Novel Missense Mutation in ALS2 Gene Results in Infantile Ascending Hereditary Spastic Paralysis

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Objective: Recessive mutations in ALS2 (juvenile amyotrophic lateral sclerosis) are causative for early-onset upper motor neuron diseases, including infantile ascending hereditary spastic paralysis (IAHSP). The goal of this study is to identify novel disease-causing ALS2 mutations.

Methods: Mutations in ALS2 were screened by direct sequencing of complementary DNA obtained from patients’ lymphoblasts.

Results: We report a novel ALS2 missense mutation in patients affected by IAHSP. This homozygous G669A mutation in exon 4 is predicted to result in a tyrosine substitution at cysteine 156 of the RCC1 (regulator of chromatin condensation)-like domain, encoding a putative guanine exchange factor for Ran guanosine triphosphatase, leading to a loss of ALS2 function due to instability of mutant protein.

Interpretation: These results highlight the important role of the RCC1-like domain in ALS2 stability and function that is essential for upper motor neuron maintenance.

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Homologous mutations in ALS2 gene are causative for autosomal recessive, early-onset forms of upper motor neuron diseases described as infantile ascending hereditary spastic paralysis (IAHSP) 1 and juvenile primary lateral sclerosis. 2, 3 “They are also rarely associated with lower motor neuron involvement (juvenile amyotrophic lateral sclerosis (ALS2).” 2, 3 The ALS2 gene has 34 exons with 2 described splice variants. The long variant (6,394 nucleotides) is expressed in various tissues with highest expression in the brain. The ALS2 protein (also referred to as Alsin) encodes three putative guanine exchange factor (GEF) domains. These include RCC1 (regulator of chromatin condensation), Dbl and pleckstrin homology (DH/PH), and vacuolar protein sorting 9 (VPS9) domains, which have highest sequence similarity to GEFs for the Ran, Rho, and Rab5 families of guanosine triphosphatases, respectively (see Fig 2A).

A total of 10 recessive ALS2 mutations spread widely across the entire coding sequence have been described with a predominant IAHSP phenotype 4–6 (see Fig 2A). All of these mutations reported thus far are predicted to result in premature termination of translation, due to a frameshift or nonsense mutation. All disease-causing ALS2 mutants have been shown to be rapidly degraded when expressed in cultured human cells, including lymphocytes and fibroblasts derived from patients with ALS2 mutations, 7 indicating that disease-causing ALS2 mutations uniformly produce loss of activity through decreased protein stability. In this article, we report a novel ALS2 missense mutation in patients from a consanguineous Turkish family affected by IAHSP, and we demonstrate the instability of this ALS2 mutant protein.

Subjects and Methods

We tested for ALS2 mutation a consanguineous Turkish family (Fig 1) in which the two affected daughters (Subjects II.1 and II.2, 22 and 20 years old, respectively) showed a classic form of IAHSP. 8 For both affected girls, the pregnancy and the neonatal period were unremarkable, whereas a delay in the motor development was observed during the first year of life. Subject II.1 sat up at the age of 12 months, was able to stand up at 18 months, and walked with support at 3 years. At 7 years old, she could walk short distances with a walker, but spasticity in lower limbs was clearly present. Subsequently, an ascending progression of motor difficulties was observed: she was wheelchair bound at 12 years, when upper limb weakness started, and she needed computer assistance to write at 16 years when her voice became weaker. Now 22 years old, she needs a talker for oral communication and has no swallowing difficulties. In contrast, cognitive functions are preserved and she works with computer aids in a sheltered workshop. Motor development impairment and disease progression were even more severe in her younger affected sister (Subject II.2): she walked with support at 6 years, was wheelchair bound with hand use difficulties at 10 years, and needed a talker at 12 years. Now 20 years old, swallowing difficulties are present. She also works with computer aids in a sheltered workshop. Cranial magnetic resonance imaging performed at 11 years old showed subtle cerebellar atrophy. Motor-evoked potentials were profoundly affected with no or significantly delayed responses. In contrast, peripheral nerve conduction velocities were normal, and electromyogram did not show signs of lower motor neuron involvement.
Results

Identification of the ALS2 Mutation

A homozygous missense mutation G669A was found in exon 4 of ALS2 by direct sequencing of the ALS2 complementary DNA, obtained from a lymphoblastoid cell line derived from affected Subject II.2 according to previously reported conditions. This is predicted to result in a tyrosine substitution at cysteine 156 (C156Y) of the protein (see Fig 1). This mutation was confirmed by direct sequencing of the exon 4 from genomic DNA extracted from blood lymphocytes. In this IAHSP affected family, segregation of this G669A mutation with disease phenotype was confirmed by direct genomic sequencing of exon 4 in the other family members: the mutation was found at an homozygote state in the identically affected sister’s (Subject II.1) DNA, but only at an heterozygote state in the healthy brother’s (Subject II.3) and parents’ (Subjects I.1 and I.2) DNA (see Fig 1). In addition, this mutation was not found by direct genomic sequencing of exon 4 in 400 chromosomes of control subjects, including subjects of Turkish origin (70 subjects), from North Africa (60 subjects), and Europe (70 subjects).

C156Y Missense Mutation on ALS2 Decreased Protein Stability

Immunoblot analysis of protein extracts from lymphoblasts of Subject II.2, carrying the C156Y mutation and similar lymphoblastoid lines previously reported from the other ALS2 mutations, showed the absence of accumulated mutant ALS2 protein compared with normal lymphoblasts (see Fig 2B). An anti-ALS2 antibody (pAb-ALS2RCC1) could detect overexpressed ALS2-C156Y mutant protein at the same efficiency as another anti-ALS2 antibody (pAb-ALS21082) (see Figs 2A, C).

The stability of the C156Y-mutated ALS2 protein was determined after expression of the mutant complementary DNA in human embryonic kidney (HEK293) cells. After incubation in the presence or absence of the proteasome inhibitor (MG132), ALS2 protein levels were analyzed by immunoblotting, as described previously. In the absence of MG132, the C156Y mutant accumulated only to low levels compared with wild-type ALS2 (see Fig 2D), a pattern similar to that previously reported for M1207stop and V1574fs (frameshift) mutants. However, after proteasome inhibition...
(MG132), all of the mutant forms accumulated to easily detectable levels, consistent with rapid turnover of each mutant. Wild-type ALS2 was stable, with its level unaffected by short-term proteasome inhibition. The protein expression level of endogenous wild-type ALS2, as well as C156Y mutants in the lymphoblasts, was not altered under the same condition of proteasome inhibition used in HEK293 cells (data not shown), likely
due to the inefficient proteasome inhibition in the lymphoblasts or yet unidentified other degradation pathway of ALS2. Because ALS2 is known to be involved in the endosomal trafficking, ALS2 might be degraded partly in the lysosome, as well as proteasome.

The C156Y mutation is located in the RCC1-like domain (RLD) of the ALS2 protein. The cysteine substituted amino-acid residue is conserved among species present in the chimpanzee (Pan troglodytes), rat (Rattus norvegicus), mouse (Mus musculus), Gallus gallus, Bos taurus, Canis familiaris, Fugu rubripes, and Danio Rerio and is partially conserved for Drosophila melanogaster and Anopheles gambia (Fig 3).

Discussion

Our evidence identifies a novel missense mutation in the ALS2 and demonstrates that this mutant also yields a disease course consistent with other IAHSP patients.5

References

Fig 3. Comparison of ALS2 (juvenile amyotrophic lateral sclerosis) sequence among species. Cross-species multiple sequence alignment of the portion 130 to 180 amino acids, containing the cysteine in position 156. A box indicates conserved cysteine in position 156 residue across species. Ag = Anopheles gambia; Cf = Canis familiaris; Dm = Drosophila melanogaster; Dr = Danio rerio; Fr = Fugu rubripes; Gg = Gallus gallus; Hs = Homo sapiens; Mm = Mus musculus; Pr = Pan troglodytes; Rn = Rattus norvegicus.
Is Prostaglandin E2 a Pathogenic Factor in Amyotrophic Lateral Sclerosis?

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Objective: To elucidate the role of cyclooxygenase-1 (Cox1) and prostaglandin E2 (PGE2) in ALS neurodegeneration.

Methods: Mutation in superoxide dismutase-1 is a cause of the fatal paralytic disorder amyotrophic lateral sclerosis. Inhibition of cyclooxygenase-2 (Cox-2) and the levels of one of its main products, prostaglandin E2 (PGE2) are elevated in postmortem spinal cord tissue of sporadic ALS cases and of affected transgenic SOD1G93A mice, which is the most widely studied model of ALS. Supportive of a pathogenic role for Cox-2, PGE2, or both in ALS are the demonstrations that inhibition of Cox-2 decreases tissue levels of PGE2 and mitigates neurodegeneration in organotypic spinal cord culture exposed to both glutamate and in transgenic SOD1G93A mice. Herein, by abrogating the constitutively expressed isoenzyme cyclooxygenase-1 (Cox-1), we show that the production of PGE2 in the spinal cord originates mainly from Cox-1, and that PGE2 is not a mediator of motor neuron degeneration caused by mutant SOD1. These results suggest that although PGE2 could still be a useful biomarker in ALS, this specific prostaglandin does not mediate Cox-2–induced neurotoxicity in this fatal disease. Moreover, our data suggests that clinical trials using selective Cox-1 inhibitors are unlikely to be beneficial for ALS patients.

Interpretation: The previously documented role of Cox-2 in ALS neurodegeneration in this particular mouse model occurs through a mechanism independent of prostaglandin E2. Furthermore, plans to use selective Cox-1 inhibitors for neuroprotection in ALS are unlikely to be fruitful.