APPENDIX A

Supplementary files

Supplementary Table S3: Control fibroblast lines used for the study

Control line	Gender/age of donation	Health status	reference
Control 1 (C1)	F/65	Neurologically healthy	[1,2]
Control 2 (C2)	M/69		
Control 3 (C3)	F/56		

Supplementary Table S4: Primers used in the study

Gene	Primer sequences	source
GAPDH	GTTCGACAGTCAGCCGCATC GGAATTTGCCATGGGTGGA	[3,4]
PLAU	CCAAAGAAATTCGGAGGGCA CTTGGACAAGCAGCTTTAGGC	Designed for this study (NCBI primer blast)
DSCR1	GCACCAGCTCAAGAAGGAACC GGGACTCAAATTTGGCCCTGG	[5]
BACE1 (exon 6F, exon 8R)	CACCACCAACCTTCGTTTGC AAGCCAATTCGTTTTCGGGC	Designed for this study (NCBI primer blast)
BACE1 (exon 3F, exon 9R)	CAACTGGGAAGGCATCCTGG GCAGGGAGATGTCATCAGCA	Designed for this study (NCBI primer blast)

Supplementary Table S5. The results of the neuropsychological assessment conducted in the proband's brother at the age of 63 when he presented with restricted behavioral repertoire and marked cognitive rigidity.

Function assessed / test	Score	Comments
General cognitive status		
ACE-III: orientation and attention / 18	17	clearly semantic errors in confrontation naming: penguin was named as "a bird"
ACE-III: memory / 26	22	rhinoceros was named as "wild boar"
ACE-III: fluency / 14	14	
ACE-III: language / 26	24	
ACE-III: visuospatial function / 16	16	
ACE-III total score / 100	93	within normal range
Mini-ACE (MACE) total score / 30	24	below cut-off
Language function & visual / verbal semantics		
Naming BNT / 30 ACE-III / 12	19 10	mild anomia
Verbal fluency (raw scores / 60s.) - semantic: animals - fruit & vegetables / fish phonemic: K / P / S	22 16 / 12 18 / 19 / 11	semantic fluency slightly lower than expected in the context of good phonemic fluency; in fish trial the patient started with aquarium fish in which he has special interest; two incidents of other personal comments during assessment (referring to the patient's dog)

Semantics
 	
Sydney Language Battery (SYDBAT):
 	
- Naming (% correct)
 	
- Word Comprehension (% correct)
 	
- Semantic Association (% correct)
 	
PALS – animal naming / comprehension
 	
   - object naming / comprehension
 	
Recognition of famous faces* / 15
 	
Naming famous faces*: spontaneous / cued
 	
Drawings: to command / copy
 	
   - guitar:
   - anchor
   - lightbulb
   - snail
   - rhinoceros
	
	19 (63%)
	
	20 (66%)
	
	21 (70%)
	
	9 / 10
	
	8/10
	
	14
	
	7 / 14
	
	both correct
	
	incorrect / correct
	
	simplified / correct
		naming, word comprehension and semantic association are similarly impaired in SYDBAT which indicates a semantic deficit
		
		in PALS "eagle" was named "flying dinosaur" (after the patient correctly named dinosaur)
		
		the rhinoceros (drawing to command) lacked any distinctive features, it was drawn as a four-legged domestic animal, resembling a sheep;
		
		anchor (drawing to command) – only the bottom part was drawn

	both correct / incorrect / correct	
Language Comprehension		
Commands from BDAE / 15	15	preserved
Repetition		
Sentence repetition from BDAE		preserved
high frequency / low frequency (max. 8)	8 / 8	
Reading		
Word reading from BDAE / 30	30	one verb distortion in sentence reading
Sentence reading from BDAE / 10	9	
Writing		
Primer level dictation from BDAE / 15	15	writing to dictation was preserved (the testing did not address surface dysgraphia)
Writing words to dictation from BDAE / 10	10	
Written confrontation naming / 10	10	three characters in the beach scene were named "man" (also the boy building a sand castle), the girl were misnamed as a boy
Written picture description (beach scene)	qualitative	
Other aspects of cognition		
Visuospatial function		mostly preserved;
VOSP: Incomplete letters / 20	20	the only visual subtest that was failed was the one with semantic involvement (Silhouettes)
Silhouettes / 30	8	
Dot counting /10	9	
Position discrimination / 20	20	
Number location / 10	10	
Cube analysis / 10	10	
CFT copy / 36	30	
Working memory		mostly preserved;
Digit span – max. forward / backward	4 / 4	verbal immediate memory was slightly deficient
Corsi – max. span forward	5	
TMT A	80 sec.; 60T	
TMT B	129 sec.; 52T ; 0 errors	
Episodic memory		prose delayed recall was relatively well preserved, but logical error (likely due to a semantic deficit) appeared (the patient recalled that the load was heavier as salt dissolved);
Address recall from ACE-III:		
- learning curve / delayed recall (max. 7)	6-5-7 / 5	
- recognition (max. 5)	5	face delayed recognition was well preserved;

10-word list learning:		word-list learning and figure recall were deficient
- learning curve	3-4-4-7-7	
- delayed recall	5	
Recognition: correct / false positives	9 / 4	
CFT – after 3 min. delay	31T	
CFT after 30 min. delay	35T	
CFT recognition	<20T	
Famous faces after a delay:		
- free recall	6	
- cued recall	8	
- recognition	15	
Story recall* (max. 10):		
- story 1: immediate (free / cued / recognition)	8 / 8 / 10	
- story 2: immediate (free / cued / recognition)	8 / 10 / -	
- story 1: delayed (free / cued / recognition)	7 / 7 / 10	
- story 2: delayed (free / cued / recognition)	9 / 9 / 9	
Executive function		poor inhibition
Sorting Weigl blocks	8 / 12	
Tower of London – DX-2: total correct SS	98	
total move SS	116	
rule violation SS	64	
Calculation		calculation was slightly impaired (problems only in complex tasks)
Serial sevens (max. 5)	4	
Calculation from WAB, multiple choice / 24	24	
Written arithmetics	few errors in complex tasks	
Arithmetic word-problem solving / 8	4	
Praxis		preserved
Alternate hand movements	correct	
Spatial praxis	correct	
Ideomotor praxis	correct	
Efferent motor praxis:		
1st sequence / 2nd sequence (max. 5)	5/5	

ACE-III- Addenbrooke's Cognitive Examination-III; BDAE- Boston diagnostic Aphasia Examination; BNT-Boston Naming Test; CFT- Complex Figure Test; PALS – Progressive Aphasia Language Scale; WAB- Western Aphasia Battery; TMT-Trail Making Test; VOSP- Visual Object and Space Perception Test

*famous face recognition (celebrity recall) and story recall tasks were adapted versions of tasks used in Manchester neuropsychological assessment battery (with permission of Prof. Julie Snowden), see [6]

Supplementary Table S6: The proband's results of Addenbrooke's Cognitive Examination-III (ACE-III) from the age of 69 till the age of 73.

ACE-III	Age at assessment			
	69 y.o.	*71 y.o.*	*72 y.o.*	*73 y.o.*
orientation and attention (max. 18)	15 (83%)	8 (44 %)	4 (22%)	1 (5%)
memory (max. 26)	7 (27%)	8 (31 %)	4 (15%)	0 (0%)
verbal fluency (max. 14)	9 (64%)	5 (36 %)	1 (7%)	0 (0%)
language (max. 26)	13 (50%)	12 (46 %)	9 (35%)	1 (4%)
visuospatial function (max. 16)	15 (94%)	12 (75%)	11 (69%)	7 (44%)
total score (max. 100)	59	45	29	9
M-ACE (max. 30)	17	7	4	0

Supplementary Table S7: The results of detailed neuropsychological assessment in the proband

	69 y.o.	*71 y.o.*	*72 y.o.*	*73 y.o.*
Language function & visual / verbal semantics				
Naming				
BNT / 30	2	0	-	-
ACE-III / 12	3	2	1	0
Verbal fluency (raw scores / 60s.)				
- semantic: animals / fruit	9 / 7	5 / 4	0 / 0	0 / -
fish / vegetables / clothes	1 / 8 / 13	0 / 0 / 10	0 / 0 / 2	-
- phonemic: K / P / S / M	17 / 23 / 24 / -	10 / 13 / 13 / -	3 / 4 / - / 3	0 / - / -/ -
Language Comprehension				
Commands from BDAE / 15	15	15	13	3
Visual and verbal semantics				
Pyramids and Palm tree test:	-	63%	42%	62%
- pictorial version (% correct)	-	71%	65%	58%
- verbal version (% correct)				
Sydney Language Battery:	30%	7%	0%	-
- Naming (% correct)	53%	37%	33%	3%
- Word Comprehension (% correct)	33%	37%	47%	-
- Semantic Association (% correct)				
Repetition				
Sentence repetition from BDAE				-
high frequency / low frequency (max. 8)	7 / 8	7 / 7	7/8	-
Reading				
Word reading from BDAE / 30	30	30	30	30
Sentence reading from BDAE / 10	10	10	10	10
Writing	-			
Primer level dictation from BDAE / 15		15	15	9
Writing words to dictation from BDAE / 10		10	10	4
Written confrontation naming / 10		9	5	-
Other aspects of cognition				
Visuospatial function				
VOSP: Incomplete letters / 20	19	19	9	-
Dot counting /10	10	9	10	9
Position discrimination / 20	20	20	-	-
Number location / 10	9	9	discontinued	-
Cube analysis / 10	8	9	7	5
JoLO / 30	-	-	22	-
CFT copy / 36	34	36	34	-
Working memory				
Digit span – max. forward / backward				discontinued
Corsi – max. span forward	5 / 3	7 / 4	6 / 3	5
TMT A			-	discontinued
TMT B	-	5	139s., >100T	-
	45s.; 57T	120s., >100T	-	
	216s., 85T, 5 errors	234s., 89T, 0 errors		
Episodic memory				
Address recall from ACE-III:				
- learning curve / delayed recall (max. 7)	5-6-7 / 0	4-4-6 / 0	3-4-4 / 0	0-1-0 / 0
- recognition (max. 5)	0	2	0	0
10-word list learning:				
- learning curve	3-5-6-6-7	5-6-3-5-6	5-3-3-3-4	-
- delayed recall	0	0	0	-
Recognition: correct / false positives	6 / 3	5 / 11	7/17	-
CFT – after 3 min. delay	<20T	<20T	-	-
CFT after 30 min. delay	<20T	-	-	-

CFT recognition	<20T	-	-	-
Executive function				
Sorting Weigl blocks	11 / 15*	7 / 9**	-	-
Tower of London – DX-2: total correct SS	-	98	-	-
total move SS	-	≤ 60	-	-
rule violation SS	-	≤ 60	-	-
Calculation				
Serial sevens (max. 14)	-	12	-	-
Calculation from WAB, multiple choice / 24	24	24	22	16***
Motor function and praxis				
9 Hole Peg test	-	-	35s. / 36s. (slowing due to compulsive tendencies)	-
Alternate hand movements	preserved	preserved	preserved	-
Spatial praxis	preserved	preserved	preserved	-
Ideomotor praxis	preserved	preserved	semantic errors	-
Efferent motor praxis:				
- 1st sequence / 2nd sequence (max. 5)	5/ 5	5 / 5	4 / 0	-

ACE-III- Addenbrooke's Cognitive Examination-III; BDAE- Boston diagnostic Aphasia Examination; BNT-Boston Naming Test; CFT- Complex Figure Test; WAB- Western Aphasia Battery; TMT-Trail Making Test; VOSP- Visual Object and Space Perception Test

*attempting 5 sorting criteria; ** attempting 3 sorting criteria; ***written calculation as the patient could not understand the multiple choice procedure

Supplementary Table S8. Human Splicing Finder Analysis of BACE1 wariant, Fig S4 C: Significant alteration of ESE / ESS motifs ratio according to Human Splice Finder Proffesional webtool (https://hsf.genomnis.com/home)[6]
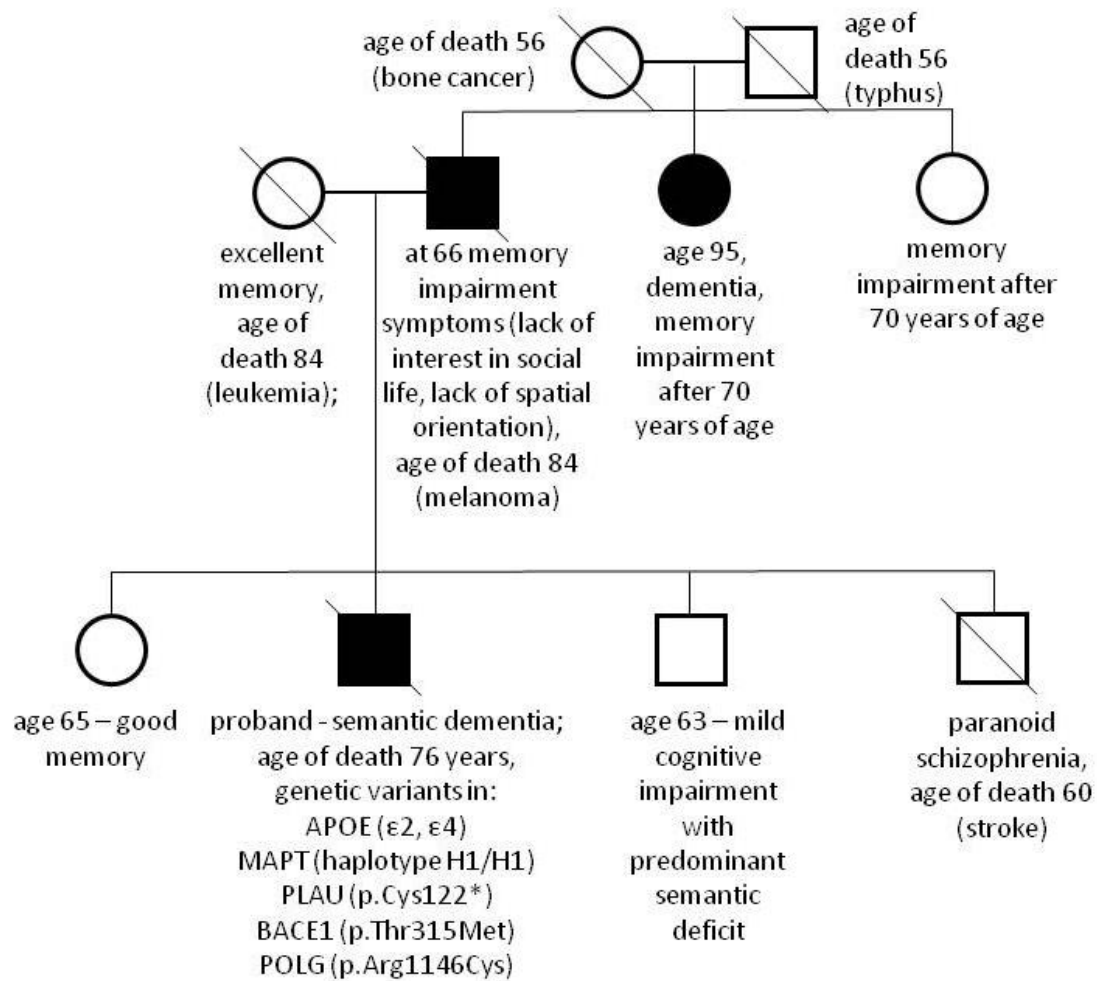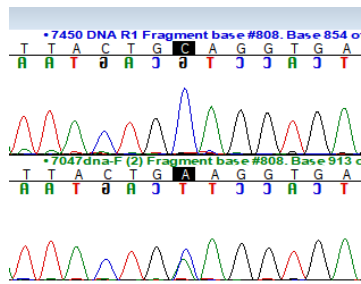
Name	Position	Sequence
ESE_ASF (ESE Site Broken)	chr11:117291048	CGGAGAA
ESE_ASFB (ESE Site Broken)	chr11:117291048	CGGAGAA
PESE (ESE Site Broken)	chr11:117291048	CGGAGAAG
PESE (New ESE Site)	chr11:117291048	TGGAGAAG
RESCUE ESE (New ESE Site)	chr11:117291050	GATGGA
Sironi_motif2 (New ESS Site)	chr11:117291050	GATGGAG
Fas ESS (New ESS Site)	chr11:117291051	AGATGG
EIE (New ESE Site)	chr11:117291052	CAGATG
PESE (ESE Site Broken)	chr11:117291052	CAGACGGA
ESE_9G8 (ESE Site Broken)	chr11:117291053	ACAGAC
ESE_SRp40 (ESE Site Broken)	chr11:117291053	ACAGACG
ESE_ASF (New ESE Site)	chr11:117291054	CACAGAT
Sironi_motif1 (New ESS Site)	chr11:117291055	ACACAGAT

Signal - Alteration of auxiliary sequences   Interpretation - Significant alteration of ESE / ESS motifs ratio (-5)
Alteration of auxiliary sequences Significant alteration of ESE / ESS motifs ratio (-5)
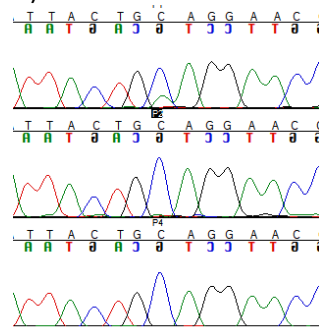
Supplementary Table S9. Human Splicing Finder Analysis of BACE1 wariant, Fig S4 C: Significant alteration of ESE / ESS motifs ratio according to Human Splice Finder Proffesional webtool (https://hsf.genomnis.com/home)[7]

Name	Position	Sequence
ESE_ASF (ESE Site Broken)	chr11:117291048	CGGAGAA
ESE_ASFB (ESE Site Broken)	chr11:117291048	CGGAGAA
PESE (ESE Site Broken)	chr11:117291048	CGGAGAAG
PESE (New ESE Site)	chr11:117291048	TGGAGAAG
RESCUE ESE (New ESE Site)	chr11:117291050	GATGGA
Sironi_motif2 (New ESS Site)	chr11:117291050	GATGGAG
Fas ESS (New ESS Site)	chr11:117291051	AGATGG
EIE (New ESE Site)	chr11:117291052	CAGATG
PESE (ESE Site Broken)	chr11:117291052	CAGACGGA
ESE_9G8 (ESE Site Broken)	chr11:117291053	ACAGAC
ESE_SRp40 (ESE Site Broken)	chr11:117291053	ACAGACG
ESE_ASF (New ESE Site)	chr11:117291054	CACAGAT
Sironi_motif1 (New ESS Site)	chr11:117291055	ACACAGAT

Signal - Alteration of auxiliary sequences   Interpretation - Significant alteration of ESE / ESS motifs ratio (-5)
Alteration of auxiliary sequences Significant alteration of ESE / ESS motifs ratio (-5)

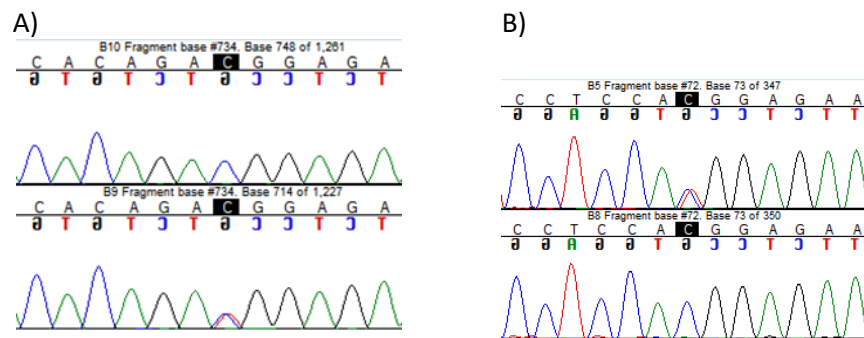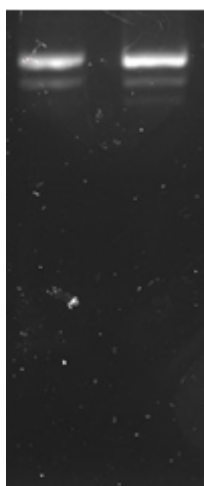**Supplementary Figure S1**: Family history of the patient;

A)

B)

Supplementary Figure S2. Sanger sequencing of patient's DNA and cDNA, A) *PLAU* - DNA from control individual and the patient (upper and lower panel, respectively); B): *PLAU* – patient's cDNA after puromycine treatment showing a re-appeareance of the allele with the PLAU mutation (upper panel), cDNA without treatment from the patient (middle panel) and from control individual (lower panel);

A)

B)

Supplementary Figure S3. Sanger sequencing of: A) *BACE1* - DNA from control individual and the patient (upper and lower panel, respectively); B) *BACE1* – patient's cDNA (upper panel); cDNA from the control individual (lower panel)

Supplementary Figure S4. Agarose gel electrophoresis of the PCR products for patient's cDNA and control's cDNA with BACE1 primers 3F-9R.

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