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RESEARCH ARTICLE

Hand-onset weakness is a common feature of ALS patients with a *NEK1* loss-of-function variant

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Abstract

Objective: The *NEK1* gene has been recently implicated in amyotrophic lateral sclerosis (ALS). This study aims to assess the influence of *NEK1* variants on the occurrence of ALS and investigate the spectrum and clinical features of *NEK1* loss-of-function (LOF) variants in a Taiwanese ALS cohort. **Methods**: We screened 325 unrelated ALS patients for coding variants in *NEK1* by targeted resequencing and queried the Taiwan Biobank database for *NEK1* coding variants in 1000 Taiwanese healthy individuals. The clinical features of the patients with a *NEK1* LOF variant were analyzed. **Results**: Six patients and two healthy individuals carried *NEK1* LOF variants. The rare missense variants with minor allele frequencies <0.1% in Taiwanese population were present in 2.8% of the ALS patients and 1.6% of the healthy subjects. *NEK1* LOF variants, but not rare missense variants, are significantly enriched in the ALS patients ($P = 0.0037$ and 0.24, Fisher's exact test). The odds ratio of an individual carrying a *NEK1* LOF variant to develop ALS is 9.39 (95% confidence interval: 1.88–46.7). All the six patients carrying a *NEK1* LOF variant had a hand-onset ALS with an onset age from 52 to 64 years. Comparing with ALS patients without a *NEK1* LOF variant, patients with a *NEK1* LOF variant tend to have a hand-onset disease ($P = 0.0008$, Fisher's exact test). **Interpretation**: Our study supports the pathogenic role of *NEK1* LOF variants and demonstrates their spectrum and clinical features in a Taiwanese cohort with ALS.

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder affecting both upper and lower motor neurons, resulting in constantly progressive weakness and muscle atrophy, bulbar function impairment and consequently respiratory failure with a short survival of 3–5 years.[1] Approximately 10% of ALS patients inherit their diseases form their parents, indicating that genetic factors substantially contribute to ALS pathogenesis. To date, more than 30 genes have been implicated in ALS pathogenesis. But only a few of them, including *C9ORF72*, *SOD1*, *FUS*, *TARDBP*, *VCP*, and *TBK1*, have been clearly demonstrated to account for a significant number of patients with ALS.[2–4] The roles of several newly identified ALS disease genes, such as *NEK1*,[5–8] are yet to be fully understood because the relevant studies are relatively sparse.

The *NEK1* gene has been recently implicated in amyotrophic lateral sclerosis (ALS). Loss-of-function (LOF) variants in *NEK1* were firstly found strongly associated with ALS in a large-scale whole-exome sequencing study. Cirulli et al. analyzed and compared the whole-exome data of 2843 ALS patients with those of 4310 controls, and additionally verified their findings using a replication cohort consisting of 1318 ALS cases and 2371 controls.[5] They found that dominant *NEK1* LOF variants were present in 0.835% of cases and 0.091% of controls, suggesting *NEK1* as a novel ALS gene with a *P*-value of 3.2×10^{-9}.[5] The strong association between *NEK1* and

ALS was further confirmed in another study by Kenna et al.[6] They performed whole-exome analyses in 1022 index cases of familial ALS (FALS) and 7315 controls, and identified a significant association between *NEK1* LOF variants and FALS risk. Moreover, they found the *NEK1* p.Arg261His variant also as a risk factor of ALS.[6] *NEK1* encodes the never in mitosis A (NIMA)-related kinase 1 (Nek1), which is a member of Serine/threonine-protein kinase Nek family. The functions of Nek1 include cell cycle control, DNA double-strand break repair, ciliogenesis and mitochondrial membrane permeability regulation.[9–11] In the modulation of the DNA damage repair pathway, Nek1 would interact with the chromosome 21 open reading frame 2 (C21orf2) protein,[12] which is encoded by the ALS risk gene *C21ORF2*.[13] Interestingly, proteomic analysis showed that Nek1 was associated with ALS2 and VAPB proteins,[5] the corresponding genes of which are both well-known ALS disease genes.[2–3]

To further understand the role of *NEK1* in ALS, we screened 325 unrelated ALS patients for coding variants in *NEK1* by targeted resequencing and queried the Taiwan Biobank database for *NEK1* coding variants in the 1000 Taiwanese healthy individuals. We analyzed the influence of *NEK1* variants on the occurrence of ALS. In addition, we also demonstrated the clinical features, the frequency and spectrum of *NEK1* LOF variants in a Taiwanese cohort with ALS.

Methods

Patients

Three hundred and twenty-five unrelated individuals with ALS diagnosed by the revised EL Escorial criteria were enrolled into this study.[14] All participants were of Han-Chinese descent and were recruited from the Neurology Service of Taipei Veterans General Hospital, Taiwan. Among the 325 ALS patients, 17 have a *SOD1* mutation, 15 have a *C9ORF72* GGGGCC hexanucleotide expansion, 12 have a *TARDBP* mutation, seven have a *FUS* mutation, two have a *CCNF* mutation, two have an *ATXN2* intermediate-length CAG repeat expansion (32 and 33 repeats) and another three carry a single mutation in *OPTN*, *MATR3*, or *TBK1* each (Table S1). The remaining 267 patients were not found to have any pathogenic mutation after screening 17 ALS causal genes, including *SOD1, C9ORF72, TARDBP, FUS, ATXN2, OPTN, VCP, UBQLN2, SQSTM1, PFN1, HNRNPA1, HNRNPA2B1, MATR3, CHCHD10, TUBA4A, TBK1,* and *CCNF*.[15–18] The mean age at disease onset of the ALS cohort was 54.3 years (range 19–89). Thirty-nine patients (12%) had a family history of ALS. Seventy patients (21.5%) were affected by a bulbar-onset ALS and 140 patients (43.1%) had an upper limb-onset disease. Hand-onset ALS was reported in 101 patients (31.1%) and was defined as ALS with the first sign appearing in the hand, usually presenting as difficulty with simple tasks such as buttoning a shirt, using chopsticks, or writing. Peripheral blood samples were collected from the patients after obtaining written informed consents. The protocols for this study were approved by the Institutional Review Board of Taipei Veterans General Hospital.

Genetic analyses

Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Mutational analysis of *NEK1* was performed by utilizing a targeted resequencing panel covering the whole-coding regions of *NEK1*. The samples were sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA). All sequenced reads were mapped to the Human Genome version 19 (hg19/GRCh37). The BaseSpace pipeline (https://basespace.illumina.com/) and the IlluminaVariantStudio software (http://variantstudio.software.illumina.com/) were utilized to do variant calling and annotate variants, respectively. The reference coding sequence of *NEK1*, NM_001199397.3, was used for annotating the variants.

For the *NEK1* coding variants identified in the ALS patients, we first checked their frequencies in general populations using the Taiwan Biobank database (TaiwanView: taiwanview.twbiobank.org.tw/search) and the genome Aggregation Database (gnomAD, version r2.0.2; http://gnomad.broadinstitute.org). Taiwan Biobank is a nationwide research database formed to collect blood samples and biomedical information of 200,000 Taiwanese participants using a population-based design.[19] Taiwan Biobank has released whole-genome sequence data generated by Illumina Hiseq platforms from 1000 unrelated Taiwanese healthy people. The presence of LOF variants and rare missense variants were further confirmed by Sanger sequencing on the patients' genomic DNA. The LOF variants were defined as nonsense, frameshift, initiation codon lost, and splice donor/acceptor sites variants, and the rare missense variants were designated for those with a minor allele frequency (MAF) lesser than 0.1% in the 1000 Taiwanese control genomes. Then, we calculated the overall frequencies of all the *NEK1* LOF variants and rare missense variants identified in the 1000 healthy Taiwanese genomes from the Taiwan Biobank database as well as those identified in the ALS patients. Fisher's exact tests were utilized to compare the distributions of *NEK1* LOF variants or rare missense variants between the ALS patients and the healthy control genomes, respectively. The frequencies of hand-onset diseases were also

compared between the ALS patients with and without a *NEK1* LOF mutation. The odds ratio (OR) and 95% confidence interval (CI) was calculated to estimate the risk for an individual carrying a *NEK1* LOF mutation or a rare missense mutation to develop ALS.

Results

Genetic analyses

Mutational analyses of *NEK1* in the 325 ALS patients revealed six LOF variants in six (1.8%) individuals and eight rare missense variants in nine (2.8%) patients (Table 1).

The six *NEK1* LOF variants include c.1127_1128delAA (p.Lys376Thrfs*7), c.1491delT (p.Pro498Leufs*10), c.1897dupA (p.Ile633Asnfs*28), c.1992delA (p.Val665Cysfs*34), c.2640delA (p.Val881Tyrfs*8), and c.2588-2A > G (Fig. 1). Among these LOF variants, *NEK1* p.Val665Cysfs*34 was present in a homozygous form in the patient and all the others are heterozygous. Two patients carrying the same *NEK1* rare missense variant, p.Arg608Cys, also had a heterozygous *TARDBP* p.Met337Val mutation. All the other thirteen patients with *NEK1* variants did not have any known mutation in other ALS disease genes.

From the genomic data of 1000 Taiwanese healthy individuals in the Taiwan biobank, we found two LOF variants in two (0.2%) individuals and 16 rare missense variants in 16 (1.6%) individuals (Table 1). Comparing the frequencies of *NEK1* LOF variants or rare missense variants in the ALS patients with those in the 1000 Taiwanese individuals, *NEK1* LOF variants but not rare missense variants are significantly enriched in the ALS patients ($P = 0.0037$ and 0.24, respectively). The odds ratio for an individual carrying a *NEK1* LOF variant to develop ALS is 9.39 (95% confidence interval: 1.88–46.7). In contrast, harboring a *NEK1* rare missense variant did not significantly increase the risk to develop ALS (odds ratio: 1.75, 95% confidence interval: 0.77–4).

Clinical information of the patients carrying NEK1 LOF variants

The clinical and genetic features of the six ALS patients harboring *NEK1* LOF variants are summarized in Table 2. These patients neither suffered from dementia nor had a family history of ALS. Patient P507 was heterozygous for the *NEK1* p.Lys376Thrfs*7 mutation. Her disease started with left hand weakness at age 63 years. The symptoms deteriorated rapidly, and she developed four limbs weakness within 1 year. Neurological examinations at age 65 years revealed weakness and atrophy with fasciculation in bilateral upper extremities (muscle strength of 4- to 4+/5 according to the Medical Research Council-scale), a milder degree of involvement in the bilateral lower limbs (4 to 4+/5), diminished deep tendon reflexes (DTRs), and normal cognitive function. The motor symptoms were more severe on left side limbs and distal parts. Her scores on the Amyotrophic Lateral Sclerosis Functional Rating Scale-revised (ALSFRSr) were 33 at age 65 years and 22 at age 66 years. She died of respiratory failure at age 67 years.

Patient E739 had a heterozygous *NEK1* p.Pro498Leurfs*10 mutation with an onset symptom of right hand weakness at age 56 years. The symptoms progressed to bilateral upper limbs, bulbar region, and then lower limbs sequentially within 2 years. He received tracheostomy at age 59 years and died of an out-of-hospital cardiac arrest at age 64 years.

Patient M797 was heterozygous for the *NEK1* p.Ile633Asnfs*28 mutation. He had a disease onset at age 54 years with left hand weakness. The symptoms rapidly extended to bilateral upper limbs, bulbar region, and then lower limbs within 6 months. Neurological examinations at age 55 years revealed severe weakness and atrophy in bilateral upper extremities (2 to 3/5), a milder degree of involvement in the bilateral lower limbs (4 to 4+/5), tongue atrophy with fasciculation, dysarthria, brisk DTRs in the upper limbs and normal knee and ankle DTRs. He died of respiratory failure at age 55 years.

Patient P399 had a heterozygous *NEK1* p.Val881Tyrfs*8 mutation and the disease began with weakness in the right hand and fingers at age 64 years. The weakness progressed to proximal right upper limb first and then right lower limb within 2 years. Neurological examinations at age 66 years revealed Mills syndrome, presenting with severe weakness in the right upper limb (0 to 1/5) and moderate weakness in the right lower limb (4- to 4/5), atrophy over right hand, increased deep tendon reflexes, right extensor plantar response, and normal cognitive function. Cervical MRI at age 66 showed mild cervical spondylosis, which mainly affected C5-6 level without cord or root compression. His ALSFRSr score was 33. The motor symptoms spread to left side limbs presenting with mild weakness (4+/5) within 4 months after first evaluation. Then, he was lost to follow-up for a period and died of pneumonia at age 68 years.

Patient Q849 is homozygous for the *NEK1* p.Val665Cys*34 mutation. He had a disease onset at the age of 55 years with left hand weakness. Neurological examinations at 6 months after the disease onset revealed weakness and atrophy in bilateral upper limbs, which was more severe in distal parts of extremities (4- to 4+/5), and decreased deep tendon reflexes. He had dyspnea on exertion at age 56 years and received tracheostomy with ventilator support soon at the 17th month after the disease onset. His ALSFRSr scores were 33 at first evaluation

Table 1. Rare *NEK1* coding variants identified in the study.

cDNA[1]	Predicted protein	dbSNP153	Allele frequency in gnomAD	ALS, *n* = 325	Controls, *n* = 1000	Fisher's exact test
Loss-of-function variants						
Patient-only variants						
c.1127_1128delAA	p.Lys376ThrfsTer7	—	—	1	—	
c.1491delT	p.Pro498LeufsTer10	—	—	1	—	
c.1897dupA	p.Ile633AsnfsTer28	—	0	1	—	
c.1992delA[2]	p.Val665CysfsTer34[2]	rs775849720	2.94E-05	1	—	
c.2640delA	p.Val881TyrfsTer8	rs760894879	1.05E-05	1	—	
Variants present in both patients and controls						
c.2588-2A > G	—	rs201769828	1.19E-04	1	1	
Controls-only variants						
c.1977T > A	p.Tyr659Ter	—	—	—	1	
Total				6 (1.8%)	2 (0.2%)	*P* = 0.0037
Rare missense variants						
Patient-only variants						
c.686A > G	p.Tyr229Cys	rs61737748	3.46E-04	1		—
c.773T > C	p.Ile258Thr	—	—	1		—
c.773T > G	p.Ile258Arg	—	—	1		—
c.1586A > G	p.Glu529Gly	rs781633610	8.20E-06	1		—
c.1822C > T	p.Arg608Cys	rs749461218	1.38E-05	2		—
c.1998G > C	p.Trp666Cys	rs746590014	8.18E-06	1		—
c.2955A > C	p.Gln985His	—	—	1		—
Variants present in both patients and controls						
c.460A > T	p.Asn154Tyr	rs756418625	1.34E-05	1	1	
Controls-only variants						
c.770T > G	p.Phe257Cys	—	—	—	1	
c.1625G > A	p.Arg542Gln	rs1032792680	1.61E-05	—	1	
c.1634T > C	p.Met545Thr	rs760674667	2.1E-05	—	1	
c.1703G > A	p.Arg568Lys	rs1561319838	—	—	1	
c.1769G > A	p.Arg590Lys	—	—	—	1	
c.1823G > A	p.Arg608His	rs551275723	6.46E-05	—	1	
c.1978G > A	p.Glu660Lys	rs1458058029	—	—	1	
c.1982G > A	p.Arg661Lys	—	—	—	1	
c.1984G > A	p.Glu662Lys	—	—	—	1	
c.2183A > C	p.Gln728Pro	—	—	—	1	
c.2195A > G	p.Asn732Ser	rs755259769	1.24E-05	—	1	
c.2287A > C	p.Thr763Pro	—	—	—	1	
c.2736G > A	p.Met912Ile	rs750788096	1.62E-05	—	1	
c.3149C > T	p.Pro1050Leu	—	—	—	1	
c.3289G > A	p.Val1097Ile	rs374890006	8.11E-05	—	1	
Total				9 (2.8%)	16 (1.6%)	*P* = 0.24

ALS, amyotrophic lateral sclerosis; dbSNP153: Single Nucleotide Polymorphism database, build 153; gnomAD, the Genome Aggregation Database; n, numbers of variant carriers in the ALS cohort or 1000 controls from Taiwan Biobank (https://www.twbiobank.org.tw).
[1]The reference coding sequence of *NEK1*, NM_001199397.3, was used for annotating the variants.
[2]In a homozygous form; other variants in the table are heterozygous.

and downhill to 2 at one year later. He denied that his parents had a consanguineous marriage.

Patient H181 had a heterozygous *NEK1* c.2588-2A > G mutation with an onset symptom of weakness in the right thumb at age 52 years. The symptoms progressed to bilateral upper limbs and then bulbar region and lower limbs within 3 years. He received percutaneous endoscopic gastrostomy feeding and tracheostomy ventilation at age 55 years. Neurological examinations at age 62 years revealed total paralysis and atrophy of four limbs (0 to 1/5), severe tongue atrophy with frequent fasciculation, and decreased deep tendon reflexes.

All the six patients carrying *NEK1* LOF mutations had a hand-onset ALS. However, only 101 of the 325 ALS patients in the study had hand weakness as the initial clinical presentation. Comparing with ALS patients

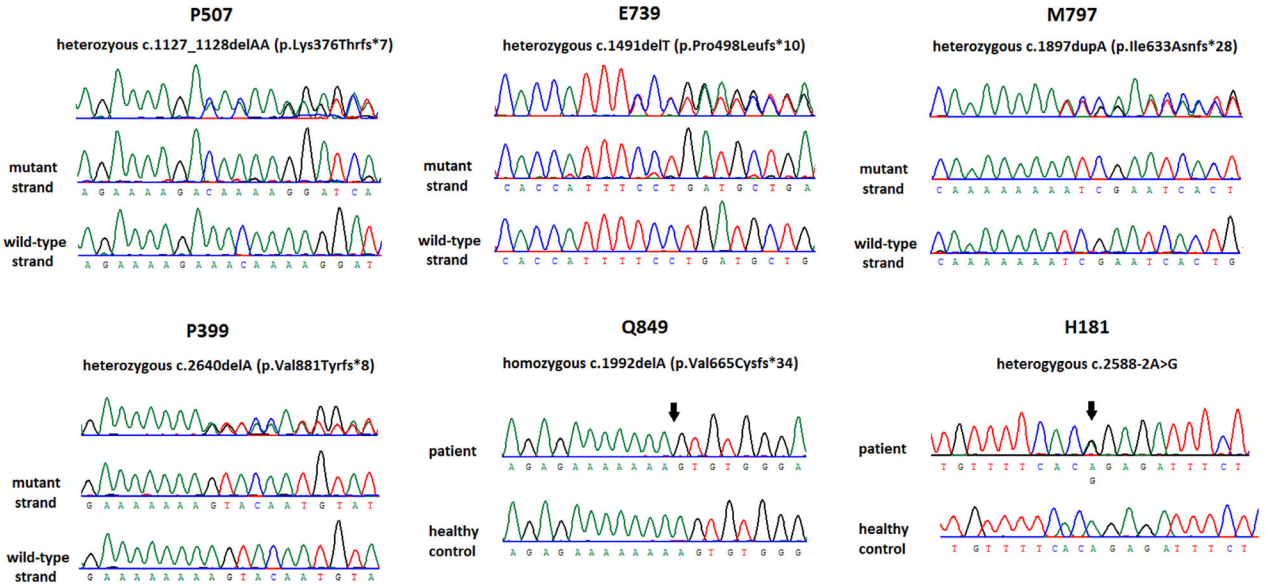

Figure 1. Sanger sequence traces of the six *NEK1* loss-of-function variants identified in the ALS patients in this study, including five frameshift variants and one splice acceptor site variant. The four heterozygous frameshift mutations are clearly demonstrated by sequencing the TA-subcloned PCR fragments. The control sequence traces for the homozygous c.1992delA and heterozygous c.2588-2A > G mutations are also shown. The arrows point to the mutation sites.

Table 2. Genetic and clinical information of the ALS patients with *NEK1* loss-of-function mutations.

Patient	Sex	Mutation	Hetero- or homozygous	Age of onset, y	Site of onset	Age at first evaluation, y	Survival, m	Clinical phenotype	Dementia
P507	F	p.Lys376Thrfs*7	Heterozygous	63	left hand	65	46	LMN	No
E739	M	p.Pro498Leufs*10	Heterozygous	56	right hand	58	98	UMN/LMN	No
M797	M	p.Ile633Asnfs*28	Heterozygous	54	left hand	55	13	UMN/LMN	No
P399	M	p.Val881Tyrfs*8	Heterozygous	64	right hand	66	43	UMN/LMN	No
Q849	M	p.Val665Cys*34	Homozygous	55	left hand	55	>18[1]	LMN	No
H181	M	c.2588-2A > G	Heterozygous	52	right thumb	55	>120	LMN	No

ALS, amyotrophic lateral sclerosis; y, years; m, months; UMN, upper motor neuron signs; LMN, lower motor neuron signs.
[1]> 18 means that more than 18 months have passed since the symptom onset and the patient was still alive on the last follow-up.

without *NEK1* LOF mutations, ALS patients with a *NEK1* LOF mutation tend to have a hand-onset disease (100% vs. 29.8%, $P = 0.0008$). The clinical and genetic information of the ALS patients harboring *NEK1* rare missense variants are summarized in Table S2.

Discussion

In this study, we evaluated the effects of *NEK1* mutations on ALS by comparing the frequencies of *NEK1* LOF variants and rare missense variants in Taiwanese ALS patients with those in healthy individuals. We also characterized the clinical and genetic features of the Taiwanese ALS patients carrying *NEK1* LOF variants. There are four major findings from this study. First, *NEK1* LOF variants are significantly enriched in the Taiwanese ALS patients. Second, harboring a *NEK1* rare missense variant does not significantly increase the risk to develop ALS. Third, approximately two percent of ALS cases in Taiwan may be attributed to *NEK1* LOF variants. Finally, comparing with ALS patients without *NEK1* LOF variants, patients with *NEK1* LOF variants tend to have a hand-onset disease. These findings may have the following implications.

First of all, our study demonstrates a clear association between *NEK1* LOF variants and ALS. In the studied population, Taiwanese individuals with a *NEK1* LOF variant pose a 9.34-fold risk to develop ALS, whereas harboring a *NEK1* rare missense variant does not significantly increase the risk of having ALS. Similar findings were also demonstrated in Caucasian populations recently by several large-scale whole-exome sequencing studies, in which *NEK1* LOF were significantly associated with ALS risks.[5–8] The

association between ALS and *NEK1* LOF mutations in multiple populations suggests that *NEK1* LOF variants are pathogenic and *NEK1* haploinsufficiency may contribute to ALS pathogenesis. It is still unclear how *NEK1* haploinsufficiency links to ALS pathogenic. Further studies are warranted to elucidate the relevant molecular mechanism.

Second, *NEK1* LOF variants may account for a certain number of ALS patients. This study identified *NEK1* LOF variants in six of 325 ALS patients, implying that the prevalence of *NEK1 LOF* variants in Taiwanese ALS patients is 1.8%. Several recent *NEK1* studies revealed that the prevalence of *NEK1* pathogenic variants in sporadic and familial ALS patients ranged from 0.8% to nearly 3% in different populations.[5–7,20,21] Comparing to the prevalence of other disease genes in ALS populations, the frequency of LOF variants in *NEK1* is not rare. The percentage of Taiwanese ALS patients carrying a *NEK1* LOF variant is lower than the rates of ALS patients harboring a mutation in *SOD1*, *C9ORF72*, *TARDBP*, and *FUS*, but higher than those of individuals with mutations in other ALS disease genes.

Third, *NEK1* LOF variants may be pathogenic variants with reduced penetrance for ALS. In this study, lack of family history of the six ALS patients carrying the *NEK1* LOF variants suggests an incomplete penetrance of these variants. Reduced penetrance of *NEK1* LOF variant has also been demonstrated in another study reporting an unaffected sibling of ALS patients carrying the *NEK1* p.Ser1036* variant.[7] Furthermore, the frequencies of healthy controls carrying the *NEK1* LOF variants are 0.2% in the Taiwanese population and 0.06–0.16% in the Caucasian populations.[5–7,20] The relatively high prevalence of *NEK1* LOF variants in control subjects implies that the penetrance of *NEK1* LOF variants is not high. Since ALS is a devastating disease, it is still important to elucidate the *NEK1*-related pathogenic mechanism and develop tailored strategies to prevent and treat ALS in individuals carrying a *NEK1* pathogenic variant.

Fourth, hand-onset weakness seems to be a common feature of ALS patients harboring a *NEK1* LOF variant. Brenner et al. reported four familial ALS patients with *NEK1* LOF variants from three families.[7] Among them, two had a hand-onset disease and another one patient had upper and lower motor neuron signs beginning from left arm.[7] Another study from Beijing China reported three ALS patients with *NEK1* LOF variants and showed that the two patients with available clinical information both had a hand-onset disease.[21] In our ALS cohort, all the six ALS patients carrying *NEK1* LOF variants manifested a hand-onset disease, whereas lesser than one third of the remaining 319 ALS patients without a *NEK1* LOF variant had symptoms beginning from hands. It remains unclear why *NEK1* LOF variants are associated with hand-onset ALS. Low cervical anterior horn cells might have a higher demand on normal Nek1 functioning than lower motor neurons in other regions. Phenotypic preferences have also been observed in ALS associated with mutations in other disease genes, such as *SOD1* mutations correlating with flail leg phenotype and *C9ORF72* expansions associating with bulbar phenotype.[22]

In conclusion, we identifies six ALS patients with *NEK1* LOF variants in 325 unrelated Taiwanese ALS patients. All the patients had a hand-onset ALS with an onset age ranged from 52 to 64 years. This study characterizes the genetic and phenotypic features of *NEK1* LOF variants and underlines their pathogenic role in ALS.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. The spectrum and frequencies of mutations in non-*NEK1* genes in the Taiwanese ALS cohort.
Table S2. Genetic and clinical information of the ALS patients with a heterozygous *NEK1* rare missense mutation.

