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LETTER TO THE EDITOR

NEK1 mutations in familial amyotrophic lateral sclerosis

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Abbreviations: ALS = amyotrophic lateral sclerosis; MAF = minor allele frequency

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

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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.Phe15SerfsTer10) (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 similar 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, myatrophic 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

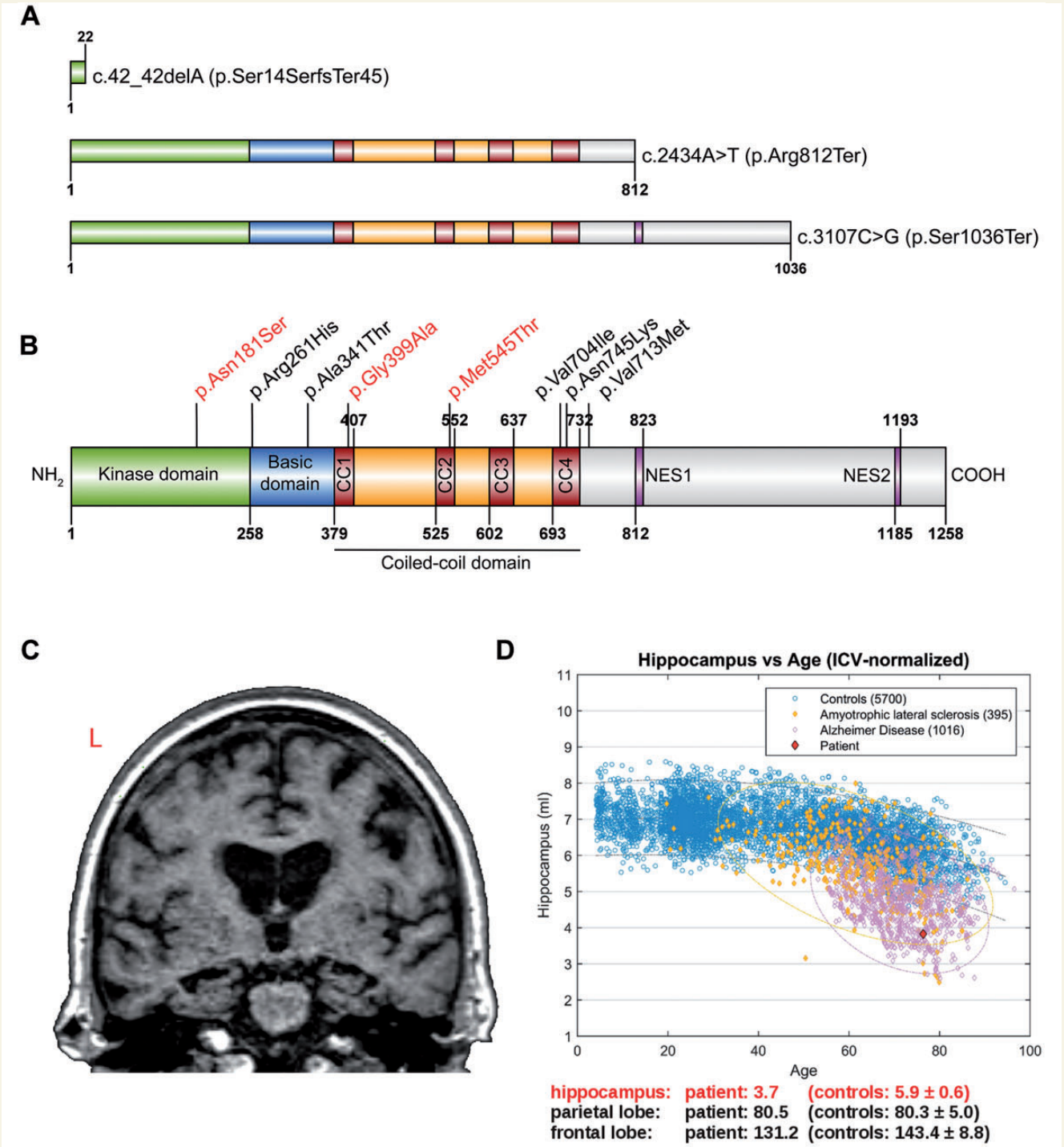

Figure 1 ***NEK1*** **variants found in familial ALS patients.** (**A**) Predicted consequences of the *NEK1* loss-of-function mutations observed in familial ALS patients at the protein level. See (**B**) for colour coding of protein domains. (**B**) Schematic drawing of the NEK1 protein showing the 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 $\leqslant$ 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 T_1-weighted MPRAGE, demonstrating bilateral hippocampal atrophy. (**D**) Atlas-based volumetry results of the hippocampus [normalized to intracranial 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 mnestic deficits as seen in the aforesaid 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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