NEK1 VARIANTS IN FRENCH PATIENTS WITH SPORADIC AND FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS.

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Background:

Variants in *NEK1* (NIMA-related kinase 1) are associated with amyotrophic lateral sclerosis (ALS) (1-3). *NEK1* encodes a multifunctional serine/threonine kinase involved in key cellular processes, including DNA damage response, mitochondrial homeostasis, and microtubule dynamics. Identified in both sporadic and familial ALS cases, the precise contribution of *NEK1* variants as genetic risk factors or causal mutations remains to be fully elucidated (3). In this study, we aimed to characterize the *NEK1* variants identified in a large French cohort of ALS patients.

Material and methods:

Blood samples were collected between 1994 and 2024 across 22 French ALS Reference Centers. The cohort comprised 550 ALS patients (300 familial index cases and 250 sporadic cases), all negative for *C9orf72* repeat expansions. Whole-exome sequencing (WES) was performed using standard protocols. WES data were screened for NEK1 variants with a minor allele frequency (MAF) < 0.005% in dbSNP and gnomAD databases (1). Clinical data were retrieved from medical records.

Preliminary results

WES of a large cohort of 450 french ALS patients → *NEK1 variants selection* → **Sanger validation** | **In silico predictions** | **Familial segregation** | **Clinical description**

I. *NEK1* variants identified in the French cohort of ALS patients

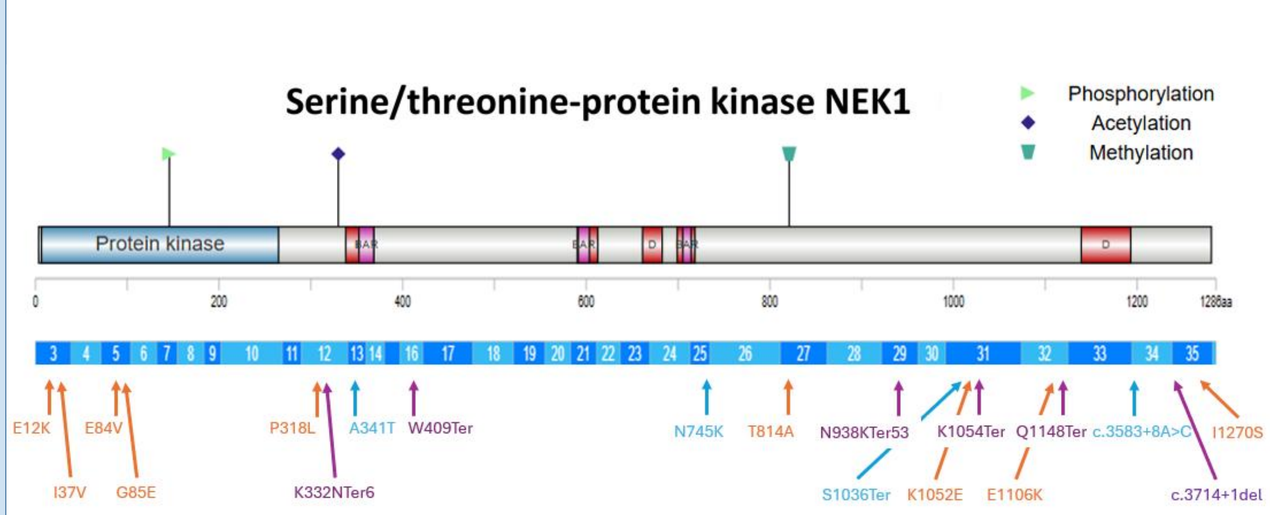

Figure 1 : Representation of the *NEK1* protein (Q96PY6) showing the positions of the variants found in French ALS patients. Rare missense variants are shown in orange, rare loss-of-function (LoF) variants are shown in violet, and frequent variants are shown in blue. Adapted from https://ibs.renlab.org

II. Sanger validation and pedigree of the NEK1 p.E84V index case

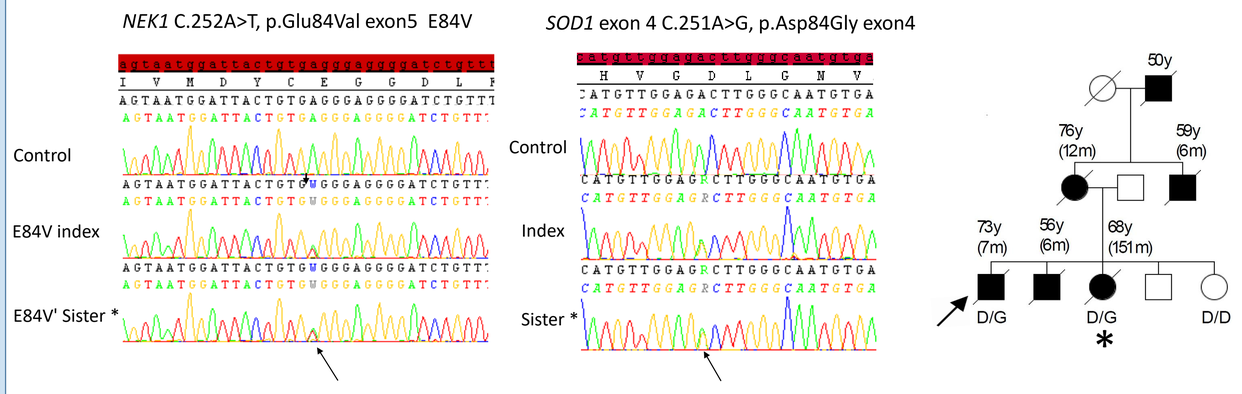

Figure 2 : Sanger validation and pedigree of the NEK1 p.E84V index case. The index patient harbouring the E84V variant carries also a SOD1 mutation we reported in Millecamps et al. (12). Both mutations were found in her affected sister (*).

III. Prediction of pathogenicity of the identified *NEK1* variants

Exon	Rare variants	Variant	Rs	Alt. Allel (%)	CADD	philoP	Polyphen	SIFT	Mutation taster	ALPH	Literature (references)
3	c.34G>A*	E12K	NA	0	27,3	NA	NA	D	B	LP	This study
3	c.109A>G*	I37V	NA	0	24,8	NA	NA	D	B	LB	4
5	c.251A>T*	E84V	rs754427159	0.00033	30	6.63	0.996	D	D	LP	This study
5	c.254G>A*	G85E	NA	0	28,3	NA	NA	D	D	LP	This study
12	c.953C>T	P318L	rs745711143	0.00067	25,6	8.79	0.996	D	B	A	This study
12	c.996_1000delGAAAC*	K332NTer6	rs1204331228	0	29,7	5.84	NA	NA	NA	NA	This study
16	c.1226G>A*	W409Ter	rs985064686	0.00144	41	8.88	NA	NA	NA	NA	This study
27	c.2440A>G*	T814A	rs939746889	0.00008	23,5	3.65	0.727	T	B	LB	This study
29	c.2814_2817del*	N938KTer53	rs752878896	0.00169	23,4	0.913	NA	NA	NA	NA	6
31	c.3154A>G*	K1052E	rs1226404048	0.00016	23,1	4.88	0.547	D	B	LB	This study
31	c.3160A>T*	K1054Ter	rs1275873455	0	25,1	3.67	0.496	NA	NA	NA	This study
32	c.3316G>A*	E1106K	NA	0			NA		B	NA	This study
33	c.3442C>T	Q1148Ter	NA	0	NA	NA	NA	NA	NA	LB	This study
intron 34	c.3714+1del *	MaxEnt 100%	NA	0	NA					LP	This study
35	c.3809T>G	I1270S	rs778919560	0.00259	28,9	6.33	0.979	D	B	NA	6,7
	Rare variants										
13	c.1021G>A*	A341T	rs189186475	0.3626	24,3	2.35	0.191	T	B	LB	4, 7, 8,9
26	c.2235T>G	N745K	rs34324114	0.5952	26,2	1.05	0.999	D	NA	NA	2, 9
31	c.3107C>G*	S1036Ter	rs199947197	0.2772		1.40	NA	NA	NA	LP	2, 4, 5, 8, 10
intron 33	c.3583+8A>C*	MaxEnt 0%	rs192372022	0.0462	NA	-0.537	NA	NA	NA	NA	This study

Figure 3 : Pathogenicity predictions of the variants of interest. Predictions were made using Alamut Visual Plus software and AlphaMissense (https://alphamissense.hegelab.org). * Validated in Sanger. Alt. Allel: Allele alternative frequency in gnomAD v.4.1.0. for European non finnish population. D: Deleterious, T: Tolerated, B: Benign. LP: likely pathogenic, LB: likely benign, A: Ambigous. ALPH: AlphaMissense. NA: not available.

IV. Clinical characteristics of ALS patients with *NEK1* variants

Rare variants	Variant	ALS	Sex	Age at onset	Site of onset	Disease duration	Other genes	variant	Other genes	variant
c.34G>A*	E12K	Fam	M	44	bulbar	11	*FUS*	R520C	*PON1*	D124N
c.109A>G*	I37V	Spo	M	21	LL	32				
c.251A>T*	E84V	Fam	M	72	UL	7	*SOD*	D84G	*GLE1*	H600R
c.254G>A*	G85E	Fam	F	49	UL	193			*DNAJB2*	D66E
c.953C>T	P318L	Fam	F	52	UL	40			*KIF1B*	I1569T
c.996_1000delGAAAC*	K332NTer6	Spo	M	52	UL	122				
c.1226G>A*	W409Ter	Fam	F	71	UL	77			*IGHMBP2*	R941W
c.2440A>G*	T814A	Fam	M	23	LL	14				
c.2814_2817del*	N938KTer53	Fam	M	56	UL	21				
c.3154A>G*	K1052E	Fam	F	73	UL	30				
c.3160A>T*	K1054Ter	Fam	M	67	UL	66				
c.3442C>T	Q1148Ter	Fam	M	69	UL	7			*ZFYVE26*	P72L
c.3316G>A*	E1106K	Fam	M	71	UL	39			*ATXN2*	22/31
c.3809T>G	I1270S	Fam	M	57	bulbar	72			*CPT1C*	H364Y
c.3809T>G*	I1270S	Fam	M	52	NA	17				
c.3809T>G*	I1270S	Fam	M	76	UL	12			*DNAJB2*	R132Ter
c.3714+1del *		Spo	M	33	UL	38				
Frequent variants										
c.1021G>A*	A341T	Fam	M	77	LL	14			*CSF1R*	c.-181+1G>T
c.1021G>A*	A341T	Spo	F	46	LL	52	*DNAJC7*	A131T		
c.3583+8A>C*		Fam	F	79	LL	18				
c.2235T>G	N745K	Spo	F	56	bulbar	27				
c.3107C>G*	S1036Ter	Spo	F	26	bulbar	11			*BSCL2*	L427P
c.3107C>G*	S1036Ter	Spo	M	74	LL	44				
c.3107C>G	S1036Ter	Fam	M	64	LL	260				

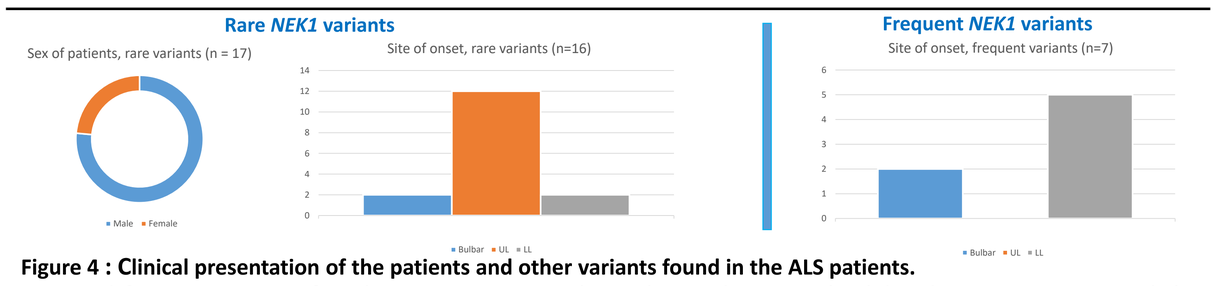

Figure 4 : Clinical presentation of the patients and other variants found in the ALS patients.
Rare and frequent variants found in our ALS patients' population. * : Sanger' validated variants. ALS : Familial history of ALS, Fam : familial ALS, Spo : sporadic ALS. M : Male, F : Female. UL : Upper Limbs, LL / Lower Limbs. Disease duration in months. NA : non available.

Results :

- We identified:
  - 15 rare *NEK1* variants in 17/550 patients (4,7% in familial cases and 1,2% in sporadic cases)
  - 3 frequent variants in 7 patients, predominantly sporadic cases.
- For the rare variants: Twelve (80%), have not been reported in the literature. Five (33%) lead to a loss-of-function.
- Of the 17 patients with rare variants:
  - Four were females (23%), 14 (82%) had a familial history of ALS.
  - Age at onset ranged from 21 to 76 years, mean: 55 years; versus a mean age of 60 years for patients with frequent variants (26-77).
  - Fifteen patients (88%) exhibited spinal onset, with upper-limbs onset being the most frequent site of onset (71%). Two patients had bulbar onset (males, mean onset age: 50 years).
  - Disease duration ranged from 7 months to 16 years, with most patients displaying typical survival patterns, mean duration: 48 months; versus mean duration of 60 months for patients with frequent variants (14-260).
  - Two patients carried an additional pathogenic variant in an ALS gene (*SOD1* or *FUS* respectively).

Discussion :

- We found rare *NEK1* variants in French ALS patients in a proportion similar to that observed in other European cohorts (2,3). Interestingly, the already described predominance of upper limb onset was observed among carriers of rare variants (3), suggesting a potential clinical characteristic associated with *NEK1* dysfunction.
- The *NEK1* p.E84V case, as well as his affected sister, carried also another pathogenic variant, in the *SOD1* gene. The observed discordance in survival: fast progressing disease in the brother and slow progressive disease in the sister, can therefore not explained by this genotypes.
- The coexistence of both rare truncating and missense variants, as well as the double mutations found in some patients support the hypothesis that *NEK1* contributes to ALS susceptibility, probably to a variable extend depending on the variant. The presence of *NEK1* variants in patients also carrying other ALS-related mutations (e.g., *SOD1*, *FUS*) highlights the complexity of ALS genetics and supports a possible oligogenic model, where multiple variants contribute to disease risk and heterogeneity.
- Further functional studies are needed to clarify the impact of specific *NEK1* variants on its expression, splicing, kinase activity and its cellular consequences. Expanding genetic analyses to larger cohorts and study of biosamples of affected carriers will be essential to better define the pathogenicity of *NEK1* variants and its possible interaction with other ALS genes.

Conclusion :

Rare *NEK1* variants are present in the French ALS population at a frequency comparable to that reported in other cohorts (2-4). Upper limb onset appears to be more frequently associated with *NEK1* variants. Further research is warranted to clarify the functional impact of these variants, and the role of *NEK1* in the complex genetic landscape of ALS.

References

1. Cirulli ET, et al. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Science. 2015 Mar27;347(6229):1436-41
2. Brenner D, et al. NEK1 mutations in familial amyotrophic lateral sclerosis. Brain. 2016 May;139(Pt 5):e28
3. Jiang Q, et al. Genetic and clinical characteristics of ALS patients with NEK1 gene variants. Neurobiol Aging. 2023 Mar;123:191-199
4. Nguyen HP, et al. NEK1 genetic variability in a Belgian cohort of ALS and ALS-FTD patients. Neurobiol Aging. 2018 Jan;61:255.e1-255.e7
5. Pensato V, et al. Exploring NEK1 genetic variability in Italian amyotrophic lateral sclerosis patients. J Neurol. 2025 Jun 19;272(7):469
6. Nguyen HP, et al. NEK1 genetic variability in a Belgian cohort of ALS and ALS-FTD patients. Neurobiol Aging. 2018 Jan;61:255.e1-255.e7
7. Gratten J, et al. Whole-exome sequencing in amyotrophic lateral sclerosis suggests NEK1 is a risk gene in Chinese. Genome Med. 2017 Nov 17;9(1):97
8. Lattante S, et al. Novel variants and cellular studies on patients' primary fibroblasts support a role for NEK1 missense variants in ALS pathogenesis. Hum Mol Genet. 2021 Mar 25;30(1):65-71
9. Kenna KP, et al. NEK1 variants confer susceptibility to amyotrophic lateral sclerosis. Nat Genet. 2016 Sep;48(9):1037-42
10. Black HA, et al. Genetic epidemiology of motor neuron disease-associated variants in the Scottish population. Neurobiol Aging. 2017 Mar;51:178.e11-178.e20. doi: 10.1016/j.neurobiolaging.2016.12.013. Epub 2016 Dec 21. Erratum in: Neurobiol Aging. 2017 Aug;56:214
11. Wang H, et al. NEK1-mediated retromer trafficking promotes blood-brain barrier integrity by regulating glucose metabolism and RIPK1 activation. Nat Commun. 2021 Aug 10;12(1):4826
12. Millecamps Set al. SOD1, ANG, VAPB, TARDBP, and FUS mutations in familial amyotrophic lateral sclerosis: genotype-phenotype correlations. J Med Genet. 2010 Aug;47(8):554-60.

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