Plasma oxysterols : diagnostic biomarkers for spastic paraplegia type 5 and application to therapeutic trial

Supplementary Method : Validation of the oxysterols method using UPLC-MS/MS

Ionization responses in LC-MS/MS can vary due to matrix components co-eluting with the analyte. Hence, matrix effect was evaluated by slopes of calibration curves made in methanol or in human plasma. Calibration standard curves were prepared in methanol or by spiking human plasma and consisted of seven standards ranging from 31.3 to 1000 µg/ml of 25-OHC, 27-OHC and 24S-OHC. The calibration curves in methanol and in plasma were not significantly different and the slopes of the calibration curves appeared to be nearly identical in methanol and in plasma as shown below.

Characteristics of the calibration regression line data prepared in methanol and plasma

Analyte	Matrix	Slope	Intercept	R^2
25-OHC	Methanol	0.00003	0.0016	0.993
	Plasma	0.00005	0.0021	0.982
27-OHC	Methanol	0,0019	0,0319	0,997
	Plasma	0,0010	0,0100	0,999
24S-OHC	Methanol	0.001	0.117	0.968
	Plasma	0.0008	0.0247	0.997

Legend: 25-OHC= 25-hydroxycholesterol; 27-OHC= 27-hydroxycholesterol; 24S-OHC= 24-hydroxycholesterol.

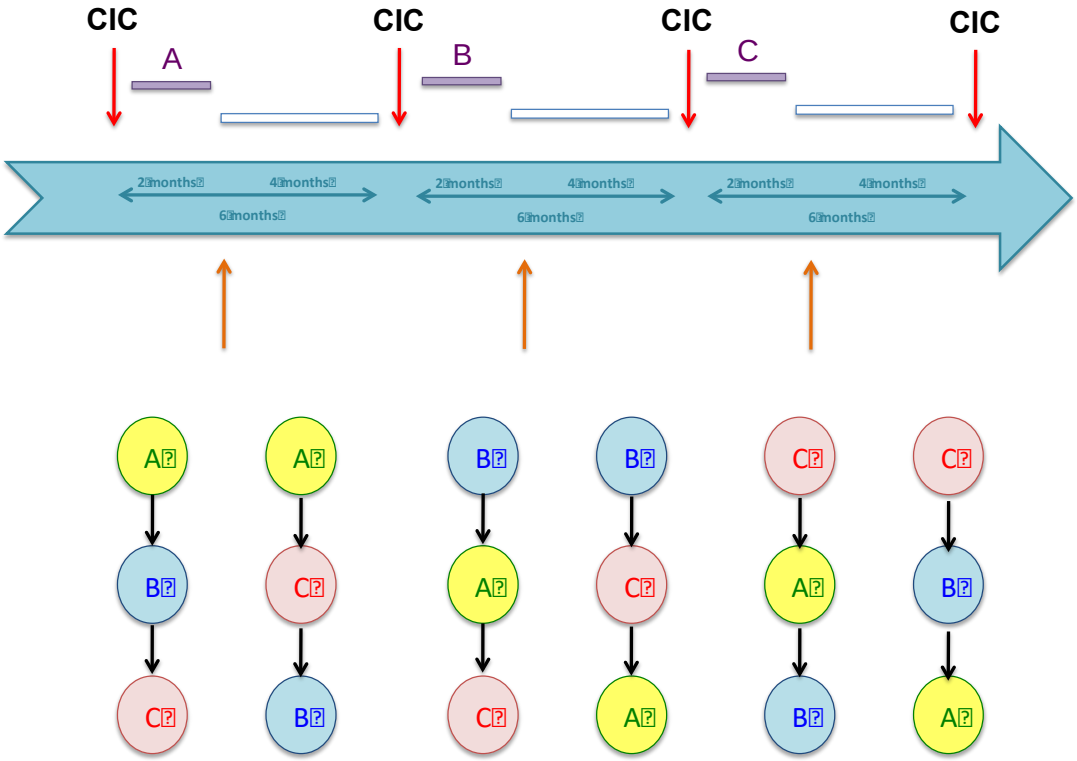
Thus, we chose methanol as surrogate matrix, which is also a simpler and well-controlled preparation method. The method is linear in the range 31.3 to 1000 µg/ml, which is completely adequate to the pathophysiological interval of values. Low limit of detection (LOD), defined as the minimum concentrations of 25-OHC, 27-OHC and 24S-OHC giving a peak area three-fold the noise, was 0.8, 2.6 and 4.9 µg/ml, respectively. Low limits of quantification (LOQ), defined as the lowest concentrations of 25-OHC, 27-OHC and 24-OHC, which can be measured in triplicate with precision higher than 20%, were 2.7, 8.5 and 16.4 µg/ml, respectively. The accuracy of the method was evaluated by analysing human plasma spiked with 100 µg of 25-OHC and 24S-OHC and with 200 µg/ml of 27-OHC. The nominal concentrations of unspiked plasma and the concentration of the spiked plasma were determined by mean of five measures. The accuracy was calculated as the percentage of nominal concentrations and was 100.1%, 101% and 95.6%, for 25-OHC, 27-OHC and 24S-OHC, respectively. The precision of the method was evaluated by analysing human plasma spiked with 100 µg of 25-OHC and 24-OHC and with 1500 µg/ml of 27-OHC corresponding to normal and pathological concentrations. Hydroxycholesterols measurement was repeated 10 times in a single run for intra-assay precision or in different runs and days for the inter-assay precision. Precision results are summarized below.

Intra- and inter-assay precision of measurement of 25-OHC, 27-OHC, and 24S-OHC

		25-OHC		27-OHC		24S-OHC	
		Low	High	Low	High	Low	High
Intra-assay (n=10)	Mean ± SD	7±0.8	247±16	238±5.3	632.2±9.2	66±4.8	194±18.2
	CV, %	11.7	6.5	2.2	1.4	7.3	9.4
Inter-assay (n=10)	Mean ± SD	18.5±4	346.7±28	241±17	1653±154	90±8	631±70
	CV, %	22	8	7	9	9	16

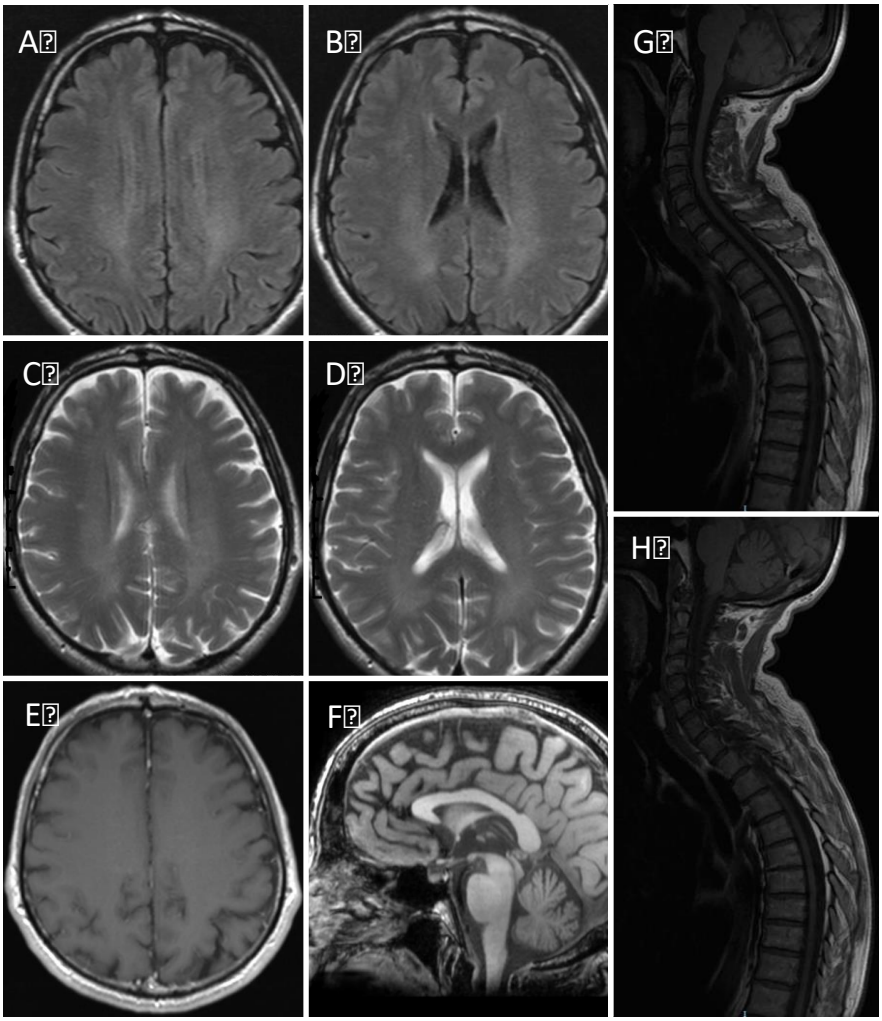
Legend: 25-OHC= 25-hydroxycholesterol; 27-OHC= 27-hydroxycholesterol; 24S-OHC= 24-hydroxycholesterol; CV=Coefficient of variation

Supplementary Figures



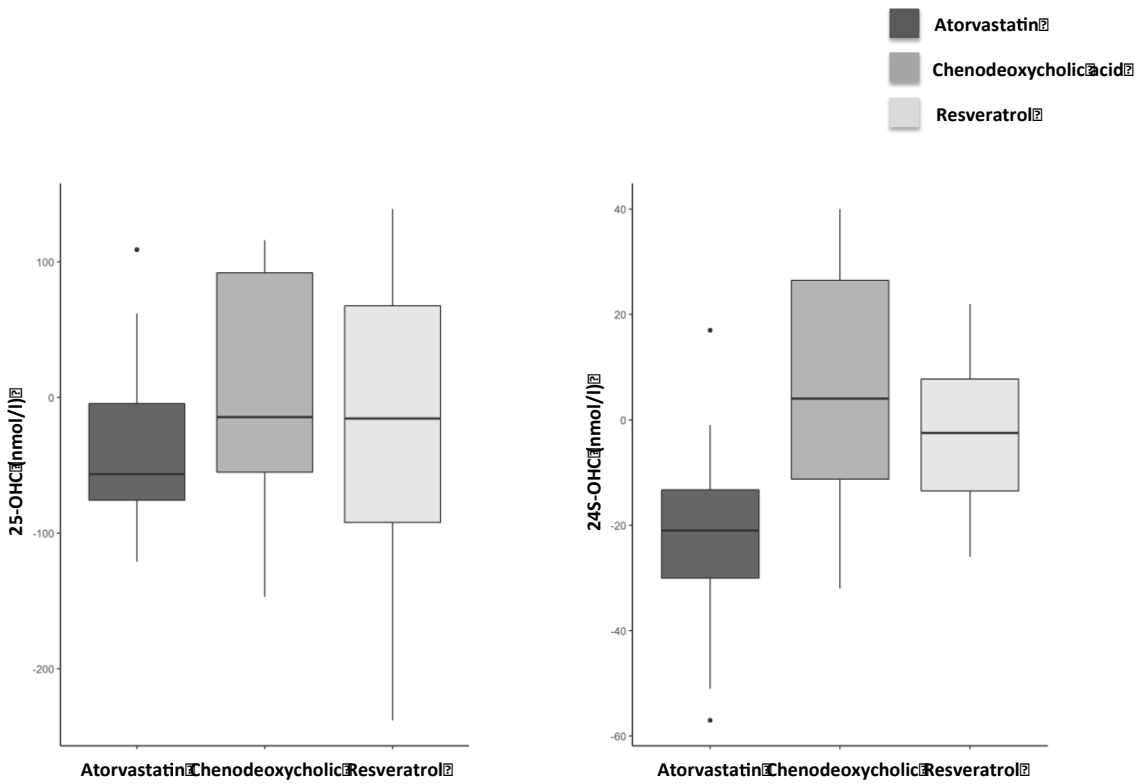
Supplementary Fig. 1 : Design of the phase II therapeutic trial

The trial consisted into a three-period, three-treatment crossover study. The six different sequences of three treatments (atorvastatin, A; chenodeoxycholic acid, B; resveratrol C), represented in the lower part of the figure, were randomized: each patient received the three treatments during three periods of two months in random order, separated by a four-months washout (upper part of the figure). The follow-up visits at the Clinical Investigation Center (CIC) were planned at a six-month interval.



Supplementary Fig. 2 : Cerebral and spinal MRI of SPG5 patients

Mild uniform posterior white matter hyper-intensity in axial FLAIR (A and B) and axial T2 weighted images (C and D), iso-intense in axial T1 (injected weighted images, E) (patient SAL-279-014); mild cerebellar atrophy, predominantly on upper vermis, in a sagittal T1 weighted images (F) (patient SAL-B-563-010); cervical (G) and dorsal (H) spinal atrophy (sagittal T1 weighted images; patient SAL-B-563-010).



Supplementary Fig. 3 : Evolution of plasma 25-OHC and 24S-OHC in SPG5 patients. Plasma 25OHC (left) levels were not impacted by any of the treatments. Plasma 24S-OHC (right) significantly decreased under atorvastatin in SPG5 patients.

Supplementary Table 1: **List of the hereditary spastic paraplegia –causing genes included from the targeted gene panel.**

Gene	SPG	References	N° OMIM	N° LRG
ALS2	-	Wakil SM *et al.,* Gene 2014	606352	‡LRG_654 (NM_020919.3)
AMPD2	SPG63	Novarino G *et al.,* Science 2014	102771	nd
AP4B1	SPG47	Bauer P *et al.,* Neurogenetics 2012	607245	nd
AP4E1	SPG51	Abou Jamra R *et al.,* Am. J. Hum. Genet. 2011	607244	‡LRG_732 (NM_007347.4)
AP4M1	SPG50	Abou Jamra R *et al.,* Am. J. Hum. Genet. 2011	602296	nd
AP4S1	SPG52	Abou Jamra R *et al.,* Am. J. Hum. Genet. 2011	607243	nd
*AP5B1**	*Candidate gene by function*	Hirst J *et al.*, PLoS Biol, 2011	614367	nd
*AP5M1**	*Candidate gene by function*	Hirst J *et al.*, PLoS Biol, 2011	614368	nd
*AP5S1**	*Candidate gene by function*	Hirst J *et al.*, PLoS Biol, 2011	614824	nd
AP5Z1	SPG48	Slabicki M *et al.,* PLoS Biol. 2010	613653	nd
ARL6IP1	SPG61	Novarino G *et al.,* Science 2014	607669	nd
ARSI	SPG66	Novarino G *et al.,* Science 2014	610009	nd
ATL1	SPG3A	Zhao X *et al.,* Nature Genet. 2001	606439	‡LRG_360 (*multiple)
B4GALNT1	SPG26	Boukhris A *et al.,* Am. J. Hum. Genet. 2013	601873	nd
BICD2	-	Novarino G *et al.,* Science 2014	609797	nd
BSCL2	SPG17	Windpassinger C *et al.,* Nature Genet. 2004	606158	‡LRG_235 (NM_001122955.3)
C12orf65	SPG55	Shimazaki H *et al.,* J. Med. Genet. 2012	613541	nd
C19orf12	SPG43	Landoure G *et al.,* Hum. Mutat. 2013	614297	nd
CCT5	-	Bouhouche A *et al.,* J. Med. Genet. 2006	610150	LRG_361 (NM_012073.3)
CPT1C	*SPG73*	Rinaldi C *et al.*, JAMA Neurol 2015	608846	nd
CYP2U1	SPG56	Tesson C *et al.,* Am. J. Hum. Genet. 2012	610670	nd
CYP7B1	SPG5A	Tsaousidou M *et al.,* Am. J. Hum. Genet. 2008	603711	nd
DDHD1	SPG28	Tesson C *et al.,* Am. J. Hum. Genet. 2012	614603	nd
DDHD2	SPG54	Schuurs-Hoeijmakers JHM *et al.,* Am. J. Hum. Genet. 2012	615003	nd
ENTPD1	SPG64	Novarino G *et al.,* Science 2014	601752	nd
ERLIN1	SPG62	Novarino G *et al.,* Science 2014	611604	nd
ERLIN2	SPG18	Alazami A *et al.,* Neurogenetics 2011	611605	nd
FA2H	SPG35	Dick KJ *et al.,* Hum. Mutat. 2010	611026	nd

FBXO7	Pallido-pyramidal syndrome	Di Fonzo A *et al.,* Neurology 2009	605648	nd
FLRT1	SPG68	Novarino G *et al.,* Science 2014	604806	nd
GAD1	-	Lynex CN *et al.,* BMC Neurol. 2004	605363	nd
GBA2	SPG46	Martin E *et al.,* Am. J. Hum. Genet. 2013.	609471	nd
GJA1	Pleiotropic phenotype of oculodentodigital dysplasia	Paznekas WA *et al.,* Am. J. Hum. Genet. 2003	121014	nd
GJC2	SPG44	Orthmann-Murphy JL *et al.,* Brain 2009	608803	nd
HSPD1	SPG13	Hansen JJ *et al.,* Am. J. Hum. Genet. 2002	118190	nd
KIAA0196	SPG8	Valdmanis PN *et al.,* Am. J. Hum. Genet. 2007	610657	nd
KIF1A	SPG30	Erlich Y *et al.,* Genome Res. 2011	601255	±LRG_367 (*multiple)
KIF1C	SPG58	Novarino G *et al.,* Science 2014	603060	nd
KIF5A	SPG10	Reid E *et al.,* Am. J. Hum. Genet. 2002	602821	nd
L1CAM	SPG1	Jouet M *et al.,* Nature Genet. 1994	308840	±LRG_14 (NM_000425.3)
MAG	-	Novarino G *et al.,* Science 2014	159460	nd
MARS	SPG70	Novarino G *et al.,* Science 2014	156560	nd
MT-ATP6	-	Verny C *et al.,* Mitochondrion 2011	516060	nd
NIPA1	SPG6	Rainier S *et al.,* Am. J. Hum. Genet. 2003	608145	nd
NT5C2	SPG65	Novarino G *et al.,* Science 2014	600417	nd
PGAP1	SPG67	Novarino G *et al.,* Science 2014	611655	nd
PLP1	SPG2	Saugier-Veber P *et al.,* Nature Genet. 1994	300401	nd
PNPLA6	SPG39	Rainier S *et al.,* Am. J. Hum. Genet. 2008	603197	nd
RAB3GAP2	SPG69	Novarino G *et al.,* Science 2014	609275	nd
REEP1	SPG31	Zuchner S *et al.,* Am. J. Hum. Genet. 2006	609139	±LRG_713 (*multiple)
REEP2	SPG72	Esteves T *et al.,* Am. J. Hum. Genet. 2014	609347	nd
RTN2	SPG12	Montenegro G *et al.,* J. Clin. Invest. 2012	603183	nd
SACS	ARSACS	Engert JC *et al.,* Nature Genet. 2000	604490	nd
SAMHD1	Aicardi-Goutières syndrome 5 (AGS5)	Rice G *et al.,* Nature Genet. 2009	606754	LRG_281 (NM_015474.3)
SETX	Juvenile Amyotrophic Lateral Sclerosis 4	Chen YZ *et al.,* Am. J. Hum. Genet. 2004	608465	LRG_268 (NM_015046.5)
SLC16A2	SPG22	Schwartz CE *et al.,* Am. J. Hum. Genet. 2005	300095	nd
SLC33A1	SPG42	Lin P *et al.,* Am. J. Hum. Genet. 2008	603690	nd
SPAST	SPG4	Hazan J *et al.,* Nature Genet. 1999	604277	±LRG_714 (NM_014946.3)

SPG11	SPG11	Stevanin G *et al.,* Nature Genet. 2007	610844	nd
SPG20	SPG20	Patel H *et al.,* Nature Genet. 2002	607111	nd
SPG21	SPG21	Simpson MA *et al.,* Am. J. Hum. Genet. 2003	248900	nd
SPG7	SPG7	Casari G *et al.,* Cell 1998	602783	nd
TECPR2	SPG49	Oz-Levi D *et al.,* Am. J. Hum. Genet. 2012	615000	nd
TFG	SPG57	Beetz C *et al.,* Proc. Nat. Acad. Sci. 2013	602498	nd
USP8	SPG59	Novarino G *et al.,* Science 2014	603158	nd
VCP	-	de Bot ST *et al.,* Brain 2012	601023	LRG_657 (NM_007126.3)
VPS37A	SPG53	Zivony-Elboum Y *et al.,* J. Med. Genet. 2012	609927	nd
WDR48	SPG60	Novarino G *et al.,* Science 2014	612167	nd
ZFR	SPG71	Novarino G *et al.,* Science 2014	615635	nd
ZFYVE26	SPG15	Hanein S *et al.,* Am. J. Hum. Genet. 2008	612012	nd

* candidate gene

Supplementary Table 2: Detailed clinical features SPG5 patients (from 1 to 10)

Patient ID	SAL-930-001	SAL-399-975	GRE 506-010	GRE 506-011	SAL-1311-001	SAL-1134-004	SAL-1465-001	NIM-001	MON-001	SAL-399-229
Age at onset/Sex	12/M	35/M	11/M	10/M	20/F	1/F	11/M	20/M	About 40/F	24/F
Symptom at onset	Stiff legs	Stiff legs, unsteadiness, cramps	Stiff legs; unsteadiness	Stiff legs; unsteadiness	Stiff legs	Stiff legs	Stiff legs; dysarthria; cramps	Unsteadiness	Stiff legs	Stiff legs
Disease duration (years)	14	30	28	26	15	28	35	35	>10	39
Disability score X/7 *	NA	6	3	3	2	5	NA	3	5	6
UL/LL reflexes**	N/++	--/ --	N/+	N/++	++/++	N/++	N/++	N/++	NA/NA	+/-
LL spasticity: action/rest	Moderate/Mild	Mild/Mild	Moderate/Absent	Present/Absent	Moderate/NA	Moderate/NA	Severe/Moderate	Mild/Moderate	Severe/Absent	Severe/NA
Babinski sign	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral
Romberg sign	Positive	Positive	Positive	Positive	Positive	NA	Positive	Positive	Positive	NA
LL weakness	Mild	Severe	Mild	Mild	No	Moderate	Moderate	No	Mild	Moderate
UL/LL Vibration sense	Decreased/abolished	Decreased/decreased	Decreased (slightly)/abolished	Decreased (slightly)/abolished	Decreased (slightly)/decreased	NA/Decreased	NA/Abolished	N/abolished	NA/Decreased	Decreased/abolished
Urinary problems	No	Mild urgency	Urgency	No	Urgency; mild incontinence	Dysuria	No	Urgency	NA	Yes (not specified)
Ocular findings	Saccadic pursuit	N	Saccadic pursuit	Saccadic pursuit	N	Saccadic pursuit	NA	NA	NA	Saccadic pursuit; familial maculopathy
Other		Scoliosis	Equinus foot and claw toes	Scoliosis	NA	Pes cavus	NA	No	NA	NA
Cognitive involvement	No	No	No	No	No	NA	No	No	No	No
ENG/EMG	NA	N	NA	NA	N	NA	N	N	N	N
PESS	NA	Altered (central) LL	NA	NA	Altered (central)	NA	NA	Altered (central) UL/LL	NA	NA
Cerebral MRI: cortical/cerebellar/ brainstem atrophy; WMH	-/-/-; Posterior diffuse	-/-/-; Posterior diffuse WMH	-/-/-; Posterior diffuse WMH	-/-/-; Posterior diffuse WMH	NA/NA/NA; Posterior diffuse WMH	NA	No/No/No; No	NA/+/NA; No	-/+/-; Posterior	NA/NA/NA; Posterior multifocal WMH

	WMH								diffuse WMH	
Spinal MRI: atrophy/signal alteration	No/syringomyelia	No/No	NA	Yes/posterior cordonal hyper-intensity	No/No	NA	NA	No/No	No/No	NA

Legend: WMH= white matter hyper-intensity; -= absent; N= normal; NA= not assessed; UL=upper limbs; LL=lower limbs; BBK: Babinski sign; nv=normal values; TChol: total cholesterol.
** for UL and LL reflexes: N= normal; += increased; ++= diffused; -= decreased; --= abolished; MS: motor and sensitive (polyneuropathy).
* for the disability score: 0= no functional handicap; 1= no functional handicap but signs at examination; 2= mild, able to run, walking unlimited; 3= moderate, unable to run, limited walking without aid; 4= severe, walking with one stick; 5= walking with two sticks; 6= unable to walk, requiring wheelchair; 7= confined to bed

Supplementary Table 3: Detailed clinical features SPG5 patients (from 11 to 21)

Patient ID	SAL-279-014	SAL-B-563-010	SAL-D-563-010	SAL-004-006	SAL-004-008	SAL-004-015	SAL-1450-001	SAL-1491-001	SAL-899-027	BEL-001	LYO-001
Age at onset/Sex	16/M	10/F	44/F	20/M	27/M	7/F	40/F	10/M	37/F	16/F	NA/F
Symptom at onset	Stiff legs; unsteadiness	Stiff legs	NA	Unsteadiness	Stiff legs	Stiff legs	Stiff legs	Stiff legs	NA	Stiff legs	Stiff legs
Disease duration (years)	42	50	25	34	NA	27	17	37	34	18	>20
Disability score X/7 *	5	4	4	6	6	6	3	3	6	5	NA
UL/LL reflexes**	++/++	+/+	++/++	+/+	++/-	-/-	++/++	+/+	+/++	+/+	NA
LL spasticity: action/rest	Severe / severe	Severe/mild	Severe/absent	Severe/NA	Severe/ NA	Severe/ mild	Moderate / absent	Present/ present	Severe/present	Severe/severe	Present/ NA
Babinski sign	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	NA
Romberg sign	Positive	Positive	Positive	Positive	Positive	NA	NA	NA	NA	Positive	NA
LL weakness	Severe	Moderate	Mild	Moderate	Severe	Moderate	Mild	No	No	No	NA
UL/LL Vibration sense	Decreased/abolished	Slightly decreased/decreased	Decreased/abolished	N/decreased	Slightly decreased/abolished	Slightly decreased/decreased	N/Slightly decrease	N/decreased	NA	Decreased/Abolished	NA/decreased
Urinary problems	No	Urgency	Urgency	Urgency	Yes (not specified)	Yes	Urgencyincontinence	Urgency	No	Urgency	NA
Ocular findings	Saccadic pursuit; ny	Saccadic pursuit	Saccadic pursuit; optic atrophy	N	Ny; saccadic pursuit	N	Saccadic pursuit	N	N	N	NA
Other	NA	Pes cavus, claw toes, scoliosis	No	NA	Mild cerebellar ataxia, myoclonus	Equinus foot	NA	NA	No	Pes cavus	Pes cavus, scoliosis
Cognitive involvement	No	No	NA	NA	NA	NA	No	No	No	No	NA
ENG/EMG	NA	N	N	N	NA	NA	N	M/S demyelinating	N	N	NA
PESS	NA	Altered (central) UL /LL	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cerebral MRI: cortical/cerebellar/	-/+/-; Posterior	+/+/-; Posterior diffuse and focal	-/NA/-; Posterior	NA/NA/NA; Posterior	+/+/-; Posterio	No/yes/ No;	-/-/-; one focal not specific	-/+/-; No	No/No/No; not specific	No/No/No: NA	NA

brainstem atrophy; WMH	diffuse WMH	WMH	diffuse WMH	diffuse WMH	r diffuse WMH	Posterior diffuse WMH	WMH		WMH		
Medullar MRI: spinal cord atrophy/signal alteration	NA	Yes/No	NA	NA	NA	NA	No/No	Yes/No	No/No	Yes/No	No/No

Legend: WMH= white matter hyper-intensity; -= absent; N= normal; NA= not assessed; UL=upper limbs; LL=lower limbs; BBK: Babinski sign; nv=normal values; TChol: total cholesterol.
** for UL and LL reflexes: N= normal; += increased; ++= diffused; -= decreased; --= abolished; M/S: motor and sensitive (polyneuropathy).
* for the disability score: 0= no functional handicap; 1= no functional handicap but signs at examination; 2= mild, able to run, walking unlimited; 3= moderate, unable to run, limited walking without aid; 4= severe, walking with one stick; 5= walking with two sticks; 6= unable to walk, requiring wheelchair; 7= confined to bed

Supplementary Table 4

Adverse events	Treatment	Causality
Urinary infection	resveratrol	unrelated
Digestive pain	chenodeoxycholic acid acid	probable
Digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Athlete's foot fungus	chenodeoxycholic acid	unrelated
Hemorrhoids	atorvastatin	unrelated
Knee pain	atorvastatin	unrelated
Lateral meniscal lesion (right knee)	resveratrol	unrelated
Synoviorthesis (right knee)	resveratrol	unrelated
Athlete's foot fungus	atorvastatin	unrelated
Constipation	atorvastatin	unlikely
Vesicular eruption (hands)	atorvastatin	unlikely
Fatigue	atorvastatin	unlikely
Muscular pain	atorvastatin	unlikely
Muscular pain	atorvastatin	unrelated
Muscular pain	atorvastatin	unrelated
Left knee pain with left calf irradiation	chenodeoxycholic acid	unrelated
Plantar arch pain	chenodeoxycholic acid	unrelated
Left hip pain	chenodeoxycholic acid	unrelated
Episodic digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Paresthesiae of the left foot fingers	chenodeoxycholic acid	unrelated
Fracture of the 3rd left metatarsal	chenodeoxycholic acid	unrelated
Dark urine (E. Coli Infection)	chenodeoxycholic acid	unrelated
Digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Headaches, epigastric pain, and vomiting	chenodeoxycholic acid	unrelated
Aphthae	chenodeoxycholic acid	unrelated
Pharyngitis	resveratrol	unrelated
Digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Leg fracture	chenodeoxycholic acid	unrelated
Left leg pain	resveratrol	unrelated
Left leg pain (after a fall)	resveratrol	unrelated
Left hip pain (after a fall)	atorvastatin	unrelated
Inguinal herniation	atorvastatin	unrelated
Digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Viral rhinopharyngitis	atorvastatin	unrelated
Back pain	atorvastatin	unrelated
Headaches	chenodeoxycholic acid	unrelated
Diarrhea	chenodeoxycholic acid	probable
Headaches	chenodeoxycholic acid	unrelated
Belly pain	chenodeoxycholic acid	probable
Flu	chenodeoxycholic acid	unrelated

Tonsillitis	chenodeoxycholic acid	unrelated
Bronchitis	resveratrol	unrelated
Gingivitis	resveratrol	unrelated
Tracheitis	resveratrol	unrelated
Digestive pain	resveratrol	unlikely
Digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Muscular pain (normal CK)	atorvastatin	unlikely
Muscular pain (normal CK)	atorvastatin	unlikely
Muscular pain (normal CK)	resveratrol	unlikely
Vomiting	chenodeoxycholic acid	unlikely
Muscular pain (normal CK)	chenodeoxycholic acid	unlikely
Digestive pain	chenodeoxycholic acid	probable
Mild fatigue	chenodeoxycholic acid	unlikely
Nasal polyp	atorvastatin	unrelated
Tendinitis (right hand)	chenodeoxycholic acid	unrelated
Digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Digestive pain	chenodeoxycholic acid	probable
Carpal tunnel syndrome	chenodeoxycholic acid	unrelated
Carpal tunnel syndrome surgery	chenodeoxycholic acid	unrelated
Headaches	chenodeoxycholic acid	unrelated
Hyperthyroidism	atorvastatin	unlikely
Fatigue	atorvastatin	unlikely
Hyperthyroidism	atorvastatin	unrelated
Seborrheic verruca (left eye)	atorvastatin	unrelated
Tonsillitis	atorvastatin	unrelated
Right knee pain (popliteal cyst and arthosis)	resveratrol	unrelated
Digestive pain	chenodeoxycholic acid	probable
Diarrhea	chenodeoxycholic acid	probable
Diarrhea	chenodeoxycholic acid	unlikely
Anxiety	chenodeoxycholic acid	unrelated
Left ear otitis	resveratrol	unrelated
Tongue aphthae	chenodeoxycholic acid	unrelated