

Effect of Simvastatin on Cognitive Functioning in Children With Neurofibromatosis Type 1

A Randomized Controlled Trial

Lianne C. Krab, MSc

Arja de Goede-Bolder, MD

Femke K. Aarsen, MA

Saskia M. F. Pluijm, PhD

Marlies J. Bouman, MA

Jos N. van der Geest, PhD

Maarten Lequin, MD, PhD

Coriene E. Catsman, MD, PhD

Willem Frans M. Arts, MD, PhD

Steven A. Kushner, MD, PhD

Alcino J. Silva, PhD

Chris I. de Zeeuw, MD, PhD

Henriëtte A. Moll, MD, PhD

Ype Elgersma, PhD

NEUROFIBROMATOSIS TYPE 1 (NF1) is a common autosomal-dominant genetic disorder (incidence 1:3000)¹ caused by a mutation in the gene encoding neurofibromin, a protein that activates the hydrolysis of RAS-bound guanosine triphosphate.² Neurofibromatosis type 1 is characterized by various neurocutaneous manifestations, problems in fine and gross motor functioning,³ as well as the frequent occurrence of cognitive disabilities. Children with NF1 have a lower mean IQ (86-94) with particular deficits in visual-spatial skills, nonverbal long-term memory, executive functions, and attention.⁴⁻⁷ These problems have a large

See also Patient Page.

Context Neurofibromatosis type 1 (NF1) is among the most common genetic disorders that cause learning disabilities. Recently, it was shown that statin-mediated inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase restores the cognitive deficits in an NF1 mouse model.

Objective To determine the effect of simvastatin on neuropsychological, neurophysiological, and neuroradiological outcome measures in children with NF1.

Design, Setting, and Participants Sixty-two of 114 eligible children (54%) with NF1 participated in a randomized, double-blind, placebo-controlled trial conducted between January 20, 2006, and February 8, 2007, at an NF1 referral center at a Dutch university hospital.

Intervention Simvastatin or placebo treatment once daily for 12 weeks.

Main Outcome Measures Primary outcomes were scores on a Rey complex figure test (delayed recall), cancellation test (speed), prism adaptation, and the mean brain apparent diffusion coefficient based on magnetic resonance imaging. Secondary outcome measures were scores on the cancellation test (standard deviation), Stroop color word test, block design, object assembly, Rey complex figure test (copy), Beery developmental test of visual-motor integration, and judgment of line orientation. Scores were corrected for baseline performance, age, and sex.

Results No significant differences were observed between the simvastatin and placebo groups on any primary outcome measure: Rey complex figure test ($\beta=0.10$; 95% confidence interval [CI], -0.36 to 0.56); cancellation test ($\beta=-0.19$; 95% CI, -0.67 to 0.29); prism adaptation (odds ratio=2.0; 95% CI, 0.55 to 7.37); and mean brain apparent diffusion coefficient ($\beta=0.06$; 95% CI, -0.07 to 0.20). In the secondary outcome measures, we found a significant improvement in the simvastatin group in object assembly scores ($\beta=0.54$; 95% CI, 0.08 to 1.01), which was specifically observed in children with poor baseline performance ($\beta=0.80$; 95% CI, 0.29 to 1.30). Other secondary outcome measures revealed no significant effect of simvastatin treatment.

Conclusion In this 12-week trial, simvastatin did not improve cognitive function in children with NF1.

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Author Affiliations: NF1 Core (Cognitive Research) Team, Departments of General Pediatrics (Ms Krab and Dr De Goede-Bolder and Moll), Neuroscience (Ms Krab and Drs Van der Geest, De Zeeuw, and Elgersma), Pediatric Neurology (Mss Aarsen and Bouman and Drs Catsman-Berrevoets and Arts); Public Health (Dr Pluijm), Pediatric Radiology (Dr Lequin), and Psychiatry (Dr Kushner), Erasmus MC University Medical Center, Sophia Children's Hospital, Rotterdam, the Netherlands;

Departments of Neurobiology, Psychiatry, and Psychology, Brain Research Institute, Los Angeles, California (Drs Kushner and Silva); and Netherlands Institute for Neuroscience, Royal Academy of Sciences (KNAW), Amsterdam, the Netherlands (Dr De Zeeuw).

Corresponding Author: Ype Elgersma, PhD, Department of Neuroscience, Erasmus MC, Dr Molewaterplein 50, 3015 GE Rotterdam, the Netherlands (y.elgersma@erasmusmc.nl).

impact on school performance of children with NF1.⁴ It has been suggested that the cognitive and motor deficits in children with NF1 are related to hyperintensities on T2-weighted magnetic resonance imaging of the brain^{3,8} that are characterized by high apparent diffusion coefficients (ADC values),⁹ but some studies failed to confirm this relationship.¹⁰

Studies using mouse models for NF1 (*Nf1* mice) revealed that increased RAS/ERK signaling is primarily responsible for the neuronal plasticity deficits as well as the spatial learning and attention deficits of these mice.¹¹⁻¹³ RAS transforming activity requires isoprenylation (ie, farnesylation or geranylgeranylation) of RAS, which can be blocked by farnesyl transferase inhibitors and by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors.^{14,15} HMG-CoA reductase is the rate-limiting enzyme in the mevalonate pathway in which cholesterol and isoprenyl groups are synthesized. Importantly, treatment of *Nf1* mice with a farnesyl transferase inhibitor or HMG-CoA reductase inhibitor for just a few days reverses the cognitive deficits of these mice.^{11,13}

These findings are not only important for NF1 but also are of great interest for other neuro-cardio-facial-cutaneous syndromes (eg, Noonan, Costello, and cardio-facio-cutaneous syndromes), which are also caused by aberrant RAS/ERK signaling, and for hamartoma syndromes (eg, Cowden disease and tuberous sclerosis complex). The genes associated with these syndromes belong to a pathway that is not only coregulated by RAS but also critically dependent on RHEB, another farnesylated protein of the RAS family.

The favorable safety profile of the HMG-CoA reductase inhibitor simvastatin in adults and children¹⁶ provided an opportunity to investigate whether the findings in the mouse model can be translated to humans. In a randomized, double-blind, placebo-controlled trial, we studied the effect of a 12-week simvastatin treatment on

cognitive function of children with NF1 using neuropsychological, neurophysiological, and neuroradiological outcome measures.

METHODS

Design

A prospective, double-blind, placebo-controlled, randomized, single-site, 12-week clinical trial was conducted in children with NF1 between January 20, 2006, and February 8, 2007. The study was approved by the medical ethical committee of the Erasmus MC, Rotterdam, the Netherlands.

Participants

All participants were recruited from the multidisciplinary NF1 outpatient clinic of the Erasmus MC–Sophia Children's Hospital, which is a university hospital and NF1 referral center in the Netherlands. Participants were enrolled by a pediatrician in the NF1 outpatient clinic (A.G.B.). Inclusion criteria were age 8 to 16 years, NF1 diagnosis according to the criteria of the National Institutes of Health,¹⁷ and oral and written informed consent from parents and children older than 12 years. Exclusion criteria were segmental NF1, pathology of the central nervous system (other than asymptomatic gliomas), deafness, severely impaired vision, use of antiepileptic drugs, insufficient comprehension or use of the Dutch language, and an IQ below 48, which was assessed at baseline using the Wechsler Intelligence Scale for Children–Revised, Dutch version.¹⁸

Protocol

Patients were randomized to simvastatin or placebo using a permuted-block, 1:1 randomization list generated by the trial statistician (S.M.F.P.) with blocks of 6 participants, in which medication numbers 1 through 62 corresponded to either simvastatin or placebo. Randomization was performed by the Erasmus MC trial pharmacist, who assigned patients a medication number in the order of their enrollment in the trial and who dispensed the medication. Patients and all other investi-

gators were blind to the treatment allocation. Patients were treated once a day in the morning for 12 weeks with simvastatin (weeks 0-4, 10 mg/d; weeks 5-8, 20 mg/d; and weeks 9-12, 20 mg/d for children aged 8-12 years or 40 mg/d [taken as two 20-mg doses] for children aged 13-16 years) or equivalent placebo. The placebo capsules were filled with microcrystalline cellulose PH102 and treatment capsules with a filler and a tablet of 10-mg (weeks 0-4) or 20-mg (weeks 5-12) simvastatin (film-coated; Alpharma Inc; Bridgewater, New Jersey). The capsules containing placebo or simvastatin were nontransparent and identical in color, shape, and size. Patients were instructed not to open the capsules. Patients were judged adherent when they took at least 80% of their study medication during the intervention period of 12 weeks, which was assessed by counting returned capsules.

Outcome Measures

Outcome measures were assessed at baseline and after 12 weeks of treatment. For the primary outcome measures, we chose 2 neuropsychological tests that were analogous to statin-responsive tests in *Nf1* mice (measuring visual-spatial memory and attention). In addition, we selected a neurophysiological and neuroradiological measure because we reasoned that these measurements would be insensitive to placebo or test-retest effects. This resulted in the following 4 primary outcome measures: performance on the Rey complex figure test (CFT) (delayed recall; assessing nonverbal long-term memory), performance on the cancellation test (speed; assessing attention), performance on a prism adaptation task (measurement of adaptation of the angle of hand movements in response to prism glass distortion,¹⁹ which is thought to be dependent on cerebellar function^{20,21}), and mean apparent diffusion coefficient (ADC value) of the brain (mean ADC value of 7 predetermined anatomic locations predominantly affected by T2-weighted hyperintensities) as previously described.⁹

For the secondary outcome measures, we selected neuropsychological tests assessing domains that are specifically affected in patients with NF1: tests for attention and tests for visual-spatial skills with baseline scores of 1 SD or more below average.^{4,9} This resulted in the following secondary outcome measures: performance on the cancellation test (standard deviation; measuring attention fluctuations), the Stroop color word test, the block design test and object assembly test of the Wechsler Intelligence Scale for Children–Revised, the Rey CFT (copy), the Beery developmental test of visual-motor integration, and the judgment of line orientation task.²²

Magnetic resonance imaging was performed by using a 1.5-tesla system (EchoSpeed; GE Healthcare, Milwaukee, Wisconsin) and a dedicated 8-channel head coil. Diffusion tensor imaging data were gathered by using a multirepetition, single-shot, echo-planar sequence with a section thickness of 3 mm with no gap. A 25-gradient directions technique was performed to obtain good diffusion tensor images (sensitivity, $b = 1000 \text{ s/mm}^2$; repetition time, 15 000 milliseconds; echo time, 82.1 milliseconds; 1 average; field of view, $240 \times 240 \text{ mm}^2$; matrix, 128×128 ; voxel size, $1.8 \times 1.8 \times 3.0 \text{ mm}^3$) as described previously.⁹

All neuropsychological tests were developed for children, administered in their Dutch versions, and scored by 1 pediatric neuropsychologist (M.J.B.). Parallel versions of tests were applied when available to reduce the impact of practice effects. For technical reasons, left-handed children ($n = 7$) were excluded from the prism adaptation task.

Treatment safety and adherence was assessed in the outpatient clinic at baseline, after 4 and 12 weeks, and with a telephone consult after 8 weeks. Patients were provided with a diary in which they were instructed to note any deviations from treatment protocol and possible adverse events. At each consult, a general pediatrician recorded any adverse events and serious adverse

events (adverse events that were life-threatening, causing disability, or requiring hospitalization) with a standardized checklist of the adverse events and serious adverse events reported with simvastatin use,²³ supported by open questions and a review of the patient's diary. All reported adverse events were scored as being not drug related, possibly drug related, or definitely drug related prior to unblinding. During the visits to the outpatient clinic, the pediatrician (A.G.B.) performed a standardized internal and neurological assessment, and blood was drawn for laboratory examination. We examined the safety parameters (levels of alanine aminotransferase, aspartate aminotransferase, and creatine phosphokinase) and efficacy parameters (levels of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides) according to standard clinical laboratory protocol. Criteria for discontinuation of study medication were a persistent increase of more than 3-fold the upper limit of normal (ULN) alanine aminotransferase or aspartate aminotransferase levels, more than 10-fold the ULN for creatine phosphokinase levels with or without muscular symptoms, or 5- to 10-fold the ULN for creatine phosphokinase levels with muscular symptoms.¹⁶

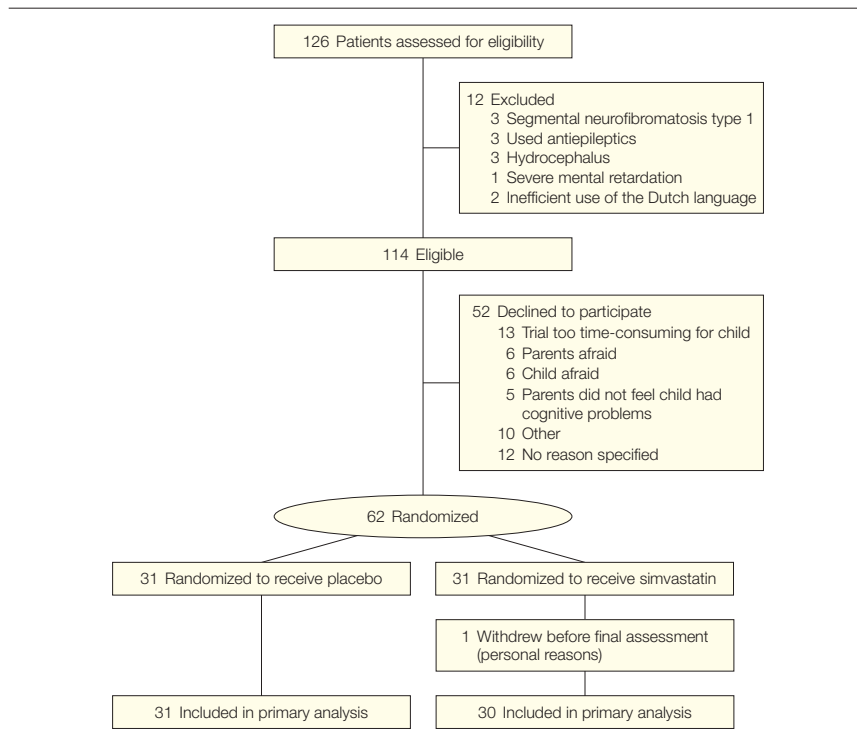
Statistical Analyses

One of the prominent effects seen in statin-treated *Nf1* mice was recovery of their deficit in visual-spatial memory.¹³ The Rey CFT (delayed recall) assesses the analogous domain of nonverbal long-term memory in humans and has good psychometric properties, and performance on this test is specifically affected in patients with NF1.²⁴ Therefore, we based our power calculation on this test. On the assumption of a correlation of 0.70 between measurement before and after treatment, and a mean (SD) z score of -1.32 (1.01) on the Rey CFT (delayed recall) at baseline,²⁴ we calculated that 30 persons were needed in both the placebo and treatment groups to ensure a power of

0.80 of detecting a significant ($\alpha = .05$) improvement in the Rey CFT (delayed recall) score up to -0.28 (difference of 1.04) in the treatment group.

All data were analyzed using SPSS 12.0 (SPSS Inc, Chicago, Illinois). For the neuropsychological tests, z scores were used (with negative values indicating performance below the normative mean and positive values performance above the normative mean), except for the cancellation test (standard deviation) (raw score for nonnormal distribution of reference values; larger negative values indicated larger attention fluctuations). Prism adaptation was scored to occur if the change (adaptation) of the angle of hand movements was significant ($P < .01$) and larger than -1 SD of the mean change of age-matched healthy controls ($n = 16$, unpublished observations). A decrease in ADC values indicates lower signal intensity.

Modified intention-to-treat analysis was performed for all patients with available 12-week test scores ($n = 61$) without imputing missing values. Differences between the simvastatin and placebo groups at baseline were analyzed with the t test, Mann-Whitney test, and χ^2 test. Differences between the simvastatin and placebo groups after 12 weeks of treatment were assessed using univariate and multivariate regression analysis. In the univariate analysis, we adjusted for baseline scores, and in the multivariate regression analysis we adjusted for baseline scores, age, and sex. Regression coefficients (β) reflect the estimated differences in mean score at follow-up between the treatment groups with 95% confidence intervals (CIs). For categorical measures (prism adaptation), the difference between the treatment groups was expressed as an odds ratio with 95% CI. Cut-off level for significance was set at $P < .05$. Effect modification of outcome parameters that were significantly different between the treatment and placebo groups after 12 weeks was examined using interaction terms between treatment and age and between treatment and baseline performance. The rationale for this

Figure 1. Flowchart of Patient Inclusion

analysis is that we expected increased plasticity in younger children and more room for improvement in children with low baseline performance, thus affecting the magnitude of response to simvastatin treatment. Subgroup analysis was performed only if effect modification was plausible ($P < .10$ to take into account the small size of the subgroups) for addition of the interaction term to the multivariate analysis. All P values reported are 2-sided. The outcome parameters and the method of statistical analysis, including the subgroup analyses, were defined before unblinding.

We did not correct for multiple comparisons for the following reasons. There are only 4 primary outcome measures, and they are specifically based on a priori assumptions. The outcome measures on the neuropsychological tests are potentially correlated, and correction would thus be inappropriate. By correcting for multiple comparisons, it would be very hard to detect a possible effect in a relatively small patient population. Thus we would run a high

risk of discarding a promising drug while in fact there is an effect (type II error).

For ethical reasons, an interim analysis was conducted by the statistician (S.M.F.P.) after 36 patients completed the study with complete maintenance of the double-blind protocol for all others. The criterion to discontinue the study was a significant difference between the simvastatin and placebo groups on Rey CFT (delayed recall) score at 12 weeks ($P < .01$). The statistician communicated that this criterion was “not reached” and the study was continued as planned.

RESULTS

Participants

One hundred fourteen children were eligible for this study. Consent to participate was obtained for 62 children (response rate, 54%). The children who participated in the trial ($n = 62$) did not differ significantly from the total eligible group ($n = 114$) on age, sex, frequency of mental retardation, or disease severity (all $P > .30$), indicating

that they were representative of the total eligible group. The 62 participants were randomly assigned to the simvastatin group ($n = 31$) or the placebo group ($n = 31$) (FIGURE 1). The baseline characteristics were similar between the simvastatin and placebo groups for all baseline parameters except for median age (TABLE 1 and TABLE 2). Mean (SD) treatment duration was 12 weeks and 3 days (6 days). There were no deviations from random allocation. One participant (2%) in the simvastatin group withdrew from the study after 10 weeks for personal reasons. Three of 62 children (5%), all in the placebo group, were not adherent according to the 80% criterion. We could not retrieve all of the medication jars for 10 of 62 children (16%; 6 in the simvastatin group and 4 in the placebo group).

Effect of Simvastatin on Outcome Parameters

After 12 weeks of treatment, we did not observe a significant difference between the simvastatin and placebo groups on the primary outcome measures (Rey CFT [delayed recall], cancellation test [speed], prism adaptation, and mean brain ADC values). We also did not observe an effect on the secondary outcome measures (cancellation test [standard deviation], Stroop color word test, block design, Rey CFT [copy], Beery developmental test of visual-motor integration, and judgment of line orientation), except for a higher score on the object assembly test in the simvastatin group using univariate analysis (adjustment for baseline scores, $\beta = 0.50$ [95% CI, 0.05 to 0.95]) as well as multivariate analysis (adjustment for baseline scores, age, and sex, $\beta = 0.54$ [95% CI, 0.08 to 1.01]) (Table 2).

Paired t tests revealed that performance after 12 weeks was similar or better than baseline for all tests in both the simvastatin and the placebo groups. In the placebo group, the improvement between baseline and 12 weeks was significant on 4 of 9 neuropsychological outcome measures (cancellation test [speed and standard deviation], Rey CFT [copy], judgment of line orienta-

tion), leading to a performance within the normal range on the first 3 tests.

Effect Modification

We found that baseline performance on the object assembly test was a modifier of the effect of simvastatin on this test ($P=.07$). Subsequent subgroup analysis showed a significant effect of simvastatin in the group with the baseline object assembly test scores -1 SD or less ($\beta=0.80$ [95% CI, 0.29 to 1.30]; $n=37$), but not in the group with the baseline object assembly test score of greater than -1 SD ($\beta=0.47$ [95% CI, -0.64 to 1.59]; $n=24$) indicating that the difference in the object assembly test results between the simvastatin and placebo groups is mostly caused by an increase in score in children with a poor baseline performance in the simvastatin group (FIGURE 2). There was no interaction between improvement on the object assembly test and age.

Safety and Effect on Cholesterol Levels

There were no laboratory adverse events and no serious adverse events. In total, 5 adverse events were reported by 3 of 31 children (10%) in the simvastatin group: hair loss (1 child after 4, 8, and 12 weeks), muscle weakness (1 child after 8 weeks), and constipation (1 child after 12 weeks) compared with 4 adverse events reported by 3 of 31 (10%) children in the placebo group (dizziness [1 child after 4 and 8 weeks] and constipation [1 child after 8 and 1 child after 12 weeks]). None of the reported adverse events were judged clinically significant.

After 12 weeks of simvastatin treatment, total cholesterol levels were reduced by a mean (SD) 21.1% (10.7%) of baseline values and low-density lipoprotein cholesterol by 39.4% (15.1%). There was no significant change in levels of high-density lipoprotein cholesterol or triglycerides. The change in low-density lipoprotein cholesterol level in the simvastatin group was not significantly related to the dose, sex, or age. The low-density lipoprotein cholesterol level of the children in

Table 1. Baseline Characteristics of the Study Groups^a

	No. (%)	
	Placebo (n = 31)	Simvastatin (n = 31)
Patient characteristics		
Age at randomization, median (IQR), y ^b	11.5 (9.4-13.5)	13.2 (11.3-15.2)
Male sex	16 (52)	19 (61)
Full-scale IQ, mean (SD)	85 (15)	88 (15)
NF1 disease severity ^c		
Minimal	10 (32)	12 (39)
Mild	13 (42)	11 (35)
Moderate	8 (26)	7 (23)
Severe	NA	1 (3)
Inheritance of NF1		
Familial	14 (45)	12 (39)
Sporadic	16 (52)	19 (61)
Unconfirmed	1 (3)	0
Socioeconomic status ^d		
Low	12 (39)	12 (39)
Middle	9 (29)	9 (29)
High	10 (32)	10 (32)
Total cholesterol, mean (SD), mg/dL	166 (31)	163 (36)
LDL cholesterol, mean (SD), mg/dL	97 (26)	96 (32)
Treatment dose in weeks 9-12		
20 mg/d	NA	12 (39)
40 mg/d	NA	19 (61)
Maximal treatment dose, mg/kg, mean (SD)	NA	0.7 (0.1)

Abbreviations: IQR, interquartile range; LDL, low-density lipoprotein; NA, not applicable; NF1, neurofibromatosis type 1.

SI conversion factors: to convert cholesterol to mmol/L, multiply by 0.0259.

^aN = 62 unless otherwise indicated.

^bP = .03 between simvastatin and placebo group.

^cDisease severity of NF1 was scored according to the Riccardi scale modified to exclude cognitive aspects of NF1.⁴

^dSocioeconomic status was determined from highest parental occupation or, if not available, highest parental education, and divided into low, middle, or high.

the simvastatin group who did not return all of their medication jars was decreased by at least 34% (1 not determined because of loss to follow-up).

COMMENT

We report the results of a randomized, double-blind, placebo-controlled trial to investigate the effect of simvastatin on cognitive function in children with NF1. We used a carefully selected set of outcomes, including tests resembling measurements shown to be responsive to statins in preclinical studies, tests reflecting the specific neuropsychological deficits in NF1, and objective outcomes such as prism adaptation and brain ADC values, which are insensitive to a placebo or test-retest effect. We did not find an effect of 12-weeks of simvastatin treatment on the primary and secondary outcome parameters except for higher scores on the object assembly test.

We can conclude post hoc that the power of our study was enough to reject a possible effect on most tests. For instance, for the Rey CFT (recall) ($\beta=0.10$, $SE=0.23$), we can reject a change larger than 0.56, and for the cancellation test (speed) ($\beta=-0.19$, $SE=0.24$), we can reject a change larger than 0.28. Furthermore, we chose to interpret an improvement of 1 SD as clinically significant, and none of the outcome measures showed a difference between the simvastatin and placebo group of 1 SD or larger. Thus, given the power of the study and the overall negative findings, this study does not provide support for prescribing simvastatin to treat the cognitive deficits of children with NF1.

The object assembly test was the only outcome measure that was significantly improved. Considering that we found an improvement only on

this test and that we did multiple statistical comparisons without adjusting the *P* value, this is probably a spurious finding.

It should be noted that the improvement in object assembly was restricted to children who performed poorly at baseline. This specific im-

provement in the subgroup of children with poor baseline scores is not likely to be related to a practice effect because children with high baseline

Table 2. Scores on Primary and Secondary Outcome Measures at Baseline and 12 Weeks

	Mean (SD)		β (95% Confidence Interval)	
	Baseline ^a	12 Weeks ^b	Univariate Difference ^{b,c}	Multivariate Difference ^{b,d}
Primary Outcome Measures				
Rey CFT (delayed recall)				
Placebo	-1.6 (0.7)	-1.5 (1.0)	0.07 (-0.37 to 0.51)	0.10 (-0.36 to 0.56)
Simvastatin	-1.7 (0.8)	-1.4 (0.8)		
Cancellation test (speed) ^e				
Placebo	-0.8 (1.6)	0.4 (1.1)	-0.27 (-0.74 to 0.20)	-0.19 (-0.67 to 0.29)
Simvastatin	-1.2 (1.8)	-0.1 (1.4)		
Prism adaptation, No. (%) ^f				
Placebo	12 (44)	10 (37)	1.57 (0.48 to 5.13) ^g	2.01 (0.55 to 7.37) ^g
Simvastatin	11 (50)	12 (48)		
Mean ADC value, $\times 10^{-3}$ mm ² /s ^h				
Placebo	8.03 (0.52)	7.97 (0.50)	0.01 (-0.12 to 0.14)	0.06 (-0.07 to 0.20)
Simvastatin	8.02 (0.44)	7.91 (0.46)		
Secondary Outcome Measures				
Cancellation test (standard deviation) (raw score) ⁱ				
Placebo	-2.7 (1.2)	-1.9 (0.9)	-0.12 (-0.65 to 0.41)	-0.26 (-0.80 to 0.28)
Simvastatin	-2.8 (1.7)	-2.0 (1.5)		
Stroop (speed) ^j				
Placebo	-0.2 (1.8)	0.2 (1.5)	0.34 (-0.36 to 1.04)	0.48 (-0.23 to 1.18)
Simvastatin	-0.5 (2.1)	0.3 (1.9)		
Block design				
Placebo	-1.1 (0.8)	-1.0 (1.0)	0.15 (-0.18 to 0.47)	0.10 (-0.24 to 0.44)
Simvastatin	-0.8 (0.9)	-0.5 (1.0)		
Object assembly				
Placebo	-1.1 (1.1)	-0.9 (1.3)	0.50 (0.05 to 0.95) ^k	0.54 (0.08 to 1.01) ^l
Simvastatin	-0.8 (1.1)	-0.1 (1.0)		
Rey CFT (copy)				
Placebo	-1.2 (1.2)	-0.7 (1.1)	-0.26 (-0.71 to 0.19)	-0.12 (-0.58 to 0.34)
Simvastatin	-1.4 (1.3)	-1.0 (1.2)		
Beery VMI				
Placebo	-1.2 (0.9)	-1.1 (0.9)	-0.01 (-0.27 to 0.26)	-0.02 (-0.30 to 0.26)
Simvastatin	-1.2 (0.7)	-1.1 (0.7)		
Judgment of line orientation test				
Placebo	-1.6 (1.4)	-1.1 (1.6)	-0.12 (-0.62 to 0.38)	-0.06 (-0.58 to 0.46)
Simvastatin	-1.1 (1.4)	-0.8 (1.6)		

Abbreviations: ADC, apparent diffusion coefficient; Beery VMI, Beery developmental test of visual-motor integration; CFT, complex figure test; NF1, neurofibromatosis type 1.

^aN = 62 (31 placebo, 31 simvastatin) unless otherwise indicated. Values indicate mean (SD) z score unless otherwise indicated in which negative values indicate performance below the normative mean and positive values performance above the normative mean.

^bn = 61 (31 placebo, 30 simvastatin) unless otherwise indicated; 1 loss to follow-up in the simvastatin group before final assessment. Values indicate mean (SD) z score unless otherwise indicated in which negative values indicate performance below the normative mean and positive values performance above the normative mean.

^cValues (regression coefficients and 95% confidence interval) indicate between-group differences in scores after 12 weeks, adjusted for baseline scores, obtained from univariate regression analysis.

^dValues (regression coefficients and 95% confidence interval) indicate between-group differences in scores after 12 weeks, adjusted for baseline scores, age, and sex, obtained from multivariate regression analysis.

^eBaseline and 12 weeks: n = 29 in the placebo group; only administered if children possessed sufficient rote memory to count groups of up to 5 dots.

^fBaseline: n = 49 (27 placebo, 22 simvastatin); 7 left-handed children excluded, 6 children excluded due to technical problems, including not understanding or adhering to task instructions (n = 4). 12 Weeks: n = 52 (27 placebo, 25 simvastatin); 6 left-handed children excluded, 3 children excluded due to technical problems including not understanding/adhering to task instructions (n = 2).

^gOdds ratio with 95% confidence interval. n = 46 (26 placebo, 20 simvastatin), 6 left-handed children excluded, 9 children excluded because of technical problems, including not adhering to task instructions (n = 6).

^hBaseline: n = 50 (25 placebo, 25 simvastatin); 2 missing due to artifacts, 10 were not scanned due to limited magnetic resonance imaging capacity (random). 12 Weeks: n = 46 (23 placebo, 23 simvastatin); 5 missing due to artifacts, 10 were not scanned due to limited magnetic resonance imaging capacity (random). A decrease in ADC values indicates lower signal intensity.

ⁱBaseline and 12 weeks: n = 29 in the placebo group; only administered if children possessed sufficient rote memory to count groups of up to 5 dots. Larger negative values indicate larger attention fluctuations.

^jBaseline: n = 59 (29 placebo, 30 simvastatin); 12 weeks: n = 58 (29 placebo, 29 simvastatin). Only administered if children could read the names of colors.

^kP = .03.

^lP = .02.

scores are expected to benefit most from a practice effect.²⁵

The object assembly test measures multiple cognitive domains, but in the context of the entire neuropsychological assessment along with the clinical behavioral observations made during the assessment, visual synthesis is probably the most damaged cognitive domain. Improved visual synthesis would affect academic performance. For instance, visual synthesis needs to be mastered for children to start reading and spelling, and visual synthesis is an important part of more advanced mathematical skills.^{26,27} However, whether the observed improvement in object assembly is a real effect and whether simvastatin would indeed improve academic achievement remain to be confirmed.

Our study has several limitations. First, the treatment duration used in our study might have been too short to observe a clinically significant cognitive

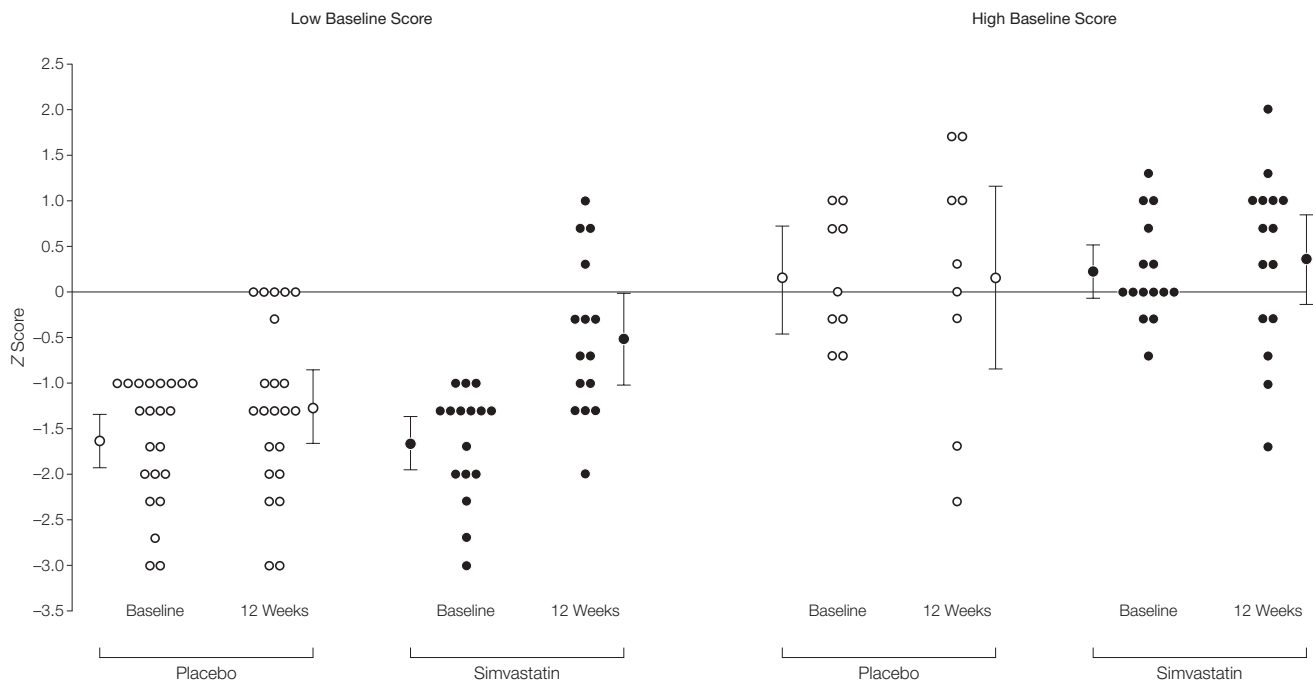
recovery in patients with NFI. We based the length of our trial on the observation that statin treatment normalized the plasticity impairment and cognitive phenotype of *Nf1* mice within days¹³ and the observation that treatment of some cognitive problems in children can be reached within days to weeks (for instance, in the treatment of attention deficits in attention-deficit/hyperactivity disorder, reviewed by Brown et al²⁸). However, because precedents for translational trials of cognition are rare, we cannot exclude the possibility that the effect of simvastatin on higher cognitive functions in humans would require a longer treatment period than 12 weeks.

Second, the placebo group showed a significant improvement between baseline and 12-week scores on 4 of 9 neuropsychological outcome measures. This resulted in a performance within normal range on 3 tests. Because preclinical studies showed that

statin treatment did not improve cognitive function in mice that already learned well,¹³ it is possible that we reached a performance ceiling that hampered detection of an effect.

Third, it is conceivable that the therapeutic effect of simvastatin on human brain function was hampered by suboptimal availability due to a first pass effect or due to inefficient crossing of the blood brain barrier. However, increasing the therapeutic dose does not seem desirable because of the lack of safety studies in children with higher doses and the increasing risk of adverse effects observed in adults.²³ Furthermore, the effect of simvastatin on low-density lipoprotein cholesterol levels at 12 weeks was similar to the decrease achieved after 48 weeks of simvastatin treatment in a previous pediatric study.¹⁶ This indicates that, at least in the liver, the treatment dose was optimal with respect to inhibition of the mevalonate pathway.

Figure 2. Interaction Between Baseline Score and Effect of Simvastatin on Object Assembly Test Results



For each subgroup, individual z scores and uncorrected group mean z scores are provided. For each subgroup, the left range shows scores at baseline and the right range, scores at 12 weeks. For the simvastatin group, $n=16$ for the low baseline score at baseline but $n=15$ for the low baseline score at 12 weeks; $n=15$ for the high baseline score. For the placebo group, $n=22$ for the low baseline score, and $n=9$ for the high baseline score. The difference between the simvastatin and placebo groups after 12 weeks is significant in the groups with low baseline performance ($\beta=0.80$; 95% confidence interval, 0.29 to 1.30; $P=.003$), but not in the groups with high baseline performance ($\beta=0.47$; 95% confidence interval, -0.64 to 1.59). Error bars represent 95% confidence intervals.

Finally, there was a relatively high amount of missing data in the neuro-radiological and prism adaptation results. Although this reduces the power on these outcome measures, there was no indication for a substantial bias because the distribution of observations that were missing did not significantly differ between the simvastatin and placebo groups. For the other outcome measures, the proportion of missing data was negligibly small.

The negative outcome of this trial suggests that simvastatin should not be prescribed to ameliorate the cognitive deficits associated with NF1. Further studies to evaluate a longer treatment period and whether the object assembly finding is spurious may be warranted.

Author Contributions: Ms Krab had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Krab, De Goede-Bolder, Aarsen, Pluijm, Lequin, Catsman-Berrevoets, Arts, Kushner, Silva, De Zeeuw, Moll, Elgersma.

Acquisition of data: Krab, De Goede-Bolder, Bouman, Lequin, Moll.

Analysis and interpretation of data: Krab, De Goede-Bolder, Aarsen, Pluijm, Van der Geest, Kushner, Moll, Elgersma.

Drafting of the manuscript: Krab, De Goede-Bolder, Van der Geest, Moll, Elgersma.

Critical revision of the manuscript for important intellectual content: Krab, De Goede-Bolder, Aarsen, Pluijm, Bouman, Lequin, Catsman-Berrevoets, Arts, Kushner, Silva, De Zeeuw, Elgersma.

Statistical analysis: Krab, Pluijm, Van der Geest.

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Study supervision: De Goede-Bolder, Aarsen, Lequin, Catsman-Berrevoets, Arts, Silva, De Zeeuw, Moll, Elgersma.

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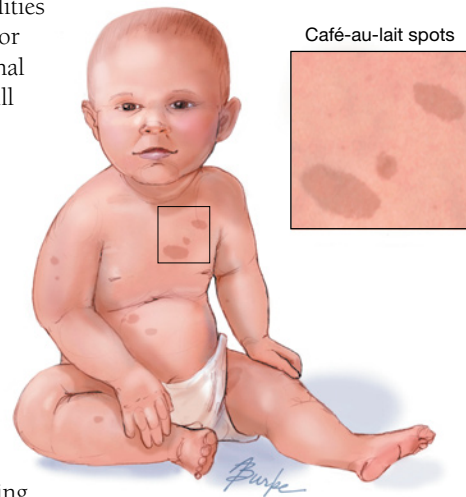
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Neurofibromatosis

Neurofibromatosis (NF) is a genetic disorder causing skin abnormalities and tumors that form on nerve tissues. These tumors can be small or large and can occur anywhere in the body, including the brain, spinal cord, large nerves, or smaller nerves. NF affects persons of both sexes and all racial groups. There are 2 types of neurofibromatosis, called NF1 and NF2. These are 2 distinct disorders that are caused by **mutations** (changes) in different genes. NF1 is also referred to as Von Recklinghausen disease and is a rather common genetic disease, affecting approximately 1 in 4000 individuals. Some patients who have NF1 only display characteristic skin abnormalities such as **café-au-lait spots**, which are flat, **hyperpigmented** (darker than surrounding skin) areas. Other patients can have severe physical complications such as **malignant** (cancerous) tumors or have mental retardation. The July 16, 2008, issue of *JAMA* includes an article about the learning deficits associated with NF. Learning disability is the most frequent complication in children with NF1.

NF2 is much more rare than NF1, affecting less than 1 in 30000 individuals, usually becoming apparent in the late teens, and typically causing hearing loss and problems with balance due to tumors on nerves to the ears.



SIGNS AND SYMPTOMS OF NF1

- Café-au-lait spots are already visible at or shortly after birth.
- Freckling in skin folds, for instance the armpit or inguinal region
- **Neurofibromas**—soft bumps on or under the skin that are tumors arising on or along nerves
- Weakness, numbness, tingling, or other symptoms may be present if the NF tumors compress the spinal cord or large peripheral nerves.
- Visual difficulties in children can indicate a tumor of the visual pathway.
- Bone deformities, including a bowed lower leg or **scoliosis** (curved spine)
- Learning disability and mental retardation are commonly associated with NF1.

DIAGNOSIS AND GENETICS

About 50% of NF patients have an affected parent, but in 50% the mutation occurs spontaneously in a family with no previous history of NF. The diagnosis of NF1 and NF2 is based on clinical symptoms. The NF1 diagnosis can usually be made before the age of 6, whereas the symptoms of NF2 often only arise in the late teens. Since the 2 genes that cause NF1 and NF2 have been discovered, genetic testing is also available.

TREATMENT

Because of the wide range of symptoms and complications that can arise in NF1 and NF2, patients should be monitored by a team of specialists. There is currently no cure for NF1 or NF2. Surgical treatment is aimed at alleviating the symptoms that arise when NF tumors compress nearby bodily tissues and can cause damage to those tissues or organs. **Chemotherapy** (anticancer drugs) may also be offered when the tumors associated with NF are malignant. This occurs in less than 10% of persons who have NF.

FOR MORE INFORMATION

- National Institute of Neurological Disorders and Stroke
www.ninds.nih.gov
- American Academy of Pediatrics
www.aap.org
- Neurofibromatosis Inc
www.nfinc.org
- The Children's Tumor Foundation
www.ctf.org

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Janet M. Torpy, MD, Writer

Alison E. Burke, MA, Illustrator

Richard M. Glass, MD, Editor

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