LRP4 LDLα repeats of astrocyte enhances dendrite arborization of the neuron

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Abstract

Low-density lipoprotein receptor-related protein 4 (LRP4) is essential for inducing the neuromuscular junction formation in muscle fibers, and LRP4 plays a role of dendritic development and synaptogenesis in the central nervous system (CNS). As a member of the low-density lipoprotein receptor family, LRP4 contains an enormously large extracellular region possessing multiple LDLα repeats in the N-terminal. LRP4 only with extracellular domain acts as a similar mechanism of full-length LRP4 in muscles to stimulate acetylcholine receptor clustering.

In this study, we elucidated that LDLα repeats of LRP4 maintained the body weight and survival rate. Dendritic branches of the neurons in Lrp4-null mice with LRP4 LDLα repeats residue were more than in Lrp4-null mice without residual LRP4 domain. Supplement with conditioned medium from LRP4 LDLα over expression cells, primary culture neurons achieved strong dendritic arborization ability. In addition, astrocytes with LRP4 LDLα repeats residue could promoted the dendrite arborization of neurons in primary co-cultured system. These observations
signify that LRP4 LDLα repeats play an underlying prominent role in dendrite arborization.

**Keywords:** LRP4, LDLα repeats, primary culture, Golgi staining, dendrite arborization

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**Introduction**

Synapses formed between neurons and target cells are the basis of brain function. Synaptic transmission is critical for thinking, learning, memory and response to environmental changes. Synaptic dysfunction involves various neuropsychiatric diseases, including autism, schizophrenia, epilepsy and addiction. LRP4 plays a vital role in neuromuscular junction (NMJ) presynaptic differentiation [1-3]. Biochemical studies have confirmed that LRP4 is a key regulator for NMJ formation [4]. It is the receptor of agrin and specifically binds to neural agrin, which promotes NMJ development [1, 3-6]. LRP4 contains an extracellular domain (ECD), intracellular domain (ICD) and transmembrane domain. ECD domain has eight LDLα domains, four β-propeller domains, and a domain for O-linked oligosaccharide modification. LRP4 ECD/ECD mice form partially functional NMJs, expressing only the ECD, without the transmembrane and ICD domain [7]. This suggested that the transmembrane domain and ICD are not required for NMJ formation [5, 8, 9], and LRP4 ECD domain plays a main role in the formation of NMJ.

LRP4 is mainly expressed in the hippocampus, olfactory bulb, cerebellum, and neocortex, especially in the postsynaptic membrane of the hippocampus [10-12]. Previous studies indicate that LRP4 is a protein present at the postsynaptic density of pyramidal neurons [12, 13]. LRP4
mRNA is present in the hippocampal dentate gyrus granulosa cell layer and hippocampal pyramidal cell layer. LRP4 plays a critical role in the central nervous system of adults, including hippocampal synaptic plasticity, maintenance of excitatory synaptic transmission, fear regulation, and long-term enhancement [13, 14]. LRP4 knockout can affect the synaptic plasticity and cognitive function of mice [13, 15]. LRP4 expressed has been shown to regulate glutamatergic synaptic transmission by regulating the release of adenosine triphosphate (ATP) in astrocytes [11]. The role of LRP4 extracellular segment in the brain is currently unclear.

In this study, two types of muscle-rescued Lrp4-null mice were used to identify LRP4 loss of function, which were muscle-rescued Lrp4\textsuperscript{LacZ} mice (mr-Lrp4\textsuperscript{LacZ}) (RRID: MMRRC_048465 -UCD) and muscle-rescued Lrp4\textsuperscript{min} mice (mr-Lrp4\textsuperscript{min}). Astonishingly, we found some intriguing differences between mr-Lrp4\textsuperscript{LacZ} mice and mr-Lrp4\textsuperscript{min} mice, which were ignored or not paid special attention in previous research. LRP4 LDL\textalpha domain not only affects the survival rate and body weight of mice. Especially, LRP4 LDL\textalpha domain manipulates dendritic arborization of neurons \textit{in vivo} and \textit{in vitro}. These results indicate LRP4 LDL\textalpha domain plays a crucial role in the development of the central nervous system during dendritic branching.

Materials and methods

Animal

All transgenic or wild type male mice were housed in ventilated cages. 5 or fewer adult mice were feed in each cage. Sufficient water and food were free intake to mice, with a 12-h light/dark cycle, room temperature at 22 to 25°C and humidity is 50-60%. All experiments involving animals were conducted according to the "guidelines for the care and use of experimental animals" issued by
Nanchang University. The Committee on the Ethics of Animal Experiments of the University of Nanchang approved the protocol (Permit Number: 2016–0002). For in vivo experiment, surgery was performed under sodium pentobarbital anesthesia (50 mg/kg, ip injection), and all efforts were made to minimize suffering. After terminal experiments, mice were euthanized by carbon dioxide inhalation followed by cervical dislocation.

**Nissl’s staining**

Brain slides were cut in 40 µm, washing with distilled water for 3 min. Dyeing in the staining buffer (0.2% Cresyl Violet solution) for 5 min in 60°C. Then dehydrating slides with 50%, 75%, 90% ethanol (Sangon Biotech, China, A500737) for 20 sec. Putting the slides into 100% ethyl alcohol 3 times, each time for 20 sec, transferring slides into xylene (Sangon Biotech, China, A530011) 3 times, each time for 10 min. Samples were mounted in Hydromount (National Diagnostics, USA, HD-106).

**Golgi staining**

Golgi staining was performed by using FD Rapid Golgi Stain™ Kit (FD NeuroTechnologies, USA, PK-401). Staining solution D, solution E and ultra-pure water were mixed in a ratio of 1:1:2. Slides were incubated with staining solution at room temperature for 10 min and washed twice with ultra-pure water, each time for 4 min. Transferring brain slides to the plate hole containing 50%, 75% and 90% ethanol for 4 min each time, then the brain slides were put into the holes containing 10 ml 90% ethanol or 100% ethanol for 3 times, 4 min for each time. Followed the samples were put into the xylene for 1 h. Images were randomly taken. Neurons with clear dendritic branches were
subjected to Sholl Analysis by using image J. The investigator who performed analysis was blinded to genotypes.

Cell culture and plasmid transfection

HEK293T (RRID:CVCL_0063) cells were cultured with media (DMEM + 10% Fetal bovine serum +1% Pens/Strep) in a cell culture incubator at 37°C and 5% CO₂, and changed the medium once every 3 days. After the cell density reached about 60%, added the plasmids (pFLAG-CMV1 vector, pFLAG-CMV1-Lrp4-LDLα, or pFLAG-CMV1-Reelin) and polyethyleneimine (Polysciences, USA, 24314) to 50 μl of serum-free DMEM culture medium according to the ratio of 3 μg of plasmid to 15 μl of PEI, then mixed together at room temperature. After 20 min, the mixture was added to HEK293T cells. After cultured in an incubator at 37°C and 5% CO₂ for 4-8 h, the culture medium was replaced with new HEK293T culture medium. After 24 h, conditioned medium collected from the supernatant was added into the primary neurons (DIV4). Primary neurons were changed half-medium every other day, and after 6 days (DIV10), the neurons were preformed to immunofluorescent staining.

Astrocyte and neuron co-culture

Primary cell separation and co-culture were performed as described previously [11] with minor modifications. The isolated hippocampi of E18 mice were cut for small pieces, and digested in 0.25% trypsin at 37°C for 10-15 min. For primary neuron culture, dissociated cells were resuspended in primary culture medium (Neurobasal+1% GlutaMax™+5%FBS+1% Pens/Strep) and plated onto poly-L-lysine-coated coverslips in 12 well-plates for 4 h. And then replacing medium with serum-
free medium (Neurobasal + 1% GlutaMAX™ + 2% B27 + 1% Pens/Strep) and cytosine arabinoside (Med ChemExpress, China, HY-13605) (10 μM) to inhibit glia proliferation. Half of the medium was changed every other day. For astrocyte culture, dissociated cells were resuspended in the plating medium (DMEM + 10% Fetal bovine serum + 1% GlutaMAX™ + 1% Pens/Strep) and plated into culture flasks for 3 d. The flasks were shaken at 250 rpm for 24 h to remove microglia and oligodendrocytes. Astrocytes were passaged every 3 d at a ratio 1:3 and seeded onto coverslips before co-culture. After neuron were cultured 8 d (DIV8), three coverslips seeded astrocytes were placed with one neuron coverslip in one 35-mm dish containing serum-free medium for incubating 7 d (DIV15) until immunofluorescent staining.

Immunofluorescent staining

The coverslips of astrocyte and neuron co-culture were fixed at room temperature for 20 min in 4% paraformaldehyde. After rinsing 10 min with phosphate buffered saline (PBS) (0.01 M, pH 7.4) at room temperature, the coverslips were immersed in antibody blocking solution (10% donkey serum, 1% calf serum albumin, 0.5% Triton X-100 in PBS) at room temperature for 2 h. After this, the coverslips were rinsed with 0.01 M PBS at room temperature for 3 times, the primary antibody (anti-β3-Tubulin antibody, Thermo Fisher Scientific, PA5-95875) was diluted 1:1000 with antibody blocking solution, and was added into the coverslips for 4°C overnight. The coverslips were washed with 0.01 M PBS at room temperature for 3 times, each time for 10 min. The secondary antibody (anti-IgG, Invitrogen, 1832035) was diluted 1:1000 with antibody blocking solution, and then the coverslips were incubated at room temperature for 2 h in dark. After washing 3 times for 10 min with PBST, samples were mounted in Hydromount (National Diagnostics). Z serial images were
collected with an Olympus fluorescence microscope (FSX100) and collapsed into a single image.

Experimental design and Statistical analysis

The study was not pre-registered. For assignment of experimental groups, no special randomization methods were employed. Sample sizes and the number of cells were determined by past experience and not by a statistical sample size calculation. The experiments reported in this work did not require institutional approval. Exclusion criteria were not pre-determined in this study.

Data were statistically analyzed using GraphPad Prism 5.0 (RRID: SCR_002798, GraphPad Software, CA, USA) and the results were expressed as mean ± standard error (Mean ± SEM). The analysis and experimental group assignments were performed by a different person than the experimenter. The measurement data were analyzed by one-way ANOVA; the comparison between groups was analyzed by independent sample T-test. The mice numbers of independent experiments were noted in the figure legend. The difference was considered statistically significant, and P < 0.05 was considered to be statistically significant, P >0.05 (#) was considered no significance.

Results

LRP4 LDLα domain was necessary to keep body weight and survival rate

Body weight and survival rate of mr-Lrp4LacZ mice, mr-Lrp4min mice and the control mice were monitored for more than 60 d. Compared with the control mice, the survival rate was significantly lower in the mr-Lrp4LacZ group (Fig1 B). The mr-Lrp4LacZ mice gained significantly less body weight and brain weight (Fig1 C-E). However, mr-Lrp4min mice appeared healthy and were indistinguishable from the control mice. There was no significantly difference between mr-Lrp4min
mice and the control mice in body weight, survival rate and brain weight (Fig 1B-1E). mr-Lrp4\textsuperscript{min} mice and mr-Lrp4\textsuperscript{LacZ} mice were all Lrp4-null mice, but there were serious differences in phenotype between mr-Lrp4\textsuperscript{LacZ} and mr-Lrp4\textsuperscript{min} mice. The mr-Lrp4\textsuperscript{min} mice remained LDL\textalpha{} domain of LRP4 theoretically, as shown in Fig 1A. Therefore, we speculate whether the LDL\textalpha{} domain played a certain function to keep mice alive and body weight, which lead to phenotypic differences between the two types of mice.

*The thickness of cerebral cortex reduced in mr-Lrp4\textsuperscript{LacZ} mice*

Nissl’s staining was carried out to observe the cerebral cortex region of mr-Lrp4\textsuperscript{LacZ} mice, mr-Lrp4\textsuperscript{min} mice and the control mice. The cerebral cortex of mr-Lrp4\textsuperscript{LacZ} mice was markedly thinner in layer I, II/III and IV than those of the control group, while the cerebral cortex of mr-Lrp4\textsuperscript{min} mice showed no difference from those of the control mice (Fig 2A, B). There was a remarkable difference in thickness of mr-Lrp4\textsuperscript{LacZ} mice compared with mr-Lrp4\textsuperscript{min} and the control mice. We speculated that the LDL\textalpha{} domain of LRP4 maintained the normal structure of the cerebral cortex.

*The dendritic branches of neurons increased in mr-Lrp4\textsuperscript{min} mice*

Golgi staining was performed to identify morphological structure of prefrontal cortex neurons in mr-Lrp4\textsuperscript{LacZ}, mr-Lrp4\textsuperscript{min} mice and the control mice. The complexity of neurons was analyzed by Sholl analysis. Sholl analysis centered on neuronal cell body, a series of concentric circles were drawn, and obtained the number of intersections of neuronal processes varying with the distance from the cell body. Compared with the control mice, there was no difference in total dendrites length presented in mr-Lrp4\textsuperscript{LacZ} mice, as shown in figure 3, but the total dendrite length in mr-Lrp4\textsuperscript{min} mice
was longer (Fig 3 B). In mr-Lrp4\textsuperscript{LacZ} mice, we observed no significant difference in total dendrites number of the prefrontal cortex neurons compared with the control group (Fig 3 C, D). Besides, more dendrite branches were showed in mr-Lrp4\textsuperscript{min} mice than the control mice (Fig 3 C, D). It indicated that the LRP4 LDL\textalpha domain may play a role of promoting dendritic arborization.

LRP4 LDL\textalpha domain increased dendrite arborization in vitro

In mr-Lrp4\textsuperscript{min} mice, the LDL\textalpha domain was secreted because of LRP4 lack of transmembrane domain. To assess whether LRP4 LDL\textalpha domain played a role in neuronal dendritic arborization, we transfected pFLAG-CMV1-Lrp4 LDL\textalpha into primary neurons of wild type mice, pFLAG-CMV1-Vector was negative control and pFlag-CMV1-Reelin plasmid was positive control. The number of dendrites branches increased in the LRP4 LDL\textalpha group compared with the negative samples (Fig 4 B, C).

Studies showed that LRP4 was expressed in neuron and astrocyte, and LRP4 knockout in astrocytes suppressed glutamatergic release by increasing ATP release [11]. To explore whether the LRP4 LDL\textalpha promoting dendritic arborization were from neuron or astrocyte, we co-cultured the astrocytes with the neurons from wild type mice and/or mr-Lrp4\textsuperscript{min} mice. Neurons of wild type mice showed more branches being co-cultured with astrocytes from mr-Lrp4\textsuperscript{min} mice than astrocytes from wild type mice. Without controversy, the Lrp4\textsuperscript{min} mice neurons boosted more dendritic arborization being co-cultured with astrocytes from Lrp4\textsuperscript{min} mice than astrocytes from wild type mice (Fig 5 B, C). The data further confirmed that free LRP4 LDL\textalpha domain in astrocytes promoted dendrite arborization of neurons.
Discussion

Here we demonstrated that the survival rate and body weight of mr-Lrp4$^{lacZ}$ mice were lower than mr-Lrp4$^{min}$ mice and the control mice. The brain tissue also was smaller. Second, the cerebral cortex was thinner in layer I, II/III and IV of mr-Lrp4$^{lacZ}$ mice than those of the control group. In mr-Lrp4$^{min}$ mice, LRP4 has the LDLα domain, but this domain was missing in mr-Lrp4$^{lacZ}$ mice. We speculated that these changes may be related to the function of the LDLα domain. Therefore, in order to clarify the role of the LRP4 LDLα domain, we performed Golgi staining to observe the branches of neuronal cells. The results showed that there were more dendritic branches in neurons of mr-Lrp4$^{min}$ mice than in the control group. On the contrary, there was no difference between mr-Lrp4$^{lacZ}$ mice and the control group. Third, neuronal cells transfected with LRP4 LDLα plasmid have more dendritic branches than the control group. When neurons were co-cultured with astrocytes of mr-Lrp4$^{min}$ mice, the number of dendritic branches increased. These results indicated that LDLα domain of LRP4 promoted more dendritic arborization in neurons.

LRP4, as a member of the low-density lipoprotein receptor family, contains a large extracellular N-terminal region, a transmembrane domain and a short C-terminal region [16]. The extracellular region has eight LDLα domains (class A repeats), four β-propeller domains (class B repeats). LRP4 has a fundamental role during formation, maintenance and regeneration of the NMJ [17]. LRP4 β1 domain binds with agrin to form the agrin-LRP4 binary complex to activate acetylcholine receptor (AChR) clustering in NMJ [5, 6]. Study results showed that treatment ecto-LRP4 (Lrp4 ECD only) into myotubes, ecto-LRP4 increased the number of agrin-induced AChR clusters. This indicates that soluble ecto-LRP4 is sufficient to serve as a receptor for agrin to initiate pathways for AChR clustering. Ecto-LRP4 acts via a similar mechanism of full-length LRP4 in
muscles to stimulate AChR clustering [1]. In this experiment, the release of LDLα domain in mr-
Lrp4\textsuperscript{min} mice may promote the increase of neuronal branches.

LRP4 plays a crucial role in CNS, including maintaining synapses, especially in synaptic
transmission [18, 19]. Studies demonstrated that in the brain glutamate release was reduced in
lacking LRP4 mice. LRP4 knockout astrocytes suppressed presynaptic glutamatergic release by
increasing ATP release. ATP released from astrocytes was converted to adenosine that activates
adenosine A1 receptors in glutamatergic pre-synapses. Synaptic plasticity was affected [11]. Besides,
LRP4 played a role in dendritic development and synaptogenesis in the CNS. Knockdown of
LRP4 in embryonic cortical and hippocampal neurons causes a reduction in density of primary
dendrites, overexpression of LRP4 in these cultured neurons had the opposite effect inducing more
but shorter primary dendrites (Karakatsani et al., 2017). Neuron-specific knockdown of LRP4 by in
utero electroporation of LRP4 miRNA also resulted in neurons with fewer primary dendrites in the
developing cortex and hippocampus in vivo (Karakatsani et al., 2017). Embryonic cortical neurons
from Lrp4\textsuperscript{min} mice had fewer but longer primary dendrites and transfection of agrin compensated
the dendritic branching deficits in LRP4-deficient neurons (Handara et al., 2019).

Overall, our data point to functional links between LRP4 LDLα domain in modulating
dendritic branching in developing CNS neurons. LRP4 LDLα domain promoted more dendritic
branches formation. LRP4 LDLα domain binds with DKK1, Sclerostin, ApoE, Gremlin1, Wise.

DKK1, Sclerostin and Wise are factors that inhibit Wnt signaling by binding LRP5/6 [20-22].

DKK1 mutation causes the phenotypes of a double ridge and polysyndactyly [23]. Mutation of
Sclerostin results in sclerosteosis [24]. Moreover, LRP4 ECD enhances sclerostin-mediated
inhibition of Wnt/β-catenin signaling [25]. LRP4 mutation increases serum sclerostin level in
osteocytes [26]. However, the significance of the LRP4 interaction with the Wnt signaling pathway in brain remains unclear [27]. LRP4 and Wise interaction is revealed to regulate the patterning and formation of gland development [28]. ApoE, as one of LRP4 ligands, is essential for the development of the nervous system, the regulation of synaptic plasticity, neuroprotection, and the innervation of the muscle [8]. LRP4 interaction with ApoE promotes Aβ uptake[29]. ApoE also interacts with Reelin [8] and inhibits Reelin boost dendritic arborization [30-33]. Therefore, we speculated that free LRP4 LDLα in mr-Lrp4mit mice may promote dendritic arborization by relieving the inhibition of ApoE on the Reelin.

References

1. Wu H, Lu Y, Shen C, Patel N, Gan L, Xiong WC, Mei L: Distinct roles of muscle and motoneuron LRP4 in neuromuscular junction formation. Neuron 2012, 75(1):94-107.

2. Wu H, Xiong WC, Mei L: To build a synapse: signaling pathways in neuromuscular junction assembly. Development 2010, 137(7):1017-1033.

3. Yumoto N, Kim N, Burden SJ: Lrp4 is a retrograde signal for presynaptic differentiation at neuromuscular synapses. Nature 2012, 489(7416):438-442.

4. Weatherbee SD, Anderson KV, Niswander LA: LDL-receptor-related protein 4 is crucial for formation of the neuromuscular junction. Development 2006, 133(24):4993-5000.

5. Kim N, Stiegler AL, Cameron TO, Hallock PT, Gomez AM, Huang JH, Hubbard SR, Dustin ML, Burden SJ: Lrp4 is a receptor for Agrin and forms a complex with MuSK. Cell 2008, 135(2):334-342.

6. Zhang B, Luo S, Wang Q, Suzuki T, Xiong WC, Mei L: LRP4 serves as a coreceptor of agrin. Neuron
7. Johnson EB, Hammer RE, Herz J: Abnormal development of the apical ectodermal ridge and polysyndactyly in Megf7-deficient mice. Hum Mol Genet 2005, 14(22):3523-3538.

8. Choi HY, Liu Y, Tennert C, Sugiura Y, Karakatsani A, Kroger S, Johnson EB, Hammer RE, Lin W, Herz J: APP interacts with LRP4 and agrin to coordinate the development of the neuromuscular junction in mice. Elife 2013, 2:e00220.

9. Gomez AM, Burden SJ: The extracellular region of Lrp4 is sufficient to mediate neuromuscular synapse formation. Dev Dyn 2011, 240(12):2626-2633.

10. Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, Boe AF, Boguski MS, Brockway KS, Byrnes EJ et al: Genome-wide atlas of gene expression in the adult mouse brain. Nature 2007, 445(7124):168-176.

11. Sun XD, Li L, Liu F, Huang ZH, Bean JC, Jiao HF, Barik A, Kim SM, Wu H, Shen C et al: Lrp4 in astrocytes modulates glutamatergic transmission. Nat Neurosci 2016, 19(8):1010-1018.

12. Tian QB, Suzuki T, Yamauchi T, Sakagami H, Yoshimura Y, Miyazawa S, Nakayama K, Saitoh F, Zhang JP, Lu Y et al: Interaction of LDL receptor-related protein 4 (LRP4) with postsynaptic scaffold proteins via its C-terminal PDZ domain-binding motif, and its regulation by Ca/calmodulin-dependent protein kinase II. Eur J Neurosci 2006, 23(11):2864-2876.

13. Gomez AM, Froemke RC, Burden SJ: Synaptic plasticity and cognitive function are disrupted in the absence of Lrp4. Elife 2014, 3:e04287.

14. Pohlkamp T, Durakoglugil M, Lane-Donovan C, Xian X, Johnson EB, Hammer RE, Herz J: Lrp4 domains differentially regulate limb/brain development and synaptic plasticity. PLoS One 2015, 10(2):e0116701.
15. Kucukdereli H, Allen NJ, Lee AT, Feng A, Ozlu MI, Conatser LM, Chakraborty C, Workman G, Weaver M, Sage EH et al: Control of excitatory CNS synaptogenesis by astrocyte-secreted proteins Hevin and SPARC. Proc Natl Acad Sci U S A 2011, 108(32):E440-449.

16. Shen C, Xiong WC, Mei L: LRP4 in neuromuscular junction and bone development and diseases. Bone 2015, 80:101-108.

17. Tintignac LA, Brenner HR, Ruegg MA: Mechanisms Regulating Neuromuscular Junction Development and Function and Causes of Muscle Wasting. Physiol Rev 2015, 95(3):809-852.

18. Mosca TJ, Luginbuhl DJ, Wang IE, Luo L: Presynaptic LRP4 promotes synapse number and function of excitatory CNS neurons. Elife 2017, 6.

19. Karakatsani A, Marichal N, Urban S, Kalamakis G, Ghanem A, Schick A, Zhang Y, Conzelmann KK, Ruegg MA, Berninger B et al: Neuronal LRP4 regulates synapse formation in the developing CNS. Development 2017, 144(24):4604-4615.

20. Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA: Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. Nat Cell Biol 2001, 3(7):683-686.

21. Balemans W, Devogelaer JP, Cleiren E, Piters E, Caussin E, Van Hul W: Novel LRPS5 missense mutation in a patient with a high bone mass phenotype results in decreased DKK1-mediated inhibition of Wnt signaling. J Bone Miner Res 2007, 22(5):708-716.

22. Semenov MV, Tamai K, Brott BK, Kuhl M, Sokol S, He X: Head inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. Curr Biol 2001, 11(12):951-961.

23. Adamska M, MacDonald BT, Meisler MH: Doubleridge, a mouse mutant with defective compaction of the apical ectodermal ridge and normal dorsal-ventral patterning of the limb. Dev Biol 2003, 255(2):350-362.
24. Brunkow ME, Gardner JC, Van Ness J, Paeper BW, Kovacevich BR, Proll S, Skonier JE, Zhao L, Sabo PJ, Fu Y et al: Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cystine knot-containing protein. Am J Hum Genet 2001, 68(3):577-589.

25. Leupin O, Piters E, Halleux C, Hu S, Kramer I, Morvan F, Bouwmeester T, Schirle M, Bueno-Lozano M, Fuentes FJ et al: Bone overgrowth-associated mutations in the LRP4 gene impair sclerostin facilitator function. J Biol Chem 2011, 286(22):19489-19500.

26. Xiong L, Jung JU, Wu H, Xia WF, Pan JX, Shen C, Mei L, Xiong WC: Lrp4 in osteoblasts suppresses bone formation and promotes osteoclastogenesis and bone resorption. Proc Natl Acad Sci U S A 2015, 112(11):3487-3492.

27. Chang MK, Kramer I, Huber T, Kinzel B, Guth-Gundel S, Leupin O, Kneissel M: Disruption of Lrp4 function by genetic deletion or pharmacological blockade increases bone mass and serum sclerostin levels. Proc Natl Acad Sci U S A 2014, 111(48):E5187-E5195.

28. Ahn Y, Sims C, Logue JM, Weatherbee SD, Krumlauf R: Lrp4 and Wise interplay controls the formation and patterning of mammary and other skin appendage placodes by modulating Wnt signaling. Development 2013, 140(3):583-593.

29. Zhang H, Chen W, Tan Z, Zhang L, Dong Z, Cui W, Zhao K, Wang H, Jing H, Cao R et al: A Role of Low-Density Lipoprotein Receptor-Related Protein 4 (LRP4) in Astrocytic Abeta Clearance. J Neurosci 2020, 40(28):5347-5361.

30. Chai X, Fan L, Shao H, Lu X, Zhang W, Li J, Wang J, Chen S, Frotscher M, Zhao S: Reelin Induces Branching of Neurons and Radial Glial Cells during Corticogenesis. Cereb Cortex 2015, 25(10):3640-3653.

31. Jossin Y, Goffinet AM: Reelin signals through phosphatidylinositol 3-kinase and Akt to control
cortical development and through mTor to regulate dendritic growth. Mol Cell Biol 2007, 27(20):7113-7124.

32. Durakoglugil MS, Chen Y, White CL, Kavalali ET, Herz J: Reelin signaling antagonizes beta-amyloid at the synapse. Proc Natl Acad Sci U S A 2009, 106(37):15938-15943.

33. Kim J, Park TJ, Kwon N, Lee D, Kim S, Kohmura Y, Ishikawa T, Kim KT, Curran T, Je JH: Dendritic planarity of Purkinje cells is independent of Reelin signaling. Brain Struct Funct 2015, 220(4):2263-2273.

Abbreviations

LRP4: Low-density lipoprotein receptor-related protein 4; CNS: Central Nervous System; AChR: Acetylcholine receptor; NMJ: neuromuscular junction; ECD: extracellular domain; ICD: intracellular domain; ATP: Adenosine Triphosphate; DIV: Days in vitro; DMEM: dulbecco's modified eagle medium; PBS: phosphate buffered saline

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Authors’ contributions
YM and WSQ initiated and designed the study. GAM and CP performed the Nissl’s staining and Golgi staining. YM and JHY performed Cell culture and plasmid transfection. RDY, ZYZ, WYQ, FEK, ZSQ and LXS analyzed data. YM and WSQ wrote the manuscript with input from all coauthors. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All experiments involving animals were conducted according to the "guidelines for the care and use of experimental animals" issued by Nanchang University. The Committee on the Ethics of Animal Experiments of the University of Nanchang approved the protocol.

Consent for publication

Not applicable.
Competing interests

The authors declare that they have no competing interests.

Figure legend

Figure 1. LRP4 LDLα domain is necessary to keep body weight and survival rate

(A) Schematic diagram of Lrp4-LacZ and Lrp4-mitt; (B-C) Comparing with mr-Lrp4
mitt and the control mice, reduced survival rate in mr-Lrp4LacZ mice (B), and reduced body weight of mr-Lrp4LacZ mice (C); (D) Representative brain of the control, mr-Lrp4LacZ and mr-Lrp4
mitt mice; (E) Brain weights of the control, mr-Lrp4LacZ and mr-Lrp4
mitt mice. Values are means ± SEM. Mice number per group no less than 10. * P < 0.05, ** P < 0.01.

Figure 2. LRP4 LDLα domain affects the thickness of mouse cerebral cortex

(A) Representative images of Nissl’s staining the cerebral cortex of the control, mr-Lrp4LacZ and mr-Lrp4
mitt mice; (B) The thickness of the cerebral cortex in layer I, II/III and IV decreased in mr-Lrp4LacZ mice. Values are means ± SEM. Mice number per group no less than 5. * P < 0.05, ** P < 0.01.

Figure 3. LRP4 LDLα domain enhances the dendrite arborization of neurons in mr-Lrp4
mitt mice

(A) Representative images of Golgi staining the neurons in cerebral cortex of the control, mr-Lrp4LacZ and mr-Lrp4
mitt mice; (B) The total dendrite length of neurons increased in mr-Lrp4
mitt mice; (C-D) The total dendrite branch number of neurons increased in mr-Lrp4
mitt
mice. Values are means ± SEM. Mice number per group no less than 3 and neuron number per group no less than 30. * P < 0.05, ** P < 0.01, # P > 0.05.

Figure 4. LRP4 LDLα domain increases the dendrites arborization in primary neurons (A-B) pFLAG-CMV1-Lrp4-LDLα, pFLAG-CMV1-Reelin and pFLAG-CMV1 plasmid were transfected into HEK293T cells, then the conditioned medium was collected to add into primary cultured wild type mice neurons; (C) LRP4 LDLα domain improved the number of dendrites branches in primary neurons. Intersections of neuron dendrites branches were scored using Image J sholl analysis. Mice number per group no less than 6 and neuron number per group no less than 30. ** P < 0.01, ***P < 0.001.

Figure 5. LRP4 LDLα domain in astrocytes promote dendrite arborization in primary co-cultured neurons (A-B) Primary neurons of mr-Lrp4milt mice or wild type mice were co-cultured with astrocytes of mr-Lrp4milt mice or wild type mice; (C) Co-cultured with astrocytes from mr-Lrp4milt mice, neuronal dendrite branch number of wild type mice was more than co-cultured with astrocytes from wild type mice; Similar difference are present in the co-cultured neurons of mr-Lrp4milt mice with astrocytes from mr-Lrp4milt mice or from wild type mice. Intersections of neuron dendrites branches were scored using Image J sholl analysis. Mice number per group no less than 6 and neuron number per group no less than 30. ** P < 0.01.