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SHORT REPORT

Novel homozygous *ALS2* nonsense mutation (p.Gln715X) in sibs with infantile-onset ascending spastic paralysis: the first cases from northwestern Europe

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We describe a previously not recognized nonsense mutation in exon 10 of the *ALS2* gene in two sibs with infantile-onset ascending spastic paralysis. The mutation predicts chain termination at amino-acid position 715 of the gene product ALSIN (p.Gln715X). The sibs' parents are descendants of a common ancestor who lived in the northern Netherlands during the eighteenth century. This is the first *ALS2* mutation detected in northwestern Europeans. The findings emphasize that mutations in *ALS2* also need to be considered in patients from northwestern Europe with early-onset spastic paralysis and amyotrophic or primary lateral sclerosis.
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Introduction

Homozygous mutations in the *ALS2* gene can result in one of three clinically distinct motor neuron diseases: (1) autosomal recessive juvenile amyotrophic lateral sclerosis (ALS2, OMIM no. 205100), (2) juvenile primary lateral sclerosis (JPLS, OMIM no. 606353), or (3) infantile-onset ascending spastic paralysis (IAHSP, OMIM no. 607225).[1–4] In ALS2 both upper and lower motor neurons are affected, whereas neurodegeneration only involves upper motor neurons in JPLS and IAHSP.[5,6] Despite this difference in neuropathology, there is considerable clinical overlap of the major signs and symptoms in the three conditions, including spasticity of the limbs and dysarthria. The age of onset is during early childhood (first or second year), in the three disorders but appears to be somewhat later in ALS2 than in JPLS and IAHSP.

The *ALS2* gene is located on the long arm of chromosome 2 (2q33) and is composed of 34 exons.[1,2] It can be transcribed into two alternative transcripts of 6.5 and 2.6 kb. The large transcript encodes ALSIN, a protein of 184 kD. ALSIN contains three putative guanine nucleotide exchange factor (GEF) domains, the N-terminal regulator of chromatin condensation (RCC1) domain, the central Db1 and pleckstrin homology (DH/PH) domains, and the C-terminal vacuolar protein sorting 9 (VPS9) domain. The RCC1 domain is characteristic of a GEF for GTPase Ran,[7] the DH/PH domains act as Rho GEFs,[8] and the VPS9 domain interacts with endosome-associated small G protein Rab5a.[9] This observation and experimental evidence points to a function of ALSIN as a GEF protein.

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It is not known whether the short transcript is physiologically translated into a functional polypeptide as well.

To date, a total of 12 different mutations have been described in the *ALS2* gene. All had occurred in people from Mediterranean countries and from Asia. Here we describe the first mutation in *ALS2* in sibs from northwestern Europe. They are the children of consanguineous parents, who are descendants of a common ancestor who lived in the eighteenth century (around 1750) in the province of Friesland, in the northern part of the Netherlands. The findings are discussed in the context of published *ALS2* mutations.

Materials and methods

DNA extraction and sequencing of the 34 exons of *ALS2* were performed according to standard procedures.

Case reports

Patient 1, the proband, is the first child of healthy parents of Dutch descent. She was born at term after an uncomplicated pregnancy and delivery. At the age of 8 months she developed distal spasticity in her legs and axial hypotonia. She was never able to sit or stand without support. During the following 3 years, spastic diplegia slowly progressed to the upper extremities. Starting at age 5, she developed tetraplegia; with the lower extremities being more severely affected than the upper extremities, soft pseudobulbar speech, and dysphagia. She has never achieved bowel or bladder control. Now aged 13 years, she suffers from drooling, receives gastroenteric feeding by Mickey button and speech has become anarthric. She is fully wheelchair dependent and voluntary movements of the hands are hampered by sudden hyperextension. Cognition, behavior, vision, and hearing have remained within the normal range.

Patient 2, the brother of the proband, is the third child born to the same parents. He was born at term, after an uncomplicated pregnancy and delivery. He developed normal gross and fine motor function during his first 18 months. He was able to grasp, sit, stand (without support), and walk (with support). However, from then onward, he progressively developed spastic diplegia with scissoring at the hips and his speech became nasal. From the age of 4 years, the volume of his voice decreased and swallowing and coughing deteriorated. His fine motor skills were assessed as being below average. He has never gained voluntary control over bladder and bowel function. Now aged 8 years, he is still able to walk short distances (walker-assisted) and he is still capable of deliberate hand movements. Like his elder sister, his cognition, behavior, vision, and hearing remain within the normal range.

In both children, neurological examination showed undisturbed extrapyramidal, lower motor neuron, and sensory function. Additional studies showed that motor and sensory conduction of peripheral nerves were normal, and needle electromyography did not reveal myopathic or neuropathic abnormalities. However, motor-evoked potentials were completely unobtainable after cortical stimulation, in either arm or leg muscles. These findings confirmed the presence of severe upper motor neuron dysfunction and absence of lower motor neuron involvement. Cerebral MRIs and blood tests (including lactate and lysosomal enzymes) were normal. On physical examination the parents showed no signs of motor neuron dysfunction, and their second child is healthy.

Molecular findings

We first sequenced all 34 exons and adjacent intronic portions of *ALS2* in one of the two index cases. We found ten homozygous sequence variants, four of which were in exons and six in introns (Table 1). Of these sequence changes, nine have been described before and are considered neutral single nucleotide polymorphisms (SNPs). One sequence change (c.2143C>T) in exon 10, however, was predicted to result in the generation of a stop codon at amino-acid position 715 (p.Gln715X) (Figure 1). We then sequenced exon 10 and those exons and introns that

Table 1 Sequence changes observed in *ALS2* in the patients and their parents

Exon/Intron	*SNP ID*	*Ref SNP(rs no.)*	*Effect*	*Genotype father*	*Genotype mother*	*Genotype sib 1*	*Genotype sib 2*
Exon 4	**c.1102G>A**	**rs3219156**	**Val368Met**	**Homozygous**	**Homozygous**	**Homozygous**	**Homozygous**
Intron 10	IVS10-62C>T	rs3731703		Heterozygous	Homozygous	Homozygous	Homozygous
Exon 10	**c.2143C>T**		**Gln 715 stop**	**Heterozygous**	**Heterozygous**	**Homozygous**	**Homozygous**
Exon 13	**c.2466G>A**	**rs2276615**	**Val822Val**	**Heterozygous**	**Homozygous**	**Homozygous**	**Homozygous**
Intron 25	IVS25+25C>T	rs3219167		Heterozygous	Homozygous	Homozygous	Homozygous
Intron 25	IVS25-76T>C	rs2882231		Heterozygous	Homozygous	Homozygous	Homozygous
Exon 26	**c.4015C>T**	**rs3219168**	**Leu1339Leu**	**Heterozygous**	**Homozygous**	**Homozygous**	**Homozygous**
Intron 26	IVS26-64G>A	rs1210940		Homozygous	Homozygous	Homozygous	Homozygous
Intron 29	IVS29-48T>C	rs3219170		Heterozygous	Homozygous	Homozygous	Homozygous
Intron 30	IVS30-69T>A	rs3219171		Heterozygous	Homozygous	Homozygous	Homozygous

Abbreviation: SNPs, single nucleotide polymorphisms.
The nonsense mutation in exon 10 is highlighted. Bold type indicates SNPs resulting in amino-acid changes.

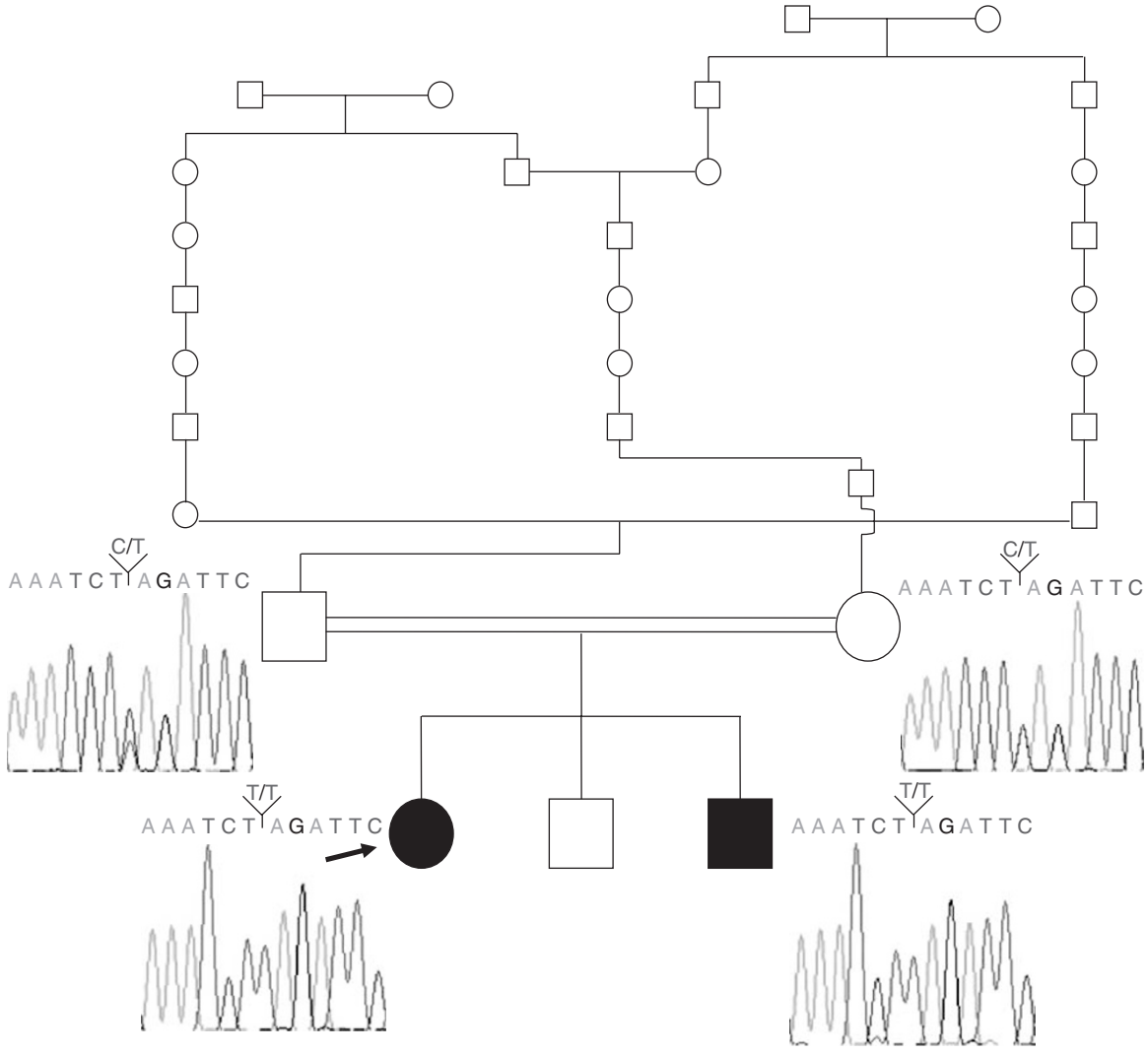

Figure 1 Pedigree of the family. The index case is marked by arrow. Sequence chromatographs are shown of both patients and their parents (for details see text).

contain SNPs in the affected sib and the patients' parents. Although the causative mutation (c.2143C>T) and the SNPs are homozygous in both sibs, the parents were heterozygous for the mutation and the father was heterozygous for most of the SNPs (Figure 1; Table 1). The mother was homozygous for all SNPs. The mutation only affects the long transcript of *ALS2*. It predicts truncation of ALSIN and the absence of most of the DH/PH domains, the eight-membrane occupation and recognition nexus motifs (MORN), and the VPS9 domain.

Discussion

We report on two sibs with clinical features of infantile-onset ascending hereditary spastic paralysis (IAHSP). The *ALS2* mutation found in these children is the first one discovered in northwestern Europeans. All previously detected *ALS2* mutations were found in people from countries surrounding the Mediterranean and from Asia (Table 2). The mutation, a previously not recognized nonsense mutation, most likely originates from one of the common ancestors of the patients' parents at least 250 years ago (Figure 1). It is the second nonsense mutation found in the *ALS2* gene. The nonsense mutation previously reported was in exon 18 (p.Arg998X) in two sisters with IAHSP from a Buchari Jewish family.[13] Altogether there are now 13 *ALS2* mutations known (Table 2).

As is evident from Table 2, there is no clear-cut genotype–phenotype correlation. However, ALS2 has not been found in persons with truncated ALSIN consisting of more than the amino acids encoded by the first four/five exons. The significance of this observation is not clear. When the first mutations were discovered in *ALS2,* it was

Table 2 Mutations in ALS2, IAHSP, and JPLS

Exon	*Nucleotide*	*Amino acid*	*Clinical phenotype*	*Age at onset*	*Origin*	*References*
Exon 3	c.138delA	p.Ala46AlafsX5 (p.A46AfsX5)	ALS2	3–10 years	Tunisia	Hadano *et al.* 2001,[1] Yang *et al.* 2001[2]
Exon 4	c.470G>A	p.Cys157Tyr (p.C157Y)	IAHSP	1 year	Turkey	Eymard-Pierre *et al.* 2006[10]
	c.553delA	p.Thr185LeufsX5 (p.T185LfsX5)	ALS2	22 months	Turkey	Kress *et al.* 2005[11]
	c.1007–1008delTA	p.Ile336ThrfsX5 (p.I336TfsX5)	IAHSP	18 months	Italy	Eymard-Pierre *et al.* 2002[3]
Exon 5	c.1425–1426delAG	p.Thr475ThrfsX72 (p.T475TfsX72)	JPLS (ALS2)	14 months	Kuwait	Hadano *et al.* 2001[1]
Exon 6	c.1471–1480delGTTTCCCCCA	p.Val491GlyfsX3 (p.V491GfsX3)	IAHSP	18 months.	France	Eymard-Pierre *et al.* 2002[3]
Exon 6	c.1619G>A	p.Gly540Glu (p.G540E)	JPLS	2 years	Italy	Panzeri *et al.* 2006[12]
Exon 9	c.1867–1868delCT	p.Leu623ValfsX24 (p.L623VfsX24)	JPLS	1–2 years	Saudi Arabia	Yang *et al.* 2001[2]
Exon 10	c.2143C>T	p.Gln715X (p.Q715X)	IAHSP	< 2 years	The Netherlands	This study
Exon 13	c.2537–2538delAT	p.Asn846IlefsX13 (p.N846IfsX13)	IAHSP	16 months	Italy	Eymard-Pierre *et al.* 2002[3]
Exon 18	c.2992C>T	p.Arg998X (p.R998X)	IAHSP	1 year	Buchari Jewish	Devon *et al.* 2003[13]
Exon 22	c.3619delA	p.Met1207Xfs1 (p.M1207Xfs1)	IAHSP	1 year	Algeria	Eymard-Pierre *et al.* 2002[3]
Exon 32	c.4721delT	p.Val1574fsX44 (p.V1574AfsX44)	IAHSP	18 months	Pakistan	Gros-Louis *et al.* 2003[4]

Abbreviations: ALS, amyotrophic lateral sclerosis; IAHSP, infantile-onset ascending spastic paralysis; JPLS, juvenile primary lateral sclerosis.
Nomenclature for nucleotide position according to Antonarakis (1998)[17] with A of start codon ATG within exon 2 being the first nucleotide. Note that some investigators calculate mutations from the A of an ATG within the untranslated exon 1 of *ALS2*. The start codon in exon 2 and the ATG within exon 1 are separated by 123 bp of exonic DNA.

thought that lower motor neurons were protected by the small transcript of *ALS2*.[1] Accordingly, ALS2 with both upper and lower motor neurons affected should always occur when exons encoding this transcript (exons 1–4) are affected. This is not the case, as IAHSP was found in a 24-year-old man with a homozygous 2 bp deletion in exon 4 (see table; reference[3]) interfering with both the long and the small transcripts. It is not yet understood why mutations affecting similar parts of ALSIN can result in either ALS2, JPLS, or IAHSP. It is also unclear why the same phenotype can occur in patients with different parts of ALSIN affected. It is likely that additional genes (modifier genes), and possibly environmental influences, shape the phenotype.

The mutation spectrum observed in *ALS2* shows that the VPS9 domain is important in normal ALSIN function. This domain is affected in all cases that result in truncation of ALSIN. Its loss alone can give rise to either JPLS or IAHSP. By binding to GTPase RAB5, VPS9 functions as a GEF for this GTPase. Furthermore, the VPS9 domain mediates binding of ALSIN to endosomes.[9] It is important in endosome fusion and trafficking.[14] Therefore, disturbance of normal endosome function, in addition to loss of GEF activity for RAB5, might be the underlying cause of motor neuron degeneration in patients with *ALS2* mutations. Missense mutations involving other domains can also cause motor neuron degeneration (Table 2). However, both the missense mutations found so far disable ALSIN function, including its VPS9 domain. Mutation of p.Gly540Glu lies within the RCC1 domain, results in delocalization of ALSIN within the cell, and appears to be neurotoxic.[12] The p.Cys157Tyr mutation is also located within the RCC1 domain and causes instability of the mutant ALSIN.[10] This observation supports the idea of Topp *et al*[15] who assumed the RCC1 domain played a structural rather than an enzymatic role in ALSIN. Furthermore, the relatively poor evolutionary conservation of the N-terminal region of ALSIN is also consistent with a structural rather than an important metabolic function of RCC1.[16]

In conclusion, we have described a nonsense mutation in exon 10 of the *ALS2* gene in two sibs of northwestern European origin. It is very likely that the mutation can be traced back to a common ancestor of the patients' parents who lived in the northern part of the Netherlands around 1750. Thus, mutations in the *ALS2* gene must also be considered in children with juvenile ALS, JPLS, and IAHSP from northwestern Europe, even when there is no immediate suspicion of parental consanguinity.

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