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Agrin and LRP4 antibodies in Amyotrophic Lateral Sclerosis Patients

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Abstract

Introduction—The prevalence and characteristics of Agrin and LRP4 antibody positive amyotrophic lateral sclerosis (ALS) patients were studied.

Methods—We tested 82 ALS patients and 59 controls for Agrin and LRP4 antibodies using ELISA.

Results—We found that 13.8% of ALS patients had Agrin antibodies, and 9.8% had LRP4 antibodies. Women ALS patients are twice as likely as men to have antibodies. Agrin-positive ALS patients are younger than Agrin-negative ALS patients.

Discussion—Antibodies to Agrin and LRP4 are found in ALS patients. It must be determined if these antibodies are pathogenic. Since antibody positive patients have upper as well as lower motor neuron findings, the antibodies' effects cannot be explained solely by their actions at the neuromuscular junction. Perhaps a breakdown in inter-neuronal signaling might cause ALS. Further research is needed to resolve this question.

Keywords

Agrin; Amyotrophic Lateral Sclerosis; Antibodies; LRP4; Low Density Lipoprotein Related Receptor Protein 4; ALS

Introduction

Amyotrophic lateral sclerosis (ALS) is a syndrome characterized by progressive motor neuron degeneration[1]. The causes of sporadic ALS may be multifactorial, similar to familial ALS which is associated with multiple gene defects[2]. However, clinical characteristics and biomarkers are lacking for different forms of ALS, which limits our ability to develop therapeutic strategies. Agrin is released by the motor neuron and binds to muscle membrane

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LRP4 (Low Density Lipoprotein Related Receptor Protein 4)[3,4]. We, along with others, recently identified Agrin and LRP4 antibodies in myasthenia gravis (MG) [5–9] and demonstrated that LRP4 antibodies are causal for MG[10]. Tzartos et al reported LRP4 antibodies in 23.1 % of ALS patients[11]. The aim of our study was to determine if ALS patients have antibodies to both LRP4 and Agrin.

Methods

Fifty-nine healthy controls and 82 ALS patients gave informed consent and participated in this IRB approved study. Patients underwent a comprehensive neurological exam and met El-Escorial criteria[12] for possible, probable, probable laboratory-supported, or definite ALS. Their blood samples were assayed by ELISA for Agrin and LPR4 antibodies as previously described [7,9]. Statistical analyses were performed using Excel (Microsoft, Redmond, WA) and QI Macros (KnowWare International, Denver, CO).

Results

Agrin and LRP4 antibody levels were not significantly different between ALS patients and controls (Agrin t=1.289, p=0.200, LRP4 t=0.192, p=0.869). The variances of Agrin and LRP4 antibody levels were significantly higher for ALS patients than for controls (Agrin F= 12.11, p< 0.001, LRP4 F= 7.04, p<0.001). A small number of ALS patients accounted for the increased variance of both Agrin and LRP4 values (Figure 1), thus identifying a subgroup of ALS patients with increased Agrin and/or LRP4 antibody levels. The normal values for Agrin and LRP4 were set at 0.265 and 0.267, respectively, representing the mean plus 2.5 standard deviations of our control population. Only 1 control subject had elevated LRP4 antibody levels and none had elevated Agrin levels. No ALS patient's antibody level was between 2.0 and 2.5 standard deviations above the mean.

Nine of 65 ALS subjects (13.8%) were positive for Agrin antibodies (Figure 1). Eight of 82 ALS subjects (9.8%) were positive for LRP4 antibodies (Figure 1). Agrin-positive ALS patients' mean value was 0.464 which was 7.71 standard deviations above the control mean. LRP4-positive ALS patients' mean value was 0.435 which was 6.99 standard deviations above the control mean. Agrin and LRP4 values were strongly correlated in ALS patients (r=0.791, r2=0.626). One subject was positive for Agrin and not LRP4.

Antibody positive ALS patients were slightly younger than negative patients. This was significant for Agrin (48.4 vs 59.7 p=0.021). Antibody positive patients had upper and lower motor neuron findings. The location of the first symptom varied among antibody positive patients; there was initial upper extremity involvement in 55.6 % of antibody-positive patients compared to 37.0% of antibody negative patients. Women with ALS were twice as likely to have antibodies as men. Approximately 15% of the women with ALS were antibody positive compared to only 8% of the men. There was no difference in race or ALS Functional Rating Scale score between antibody positive and negative ALS patients.

Discussion

In our population of ALS patients 13.8% had Agrin antibodies and 9.8% had LRP4 antibodies, which indicates that there is a significant subgroup of patients who are positive for these antibodies. Tzartos et al. described LRP4 antibodies in 23.1% of an Italian and Greek population of ALS patients[11]. Our values may differ from theirs for several reasons. First, our assay was a quantitative ELISA assay with purified LRP4 while Tzartos et al. used a qualitative cell-based assay. When a radio-immunoprecipitation assay was used on the same population, only 11.5% were positive for LRP4 [11]. Interestingly 4 of the antibody-positive subjects were not positive using the cell-based assay. Second, the prevalence might depend on patient demographics. In a later study of 87 Israeli subjects, Tzartos et al. found 14.9 % to be positive using the cell based assay [13]. The prevalence of LRP4 antibodies might be higher in Europe compared to that of the Southeastern US.

The question remains whether Agrin and LRP4 antibodies present in a significant portion of our ALS patients are pathogenic. Future studies are warranted to determine whether these antibodies will produce disease in experimental animals. Previous studies, including ours, have shown antibodies to Agrin and LRP4 in patients with MG [9,5–8]. The positive ALS patients in this study do not show clinical symptoms of MG. The actions of Agrin and LRP4 antibodies at the neuromuscular junction cannot explain the significant upper motor neuron findings seen in our ALS patients. Therefore, some actions of these antibodies probably occur elsewhere in the motor system. LRP4 has been shown to play a role in hippocampal neuronal plasticity[14] and astrocyte ATP release[15], and LRP4 antibodies are seen in the cerebrospinal fluid of ALS patients[13,11]. It is likely that Agrin and LRP4 antibodies play a role in neuronal function elsewhere in the nervous system. At the neuromuscular junction, Agrin acts as a motor neuron-derived signal. When Agrin binds to muscle LRP4, it triggers a complex sequence of events which lead to neuromuscular junction development[16]. Likewise, elsewhere in the nervous system, Agrin might act as a messenger in inter-neuronal communication by binding to LRP4. Perhaps the breakdown in intercellular communication leads to degeneration of the motor system as seen in ALS. This might explain the contiguous spread of symptoms in ALS and why degeneration is largely confined to motor neurons.

Why antibodies to Agrin and LRP4 produce MG in some patients and possibly produce ALS in others is not yet known. Perhaps different serotypes of these antibodies might be an explanation. It is hoped that LRP4 and Agrin antibodies might be a biomarker that identifies a subpopulation of ALS patients who can be studied further. Even if they do not contribute to the pathogenesis of ALS, the presence of these antibodies is of interest and warrants further study.

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Abbreviations

ALS	Amyotrophic Lateral Sclerosis
ALSFRS	ALS Functional Rating Scale
ELISA	Enzyme-Linked Immunosorbent Assay
LMN	Lower Motor Neuron
LRP4	Low Density Lipoprotein Related Receptor Protein 4
MG	Myasthenia Gravis
OD	Optical Density
UMN	Upper Motor Neuron

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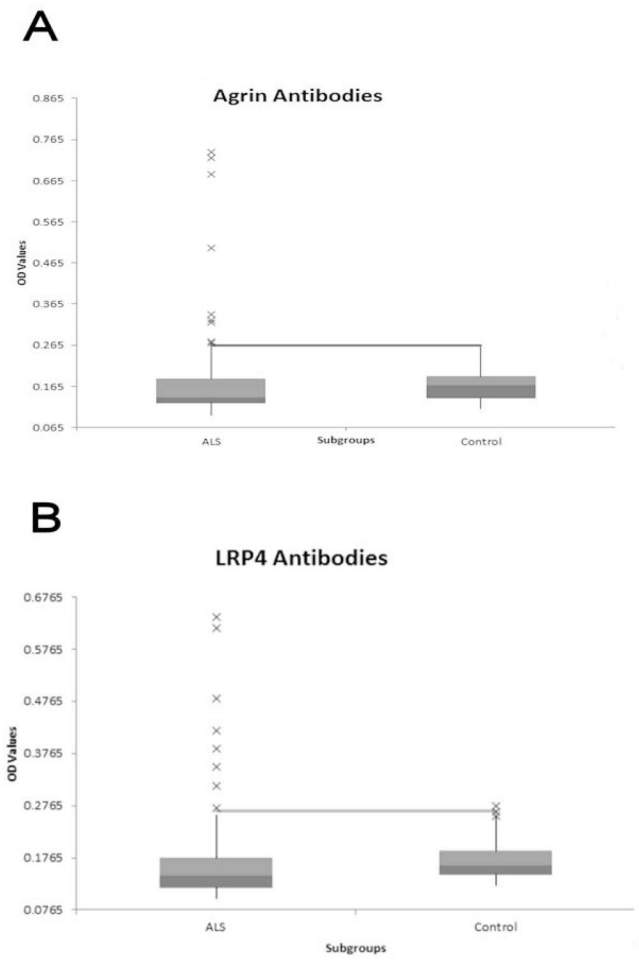

Figure 1. Distribution of Agrin and LRP4 antibodies<

br>Bar and whisker graphs show the median, minimum, and maximum values and upper and lower quartiles of the study population. Outliers are indicated by x on the graph. The line indicates the upper limit of normal. A: Distribution of Agrin antibodies in the ALS population compared to normal controls. B: Distribution of LRP4 antibodies in the ALS population compared to normal controls. OD: Optical Density.