gutmann DH. Optic nerve glioma in mice requires astrocyte Nf1 gene inactivation and Nf1 brain heterozygosity. Cancer Res. 2003; 63:8573–8577. [PubMed: 14695164]

Barton B, North K. Social skills of children with neurofibromatosis type 1. Dev Med Child Neurol. 2004; 46:553–563. [PubMed: 15287247]

Brown JA, Emnett RJ, White CR, Yuede CM, Conyers SB, O'Malley KL, Wozniak DF, Gutmann DH. Reduced striatal dopamine underlies the attention system dysfunction in neurofibromatosis-1 mutant mice. Hum Mol Genet. 2010a; 19:4515–4528. [PubMed: 20826448]

Brown JA, Gianino SM, Gutmann DH. Defective cAMP generation underlies the sensitivity of CNS neurons to neurofibromatosis-1 heterozygosity. J Neurosci. 2010b; 30:5579–5589. [PubMed: 20410111]

Brown JA, Xu J, Diggs-Andrews KA, Wozniak DF, Mach RH, Gutmann DH. PET imaging for attention deficit preclinical drug testing in neurofibromatosis-1 mice. Exp Neurol. 2011; 232:333–338. [PubMed: 21963652]

Brown JA, Diggs-Andrews KA, Gianino SM, Gutmann DH. Neurofibromatosis-1 heterozygosity impairs CNS neuronal morphology in a cAMP/PKA/ROCK-dependent manner. Mol Cell Neurosci. 2012; 49:13–22. [PubMed: 21903164]

Chabernaud C, Mennes M, Kardel PG, Gaillard WD, Kalbfleisch ML, VanMeter JW, Packer RJ, Milham MP, Castellanos FX, Acosta MT. Lovastatin regulates brain spontaneous low-frequency brain activity in neurofibromatosis type 1. Neurosci Lett. 2012; 515:28–33. [PubMed: 22433254]

Costa RM, Yang T, Huynh DP, Pulst SM, Viskochil DH, Silva AJ, Brannan CI. Learning deficits, but normal development and tumor predisposition, in mice lacking exon 23a of Nf1. Nat Genet. 2001; 27:399–405. [PubMed: 11279521]

Costa RM, Federov NB, Kogan JH, Murphy GG, Stern J, Ohno M, Kucherlapati R, Jacks T, Silva AJ. Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. Nature. 2002; 415:526–530. [PubMed: 11793011]

Cui Y, Costa RM, Murphy GG, Elgersma Y, Zhu Y, Gutmann DH, Parada LF, Mody I, Silva AJ. Neurofibromin regulation of ERK signaling modulates GABA release and learning. Cell. 2008; 135:549–560. [PubMed: 18984165]

Cutting LE, Levine TM. Cognitive profile of children with neurofibromatosis and reading disabilities. Child Neuropsychol. 2010; 16:417–432. [PubMed: 20521183]

Dasgupta B, Dugan LL, Gutmann DH. The neurofibromatosis 1 gene product neurofibromin regulates pituitary adenylate cyclase-activating polypeptide-mediated signaling in astrocytes. J Neurosci. 2003; 23:8949–8954. [PubMed: 14523097]

Denayer E, Ahmed T, Brems H, Van WG, Borgesius NZ, Callaerts-Vegh Z, Yoshimura A, Hartmann D, Elgersma Y, D'Hooge R, Legius E, Balschun D. Spred1 is required for synaptic plasticity and hippocampus-dependent learning. J Neurosci. 2008; 28:14443–14449. [PubMed: 19118178]

Descheemaeker MJ, Roelandts K, De RT, Brems H, Fryns JP, Legius E. Intelligence in individuals with a neurofibromatosis type 1 microdeletion. Am J Med Genet Part A. 2004; 131A:325–326. [PubMed: 15472997]

Edison RJ, Muenke M. Central nervous system and limb anomalies in case reports of first-trimester statin exposure. N Engl J Med. 2004; 350:1579–1582. [PubMed: 15071140]

Ehninger D, Han S, Shilyansky C, Zhou Y, Li W, Kwiatkowski DJ, Ramesh V, Silva AJ. Reversal of learning deficits in a Tsc2+/− mouse model of tuberous sclerosis. Nat Med. 2008a; 14:843–848. [PubMed: 18568033]

Ehninger D, Li W, Fox K, Stryker MP, Silva AJ. Reversing neuro-developmental disorders in adults. Neuron. 2008b; 60:950–960. [PubMed: 19109903]

Ehninger D, Sano Y, de Vries PJ, Dies K, Franz D, Geschwind DH, Kaur M, Lee YS, Li W, Lowe JK, Nakagawa JA, Sahin M, Smith K, Whittemore V, Silva AJ. Gestational immune activation and Tsc2 haploinsufficiency cooperate to disrupt fetal survival and may perturb social behavior in adult mice. Mol Psychiatry. 2012; 17:62–70. [PubMed: 21079609]

Guo HF, Tong J, Hannan F, Luo L, Zhong Y. A neurofibromatosis-1-regulated pathway is required for learning in Drosophila. Nature. 2000; 403:895–898. [PubMed: 10706287]

Gutmann DH, Geist RT, Rose K, Wright DE. Expression of two new protein isoforms of the neurofibromatosis type 1 gene product, neurofibromin, in muscle tissues. Dev Dyn. 1995; 202:302–311. [PubMed: 7780179]

Gutmann DH, Zhang Y, Hirbe A. Developmental regulation of a neuron-specific neurofibromatosis 1 isoform. Ann Neurol. 1999; 46:777–782. [PubMed: 10553997]

Ho IS, Hannan F, Guo HF, Hakker I, Zhong Y. Distinct functional domains of neurofibromatosis type 1 regulate immediate versus long-term memory formation. J Neurosci. 2007; 27:6852–6857. [PubMed: 17581973]

Hoeffer CA, Klann E. mTOR signaling: At the crossroads of plasticity, memory and disease. Trends Neurosci. 2010; 33:67–75. [PubMed: 19963289]

Hyman SL, Shores A, North KN. The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. Neurology. 2005; 65:1037–1044. [PubMed: 16217056]

Hyman SL, Arthur SE, North KN. Learning disabilities in children with neurofibromatosis type 1: Subtypes, cognitive profile, and attention-deficit-hyperactivity disorder. Dev Med Child Neurol. 2006; 48:973–977. [PubMed: 17109785]

Hyman SL, Gill DS, Shores EA, Steinberg A, North KN. T2 hyperintensities in children with neurofibromatosis type 1 and their relationship to cognitive functioning. J Neurol Neurosurg Psychiatry. 2007; 78:1088–1091. [PubMed: 17299016]

Jain M, Velez JI, Acosta MT, Palacio LG, Balog J, Roessler E, Pineda D, Londono AC, Palacio JD, Arbelaez A, Lopera F, Elia J, Hakonarson H, Seitz C, Freitag CM, Palmason H, Meyer J, Romanos M, Walitza S, Hemminger U, Warnke A, Romanos J, Renner T, Jacob C, Lesch KP, Swanson J, Castellanos FX, Bailey-Wilson JE, Arcos-Burgos M, Muenke M. A cooperative interaction between LPHN3 and 11q doubles the risk for ADHD. Mol Psychiatry. 2011:1–7. [Epub ahead of print]. [PubMed: 21483438]

Johnson CY, Rasmussen SA. Non-genetic risk factors for holoprosencephaly. Am J Med Genet Part C. 2010; 154C:73–85. [PubMed: 20104598]

Krab LC, Aarsen FK, de Goede-Bolder A, Catsman-Berrevoets CE, Arts WF, Moll HA, Elgersma Y. Impact of neurofibromatosis type 1 on school performance. J Child Neurol. 2008a; 23:1002–1010. [PubMed: 18827266]

Krab LC, de Goede-Bolder A, Aarsen FK, Pluijm SM, Bouman MJ, van der Geest JN, Lequin M, Catsman CE, Arts WF, Kushner SA, Silva AJ, De Zeeuw CI, Moll HA, Elgersma Y. Effect of simvastatin on cognitive functioning in children with neurofibromatosis type 1: A randomized controlled trial. JAMA. 2008b; 300:287–294. [PubMed: 18632543]

Kwon CH, Luikart BW, Powell CM, Zhou J, Matheny SA, Zhang W, Li Y, Baker SJ, Parada LF. Pten regulates neuronal arborization and social interaction in mice. Neuron. 2006; 50:377–388. [PubMed: 16675393]

Lee YS, Silva AJ. The molecular and cellular biology of enhanced cognition. Nat Rev Neurosci. 2009; 10:126–140. [PubMed: 19153576]

Li W, Cui Y, Kushner SA, Brown RA, Jentsch JD, Frankland PW, Cannon TD, Silva AJ. TheHMG-CoA reductaseinhibitor lovastatin reverses thelearningand attention deficitsina mousemodel of neurofibromatosis type 1. Curr Biol. 2005; 15:1961–1967. [PubMed: 16271875]

Li Y, Li Y, McKay RM, Riethmacher D, Parada LF. Neurofibromin modulates adult hippocampal neurogenesis and behavioral effects of antidepressants. J Neurosci. 2012; 32:3529–3539. [PubMed: 22399775]

Listernick R, Louis DN, Packer RJ, Gutmann DH. Optic pathway gliomas in children with neurofibromatosis 1: Consensus statement from the NF1 Optic Pathway Glioma Task Force. Ann Neurol. 1997; 41:143–149. [PubMed: 9029062]

Lorenzo J, Barton B, Acosta MT, North K. Mental, motor, and language development of toddlers with neurofibromatosis type 1. J Pediatr. 2011; 158:660–665. [PubMed: 21094952]

Medeiros R, Prediger RD, Passos GF, Pandolfo P, Duarte FS, Franco JL, Dafre AL, Di GG, Figueiredo CP, Takahashi RN, Campos MM, Calixto JB. Connecting TNF-alpha signaling pathways to iNOS expression in a mouse model of Alzheimer's disease: Relevance for the behavioral and synaptic deficits induced by amyloid beta protein. J Neurosci. 2007; 27:5394–5404. [PubMed: 17507561]

Messiaen L, Yao S, Brems H, Callens T, Sathienkijkanchai A, Denayer E, Spencer E, Arn P, Babovic-Vuksanovic D, Bay C, Bobele G, Cohen BH, Escobar L, Eunpu D, Grebe T, Greenstein R, Hachen R, Irons M, Kronn D, Lemire E, Leppig K, Lim C, McDonald M, Narayanan V, Pearn A, Pedersen R, Powell B, Shapiro LR, Skidmore D, Tegay D, Thiese H, Zackai EH, Vijzelaar R, Taniguchi K, Ayada T, Okamoto F, Yoshimura A, Parret A, Korf B, Legius E. Clinical and mutational spectrum of neurofibromatosis type 1-like syndrome. JAMA. 2009; 302:2111–2118. [PubMed: 19920235]

Pride N, Payne JM, Webster R, Shores EA, Rae C, North KN. Corpus callosum morphology and its relationship to cognitive function in neurofibromatosis type 1. J Child Neurol. 2010; 25:834–841. [PubMed: 20142468]

Raichle ME. Two views of brain function. Trends Cogn Sci. 2010; 14:180–190. [PubMed: 20206576]

Rauen KA, Schoyer L, McCormick F, Lin AE, Allanson JE, Stevenson DA, Gripp KW, Neri G, Carey JC, Legius E, Tartaglia M, Schubbert S, Roberts AE, Gelb BD, Shannon K, Gutmann DH, McMahon M, Guerra C, Fagin JA, Yu B, Aoki Y, Neel BG, Balmain A, Drake RR, Nolan GP, Zenker M, Bollag G, Sebolt-Leopold J, Gibbs JB, Silva AJ, Patton EE, Viskochil DH, Kieran MW, Korf BR, Hagerman RJ, Packer RJ, Melese T. Proceedings from the 2009 genetic syndromes of the Ras/MAPK pathway: From bedside to bench and back. Am J Med Genet Part A. 2010; 152A:4–24. [PubMed: 20014119]

Roessler E, Muenke M. The molecular genetics of holoprosencephaly. Am J Med Genet Part C. 2010; 154C:52–61. [PubMed: 20104595]

Shilyansky C, Karlsgodt KH, Cummings DM, Sidiropoulou K, Hardt M, James AS, Ehninger D, Bearden CE, Poirazi P, Jentsch JD, Cannon TD, Levine MS, Silva AJ. Neurofibromin regulates corticostriatal inhibitory networks during working memory performance. Proc Natl Acad Sci USA. 2010; 107:13141–13146. [PubMed: 20624961]

Silva AJ, Frankland PW, Marowitz Z, Friedman E, Laszlo GS, Cioffi D, Jacks T, Bourtchuladze R. A mouse model for the learning and memory deficits associated with neurofibromatosis type I. Nat Genet. 1997; 15:281–284. [PubMed: 9054942]

Solomon BD, Lange E, Shubrook J, Service FJ, Herman G, Karne RJ, Gorden P, Muenke M, Stratakis CA. Deletion of 8q24 in an adult with mild dysmorphic features, developmental delay, and ketotic hypoglycemia. Am J Med Genet Part A. 2010; 152A:1545–1549. [PubMed: 20503333]

Williams JA, Su HS, Bernards A, Field J, Sehgal A. A circadian output in Drosophila mediated by neurofibromatosis-1 and Ras/MAPK. Science. 2001; 293:2251–2256. [PubMed: 11567138]

Zhao Y, Fung C, Shin D, Shin BC, Thamotharan S, Sankar R, Ehninger D, Silva A, Devaskar SU. Neuronal glucose transporter isoform 3 deficient mice demonstrate features of autism spectrum disorders. Mol Psychiatry. 2010; 15:286–299. [PubMed: 19506559]

Zhou J, Blundell J, Ogawa S, Kwon CH, Zhang W, Sinton C, Powell CM, Parada LF. Pharmacological inhibition of mTORC1 suppresses anatomical, cellular, and behavioral abnormalities in neural-specific Pten knock-out mice. J Neurosci. 2009; 29:1773–1783. [PubMed: 19211884]

Zhu Y, Harada T, Liu L, Lush ME, Guignard F, Harada C, Burns DK, Bajenaru ML, Gutmann DH, Parada LF. Inactivation of NF1 in CNS causes increased glial progenitor proliferation and optic glioma formation. Development. 2005; 132:5577–5588. [PubMed: 16314489]