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# Cognitive and behavioral problems in children with neurofibromatosis type 1: challenges and future directions

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Cognitive and behavioral disorders affect nearly 80% of all children with the neurofibromatosis type 1 inherited cancer syndrome, and are among the most significant clinical manifestations for patients and their families. One of the barriers to successful therapeutic intervention is the wide spectrum of clinical phenotypic expression, ranging from visuospatial learning problems to social perceptual deficits (autism). Leveraging numerous small-animal models of neurofibromatosis type 1, several promising targets have been identified to treat the learning, attention, and autism spectrum phenotypes in this at-risk population. In this review, we provide an up-to-date summary of our current understanding of these disorders in NF1, and propose future research directions aimed at designing more effective therapeutic approaches and clinical trials.

**KEYWORDS:** attention • autism • cognitive and behavioral • learning and memory • monogenic • mouse models • neurofibromatosis type 1 • NF1 • preclinical • single gene

## Preface

Cognitive and behavioral disorders in any population are, by their very nature, difficult to study. For example, we employ operational definitions of observed clinical traits, like major depressive disorder, in which observable cognitive and behavioral patterns classified as ‘major depressive disorder’ likely have multiple independent etiologies which result in similar phenotypes operationally defined using diagnostic criteria. Identifying the molecular and cellular basis for these clinical problems is a daunting task in the general population where numerous other factors (e.g., genomic variation, environmental effects) co-exist.

One approach to understanding these complex cognitive and behavioral disorders is to employ genetic conditions in which learning, memory, attention and social deficits predominate (TABLE 1). As such, conditions like Rett syndrome, tuberous sclerosis complex, Williams syndrome, Fragile X syndrome and

neurofibromatosis type 1 (NF1) can all be modeled using *in vitro* and *in vivo* techniques to elucidate the responsible cellular and molecular defects. Employing these neurogenetic syndromes, we have the opportunity to identify the relationship between specific human phenotypes and particular molecular and cellular abnormalities (FIGURE 1). This information is next used to discover potential therapeutic targets for preclinical evaluation and, following validation and optimization, testing in affected individuals (reviewed in [1–4]). Using this translational research paradigm, the insights garnered from the study of these rare genetic disorders may be applied to achieve a more complete understanding of more common non-syndromal cognitive and behavioral conditions. In this review, we describe the use of one illustrative single-gene disorder, NF1, as a tractable experimental platform to unravel the underlying causative etiologies for cognitive and behavioral disorders.

**Table 1. Monogenic/oligogenic syndromes characterized by cognitive and behavioral deficits.**

Syndrome	Gene(s)	Cognitive/behavioral deficits
Neurofibromatosis 1	<i>NF1</i>	Learning and memory disabilities, attention deficit, visuospatial deficits, ASD
Tuberous sclerosis complex	<i>TSC1</i> , <i>TSC2</i>	Deficits in memory, attention and executive function, ASD, intellectual disability
Fragile X syndrome	<i>FMR1</i>	ASD, attention deficit, intellectual disability
Rett syndrome	<i>MECP2</i>	Loss of verbal abilities, social withdrawal, autistic-like behaviors
Williams syndrome	1.5 Mb deletion of the Williams–Beuren syndrome critical region at 7q11.23	Deficits in visuospatial orientation, verbal and linguistic defects, hypersocialization

ASD: Autistic spectrum disorder.

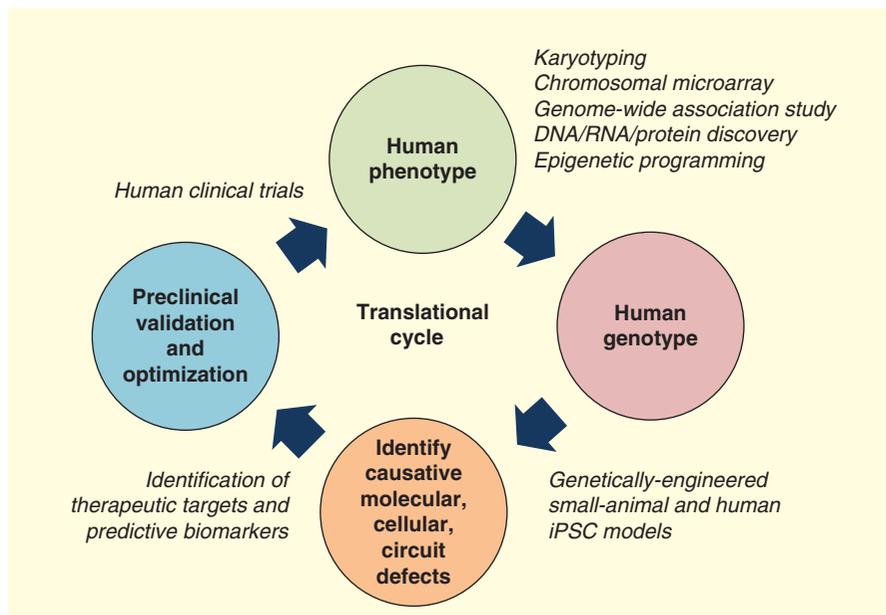
### NF1: genetic & clinical features

NF1 is a multisystemic autosomal dominant genetic disorder with an estimated prevalence of 1 in 2500 worldwide. The diagnosis of NF1 is based on clinical criteria established by the 1987 National Institutes of Health Consensus Development Conference [5]. Using these criteria, a clinical diagnosis requires that two of the following criteria be present (TABLE 2): greater than 5 café-au-lait macules, skinfold (underarm or groin) freckling, iris hamartomas (Lisch nodules), two of more neurofibromas (or one plexiform neurofibroma), an optic pathway glioma, tibial dysplasia or another distinct bony abnormality or

a first-degree relative with NF1. Other manifestations include cognitive and behavioral problems, delayed gross and fine motor function, T2 hyperintensities on MRI macrocephaly and short stature, as well as other benign and malignant cancers.

Using positional cloning strategies, the *NF1* gene on chromosome 17 (17q11.2) was identified [6], which encodes a 250-kDa protein product, neurofibromin [7]. Examination of people with NF1 has revealed a large number of non-recurrent germline *NF1* gene mutations, which are all hypothesized to impair the function of neurofibromin. While NF1 is a genetic disorder with complete penetrance, approximately 50% of individuals with NF1 are the first person in their families to be diagnosed. These individuals are thought to represent spontaneous mutations that arise *de novo* either in a parental gamete or in the zygote [8].

In general, there are no obvious genotype–phenotype relationships in NF1, owing to the large number of unique mutations. However, recent studies have begun to reveal specific phenotypes that result from particular mutation subtypes. First, individuals harboring *NF1* locus microdeletions (850–1000 MB chromosomal deletions) tend to have increased numbers of neurofibromas in early childhood, more pronounced cognitive abnormalities, distinctive cranio-facial features and an increased cancer risk [9–15]. These children exhibit severe learning disabilities and development delays as well as frank mental retardation (IQ <70) [16]. This microdeletion phenotype is attributed to loss of genetic material adjacent to the *NF1* gene [17]. Second, individuals from different families harboring the c.2970-2972\_delAAT mutation do not develop cutaneous, subcutaneous or clinically obvious plexiform neurofibromas



**Figure 1. The translational research paradigm, beginning with the human phenotype, and culminating in the development of preclinical trials to validate and optimize therapies.** A complete understanding of the human condition requires detailed clinical characterization, molecular analyses and genetic/genomic level studies.

The resulting information is then used to select affected individuals for iPSC generation and the development of accurate small-animal models useful for therapeutic drug discovery and validation. These new optimized drugs and potential predictive biomarkers can finally be evaluated in people with NF1.

NF1: Neurofibromatosis type 1.

despite having the other features of NF1 [18]. Last, individuals with 5' end *NF1* gene frameshift and premature truncation mutations are prone to develop optic gliomas [19,20]. Additional studies are ongoing to explore these potential genotype–phenotype correlations.

Although NF1 is a monogenic disorder, there is significant variation in phenotypic expression, even within individuals from the same family harboring an identical germline *NF1* gene mutation [21–23]. While the sources for this clinical variability have yet to be elucidated, they may be attributable to a number of different factors, including modifying genes, epigenetic phenomena, allelic heterogeneity, somatic mosaicism, deletion of contiguous genes and environmental influences [24].

### Cognitive & behavioral problems in NF1

Children with NF1 often experience cognitive and behavioral difficulties; as many as 80% of children diagnosed with NF1 will manifest some delay in these domains. Although these features are not part of the established diagnostic criteria, developmental challenges are the most common complication of the syndrome, and one of most concerning issues for parents of children with NF1 [25].

Studies examining the intelligence of children with NF1 using standard measures have demonstrated that only 4–8% of individuals with NF1 have full-scale IQs below 70 determined using the Wechsler intelligence scale for children-revised [26]. However, as a population, children with NF1 exhibit a left shift in the distribution of IQ scores that falls within one standard deviation (SD) of the general population (mean IQ scores ~ 85). While children with NF1 perform worse than their unaffected siblings, with as many as 45% performing 1 SD below a sibling on tests of verbal concept formation [27–30], abnormalities vary on tests of reasoning skills, picture arrangement/completion, arithmetic, or comprehension [28,29,31]. Unfortunately, as measures of intelligence, these standardized evaluations do not fully capture the contributions of other co-morbid factors, such as executive dysfunction or behavioral perceptual deficits.

Despite the fact that over 90% of children with NF1 possess near normal-range intelligence, specific learning disabilities are common. Specific learning disabilities are

defined as a major discrepancy between ability (intellect or aptitude) and achievement (performance). Early studies revealed that 30–45% of children with NF1 have specific learning disabilities, which is approximately three to fourfold more than observed in the general population [32–34]. However, greater than 70% of children with NF1 perform more poorly at school than their intellectual abilities would predict [29,35–39]. This finding suggests that the academic struggles experienced by children with NF1 likely reflect the interdependence of various cognitive and behavioral domains that impact on academic performance.

**Table 2. Clinical features and diagnostic of neurofibromatosis type 1 and phenotypic findings.**

Clinical feature	Diagnostic criteria (fulfillment >2 <sup>†</sup> )	Prevalence (% of NF1 population)
Family history	First-degree relative	50
<b>Neurocutaneous</b>		
Café-au-lait spots	≥6	99 in adults
Axillary/inguinal freckling	≥2	85 in adults
Lisch nodules	≥2	95 in adults
Neurofibromas	≥2	99 in adults
Plexiform neurofibroma	≥1	20–45
<b>Skeletal</b>		
Bone dysplasia		1–5
Pseudarthrosis		2
Scoliosis	ND	14
Short stature	ND	10–26
Macrocephaly	ND	45
<b>Tumors</b>		
Optic pathway glioma		15
Low-grade glioma, other <sup>‡</sup>	ND	3.5
MPNST <sup>§</sup>	ND	2–10
Malignant glioma	ND	2–3
Pheochromocytoma	ND	0.1–5 (10% malignant)
GIST <sup>¶</sup>	ND	–
Neuroendocrine tumors	ND	–
<b>Cardiovascular</b>		
Congenital heart disease	ND	–
Arterial vasculopathy	ND	Renal artery stenosis, 2

<sup>†</sup>ND, features associated with NF1, but not required for diagnosis.  
<sup>‡</sup>Brainstem, diencephalon and cerebellum.  
<sup>§</sup>Malignant peripheral nerve sheath tumor.  
<sup>¶</sup>Gastrointestinal stromal tumor.  
 ND: Non-diagnostic; NF1: Neurofibromatosis type 1.

### **Executive function**

Children with NF1 perform significantly more poorly than their unaffected siblings on tasks of executive functioning, such as the Tower of London and the Children's Category Test [29,40]. In this regard, over 70% of children with NF1 were more than 1 SD below the norm on the Tower of London test [41]. As a composite measure of selective attention, cognitive flexibility and processing speed, approximately 20% of children with NF1 performed 2 SD below the mean on the Stroop Task [27] in one study, while were only mildly impaired on the Wisconsin Card Sorting Task and Stroop Task in another [31,42].

Planning and working memory are also frequently abnormal in children with NF1. Using functional MRI (fMRI), young adults with NF1 showed spatial working memory impairments that correlated with decreased neural activity in frontal corticostriatal networks [43]. Similarly, poor organizational skills are often reported in children with NF1 [40,44,45].

### **Attention**

Problems with attention are frequently reported in children with NF1 [35–38,46]. Using questionnaire-based methods, nearly half of children with NF1 meet criteria for ADHD [28,41,47–53]. The conclusions from these indirect assessment studies have been substantiated using more direct methods of evaluating attention deficits. Children with NF1 are impaired on tasks that measure attention [27,29–31,42,45,54]. Specifically, switching and sustaining attention are particularly affected in people with NF1 [29,40,54]. In addition, children with NF1 more frequently display inattentive behaviors than their unaffected sibling controls [45,55]. These deficits were highlighted using a computer-based virtual reality testing paradigm, in which children with NF1 made more commission errors and had fewer total correct responses [55]. A recent study on children with NF1 also demonstrated deficits in sustained and divided auditory attention, sustained visual attention and response inhibition [46].

### **Memory**

Memory functions are often divided into declarative memory (explicit, semantic and episodic memories) and implicit memory (procedural memory and implicit item-specific memory) or alternatively into verbal and non-verbal memory. Deficits in both sequential verbal and non-verbal memory have been reported in patients with NF1 [56], attributed to deficits in temporal ordering, which can result in poor organizational skills, poor general sequencing, difficulty in following directions and instructions and poor spelling and writing skills. Using more specific tests of recall and delayed recall, impairments have been reported in children with NF1 [28,31,42,57]. As such, individuals with NF1 made more errors on a test of working memory [27] and performed worse than their unaffected sibling controls on spatial working memory tasks [53,58]. In addition, using a computerized virtual reality task equivalent to the Morris Water Maze used to assess spatial learning and memory in rodents [59–63], children with NF1 spent significantly less time searching the correct quadrant for the hidden object following training [53].

### **Visuospatial & visuo-perceptual skills**

Visuospatial and visuo-perceptual skills are also frequently impaired in children with NF1, and are found in over 50% of affected individuals [32]. Using a variety of metrics, such as the judgment of line orientation (JLO) test, the Hooper Visual Organization Test, the Benton Visual Form Discrimination Test and the Birmingham Object Recognition Battery [28,29,31,50,53,64–69], multiple studies have confirmed the presence of these abnormalities in children with NF1. In particular, the JLO test seems to be a sensitive and discriminant tool in children with NF1 [29,31,69]. Results from a more recent study using the Peabody Picture Vocabulary Test likewise support the observation that visuospatial deficits are common in children with NF1 [46].

While the neuroanatomical basis for these visuospatial problems is not entirely understood, fMRI revealed differences in the regions of the brain activated during the performance of the JLO task between children with NF1 and unaffected controls [47]. In another study, individuals with NF1 showed deficient activation of the low-level visual cortex [70], suggesting circuit-level dysfunction for these higher-order cognitive deficits in NF1. This hypothesis is further supported by EEG/event-related potential (ERP) studies, which also revealed abnormalities in visual processing and alpha oscillations [71].

### **Emotionality, behavior problems & social competence**

Children with NF1 display higher frequencies of emotional and behavioral problems than unaffected children. Using parental rating tools, children with NF1 have significantly higher scores on the total problems scale [65,72–74] and exhibit more problems with anxiety and depression [65]. Using a different measurement tool, two studies also found that children with NF1 had significantly more conduct and emotional problems [75,76].

### **Autistic spectrum disorder**

Autistic spectrum disorder (ASD) is a pervasive developmental disorder characterized by impairments in reciprocal social interaction, social communication and restricted interests, often with associated rigid, repetitive behaviors. The onset is typically in early childhood, and there is a male predominance. With changes in the diagnostic criteria for ASD using the newly published DSM-V, there has been an increasing recognition that approximately one-third of children with NF1 have ASD symptomatology [77,78]. Early studies suggested that a small fraction of children with NF1 met criteria for an autistic disorder; the earliest reported cases were identified among a cohort with a previous diagnosis of autism and secondary identification of NF1, in which up to only 15% of patients had concurrent NF1 and autism [79,80]. Other studies in small cohorts showed a frequency of less than 5% [81,82], including a cohort of children with NF1 evaluated using DSM-III and DSM-IV criteria [83]. However, using newer tools, more recent studies have demonstrated a much higher prevalence of ASD traits in children with NF1; a recent population-based epidemiological study using an NF1 registry in the UK found that approximately 45% of children with NF1 show some ASD spectrum phenotype as assessed

by a combination of the Autism Diagnostic Interview-Revised, Autism Diagnostic Observation Scale-Generic and verbal IQ using standard criteria [77]. As assessed using the social responsiveness scale, a similar frequency of ASD symptomatology (~40%) was found in an NF1 clinic-based study in the USA, with 15–30% meeting criteria for ASD [78,84]. In these studies, a male predominance was noted. Additionally, significant interactions were noted between attention deficits and impairments in social awareness and social motivation, suggesting a link between ADHD and ASD in the setting of NF1 [78]. In terms of the specific clinical features of ASD symptomatology in this population, ‘autistic mannerisms’ were most common (44%), including problems with flexibility and transitions, perseverative behaviors, and being regarded by others as atypical.

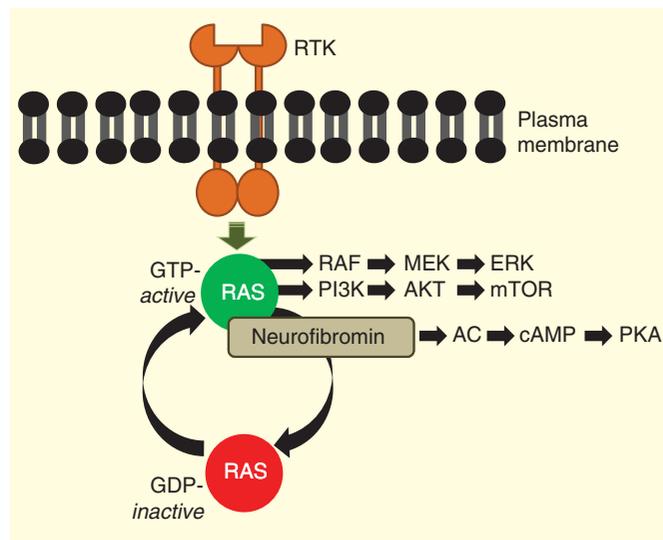
Clinically impaired social communication, motivation, awareness and cognition were likewise noted in 30% of children with NF1 [78]. Previous work has revealed that general cognitive abilities impact on ASD, as measured using tests of processing speed, social information processing and cognitive control [75]. Taken together, NF1 as a monogenic disorder in which the clinical expression of autistic traits is similar to idiopathic cases provides a tractable platform to elucidate the general mechanisms underlying ASD symptomatology [3,85].

### Language, reading & spelling

Early in life, children with NF1 group tend to be more globally impaired in language than their unaffected counterparts; a study of toddlers aged 21–30 months with NF1 demonstrated poorer basic language abilities than control children, with over 70% demonstrating below-average performance in expressive language domains [86]. As such, two-thirds of children with NF1 have problems in at least one reading subskill, and 75% fulfill criteria for phonological dyslexia [54]. The neuroanatomical basis for these differences has been explored using fMRI; People with NF1 show unique regional patterns of hemodynamic activity associated with phonologic processing compared to controls, specifically in the use of inferior frontal cortices relative to posterior (temporal, parietal and occipital) cortices to discriminate distinctive speech elements [87]. Additionally, the visual processing component of reading skills may also contribute to these observed differences: in a task requiring mental rotation of numbers and letters, multiple differences were observed between NF1 and control participants in regional cortical contributions as measured by both the volume and magnitude of activation [88].

### Molecular & neurobiological mechanisms underlying the cognitive & behavioral deficits in NF1

Neurofibromin is a large, 2818-amino acid RAS GTPase-activating protein that enhances the rate at which the active GTP-bound form of RAS is converted into its inactive GDP-bound form (FIGURE 2). RAS is anchored to the plasma membrane by a lipid modification (farnesyl or geranyl modification), where it can bind to guanine nucleotides. The RAS-regulatory activity of neurofibromin is mediated by its GTPase-activating protein domain, located in the middle of



**Figure 2. Signaling pathways relevant to cognitive and behavioral dysfunction in neurofibromatosis type 1.**

Neurofibromin functions as a negative RAS regulator, accelerating the inactivation of GTP-bound RAS. Active, GTP-bound RAS controls cell growth through its downstream RAF/MEK/ERK and PI3K/AKT/mTOR effector pathways. In addition, neurofibromin is a positive regulator of cAMP and activates AC function. AC: Adenylyl cyclase.

the protein, which binds to RAS and accelerates its conversion to an inactive conformation. Active GTP-bound RAS largely transmits its intracellular signal through the Raf/MEK/ERK and PI3K/Akt/mTOR pathways. In addition, neurofibromin is also a positive regulator of cAMP [89–96]. While not yet fully elucidated, both RAS-dependent and RAS-independent mechanisms for cAMP regulation in the setting of reduced neurofibromin expression have been reported [90,94,97,98].

Multiple fly and mouse models have been generated to explore the molecular and cellular pathogenesis responsive for NF1-associated cognitive and behavioral phenotypes (TABLE 3).

### Role of specific NF1 isoforms in learning & memory

The processed full-length messenger RNA transcript of the *NF1* gene is 11–13 kb in length, with three alternatively spliced exons, each with a tissue-specific expression pattern [99–107]. Of these, exons 9a and 23a are the most thoroughly investigated in mouse models. Exon 9a is expressed specifically in brain, enriched in forebrain neurons of the septum, striatum, cortex, hippocampus and olfactory bulb, with significantly less expression in brainstem, cerebellum and spinal cord [103]. Furthermore, the expression of exon 9a neurofibromin is first detected after postnatal day 2, suggesting a potential role in the cognitive and behavioral deficits in children with NF1 [108]. Similarly, developmental and tissue-specific regulation of exon 23a is conserved across vertebrates [99,105,107,109–111]. Although neuronal expression of exon 23a in *Nf1* mRNA transcripts is less common than in other cell types [107,110,112], the importance of exon 23a to learning and memory function is underscored by findings in a

**Table 3. Small-animal models of NF1 and phenotypic findings.**

Species	<i>Nf1</i> gene mutation	Cognitive/behavioral abnormalities	Ref.
Fly	<i>Nf1</i> <sup>c00617</sup> (insertion of transposon in the seventh intron of <i>Drosophila Nf1</i> gene)	Memory acquisition, but not memory stability, defects in olfactory associative learning	[113]
Fly	<i>Nf1</i> <sup>P1</sup> - and <i>Nf1</i> <sup>P2</sup> -null alleles	C-terminal region of <i>Nf1</i> gene controls immediate memory; GTPase-activating protein -related domain required for long-term olfactory associative memory formation	[118]
Fly	<i>Nf1</i> <sup>P1</sup> - and <i>Nf1</i> <sup>P2</sup> -null alleles	Deficits in olfactory associative learning	[93]
Fly	<i>Nf1</i> <sup>P1</sup> - and <i>Nf1</i> <sup>P2</sup> -null alleles	Reduced escape response; larval neuromuscular junction defect maintained by cAMP-PKA signaling	[95]
Mouse	<i>Nf1</i> <sup>+/-</sup> ( <i>Nf1</i> gene reduction)	Spatial learning and memory deficits No simple associative learning deficits	[60,63]
Mouse	<i>Nf1</i> <sup>23a-/-</sup> (deletion of <i>Nf1</i> exon 23a)	Spatial learning and memory and contextual discrimination defects Delayed acquisition of motor skills	[61]
Mouse	<i>Nf1</i> <sup>+/-</sup> <sup>GFAP</sup> CKO	Non-selective and selective attention deficits Mild-spatial learning deficits	[59]
Mouse	<i>Nf1</i> <sup>flox/wt</sup> ; synapsin 1-Cre CKO ( <i>Nf1</i> gene reduction in excitatory and interneurons)	Spatial learning defects	[62]
Mouse	<i>Nf1</i> <sup>flox/wt</sup> ; Dlx5/6-Cre CKO ( <i>Nf1</i> gene reduction in inhibitory interneurons)	Spatial learning deficits	[62]
Mouse	<i>Nf1</i> <sup>flox/wt</sup> ; CamKII-Cre CKO ( <i>Nf1</i> gene reduction in forebrain pyramidal neurons)	No spatial learning deficits	[62]

NF1: Neurofibromatosis type 1.

genetically engineered mouse strain lacking this alternatively spliced exon. Deletion of exon 23a results in deficits in spatial learning, impaired contextual discrimination and delayed acquisition of motor skills, but does not lead to tumorigenesis [61].

#### **cAMP in memory & learning**

Elegant studies in *Drosophila* homozygous for null *Nf1* gene mutations have revealed defects in cAMP generation [92,95], which were later confirmed in mammalian species [89–91,93,96]. In these studies, reduced or absent neurofibromin function leads to decreased cAMP generation. Important to NF1-associated learning and memory deficits, olfactory learning in *Nf1*-mutant flies requires neurofibromin-regulated adenylyl cyclase activity [93], a function localized to a particular set of *Drosophila* brain neurons [113].

In mammals, the role of neurofibromin-cAMP regulation in cognitive or behavioral dysfunction is less clear. While conditional *Nf1* inactivation in neural stem cells resulted in decreased neuronal arborization and cortical thickness, which is corrected by elevating cAMP levels [94], attention dysfunction is not similarly corrected in *Nf1*-mutant mice [114]. In addition, neurofibromin-cAMP regulation is important for both dendritic spine formation [115,116] and axonal lengths [59,89,90]. Further investigation will be required to clarify the relationship between

neurofibromin regulation of cAMP, neuronal process formation, synaptic function and cognitive or behavioral abnormalities seen in children with NF1.

#### **RAS in memory, learning & attention**

*Nf1*<sup>+/-</sup> mice (harboring one mutant *Nf1* allele) show impairments in spatial learning and memory in the Morris water maze, which reflect increased GABA-mediated inhibition [62] and decreased long-term potentiation (LTP) [60,63]. While *Nf1*-mutant mice have defects in both early- (immediate learning) and late- (long-term memory formation) phase memory [60,117], these functions are separable in *Drosophila*: long-term memory in *Nf1*-mutant flies results from impaired neurofibromin RAS regulation, whereas immediate learning requires a functional carboxyl terminus of neurofibromin, important for mediating neuropeptide and neurotransmitter stimulation of adenylyl cyclase activity [118].

Studies pioneered by Alcino Silva have revealed that the above learning and memory deficits in mice are due to impaired neurofibromin RAS regulation. In these studies, genetic and pharmacologic manipulations that decrease RAS signaling rescue the spatial learning and LTP deficits in *Nf1*<sup>+/-</sup> mice [60]. Blocking RAS activation with a farnesyltransferase inhibitor (lovastatin) was sufficient to reverse the learning and memory impairments

**Table 4. Current neurofibromatosis type 1 cognitive and social clinical studies.**

Clinical trial number	Title	Description	Ref.
NCT01410006	NF1 patient registry: NF1 patient registry initiative	Web-based registry to define the spectrum of medical and social problems experienced by children and adults with NF1	[129]
NCT01650142	Study of the NF1 expression: identification of the modifying genes	Expansion of a data bank from 1099 patients and 575 families with genotypes, phenotypes and DNA samples to detect genomic variants potentially use	[130]

NF1: Neurofibromatosis type 1.

in *Nf1*<sup>+/-</sup> mice [119], prompting two clinical trials in children with NF1. While lovastatin appears to improve synaptic plasticity in individuals with NF1 [119,120], there were no significant improvements in learning in these clinical studies [121,122].

Some attention deficits in *Nf1*<sup>+/-</sup> mice have also been shown to be, at least in part, due to defects in RAS regulation. Using the lateralized-reaction time test, attention was impaired at the most difficult target stimulus duration, an effect also reversed by lovastatin [119]. In addition, the ability to gate intrusive, external stimuli is an important aspect of attention deficit disorder in the general population [123]. In *Nf1*<sup>+/-</sup> mice, RAS hyperactivation may also underlie these gating deficits: impaired sensorimotor gating is improved following lovastatin treatment [119].

#### Dopamine in learning & attention

Using a different *Nf1*-mutant mouse model in which *Nf1*<sup>+/-</sup> mice have complete neurofibromin loss in neuroglial progenitor cells (*Nf1*<sup>+/-</sup><sup>GFAP</sup>CKO mice), the observed defects in exploratory behaviors (attention) were not reversed by RAS inhibition [114]. Instead, these mice had reduced dopamine levels in the striatum [59]. Correction of this dopamine defect resulted in rescue of the exploratory behavior deficits [59]. Additional studies using the same *Nf1*<sup>+/-</sup><sup>GFAP</sup>CKO mouse strain found low dopamine levels in the hippocampus as well as defective learning and memory. As such, treatment with L-DOPA or a dopamine receptor agonist rescued the learning and memory deficits [90,124] and resulted in correction of the LTP and Morris Water Maze performance defects.

#### Mouse sex & learning

Re-analysis of the performance deficits of *Nf1*<sup>+/-</sup><sup>GFAP</sup>CKO mice in the Morris Water Maze revealed differences between male and female littermates. While sex did not influence performance during the cued and place trials, only male mice showed a lack of spatial preference during both the memory acquisition and retention trials and exhibited a 25% reduction in time spent and number of entries into the target quadrant [125]. Consistent with this sexually dimorphic result, only male *Nf1*<sup>+/-</sup><sup>GFAP</sup>CKO mice had reduced hippocampal dopamine levels, decreased DARPP32 phosphorylation and increased hippocampal RAS activation [125]. These results suggest that sex may be an important variable in preclinical and clinical testing for treatments targeting learning and memory deficits. Future research in both human patients and model systems should consider the role of sex in various cognitive and behavioral domains.

#### Therapy for NF1-associated cognitive & behavioral deficits

While these exciting advances in molecular and cell biology have provided new insights into the pathogenesis of NF1-associated learning, memory and attention in lower organisms, there remains a paucity of therapeutic intervention studies for this affected population. In this regard, the majority of clinical studies have focused on further characterizing the cognitive and social problems in children with NF1 (TABLES 4-6). While some studies have employed behavioral therapy approaches, there have been few treatment trials that directly derive from mouse

**Table 5. Current neurofibromatosis type 1 clinical trials: social and emotional domains.**

Clinical trial number	Title	Description	Ref.
NCT00508235	Quality of friendships in children with neurofibromatosis: relationship to disease severity	Assess disease severity scale using the friendship quality questionnaire	[131]
NCT01626846	How NF1 affects schoolwork and self-esteem	Assess the effect of NF1 on self-esteem	[132]
NCT00684398	Adaptation and quality of life among adults with NF1	Understand predictors of adaptation and quality of life among adults affected with NF1 using a web-based, self-administered survey	[133]
NCT01851135	Neuropsychological impairment and quality of life in NF1	Assess the quality of life as measured by questionnaire	[134]

NF1: Neurofibromatosis type 1.

**Table 6. Current neurofibromatosis type 1 clinical trials: learning and memory domains.**

Identifier	Title	Description	Ref.
NCT00624234	Acceptance and commitment therapy for adolescents and adults with NF1 and chronic pain: a pilot study	To evaluate whether acceptance and commitment therapy results in improvements on the modified brief pain inventory scale from baseline to 3 months in adolescents and young adults with NF1 and chronic pain	[135]
NCT00169611	Neurobiology and treatment of reading disability in NF1  Behavioral and neuropsychological study of children with NF1 treated by methylphenidate. a double-blind randomized study methylphenidate versus placebo	To determine whether children with NF1 and reading disabilities respond to treatment programs effective in children with idiopathic reading disabilities  To determine the effect of methylphenidate compared to placebo on ADHD as well as specific neuropsychological, depression and anxiety scales	[136]
NCT00352599	Trial to evaluate the safety of lovastatin in individuals with NF1	To determine the effect of lovastatin on non-verbal learning as well as attention	[137]
NCT00853580	A randomized placebo-controlled study of lovastatin in children with NF1 (Phase II)	To determine the effect of lovastatin on visual-spatial learning and memory	[138]

NF1: Neurofibromatosis type 1.

preclinical studies focused on inhibiting RAS activity using statin-based drugs that target RAS post-translational modification [119]. In these studies, lovastatin treatment of children with NF1 resulted in improvements in long-range positive resting state functional connectivity [126] and decreased intracortical inhibition in adults with NF1 [120]. Moreover, lovastatin treatment also improved synaptic plasticity using transcranial magnetic stimulation and enhanced phasic alertness on the test of attention performance [120]. Unfortunately, a related statin compound, simvastatin, showed no efficacy in two independently conducted clinical trials [121,122]: in these studies, no significant differences were reported between simvastatin and placebo groups on any primary outcome measure; however, some improvement was noted in the object assembly scores (visual analysis measure) following simvastatin treatment [121].

In the realm of NF1-associated attention deficits, only one small study has been performed to date. Following low-dose methylphenidate (Ritalin) treatment (5–15 mg/day), significant improvement was documented using the tests of variables of attention instrument and the child behavior checklist [52]. These promising results have not resulted in additional studies, but, in combination with preclinical observations [59,124] do support the use of stimulant medication in this population of affected children.

The paucity of therapeutic investigations in this area coupled with the disappointing outcomes in the statin treatment trials argues that further investigations might consider enrolling children better stratified for specific neurocognitive problems (e.g., spatial learning deficits). In addition, future human clinical studies may need to employ behavioral measures that accurately mirror the tests used in the preclinical experiments. For example, lovastatin improved spatial learning and memory in *Nf1*-mutant mice in the Morris Water Maze [119], which could be

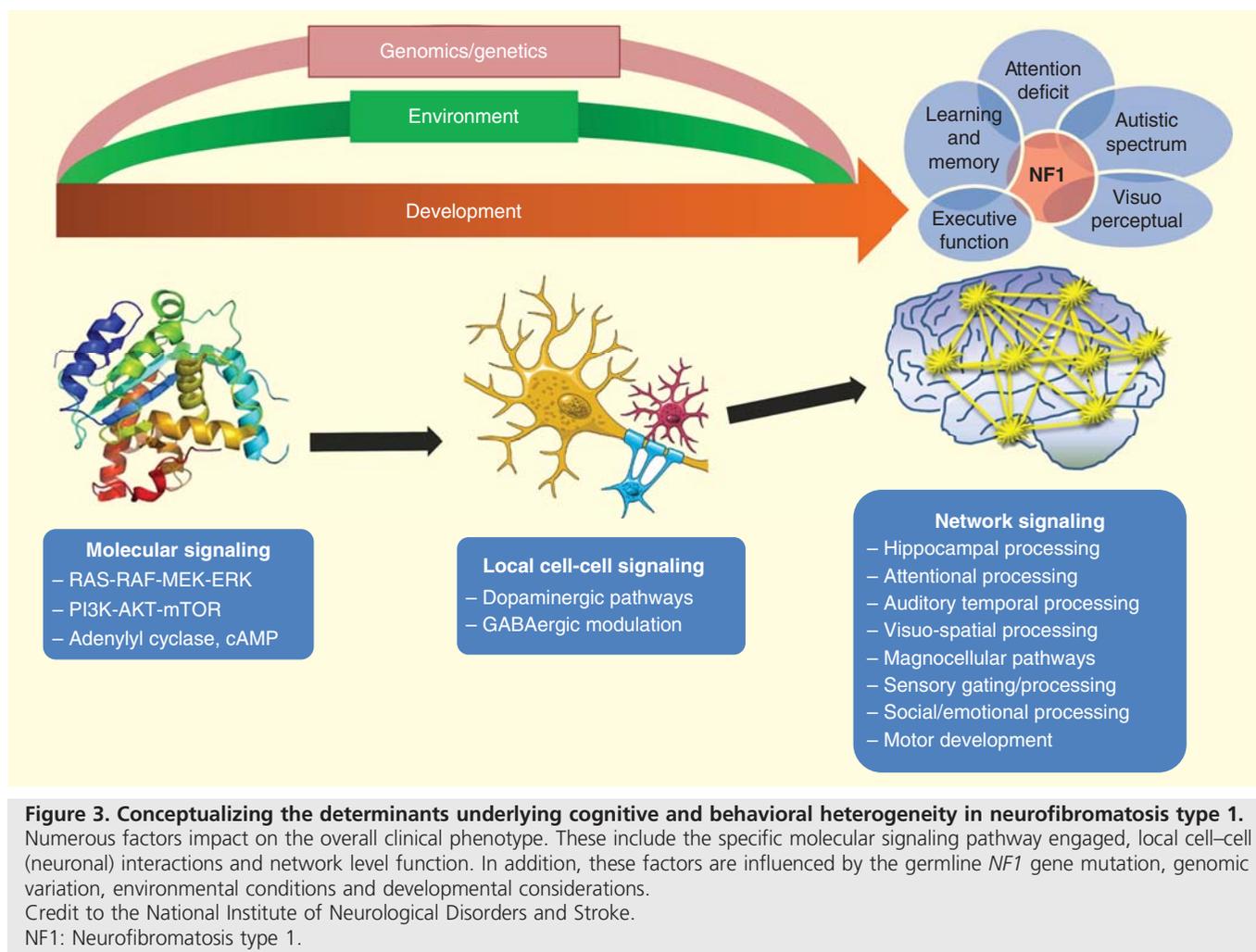
validated in human trials using computerized environmental tasks that simulate this behavioral paradigm [53].

### Expert commentary

Children with NF1 are frequently noted to have difficulties with academic performance, which likely reflect a myriad of etiologies, including problems with executive function, attention, social perception, learning and memory. When formally assessed for cognitive and behavioral abnormalities using standard psychological measurements, these children often qualify for services, such as individualized educational plans as well as occupational, physical and speech therapy. However, given the incomplete appreciation for the underlying pathogenesis of these impairments in children with NF1, no effective medical therapies have emerged. As we move forward into an era of personalized (precision) medicine, it will become critical to more clearly define the learning and behavioral deficits in children with NF1 and to define how these deficits impact on each other and on overall scholastic performance. In addition, we need to leverage the power of mouse genetic engineering to develop a diverse collection of *Nf1*-mutant mice that more fully represent the spectrum of learning and behavioral abnormalities observed in children with NF1. Last, the optimization of cellular engineering, such as the use of induced pluripotent stem cell-derived neurons [127], may elucidate how neurofibromin dysfunction impairs human neuron signaling and function. The convergence of human reprogrammed neurons, next-generation mouse models and more robust clinical information should pave the way to the actualization of these individualized therapies.

### Five-year view

The successful employment of translational approaches hinges on the fidelity of small-animal models in representing the



human condition. In this regard, these successes will require several key advances. First, multilevel analyses should be conducted on large cohorts of children with NF1 to assess how impairments in one sphere of functioning impact on other spheres. For example, what is the relationship between disordered planning (executive function) and fine motor skills, or how do attention deficits impact on sleep? Each of these factors may influence another realm of functioning in an unanticipated manner to disturb overall academic performance, and, as such, reveal new treatment strategies, like physical therapy focusing on motor delays to improve spatial learning.

Second, it is time to assemble a toolbox of complementary translational research resources, including iPSCs and novel genetically engineered mouse strains. The availability of actual patient-derived reprogrammed brain neurons offers unprecedented opportunities to determine how observations made in lower organisms operate in humans. In addition, given the heterogeneity inherent in this monogenic syndrome, the generation of *Nf1*-mutant mice with specific germline *NF1* gene mutations, genetic backgrounds and spectrum of deficits will enable the identification of drug therapies most likely to benefit specific subgroups of children with NF1-related cognitive and behavioral problems.

Third, efficient translation of preclinical observations to the clinical workplace necessitates that we employ similar measurement tools to assess efficacy. If we use tasks and tests in rodents that do not have counterparts in humans, we may reject a promising pharmacologic agent because it did not correct the abnormality against which it was evaluated in rodents. The development of a virtual Morris Water Maze represents a step forward in this direction [53]. Similarly, efforts should be made to employ analogous tests in rodents that more closely parallel the types of measurement tools used to assess these deficits in children with NF1.

Last, NF1 represents a model genetic condition to look for genomic modifiers through genome-wide association studies. Using a combination of human genomics and mouse genetics, it might be possible to identify genetic predictors for risk assessment and potentially treatment stratification. Together, the advances and insights that derive from NF1 will have applicability to non-syndromic learning and behavior deficits (FIGURE 3). In this regard, there are other monogenic syndromes characterized by neurocognitive and behavioral deficits in which de-regulated RAS signaling represents the causative molecular etiology: patients with Noonan syndrome (*KRAS* and *PTPN11* mutations) and Costello syndrome

(*HRAS* mutation) exhibit increased RAS activation and exhibit a spectrum of cognitive delays and behavioral problems [84]. Lessons learned from the NF1 statin clinical trials have already been incorporated into the design of planned RAS-opathy patient studies, including the use of defined outcome measurements and targeted patient stratification strategies [128].

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#### Key issues

- Cognitive and behavioral disorders affect the vast majority of children with neurofibromatosis type 1 and account for much of the lifetime morbidity and societal impact associated with the condition.
- Most current treatment strategies are based on approaches developed for the general population.
- Small-animal models and *in vitro* studies have revealed molecular pathways (RAS, cAMP and dopamine) affected by impaired neurofibromin function.
- Clinical trials are currently underway to evaluate some of these discovered targeted therapies.
- Successful development of targeted therapeutics will require further studies using tractable experimental models and clinical trials designed to test specific hypotheses in pre-selected patient cohorts.

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