CASE PRESENTATION

In view of future gene-specific therapies for genetic ALS patients, understanding not only familial but also sporadic ALS genetics is becoming increasingly important. Heritability seems to be high also in patients with a negative family history of the disease (Mejzini et al., 2019). Genetic studies suggest that a proportion of sporadic ALS cases has an oligogenic origin due to the combination of lower-effect size mutations in more than one ALS gene. Maximally three mutations in different ALS disease genes have indeed been described in isolated ALS patients. Here, we report for the first time the co-occurrence of rare nonsynonymous variants in four known ALS genes in a SALS patient (c.859G > A/p.Gly287Ser in TARDBP, c.304G > T/p.Glu102* in NEK1, c.3446C > A/p.Gly1149Val in ERBB4, and c.1015C > T/p.Arg339Trp in VEGFA). All four variants were unique for the patient, whereas up to three of these variants were detected in the unaffected family members, all older than the patient.

DISCUSSION: Our study suggests that SALS can be caused by the additive or synergistic action of low-effect size mutations. Broader use of gene panel analysis or whole exome/genome sequencing may reveal a potentially treatable oligogenic causation in a higher percentage of SALS than previously thought.
A currently between 50 and 55 years old patient presented with myatrophic paresis of the right thumb 5 years ago, which spread first proximally and then contralaterally to a flail arm syndrome. At present, the patient suffers from myatrophic proximal grade 3–4 and distal grade 0–1 paresis (extensors more affected than flexors) of the upper limbs while lower limb muscles are still spared (Vulpian-Bernhardt variant of ALS/flail arm syndrome). Fasciculations were noted in all four extremities. The disease is lower motor neuron dominant. The patient reports a slightly coarse voice, but otherwise no bulbar symptoms or evidence for respiratory impairment. Electromyographic and neurographic studies were consistent with a motor neuron disease.

Despite a negative family history for neuromuscular diseases, the patient asked for genetic testing. An ALS gene panel including the most frequently mutated ALS disease genes (in the alphabetic order: ALS2, ANG, ARHGEF28, ATXN2, BSCL2, C9orf72, CCFN, CHCHD10, CHMP2B, DCTN1, ERBB4, FIG4, FUS, GBE1, GLE1, GRN, HNRNPA1, HNRNPA2B1, HSPB1, HSPB8, MAPT, MATR3, MME, NEFH, NEK1, OPTN, PFN1, PRPH, SETX, SIGMAR1, SOD1, SPG11, SPG20, SQSTM1, TAF15, TARDBP, Tbk1, TUBA4A, UBQLN2, VAPB, VCP, VEGFA, VPS54) revealed four rare variants in four genes previously linked to ALS: c.859G>A/p.Gly287Ser in TARDBP (OMIM 605078), c.304G>T/p.Glu102* in NEK1 (OMIM 604588), c.3446C>A/p.Gly1149Val in ERBB4 (OMIM 600543), and c.1015C>T/p.Arg339Trp in VEGFA (OMIM 192240) (Figure 1a) (TARDBP: Accession: NM_007375.4, GI: 1777456621; NEK1: Accession: NM_001199397.3 GI: 1751363252; ERBB4: Accession: NM_005235.3 GI: 1519245003; VEGFA: Accession: NM_003376.6 GI: 1677500543).

The TARDBP/TDP-43 variant has been previously reported in sporadic ALS cases (Cady et al., 2015; Corrado et al., 2009; Kabashi et al., 2008; Kirby et al., 2010). It has an allele frequency of 1.59e-5 in the gnomAD dataset and is located in the low-complexity domain of TDP-43. It is thus very likely pathogenic, albeit with reduced penetrance (Vanden Broeck et al., 2015). The NEK1 variant has not been detected in gnomAD dataset in more than 248,000 alleles. It results in a premature termination at position 102 of the otherwise 1286 amino acids long protein. Thus, it represents a loss-of-function mutation and is most likely pathogenic, since NEK1 is haploinsufficient. The ERBB4 variant has allele frequency of 1.13e-4 in the gnomAD dataset. Two additional ERBB4 variants affecting the same codon are also listed, namely p.Gly1149Cys and p.Gly1149Ser, but even at a lower frequency. The VEGFA variant has an allele frequency of 2.83e-5 in gnomAD. Two less frequent variants, p.Arg339Ser and p.Arg339Gln, affecting the same codon have been reported there. Both the ERBB4 and the VEGFA variants we detected are predicted to be damaging by bioinformatic analyses. Further genetic testing of the patient's relatives showed that the NEK1 and VEGFA variants are paternally transmitted, while the TARDBP and ERBB4 variants are inherited from the mother. Genetic testing of five first- and second-degree relatives without ALS or FTLD (all older than the patient, aged 55–60, and 80–85) revealed that the combination of all four variants was unique for the patient with manifest ALS (Figure 1b). The cause of death for the father of the proband was cancer at the age of 78.

2 | DISCUSSION

In a low percentage of the ALS patients with the sporadic disease a monogenic cause due to a highly penetrant mutation can be identified. A combination of several variants with low and/or moderate effect size could be cause for the suggested missing heritability of ALS. Here, we report for the first time a patient with four genetic variants in four different known ALS genes. Missense variants in TARDBP and loss-of-function variants in NEK1 have repeatedly been shown to be associated with ALS, but exhibit incomplete penetrance. Although the causality of the variants in the putative ALS genes ERBB4 and VEGFA cannot be proven, they nevertheless might contribute to a polygenic inheritance. NEK1 encodes a serine/threonine kinase, the mutations in which lead to abnormal DNA damage response and repair (Higelin et al., 2018). VEGFA (vascular endothelial growth factor A) is involved in neuronal migration, neurogenesis, and axon guidance (Mackenzie & Ruhrberg, 2012). ERBB4 codes for a tyrosine kinase that activates signaling required for neural crest cell migration and normal axonal guidance (Kuo & Erickson, 2010). ERBB4 binds to neuregulins and the disruption of this pathway is linked to the ALS pathogenesis (Takahashi et al., 2013). Finally, TARDBP is involved in regulation of splicing, transcriptional repression, and DNA repair (Mitra & Hegde, 2019). Therefore, ERBB4 and VEGFA may be acting on signaling pathways regulating neurogenesis, whereas NEK1 and TARDBP both are involved in DNA repair. It is likely that the defects in single gene in these pathways do not have a detrimental effect, which is only revealed when two components in these pathways are affected. The observation that all four ALS gene variants accumulated solely in the patient but not in several older relatives without neurodegenerative disease corroborates the hypothesis that sporadic ALS can be caused by the additive or synergistic action of low-effect size mutations. Broader use of gene panel analysis or whole exome/genome sequencing may reveal a potentially treatable oligogenic causation in a higher percentage of sporadic ALS than previously thought.
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**CONFLICT OF INTEREST**

There are no competing interests for any author.

**AUTHOR CONTRIBUTION**

Rüstem Yilmaz, David Brenner, and Jochen H. Weishaupt carried out drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. Antje Knehr, Kanchi Weishaupt, and Ivan Valkadinov played a major role in the acquisition of data; study concept or design. All authors have read and approved the final version of the manuscript.

**ETHICAL COMPLIANCE**

The study was approved by the medical ethical review board of the Medical Faculty Mannheim of the University of Heidelberg (ID: 2017-589-N-MA).

**DATA AVAILABILITY STATEMENT**

Data available on request due to privacy/ethical restrictions.

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**FIGURE 1** Variation in four different ALS genes in a sporadic ALS case. (a) the variants and their allele frequencies. **TARDBP**: Accession: NM_007375.4, GI: 1777456621; **NEK1**: Accession: NM_001199397.3 GI: 1751363252; **ERBB4**: Accession: NM_005235.3 GI: 1519245003; **VEGFA**: Accession: NM_003376.6 GI: 1677500543. *number of homozygotes/allele count/allele number are listed. (b) Segregation of the variants within the family. Variant alleles are shown in red.
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