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Neuronal LR11/SorLA Expression Is Reduced in Mild Cognitive Impairment

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Abstract

Objective—LR11 (aka sorLA) is a multifunctional neuronal receptor that binds apolipoprotein E and interacts with amyloid precursor protein to regulate amyloidogenesis. Reduced expression of LR11, as occurs in the brains of individuals with Alzheimer's disease (AD), increases amyloidogenesis, and variants in the gene that encodes LR11, *SORL1*, have recently been linked to risk for late-onset AD. In this study, we sought to determine whether reduced expression of LR11 occurs early in the disease process and whether protein levels in cortical neurons are associated with clinical and pathological changes in mild cognitive impairment (MCI), a condition that may represent prodromal AD.

Methods—A novel quantitative immunohistochemical approach was used to measure LR11 levels in brain tissue collected from subjects diagnosed antemortem with either no cognitive impairment, MCI, or AD from the Rush University Religious Orders Study.

Results—LR11 levels in MCI were intermediate between no cognitive impairment and AD. LR11 expression was heterogeneous in MCI, forming low- and high-level LR11 subgroups. MCI subjects with low LR11 were significantly more cognitively impaired than the high LR11 subjects. We also found a significant correlation between cognitive performance and LR11 levels across all clinical groups examined. There was no association between LR11 and plaque and tangle pathology.

Interpretation—Neuronal LR11 levels are reduced in prodromal AD. The correlation between LR11 expression and cognitive performance indicates that reduced LR11 levels reflect disease severity and may predict progression to AD in a subgroup of individuals with MCI.

Alzheimer's disease (AD) is the most common form of dementia in the elderly. Clinical manifestations include memory loss, deterioration of language skills, and impaired ability to perform daily activities.¹ Although the exact mechanisms remain unknown, it is widely accepted that amyloid-beta peptide (A β) plays a key role in AD pathogenesis. Thus, factors that regulate A β accumulation are of particular interest.^{2,3}

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We originally identified reduced expression of LR11 (aka sorLA and SORL1) in a microarray study and confirmed consistent loss of LR11 in AD brains, particularly in vulnerable regions.^{4,5} LR11 is an unusual mosaic receptor that is highly enriched in brain and possesses features of both low-density lipoprotein receptors and the sortilin family of receptors.⁶ We and others have shown that LR11 interacts with the amyloid precursor protein, influences amyloid precursor protein trafficking, and regulates A β levels.^{4,7,8} In addition, a recent study has identified a relation between variants in the *SORL1* gene encoding LR11 and risk for AD,⁹ adding considerable support to the hypothesis that LR11 is causally linked to AD pathogenesis.

To better understand the role of LR11 in the molecular pathogenesis of AD, we investigated neuronal LR11 expression in an early stage of this disease by examining brain tissue collected from people who died with an antemortem clinical diagnosis of mild cognitive impairment (MCI). MCI represents individuals whose cognition is not normal, but whose declines in cognitive and/or functional abilities are not sufficiently severe to meet criteria for dementia.¹⁰ Patients with MCI often progress to overt dementia, with a 12% annual conversion rate to AD.¹¹ An MCI subset with memory loss also display AD neuropathology, suggesting that these MCI cases in part represent prodromal stages of AD.^{10,12} However, a substantial percentage of MCI cases do not progress to AD, and some may revert to normal cognitive function.^{13,14}

To determine whether a reduction in neuronal LR11 occurs early in the progression of AD, we employed a novel quantitative immunohistochemical approach to tissue collected from participants in the Religious Orders Study, a longitudinal investigation of aging and AD,¹⁵⁻¹⁸ including subjects diagnosed with no cognitive impairment (NCI), MCI, and AD. We observed reduced cortical neuronal LR11 expression comparable with the loss seen in AD in a subset of MCI cases. Moreover, LR11 expression was found to correlate significantly with cognitive performance, with those cases with the lowest LR11 levels showing the greatest impairment. Together, these results support the hypothesis that altered expression of LR11 is an early event in AD pathogenesis.

Subjects and Methods

Case Material

Religious Orders Study participants undergo an annual clinical evaluation as described previously.¹⁵ Diagnosis of AD follows criteria implemented by the Consortium to Establish a Registry for Alzheimer's Disease, with MCI and NCI classifications following the logic of these criteria.¹⁹ Neuropathological evaluation is based on the National Institute on Aging/Reagan Consensus criteria,¹⁹ and all cases received a Braak stage.²⁰ Our study consisted of 9 NCI, 15 MCI, and 10 AD cases chosen from the Religious Orders Study cohort and matched on sex, education, and postmortem interval (Table 1). Apolipoprotein E (ApoE) genotyping was performed as described previously,^{21,22} although ApoE genotype was not considered in case selection. To ensure that the NCI group did not include cases at preclinical stages of AD, we included only control cases lacking significant amyloid pathology. Every attempt was made to match for age, though the exclusion of control cases with significant amyloid pathology resulted in a younger NCI group (see Table 1).

Immunohistochemistry

Free-floating, frozen cut 40 μ m-thick sections from superior frontal cortex (BA 9) were labeled with a polyclonal antibody to LR11²³ (a gift from Dr Chica Schaller). Sections were blocked with 8% normal goat serum, 0.1% Triton X-100 (Sigma Labs, St. Louis, MO), and 10 μ g/ml avidin in Tris-buffered saline; incubated overnight with anti-LR11; incubated for 1 hour with biotinylated goat anti-rabbit antibody (Vector Laboratories, Burlingame, CA) followed by

avidin-biotinylated horseradish peroxidase (ABC reagent; Vector Laboratories) for 1 hour; and developed in 3,3'-diaminobenzidine.

LR11 Quantitative Immunohistochemistry

We developed a novel quantitative approach to measure LR11 neuronal immunostaining to overcome observer bias²⁴ and allow more powerful statistical analyses. Five distinct regions from a single section of superior frontal cortex were randomly selected and 20 neurons were imaged from each region, for a total of 100 cells per case. Images were captured using a 100X oil immersion lens and a Hamamatsu digital camera (Hamamatsu, Bridgewater, NJ). Images of 20 successive cells per region were taken, with an average of 1 to 2 and a maximum of 6 cells per image to ensure equal sampling across the section. The selection of cells and quantitation were performed in a blinded manner. We used the Metamorph image analysis program, adapting a method that we previously developed to measure antigen colocalization.²⁵ Each cell in the field was outlined, and a threshold was set at the most intense staining in the local background. All pixels stained more intensely than this threshold level were considered positively stained, allowing us to calculate the percentage stained surface area. A percentage surface area was calculated from each of the 100 cells from each case.

Statistical Analyses

Clinical, demographic, and neuropathological characteristics were summarized and compared by Kruskal–Wallis or Fisher's exact test, with Bonferroni corrections for pairwise comparisons. LR11 measurements are shown in the original scale for summary statistics, with square-root transformations applied in statistical testing to correct for skewed (nonnormal) distribution. Because LR11 intensity was measured in each cell, and there were multiple cells per image and multiple images per case, three possible sources of variability were examined by variance component analysis. Image-to-image variability was small (8% of total) and therefore excluded from the analyses. The difference in LR11 among diagnosis groups was analyzed using mixed models with random intercept, fixed effect for diagnosis, Kenward–Roger denominator degrees of freedom, and unstructured covariance structure. Similar mixed models analyses were performed to assess the association between LR11 and clinicopathological variables. Mixed models for repeated measures take into account the correlation among observations from the same subject and give appropriate weighting to between-subject versus within-subject variation. Interrater reliability in the semiquantitative scorings of LR11 was examined by generalized weighted κ , and their consistency with quantitative LR11 measurements was assessed by Spearman's rank correlation. Levene's test was employed to test for the homogeneity of variances among the three diagnostic groups.²⁶ In addition, we performed a single-linkage agglomerative hierarchical clustering of LR11 values in the MCI subjects to determine whether the values clustered.²⁷ The distance matrix showing distances between each pair of individuals was derived from the Man–n–Whitney U statistic (the absolute value of $U - 1/2$). Statistical analyses were performed using SAS 9.1.3 (SAS Institute, Cary, NC). To account for the large number of analyses performed in this study, we set the level of statistical significance at 0.01 (two sided).

Results

General Staining Characteristics

The localization of LR11 was the same as we have described previously,^{4,5} with immunoreactivity in the soma and proximal dendrites of pyramidal neurons. Neuronal LR11 labeling appeared punctate, with some cases displaying strong and others little to no detectable LR11 immunoreactivity. Our quantitative approach easily distinguished between varying levels of neuronal LR11 labeling (Fig 1). In addition, three blinded raters also scored each case semiquantitatively, with a score of 1 denoting no discernible LR11 staining and a score of 4

representing strong immunostaining. Spearman's rank correlation demonstrated a significant correlation between the semiquantitative scorings and quantitative measures of LR11, indicating that the two approaches are generally consistent (Spearman's $r = 0.73-0.80$, $p < 0.001$ for all three raters). The three raters showed only moderate agreement (generalized weighted kappa, $\kappa = 0.46$), reflecting possible observer bias and demonstrating the benefits of quantitative approaches over semiquantitative rating scales.

Reduction of LR11 Expression in a Subset of Mild Cognitive Impairment Cases

There was strong punctate neuronal LR11 staining in NCI ($28.6 \pm 2.7\%$ surface area) that was markedly reduced in AD ($13.0 \pm 2.5\%$; $p = 0.020$; Fig 2A). The mean LR11 level in the MCI group ($22.8 \pm 4.7\%$) was intermediate between NCI and AD, but not statistically different from either group. The mean distribution of LR11 levels was significantly more variable in MCI compared with NCI and AD ($p = 0.003$, Levene's test for equal variances; see Fig 2B).

Hierarchical cluster analysis of a distance matrix between pairs of MCI subjects demonstrated high LR11 expression (MCI-H) and low LR11 expression (MCI-L) MCI subgroups (Fig 3). A series of pairwise comparisons were performed to test our hypotheses that LR11 expression in the MCI-H subgroup was similar to that seen in NCI, and that LR11 expression in the MCI-L subgroup was similar to that seen in AD. As predicted, there was no significant difference between MCI-L ($11.3 \pm 2.5\%$) and AD ($p = 0.43$). LR11 expression was significantly greater in the MCI-H subgroup ($45.8 \pm 3.6\%$) than in the NCI group ($p = 0.0078$). This can be attributed to the lack of cases with lower LR11 expression as a consequence of splitting the MCI group into two subgroups. Finally, this analysis confirmed a significant difference between LR11 expression in MCI-L and MCI-H ($p < 0.0001$).

Demographic features of our cases were examined for potentially confounding factors. The MCI-H and MCI-L groups did not differ in age at death ($p = 0.8$, Wilcoxon rank-sum test), and although the NCI group was younger ($p = 0.0034$, Kruskal-Wallis test), the four diagnostic groups did not differ in sex ($p = 0.72$, Fisher's exact test), years of education ($p = 0.2$, Kruskal-Wallis test), or postmortem interval ($p = 0.99$, Kruskal-Wallis test).

Because *APOE* genotype is a risk factor for AD,^{28,29} we compared the distribution of $\epsilon 4$ allele carriers in each diagnostic group. There were no (0/9) $\epsilon 4$ carriers in the NCI group, whereas 50% (5/10) of AD cases had at least one $\epsilon 4$ allele. Interestingly, similar to AD, 40% of the MCI-L cases were $\epsilon 4$ carriers, compared with only 20% in the MCI-H subgroup.

LR11 Expression Correlates with Cognitive Impairment

We examined the relation between LR11 neuronal levels and cognitive performance based on Mini-Mental State Examination (MMSE)³⁰ and the global cognitive score (GCS) in all four subgroups. The GCS is a composite Z-score compiled from 19 neuropsychological tests of cognition.³¹ A GCS of zero indicates cognitive function similar to the average of the reference population.³² A GCS of +1 (or -1) indicates cognitive function that is 1 standard deviation above (or below) the average of the reference population. MMSE scores were similar between both MCI subgroups (MCI-H: 26.8 ± 0.8 ; MCI-L: 26.2 ± 2.3) and NCI group (27.6 ± 1.8) but were greater than the AD group (20.0 ± 6.0 ; $p = 0.0006$ for comparison among the four groups; $p < 0.003$ for pairwise comparisons). Notably, the average MMSE score of the AD cases was 20, and the narrow spread of scores across all cases suggests that the degree of difference in cognitive impairment is too minor to be detected on the less sensitive MMSE. In contrast, there was a significant difference in the mean GCS between the two MCI subgroups. Although the mean GCS for MCI-H (0.4 ± 0.2) was similar to NCI (0.7 ± 0.2), the mean GCS for the MCI-L subgroup (0.0 ± 0.2) was significantly lower compared with the MCI-H subgroup. The MCI-L subgroup did, however, perform better than the AD group (-0.8 ± 0.5 ; $p < 0.001$ for pairwise

comparisons). We also examined the relation between LR11 and cognitive performance across all diagnostic groups using repeated-measures analysis, and LR11 expression was found to be significantly associated with the GCS ($p = 0.002$; Fig 4) and, to a lesser extent, with the MMSE ($p = 0.055$; data not shown). In other words, pooling across all diagnosis groups, those cases with the highest LR11 expression performed best on the cognitive tests, whereas those with the greatest reduction in LR11 were the most impaired. These findings suggest that neuronal LR11 expression levels relate to cognitive ability.

LR11 Expression and Alzheimer's Disease Neuropathology

Because LR11 is involved in regulating amyloid precursor protein processing and A β accumulation,⁴ we compared amyloid and tau pathology in each of the four groups. Whereas all AD and MCI cases were Braak stage III and above, all of the NCI cases but one were Braak stages 0, I, or II. Both MCI subgroups tended to have less extensive tangle pathology than AD based on the percentage of cases with Braak stage V/VI. The AD cases had high frequencies of both neuritic and diffuse plaques (see Supplementary Fig 1). Interestingly, both MCI subgroups showed pathology similar to the AD group (Table 2). Thus, unlike our findings correlating LR11 expression and cognitive decline, we did not detect any trend for increasing AD pathology from MCI-H to MCI-L to AD.

Discussion

A growing body of evidence suggests that LR11 is intricately involved in the pathogenesis of AD.^{4,5,7-9} This study characterized the expression of LR11 in MCI using a novel quantitative immunohistochemical procedure, which avoids limitations of semiquantitative methods and allows more powerful statistical analyses. We confirmed and extended our earlier findings that LR11 expression is reduced in AD compared with age-matched control cases with an independent and more mildly affected cohort.^{4,5} Surprisingly, we also found highly variable LR11 expression among MCI cases, showing two distinct MCI subgroups. The MCI-H subgroup is characterized by robust, control-like LR11 neuronal immunostaining, whereas the MCI-L subgroup exhibited a marked reduction in LR11, similar to that seen in AD. Further examination demonstrated that although both MCI subgroups showed significant AD pathology, the MCI-L subgroup showed more severe cognitive impairment. These findings suggest that LR11 expression distinguishes two MCI subgroups and that the low LR11 subgroup has greater cognitive impairment, reflecting a phenotype similar to AD.

Unlike the consistently high levels of LR11 in NCI and consistently low levels in AD, our analyses demonstrated a bimodal distribution in MCI. Although the identification of two distinct subgroups was unexpected, it is logical considering the clinical heterogeneity of MCI. Because MCI is a relatively new clinical entity with widely varying criteria^{11,13,33} and individuals with MCI convert to AD at a greater rate than those with normal cognition, it is often considered a prodromal stage of AD.^{10,14} However, individuals with MCI have heterogeneous neuropsychological profiles, and not all MCI cases progress to AD.³⁴ The highly variable LR11 expression seen in MCI and the stratification of cases into distinct subgroups by LR11 expression is a particularly exciting finding with implications for early diagnosis of AD.

Of the demographic, clinical, and pathological measures examined, the MCI subgroups defined by LR11 expression differed only in GCS, a general measure of cognitive performance. Moreover, LR11 levels strongly correlated with GCS across all cases, suggesting that LR11 expression may serve as a marker of disease severity. Much effort has been devoted to the identification of pathological correlates for symptom severity in AD.³⁵⁻³⁷ However, the lack of correlation with amyloid pathology and the weak correlation with neurofibrillary tangles have been disappointing.³⁸⁻⁴¹ The best known correlate of cognitive dysfunction in AD is

synaptic density,⁴²⁻⁴⁴ and it is interesting to note that the loss of other low-density lipoprotein receptor family members may contribute to synaptic loss and the resultant cognitive impairment.^{45,46} Our findings provide evidence that reduced LR11 expression may predispose individuals to cognitive impairment and the development of AD. Given the growing body of evidence linking LR11 to amyloid pathology, additional examination of the relation between LR11 and cognitive ability should be illuminating.

Because MCI is highly heterogeneous, and not all MCI cases will ultimately progress to AD, lower LR11 levels may identify cases likely to develop AD. Memory impairment is the hallmark symptom of AD, and it has been proposed that the amnesic subtype of MCI represents the earliest stage of AD, whereas the other subtypes may presage other forms of dementia.^{11,13,14,34,47} However, studies focusing exclusively on amnesic MCI^{37,48,49} have been shown to have low predictive value,^{29,50,51} suggesting that concentrating on this group may lead to inadvertent exclusion of cases that progress to AD. This cohort includes a heterogeneous mix of both amnesic and nonamnesic MCI subtypes. Given the range of MCI subtypes represented in our cohort, it is compelling that the subset of MCI cases with low, AD-like LR11 expression also shows the greatest degree of cognitive impairment. Moreover, LR11 neuronal expression in MCI displayed a bimodal distribution, suggesting that those cases with lower levels of LR11 expression may be at greater risk for progression to AD.

In summary, we have shown that LR11 expression can be measured quantitatively, that LR11 expression demonstrates two distinct MCI subgroups, and that LR11 levels are related to cognitive impairment. The recent report that LR11 is genetically linked to sporadic AD provides direct evidence that LR11 is causally linked to the development of the disease.⁹ When combined with evidence that LR11 modulates A β production^{4,8} and our current findings that LR11 expression is reduced in a subset of MCI cases with more cognitive impairment, it appears increasingly likely that the loss of LR11 is a causal event in the AD pathological cascade.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.

Quantitative immunohistochemistry can detect different levels of LR11 expression. Shown are representative images from cases with very high (A), high (B), medium (C), and low levels of LR11 expression. The red overlay shown in the inset of each image represents the pixels determined to be stained positive for LR11 for each cell. The number of pixels stained positively for LR11 is expressed as a percentage of the total number of pixels present within the outlined cell in the image.

Fig 2.

LR11 expression in mild cognitive impairment (MCI) is variable relative to no cognitive impairment (NCI) and Alzheimer's disease (AD). (A) Representative images from an NCI case (a), showing robust LR11 expression (46.5% cell-surface area stained positive for LR11); an AD case (b), showing weak LR11 expression (8.29% LR11); and two MCI cases, one showing NCI-like LR11 expression (c, 45.06% LR11) and one showing AD-like LR11 expression (d, 4.58% LR11). (B) The distribution of the case means for each diagnostic group showing that although there is an intermediate level of LR11 expression in the MCI group (22.75 ± 4.7) relative to NCI (28.6 ± 2.7) and AD (13.0 ± 2.5), the MCI group is significantly more variable ($p = 0.003$, Levene's test for equal variances) as a result of the bimodal distribution of LR11 expression in the MCI group.

Fig 3.

LR11 expression shows two distinct subgroups of mild cognitive impairment (MCI) cases. Independent statistical analysis using hierarchical clustering of 105 test statistics generated from the distribution-free, two-sample Wilcoxon rank-sum test demonstrated that MCI with high (MCI-H) and low LR11 expression (MCI-L) form two distinct clusters.

Fig 4.

LR11 expression is related to cognitive performance. LR11 expression was found to be strongly correlated with global cognitive score across all cases by repeated-measures analysis ($F_{(1,29)} = 11.48$; $p = 0.0020$). Diamonds designate Alzheimer's disease; filled squares designate mild cognitive impairment (MCI) with low LR11 expression; open squares designate MCI with high LR11 expression; triangles designate no cognitive impairment.

Table 1

Demographic Characteristics by Group

Characteristics	NCI (n = 9)	MCI (n = 15)	AD (n = 10)	Total (N = 34)	Comparison by Group, <i>p</i>
Mean age at death ± SD (range), yr ^a	75.4 ± 5.2 (67–82)	83.6 ± 5.1 (75–97)	82.6 ± 4.8 (80–94)	82.6 ± 6.9 (67–97)	0.0034 ^b
Male sex, n (%)	6 (67%)	7 (47%)	4 (40%)	17 (50%)	0.72 ^c
Mean education ± SD (range), yr	19.2 ± 4.2 (12–26)	17.4 ± 5.6 (8–30)	16.3 ± 3.9 (6–20)	17.9 ± 4.4 (6–30)	0.20 ^b
Mean postmortem interval ± SD (range), hr	11.3 ± 9.7 (2.2–33.5)	7.5 ± 4.3 (3.5–16)	6.4 ± 3.0 (3–10.7)	8.3 ± 6.4 (2.2–33.5)	0.51 ^b
Subjects with APOE ε4, n (%)	0 (0%)	5 (33%)	5 (50%)	10 (29%)	0.086 ^c

^aThe preclusion of control cases with significant amyloid pathology entailed a younger no cognitive impairment (NCI) group.

^bKruskal–Wallis test.

^cFisher’s exact test.

MCI = mild cognitive impairment; AD = Alzheimer’s disease; SD = standard deviation.

Mild Cognitive Impairment with High and Low LR11 Expression Subgroups Are Both Pathologically Alzheimer’s Disease-like

Table 2

Characteristics	NCI (n = 9)	MCI-H (n = 5)	MCI-L (n = 10)	AD (n = 10)	Comparison by group, p^a
Mean postmortem interval $\pm$ SD (range), hr	6.5 $\pm$ 3.9 (3.5–16)	6.5 $\pm$ 3.7 (4–13)	6.7 $\pm$ 4.6 (3–16)	6.1 $\pm$ 2.7 (3–10.7)	0.99
Distribution of Braak scores					0.0001 ^b
0	1	0	0	0	
I/II	7	0	0	0	
III/IV	1	3	9	4	
V/VI	0	2	1	6	
Frequency of neuritic plaques ^c	0	3.4 $\pm$ 0.9 (2–4)	1.9 $\pm$ 2.0 (0–5)	3.7 $\pm$ 1.2 (2–5)	0.0003 ^b
Frequency of diffuse plaques ^c	0	3.6 $\pm$ 2.2 (0–5)	2.9 $\pm$ 2.2 (0–5)	3.9 $\pm$ 1.4 (1–5)	0.0010 ^b

^aKruskal–Wallis test.

^bPairwise comparisons (with Bonferroni correction) between groups showed that only the no cognitive impairment (NCI) group was found to have significantly different Alzheimer’s disease (AD) pathology compared with the other groups.

^cFrequency scores; 0 = none, 1 = sparse (1–2), 2 = sparse to moderate (3–5), 3 = moderate (6–12), 4 = moderate to frequent (13–19), 5 = frequent (20+).

MCI-H = mild cognitive impairment with high LR11 expression; MCI-L = mild cognitive impairment with low LR11 expression; SD = standard deviation.