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Jagged1 is essential for radial glial maintenance in the cortical proliferative zone

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Abstract

Radial glial maintenance is essential for the proper development of the cortex. It is known that the evolutionarily conserved Notch signaling pathway is required for maintaining the pool of radial glial stem cells although the mechanisms involved are not entirely understood. Here, we study the Notch ligand, *Jagged1*, in the mouse ventricular zone at a late stage of embryonic development. We use a conditional loss of function allele to show that *Jagged1* is required for maintaining radial glial cells and when absent, leads to defects in the cortical proliferation zone and expression of intermediate progenitor cells. Using *in-vitro* approaches, we found that depletion of *Jagged1* reduced the size of primary neurospheres and their capacity to self-renewal. Finally, *Jagged1* mutants also showed precocious neuronal differentiation and cortical defects. Together, these data support a role for *Jagged1* in radial glia maintenance in the neocortex.

Keywords

Notch; *Jagged1*; corticogenesis; neocortex; *Pax6*; *Tbr2*; *Tbr1*; *glia*

Introduction

At the onset of neocortical neurogenesis, neuroepithelial cells become radial glial cells (RGCs) (Kriegstein et al., 2006). RGCs likely act as stem cells that divide in an asymmetric manner to self-renew and generate restricted intermediate progenitor cells (IPCs) and cortical neurons (Englund et al., 2006; Kriegstein et al., 2006; Merkle and Alvarez-Buylla, 2006; Miyata et al., 2001; Tamamaki et al., 2001). Although the ability of RGC to generate neurons while self-maintaining is one of their essential functions during corticogenesis, the basic mechanisms that regulate this process are not fully understood.

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Author Contributions

C.A.B. conceptualized, designed, performed all experiments, analyzed data, and wrote the manuscript.

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Notch signaling has been implicated in neurodevelopmental, neurodegenerative, and psychiatric disorders (Dieset et al., 2012; Masek and Andersson, 2017; Oda et al., 1997; Song et al., 1999). For example, Notch signaling can alter neurogenic potential of RGCs and promote neuronal differentiation, cell division, and neuronal migration (Dang et al., 2006; Gaiano and Fishell, 2002; Justice and Jan, 2002). Loss of Notch signaling leads to the premature differentiation of neural stem cells into neurons in most regions of the CNS (Hatakeyama et al., 2004). However, the role of Notch signaling in the regulation of RGCs during development has not been completely clarified.

In mammals, there are four Notch receptors (Notch1-4) that are activated by five ligands (Delta-like1, 3, and 4, and Jagged1 and 2) (Artavanis-Tsakonas et al., 1995; Kopan and Ilagan, 2009). Ligand binding to a Notch receptor leads to a series of cleavage events that results in the release of the Notch intracellular domain (NICD). Translocation of the NICD to the nucleus leads to the formation of a transcriptional complex that drives the expression of Notch target genes. Mutation of Foxp1 was previously suggested to promote neuronal differentiation in neurosphere cultures by suppressing Jag1 transcriptionally (Braccioli et al., 2017), however, a role for Jag1 in neurogenesis in vivo has yet to be established. Moreover, *Jag1* is essential for maintaining progenitor division in the postnatal and adult subventricular zone (Nyfeler et al., 2005). However, whether Jag1 plays a similar role during embryonic development, and whether or not Jag1 is involved in the radial glia maintenance is unknown.

We utilize the $Foxg1^{Cre}$ driver (Hebert and McConnell, 2000) and a conditional loss-of-function allele of *Jag1* (Kiernan et al., 2006) to drive a decrease in the expression of *Jag1*. We show that decrease *Jag1* is associated with reduced numbers of RGCs and proliferation in the Ventricular zone (VZ). We further show the loss of *Jag1* causes precocious intermediate progenitor cells, which in turn leads to defects in cortical migration during late embryonic development. Our results demonstrate that *Jag1* is required for maintaining RGCs and for the proper development of the neocortex.

Materials and methods

2.1 Mice

All animal procedures were performed in accordance with the guidelines outlined in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (eighth Edition, https://guide-for-the-care-and-use-of-laboratory-animals.pdf) and were approved by Cornell University's Animal Care and Use Committee (IACUC). $Jagged1^{flox}$ ($Jag1^{flox}$) (Kiernan et al., 2006b) and $Foxg1^{Cre}$ (Hebert and McConnell, 2000b) mice were maintained on a mixed 129Sv/C57BL/6 background.

2.2 RNA *in situ* hybridization

Embryonic day 17.5 (E17.5; E0.5 was defined as the first detection of the vaginal plug) embryos were dissected and decapitated. Heads were embedded in OCT (Tissue Tek) and fresh-frozen in liquid nitrogen cooled isopentane. Briefly, RNA *in situ* hybridization was done as previously described (Rodriguez et al., 2008). Probes for *Notch1-3* and *Jag1* were identical to those previously reported (Rodriguez et al., 2008; Williams et al.,2011). Other

probes (*Dll1, Jag2, Vimentin, Tbr1*) were derived from the BMAP (Brain Molecular Anatomy Project) or NIA (National Institute of Aging) 15k or 7.4k clone sets.

2.3 Histology and immunohistochemistry

Embryos (E17.5) were fresh-frozen in Optimal Cutting Temperature OCT compound (Tissue-Tek) using liquid nitrogen cooled isopentane and sectioned (20 micron). Sections were dried for two hours and fixed in 4% phosphate buffered formaldehyde (pH 7.4). After three rinses with distilled water and treatment with 0.2% acetate buffer (pH 4.0) (2 min), slides were stained using 0.1% cresyl violet (5-10 min) before rinsing with water and mounting using 70% glycerol.

Next, antigen retrieval was performed by incubation with citrate buffer (10 mM sodium citrate, pH 6.0) and microwaved (2 min, 70% power followed by 8 min, 20% power). Slides were cooled to room temperature for 3-5 minutes, washed with 1x PBS, and blocked in 10% heat-inactivated normal goat serum in PBS. Primary antibody was applied overnight at 4 °C, washed with PBS, and hybridized with secondary antibody in 5% goat serum/PBS for two hours. After washing, samples were mounted in 70% glycerol/PBS with 10 μg/ml DAPI. Slides were imaged on a Zeiss Axioskop2 Plus fitted with appropriate filter cubes. Primary antibodies used were: Ki67 (1:100, NovoCastra, NCL-Ki67p); rabbit anti-phospho-HistoneH3 (1:200, Millipore, 06570); rabbit TBR2 (1:1:000, Millipore, 42-6600); rabbit Anti-PCNA (1:500, Abcam, ab18197); and mouse anti-Pax6 (1:250, Abcam; ab78545). Secondary antibodies used were rabbit and mouse Alexa Fluor 568 conjugated (Thermo-Fisher; A-11036), goat anti-rabbit-HRP (1:500; Santa Cruz; sc-2054). TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labeling) staining was performed following the manufacturer's protocol (Roche).

2.4 Neurosphere and Differentiation Assays

Dorsal proliferative zone tissue from E17.5 mice were dissected and mechanically dissociated. 10,000 cells were placed in 48 well plates. Cells were grown for 5-7 days *in vitro* (DIV) with medium containing DMEM (ThermoFisher Scientific; 11320033), B27 (ThermoFisher Scientific; 875713), and 10 ng/ml EGF (ThermoFisher Scientific; PHG0311). To generate secondary neurospheres, primary neurospheres were passaged on sixth DIV and incubated in 0.25% Trypsin/EDTA (ThermoFisher; 25200-0056) for 5-10mins. They were then incubated in an equal volume of Trypsin inhibitor (Sigma-Aldrich; T6522) and triturated with gauge needles (Becton Dickinson 305190), and plated at 10,000 cells per well (48 well plate) and DMEM/F12 (ThermoFisher Scientific; 11320-033) containing 10% FBS (ThermoFisher Scientific; 26400044) was added every three DIV. Neurospheres were induced to differentiate by plating on poly-L-lysine-coated coverslips in DMEM/F12 without B27 or EGF.

2.5 Immunofluorescence

Neurospheres were fixed with 4% paraformaldehyde, and blocked in goat serum containing 0.5% Triton. Immunofluorescent analysis of protein expression was performed using mouse anti-PSA-NCAM (Millipore; MAB5324; 1:100). Secondary antibodies used were

biotinylated goat anti-mouse (Abcam; ab64255; 1:1000), and streptavidin texas 568 conjugate (ThermoFisher Scientific; S11226; 1:500).

2.6 Data acquisition and statistics

Nissl, *Vimentin, Pax6, Tbr1*, and *Tbr2* quantitation were carried out using imageJ64 (version 1.48a; Bethesda, MD) and Adobe (version CS3; San Jose, CA) software. Images were imported; a standardized region of interest of the same size was selected for all images. Using the ruler tool function the area were calculated by measuring the length of the positive signaling and the width of the expression per picture view. Ki67, p-HH3, and PCNA quantitation were carried manually by counting the number of positive cells in the VZ. The VZ was defined as 4-5 cells in length and a minimal of 3mm in width from the first cell layer of the dorsal VZ and dividing by the number of DAPI-positive cells to generate a percentage (Ki67 and p-HH3). Neurosphere and differentiation assays quantitation were carried out using Adobe CS3. Size measurements or neuron counts were taken by measuring the diameter using the ruler tool or the count function. A minimal of 3 random wells were selected and pictured using a Cannon EOS Digital Rebel XTi digital camera. Data were analyzed using PRISM 8 (GraphPad Software; San Diego, CA). All data are based on a minimum of three independent experiments and expressed as means ± SEM and regarded statistically significant if $p<0.05$ by performing student's t-test.t-test.

Results

3.1 *Jag1* expression is maintained in the ventricular zone at E17.5

Notch signaling is critical for maintaining stem and progenitor cells in the VZ during corticogenesis (Dave et al., 2011). While virtually no radial glial stem cells are present at E10-E11 their numbers begin to peak at later stages of corticogenesis. Radial glia generates intermediate progenitor cells (IPC) expressing *Tbr2* (Englund et al., 2005). *Tbr2*$^{+}$ are first expressed from E10.0/12.5 and most abundant from E13.5-16.5, during the peak of neurogenesis and their expression is essential for the sequential progression into migrating cortical neuron (Englund et al., 2005; Nadarajah and Parnavelas, 2002; Turrero Garcia and Harwell, 2017). We examined the expression of Notch receptors and ligands at E17.5, after IPC have peak. We found that *Notch1, Notch2*, and *Notch3* receptors were all expressed (Fig. 1A-C), as were *Jagged1 (Jag1), Jagged2 (Jag2), Delta-like1* (Dll1) (Fig. 1D-F). In addition, *Jag1* expression is maintained in the VZ (Fig 1D; arrow) and RNAseq studies have revealed higher *Jag1* than *Delta1* mRNA expression in neurons (Zhang et al., 2014). We thus investigated the potential roles of *Jag1* during late embryonic development.

3.2 Deletion of Jag1 reduces proliferative zone size

Null mutations of *Jag1* are embryonic lethal at E10 (Xue et al., 1999). Therefore, we crossed a conditional *Jag1* allele (*Jag1*$^{f/f}$) (Kiernan et al., 2006) with the *Foxg1*^{Cre} driver (Hebert and McConnell, 2000) to ablate *Jag1* from telencephalic cells around embryonic day 8.5/10 (Fig. 2A). We used a lacZ reporter strain to confirm that *Foxg1*^{Cre} was active in tissue that will give rise to the VZ (data not shown). Using this previously established conditional loss-of-function model of Jag1 (Brooker et al., 2006; Pan et al., 2010) and the chi square test (26 *Jag1* mutants out of 120 total embryos; $p < 0.05$), we found that the Mendelian ratios of

homozygous (*Foxg1*$^{Cre/+}$; *Jag1*$^{fl/fl}$) embryos were obtained at E17.5. However, homozygous *Jag1* mutant mice die shortly after birth for undefined reasons.

We first used Nissl staining to examine the overall structure of the neocortex at E17.5. We measured the area of the cortex between controls (234.8mm^2 ± 2; n=5) and mutants (228.8mm^2 ± 2; n=5) and found no significant differences (p=0.0545) (data not shown). In contrast, we observed a significant decrease in the cortical proliferative zone size in the *Jag1* mutants (9.74mm^2 ± 1; n=5) relative to the controls (13.14mm^2 ± 0.5; n=5) (defined as littermates without the *Foxg1*^{Cre} driver bearing one or two copies of the *Jagged1* floxed allele) (Fig. 2B-D). Examination of the brains of mutants revealed that the mutant has a thinner proliferative zone (~ 25.8% decrease compared with control; (p=0.009). However, no significant (p=0.615) changes were observed in proliferative zone size of Foxg-cre/+ in comparison to wild-type mice (Fig. 2E).

3.3 Loss of *Jag1* leads to reduced numbers of radial glial stem cells and proliferation in the VZ

The VZ gives rise to RGCs, which have been shown to be essential for the generation of cortical neurons in the neocortex (Gotz et al., 1998). To quantify and confirm these findings, we examined whether depletion of *Jag1* would have an effect on *Vimentin* and *Pax6* expression of RGCs. Using a probe for *Vimentin* we found a ~52% reduction (p=0.0005) in mutants (26.3mm^2 ± 4; n=5) compared to control (55.6mm^2 ± 3; n=5) (Fig. 3A-B, I). We also observed a ~58% reduction (p=0.0007) in the area of *Pax6* expression in mutants (54.29mm^2 ± 11; n=3) compared to controls (130.5mm^2 ± 7; n=5) (Fig. 3C-D, J).

Because RGCs undergo division, we examined whether cell proliferation was affected in the *Jag1* mutant. We found that expression of phosphohistone H3 (p-HH3), a mitotic marker (Hendzel et al., 1997) (Fig. 3E-F, K) showed reduction(−67%; p=0.0007) in mutants (27% ± 2 cells/Dapi; n=4) compared to controls (9% ± 1 cells/Dapi; n=4). Similarly, using Ki67, a proliferation marker (Gerlach et al., 1997) (Fig. 3G-H, L), we observed a substantial reduction (−23%; p=0.0456) in mutants (155 ± 7 cells/Dapi; n=4) compared to controls (119 ± 2 cells/Dapi; n=4). Moreover, using an anti-PCNA antibody to label dividing cells (Bravo, 1986), we found significantly (~59%; p <0.0001) reduced proliferation in the mutants (185 ± 20 cells / μm^2; n=4) compared to controls (454 ± 16 cells / μm^2; n=4) in the VZ (Figs. 6A-B and 3M). Next, we asked whether or not programmed cell death could contribute to the reduced numbers of RGCs in the VZ. To this end, no significant differences (p= 0.9778) were found in the number of apoptotic cells between mutants (4 ± 1 cells; n=6) and controls (4 ± 1 cells; n=6) cells in the VZ at E17.5 (Fig. 3M).

3.4 Jag1 deficiency leads to reduced large neurospheres

Because we observed a reduction in proliferation and in RGCs in the VZ, we next examined the capacity of stem/progenitor cells to grow into spheres *in vitro*. To achieve this goal, we isolated tissue from the dorsal proliferative zone and cultured the cells, then monitored spheres growth using the neurospheres assay (Louis and Reynolds, 2005) for 12 days *in vitro*. We measured the sizes of the neurospheres because large-size neurospheres are presumably derived from stem cells due to the unlimited ability to divide (Golmohammadi et

al., 2008; Louis et al., 2008) *in vitro*, we found that spheres derived from control mice showed substantially increased (~72% and ~49%, respectively) number of neurospheres that had diameters that were greater than 300μm (control, 5 ± 1 spheres, n=9; and mutant, 1 ± 0 spheres, n=9; p<0.0001) (Fig. 4A-B, E) and diameters that were between 200-300μm (control, 4 ± 0 spheres, n=9; and mutant, 2 ± 0 spheres, n=9; p <0.0006) (Fig. 4A-B, D) in size compared to the diameters of mutant mice. In contrast, the control mice showed a significantly decreased number of neurospheres that had diameters that were less than 200μm (control, 2 ± 0 spheres, n=9; and mutant, 5 ± 0 spheres, n=9; p<0.0001) (Fig. 4-B, C).

3.5 Jagged1 mutants showed decreased formation of secondary neurospheres

RGCs are known to undergo self-renewal. We therefore dissociated primary neurospheres and tested whether the neurospheres have ability to self-renew and generate secondary neurospheres. After 5-7 days *in vitro*, we found no significances (p = 0.2480) between the number of primary (23 ± 1 spheres; n=10) and secondary (21± 1 spheres; n=10) control neurospheres (Fig. 5A-C, E). In contrast, we observed a substantial decrease (−56%; p=0.0042) between the numbers of primary (9 ± 1 spheres; n=9) and secondary (4± 1 spheres; n=9) mutant neurospheres (Fig. 5B-D, F).

3.6 *Jag1* mutant shows defects in IPCs and *Tbr1*$^+$ neurons

Given the earlier decrease in the numbers of *RGCs* (Fig. 3), we tested whether its downregulated expression may affect neurons in the cortical intermediate zone (IZ) layer. Therefore, we tested whether the loss of *Jag1* leads to decreases in proliferating cells in the IZ. Using an anti-PCNA antibody, we found a −61.8% reduction (p = 0.0048) in mutants (91 ± 3 cells / μm^2; n=4) compared to controls (239 ± 34 cells / μm^2; n=4) (Fig. 6A-B, G; yellow arrow head). Next, we examined if the decreased proliferation in the IZ affected the production of proliferating IPC expressing *Tbr2*$^+$. We chose to focus *Tbr2*$^+$ because they are derived from RGCs expressing *Pax6*$^+$ (Englund et al., 2005). Interestingly, the *Jag1* mutants showed disruptions in the expression of *Tbr2* (Fig. 6D; yellow arrow head) in the IZ layer. Surprisingly, we found a dramatically increased (p < 0.0001) *Tbr2* expression in mutant mice (943 ± 32 μm^2; n=4) relative to control mice (2 ± 1 μm^2; n=3) in the VZ (Fig. 6C-D, H; yellow box). *Tbr2*$^+$ neurons progress into *Tbr1*$^+$ cortical neurons (Englund et al., 2005). Although *Tbr1*$^+$ neurons are generated at ~E12.5/13.5 and by E17.5 their migration to their final destination are vastly complete. We therefore, investigated whether or not *Tbr1*$^+$ cortical neurons had any apparent cortical defects by labeling its expression at E17.5. In the IZ, we observed significant increase (~64%; p= 0.003) in expression of *Tbr1* in *Jag1* mutants (132.88 ± 4 mm^2; n=4) (6F; arrows) compared to controls (80 ± 6 mm^2; n=4) (Fig. 6E-F, I). In contrast, in the cortical plate, we found a substantial decrease (~24%; p= 0.001) in expression of *Tbr1* in mutants (145.72 ± 7 mm^2; n=4) compared to controls (193.56 ± 8 mm^2; n=4) (6D; arrowheads). Using the same *Tbr1* probe, we also confirmed that the Foxg-cre/+ mice showed no changes (p=0.9988) in *Tbr1* expression in the cortical plate (CP) in comparison to wild-type mice (data not shown).

3.7 Jagged1 mutant neurospheres show premature neuronal differentiation

To test *in vitro* whether the loss of *Jag1* from neurospheres have an effect on neuronal differentiation. We cultivated neurospheres for 5-7 DIV under normal conditions. Afterwards, neurospheres were cultivated without supplement and growth factors to induce differentiation for 2 DIV and the numbers of differentiated neurons were quantified. We found substantial increase (~67%; $p < 0.0001$) in differentiated neurons in the mutants (87 ± 10 neurons; n=17) compared to controls (28 ± 4 neurons; n=17) (Fig. 7A-B; black arrow heads and 7E). We used NCAM to visualize the differentiated neurons (Fig. 7C-D; white arrow head).

Discussion

This study provides new insights into Notch signaling by examining the loss of *Jag1* in corticogenesis. The depletion of *Jag1* led to a thinner proliferative zone and decreased numbers of radial glial stem cells. Moreover, we showed that *Jag1* promotes growth and self-renewal of neurospheres. Lastly, we observed that *Jag1* deficiency is associated with precocious generation of *Tbr2*$^{+}$ intermediate progenitors and cortical defects. These data detail a key role of *Jag1* in the neocortex.

Our data that the loss of *Jag1* leads to reduced numbers of RGCs (Fig. 3). Interestingly, RGCs are known to divide asymmetrically, giving rise to another RGC and either an intermediate progenitor cell or a neuron (Englund et al.2005; Gotz and Huttner, 2005; Noctor et al., 2004; Shitamukai et al., 2011). A possible explanation for the reduction of RGCs is that *Jag1* promotes self-renewal. Our findings that the loss of *Jag1* reduces the formation of secondary neurospheres support the role of *Jag1* in promoting self-renewal (Fig. 5). This notion is consistent with previous reports demonstrating that *Jag1* is essential for self-renewal of neural stem cells both in the postnatal subventricular zone (Nyfeler et al., 2005) and in the adult dentate gyrus (Lavado and Oliver, 2014). This idea is further supported by studies that demonstrate that inhibition of Notch can reduce the self-renewal of neural stem cells (Imayoshi et al., 2010) and diminish radial glial identify (Gaiano et al., 2000).

Studies have shown that continuous growing (large) neurospheres are derived from neural stem cells (Louis et al., 2008). In their report, the authors demonstrated that large neurospheres still maintained their unlimited growth potential after several passaged *in vitro* and can reform into a large neurosphere. Our results demonstrating that *Jag1* mutants showed reduced numbers of large neurospheres (200-300μm or <300μm) after 12 days *in vitro* (DIV) suggests the possibility that there may be less neural stem cells in the proliferative zone (Fig. 4). This is in line with our observation that the loss of *Jag1* leads to reduce RGCs (Fig. 3). Taken together, this suggests that *Jag1* is essential to prevent the exhaustion of radial glial stem cells pool presumably by promoting self-renewal.

Our findings of precocious intermediate progenitor cells (*Tbr2*$^{+}$) in the VZ of *Jag1* mutants suggest that *Jag1* may be, in part, required to prevent premature differentiation of RGCs. This is consistent with our observation that *Jag1* mutants show early neuronal differentiation *in vitro* (Fig. 7). This is further supported by studies that *Jag1 has* been shown to rescues

neuronal differentiation in other animal models (Braccioli et al., 2017) and reports that have shown that Notch activation promotes the expression of transcription factors that inhibit pro-neural differentiation, thereby acting to maintain the stem cell population (Kageyama et al., 2009). Thus, *Jag1* may also play a role in preventing premature differentiation.

Our data implicate *Jag1* as an important agent for migration of cortical neurons. *Tbr1*$^{+}$ neurons that are normally expressed in the cortical plate were also found misplaced in the intermediate zone of the neocortex in *Jag1* mutants even though *Jag1* loss does not completely impact all *Tbr1*$^{+}$ neurons (Fig. 6C). This discussion suggests that there may be additional Jag1-independent signals that may also participate in the migration of these neurons. Interestingly, our *in situ* hybridization studies show that *Jag2* is also expressed in the VZ (Fig. 1E; arrow). Although not tested, it is possible that *Jag2* or other Notch ligands may, in part, compensate for the loss of *Jag1* in the migration of cortical neurons.

Given the known compensatory role of Notch ligands in Notch signaling, it is of interest to note that *Jag1* mutants do not exhibit identical phenotype to *delta1* mutants during development of the forebrain. Specifically, conditional deletion of *Jag1* led to decreased *Pax6* expression (Fig. 3) and premature neuronal differentiation (Fig. 7). In contrast, conditional deletion of *Delta1* led to increased *Pax6* expression and suppression of neuronal differentiation (Kawaguchi et al, 2008). Taken together, these observations suggest that *Jag1* and *Delta1* may play distinct roles during corticogenesis. *Jag1* expression has been observed at earlier stages of development (E12.5) (Braccioli et al., 2017) resulting in the prediction that *Jag1* may be essential throughout corticogenesis.

In humans, mutations in Jag1 cause Alagille Syndrome (ALGS) (Alagille et al., 1975; Krantz et al., 1997). Complications associated with ALGS patients that suffer from neurological defects including intellectual disability (Alagille et al., 1975; Krantz et al., 1997; Oda et al., 1997). Interestingly, Jag1 null mice provide a partial phenocopy of ALGS syndrome in human (Xue et al., 1999). Unfortunately, in the null mice identifying the role for Jag1 in the central nervous system has been challenging because the complete loss of Jag1 is embryonically lethal (Xue et al., 1999). Our approach using a conditional loss-of-function of Jag1 may begin to shed some light onto the possible mechanisms associated with ALGS. Finally, a clearer understanding of the factors involved in the maintenance of radial glial stem cells might inform delivery approaches that target neurons in brain regions that show neurodegeneration.

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Highlights

- Conditional loss-of-function of Jagged1 shows reduced cortical proliferative zone
- Loss of Jagged1 decreases the number of radial glial stem cells
- Jagged1 deficiency leads to defects in self-renewal and growth of neurospheres
- Jagged1 mutants display cortical defects

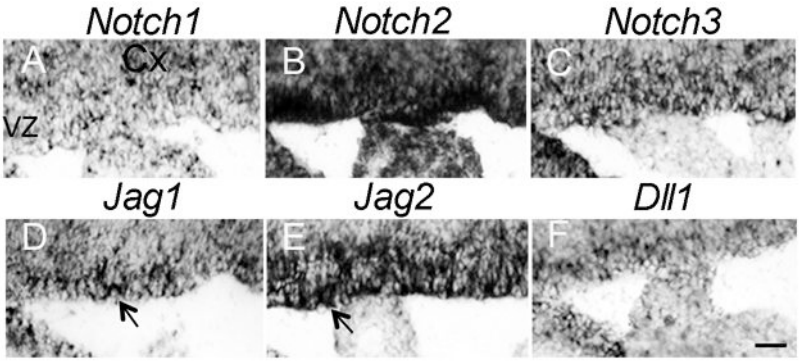

Figure 1. Notch receptor and ligand expression patterns in the ventricular zone.

(A-F) RNA *in situ* hybridization of sagittal brain sections at embryonic day 17.5. Arrows in D and E indicates positive expression of *Jag1 and Jag2* in the *VZ*. Scale bar = 30μm. Cx, cortex; VZ, ventricular zone.

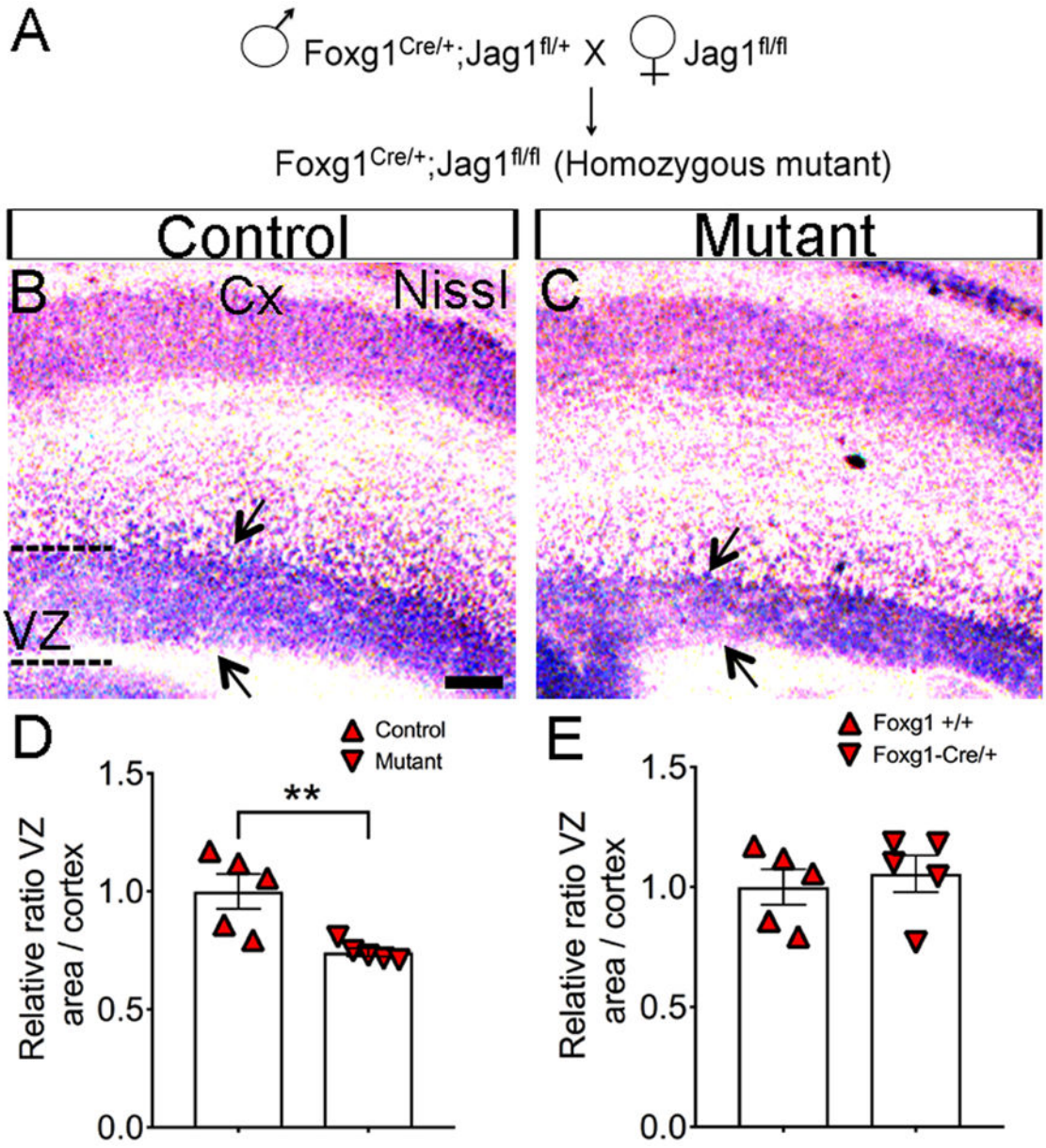

Figure 2. Loss of Jag1 leads to thinner cortical proliferative zone.

(A) Schematic cross for the generation of *Jag1* homozygous mutants. (B-C, E) Reduction of VZ size as demonstrated by Nissl staining performed on sagittal brain sections at E17.5 (Arrows). (D) Quantification of the VZ area compared to control. Scale bar = 100μm. (E) Nissl Quantification of the VZ size in *Foxg1* and *Foxg1*$^{Cre/+}$ mice. All graphs show means ± SEM. **, p<0.01; Cx, cortex;

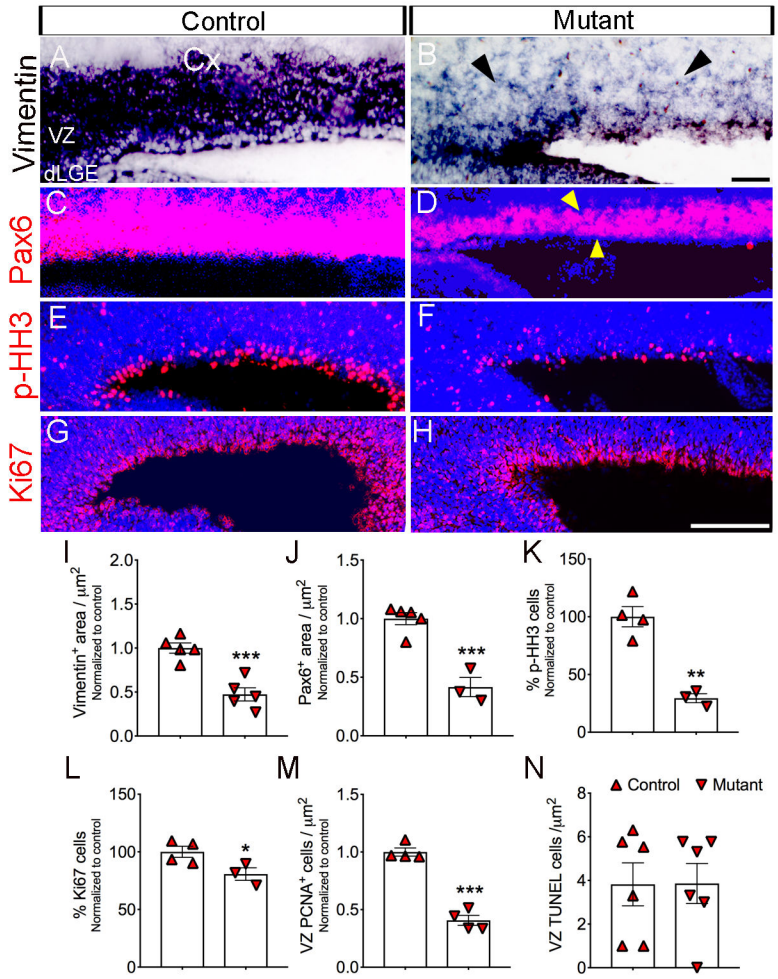

Figure 3. Reduce expression of *Vimentin, Pax6,* and proliferation suggest a loss of radial glial stem cell in Jag1 mutants.

(A-B) RNA *In situ* hybridization using a *Vimentin* probe and (C-D) Immunohistochemistry using anti-Pax6 antibody on sagittal sections show thinner expression in *Jag1* mutant. Quantification of Vimentin (I) and *Pax6*$^{+}$ (J) labeling shows decrease expression in mutants compared to control. Antibodies against p-HH3 (E-F, K) and Ki67 (G-H, L) show decrease expression in the VZ. Scale bar = 200µm. All graphs show means ± SEM. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Cx, cortex; dLGE, dorsal lateral ganglionic eminence.

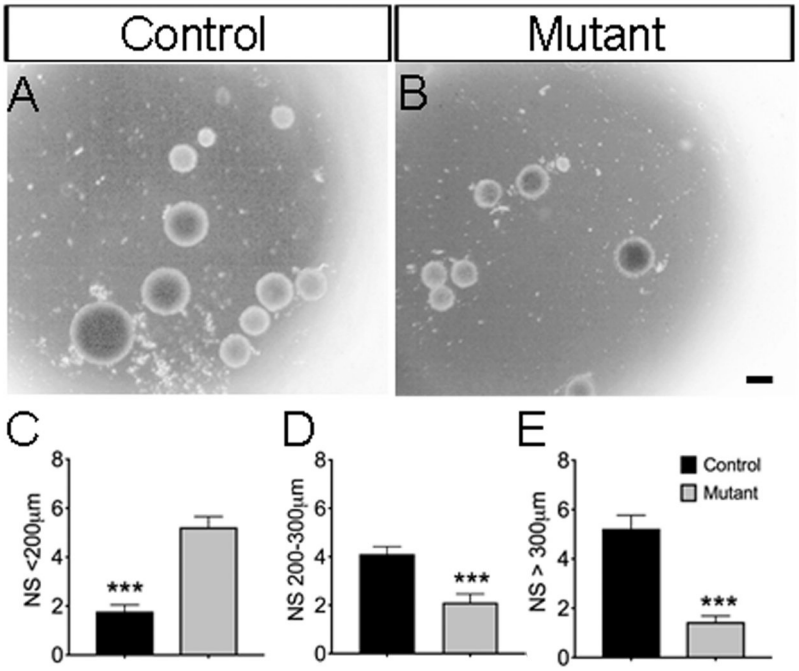

Figure 4. Jag1 mutants show fewer large size neurospheres after 12 days *in vitro*.

(A) Control and (B) *Jag1* mutant primary neurospheres (NS) after 12 days *in vitro*. (C-E) Quantification of the NS that were less than 200µm, between 200-300µm, and greater than 300µm. Scale bar = 200µm. All graphs show means ± SEM; ***, p<0.001.

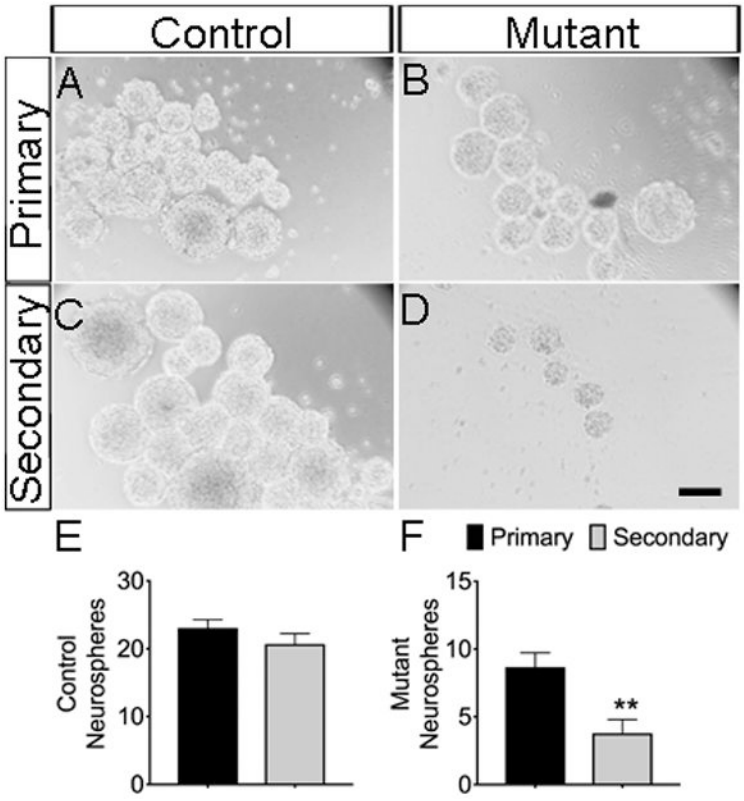

Figure 5. Loss of Jag1 affects self-renewal of neurospheres.

The growth of primary and secondary neurospheres after 5-7 days *in vitro* derived from control (A, C) and *Jag1* mutant (B, D). Quantification of the primary and secondary neurospheres in control (E) and *Jag1* mutant (F). Scale bar = 100μm. All graphs show means ± SEM; **, $p<0.01$.

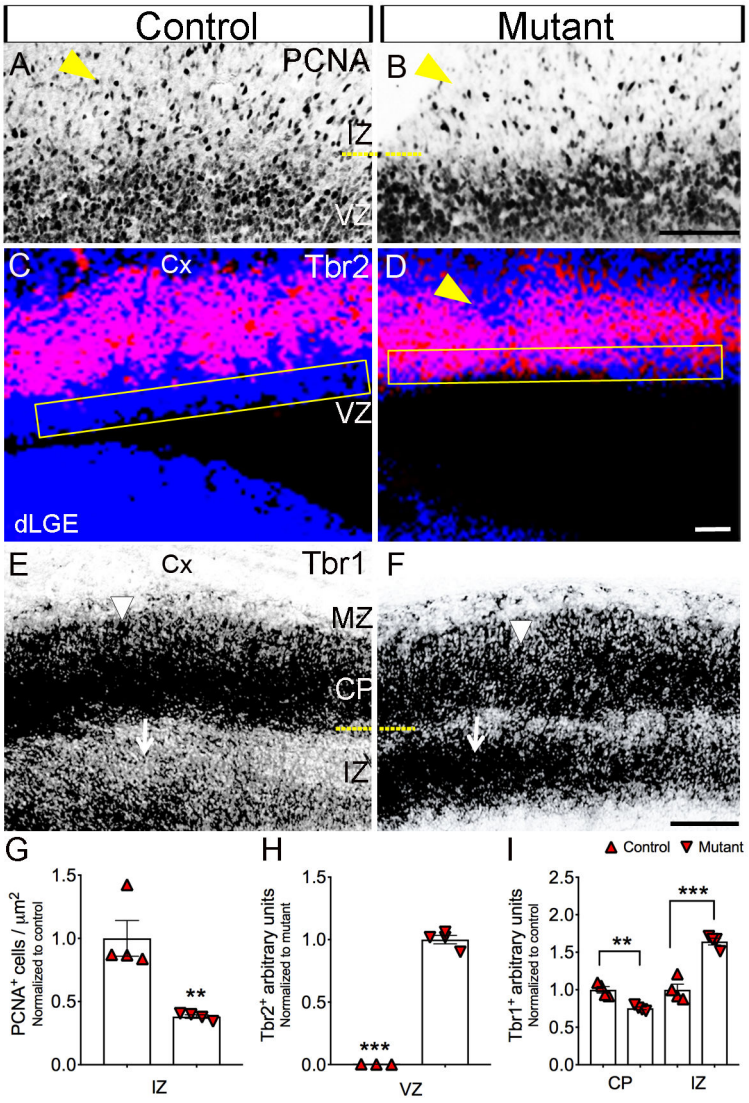

Figure 6. *Tbr2*$^{+}$ progenitor and *Tbr1*$^{+}$ neuron show a defects in the neocortex.

(A-B, E) Immunohistochemistry on using anti-Tbr2 antibody shows increase expression in the VZ (yellow box). Scale bar = 200µm. (C-D, H) *In situ* hybridization using a probe for *Tbr1* shows decreased expression in the cortical plate (arrow head) and increased expression in IZ (arrows) compared to control. Scale bar = 200µm. (E-F, I) Immunolabeling with anti-PCNA antibody shows decease proliferation in IZ (yellow arrow head). Scale bar = 100µm. All graphs show means ± SEM. *, p<0.05; **, p<0.01; ***, p<0.001. All sections are sagittal. VZ, ventricular zone Cx, cortex; MZ, marginal zone; dLGE, dorsal lateral ganglionic eminence, CP; cortical plate, IZ; intermediate zone.

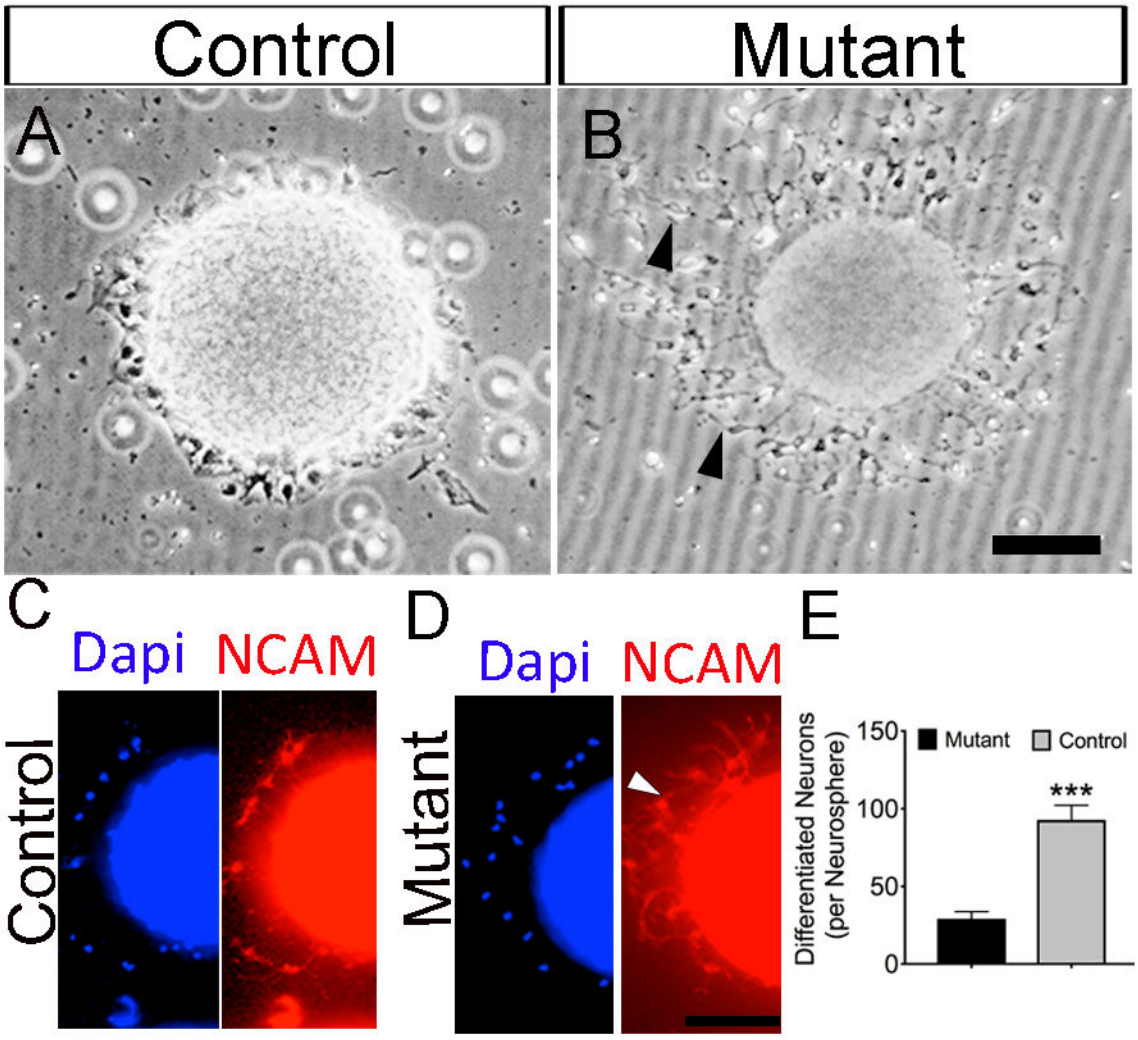

Figure 7. Loss of Jag1 leads to neuronal differentiation *in vitro*.

Visual representative images of neuronal differentiation of (A) control and (B) mutant neurospheres after 12 days *in vitro*. Visual representation of immunocytochemistry staining of (C) control and (D) mutant neurospheres using anti-NCAM co-stained with Dapi. (E) Quantification shows increase neuronal differentiation in mutants compared to control. Graphs shows means ± SEM. ***, $p<0.001$. Scale bar = 100μm.

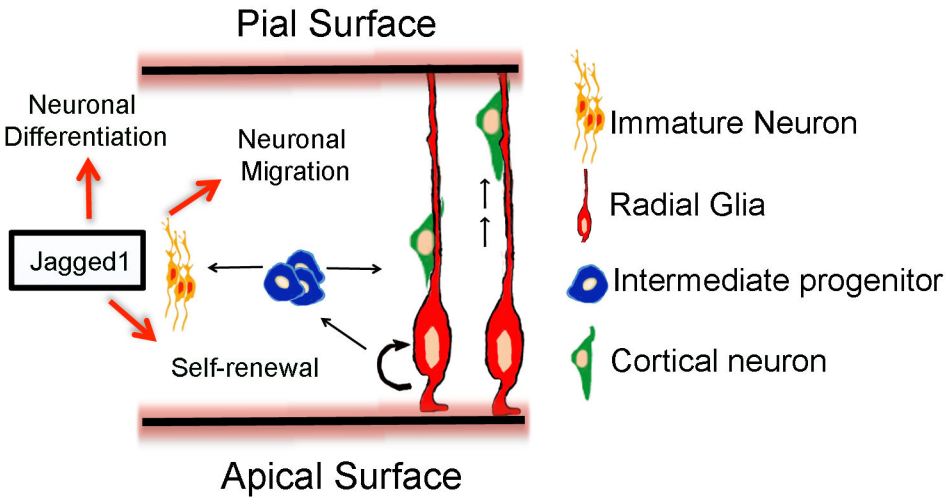

Figure 8. Schematic illustration: the possible roles of *Jagged1* in radial glia maintenance in corticogenesis.

The possible roles of Notch ligand, *Jag1* during late cortical development. *Jag1* promotes self-renewal of radial glial stem cells, prevents premature neuronal differentiation, and may potentially play a role in cortical migration.