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ORIGINAL COMMUNICATION

The epidemiology of CuZn-SOD mutations in Germany: a study of 217 families

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Abstract We screened 217 patients from Germany ($n = 213$), Austria ($n = 2$) and Switzerland ($n = 2$) with a positive family history for amyotrophic lateral sclerosis (ALS) for mutations in the copper/zinc superoxide dismutase (SOD1) gene. We found that 13% of the families tested carried mutations. By analyzing inheritance, we detected a clear-cut co-segregation in 5 of the 28 families; however, in two families with an established mutation, co-segregation was absent. In Germany, the R115G mutation is comparatively frequent and exhibits a specific aggressive phenotype. The L144F mutation, which is the most prevalent mutation in the Balkan countries, and the D90A mutation which is the most frequent SOD1 mutation globally, seem to be the second most common disease-causing mutations in Germany.

Keywords Amyotrophic lateral sclerosis · Genetics · Cu/Zn SOD1 · Epidemiology

Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset, fatal neurodegenerative disorder characterized by progressive dysfunction of the upper and lower motor neurones. Most cases are sporadic, but about 5–10% have a positive family history (fALS) [3]. The pattern of inheritance seems to be autosomal-dominant in most identified pedigrees. The clinical phenotype of familial and sporadic ALS is usually similar. One hundred fifty mutations in the Cu/Zn superoxide dismutase (SOD1) gene in ALS have been described [8]. SOD1 is located on the chromosome 21q22.1 and the mutation has been found in all of the five exons. The frequency of SOD1 gene mutations varies in different countries from 12 to 23.5% of diagnosed fALS cases [4].

In our study we analysed 217 families with a positive family history of ALS for the presence of SOD1 mutations in all five exons. The SOD1 screening was done in the Departments of Medical Genetics of the University of Giessen and Ulm. Since no other major German laboratories were involved in SOD1 screening in the past decade, the results described in this paper are currently the best mirror of the epidemiology of SOD1 mutations in fALS in Germany. We find that the R115G mutation is the most frequent mutation in Germany and exhibits a specific phenotype.

Methods

Two hundred and seventeen Caucasian German, Austrian and Swiss patients were tested in our study. We included only patients who suffered from definite ALS according to the revised El Escorial criteria and had a positive family history. We excluded all definite ALS cases which had no first or second degree relatives with probable and/or definite ALS. We analysed all those patients who had been diagnosed with familial ALS between 1998 and 2008 and whose DNA had been sent for analysis to the Department of Medical Genetics of the University of Giessen and Ulm. If we had not seen the patients personally, we verified the diagnosis of ALS by clinical and electrophysiological criteria by evaluation of the patient's chart and by taking the history with the patient or his/her relatives. If necessary, we gathered additional information about the family history by contacting and visiting the relatives of the patients.

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The study adhered to the principles of the Declaration of Helsinki (WMA, 1964): we obtained blood samples after all participants gave informed oral and written consent. The study was approved by the Ethics Committee of the Medical Faculty of the University of Ulm and Giessen. All exons 1–5 of the SOD1 gene and 30–50 bp flanking intronic sequences were amplified by polymerase chain reaction (PCR). This was followed by direct sequencing of both DNA strands of PCR products on an ABI-Prim 3100 DNA sequencer.

Results

Through sequencing of all five exons in all 217 fALS cases, we identified 16 different SOD1 point mutations in 28 fALS families.

All mutations are located in exon 1, 2, 4 or 5 and have been reported before. Since this cohort is likely to be the best available to mirror the German fALS population, we may conclude that approximately 13% of German fALS patients carry a SOD1 mutation.

In five families of the 28 pedigrees with SOD1 mutations, we analysed a number of unaffected family members and detected a clear-cut pattern of co-segregation. All unaffected members do not carry the SOD1 mutation, whereas all patients suffering from ALS have a mutation. In this cohort, we could prove unequivocal co-segregation for the SOD1 mutations R115G, I104F and D90A.

An apparently specific finding for the German population is the frequency of the R115G mutation. Eight families of the 28 fALS cases carry the same SOD1 mutation R115G on exon 4. All reported families are seemingly unrelated to each other and originate either from the East (Berlin, Saxony, Saxony-Anhalt) or the North West (Lower Saxony) areas (Fig. 1).

In sum, in our study 29% of all German familial amyotrophic lateral sclerosis patients with a SOD1 mutation carry the R115G mutation.We found that the R115G mutation exhibits a specific stereotyped aggressive phenotype. We recorded an age at disease onset between 42 and 74 years. The site of disease onset in patients with R115G mutations was consistently at the lower limbs. Pareses progressed very fast and resulted in paralysis of all limbs and finally affected the bulbar muscles as well. The average survival time was 2–3 years.

Representative case reports

A 66-year-old female patient carried the R115G mutation on exon 4. She was born in Lower Saxony and six members of her family have been affected with ALS. Besides herself, her father, sister, niece and two sons suffered from ALS. This patient initially complained about difficulty walking, weakness and atrophy of the right thigh. At neurological examination, the patellar tendon reflex was absent. After 1 year the patient developed identical pareses and amyotrophy of the proximal left leg. Pyramidal signs were absent and sensory examination was normal. Then the patient developed distal paresis and amyotrophy of the right and, later, the left arm. The muscle reflexes of the upper limbs were normal and symmetrical. After 2 years the patient developed dysphagia and dysarthria. She died at the age of 68. One of her sons showed an identical course of the disease, and he died at the age of 45, 2 years after ALS has been diagnosed.

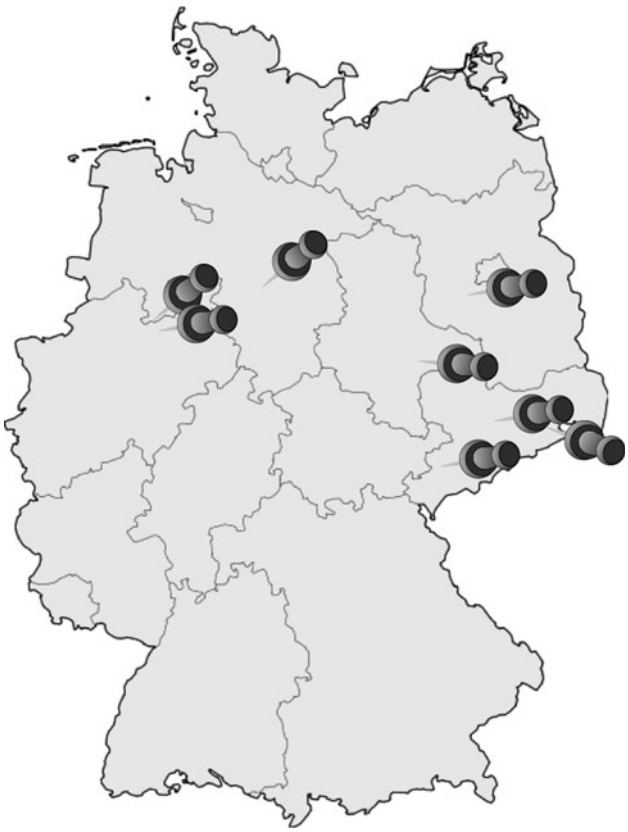

Fig. 1 The place of origin of the R115G families in Germany. Most of the families come from the east and the northwest of Germany but they are not related in an obvious manner

The SOD1 mutation E100K on exon 4 was identified in two families. In one, 14 members (2 men and 12 women) were affected and in the other family four affected individuals were identified. The onset of the disease was between 33 and 41 years and the course was comparatively benign, with a duration of disease of 10–20 years. Here also, early motor deficits were restricted to the lower limbs. Later on, skilled finger movements were impaired. Lower motor neuron dysfunction dominated the disease. During the course of the disease, all affected family members developed bulbar symptoms. An autopsy was performed in

one single patient which showed classical TDP-43 negative ALS neuropathology. This family was of special interest because co-segregation was absent. We found unaffected family members which nonetheless carried the E100K mutation. On the other hand, we could identify affected family members who had no SOD1 mutation. This finding is being reported separately (Felbecker et al., submitted JNNP, 2009). The other E100K family showed a slowly progressive disease as well. A dysfunction of the lower motor neuron dominated the disease and the patients suffered from the same segmental symptoms as described in the first family. After motor deficits started in the lower limbs, paresis of the upper limbs developed. Later, the patients suffered from bulbar symptoms such as dysarthria and dysphagia. In this family we could not analyse unaffected family members, so we have no evidence on co-segregation in this E100K family.

In three families we verified the presence of the SOD1 mutation L144F. This mutation causes a change of Leu to Phe on exon 5. In those three families ten members were affected. The age at onset was between 46 and 57. All affected patients showed a similar course. Generalised fasciculation and cramps in the calves were noticed first. Further symptoms of the disease resulted in paralysis and atrophy of all limbs, with the lower limbs predominantly affected. The process was slowly progressive. The paralysis of the lower limbs caused difficulties in walking, and some of the patients became wheelchair-bound. Muscle reflexes were absent at the lower limbs; at the arms they were brisk on both arms. All patients showed bulbar symptoms, however the intensity was different. Whereas some presented atrophy and fibrillation of the tongue, others had some swallowing difficulties. The patients showed weight loss after 2 years of progression. There was no geographical pattern of the three families.

The SOD1 mutation I104F on exon 4 results in an amino acid change of Iso to Phe. The disease caused by this mutation is slowly progressive and lasts over 15 years. In each of the three affected members, the symptoms began at the age of about 60 with generalised fasciculations and muscular cramps. Weakness and atrophy, especially of the distal limbs, dominated the course of the disease. After slow progression of close to 10 years, the patients showed difficulties walking and needed wheelchairs for longer distances. Involvement of the upper motor neuron was demonstrated by increased muscle tone of the lower limbs and brisk reflexes in the presence of amyotrophy of the upper limbs. The Achilles tendon reflex was absent and generalised fasciculations were found. Bulbar symptoms were characterised by dysarthria and dysphagia.

In three families we could identify the SOD1 mutation D90A. We saw the D90A mutation in the homozygous form with recessive inheritance and its phenotype was largely identical with the stereotyped picture and disease course described previously by Andersen [4]. The incidence of D90A in Germany was previously reported [21].

We identified the SOD1 mutation L38V on exon 2 in one family with three affected patients. The phenotype caused by this mutation had an early onset at the age of 31 years and resulted in symmetric paresis and atrophy of all limbs with no cramps. The symptoms started with a weakness of the distal lower extremities, especially in the left leg, that caused difficulties in walking. After 1–2 years the distal upper limbs were affected as well. Whereas muscle reflexes of the upper limbs were normal, the patella and Achilles tendon reflexes were absent or weak. Later on, the disease resulted in bulbar symptoms such as dysphagia and dysarthria and also respiratory dysfunction, although one affected family member seems to have had no bulbar symptoms. The survival life time averages between 3 and 4 years.

The SOD1 mutation I149T on exon 5 caused ALS in two patients in one family with an onset of about 50 years. The disease typically began with a slowly progressive paralysis of the lower limbs in the presence of brisk reflexes. The upper limbs did not show major deficits during the first year of disease. The SOD1 mutation L84F causes an amino acid change from Leu to Phe on exon 4. The mutation is associated with an early disease onset of 33 years and a very rapid progression of disease resulting in a survival time of only 1–2 years. In this family two members were affected. Bulbar symptoms dominate the disease and were first noticed by an inability to pronounce words correctly and dysphagia. The patient suffered from early weight loss and generalised fasciculations. After only 1 month, the patient realised proximal weakness of the right arm and half a year later he developed symmetric paralysis of both legs. Only 7 months after the first symptoms appeared, the patient was wheelchair-bound. Reflexes were hyperactive.

The H48R SOD1 mutation was detected in one person. The survival time was more than 20 years. The main symptoms were restricted to the lower limbs. There were no muscle cramps and only a few fasciculations. Not until a course of a 17-year-lasting disease did the patient become wheelchair-bound. Weakness in the upper limbs affected in particular the distal muscles. After a course of 7 years of disease the patient became wheelchair bound. The distal muscles were predominantly affected at the upper limbs. Mild bulbar symptoms developed after 20 years of duration of the disease.

A Val to Gly amino acid change in codon 148 of exon 5 was identified in one family with four affected members. The onset of disease caused by this V148G mutation was between the age of 25 and 55. At the beginning of the disease the main symptoms consisted of weakness of both upper and lower limbs and loss of weight. Weakness and

Table 1 Clinical and molecular data of patients with familial amyotrophic lateral sclerosis who have SOD1 mutations

Codon	Mutation	Amino acid change	Exon	Age at onset (years)	Affected family members	Principal reference
22	Q22L	Gln-Leu	1	48	2	[3]
38	L38V	Leu-Val	2	31	3	[16]
46	H46R	His-Arg	2	53	2	[5]
48	H48R	His-Arg	2	53	1	[3]
84	L84F	Leu-Phe	4	33	2	[17]
86	N86D	Asn-Asp	4	55	2	[4]
90	D90A	Asp-Ala	4	65	2	[2]
90	D90A	Asp-Ala	4	61	2	[2]
90	D90A	Asp-Ala	4	45	2	[2]
93	G93A	Gly-Ala	4	61	2	[13, 16]
100	E100K	Glu-Lys	4	33	14	[19]
100	E100K	Glu-Lys	4	41	4	[19]
104	I104F	Iso-Phe	4	60	3	[1]
113	I113T	Ile-Thr	4	56	1	[16]
115	R115G	Arg-Gly	4	66	6	[11]
115	R115G	Arg-Gly	4	67	2	[11]
115	R115G	Arg-Gly	4	64	2	[11]
115	R115G	Arg-Gly	4	42	5	[11]
115	R115G	Arg-Gly	4	52	2	[11]
115	R115G	Arg-Gly	4	60	5	[11]
115	R115G	Arg-Gly	4	74	3	[11]
115	R115G	Arg-Gly	4	56	3	[11]
144	L144F	Leu-Phe	5	46	3	[7]
144	L144F	Leu-Phe	5	57	5	[7]
144	L144F	Leu-Phe	5	47	2	[7]
148	V148G	Val-Gly	5	55	4	[7]
149	I149T	Ile-Thr	5	51	2	[14]
151	I151T	Ile-Thr	5	48	2	[12]

atrophy was more pronounced on the right side of the upper limbs. Fine motor skills were impaired on both sides, while reflexes on the upper and lower limbs were brisk. The weakness and atrophy eventually resulted in bulbar symptoms such as dysphagia and dysarthria and led to respiratory dysfunction. Patients survived about 4 years. However, one patient suffered from ALS with the age of onset of only 25 years. He showed a rapidly progressive bulbar paralysis (Table 1).

Discussion

In this cooperative study of two groups in Ulm and Giessen, we analysed 217 fALS patients and identified a SOD1 mutation in 13% of these patients. This result is broadly consistent with the proportion of 12–23.5% in other populations [4, 18, 20]. We could show the presence of 16 different SOD1 mutations and think that these numbers mirror the epidemiology of SOD1 mutations in Germany, since to the best of our knowledge, in the previous decade no other genetic laboratory has done systematic SOD1 testing in Germany in larger numbers.

In Germany, the most common SOD1 mutation is the R115G. Twenty-nine percent of all German fALS patients with a SOD1 mutation carry this mutation, which we found co-segregating in each of the families tested. The R115G mutation has not been described in any other population so far [11]. We find that it is remarkable that the R115G is so frequent within Germany and not in the surrounding countries or in countries where many Germans have emigrated, such as the USA, Canada or Australia. Other mutations that seem to play an important role in fALS cases in Germany are the L144F, D90A and E100K. Both, L144F and D90A were detected in 11% of all fALS cases.

A clear-cut pattern of co-segregation could be shown in five families with R115G, I104F and D90A mutations. The homozygous form of the D90A has previously been shown to be quantitatively the most important SOD1 mutation [4, 6, 9, 10, 15]. In two families we could not show

co-segregation, indicating that the mutation is rather a risk factor than disease-causing. In all of the other 21 families we could not analyse unaffected family members because of the missing consent of the probands or of our inability to contact them.

The clinical phenotype of the most frequent mutation, the R115G mutation, is represented by a specific aggressive course of leg onset and a predominant affection of anterior horn cells and bulbar motor neurons. The average age at onset of fALS caused by the R115G mutation including all previous studies [11, 13] is 60.

In our cohort, the age of onset seems to be linked to a specific SOD1 mutation. Whereas E100K, L38V and L84F have an early onset, all the other mutations cause a later onset of the disease. The mean survival time also seems to be dependent upon the specific SOD1 mutation. While patients with E100K, I104F and H48R SOD1 mutations have a survival time of over 10 years, R115G and L84F mutations present with very rapid progression of the disease.

In conclusion, the epidemiology of SOD1 mutations in Germany has features which represent the pattern seen all over the world, such as the frequency of the mutations and the quantitative contribution of the D90A, but also has specific aspects, such as the importance of the aggressive R115G and the L144F. Most interestingly, we could not demonstrate convincing co-segregation of two SOD1 mutations which were previously seen as disease-causing.

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