Sir,

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by adult-onset loss of motor neurons. Five to 10% of all ALS cases are familial ALS. To date, more than 20 genes have been implicated in causing familial ALS, with the discovery of mutations in CHCHD10 (Bannwarth et al., 2014) and TBK1 (Cirulli et al., 2015; Freischmidt et al., 2015) representing the latest examples for monogenic causes of ALS. Most recently, whole exome sequencing of ALS patients suggested an association of heterozygous loss-of-function mutations in NEK1 with ALS. However, this observation was made in a cohort of mostly sporadic patients, and the result was only significant in a combined analysis of the discovery and the replication cohort (Cirulli et al., 2015), making further validation essential.

To assess the association between NEK1 variants and familial ALS we analysed whole exome sequence data of 265 familial ALS index patients and 827 control individuals. A subset of these exome sequence data has recently led to the discovery of mutations in TBK1 as a cause for ALS in an exome-wide mutational burden analysis (Freischmidt et al., 2015). The patients with familial ALS were selected from families with two or more affected individuals from European countries (Germany, Sweden, Finland, Denmark, Switzerland, and Portugal) following a negative screen for SOD1 and C9orf72 mutations as described previously (Freischmidt et al., 2015). In-house control exomes (n = 827) from Germany were used to compare the variant burden in NEK1. All ALS patients were diagnosed according to the EFNS Consensus criteria (Andersen et al., 2012). Control subjects were comprised...
of healthy parents of children with various diseases, healthy control tissues of individuals with tumour diseases and 200 individuals of the KORA study. With informed written consent and approval by the national medical ethical review boards in accordance with the Declaration of Helsinki (WMA, 2013), EDTA blood samples were drawn from control individuals, ALS patients and their healthy relatives. DNA was extracted from EDTA blood samples according to standard procedures (Erdmann et al., 2013). Sequencing, read mapping and variant calling was performed on HiSeq2000/2500 systems (Illumina) as described previously (Freischmidt et al., 2015).

We analysed loss-of-function variants, defined as nonsense, canonical splice site (within two nucleotides of exon boundary), read-through and frameshift variants [minor allele frequency (MAF) < 5%]. In addition, missense variants at a MAF threshold of < 0.01 or 0.001 were investigated separately. We identified a statistically significant enrichment of loss-of-function variants of NEK1 in the familial ALS patient group (allele frequency 0.57%) compared to the in-house control group (0.06%) (P-value = 0.0474; Fisher’s exact test) (Fig. 1), with three NEK1 loss-of-function carriers in the familial ALS patient group (c.42_42delA/p.Ser14SerfsTer45, c.2434A > T/ p.Arg812Ter and c.3107C > G/p.Ser1036Ter) and one in the control cohort (c.3044_3044delT/p.Phe115SerfsTer10) (Fig. 1A). Mutations in known ALS disease genes were not detected in any of the NEK1 loss-of-function mutation carriers. The presence of one loss-of-function mutation in the control group (allele frequency for NEK1 loss-of-function mutations = 0.06%) is in agreement with the high frequency of NEK1 loss-of-function mutations in the ExAC dataset (allele frequency 0.05%; http://exac.broadinstitute.org). This suggests a reduced penetrance of heterozygous NEK1 loss-of-function mutations. Along this line, non-penetrance of the p.Ser1036Ter mutation was observed in a brother of the respective index patient, who is without signs of ALS 4 years after death of the index patient and 9 years after onset of disease. Unavailability of DNA samples precluded further segregation analysis in other families with a NEK1 loss-of-function mutation.

Furthermore, we observed a trend towards enrichment of rare missense variants (MAF < 0.01) in the patient group (allele frequency 3.2%) compared to the in-house control group (1.87%) or the allele frequency in the ExAC dataset (1.84%) (overview of observed missense variants in Fig. 1B). However, this association did not reach statistical significance when comparing the patient group with our in-house control dataset (P-value = 0.088; Fisher’s exact test). Using a MAF threshold of < 0.001, we detected missense variants at an allele frequency of 1.13% and 0.6% in the patient and control cohort, respectively (P-value = 0.242; Fisher’s exact test). In line with the hypothesis that only a subset of NEK1 missense variants is causative, non-segregation could be demonstrated for the NEK1 variants p.Arg261His (in one pedigree; MAF = 0.23% in the ExAC reference dataset) and p.Asn745Lys (found in two pedigrees; MAF = 0.63%) (Fig. 1B). Available DNA samples allowed us to exclude the respective variants in at least one ALS patient in each of the three families. No DNA was available for segregation analysis of the rarer p.Met545Thr, p.Gly399Ala or p.Asn181Ser variants, all of which had a MAF < 0.005 (Fig. 1B).

The female patient with the loss-of-function variant p.Ser14SerfsTer45 showed upper and lower motor neuron signs in the left arm at the age of 54. Symptoms spread to the leg and right side, and within 2 years bulbar signs developed. The patient is still alive. The Edinburgh Cognitive Assessment (ECAS) (Lulé et al., 2015) was performed twice with normal results.

The first symptom of the male index patient carrying the p.Ser1036Ter mutation was dysphagia at the age of 59. Within 2 years the patient developed dysarthria, myotrophic paresis of the left arm and respiratory insufficiency while the lower extremities were not affected. The patient showed hyperreflexia in the atrophic limb. Affective lability or cognitive deficits were not noted, and a detailed neuropsychological testing or brain MRI was not performed. The patient died aged 63. The patient’s mother died from ALS at the age of 62, after a 5-year disease duration with onset in the hands and progression to tetraparesis. Late in the disease she developed bulbar symptoms and had a gastrostoma. Cognitive impairment or aphasia has not been reported.

The female index patient carrying the loss-of-function mutation p.Arg812Ter had first noted an asymmetric amyotrophic paresis of the first dorsal interosseous muscle of the left hand at the age of 75. Within 1.5 years the disease progressed to an asymmetric amyotrophic tetraparesis with asymmetric hyperreflexia without spasticity in all limbs, beginning (pseudo-) bulbar dysarthria, dysphagia and mild respiratory insufficiency. The patient is still alive 18 months after disease onset. No affective lability is present. The second affected family member is the uncle of the index patient who died of ALS at the age of 70. His detailed medical history is not available. The father of the index patient, who may have transmitted the NEK1 mutation, died at the age of 43 years of cardiac complications reportedly secondary to an infection. While overt cognitive deficits were absent at clinical examination of the index patient, neuropsychological testing revealed no difficulties in ALS-specific domains such as executive functions, apart from design fluency. Instead, ALS untypical hippocampal/temporal lobe functions, such as non-verbal memory, were impaired. This was in line with the automated atlas-based 1.5 T MRI volumetry (Huppertz et al., 2010), which showed severe atrophy of the hippocampus, and some atrophy of the caudate and thalamus that did not extend to other brain regions. This is a finding not characteristic of ALS/frontotemporal dementia (FTD) and rather reminiscent of Alzheimer’s disease (Fig. 1C and D). However, substantial atrophy of the parietal and frontal lobes, as expected in cases of Alzheimer’s disease or frontotemporal dementia, was absent. Moreover, while the ALS biomarkers neurofilament (NF)-light chain and phosphorylated NF-heavy
Figure 1  NEK1 variants found in familial ALS patients. (A) Predicted consequences of the NEK1 loss-of-function mutations observed in familial ALS patients at the protein level. See (B) for colour coding of protein domains. (B) Schematic drawing of the NEK1 protein showing the kinase domain, the basic domain, the four coiled-coil domains (CC) and the two nuclear export sequences (NES). The positions of NEK1 missense variants with a MAF < 1/100 that were observed in familial ALS patients are indicated. Missense variants with a MAF < 1/10 000 are coloured in red. (C) Brain 1.5 T MRI analysis of the patient carrying the NEK1 loss-of-function mutation p.Arg812Ter. Coronal slice (mesiotemporal) of the T1-weighted MPRAGE, demonstrating bilateral hippocampal atrophy. (D) Atlas-based volumetry results of the hippocampus [normalized to intra-cranial volume (ICV)]; the patient is clearly categorized within the control sample of Alzheimer’s disease patients, separate from the age-specific control group and the vast majority of individuals in an unselected cohort of ALS patients.
chain (Steinacker et al., 2016) were clearly elevated in the CSF of this patient (3808 pg/ml and 1938 pg/ml, respectively), CSF markers for Alzheimer’s disease were completely normal (total tau protein = 307 pg/ml and amyloid-β protein = 1173 pg/ml).

In summary, analysis of our whole exome sequence data from a large cohort of familial ALS patients, in combination with the suggestive association between NEK1 mutations and mostly sporadic ALS in a previous independent study (Cirulli et al., 2015), show that NEK1 is a novel ALS gene. Nevertheless, confirmation of NEK1 as an ALS-linked disease gene in additional cohorts is desirable. The significant enrichment of heterozygous loss-of-function mutations, but not missense mutations, in both the familial (this work) and mostly sporadic (Cirulli et al., 2015) ALS cohorts argues in favour of NEK1 haploinsufficiency as the molecular genetic mechanism. The nominal trend towards an enrichment of rare missense variants suggests the possibility of a subset of pathogenic missense variants resulting in a partial loss function.

Moreover, the comparatively high prevalence of NEK1 loss-of-function and missense variants in public control datasets and our in-house control cohort together with the observation of non-penetrance in a living, asymptomatic 71-year-old NEK1 loss-of-function mutation carrier suggest a greatly reduced penetrance of NEK1 loss-of-function mutations. The contribution of genetic factors is most likely higher in familial ALS when compared to sporadic ALS. In agreement with this assumption, the frequency of NEK1 loss-of-function mutation carriers was higher in our cohort of familial ALS patients if compared to the recently published frequency of patients with NEK1 loss-of-function mutations in mostly sporadic ALS patients (Cirulli et al., 2015) (1.13% versus 0.84%, respectively). We thus provide here the first demonstration that NEK1 mutations cause specifically familial ALS. As our study results, together with the previous discovery data (Cirulli et al., 2015), provide solid evidence that haploinsufficiency of NEK1 contributes to ALS pathogenesis, future studies are now necessary to illuminate the underlying molecular pathomechanisms. In light of the reduced penetrance of NEK1 mutations, the possible synergistic interaction of NEK1 mutations with additional genetic or exogenous factors will be of interest. Furthermore, it has to be reassessed whether NEK1-linked ALS is commonly associated with mesiotemporal atrophy and mnemonic deficits as seen in the aforementioned patient. The normal CSF tau and amyloid-β protein levels in this patient in combination with the brain MRI results argue against coincidental Alzheimer’s disease or typical frontotemporal dementia comorbidity.

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**References**


