RANDOMISED CONTROLLED TRIAL OF SIMVASTATIN TREATMENT FOR AUTISM IN YOUNG CHILDREN WITH NEUROFIBROMATOSIS TYPE 1 (SANTA). Stivaros et al.

Additional file 1

Details of analytic techniques

MAPK Assay Peripheral Blood Mononuclear Cells (PBMCs) were isolated from 10ml venous blood samples and spun into freeze-dried cell pellets (Manchester Biobank). Dried cell pellets were shipped with dry ice to UCLA for analysis using western blot electrophoresis. Protein concentration was determined using a BCA assay kit and equal amounts of proteins (10 ug) were separated by electrophoresis on 4-12% SDS-PAGE gels. After transferring to nitrocellulose membrane, membranes were blocked with 5% BSA in TBS-T for 1 hour then hybridized with a primary antibody (p-MAPK) overnight at 4^0C. After washing with TBS-T, the membrane was incubated with a secondary antibody in 5% nonfat milk and TBS-T for 1 hour. Signals were visualized by ECL. After detecting p-MAPK, the membranes were striped and re-probed with a total MAPK antibody. The total MAPK were used to normalize with each sample. The following primary antibodies were used: p-MAPK (#9101S, Cell Signaling, 1:1,000), anti-total MAPK (#9102S, Cell Signaling, 1:5,000)

Imaging preparation Participating families were provided with a two-week imaging habituation protocol and an audio CD of study-specific scanner noise. The patient cohort was acclimatised to awake MRI scanning using a social story preparation and two weeks of graded exposure to mp3 recordings of the sounds made by the MRI

scanner. This was initially at any time of the day, then specifically at bedtime. These recordings were prioritised in the same order as each specific scan sequence in the imaging protocol. Children and families were assisted through the scanning procedure with a clinical protocol led by a play specialist, clinical nurses and psychiatrists.

Details of imaging protocol

Table S1: *MRI sequence parameters and scan time duration for a complete imaging acquisition lasting approximately 45 minutes**

Sequence Order	Sequence Name	Duration (min)	Parameters
1	Resting State fMRI with Echo planar imaging (EPI) readout	8.25	FOV 224mm, slice thickness 3mm, Slices 42, TE 35ms, TR 3000ms, Flip angle 90^{o} degrees, number of dynamic scans 160, acq matrix 80×76, recon matrix 80.
2	T1 Inversion-recovery fast gradient echo 3-D volume	5.75	FOV 240mm, Voxel size 1x1x1mm^{3}, TR 6.9ms, TE 3.2ms, flip angle 9^{o}, acq matrix 240×188, recon matrix 240.
3	T2 Axial Turbo Spin Echo	1.77	FOV 230mm, Slice thickness 4mm, slices 28, slice gap 1mm, TE 80ms, TR 3000ms, acq matrix 400×255, recon matrix 512.
4	Diffusion Tensor Imaging with EPI readout	1.87	FOV 230mm, Slice thickness 2.5mm, slices 55, b value 0 and 1000 smm^{-2}, Directions 6, TR 5353ms, TE 73ms, slice gap 0, acq matrix 112×86, recon matrix 128.
5	Arterial Spin Labelling with EPI readout	5.5	Labelling Slab 150mm, with a gap of 15mm and increasing delay times from 600 ms to 3050 ms - constant interval of 350 ms. Flip angle 40°; 3.5 × 3.5 × 6 mm3 voxels with a 1 mm gap between slices; 15 slices covering the cerebrum, TR 3500ms, TE 11ms, acq matrix 64×61, recon matrix 64.
6	GABA spectroscopy Frontal White Matter	8.87	Single Voxel MEGA-PRESS, voxel size 3x3x3cm3, TR 2000ms, TE 68ms, 44 blocks of 4 averages,

			interleaving decoupling and control MEGA pulses.
7	GABA spectroscopy Deep Grey Nuclei	8.87	As per 6

* includes scout sequences, planning and time for shim. GABA spectroscopy for sequence 6 and 7 related to differing voxel positioning.

GABA Spectroscopy GABA was measured in two voxels of the brain; i) frontal white matter (FWM) and ii) deep grey nuclei (DGN) using the localized spectroscopy sequence MEGA-PRESS,[1] in which the dominant signal from creatine + phosphocreatine at 3.03 ppm, which overlaps the GABA C4 signal at 3.02 ppm, is suppressed by spectroscopic editing (Fig 1). The resultant edited spectrum consists of signals from glutamate and glutamine centred at 3.78 ppm (Glx), GABA at 3.02 ppm and an inverted signal from N-acetyl aspartate (NAA) at 2.02 ppm. For signal quantification, the AMARES algorithm implemented in jMRUI software[2] was utilized to calculate the concentrations of GABA+ (consisting mainly of GABA but with contributions from co-edited macro-molecule signals), the sum of glutamate plus glutamine (Glx) and NAA. The AMARES approach is a prior-knowledge based quantification method in the time domain, applied via nonlinear least square fitting. Regarding voxel localization, in order to maximize the signal to noise ratio in these studies we faced a necessary trade-off between the size of the voxel that could be interrogated against the time taken in the scanner to acquire the spectroscopic data. Our preliminary work when designing the study indicated that the best trade off was a 3cm x 3cm x 3cm voxel of interest which returned usable signal after an 8 minute acquisition. . Since we would not have been able to specifically acquire GABA data solely from the cortex given the lack of signal, we enlarged the interrogation voxel to that described above, and by necessity this would concentrate signal returen

fromconcentrate the frontal white matter. Given previous studies have used similar voxel sizes [3, 4] and also concerntrated on the left frontal lobe in a similar region within the ASD population [3, 5, 6] we felt this voxel placement and size was appropriate[3, 4, 6].

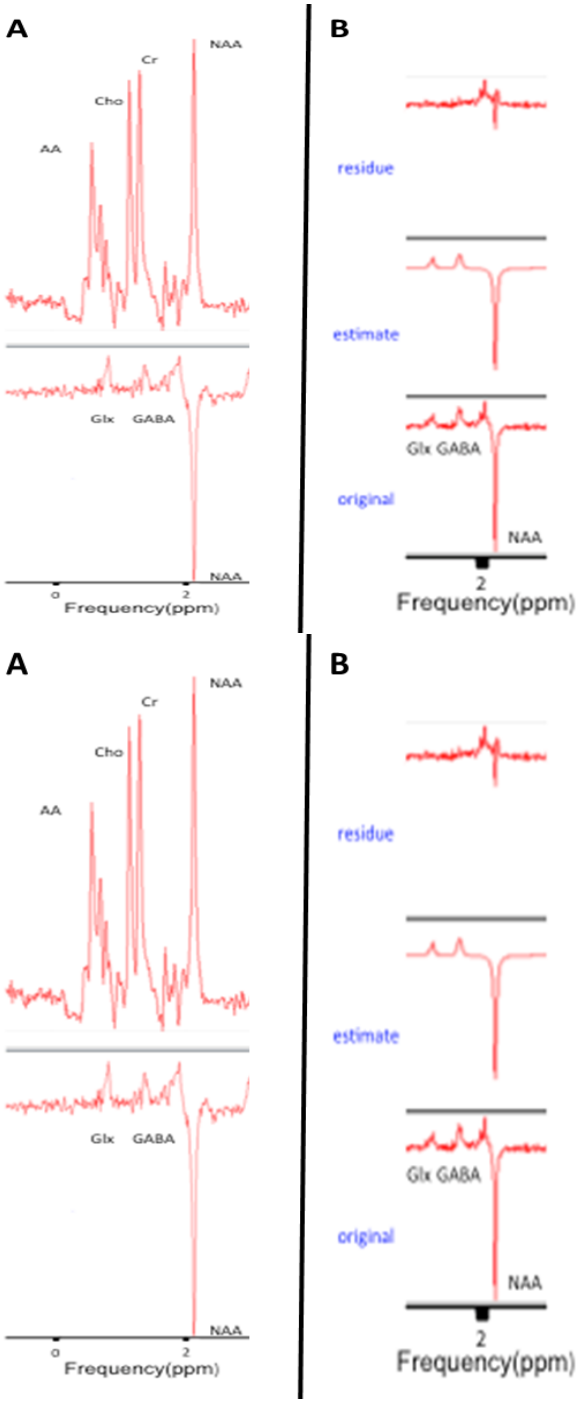


Figure S1 a) ***S****pectrum obtained from 3x3x3 voxel placed in deep gray matter of a 5 year old child using MEGA-PRESS suppression scheme at 3 T (top, non-edited sub-spectrum; bottom, GABA-edited spectrum) showing signals from amino-acid protons (AA), choline-containing compounds (cho), creatine + phosphocreatine (cr), N-acetylaspartate (NAA), GABA and glutamate + glutamine (glx).* **b)** *Figure depicting example output of AMARES Model fitting in jMRUI*

The semi-automatic post-processing steps for metabolite quantification within each VOI (Fig 1) included manual averaging of spectra, removal of water peak using the Hankel-Lanczos Singular Value Decomposition filter,[7] reference the NAA peak to 2.02 ppm, use of approximately 5 Hz apodization, and application of phase correction prior to jMRUI fitting. Prior knowledge on spectral parameters incorporated GABA+, which was modeled as a singlet with gaussian lineshape at 2.99 ppm, and glutamate plus glutamine (Glx), modeled as a doublet, with peaks at 3.71 and 3.78 ppm, and NAA set at 2 ppm as a single inverted Lorentzian peak. As contributions of macromolecules are expected when using MEGA-PRESS, the final quantification can only be described as GABA+ rather than GABA. A similar procedure to the aforementioned was undertaken to quantify NAA and other metabolite concentrations. The reference spectrum, used to convert from model-based to absolute concentration, was the signal from the non-suppressed water voxel. Participant datasets were excluded from analysis if there was severe extracranial lipid contamination, motion-related artefacts or if the post-fitting assessment indicated that standard deviation of the amplitude was greater than a threshold of 30%. Exclusion was also made if there was significant NF1 related T2 signal hyperintensity in the proposed region of interest. Finally, a procedure was followed in order to overlay each acquired MRS voxel onto the corresponding T1 weighted image so as to correct for cerebrospinal fluid (CSF) contamination. The partial volume segmentation software that was used was created by Dr. Nia Goulden and Dr. Paul Mullins of Bangor University (https://www.bangor.ac.uk/psychology/biu/Wiki.php.en). The software generates a GM, WM and CSF image and calculates the percentages of the aforementioned tissue types within each MRS voxel. Statistical analyses were performed with SPSS version 22.0 (SPSS Inc., Chicago, IL).

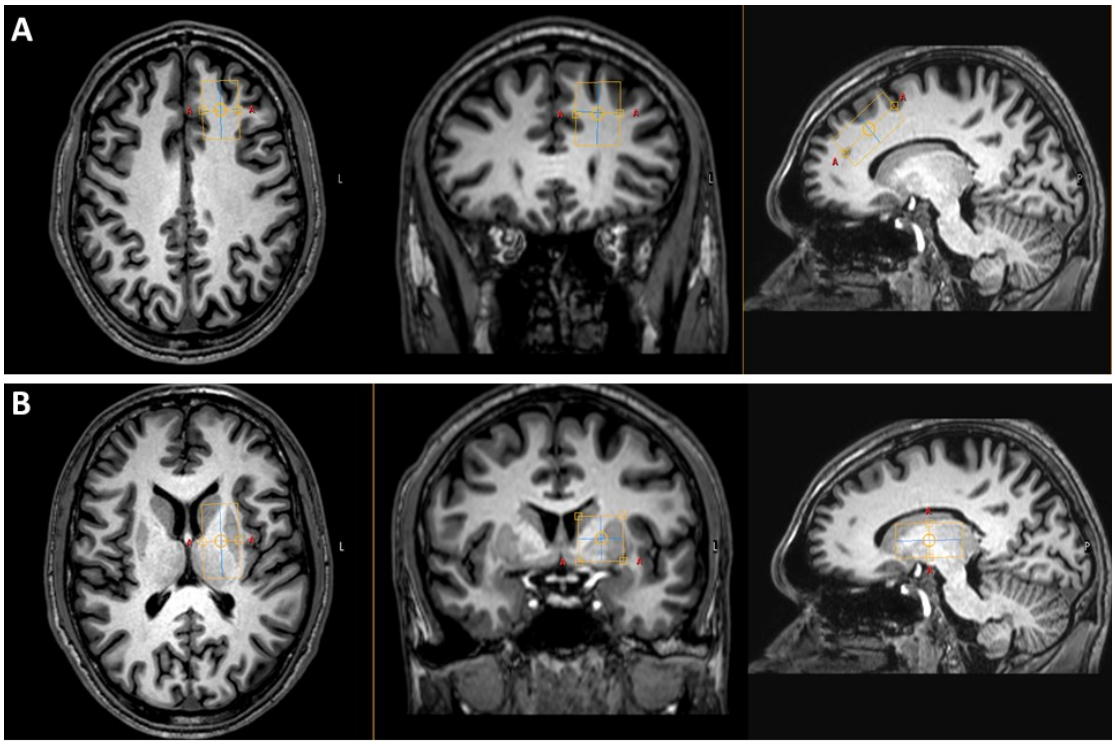


Figure S2 *Example locations of VOI (3x3x3 cm^3) acquired from a) left fontal white matter and b) deep grey matter (including caudate, lentiform nucleus, thalamus and putamen)*

We considered both the absolute change in values of the various metrics measured in Table 1 from week zero to week twelve as well as the change in value of these features between the two time points, the null hypothesis being that if there was no effect of simvastatin on the brain then the distribution of changes should be the same between the two groups. This also allowed for the control of the wide variation in baseline measures. A student T-test was used for the absolute assessment of GABA values. With regards to the distribution of change two types of test were run. Firstly we ran an ANCOVA-type test, where an ordinary least-squares model of the form y = ax + bc was fit. Here x refers to the covariate of the initial value at week 0 and c refers to the group. Such a model makes normality and equal variance assumptions, therefore, we also applied the tests proposed by Vermeulen [8](a Mann-Whitney test with covariate adjustment) where the normality assumption is null.

Resting state fMRI Table S1 shows the parameters of the multi-slice whole brain acquisition of these data. Spatial networks demonstrating strong temporal coactivation in the resting BOLD fMRI responses were defined using probabilistic independent component analysis (ICA). Data analysis was performed using the FSL software library[9] with MELODIC ICA decomposition.[10] Preprocessing included motion correction for each 4D time series, [11] brain extraction, spatial smoothing (gaussian full-width half maximum 4 mm), high pass temporal filtering, and registration to an age-specific paediatric template (Cincinnati Children's Hospital, Imaging Research Center, irc.cchmc.org). Identification and removal of motion related independent components was performed with ICA-AROMA.[12] Temporal concatenation group ICA decomposed the pre-processed data into 25 independent components, performed separately on data acquired at week zero and week twelve (n=21, n=17 respectively). The default mode network (DMN) was identified by the characteristic pattern of coactivation involving medial prefrontal, posterior cingulate/precuneus, lateral parietal, lateral temporal, and cerebellar regions.[13, 14] For comparison, sensorimotor and medial visual networks were identified by characteristic coactivation patterns including perirolandic and medial occipital regions respectively.[14] The analysis for differences between groups was performed using a dual regression technique which allowed for voxel-by-voxel comparisons of functional connectivity.[15] Briefly, this involved using the spatial group ICA maps to derive subject specific temporal responses and thus estimate subject specific spatial maps. Component maps from different subjects could then be compared between groups using non-parametric permutation testing (5,000 permutations).

Perfusion Imaging Perfusion imaging was achieved using arterial spin labelling (ASL). A pulsed STAR (signal targeting with alternating radiofrequency) labeling technique with Look-Locker readout[16] at eight timepoints was used for ASL-based perfusion imaging.[17] A series of excitation pulses were applied, with increasing delay times from 600 ms to 3050 ms, and a constant interval of 350 ms between them, followed by Look-Locker readout (TR/TE 350/11 ms,). To allow quantification of CBF a M0 (proton density) scan was acquired using a very similar multiphase ASL sequence but with TR of 10sec. The ASL analysis was carried out using in-house MATLAB (www.mathworks.com) routines. The raw images were qualitatively assessed for motion and artefacts before being processed with analysis using our previously published technique.[18] A single blood compartment model, was fit to the averaged images on a voxel-by-voxel basis, producing maps of Cerebral Blood Flow (CBF) and Arterial Arrival Time (AAT, without vascular crushing). Structural T1 images were processed using FreeSurfer v5.3.0 using a standardized and automated pipeline - recon all - of cortical surface-based analysis.[19] The output of this analysis generated forty five separate regions of interest which were used to guide the calculation of regional perfusion values using the 'Destrieux' cortical atlas [20] as per diffusion section.

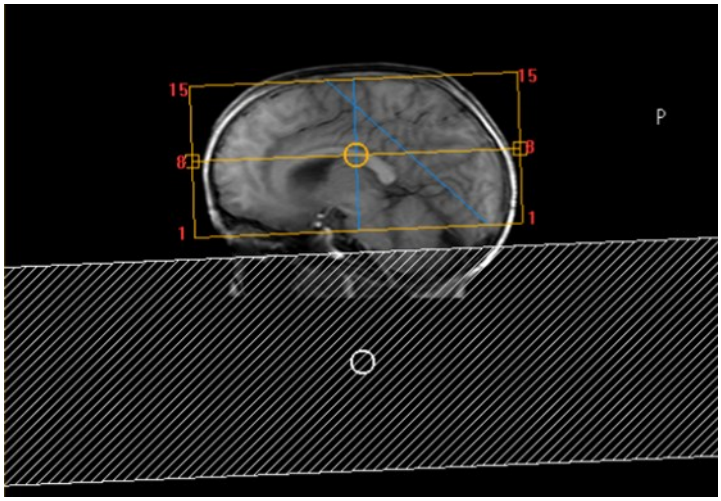


Figure S3 *Example illustrating in sagittal view the position of the perfusion-imaging slices, which were planned above the ventricles and the labelling slab (150 mm) that was set 10 mm below the imaging slices*

Diffusion Imaging The T1 images of each participant were preprocessed using the open source FreeSurfer v5.3.0 image analysis suite, which was downloaded and installed onto a Linux-based workstation. The image analysis suite encompasses a set of CT computational tools, which provide a standardized and automated pipeline - recon all - of cortical surface-based analysis.[21] The output of the analysis was used to guide the calculation of regional ADC values. FSL v.5 software was used for preprocessing the raw diffusion MR datasets. Eddy current correction was applied to the data, using the appropriate tool via the FSL software; [9] this tool is used to correct eddy current induced distortions and simple head motion by affine registration to the b0 image. The median ADC value of each of the fourty five areas was calculated via registration of ADC maps to freesurfer output; this was computed via FLIRT. [11]

Judgement of Line Orientation (JLO) This test was used for assessment of visuospatial skills as reported in previous studies in NF1 cohorts. However, compliance with this test was low due to poor attentional skills and the younger age of the cohort. Due to these reasons and the amount of missing data, we do not report the results from this assessment.

Table S2 *Baseline Descriptive Data*

	Sample N=30	**Placebo N=16**	**Simvastatin N=14**
Sex			
Male	24 (80%)	14 (87.5%)	10 (71.4%)
Female	6 (20%)	2 (12.5%)	4 (28.6%)
Mutation*			
Inherited	13 (43.3%)	10 (62.5%)	3 (21.4%)
De novo	16 (53.3%)	5 (31.3%)	11 (71.4%)
Age (years)	8.10 (1.80)	8.28 (1.76)	7.90 (1.90)
Weight (kg)	27.88 (7.59)	29.85 (8.51)	25.76 (6.08)
Social Responsiveness Scale (SRS) T Score (Mean, SD)	83.00 (7.96)	83.06 (7.58)	82.93 (8.67)
Autism Diagnostic Interview (ADI-R)			
Social Interaction (Total A)	17.67 (5.16)	18.88 (5.08)	16.29 (5.08)
Social Communication (Total B)	14.10 (4.14)	14.19 (4.37)	14.00 (4.02)
Restricted Repetitive Behaviours (Total C)	5.90 (2.67)	5.88 (2.78)	5.93 (2.64)
Autism Diagnostic Observation Schedule (ADOS)	9.73(3.23)	10.13(3.26)	9.29 (3.24)
Social Affect	2.00(1.67)	1.88(1.63)	2.14(1.74)
RRB	11.80(3.82)	12.00(3.81)	11.57(3.96)
Total			
WASI verbal IQ (n=26)	85.46 (12.72)	81.57 (12.99)	90.00 (11.24)
Aberrant Behaviour Checklist (ABC) †			
Irritability	21.72 (10.03)	19.40 (10.38)	24.21 (9.36)
Lethargy	15.07 (7.62)*	14.20 (8.36)	16.08 (6.85)*
Stereotypy	6.03 (4.44)	4.87 (3.56)	7.29 (5.04)
Hyperactivity	27.03 (11.42)	24.07 (13.04)	30.21 (8.75)
Inappropriate speech	6.66 (3.40)	5.93 (3.15)	7.43 (3.61)
Clinical Global Impression (CGI) Severity of Illness; mean (SD)	3.73(0.785)	3.88 (0.885)	3.57(0.646)
Parent-Defined Target symptoms			
Hyperactivity	13	6	7
Aggression	13	6	7
Social inappropriateness	18	9	9

Problems with communication	5	3	2
Inflexibility/obsessionality	9	7	2
Learning problems	3	1	2
Conners 3 Parent Rating Scale†			
Inattention	80.21 (10.70)	79.73 (12.13)	80.71 (9.36)
Hyperactivity	76.34 (12.95)	71.87 (14.75)	81.14 (8.88)
Riccardi Scale			
1	7 (23.3%)	3 (18.8%)	4 (28.6%)
2	17 (56.7%)	12 (75%)	5 (35.7%)
3	4 (13.3%)	0 (0.0%)	4 (28.6%)
4	2 6.7%)	1(6.3%)	1 (7.1%)

Mutation status for 1 participant in placebo group unknown as participant was adopted
†1 observation missing for Aberrant Behaviour Checklist and Conners: N=29 overall, N=15 in Group A. ADOS, Autism Diagnostic Observation Scale. SRS T score >75 = autism symptoms within clinical range; ADOS total score >7 = meets diagnostic threshold for autism spectrum disorder.
High scores on WASI indicate better performance. High scores on SRS, ADI-R, ADOS, ABC and Conners are indicative of higher levels of impairment. Parent defined target symptoms- frequency of the problem as rated by the parent.

Table S3: *Baseline clinical findings*

	Week 0 (N=30)	**Placebo (N=16)**	**Simvastatin (N=14)**
ABC†			
Irritability	21.72 (10.03)	19.40 (10.38)	24.21 (9.36)
Lethargy	15.07 (7.62)*	14.20 (8.36)	16.08 (6.85)*
Stereotypy	6.03 (4.44)	4.87 (3.56)	7.29 (5.04)
Hyperactivity	27.03 (11.42)	24.07 (13.04)	30.21 (8.75)
Inappropriate speech	6.66 (3.40)	5.93 (3.15)	7.43 (3.61)
Conners†			
Inattention	80.21 (10.70)	79.73 (12.13)	80.71 (9.36)
Hyperactivity	76.34 (12.95)	71.87 (14.75)	81.14 (8.88)
Learning problems	72.66 (12.78)	68.53 (10.78)	77.07 (13.63)
Executive function	76.66 (11.29)	74.40 (12.15)	79.07 (10.16)
Aggression	72.17 (17.85)	69.87 (17.92)	74.64 (18.10)
Peer relations	85.28 (9.62)	83.53 (11.36)	87.14 (7.27)
CGI			
Severity of illness (mean (SD)	3.73(0.785)	3.88 (0.885)	3.57(0.646)

†1 observation missing for ABC and Conners: N=29 overall, N=15 in Group A
*1 additional observation missing
Higher scores on ABC, Conners & CGI are indicative of higher levels of impairment

Table S4 *Adverse events*

Adverse events by body system	Simvastatin		Placebo	
	Grade 1-2	Grade 3	Grade 1-2	Grade 3
Gastrointestinal system disorders	1 (1)	0	4 (4)	2 (2)
General, whole body system disorders	1 (1)	0	2 (2)	0
Neurologic system disorders	6 (5)	1 (1)	12 (4)	1 (1)
Musculoskeletal system disorders	4 (2)	0	4 (4)	1 (1)
Respiratory system disorders	2 (1)	0	4 (4)	2 (2)
Cardiovascular system disorders	0	0	0	0
Psychiatric disorders	7 (7)	2	13 (5)	4 (3)
Visual system disorders	0	0	0	0
Dermatologic system disorders	3 (3)	2 (2)	6 (4)	1 (1)
Urinary system disorders	0	0	1 (1)	0

No of events (no of patients who reported the event)

Table S5 *Week 4 intermediate outcomes*

Week 4 outcomes	**Summary statistics**			**Mean difference**			
	Sample	**Placebo**	**Simvastatin**	**Adjusted mean difference (95% CI)**	**Bootstrap SE**	**Effect Size (95% CI)**	**Number in analysis**
ABC	**N=29**	**N=16**	**N=13**				
Irritability*	18.89 (10.02)	17.06 (9.90)	21.33(10.08)*	0.93 (-3.95, 5.82)	2.49	0.09 (-0.39, 0.58)	27
Lethargy*	12.46 (8.95)	11.56 (9.19)	13.67 (8.86) *	0.66 (-5.58, 6.91)	3.19	0.07 (-0.62, 0.77)	26
Stereotypy*	6.00 (4.78)	5.06 (4.36)	7.25 (5.21) *	0.42 (-2.21, 3.04)	1.34	0.09 (-0.46, 0.64)	27
Hyperactivity	24.41 (12.74)	21.25 (12.82)	28.31 (11.97)	0.66 (-4.16, 5.48)	2.46	0.05 (-0.33, 0.43)	28
Inappropriate speech	5.69 (3.08)	5.06 (2.82)	6.46 (3.33)	0.52 (-0.88, 1.92)	0.71	0.17 (-0.29, 0.62)	28
25% reduction irritability subscale	N=12	7	5				
Conners	**N=26**	**N=16**	**N=10**				
Inattention	76.88 (12.91)	75.94 (12.97)	78.40 (13.36)	2.65 (-2.85, 8.16)	2.81	0.21 (-0.22, 0.63)	25
Hyperactivity	73.04 (16.00)	70.31 (17.78)	77.40 (12.22)	-2.67 (-9.02, 3.69)	3.24	-0.17 (-0.56, 0.23)	25
Learning problems	68.38 (14.17)	64.63 (11.19)	74.40 (16.83)	3.19 (-1.76, 8.15)	2.53	0.23 (-0.12, 0.58)	25
Executive function	74.19 (15.66)	72.13 (17.55)	77.50 (12.17)	-1.05 (-9.22, 7.12)	4.17	-0.07 (-0.59, 0.45)	25
Aggression	71.04 (19.22)	72.44 (19.67)	68.80 (19.29)	-4.43 (-14.98, 6.11)	5.38	-0.23 (-0.78, 0.32)	25
Peer relations	84.58 (11.06)	83.44 (12.23)	86.40 (9.19)	1.10 (-3.15, 5.35)	2.17	0.10 (-0.28, 0.48)	25

Parent defined target symptoms (PDTS)	**N=29**	**N= 16**	**N=13**				
Mean (SD)	4.38(1.179)	4.46 (0.718)	4.28 (1.577)				
Responders (PDTS score <3)	3	2	1				
CGI*	**N=29**	**N=16**	**N=13**				
Severity of illness Mean (SD)	3.69(0.712)	3.69 (0.793)	3.69(0.630)				
Treatment responder	N=1	N=1	N=0				

*1 additional observation missing. Treatment responder defined as >25% reduction in ABC Irritability sub-scale and a score of improved or much improved on CGI. High scores on ABC, Conner's, CGI & PDTS are indicative of higher levels of impairment.

Table S6: *Quantification of MAPK outcomes at baseline and endpoint*

Participant number	Group	MAPKKinase_Wk_0	MAPKinase_Wk_12
S0001	Placebo	50.3114126	.2188209
S0002	Placebo	2.9527485	4.8079458
S0006	Placebo	3.8926110	61.8679608
S0020	Placebo	27.5060898	142.9943580
S0023	Placebo	3.0623875	42.2400651
S0029	Placebo	63.7154251	70.4316268
S0033	Placebo	96.9558036	
S0034	Placebo	35.0355789	92.9567297
S0035	Placebo	56.1097401	64.3669527
S0036	Placebo	45.2664300	89.7891294
S0037	Placebo	62.6400898	
S0046	Placebo	10.9267266	48.8793436
S0056	Placebo	15.7506385	31.6528862
S0057	Placebo	119.4835133	376.8717635
S0060	Placebo	167.6537279	56.7577496
S0063	Placebo		
S0005	Simvastatin	4.5859668	12.1169060
S0010	Simvastatin	24.1460914	9.0388298
S0011	Simvastatin	2.2530488	18.8252183
S0021	Simvastatin		
S0025	Simvastatin	4.5638149	85.4512618
S0027	Simvastatin	54.9854703	
S0031	Simvastatin	25.6439249	31.2619203
S0038	Simvastatin	62.7051706	61.4602927
S0039	Simvastatin	51.8592134	
S0043	Simvastatin	355.4792373	47.5177311
S0051	Simvastatin		
S0058	Simvastatin	172.9821432	75.6083354
S0061	Simvastatin	47.9587414	72.1282853
S0062	Simvastatin	86.8384461	

Table S7: *A comparison of the mutation data in the SANTA sample to previously reported data from a clinic referred NF1 sample (see text)*

Study Ref	Mutation	Type	Exon
S0001	WGD	large re	EGD
S0003	c.3911T>A p. (Leu 1 304Ter)	nonsense	exon 29
S0005	c.2041C>T	nonsense	exon 18
S0006*	c.3741delT	frameshift	exon 28
S0010	c.3916C>T	nonsense	exon 29
S0011	c.5749+332A>G	splice	intron 38
S0020	c.3497-4T>G	splice	intron 26
S0021	c.203delins6	frameshift	exon 02
S0023	c.2504_2505delAG	frameshift	exon 21
S0025	c.2953C>T p.(Gln985Ter)	nonsense	exon 22
S0027	c.174delC	frameshift	exon 02
S0029**	C.7908-321C>G	Splice deep intronic	intron 54
S0031	c.5546G>A p.(Arg1849G1n)	missense	exon 37
S0033	c.4537C>T(p.Arg1513Ter)	nonsense	exon 35
S0034	c.1246C>Tp.(Arg416Ter)	nonsense	exon 11
S0035*	c.3741delT	frameshift	exon 28
S0036	c.7239_7240insTT	frameshift	exon 49
S0037	c.1381C>Tp.(Arg461Te	nonsense	exon 12
S0038	c.2087G>A p.(Trp696Ter)	nonsense	exon 18
S0039	c.4306A>G p.(Lys1436GIu)	missense	exon 32
S0043	c.1260+1G>A	splice	intron 11
S0046**	C.7908-321C>G	Splice deep intronic	intron 54
S0051	Not done		
S0056	c.479G>Tp.(Arg160Met)	missense	intron 4
S0057	C7404_7405insTA. Glu2469Ter	frameshift	Exon 51

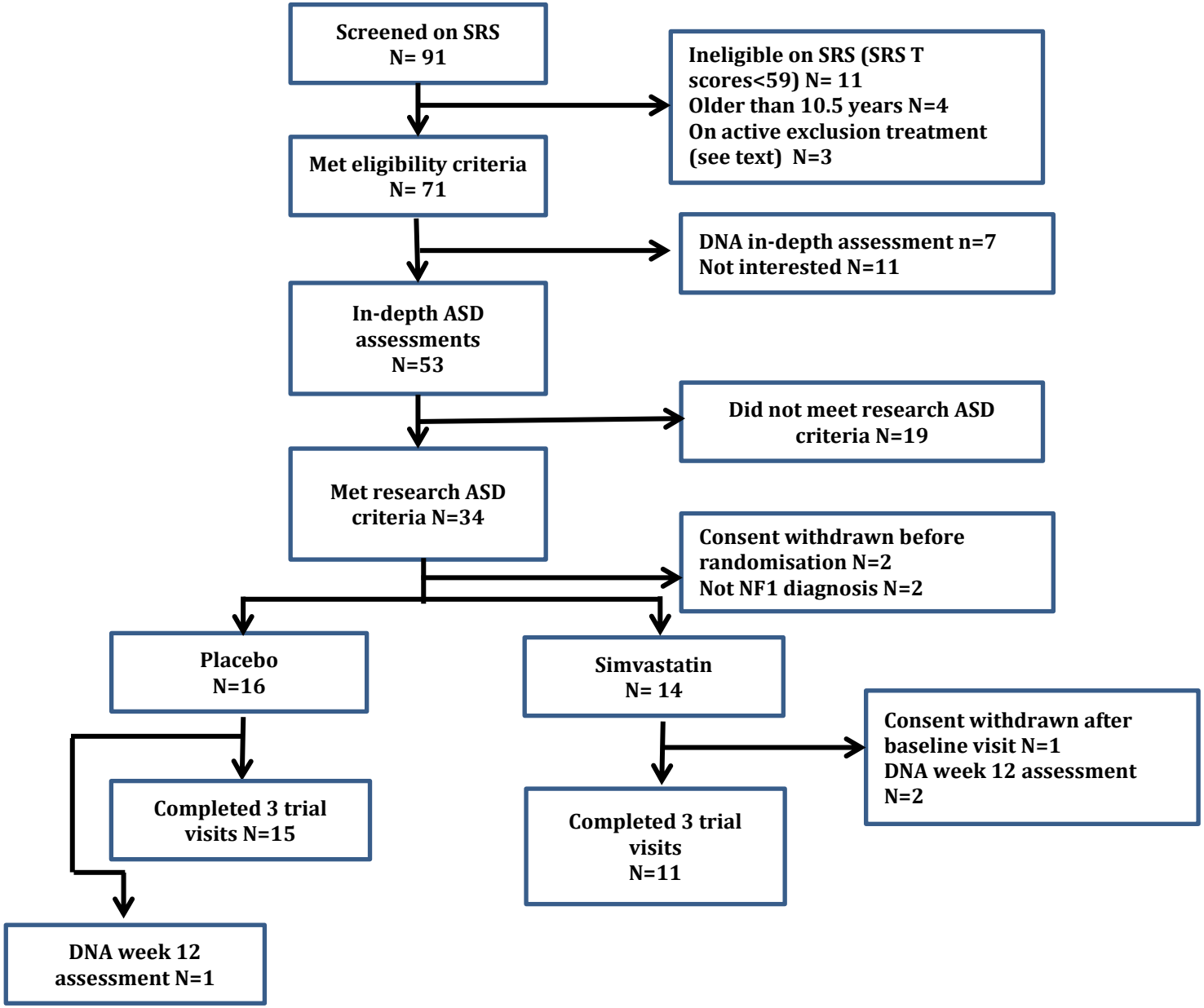
S0058	c.7096_7101del6p.(Asn2366_Phe2367del)	in-frame deletion	exon 48
S0060	c.1756_1759delACTA	frameshift	exon 16
S0061	c.1186-1G>T	splice	Exon 11
S0062	Not done		
S0063	Not done		

Data was not available for 3 participants
* & ** sibling pairs

	SANTA	**%**	**Controls+**	**%**
splice	5	19.23%	80	22.99%
frameshift	8	30.77%	114	32.76%
nonsense	8	30.77%	76	21.84%
missense	3	11.54%	31	8.91%
large re	1	3.85%	28	8%
ifd	1	3.85%	13	3.74%
	26		342	

+ Evans et al European Journal of Human Genetics 2016 [22]

Figure S4 *SANTA CONSORT flow diagram (see text; SRS, Social Responsiveness Scale)*



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