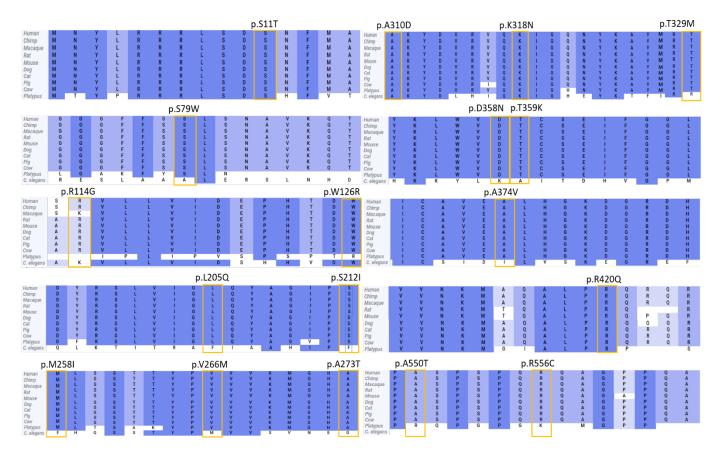


## Supplementary Material



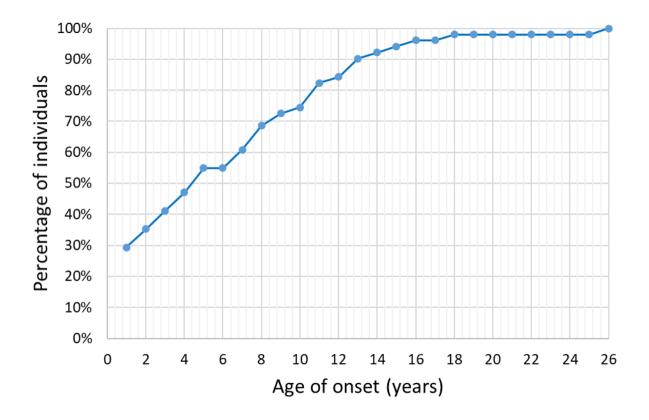
## Supplementary Figure 1. Conservation of the affected amino acids across different species.

Alignment of the SYN1 protein sequence in 11 species (human, chimp, macaque, rat, mouse, dog, cat, pig, cow, platypus, and C. elegans) was performed with the Alamut Visual Plus<sup>TM</sup> software. This analysis shows that almost all residues affected by likely pathogenic or VUS missense substitutions are evolutionary conserved. p.Arg114 represents the only exception.

	p.S11T			p.A310D p.K318N p.T329M	
SYN3	-MNFLRRRLSDSSFMANLPNGYMTDLQRPDSSTSSPASPAME	RR 43	SYN3	VVAMAKTYATTEAFIDSKYDIRIQKIGSNYKAYMRTSISGNWKANTGSAMLEQVAMTERY 332	
SYN1a	-MNYLRRRLSDSNFMANLPNGYMTDLQRPQPPPPPGAHSPGATPGF		SYN1a	VVALTKTYATAEPFIDAKYDVRVQKIGQNYKAYMRTSVSGNWKTNTGSAMLEQIAMSDRY 353	
SYN1b	-MNYLRRRLSDSNFMANLPNGYMTDLQRPQPPPPPGAHSPGATPGF		SYN1b	VVALTKTYATAEPFIDAKYDVRVQKIGQNYKAYMRTSVSGNWKTNTGSAMLEQIAMSDRY 35	
SYN2a	MMNFLRRRLSDSSFIANLPNGYMTDLQRPEPQQPPPPPPGPGAASASAAF		SYN2a SYN2b	VVALTQTYATAEPFIDSKYDIRVQKIGNNYKAYMRTSISGNWKTNTGSAMLEQIAMSDRY 35: VVALTQTYATAEPFIDSKYDIRVQKIGNNYKAYMRTSISGNWKTNTGSAMLEQIAMSDRY 35:	
SYN2b	MMNFLRRRLSDSSFIANLPNGYMTDLQRPEPQQPPPPPPGPGAASASAAP	PTASPGPER 60	511/20	***********************************	>
	p.S79W	p.R114G		p.D358N p.T359K p.A374V	
SYN3	HPQPLAASFSSPGSSLFS <mark>S</mark> LSSAMKQAPQATSGLMEPPGP	STPIVQRPR 92	SYN3	RLWVDSCSEMFGGLDICAVKAVHSKDGRDYIIEVMDSSMPLIGEHVEEDRQLMADLVVSK 392	1
SYN1a	GVAPA-ASPAAPSPGSSGGGGFFS <mark>S</mark> LSNAVKQTTAAAAATFSEQVGGGSGG		SYN1a	KLWVDTCSEIFGGLDICAVEALHGKDGRDHIIEVVGSSMPLIGDHQDEDKQLIVELVVNK 413	ł
SYN1b	GVAPA-ASPAAPSPGSSGGGGFFSSLSNAVKQTTAAAAATFSEQVGGGSGG		SYN1b	KLWVDTCSEIFGGLDICAVEALHGKDGRDHIIEVVGSSMPLIGDHQDEDKQLIVELVVNK 413	
SYN2a	RPPPASAPAPQPAPTPSVGSSFFS <mark>S</mark> LSQAVKQTAASAGLVDAPAP		SYN2a	KLWVDTCSEMFGGLDICAVKAVHGKDGKDYIFEVMDCSMPLIGEHQVEDRQLITELVISK 413	
SYN2b	RPPPASAPAPQPAPTPSVGSSFFSSLSQAVKQTAASAGLVDAPAP		SYN2b	KLWVDTCSEMFGGLDICAVKAVHGKDGKDYIFEVMDCSMPLIGEHQVEDRQLITELVISK 413	ě.
	p.W126R			p.R420Q	
SYN3	ILLVIDDAHTDWSKYFHGKKVNGEIEIRVEQAEFSELNLAAYVTGGCMVDM	VVRNGTKV 152	SYN3	MSOLPMPGG-TAPSPLRPWAPOIKSAKSPGOAOLGPOLGOPOPRPPPOGGPROA 445	5
SYN1a	VLLVIDEPHTDWAKYFKGKKIHGEIDIKVEQAEFSDLNLVAHANGGFSVDM	EVLRNGVKV 174	SYN1a	MAQALPRORORDASPGRGSHGQTPSPGALPLGRQTSQQPAGPPAQQRPPPQGGPPQP 470	3
SYN1b	VLLVIDEPHTDWAKYFKGKKIHGEIDIKVEQAEFSDLNLVAHANGGFSVDM	EVLRNGVKV 174	SYN1b	MAQALPRQRQRDASPGRGSHGQTPSPGALPLGRQTSQQPAGPPAQQRPPPQGGPPQP 470	3
SYN2a	VLLVVDEPHADWAKCFRGKKVLGDYDIKVEQAEFSELNLVAHADGTYAVDM	QVLRNGTKV 174	SYN2a	MNQLLSRTPALSPQRPLTTQQPQSGTLKDPDSSKTPPQRPPPQGGPGQP 462	
SYN2b	VLLVVDEPHADWAKCFRGKKVLGDYDIKVEQAEFSELNLVAHADGTYAVDM		SYN2b	MNQLLSRTPALSPQRPLTTQQPQSGTLKDPDSSKTPPQRPPPQGCLQYI 462	2
	:***:*: *:* <mark>*</mark> :* *:***: *: :*:*******:**:***	* * *** **		* * * * * * * * *****	
	p.L205Q p.S212I			p.A550T p.R556C	
SYN3	VSRSFKPDFILVRQHAYSMALGEDYRSLVIGLQYGGLPAVNSLYSVYNFCSI		SYN3	504	£
SYN1a	V-RSLKPDFVLIRQHAFSMARNGDYRSLVIGLQYAGIPSVNSLHSVYNFCD		SYN1a	GRQSRPVAGGPGAPPAARPPASPSPQRQAGPPQATRQTSVSGPAPPKASGAPPGGQQRQG 589	)
SYN1b	V-RSLKPDFVLIRQHAFSMARNGDYRSLVIGLQYAGIPSVNSLHSVYNFCD		SYN1b	GRQSRPVAGGPGAPPAARPPASPSPQRQAGPPQATRQTSVSGPAPPKASGAPPGGQQRQG 589	
SYN2a	V-RSFRPDFVLIRQHAFGMAENEDFRHLIIGMQYAGLPSINSLESIYNFCD		SYN2a	GPSLPPSSSSSSSSSSSSSSSS	
SYN2b	V-RSFRPDFVLIRQHAFGMAENEDFRHLIIGMQYAGLPSINSLESIYNFCD		SYN2b	S 478	
	p.M2581 p.V266M p.A273T				
SYN3	KIFHSLGPEKFPLVEQTFFPNHKPMVTAPHFPVVVKLGHAHAGMGKIKVEN	DLDFODITS 272			
SYN1a	RLHKKLGTEEFPLIDQTFYPNHKEMLSSTTYPVVVKMGHAHSGMGKVKVDNG				
SYN1b	RLHKKLGTEEFPLIDQTFYPNHKEMLSSTTYPVVVKMGHAHSGMGKVKVDNC			Likelu nathagania	
SYN2a	AIYKTLGGEKFPLIEQTYYPNHKEMLTLPTFPVVVKIGHAHSGMGKVKVENH			VUS Likely pathogenic	
SYN2b	AIYKTLGGEKFPLIEQTYYPNHKEMLTLPTFPVVVKIGHAHSGMGKVKVENH				
	····** *·***··*** ***** **************	****			

## Supplementary Figure 2. Conservation of the affected amino acids across synapsin isoforms.

Alignment of the protein sequence of all synapsin isoforms (SYN1a, SYN1b, SYN2a, SYN2b, SYN3) was performed with ClustalOmega. VUS missense substitutions are highlighted in yellow and likely pathogenic variants in red. Eleven missense variants (p.S11T, p.S79W, p.W126R, p.M258I, p.V266M, p.A273T, p.K318N, p.T329M, p.D358N, p.T359K, and p.A374V) alter residues that are conserved across all synapsin isoforms. The following substitutions affect amino acids that are common to two out of three synapsins: p.L205Q (SYN1 and SYN3), p.S212I (SYN1 and SYN2), p.R420Q (SYN1 and SYN2), and p.R556C (SYN1 and SYN3). The two remaining missense variants, p.A310D and p.A550T, affect residues that are specific to SYN1a and SYN1b.



**Supplementary Figure 3. Trajectory of epilepsy onset in individuals with** *SYN1* **variants.** Seizures onset ranges from six months to 26 years of life in individuals with *SYN1* variants, with a median age of onset of seven years. For those patients for whom seizures are reported, seizures manifest for the first time before the second year of life in 29% of the cases, and within the first five years of life in 55% of the cases. Before the 13<sup>th</sup> year of age, 90% of the individuals has had the first seizure.