Chapter 2

Simvastatin for cognitive deficits and behavioural problems in patients with neurofibromatosis type 1: a randomised placebo-controlled trial

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Summary

Background

Neurofibromatosis type 1 is a common genetic disorder characterised by neurocutaneous manifestations and cognitive and behavioural problems. Statins were shown to reduce analogous learning deficits in a mouse model of the disease, but a short-term trial in humans was inconclusive. We aimed to assess the use of simvastatin for the improvement of cognitive and behavioural deficits in children with neurofibromatosis type 1 for 12 months.

Methods

In this randomised, double-masked, placebo-controlled trial, we recruited children with genetically confirmed neurofibromatosis type 1 aged 8–16 years from two national referral centres in the Netherlands and Belgium. Those with symptomatic CNS abnormalities or on neurotropic medication, including stimulants, were excluded. Eligible patients were randomly assigned (1:1) via a computer-generated, permuted-block list to simvastatin (10 mg per day in month 1, 20 mg per day in month 2, and 20–40 mg per day in months 3–12) or placebo for 12 months. Investigators, participants, and parents were masked to treatment assignment. Primary outcome measures were full-scale intelligence (Wechsler intelligence scale for children), attention problems (child behaviour checklist, parent-rated [CBCL]), and internalising behavioural problems (CBCL). We did intention-to-treat analyses (of all patients who had outcome data) using linear regression of the 12 month outcome scores, adjusted for baseline performance. This trial is registered with the Netherlands Trial Register, number NTR2150.

Findings

We randomly assigned 84 children to a treatment group (43 to simvastatin, 41 to placebo) between March 9, 2010, and March 6, 2012. We did not assess outcomes in two patients in the placebo group because they needed additional drug therapy. Simvastatin for 12 months had no effect on full-scale intelligence (treatment effect compared with placebo –1•3 IQ points [95% CI –3•8 to 1•3]; p=0•33), attention problems (–1•6 T-score points [–4•3 to 1•0]; p=0•23), and internalising behavioural problems (–0•1 T-score points [–3•3 to 3•1]; p=0•96). 38 (88%) of 43 patients on simvastatin and 39 (95%) of 41 patients on placebo reported adverse events, which were serious in two and four patients, respectively.

Interpretation

12 month simvastatin treatment did not ameliorate cognitive deficits or behavioural problems in children with neurofibromatosis type 1. The use of 20–40 mg simvastatin per day for cognitive enhancement in children with neurofibromatosis type 1 is not recommended.

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Introduction

Neurofibromatosis type 1 is a common autosomal-dominant disorder, with a prevalence of 1 in every 2500–3000 births.[1] It is caused by loss-of-function mutations in the NF1 gene, which encodes neurofibromin, a negative regulator of rat-sarcoma viral oncogene homologue (Ras). Neurofibromatosis type 1 is characterised by cutaneous café-au-lait spots, neurofibromas, and cognitive and behavioural problems.[2] Up to 80% of children aged 6–18 years with neurofibromatosis type 1 present with moderate to severe impairment in one or more areas of cognitive functioning, and 40% attend special education.[3,4] Moreover, 30–40% of children with neurofibromatosis type 1 fulfil criteria for attention deficit hyperactivity disorder and up to 60% have problems with executive functioning.[3,5] The average intelligence quotient (IQ) is 10–15 points lower in these children than in population or sibling control groups.[3,6] Parents of children with neurofibromatosis type 1 frequently report difficulties in their child's social daily life activities and a high rate of internalising behavioural problems, such as anxiety or mood disorders.[7] Taken together, cognitive and behavioural deficits lead to lower academic achievement and loss of quality of life,[4,8,9] persisting into adulthood.[10] The learning and attention deficits noted in patients with neurofibromatosis type 1 are reported in the *Nf1*$^{+/-}$ mouse model,[11–13] accompanied by a decrease in synaptic plasticity.[11–13] These animal studies have shown that the plasticity and behavioural deficits are reversed by reducing Ras activity.[11,14] Ras activity requires farnesylation, which allows Ras to anchor to the plasma membrane where it can be activated by growth-factor receptors and their adaptor proteins. Since cholesterol is an obligate precursor of farnesyl, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase have been suggested as a potential therapy for neurofibromatosis type 1. Indeed, lovastatin normalised Ras activity, rescued synaptic plasticity deficits, and restored learning and attention deficits in the *Nf1*$^{+/-}$ mouse model.[14] Results of a small, open-label, single-arm study of lovastatin in children with neurofibromatosis type 1 suggested that lovastatin improved memory and attention, and normalised default network functional connectivity measured with resting state functional MRI.[15,16]

However, lovastatin is not approved or marketed in many parts of the world, including the European Union. The closest approved alternative, simvastatin, is similar in structure, pharmacokinetics, and blood–brain barrier permeability.

Moreover, simvastatin is a slightly more potent inhibitor of HMG-CoA reductase and is better at reducing HMG-CoA reductase activity in neurons than is lovastatin.[17,18] Although findings of a randomised controlled trial reporting the short-term effect of simvastatin in children with neurofibromatosis type 1 showed no effect after 12 weeks on a set of primary outcome measures,[6] a significant improvement was reported for a secondary outcome measure, the object assembly subtask of the Dutch translation of the third edition of the Wechsler intelligence scale for children (WISC-III-NL).[6] Although this trial had an overall negative outcome, it had some limitations that might have affected its results: children on stimulant- medication were not excluded, and 12 week treatment was short, with only 4 weeks at the highest target dose. A longer treatment duration would have allowed the assessment of the effects on global cognitive functioning, daily life functioning, and behaviour, and might have been necessary to show clinical benefits.

Given the large amount of safety data in children[6,19] and worldwide marketing authorisation of simvastatin, we aimed to improve upon the limitations of this previous trial by assessing the use of simvastatin for the treatment of cognitive and behavioural deficits in children with neurofibromatosis type 1 for 12 months.

Methods

Study design and participants

We undertook this randomised, parallel-group, placebo-controlled trial in two national referral centres: Erasmus MC (Rotterdam, Netherlands) and UZ Leuven (Leuven, Belgium). We screened patients aged 8–16 years with genetically confirmed neurofibromatosis type 1 for eligibility. Genetic counselling and testing for neurofibromatosis type 1 is part of routine care and was done independently of this trial. The rationale for genetic confirmation was the substantial overlap in phenotypes between neurofibromatosis type 1 and related disorders (eg, Legius syndrome).[20] Exclusion criteria were: use of neurotropic medication, including stimulant, anti-psychotic, antiepileptic, antianxiety, and antidepressant drugs, or current simvastatin use; symptomatic CNS abnormalities; insufficient comprehension of the Dutch language; severely impaired vision or deafness; segmental neurofibromatosis type 1; or an IQ below 48.

We obtained informed oral and written consent from parents and assent from children of 12 years and older. Local and national institutional review boards approved the

protocol. The trial was done in agreement with the Declaration of Helsinki (version 2008) and Good Clinical Practice guidelines.

Randomisation and masking

Eligible patients were randomly assigned (1:1) by the local hospital pharmacist to simvastatin or matched placebo according to computer-generated, permuted block randomisation lists (ten participants per block, stratified by centre) that were provided by the Department of Biostatistics, Erasmus MC, with medication numbers in the order of enrolment. All investigators, participants, and their parents were masked to treatment allocation. We achieved blinding by using capsules of identical colour, shape, size, weight, smell, and taste.

Procedures

Participants took 10 mg per day of simvastatin or matched placebo once daily in the morning during the first month and 20 mg per day once daily in the morning during the second month. During months 3–12, dosing was fixed at 20 mg per day for children aged 12 years and younger and 40 mg per day for adolescents older than 12 years. We assessed efficacy outcome measures at baseline and at the end of month 12 of treatment. Since no standard measure exists to assess improvement of cognition in patients with neurofibromatosis type 1, we included a broad range of validated tests and questionnaires that are sensitive to the cognitive and behavioural deficits in this group of patients. Outcome measures included constructs that were similar to those that improved in mouse models receiving statins:[14] visual-spatial memory and attention; improvements in daily life behavioural problems rated by parents; and global cognitive functioning. We used three primary outcome measures that are relevant to daily life functioning and academic achievement: full-scale intelligence (WISC-III-NL),[4,6] parent-reported attention problems (child behaviour checklist [CBCL][21]), and parent-reported internalising behavioural problems (CBCL). The attention problems scale of the CBCL consists of items screening for problems in directing and sustaining attention, controlling impulsivity, and hyperactivity. Secondary outcomes were visual-spatial memory (Rey complex figure test–delayed recall),[6] attention (Stroop colour–word interference test),[6] teacher-reported school performance (teacher report form),[21] parent-reported psychosocial quality of life (child health questionnaire–parent form 50 [CHQ-PF50]),[9] patient-reported internalising behavioural problems (youth self-report [YSR] form, completed by patients aged ≥11 years),[21] and fine motor coordination (grooved pegboard test).[8] All

neuropsychological tests were developed for children and were written or presented in Dutch. For most outcome measures, we used age-standardised scores. The mean average IQ for the general population is 100 (SD 15), with higher IQ WISC-III-NL test scores indicating higher intelligence. For CBCL and YSR, data were represented as T scores, with a mean average of 50 and an SD of 10 in the general population, with higher scores indicative of more problems. The Rey complex figure test (for which a higher score suggests a better visual-spatial memory) and CHQ-PF50 (for which a higher score suggests a better quality of life) are presented using Z scores, with 0 representing the mean for the normal sample with an SD of 1. Teacher-reported school performance was calculated on a scale from 2 to 10, by summation of 5-point scores on topics of language and arithmetic, in which higher scores were given for greater ability in each area. For teacher-reported school performance, Stroop colour–word test (for which a lower score suggests better attention), and grooved pegboard test (for which a lower score suggests better fine motor coordination), raw scores were used, since no appropriate normal groups are available for the entire age range. Measurements taken before and after administration of study drug were done by the same neuropsychologist (either ABR or EP). Adverse events and study compliance were monitored by monthly telephone contact and by visits to the outpatient clinic at baseline and at 1, 3, 6, 9, and 12 months. Adverse events were classified according to WHO adverse reaction terminology and graded according to the National Cancer Institute common terminology criteria for adverse events. Blood was drawn at baseline and at 1, 6, 9, and 12 months to measure: alanine aminotransferase, aspartate aminotransferase, and creatine phosphokinase to screen for laboratory adverse events; and total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides to assess lower limits of lipid concentrations and to monitor compliance. Further details on procedures are presented in the appendix.

Statistical analysis

We used data from the intention-to-treat population—which consisted of all participants with outcome data—for all primary and secondary analyses, without imputation of missing values. Data from all patients were used for safety analyses—even those without efficacy outcome data. We analysed primary and secondary

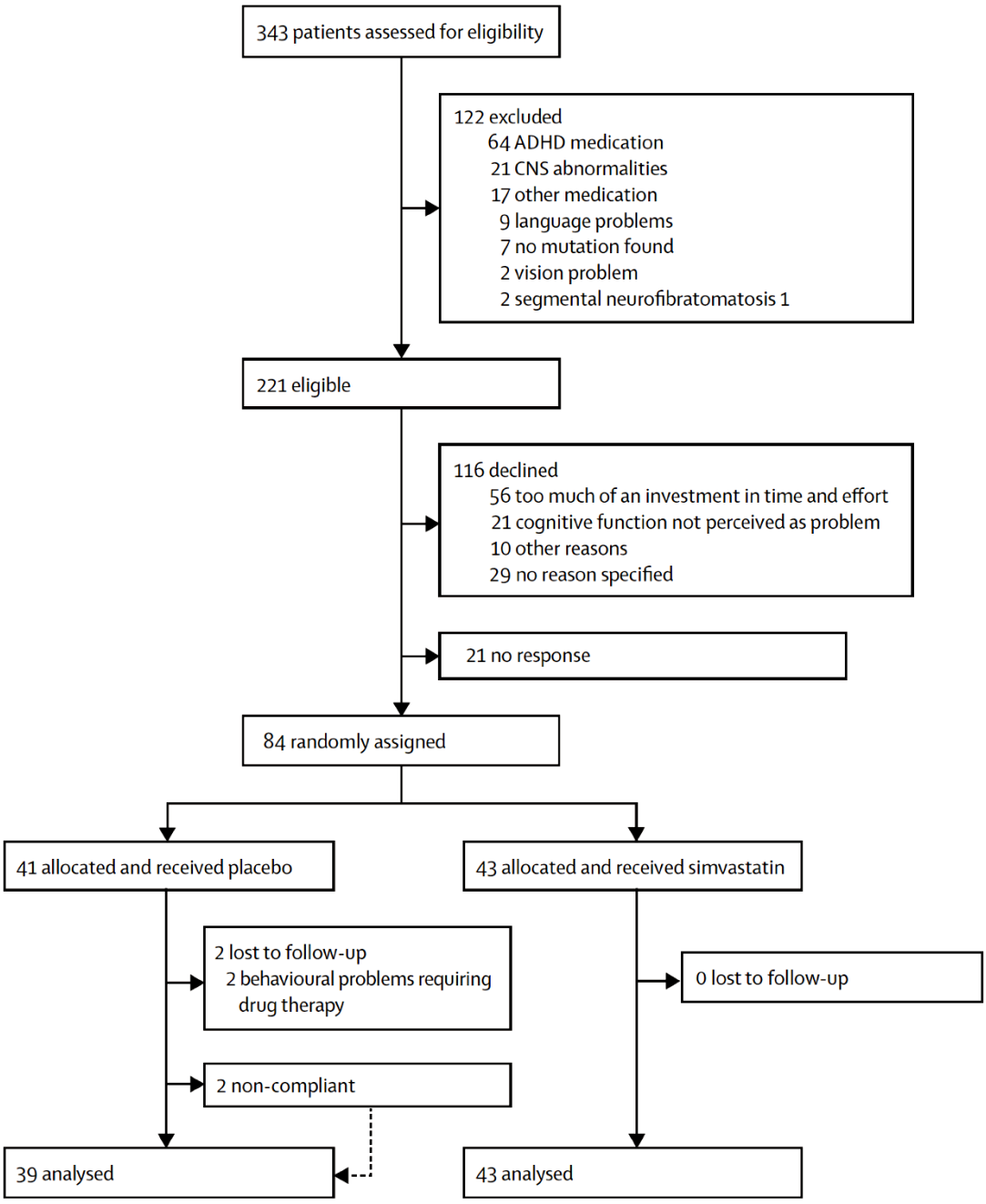

Figure 1: Trial profile ADHD = attention deficit hyperactivity disorder

outcome measures using linear regression for the effect of treatment group on the score at 12 months, adjusted for baseline performance in the bivariable analysis and adjusted for baseline performance, age, and sex in the multivariable analysis. The cutoff level for significance was set at $p<0•05$, ignoring multiple testing. We analysed lipid blood concentrations using the generalised linear mixed model procedure with the interaction of time and treatment as the variable of interest. Sample size calculation suggested that inclusion of 84 participants (85% power; $\alpha=0•05$) would be

sufficient to detect a clinically relevant treatment effect of 7•5 full-scale intelligence points (0•5 SD), adjusted for baseline performance, and an increase or decrease of 5 T-score points (SD 0•5) for attention problems and internalising behavioural problems. Inclusion of 84 participants would lead to greater than 80% power on the coprimary outcome measures of attention problems and internalising behavioural problems. Because of low inclusion rates, the protocol was amended from 90% power and 106 participants to 85% power and 84 participants in the second recruitment year, without outcome knowledge and with approval from review boards. We planned the analysis before unmasking according to the study protocol. All data were analysed using IBM SPSS Statistics for Windows (version 20.0).

This trial is registered with the Netherlands Trial Register, number NTR2150.

Role of the funding sources

The sponsors of the study had no role in the conception and design of the trial, the collection, analysis, and interpretation of the data, the writing of the manuscript, or the decision to publish the results. All authors had full access to all of the data in the study, and EL, YE, and HAM had final responsibility for the decision to submit for publication.

For the study protocol see http://www.erasmusmc.nl/nf1-simcoda

Results

We screened 343 patients for eligibility, of whom 221 were eligible. Between March 9, 2010, and March 6, 2012, we obtained informed consent from 84 patients or their parents. They were randomly assigned to 12 months of treatment with simvastatin (n=43) or placebo (n=41). Two patients in the placebo group were lost to follow-up before outcome could be assessed because they had behavioural problems that required drug therapy. Two participants in the placebo group discontinued study medication, but were available for outcome assessment (figure 1). Median compliance per patient was 96% (IQR 93–100), measured by counting returned capsules. Baseline demographic and disease characteristics were generally balanced between both treatment groups, although more patients in the simvastatin group were male than in the placebo group (table 1). At baseline, average full-scale intelligence was 83·3 points (SD 15·6) and 46 (55%) participants had attention problems scored on the CBCL of more than 1 SD above the mean of the general population. Median age was 11·5 years (range 7·9–16·0). 12 months of simvastatin had no significant effect on

	Simvastatin (n= 43)	Placebo (n= 41)
Age, years	11·1 (9·2 – 13·0)	11·8 (10·2 – 14·7)
Male sex	26 (61%)	13 (32%)
Full-scale intelligence (WISC-III-NL)*	83·8 (16·1)	82·7 (15·3)
Attention problems (CBCL), *T*-score†	61·1 (8·9)	62·8 (8·3)
Internalizing behavioural problems (CBCL), *T*-score†	55·2 (10·7)	56·7 (10·1)
NF1 disease severity‡		
Minimal	18 (42%)	21 (51%)
Mild	7 (16%)	4 (10%)
Moderate	17 (40%)	15 (37%)
Severe	1 (2%)	1 (2%)
Genetic mutation type		
Truncating mutation	24 (56%)	28 (68%)
In-frame del-dup or missense mutation	18 (42%)	11 (27%)
Microdeletion	1 (2%)	1 (2%)
Unclassified variant	0	1 (2%)
NF1 inheritance		
Familial	22 (51%)	19 (46%)
Sporadic	21 (49%)	20 (49%)
Unknown	0	2 (5%)
Education type		
Regular	20 (46%)	24 (58%)
Special	23 (54%)	17 (42%)
Parental Occupation§		
Lower	18 (42%)	13 (32%)
Middle	13 (30%)	15 (37%)
Higher	12 (28%)	13 (32%)
Total cholesterol, mmol/L	4·17 (0·57)	4·30 (0·75)
LDL cholesterol, mmol/L	2·33 (0·54)	2·41 (0·65)
Dose group in months 3-12¶		
20 mg/d or placebo	29 (67%)	23 (56%)
40 mg/d or placebo	14 (33)%	18 (44%)

Table 1: Baseline characteristics. Data are median (IQR), number (%), or mean (SD). WISC-III-NL=Wechsler intelligence scale for children, third edition, Dutch translation. CBCL=parent-reported child behaviour checklist. NF1=neurofibromatosis type 1. LDL=low-density lipoprotein. *Higher is better. †Lower is better. ‡NF1 disease severity was scored according to the Riccardi scale, modified to exclude cognitive aspects of NF1.[6] §Classification of parental occupation was done according to data from the Dutch central bureau of statistics (which uses a five-level scale), which we used to apply our own three-level scale. ¶Doses were decided based on the patient's age.

full-scale intelligence (treatment effect –1·3 IQ points [95% CI –3·8 to 1·3]; p=0·33), attention problems (–1·6 T-score points [–4·3 to 1·0]; p=0·23), or internalising behavioural problems (–0·1 [–3·3 to 3·1]; p=0·96) when adjusted for baseline performance (table 2). Additional adjustment for age and sex produced similar results (table 2). Simvastatin had no significant effects on any of the secondary outcome

measures, including visual-spatial memory and attention (table 2). Figure 2 shows the standardised treatment effects on primary and secondary outcome measures.

After 1 month (10 mg simvastatin per day), mean total cholesterol in the simvastatin group had decreased by 0·78 mmol/L (95% CI 0·54–1·03) more than it had in the placebo group and LDL cholesterol decreased by 0·79 mmol/L (0·56–1·01). Cholesterol concentrations had decreased no further at 6, 9, or 12 months. HDL cholesterol and triglycerides remained stable over the course of the study (appendix).

Most adverse events were mild or moderate and frequency was similar between groups (table 3). 38 (88%) of 43 patients in the simvastatin group and 39 (95%) of 41 patients in the placebo group reported at least one adverse event. No increased incidence of myalgia, myopathy, or rhabdomyolysis was reported in patients given simvastatin compared with patients given placebo (appendix). Serious adverse events occurred in six patients: two in the simvastatin group and four in the placebo group. These events included continuing growth of plexiform neurofibromas (in two patients receiving simvastatin and one patient receiving placebo) and progressive scoliosis (two patients receiving placebo), all requiring surgery, and hospital admission for gastritis (one patient receiving placebo).

Results of laboratory screens showed a few mild and transient increases in liver enzymes and creatine kinase in both groups (table 3); none led to cessation of treatment. No participants reached the predefined lower limits for total cholesterol, HDL-cholesterol, or triglycerides (non-fasting). In the simvastatin group, seven children had one (n=3) or more (n=4) LDL cholesterol measurements below the predefined lower threshold, but no action was recommended by the data and safety monitoring board, since other values were within the normal range. Nine (53%) of 17 girls receiving simvastatin advanced one or more Tanner stages of puberty during the trial, compared with 16 (67%) of 24 receiving placebo. 14 (54%) of 26 boys receiving simvastatin and seven (54%) of 13 receiving placebo advanced one or more Tanner stages. Two girls in the placebo group were not included in this analysis because they did not undergo postbaseline Tanner stage assessments.

	Simvastatin	Placebo	Adjusted for baseline score		Adjusted for baseline score, age and sex	
			Treatment effect (95% CI)	*p*	Treatment effect (95% CI)	*p*
Primary outcome measures						
Full-scale intelligence (WISCIII-NL)*	n = 43	n = 39	-1·3 (-3·8 – 1·3)	0·33	-0·8 (-3·4 – 1·8)	0·56
Baseline IQ	83·8 (16·1)	82·3 (15·5)	"	"	"	"
12 months IQ	85·7 (18·0)	85·4 (16·4)	"	"	"	"
Attention problems (CBCL)†	n = 42‡	n = 39	-1·6 (-4·3 – 1·0)	0·23	-2·2 (-5·0 – 0·5)	0·11
Baseline *T*-score	61·1 (9·0)	62·0 (7·6)	"	"	"	"
12 months *T*-score	58·8 (7·4)	60·9 (9·0)	"	"	"	"
Internalizing behavioural problems (CBCL)†	n = 42‡	n = 39	-0·1 (-3·3 – 3·1)	0·96	0·0 (-3·4 – 3·4)	0·99
Baseline *T*-score	54·9 (10·6)	56·1 (10·0)	"	"	"	"
12 months *T*-score	54·0 (9·0)	54·9 (10·0)	"	"	"	"
Secondary outcome measures						
Visual-spatial memory (Rey Complex Figure test – delayed recall)*	n = 42§	n = 39	-0·2 (-0·6 – 0·2)	0·34	-0·1 (-0·6 – 0·3)	0·50
Baseline *Z* score	-2·0 (0·9)	-2·0 (1·1)	"	"	"	"
12 months *Z* score	-1·9 (1·0)	-1·7 (1·2)	"	"	"	"
Attention (Stroop Colour Word Interference)†	n = 41¶	n = 37¶	7·5 (-1·3 – 16·2)	0·14	2·8 (-5·9 – 11·7)	0·55
Baseline raw score	72 (39)	64 (45)	"	"	"	"
12 months raw score	59 (31)	47 (27)	"	"	"	"
Teacher rated school performance*	n = 34‖	n = 30‖	0·2 (-0·6 – 0·9)	0·64	0·1 (-0·7 – 1·0)	0·74
Baseline raw score	5·8 (2·2)	5·7 (2·4)	"	"	"	"
12 months raw score	6·2 (1·9)	6·0 (1·9)	"	"	"	"
Psychosocial Quality of Life (CHQ-PF50)*	n = 40**	n = 38**	0·02 (-0·22 – 0·25)	0·89	0·04 (-0·20 – 0·29)	0·72
Baseline	-0·06 (0·80)	-0·07 (0·74)	"	"	"	"
12 months	0·15 (0·69)	0·13 (0·80)	"	"	"	"
Internalizing behavioural problems (youth self-report)†	n = 23††	n = 24††	-1·7 (-6·5 – 3·1)	0·48	-2·5 (-8·1 – 3·1)	0·37
Baseline *T*-score	56·4 (11·9)	53·0 (8·3)	"	"	"	"
12 months *T*-score	51·9 (9·9)	51·7 (9·8)	"	"	"	"
Fine motor coordination (grooved pegboard test, dominant hand)†	n = 43	n = 39	-3·8 (-8·8 – 1·3)	0·14	-4·9 (-10·2 – 0·3)	0·07
Baseline	94 (29)	84 (23)	"	"	"	"
12 months	80 (18)	79 (18)	"	"	"	"

Table 2: Primary and secondary outcome measures at baseline and 12-month follow-up. Data are mean (SD), unless otherwise specified. WISC-III-NL=Wechsler intelligence scale for children, third edition, Dutch translation. CBCL=parent-reported child behaviour checklist. CHQ-PF50=child health questionnaire–parent form 50. *Higher is better. †Lower is better. ‡Data missing for one patient in the simvastatin group because the questionnaire was not returned by the parents. §Data missing for one patient in the simvastatin group because they were omitted from the test battery erroneously. ¶Data missing for two patients in each group because they were unable to take the test because of reading disability. ‖Data missing for nine patients in each group because arithmetic and language topics were classified by teachers as not applicable to these patients. **Data missing for one patient in the simvastatin group because the questionnaire was not returned, and for two patients in the simvastatin group and one in the placebo group because essential items were not completed on the checklist. ††Children younger than 11 years were deemed too young to be given the youth self-report form (20 patients in the simvastatin group; 15 patients in the placebo group).

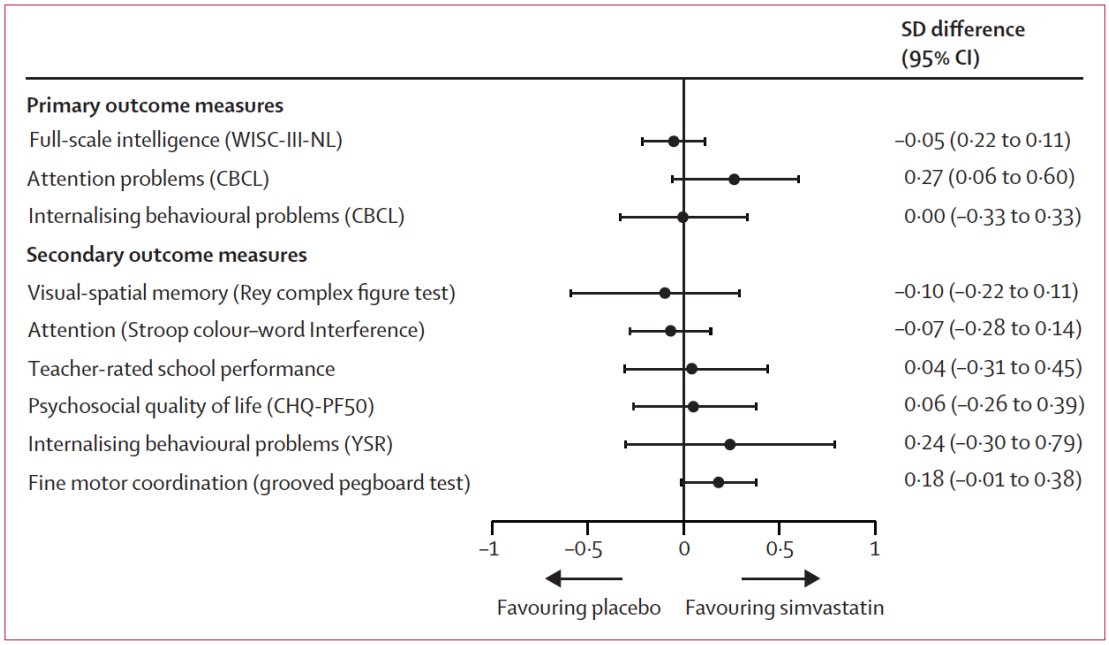

Figure 2: Standardised treatment effects. The effect of simvastatin on primary and secondary outcome measures, adjusted for baseline performance, age, and sex. Treatment effects have been converted to SD difference and are accompanied by the corresponding 95% CI. WISC-III-NL = Wechsler intelligence scale for children, third edition, Dutch translation. CBCL = parent reported child behaviour checklist. CHQ-PF50 = child health questionnaire–parent form 50. YSR = youth self-report.

Discussion

Here we present the outcome of our randomised, doublemasked, placebo-controlled trial aimed at improving cognitive deficits in children with neurofibromatosis type 1. Our results showed that simvastatin treatment for 12 months had no effect on full-scale intelligence, attention problems, or internalising behavioural problems. Moreover, we found no indications of efficacy on a carefully selected range of predefined secondary outcome measures. Hence, this trial refutes a role for simvastatin in treatment of cognitive or behavioural problems in children with neurofibromatosis type 1. Unfortunately, despite the many promising drugs that have been identified in mouse models of cognitive disorders, translational studies with placebo-controlled trial designs are rare for cognitive disorders caused by single-gene mutations. This situation is also true for neurofibromatosis type 1 (panel). The absence of good clinical studies encourages off -label prescription, which is a major concern, particularly when the drug is readily available to the patient. In this study, the cognitive and behavioural profile of the study population at baseline (table 1) was

fairly representative of the cognitive profile in the general neurofibromatosis type 1 population.[3–5,8] Sample size was adequate, because we could confidently rule out a

	Simvastatin (n=43)		Placebo (n=41)	
	Grade 1-2	**Grade 3**	**Grade 1-2**	**Grade 3**
Adverse events by system organ class				
Gastrointestinal system disorders	23 (17)	0	25 (21)	1 (1)
General, whole-body disorders	16 (16)	0	25 (20)	0
Skin and appendage disorders	12 (10)	0	11 (10)	0
Musculoskeletal system disorders	8 (7)	0	13 (11)	0
Respiratory system disorders	12 (11)	0	5 (5)	0
Central and peripheral nervous system disorders	9 (8)	0	6 (6)	0
Neoplasms (eg, aggravated neurofibroma)	2 (2)	2 (2)	3 (2)	1 (1)
Psychiatric disorders	2 (2)	0	4 (4)	0
Urinary system disorders	2 (2)	0	4 (4)	0
Secondary events (eg, postoperative pain)	3 (3)	0	1 (1)	2 (2)
Resistance mechanism disorders	4 (4)	0	2 (2)	0
Vision disorders	1 (1)	0	3 (3)	0
Other systems	1 (1)	0	3 (3)	0
Laboratory Adverse Events				
Raised alanine transaminase	6 (6)	0	1 (1)	0
Raised aspartate transaminase	3 (3)	0	5 (5)	0
Raised creatine kinase (CK)	1 (1)	0	1 (1)	0

Table 3: Adverse events. Data are number of events (number of patients who had an event). Adverse events are grouped by system organ class according to WHO adverse reaction terminology. A complete list of adverse events is presented in the appendix.

positive change of more than 1·3 points in full-scale intelligence, a reduction of attention problems of more than 4·3 T-score points, and a reduction of internalising behavioural problems of more than 3·3 T-score points (table 2). Furthermore, we achieved a low attrition rate and high medication compliance, which suggests that medium-term to long-term trials for cognitive dysfunction are feasible in this population. The dosing was based on the maximum recommended daily dose for treatment of children with familial hypercholesterolaemia.[19] At least in the liver, maximal inhibition of the HMG-CoA reductase pathway was achieved in patients on simvastatin, shown by the substantial reduction of blood cholesterol concentrations after 1 month (appendix). Whether similar inhibition of the HMG-CoA reductase pathway was achieved in the brain is unknown. It is possible that higher doses are necessary to achieve biological effects in human beings. However, increasing the dose would increase safety concerns, including the risk of myopathy, which was 30 times higher (0·9%) in adults on 80 mg per day of simvastatin than in those on 20 mg per day.[23] Although 12 months of simvastatin was not related to any adverse events, this study was not powered to detect rare effects. Of note, a lower proportion of girls

receiving simvastatin advanced one or more pubertal stages than did those receiving placebo, which was non-significant and might simply be attributed to age differences between the groups. Nonetheless, future studies of statin treatment in other populations of normocholesterolaemic children and adolescents should monitor puberty development.

We assumed 12 months of treatment was long enough to measure effects on full-scale intelligence. In support of this view, results of 1 year randomised studies showed that full-scale intelligence can improve in children with attention deficit hyperactivity disorder who receive stimulant medication24 and in healthy children taking music lessons.[25] However, how much time a human brain would need to show a discernible effect on full-scale intelligence or other neuropsychological tests is unknown. In view of the broad range of tests and validated questionnaires in this study, selection of different outcome measures would have been unlikely to change the conclusions on the effect of simvastatin treatment.

Our study population was selective in two ways. First, it was limited to children aged 8–16 years, so a therapeutic benefit in younger children cannot be excluded. Second, of 343 children who were screened, 64 (19%) were excluded from the trial because they had been taking stimulant medication. Despite this selection, 46 (55%) participants had attention problems of more than 1 SD above population norms, suggesting that attention problems were prevalent in the study population. Children were eligible for this study irrespective of their baseline neuropsychological test scores, since several difficulties are associated with selecting participants according to baseline performance. First and most important, the subgroup of children with neurofibromatosis type 1 that might benefit most from drug treatment is unknown. Also, any upper or lower limit of functioning would be arbitrary. Therefore, we chose to recruit children irrespective of baseline deficits and to do subgroup analyses if any benefits were noted in primary analysis. Differences between lovastatin and simvastatin are unlikely to explain our negative results, since the rationale for using statins in neurofibromatosis type 1 is their ability to reduce Ras farnesylation, for which mevalonate is an obligate precursor in the synthesis of both farnesyl moieties and cholesterol. However, we cannot completely exclude off -target effects that are exclusive to lovastatin. A phase 2 randomised trial of lovastatin for 16 weeks is underway to assess its effects on visual spatial learning and sustained attention in children with neurofibromatosis type 1.[26] The preclinical studies on which this study

was predicated were done exclusively in a mouse model of neurofibromatosis type 1, for which underlying human pathophysiological changes might not be sufficiently analogous. For instance, we cannot exclude that certain pathological changes frequently reported in patients with neurofibromatosis type 1, such as microstructural changes of white brain matter identified with diffusion tensor imaging[27] or changes in corpus callosum thickness,[28] contribute to cognitive deficits and might not be responsive to statins. However, important similarities in neurophysiology are reported between patients with neurofibromatosis type 1 and the *Nf1*$^{+/-}$ mouse model. For example, neurofibromatosis type 1 seems to affect working memory and attention in both human beings and rodents through cortical inhibition of corticostriatal pathways.[13] Additionally, behavioural deficits in patients with neurofibromatosis type 1 and mice are very similar: most notably in their analogous deficits in (virtual) watermaze performance.[11,29,30] Mechanistically, GABAergic dysfunction has been observed in both the mouse model and patients.[11,12,31] Nevertheless, in view of the results of our trial, further insight into the pathophysiology of neurofibromatosis type 1 will be necessary to explore other targetable disease mechanisms.

Panel: Research in context

Systematic review

We did a systematic search of PubMed on July 8, 2013, for additional cognitive trials in neurofibromatosis type 1. Search terms included "neurofibromatosis", "cognition", "attention", "behaviour", and "clinical trial". Of 25 articles found, four described three clinical trials in patients with neurofibromatosis type 1. A 12 week randomised placebo-controlled trial in 61 children with neurofibromatosis type 1 showed no effect of simvastatin on cognitive function and MRI abnormalities, with the notable exception of the significant effect on one secondary outcome measure: the object assembly subtask of the Wechsler intelligence scale for children.[6] Furthermore, results of a phase 1 single-arm open-label study of lovastatin in 23 children with neurofibromatosis type 1 suggested lovastatin improved memory and attention, accompanied by normalisation of default network functional connectivity measured with resting-state functional MRI in a subset of the participants.[15,16] These seemingly encouraging results might be attributable to normal cognitive development, test–retest improvements, or placebo effects. A third study was a single-arm 1 year study of

methylphenidate to treat attention problems in children with neurofibromatosis type 1 and comorbid attention deficit hyperactivity disorder, and results showed a decrease in attention problems in children who received the drug.[22]

Interpretation

In this 12 month trial, use of simvastatin provided no benefit over placebo on full-scale intelligence, behavioural problems, visual-spatial memory, attention, motor coordination, school performance, and quality of life. These findings are in contrast with results from the previous single-arm study,[15,16] but largely consistent with the smaller randomised controlled trial that measured short-term effects of simvastatin on neuropsychological test scores and MRI abnormalities.[6] We conclude that the number of trials is limited, and more studies are needed to identify effective treatments for cognitive and behavioural problems in children with neurofibromatosis type 1.

Supplementary methods (published Online)

Participants, treatment and follow-up

Patients were eligible for randomization when they were 8·0 to 16·0 years of age and had a genetic confirmation of NF1. Genetic counseling and testing for NF1 is offered routinely at our centers, minimizing selection. Informed oral and written consent was obtained from parents or guardians and oral and written informed assent was obtained from participants aged 12 years and older. Exclusion criteria were use of neurotropic medication or current simvastatin use; symptomatic central nervous system abnormalities; insufficient comprehension of the Dutch language; severely impaired vision or deafness; segmental NF1 and IQ below 48, which is the detection limit for Wechsler Intelligence Scales for Children.

Study design and setting

We performed an investigator-initiated randomized, parallel group, double-masked, placebo-controlled, one-year clinical trial in children with NF1 between March 9, 2010 and March 5, 2013. This was a two-center study at Erasmus University Medical Center, Rotterdam, the Netherlands, and University Hospital Leuven, Belgium, both national referral centers for Neurofibromatosis type 1. Approval was obtained from the Central Committee on Research involving Human Subjects (The Hague, The Netherlands) and the Ethical Committee of University Hospital Leuven (Belgium)

and performed in agreement with Declaration of Helsinki (2008 version) and Good Clinical Practice guidelines. Full source data verification was performed and all data queries had been solved before unmasking. All authors subscribe to adherence to the study protocol.

Intervention

Participants were treated with simvastatin or identical placebo once daily in the morning. The doses were carefully selected at the maximal daily dose recommended for children with familial hypercholesteremia: 10 mg/d in the first month, 20 mg/d in the second month, and fixed at 20 mg/d for children aged ≤ 12 years or 40 mg/d for adolescents aged 13·0 years and older in months 3 – 12. Treatment group assignments were masked for participants, investigators, and outcome assessors. Simvastatin and placebo capsules were produced by the hospital pharmacy. Capsules were identical in color, shape, size, weight, smell, and taste. The simvastatin capsules contain the active substance, simvastatin (Spruyt hillen bv, IJsselstein, The Netherlands), and as excipients siliciumdioxide colloidal (as glidant), magnesium stearate (as lubricant, diluent), cellulose microcrystalline (as binder, diluent) and lactose (as volume filler). The placebo capsules contain all of the abovementioned components, except the active substance. They were dispensed by the hospital pharmacy in containers consisting of 35 capsules per month, allowing some flexibility in the planning of follow-up visits. Left-over capsules had to be returned and counted for compliance. To avoid unmasking of investigators during the trial, the independent Data and Safety Monitoring Board (Drs. Hop, de Rijke, de Klerk, de Heus, Erasmus MC) reviewed cholesterol levels during the study and primary analysis phase. Randomization was generated by the department of Biostatistics at Erasmus MC (dr. Hop) and implemented by the local hospital pharmacist (Erasmus MC: dr. Zaal; UH Leuven: dr. de Gieter) using computer generated, permuted block randomization lists, using blocks of 10 participants stratified by center. Patients were assigned a medication number in the order of their enrollment. Treatment allocation was concealed from all participants and investigators.

Sample size calculation indicated that inclusion of 84 participants had a power of 85% with an alpha of 0.05 of detecting a clinically relevant effect of 7.5 IQ-points (equivalent to 0.5 standard deviation) difference between simvastatin and placebo on the primary outcome measure. The before-after design of the trial allows for the incorporation of test-retest correlation. No data was available on the one-year test-

retest correlation of the WISC-III-NL, but 2-year correlation is 0.91[s1]. We estimated correlation after one-year at a conservative 0.69.

Outcome measures

Outcome measures were assessed at baseline and after 12 months of treatment. All neuropsychological tests were developed for children and were administered in their Dutch versions. For most outcome measures, age standardized scores were used. Population average for IQ is 100 and the standard deviation is 15, with higher scores indicating better performance. For CBCL, and YSR, data were represented as T-scores, with a population average of 50 and standard deviation of 10, with a higher score indicating more problems. Teacher reported school performance was calculated on a scale from 2 to 10, by summation of five-point scores on topics of Language and Arithmetic. Higher scores indicate better performance. Health-related Quality of life (CHQ-PF50) and Rey complex figure test are presented using z-scores, with 0 indicating the mean for the norm sample with a standard deviation of 1. For teacher reported school performance, Stroop Color Word test and grooved pegboard test, raw scores are used, since no appropriate norm groups are available for the entire age-range. Before and after measurements were performed by the same neuropsychologist AR or EP. Any age or gender confounding effects in the estimation of treatment effects are accounted for by multivariable analysis. Harms were monitored during monthly contacts with the investigators. Outpatient visits were scheduled at baseline and after 1, 3, 6, 9 and 12 months. In the intervening months, harms and study compliance were monitored by telephone interviews. Participants were provided with a diary in which they were instructed to note any deviations from treatment protocol and possible adverse events. At each consult, one of the study physicians recorded any adverse events and serious adverse events (adverse events that were life-threatening, causing disability, or requiring hospitalization) with a standardized checklist containing simvastatin associated sideeffects, supported by open questions and a review of the participant's diary. Standard internal and neurological clinical exams were performed and blood was drawn by phlebotomy for laboratory examination at visits after 1, 6, 9 and 12 months of treatment. Hypothetically, cholesterol reduction could influence sex hormone production. Therefore, Tanner stages for puberty development were noted. Laboratory screening parameters were measured according to standard hospital laboratory protocol; alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine phosphokinase (CK) to screen

for laboratory adverse events; total cholesterol (tChol), highdensity lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides to assess lower limits of lipid levels and to monitor compliance. Criteria for discontinuation of study medication were a persistent increase of more than 3-fold the upper limit of normal (ULN) ALT or AST levels, more than 10- fold the ULN for CK levels with or without muscular symptoms, or 5- to 10-fold the ULN for CK levels with muscular symptoms. Lower limits of cholesterol in blood in children do not exist, but children would stop study medication if these levels decreased with 3x standard deviation of the population norms, as assessed by the independent Data and Safety Monitoring Board to avoid premature unmasking. Adverse events were categorized according to WHO-ART nomenclature[s2], and tabulated. No significance testing has been performed on adverse events, since statistical power to detect significant differences is low. All adverse events are therefore displayed using counts. For puberty development, Tanner stage change was defined as any change during the study versus no change during the study. Logistic regression analysis was performed by adjusting for lowest baseline Tanner scale. Adjusting for lowest baseline scale, age and sex were used to reveal significant changes. Lipid blood levels were analyzed over the course of the trial using the generalized linear mixed model procedure with time x treatment as the variable of interest. The statistical analysis plan and any exclusion from the intention to treat set were finalized before unmasking. All reported adverse events were scored as being not drug related, possibly drug related, or definitely drug related prior to unmasking.

Statistical analysis

No statistical testing was performed for baseline study group differences. Intention to treat analysis was performed for all participants of whom post-baseline data was available, without imputation of missing values. Primary and secondary outcome measures were analyzed using bivariable linear regression for the effect of treatment group on the score at 12-month visit, adjusting for baseline performance. Multivariable regression was performed by adjusting treatment effects for baseline performance, age and sex. We planned to determine effect modification of outcome parameters using interaction term of treatment and age and baseline, only if main effects were present. If interaction terms treatment x baseline score would have shown significant effect modification, subgroup analysis was planned for groups with -1SD lower scores at baseline. The analysis plan was determined before unmasking and

compiled according to the study protocol. All data were analyzed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. All authors had full access to all trial data and assume final responsibility for the decision to submit the manuscript for publication.

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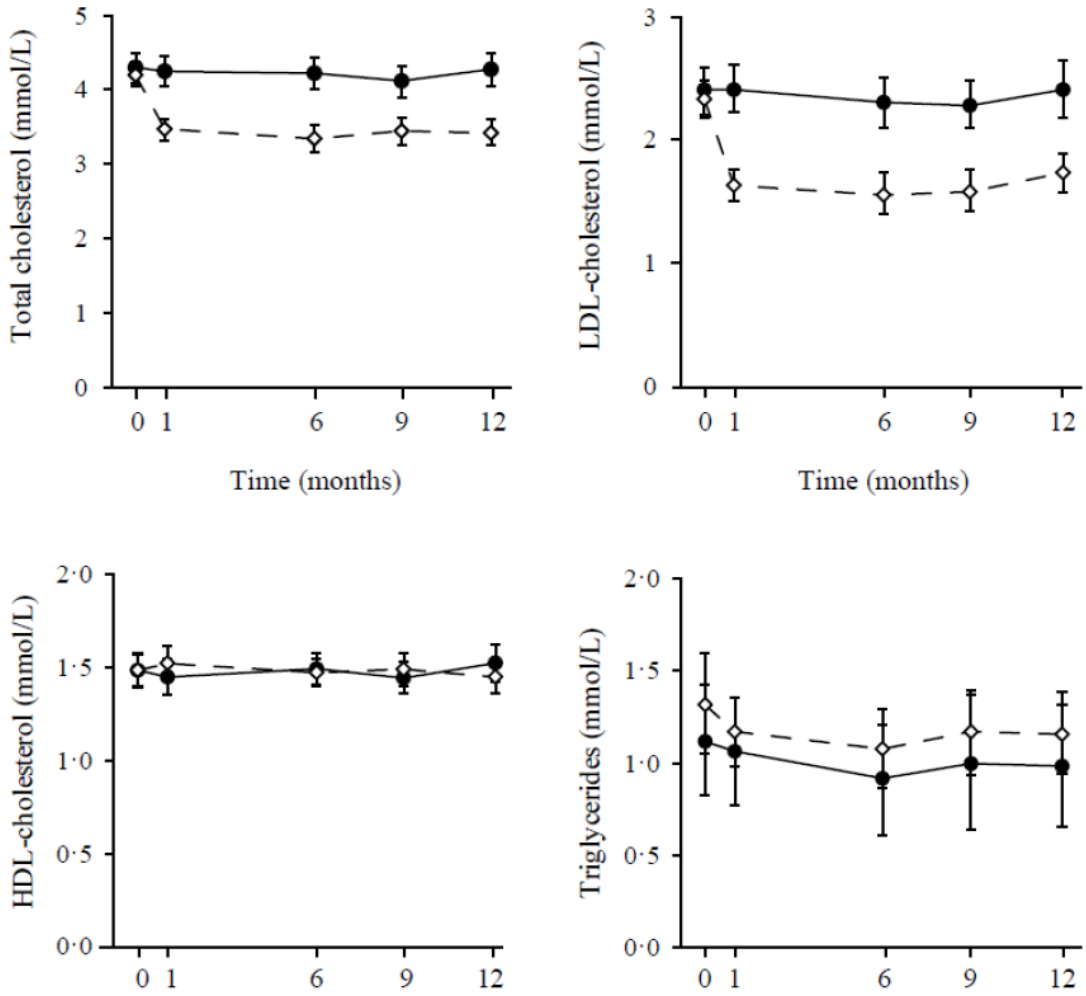

Online supplementary figure 1: Estimated means for total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. Black circles = placebo. White diamonds = Simvastatin. Error bars represent 95% confidence intervals.

Online supplementary table 1: Adverse reactions ordered to World Health Organisation – Adverse Events Terminology on the level of preferred terms[s2].

Contributors

TvdV contributed to study design, grant writing, study planning, data collection, data analysis, data interpretation, writing of the first draft of the report, and report revision. EP contributed to study planning, data collection, neuropsychological testing, data analysis, data interpretation, writing of the report, and report revision. ABR contributed to study design, data collection, neuropsychological testing, data analysis, data interpretation, writing of the report, and report revision. MR contributed to data collection and clinical follow-up. RO contributed to study design, grant writing, data collection, clinical follow-up, data interpretation, and report revision. AV contributed to data collection, clinical follow-up, data interpretation, and report revision. M-CYdW contributed to data collection, clinical follow-up, data interpretation, and report revision. M-JD contributed to data interpretation and report revision. YV contributed to data analysis, data interpretation, and report revision. CEC-B contributed to study design, grant writing, clinical follow-up, data interpretation, and report revision. EL is a coprincipal investigator and contributed to grant writing, study planning, data interpretation, and report revision. YE is a coprincipal investigator and contributed to conception of the study, study design, grant writing, study planning, data interpretation, and report revision. HAM is a coprincipal investigator and contributed to conception of the study, study design, grant writing, study planning, patient follow-up, data interpretation, and report revision.

Conflicts of interest

All authors declare that they have no conflicts of interest.

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References

1 Evans DG, Howard E, Giblin C, et al. Birth incidence and prevalence of tumor-prone syndromes: estimates from a UK family genetic register service. Am J Med Genet A 2010; 152A: 327–32.

2 Ferner RE, Huson SM, Thomas N, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. J Med Genet 2007; 44: 81–88.

3 Hyman SL, Shores A, North KN. The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. Neurology 2005; 65: 1037–44.

4 Krab LC, Aarsen FK, de Goede-Bolder A, et al. Impact of neurofibromatosis type 1 on school performance. J Child Neurol 2008; 23: 1002–10.

5 Payne JM, Hyman SL, Shores EA, North KN. Assessment of executive function and attention in children with neurofibromatosis type 1: relationships between cognitive measures and real-world behavior. Child Neuropsychol 2011; 17: 313–29.

6 Krab LC, de Goede-Bolder A, Aarsen FK, et al. Effect of simvastatin on cognitive functioning in children with neurofibromatosis type 1: a randomized controlled trial. JAMA 2008; 300: 287–94.

7 Garg S, Lehtonen A, Huson SM, et al. Autism and other psychiatric comorbidity in neurofibromatosis type 1: evidence from a population-based study. Dev Med Child Neurol 2013; 55: 139–45.

8 Hyman SL, Arthur Shores E, North KN. Learning disabilities in children with neurofibromatosis type 1: subtypes, cognitive profile, and attention-deficit-hyperactivity disorder. Dev Med Child Neurol 2006; 48: 973–77.

9 Krab LC, Oostenbrink R, de Goede-Bolder A, Aarsen FK, Elgersma Y, Moll HA. Health-related quality of life in children with neurofibromatosis type 1: contribution of demographic factors, disease-related factors, and behavior. J Pediatr 2009; 154: 420–25.

10 Descheemaeker MJ, Plasschaert E, Frijns JP, Legius E. Neuropsychological profile in adults with neurofibromatosis type 1 compared to a control group. J Intellect Disabil Res 2013; 57: 874–86.
11 Costa RM, Federov NB, Kogan JH, et al. Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. Nature 2002; 415: 526–30.
12 Cui Y, Costa RM, Murphy GG, et al. Neurofibromin regulation of ERK signaling modulates GABA release and learning. Cell 2008; 135: 549–60.
13 Shilyansky C, Karlsgodt KH, Cummings DM, et al. Neurofibromin regulates corticostriatal inhibitory networks during working memory performance. Proc Natl Acad Sci USA 2010; 107: 13141–46.
14 Li W, Cui Y, Kushner SA, et al. The HMG-CoA reductase inhibitor lovastatin reverses the learning and attention deficits in a mouse model of neurofibromatosis type 1. Curr Biol 2005; 15: 1961–67.
15 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 2011; 45: 241–45.
16 Chabernaud C, Mennes M, Kardel PG, et al. Lovastatin regulates brain spontaneous low-frequency brain activity in neurofibromatosis type 1. Neurosci Lett 2012; 515: 28–33.
17 Chong PH, Seeger JD, Franklin C. Clinically relevant differences between the statins: implications for therapeutic selection. Am J Med 2001; 111: 390–400.
18 Sierra S, Ramos MC, Molina P, Esteo C, Vazquez JA, Burgos JS. Statins as neuroprotectants: a comparative in vitro study of lipophilicity, blood-brain-barrier penetration, lowering of brain cholesterol, and decrease of neuron cell death. J Alzheimers Dis 2011; 23: 307–18.
19 de Jongh S, Ose L, Szamosi T, et al. Efficacy and safety of statin therapy in children with familial hypercholesterolemia: a randomized, double-blind, placebo-controlled trial with simvastatin. Circulation 2002; 106: 2231–37.
20 Brems H, Chmara M, Sahbatou M, et al. Germline loss-of-function mutations in SPRED1 cause a neurofibromatosis 1-like phenotype. Nat Genet 2007; 39: 1120–26.
21 Verhulst FC, Van der Ende J, Koot HM. Handleiding voor de CBCL/4–18. Rotterdam: Academic Medical Center Rotterdam/Erasmus University, Sophia Children's Hospital, Department of Child and Adolescent Psychiatry, 1996.

22 Mautner VF, Kluwe L, Thakker SD, Leark RA. Treatment of ADHD in neurofibromatosis type 1. Dev Med Child Neurol 2002; 44: 164–70.
23 Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) Collaborative Group. Intensive lowering of LDL cholesterol with 80 mg versus 20 mg simvastatin daily in 12 064 survivors of myocardial infarction: a double-blind randomised trial. Lancet 2010; 376: 1658–69.
24 Gillberg C, Melander H, von Knorring AL, et al. Long-term stimulant treatment of children with attention-deficit hyperactivity disorder symptoms. A randomized, double-blind, placebo-controlled trial. Arch Gen Psychiatry 1997; 54: 857–64.
25 Schellenberg EG. Music lessons enhance IQ. Psychol Sci 2004; 15: 511–14.
26 ClinicalTrials.gov. A randomized placebo-controlled study of lovastatin in children with neurofibromatosis type 1 (STARS). http://clinicaltrials.gov/show/NCT00853580 (accessed Aug 5, 2013).
27 van Engelen SJ, Krab LC, Moll HA, et al. Quantitative differentiation between healthy and disordered brain matter in patients with neurofibromatosis type I using diffusion tensor imaging. AJNR Am J Neuroradiol 2008; 29: 816–22.
28 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: 834–41.
29 Silva AJ, Frankland PW, Marowitz Z, et al. A mouse model for the learning and memory deficits associated with neurofibromatosis type I. Nat Genet 1997; 15: 281–84.
30 Ullrich NJ, Ayr L, Leaff er E, Irons MB, Rey-Casserly C. Pilot study of a novel computerized task to assess spatial learning in children and adolescents with neurofibromatosis type 1. J Child Neurol 2010; 25: 1195–202.
31 Violante IR, Ribeiro MJ, Edden RA, et al. GABA deficit in the visual cortex of patients with neurofibromatosis type 1: genotype-phenotype correlations and functional impact. Brain 2013; 136: 918–25.

Online supplementary references

s1. Kort W, Schittekatte M., Dekker, P.H., Verhaeghe, P., Compaan, E.L., Bosmans, M., Vermeir, G. WISC-III NL Wechsler Intelligence Scale for Children.

Derde Editie NL. Handleiding en Verantwoording. Amsterdam: Hartcourt Test Publishers/Nederlands Instituut voor Psychologen; 2005.

s2. Edwards IR, Aronson JK. Adverse drug reactions: definitions, diagnosis, and management. Lancet 2000; 356: 1255-9.