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FULL RESEARCH PAPER
Prevention in practice

Evolocumab in paediatric heterozygous familial hypercholesterolaemia: cognitive function during 80 weeks of open-label extension treatment

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Aims PCSK9 inhibition intensively lowers low density lipoprotein cholesterol and is well tolerated in adults and paediatric patients with familial hypercholesterolaemia (FH). HAUSER-RCT showed that 24 weeks of treatment with evolocumab in paediatric patients did not affect cognitive function. This study determined the effects of 80 additional weeks of evolocumab treatment on cognitive function in paediatric patients with heterozygous FH.

Methods and results HAUSER-OLE was an 80-week open-label extension of HAUSER-RCT, a randomized, double-blind, 24-week trial evaluating the efficacy and safety of evolocumab in paediatric patients (ages 10–17 years) with FH. During the OLE, all patients received monthly 420 mg subcutaneous evolocumab injections. Tests of psychomotor function, attention, visual learning, and executive function were administered at baseline and Weeks 24 and 80 of the OLE. Changes over time were analysed descriptively and using analysis of covariance. Cohen's *d* statistic was used to evaluate the magnitude of treatment effects. Analysis of covariance results indicated no decrease in performance across visits during 80 weeks of evolocumab treatment for Groton Maze Learning, One Card Learning accuracy, Identification speed, or Detection speed (all $P > 0.05$). Performance on all tasks was similar for those who received placebo or evolocumab in the RCT (all $P > 0.05$). For all tests, the least square mean differences between patients who received placebo vs. evolocumab in the parent study were trivial (all Cohen's *d* magnitude < 0.2).

Conclusion In paediatric patients with FH, 80 weeks of open-label evolocumab treatment had no negative impact on cognitive function.

Registration ClinicalTrials.gov identifier: NCT02624869

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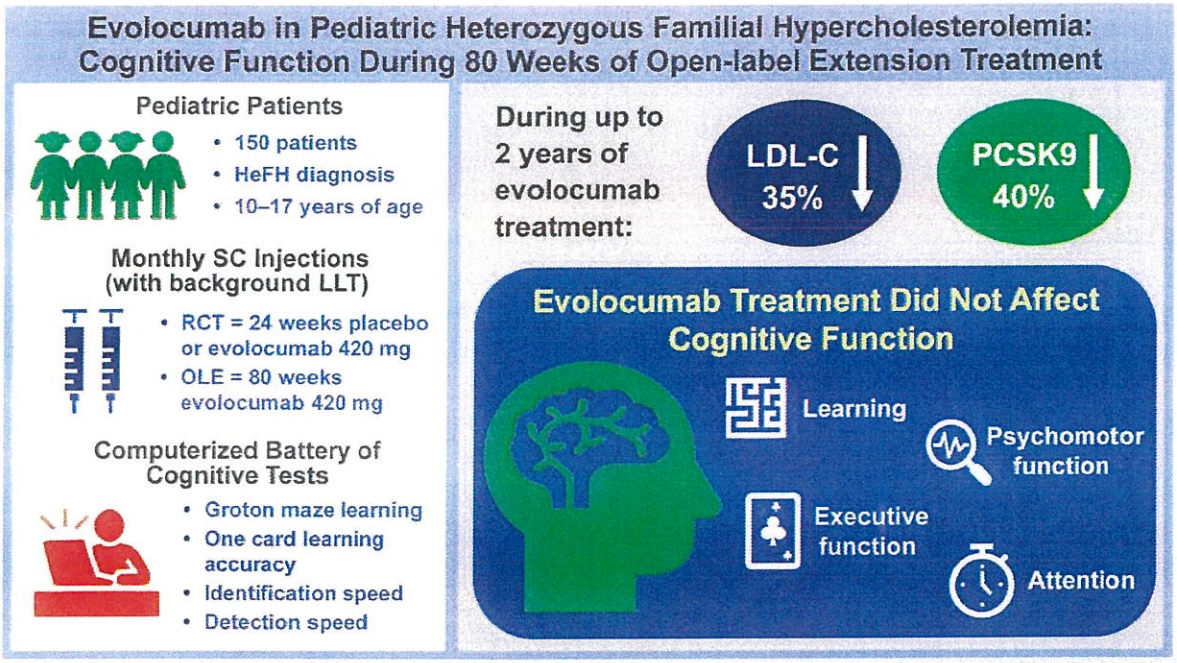
Lay summary

- Some children are born with a genetic disorder that causes high cholesterol, which leads to heart disease. Children with high cholesterol can be treated with evolocumab, a medication that lowers blood cholesterol. Because cholesterol is important for development and adequate function of the brain, there is a concern that lowering cholesterol in children may affect mental ability. In this study, we tested whether treating children with evolocumab for 80 weeks affected mental ability in performing several tasks. A battery of tests that measure executive function (Groton Maze Learning Test), visual learning (One Card Learning Test), visual attention (Identification Test), and psychomotor function (Detection Test) showed no decrease in performance across visits during 80 weeks of evolocumab treatment.
- Performance on all tasks was similar for the children who received placebo for the first 24 weeks then received evolocumab for an additional 80 weeks (placebo/evolocumab) and those who received evolocumab for 24 weeks then received evolocumab for an additional 80 weeks (evolocumab/evolocumab).

Graphical Abstract



HeFH, heterozygous familial hypercholesterolaemia; LDL-C, low density lipoprotein cholesterol; LLT, lipid-lowering therapy; OLE, open-label extension; PCSK9, proprotein convertase subtilisin/kinase 9; RCT, randomized controlled trial; SC, subcutaneous.

Keywords Atherosclerotic cardiovascular disease • Central nervous system • Children • Low density lipoprotein cholesterol • Proprotein convertase subtilisin kexin 9 (PCSK9) • Safety

Introduction

Familial hypercholesterolaemia (FH), if left untreated, is characterized by early onset of atherosclerotic cardiovascular disease (ASCVD).[1] Children and adolescents with heterozygous FH should receive early pharmacological lipid-lowering treatment to reduce the burden of exposure to high levels of low density lipoprotein cholesterol (LDL-C).[2,3] Long-term therapy with statins reduces progression of subclinical vascular disease in children and adolescents[4] and has been shown to reduce the long-term risk of ASCVD when treatment begins in adolescence.[5] However, many individuals with FH have elevated LDL-C despite treatment with statins and ezetimibe.[6] Evolocumab is a monoclonal antibody that inhibits proprotein convertase subtilisin kexin 9 (PCSK9) and is approved for use in children and adolescents (10 to 17 years of age) with FH for whom LDL-C levels remain high despite standard lipid-lowering therapy. Evolocumab provides robust additional LDL-C lowering and has been shown to be effective, safe, and well tolerated in both the randomized controlled trial (RCT) and open-label extension (OLE) phases of the HAUSER study.[7,8]

Cholesterol is important for development, maturation, and adequate function of the central nervous system (CNS). Consequently, there has been concern that cholesterol-lowering therapies could impair CNS function, especially during CNS maturation in children and adolescents.[9] Experimental evidence indicates that PCSK9 may influence CNS function by not only regulating blood cholesterol levels but also by acting on neuronal differentiation and apoptosis.[9] In 2014, the US Food and Drug Administration requested developers of PCSK9 inhibitors to evaluate possible neurocognitive effects in a subset of patients in

ongoing clinical trials.[10] Although a 2015 meta-analysis of smaller trials in adults suggested an association between PCSK9 inhibition treatment and cognitive adverse events,[11] several types of evidence have refuted this conclusion, including more robust, randomized trials with validated assessments of cognitive function,[12,13] trials that included patient self-reports of everyday cognition,[14] and analyses of neurocognitive adverse events in trials with larger patient populations, pooled trials, or meta-analyses.[12,15–17]

Although multiple studies now support the absence of any association between PCSK9 inhibition and impairment in cognitive function in adults, only one trial has investigated this issue in a paediatric population. The HAUSER-RCT study found no adverse impact of PCSK9 inhibition on cognitive function in children and adolescents with FH who were treated monthly with 420 mg evolocumab or placebo for up to 24 weeks.[10] However, longer-term data would help allay any concerns about treatment with PCSK9 inhibitors during CNS maturation. Here, we report data on cognitive function during the HAUSER-OLE study, in which children and adolescents received 80 additional weeks of treatment with evolocumab.

Methods

The study was performed in accordance with the principles of the Declaration of Helsinki, the Good Clinical Practice guidelines of the International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, and relevant regulatory requirements. Written informed consent was obtained from legal guardians, and patient assent was obtained as per local guidelines. The study protocol and informed consent forms were approved by the local institutional review boards at each site.[7]

Study design and patients

HAUSER-OLE (registered with ClinicalTrials.gov, NCT02624869) was an 80-week OLE that followed HAUSER-RCT (ClinicalTrials.gov, NCT02392559), a randomized, double-blind trial in which patients received monthly subcutaneous injections of 420 mg evolocumab or placebo for 24 weeks.[18] Patients were eligible to enter the OLE if they completed 24 weeks of evolocumab or placebo treatment in the RCT with no serious treatment-emergent adverse events. All patients received 420 mg evolocumab monthly in the OLE.

Inclusion and exclusion criteria for patients enrolled in the RCT have been described previously.[7,18] Briefly, patients were aged 10 to 17 years; had a diagnosis of heterozygous FH [per genetic testing or criteria outlined by the Simon Broome Register Group, the Dutch Lipid Clinic Network, or the Make Early Diagnosis To Prevent Early Death (MEDPED) programme]; were on stable, optimized lipid-lowering therapy for a minimum of 4 weeks before screening; were following a low-fat diet; had LDL-C levels of 130 mg/dL (3.4 mmol/L) or greater; and had triglyceride levels of 400 mg/dL (4.5 mmol/L) or less.

Study procedures and outcomes

Procedures for the OLE included measurement of lipid parameters and a number of safety assessments including adverse events, Tanner stages of physical and sexual development, hormone levels, biochemical assessments, and carotid intima media thickness. These results have been reported previously.[8]

The focus of the current study is to evaluate the effect of evolocumab on cognitive function, which was measured using a Cogstate computerized cognitive test battery, as previously described by Gaudet *et al.*[18] This battery of tests was selected because it provided assessments of psychomotor function, attention, learning, and executive function, which are areas of cognitive function that provide sensitive indices of normal neurodevelopment. The design, administration, and psychometric properties of the four Cogstate tests used in this study have been described in detail[19,20] and are briefly described here.

The Groton Maze Learning Test (GMLT) measures executive function. The GMLT requires participants to find and learn a 28-step pathway hidden in a 10 × 10 grid of boxes during five learning trials. On each trial, participants must begin in the top left-hand corner of the grid and then find the pathway one step at a time, by applying a set of rules trained before starting the test and by using feedback on errors made during the test. Once the pathway is found, it is hidden again, and participants must repeat their search. Performance is measured as the total number of errors made in finding the pathway over the five attempts.

The One Card Learning Test measures visual learning. This test is based on the pattern separation memory paradigm. Participants are presented with a stream of 48 visual stimuli (playing cards) that appear one at a time in the centre of the display. Upon the presentation of each visual stimulus, participants must decide, 'Yes' or 'No' as to whether they have seen that stimulus previously and give a manual response. The order of the visual stimuli is controlled so that a subset of four stimuli is presented repeatedly (six times each), while the remainder are seen only once. The computer software measures the speed and accuracy of each response and performance is defined as the proportion of correct responses made on 48 trials.

The Identification Test evaluates visual attention. The test is based on the choice reaction time test paradigm. Participants are presented with a stream of visual information (playing cards). Upon the presentation of each card, participants must decide, 'Yes' or 'No' as to whether the colour of the card is red and indicate this with a manual response. The computer software measures the speed and accuracy of each response, and performance is defined as the average speed of correct responses on 35 trials.

The Detection Test measures psychomotor function. The test is based on a simple reaction time test paradigm. Participants are presented with a stream of visual information (playing cards). With the presentation of each card, participants must indicate 'Yes' with a manual response as soon as the card is presented. The computer software measures the speed and accuracy of each response, and performance is defined as the average speed of correct responses on 35 trials.

In the parent study, the Cogstate battery was administered at screening, baseline, and at Week 24 (corresponding to Week 0 of the OLE). In the OLE, the battery was administered at Weeks 24 and 80 (i.e. Weeks 48 and 104, respectively, from baseline of the parent study).

LDL-C and free PCSK9 were measured at multiple points during the study and are reported as change from baseline to Week 80 of OLE.

Statistical analysis

The sample size for HAUSER-OLE was determined by the number of patients who continued to the OLE from the RCT. All patients who were enrolled in the OLE and received at least one dose of study treatment were included in the full analysis set, which was used for all analyses presented here. Patients who received placebo in HAUSER-RCT are referred to as the placebo/evolocumab group, and patients who received evolocumab in the RCT are referred to as the evolocumab/evolocumab group. Baseline refers to the baseline of the RCT.

Performance on each of the cognitive tasks was analysed at each visit. Because the distribution of reaction times for individuals is typically skewed positively (i.e. by rare, but high, values), distributions of reaction times for the Identification Test and the Detection Test were normalized using a logarithmic base 10 transformation. Similarly, because distributions of accuracy scores on the One Card Learning tests can be skewed negatively (i.e. by rare, but low, values), they were normalized through an arcsine transformation. Missing data were not imputed.

The key outcomes for each treatment group on each of the cognitive tasks were summarized descriptively at each visit and for the change from baseline to Week 24 and Week 80 of the OLE.

To evaluate changes over time and the effects of treatment group, scores from each test were analysed using an analysis of covariance (ANCOVA) model with factors of treatment group (placebo/evolocumab, evolocumab/evolocumab), visit (OLE Week 24, Week 80), and interaction of treatment group and visit, and with baseline score as a covariate. Alpha level was set at $P = 0.05$. No correction was done for multiplicity.

Using the modelled scores, differences between the treatment groups on each of the cognitive tests were evaluated at Week 24 and Week 80 of the OLE. Cohen's *d* statistic was used to evaluate the magnitude and direction of treatment effects at each assessment point. The difference between treatment groups was considered trivial if the absolute value of Cohen's *d* was <0.2, small if it was between 0.2 and 0.5, moderate if it was between 0.5 and 0.8, and large if it was >0.8.[21]

Several sensitivity analyses were conducted to determine whether any moderating effects occurred when ANCOVA models included age, sex, baseline LDL-C, and achieved LDL-C. For the sensitivity analysis, alpha level was also set at $P = 0.05$, and no correction was done for multiplicity.

To contextualize the results for the OLE study in terms of complete period of treatment with evolocumab, group data from the OLE and the parent RCT were combined for each of the four cognitive tests and group performance presented across the four assessment points during the entire 104 weeks (24 weeks during the RCT and 80 weeks during the OLE) of treatment. An additional ANCOVA model was performed, which was identical to the base model except that it included performance at all four visits.

To examine the relationship between LDL-C reduction and cognitive test scores, Pearson correlation coefficients (r) were calculated for the relationship between change from baseline to Week 80 in each cognitive test score and change from baseline to Week 80 in LDL-C. Pearson correlation coefficients were also calculated for the relationship between change in each cognitive test score and change in free PCSK9.

All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results

Disposition and exposure

Of the 157 patients in the RCT who were eligible for the OLE, 150 enrolled. During the RCT, 101 of these patients received evolocumab and 49 received placebo, as reported previously.[7] During the OLE, all 150 patients received at least one dose of evolocumab and were included in the full analysis set, as reported previously.[8] Four patients discontinued treatment due to withdrawal of consent during the OLE (one in the placebo/evolocumab group and three in the evolocumab/evolocumab group); no patients withdrew due to adverse events. Mean exposure to evolocumab during the OLE was 78.1 weeks (SD = 10.1). Overall exposure to evolocumab across the RCT and OLE was 77.6 (SD = 11.2) weeks for the placebo/evolocumab group and 102.4 (SD = 9.6) weeks for the evolocumab/evolocumab group.

Baseline characteristics

Baseline characteristics of the patients in the OLE ($n = 150$) were similar across assigned treatment groups from the RCT as reported previously.[8] The median [quartile 1, quartile 3 (Q1, Q3)] age of patients was 14.0 (12, 16) years, and 55% were female. Detailed information on the diagnosis of heterozygous FH has been reported previously.[8] All 150 patients had a diagnosis of heterozygous FH at enrolment, with 99 (66%) having a molecular diagnosis. LDL receptor mutation was detected in 96 (64%) patients [placebo/evolocumab, 29 (59%); evolocumab/evolocumab, 67 (66%)]. Diagnosis of heterozygous FH for the remaining 51 (34%) patients was based on clinical criteria: the Simon Broome Register Group criteria in 16 (11%) patients [placebo/evolocumab, 7 (14%); evolocumab/evolocumab, 9 (9%)]; the Dutch Lipid Clinic criteria in 32 (21%) patients [placebo/evolocumab, 10 (20%); evolocumab/evolocumab, 22 (22%)]; and the MEDPED criteria in 3 (2%) patients [placebo/evolocumab, 2 (4%); evolocumab/evolocumab, 1 (1%)]. Statin therapy was received by 149/150 patients (99%), with 78% (117/150) receiving moderate- or high-intensity statins according to the 2018 American College of Cardiology/American Heart Association Multi-society Blood Cholesterol Guidelines[22] definitions.

LDL-C and free PCSK9 levels

Median (Q1, Q3) LDL-C at baseline was 173 mg/dL (154.0, 207.5) (see Supplementary material online, *Table S1*). At the end of the 80-week OLE, median (Q1, Q3) LDL-C was 101.5 mg/dL (77.0, 149.0), reflecting a mean percentage change from baseline of −35.3% (SD = 28.0). Free PCSK9 concentrations were reduced by a mean of −40.0% (SD = 46.7), from a median (Q1, Q3) of 269.0 (214.0, 339.0) ng/mL at baseline to 134.0 (58.4, 238.0) ng/mL at Week 80.

Cognitive test performance

Medians and interquartile ranges for each cognitive test score at baseline and Weeks 24 and 80 of the OLE by treatment group are shown in *Table 1*. The two treatment groups (placebo/evolocumab vs. evolocumab/evolocumab) had similar performance during the OLE on all tasks and showed no decrease in performance from baseline to Week 80. Changes from baseline to Week 24 and Week 80 (*Figure 1*) showed numerically similar levels of improvement across visits for the two treatment groups, consistent with the expected effects of maturation in the study sample.

The base model ANCOVA results confirmed no decrease in performance across visits on any of the cognitive tests, with no statistically significant effect of visit, treatment group, or interaction between visit and treatment group observed for Groton Maze Learning (all $P > 0.05$), One Card Learning accuracy (all $P > 0.05$), Identification speed (all $P > 0.05$), and Detection speed (all $P > 0.05$) (*Table 2*, Supplementary material online, *Table S2*).

At both Weeks 24 and 80, performance differences between the treatment groups were trivial according to predefined criteria for Cohen's d for effect sizes (absolute value of $d < 0.2$) for performance on Groton Maze Learning ($d = 0.02$ and -0.17 at Weeks 24 and 80, respectively), One Card Learning accuracy ($d = 0.06$ and 0.14), Identification speed ($d = 0.05$ and -0.05), and Detection speed ($d = -0.13$ and -0.04) (*Figure 1*).

Sensitivity analyses explored whether any treatment effects emerged when age, sex, baseline LDL-C, and achieved LDL-C were added to the ANCOVA models. These sensitivity analyses for each cognitive test yielded no statistically significant effects for treatment group or any interactions with treatment group (see Supplementary material online, *Table S3*). The main effect of visit, indicating improvement over time, was significant at $P < 0.05$ for the Identification and Detection tests in the models that added baseline LDL-C ($P = 0.0129$ for the Identification Test; $P = 0.0119$ for the Detection Test) and achieved LDL-C as factors ($P = 0.0105$ for the Identification Test; $P = 0.0096$ for the Detection Test). Baseline LDL-C had a significant main effect at $P < 0.05$ for the Identification and Detection tests, such that performance was better with lower LDL-C. No other main effects or interactions occurred.

When results from the RCT and the OLE were combined to show performance at each visit at which the Cogstate battery was administered across the entire study, no decrease in cognitive performance was evident in either the randomized or open-label portion of the study (*Figure 2*). Additional sensitivity analyses that repeated the base model ANCOVA for each cognitive test and included all four assessment points also found no statistically significant effects for visit, treatment group, or their interaction (see Supplementary material online, *Table S4*).

No correlation was observed for change from baseline to Week 80 in each of the cognitive test scores with change from baseline to Week 80 in either LDL-C or free PCSK9 (see Supplementary material online, *Figures S1 and S2*). These results show no association between reduction in LDL-C or PCSK9 levels and cognitive function.

Discussion

HAUSER-RCT and HAUSER-OLE demonstrated that 2 years of treatment with evolocumab was associated with clinically important and sustained reduction in pro-atherogenic lipids and lipoproteins with no negative effects on physical or sexual development, hormone levels, or blood biochemistry.[7,8] The current report increases our understanding of longer-term treatment with evolocumab in children and

Table 1 Median scores on each cognitive test by treatment group

Cognitive Domain Test—outcome measure		Placebo/evolocumab ($n = 49$)					Evolocumab/evolocumab ($n = 101$)				
		Baseline	OLE Wk 24	Change BL to Wk 24	OLE Wk 80	Change BL to Wk 80	Baseline	OLE Wk 24	Change BL to Wk 24	OLE Wk 80	Change BL to Wk 80
Executive function	*n*	49	43	43	42	42	100	95	95	86	86
Groton Maze Learning	Median	49.00	42.00	−6.00	38.00	−8.50	51.00	44.00	−8.00	42.50	−9.00
Test— total errors	Q1, Q3	42.00, 61.00	34.00, 51.00	−15.00, 4.00	30.00, 49.00	−19.00, −2.00	42.00, 58.50	34.00, 54.00	−19.00, 1.00	37.00, 51.00	−16.00, 1.00
Visual learning	*n*	49	43	43	44	44	101	95	95	87	87
One Card Learning Test—Accuracy	Median	1.01	1.04	0.02	1.08	0.01	1.02	1.05	0.02	1.08	0.07
of Performance (arcsine square root proportion correct)	Q1, Q3	0.92, 1.11	0.91, 1.19	−0.07, 0.10	0.89, 1.16	−0.06, 0.10	0.90, 1.12	0.93, 1.17	−0.02, 0.11	0.91, 1.17	−0.06, 0.12
Attention	*n*	49	43	43	44	44	101	95	95	86	86
Identification Test—Speed of	Median	2.72	2.74	0.01	2.69	−0.03	2.74	2.72	−0.02	2.71	−0.03
Performance, $\log_{10}$ ms	Q1, Q3	2.63, 2.80	2.63, 2.80	−0.07, 0.05	2.59, 2.76	−0.07, 0.04	2.67, 2.82	2.65, 2.80	−0.07, 0.03	2.64, 2.77	−0.09, 0.03
Psychomotor function	*n*	49	43	43	44	44	101	95	95	86	86
Detection Test—Speed	Median	2.50	2.50	0.00	2.46	−0.04	2.53	2.53	0.00	2.50	−0.01
of Performance, $\log_{10}$ ms	Q1, Q3	2.43, 2.61	2.42, 2.64	−0.04, 0.03	2.41, 2.61	−0.07, 0.04	2.47, 2.64	2.45, 2.67	−0.06, 0.05	2.44, 2.60	−0.08, 0.05

BL, baseline; OLE, open-label extension; Q1, quartile 1; Q3, quartile 3; Wk, week.

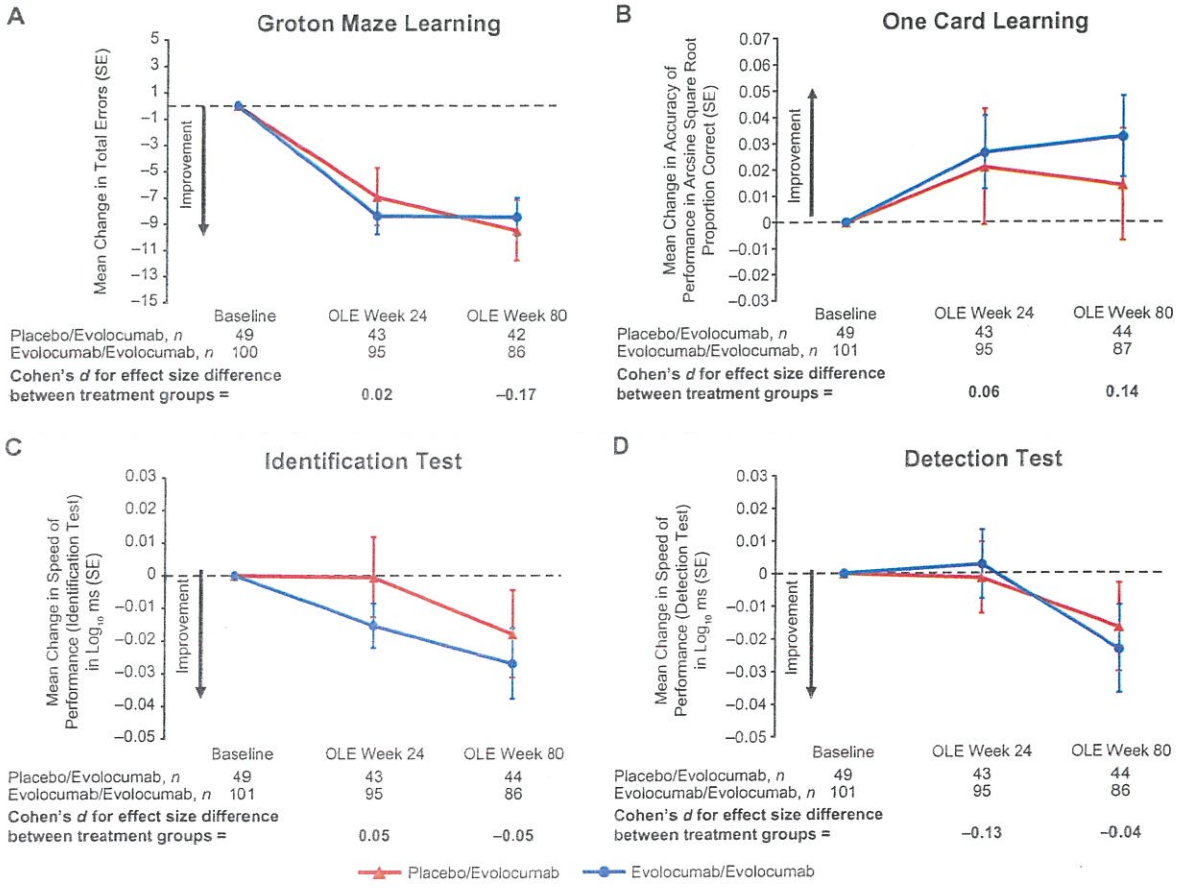


Figure 1 Change from baseline performance during HAUSER-OLE for each cognitive test. (*A*) Groton Maze Learning, (*B*) One Card Learning, (*C*) Identification Test, (*D*) Detection Test. The figure shows the mean change from baseline performance for each of the cognitive tests during the OLE Weeks 24 and 80. The arrow in each figure indicates the direction of change expected for improvement in performance over time. OLE, open-label extension; SE, standard error.

Table 2 Analysis of covariance results for base model for each cognitive test

Cognitive Domain Test—outcome measure	Effect	*F* value	*P* value
Executive function	Visit	1.13	0.2879
Groton Maze Learning Test—total errors	Treatment group	0.34	0.5576
	Visit × treatment group	0.51	0.4747
Visual learning	Visit	0.02	0.8838
One Card Learning Test—Accuracy of Performance	Treatment group	0.59	0.4437
(arcsine square root proportion correct)	Visit × treatment group	0.09	0.7672
Attention	Visit	2.8	0.0952
Identification Test—Speed of Performance, log_{10} ms	Treatment group	0	0.9983
	Visit × treatment group	0.13	0.7188
Psychomotor function	Visit	3.61	0.0587
Detection Test—Speed of Performance, log_{10} ms	Treatment group	0.43	0.5103
	Visit × treatment group	0.12	0.7284

The base model has factors of visit (OLE Week 24 and Week 80) and treatment group (placebo/evolocumab and evolocumab/evolocumab), and baseline score is the covariate.
OLE, open-label extension.

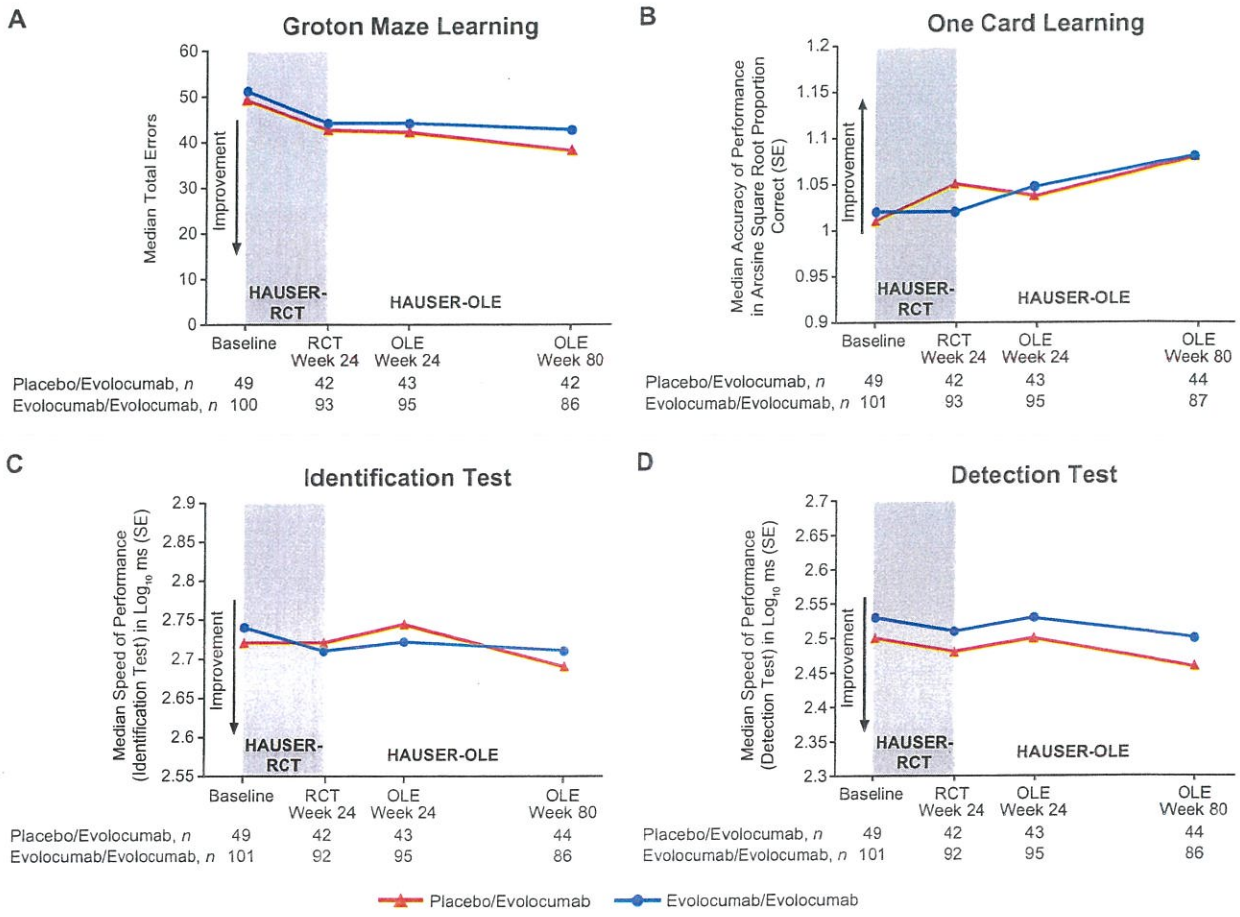


Figure 2 Performance on each cognitive test during HAUSER-RCT and HAUSER-OLE. (*A*) Groton Maze Learning, (*B*) One Card Learning, (*C*) Identification Test, (*D*) Detection Test. The figure shows the median performance on each cognitive test for all time points at which the cognitive battery was performed during the RCT and OLE. The arrow in each figure indicates the direction of change expected for improvement in performance over time. RCT Week 24 was the end of the randomly assigned treatment period and marked the start of the OLE, during which all patients received evolocumab. OLE, open-label extension; RCT, randomized controlled trial; SE, standard error.

adolescents by showing that treatment was not associated with any negative effects on psychomotor function, attention, learning, or executive function during up to 2 years of treatment. Because CNS maturation occurs over a period of years in children and adolescents, it was important to evaluate the effects of longer-term treatment with evolocumab using rigorous assessments of cognitive function. The HAUSER studies were designed to include a battery of tests that measure cognitive functions that are known to improve with normal CNS maturation[20] and are sensitive to pharmacological or physiological disruption to the CNS.[19,23–27] HAUSER-RCT showed that 24 weeks of treatment with evolocumab achieved desired reduction in cholesterol levels without negative effects on these four rigorous tests of cognitive function.[10] HAUSER-OLE extends these findings to 2 years of evolocumab treatment. Given the importance of cholesterol and PCSK9 for optimal CNS function and development,[9] the data reported here are crucial to understanding that the substantial effects of evolocumab in reducing blood cholesterol and circulating PCSK9 levels do not impair cognitive function in the developing brain.

The HAUSER study results are consistent with those of large, randomized studies of adults who received PCSK9 inhibitors for both short (months) and long (years) periods of time with no demonstrable adverse CNS effects.[12–14,28] Similarly, Mendelian randomization studies have tested genetic variants as proxies of pharmacological intervention and found that PCSK9 inhibition was not related to adverse cognitive outcomes.[29,30] Although a recent genetic analysis of the UK Biobank suggests the association of lipid-lowering PCSK9 allele variants with mood instability and neuroticism,[31] a recent Mendelian randomization study showed no relationship between PCSK9 inhibition and mood,[30] and there is no current evidence that pharmacological PCSK9 inhibition influences mood or is associated with psychiatric disorders. Randomized clinical trials would be needed to appropriately evaluate this issue.

Across the four tests of cognitive function in HAUSER-OLE, the pattern of performance over 80 weeks was similar, with all aspects of cognitive function improving numerically over time. This improvement occurred in patients treated with evolocumab from the start of the RCT and in those who were randomized to placebo in the RCT and then switched to evolocumab after 24 weeks. Although both the raw scores (*Table 1*) and modelled scores (*Figure 2*) indicate improvement on each cognitive test over time, the effect of visit was not sufficient

to reach statistical significance in ANCOVA analyses for any of the tests (*Table 2*). The lack of statistical significance was likely because the statistical model was designed to test the effect of treatment with evolocumab over time and not to characterize the effect of maturation on the different cognitive tests. Given the absence of differences in cognition between the evolocumab and placebo groups and the presence of numeric improvement in performance over the 80-week study period, it is parsimonious to attribute these improvements to normal maturation. In addition, the general improvement in scores across visits is consistent with that observed with increasing chronological age in healthy children performing the cognitive tests used in this study.[20,32–34] The mean age of the HAUSER participants was ~14 years, and the general improvement on each of the cognitive tests over the 18 months of the OLE is consistent with typical cognitive maturation, suggesting that maturation of those CNS areas necessary for attention, memory, and executive function occurred normally.

Within the context of this general cognitive maturation, the timing of evolocumab treatment did not influence cognitive function. This was evident in the similarity of the group performance at each time point (*Figure 2*, *Table 1*), the consistency and trivial magnitude of differences in mean performance between the treatment groups across the study time points (*Figure 1*), and the absence of any statistically significant treatment effect or interaction involving treatment in the ANCOVA models (*Table 2*).

To ensure that the absence of cognitive differences between treatment groups was not a consequence of uncontrolled factors decreasing the statistical power of analyses, a series of sensitivity analyses was conducted using age, sex, baseline LDL-C, and achieved LDL-C as added factors in the models. Reassuringly, the results of these sensitivity analyses did not change the conclusion from the main statistical analyses that longer-term treatment with evolocumab did not disrupt CNS function or development. Additionally, determination of Pearson correlation coefficients showed no statistical association between reductions in LDL-C or PCSK9 levels and cognitive function (see Supplementary material online, *Figures S1 and S2*). This further reinforces that there are no negative effects on cognition from reductions in LDL-C or free PCSK9 secondary to long-term treatment with evolocumab.

A strength of the current study is that it includes a relatively large sample of individuals with paediatric heterozygous FH who were studied over 2 years with minimal attrition in the sample size. The design of HAUSER included validated tests of several different aspects of cognitive function that were appropriate for the paediatric population.

The study is limited in that the detailed inferential statistical analyses conducted here were exploratory and should be confirmed in additional long-term studies. It is notable that the mean percentage reduction in LDL-C with PCSK9 inhibition was observed to be less pronounced in paediatric patients (~35%) from that typically reported in many studies in adult populations (~60%[11,17]). This observed difference may reflect the variability in this relatively small paediatric sample, high LDL-C levels at baseline in our study population (contributing to a higher absolute mean LDL-C reduction than in some studies of adult patients), variations in adherence to background LLT regimens, or potentially due to a less developed LDL removal system in paediatric patients. Additional studies are warranted to address this issue. In this study, the conclusion that evolocumab had no negative effect on neurodevelopment was based on the neuropsychological assessment of cognition. Future work using structural and functional neuroimaging technologies may provide further information on the role of cholesterol metabolism in CNS development and function.

In conclusion, treatment with evolocumab for up to 2 years in 150 patients with heterozygous FH aged 11–17 years had no negative effects on tests of executive function, attention, visual learning, or psychomotor function.

Authors' contributions

R.D.S., A.R., P.M., A.K.B., D.G., and F.J.R. contributed to the conception or design of the work. R.D.S., A.R., B.W., P.M., A.S., F.M., J.B., I.G., J.S.P., J.J.P.K., G.K.H., A.W., D.G., and F.J.R. contributed to the acquisition, analysis, or interpretation of data for the work. R.D.S., A.R., B.W., P.M., A.K.B., D.G., and F.J.R. drafted the manuscript. R.D.S., A.R., B.W., P.M., A.S., A.K.B., F.M., J.B., I.G., J.S.P., J.J.P.K., G.K.H., A.W., D.G., and F.J.R. critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy of the manuscript content.

Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology*.

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Data availability

Qualified researchers may request data from Amgen clinical studies. For details, visit http://www.amgen.com/datasharing.

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