Title: Reelin Signalling Pathway and Mesial Temporal Lobe Epilepsy: A Causative Link?

Running title: Mesial Temporal Lobe Epilepsy

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Abstract

Mesial temporal lobe epilepsy (MTLE) is the most frequent form of partial epilepsy. Granule cell dispersion, resulting from aberrant neuronal migration in the hippocampus, is pathognomonic of MTLE. Reelin, a secreted neurodevelopmental glycoprotein has a crucial role in controlling the radial migration of neurons. Several animal studies have implicated Reelin in the MTLE pathogenesis. The aim of this study was to investigate the Reelin signaling pathway in the MTLE patients. Therefore, we studied each step in the Reelin signalling pathway for the gene and protein expressions, in the hippocampal tissue obtained from patients undergoing surgery for MTLE and compared it with age matched normal autopsy cases. We found statistically significant decrease ($P<0.001$) in the Reelin mRNA expression in MTLE patients. Among the two reelin receptors, apolipoprotein E receptor 2 (ApoER2) was significantly increased whereas very low density lipoprotein receptor (VLDLR) was decreased among the patients. Disabled 1 (Dab1), the downstream target of reelin, was found to be decreased. Dab1 in turn inhibits Cofilin, which is responsible for cytoskeletal reorganization, thus limiting aberrant neuronal migration. Statistically significant over expression of Cofilin protein was found in the patient group. Matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of metalloproteases-1 (TIMP-1), both of which are involved in processing of Reelin, were down regulated in 70-85% of cases. In summary, the whole pathway was found to be deranged in MTLE. These results indicate that Reelin signaling pathway is disturbed at various points in the MTLE patients and might be involved in the pathogenesis & progression of MTLE. Our results extend the existing information regarding the components of the Reelin pathway and further, establish a link between pathway disturbance and MTLE.

Keywords: Reelin; ApoER2; VLDLR; Dab1; MMP-9; TIMP-1
Introduction

Epilepsy is a common, chronic and serious neurological problem affecting more than 60 million people worldwide (Cavarsan, Malheiros, Hamani, Najm, & Covolan, 2018). Mesial temporal lobe epilepsy (MTLE) is the most prevalent form of refractory partial epilepsy, characterized by recurrent seizures with a prevalence of about 1% worldwide. Hippocampal sclerosis is typically present in more than 80% of the MTLE cases. Histologically it is characterized by neuronal loss, granule cell dispersion and mossy fiber sprouting in the hippocampal cortex. Granule cell dispersion (GCD), is due to abnormal migration of neurons from the granule cell layer to the molecular layer in the dentate gyrus (Schmeiser, Zentner, Prinz, Brandt, & Freiman, 2017).

Reelin, a neuronal glycoprotein plays an important role in adult neuronal migration. Through its signalling pathway, it acts as a stop-signal and controls abnormal neuronal migration (Frotscher, 2010). In the adult hippocampus, Reelin is expressed by GABAergic interneurons in the stratum oriens and stratum radiatum of CA1, CA3 and in the hilus of the dentate gyrus (Doco-fenzy et al., 2006; Fatemi et al., 2005). Reelin acts through two receptors: Apolipoprotein E receptor 2 (ApoER2) and Very low density lipoprotein receptor (VLDLR) (Lane-Donovan & Herz, 2017). These are single membrane spanning receptors, having the extracellular as well as the intracellular ligand binding regions. VLDLR acts as a stop signal for migrating neurons whereas ApoER2 helps to move the late generated neocortical neurons (Hack et al., 2007). Active Reelin molecule attaches to their extracellular domains leading to the phosphorylation of the Disabled 1 (Dab1) protein attached to the intracellular domain. Dab1 is a cytoplasmic adaptor protein (Benhayon, Magdaleno, & Curran, 2003). Phosphorylated Dab1 inhibits the Cofilin, final target protein of this cascade. Cofilin is required for neuronal migration, as it depolymerizes F-actin to supply actin monomers for the formation of new actin filaments (Chai & Frotscher, 2016). Thus, Reelin induced inhibition of cofilin stabilizes F-actin cytoskeleton and reduces the neuronal migration. As Reelin is a secreted protein, it is cleaved into smaller active isoforms with the help of matrix metalloproteinases (MMPs) (Quirico-Santos et al., 2013). Epileptic conditions impair Reelin processing by inhibiting MMP-9 activity and lead to the deficiency of active Reelin. The activity of the MMP-9 is naturally inhibited by tissue inhibitor of metalloproteinases-1 (TIMP-1). Previous animal studies have shown that TIMP -1 reduces with the disease progression (Acar, Tanriöver, Acar, & Demir, 2014).

We could not come across any study describing the complete signalling pathway of Reelin, its membrane receptors, its downstream molecule Dab-1 and Cofilin and its upstream inhibitor TIMP-1 and MMP-9, in the same human tissue.
Most of the related literature is on findings in the animal tissue, with very few human studies (Boyle et al., 2011; Heinrich, 2006; Imai et al., 2017). Elucidating the reelin signaling pathway in human tissue is necessary as significant differences between animal and human samples have been reported (Roberts, Xu, Roche, & Kirkpatrick, 2005). Another limitation of the few human studies available is the lack of normal control tissue. Therefore, the present study was designed to overcome these lacunae and to study complete Reelin signalling pathway including Reelin, ApoER2, VLDLR, Dab1, Cofilin, MMP-9 and TIMP-1 in hippocampal tissue from MTLE surgery patients and compare the results with those obtained from normal age matched autopsy specimens.

Materials and Methods

Patient selection

We have studied the hippocampal tissue of the refractory MTLE patients (n=15) who had undergone selective amygdalo-hippocampectomy surgery in the department of neurosurgery, PGIMER, Chandigarh, India (Table 1). Presurgical evaluation was done including high-resolution magnetic resonance imaging (MRI) and continuous non-invasive video-encephalographic (EEG) monitoring with superficial electrodes. Long Term Video EEG Monitoring was undertaken with recording of ictal events in all patients. Temporal Lobe Epilepsy was diagnosed on basis of ictal EEG onset from the mesial temporal region; along with temporal lobe interictal discharges where present. Only post-operative histologically proven hippocampal sclerotic cases were selected. The cases with any other type of seizures like focal cortical dysplasia, absence seizures, tonic-clonic seizures, atonic seizures, clonic seizures, & tonic or myoclonic seizures and with structural intracranial lesion like gliomas, meningitis, and neurofibromatosis were not incorporated.

Control selection

Hippocampal tissue was collected from the autopsies (n=15) being done in the department of forensic medicine, PGIMER, Chandigarh, India (Table 1). The autopsies performed within 2-4 hours after death were considered eligible for sample collection. Patients who died because of non-neurological causes were selected, whereas cases with head injury, brain haemorrhage, or any history of neurological disease were excluded.
Ethical approval

Tissue collection and sampling was started after getting approval from the Institutional Ethics Committee of PGIMER, Chandigarh, India, vide no. INT/IEC/000931 dated: 25/06/2018. Informed consent from patients and families of controls was taken prior to the tissue collection.

Sample Processing

The tissue samples were collected in the isotonic saline solution and placed in ice for transportation to the laboratory. The tissue was divided into three parts: one part was kept in 10% formalin overnight; one part was immersed in the RNA later (stabilization reagent) for overnight at 4°C & then at -20°C for further processing; and another part was kept frozen at -80°C.

Histology

Hematoxylin and Eosin (H&E) staining was done. Sections were used to diagnose hippocampal sclerosis and to identify GCD (fig.1a and 1b).

Quantitative real time (qPCR)

Around 0.5 g of tissue frozen in RNA later was processed. Total RNA was isolated by using Trizol method (Ambion). The yield and purity of the RNA was assessed by spectrophotometric measurements (Biotek Epoch) by measuring absorbance at \( A_{260} / A_{280} \) using microplate reading. Synthesis of cDNA was done using a commercially available kit (BioradiScript cDNA synthesis kit) according to the manufacturer’s instructions.

To find out the relative gene expression, quantitative real time PCR was performed using specific primers using SYBR Green chemistry (Abi SYBR Select Master Mix) on a Abi Step One Plus RT-PCR system (Applied Biosystems). Primers were used at 250 nM concentration – human reelin left 5’-TTGGAAGCGGATCAGTCT-3’; right 5’-GCATCATAATCCCTGCT-3’, human ApoER2 left 5’-GGAACAAAAGGCTCAAGGG-3’; right 5’-CTTTGGCCACTGGAAGCT-3’, human VLDLR left 5’-CAGCCGATGGAATGTG-3’; right 5’-GTGAATCGTCCGGACTACA-3’, human Dab1 left 5’-CTTCAACAAAGTCGG-3’; right 5’-GTAGGATCACTGGGACTACA-3’, human Cofilin left 5’-AAGTCTTCAACGCAGAGGA-3’; right 5’-GCATAGCGGCGTCTTATC-3’, human MMP-9 left 5’-TTGACAGCGACAAGAGTTG-3’; right 5’-
TCACGTCGCTTATGCAAG-3’, human TIMP-1 left, 5’-CCTTCTGAATTCGACCTC-3’; right 5’-
GTATCCGCAGACACTCTCCA-3’, human GAPDH left, 5’-TGAACGGGAAGCTCAGTGG-3’; right 5’-
TCCACCACCTGTGCTGTA-3’. Following cycling parameters were used- 7 minutes for 95°C followed by 40
cycles of 10 seconds at 95°C and 30 seconds at 62°C. Reaction volume used was 10 µl.

Quantitative PCR was performed in duplicate to quantify the mRNA expression. In order to nullify the due variation
in samples, the data has been normalized to the housekeeping gene, GAPDH, which served as an internal control, and
again normalized to control which was followed by final data presented in the form of relative fold change by 2−∆∆Ct
formula (Schmittgen & Livak, 2008). Ct value is inversely proportional to the gene level, thus Ct has an indirect
relationship with the relative levels of the target gene; greater the value less is the expression level. Representative
plots of relative expression for each gene were plotted. The fold change observed in patients was given from serial no.
1 to 15 respectively for each patient. The standard criterion was used to define the up regulation (fold change >1.5)
and down-regulation (fold change <0.5) of all the genes.

Western Blot

Tissue was washed with cold PBS buffer and total protein was extracted using 1ml RIPA lysis buffer & 10µL protease
inhibitor (Sigma). The concentration for all samples was analysed by bicinchoninic acid (BCA) method using BCA
kit (BioRad) and albumin (conc. 2mg/ml) as standard. Each sample was heated for 3–5min & resolved. The protein
were separated on sodium-dodecyl sulphate polyacrylamide gel (SDS-PAGE) with 4% stacking gel and 6-12%
separating gel. Proteins were blotted onto polyvinylidene difluoride (PVDF) membranes. After blocking with bovine
serum albumin at room temperature for 2 hours, membranes were incubated with primary antibodies: mouse
monoclonal reelin (1:1000, Abcam), rabbit polyclonal ApoER2 (1:1000, Sigma), mouse monoclonal VLDLR (1:1000,
Novus), rabbit monoclonal dab1 (1:1000, Sigma), mouse monoclonal coflin (2 µg/ml, Abcam), rabbit monoclonal
MMP-9 (1:18000, Abcam), rabbit monoclonal TIMP-1 (1:8000, Abcam) and mouse monoclonal β-actin (1:5000,
Thermo fisher) overnight at 4°C. The membranes were incubated with respective secondary antibodies: rabbit anti-
mouse (1:10,000, Abcam) and goat anti-rabbit (1:1000, Santa-Cruz) at room temperature for 1 hour. β-actin was used
as an internal control. Visualization of the membrane was done using enhanced chemiluminescence (ECL) kit
(BioRad). Densitometry analysis was done using Image J Plus software. Width and intensity of the band were
measured and quantification was performed. The ratio of area of sample/ β-actin of the band intensities was taken for quantification.

**Statistical analysis**

Statistical significance was determined using Student’s unpaired t-test (parametric) by comparing mRNA expression of Reelin, its receptors, dab-1, cofilin, MMP-9 and TIMP-1 in patients with that found in controls. Mean and standard deviation were calculated. A p-value of ≤0.05 was considered as significant. Normality test - D’Agostino-Pearson omnibus for diseased and control groups was performed to check whether the data was normally distributed or not. Correlation was determined by calculating Spearman’s rank correlation coefficient, ρ. A strong positive or negative correlation was assumed when the coefficient was more than 0.5 and less than -0.5. The statistical analysis was done using softwares GraphPad Prism version 8.0 and SPSS version 23.0.

**Results**

We investigated the gene expression of Reelin signalling pathway in the surgically resected hippocampal tissue (n=15) from the MTLE patients and normal controls (n=15) from autopsy. The demographic and clinical data of the patients was collected (Table 1). The patient group was comprised of 9 male and 6 female. The mean age of the patients was 29.26±10.11. The onset age of seizures was 12.13±6.26 and duration of epilepsy was 15.13±10.70. The control group was comprised of 13 male and 2 female. The mean age of the controls was 40.46±13.68. The data of patients is expressed in comparison with age matched controls.

**Reelin** - The mRNA expression of Reelin was found to be decreased in all the patients except one, as compared to normal control (fig.2a). The fold change calculated for each patient is shown in fig.2a. Relative expression for Reelin mRNA was calculated in the patients in comparison to controls (fig.2b), the mean±SD of patient group was 0.05±0.13 and of control group was 1.64±4.37. The results were statistically highly significant (P-value was <0.0001). The calculated R² value to fit regression line was 0.12. The correlation coefficient calculated to check the efficacy of pairing, was -0.15.

Western blot results showed that Reelin protein was reduced in all patient samples as compared to normal. Taking the reference value of control cases as 1, the mean value for Reelin protein was 0.44±0.16 in patients (fig.2c). The data of
protein expression of each patient was represented graphically as shown in fig.2d. The results were statistically highly significant with P-value of <0.0001. D’Agostino-Pearson omnibus test was passed with alpha value of less than 0.05.

**Receptors** – The mRNA expression of receptor ApoER2 was up regulated in patient group, as compared to the controls (fig.3a). The relative ApoER2 gene expression was 5.50±13.4 in the patient group and was 0.92±2.6 in the control group (fig.3c). The correlation coefficient was 0.70 and was statistically significant. On analysing fold change for each case 40% of patients showed up regulation. Another membrane receptor VLDLR mRNA was down regulated in the patients group. Its relative expression was decreased in patients group (0.61±1.2) as compared to the controls group (1±0) (fig.3b and 3d). On analysing fold change for each case, 46% of patients showed down regulation. The results for both receptors were not statistically significant. P-value was 0.15 and 0.22 for ApoER2 and VLDLR respectively.

Western blot analysis showed increased relative expression of ApoER2 protein with mean±SD 1.2±0.19 and decreased relative expression of VLDLR protein with mean±SD 0.71±0.09 in all patients as compared to controls (fig.6, fig.7a and 7b). The results for both receptors were statistically significant with P-value was <0.0001. D’Agostino-Pearson omnibus test was passed (alpha value > 0.05).

**Dab1 and Cofilin** – The Dab 1 gene was down regulated in the patients as the relative expression in the patient group was 1±0 while the control group had the mean value of 1.92±2.61. On individual case wise analysis, down regulation of the Dab-1 was seen in nine out of fifteen cases (fig.4a and 4c). The data was normalized as per normality test D’Agostino-Pearson omnibus (3.6). Up regulation of the Cofilin was found in the patient group, with mean±SD of 3.12±6.5 in the patient group and 0.44±1.26 in the control group. The calculated R2 value to fit regression line was 0.19. The relative expression was increased in eight out of fifteen cases (fig.4b and 4d).

The protein expression Dab1 was decreased with mean±SD 0.65±0.19 and Cofilin was increased with mean±SD 1.23±0.28 in all the patients as compared to controls (fig.6, fig.7c and 7d). The results were statistically significant with P-value was <0.0001. Normality check tests were passed (alpha value > 0.05).

**MMP-9 and TIMP-1** - The MMP-9 gene was down regulated in 60% cases as compared to the control (fig.5a and 5c). The mean±SD of patient group was 0.65±1.29 and of control group was 1±0. The TIMP-1 was also down regulated
in 86% of the cases as compared to the controls (fig.5b and 5d). The mean±SD of patient group was 0.03±0.07 and of control group was 0.56±0.90. The results were statistically significant with P-value 0.04 and R2 was 0.26.

The gene expression results were validated by western blotting and found to be similar. The protein expression of MMP-9 and TIMP-1 were reduced with mean±SD 0.51±0.17 and 0.30±0.15 respectively in all patients as compared to controls (fig.6, fig.7e and 7f). The results were statistically significant with P-value was <0.0001. Normality check tests were performed. D'Agostino-Pearson omnibus test was passed for MMP-9 (alpha value > 0.05) but not for TIMP-1 (alpha value < 0.05). The gene and protein expression in the components of Reelin signalling pathway is summarized in the fig.8 and fig.9 respectively.

Correlation – We correlated the Reelin levels with different parameters including age of patient, age at epilepsy onset, duration of epilepsy and seizure frequency. Spearman’s rank correlation, ρ, was calculated. Age of patient (ρ was -0.57 and p-value was 0.20) (fig.10a), epilepsy onset age (ρ was -0.01 and p-value was 0.96) (fig.10b), duration of epilepsy (ρ was -0.35 and p-value was 0.19) (fig.10c) and seizure frequency (ρ was 0.27 and p-value was 0.32) (fig.10d) did not show any correlation with the decreased Reelin. Further we also divided all the patients into two groups according to the duration of epilepsy; up to 10 years and more than 10 years. We compared these two groups but no correlation was found (ρ was -0.47 and p-value was 0.21) (fig.10e).

Discussion

Granule cell dispersion:

Granule cell dispersion (GCD), which is pathognomonic of hippocampal sclerosis of MTLE (Maria Thom, 2014), was found in tissue obtained from MTLE patients in all the cases included in the present study. Several studies have observed a correlation between severity of GCD and the extent of neuronal loss in hippocampus, as well as with early onset of disease and with longer duration of epilepsy in MTLE (Blümcke et al., 2009; Silva et al., 2007; M Thom, Martinian, Williams, Stoebier, & Sisodiya, 2005). These observations indicate that GCD has a pivotal role in MTLE pathophysiology. Muller et al, 2009 demonstrated that infusion of exogenous Reelin significantly decreased the development of GCD in the epileptic mice model and showed that Reelin deficiency was causally involved in GCD development (Müller et al., 2009). There are two modes of migration of cortical neurons - somal translocation and glia-dependent migration; in the latter the migrating neurons use radial glial fibers as a guiding scaffold. In both modes,
the nucleus is moved towards the leading process and trailing process is retracted. It is important that the leading process is stable and tense during this migration; Reelin attaches the leading process to the cortical surface and helps to orient it (Frotscher, Zhao, Wang, & Chai, 2017) and maintain the normal glial scaffold. In the deficient Reelin signalling seen in the MTLE, many of the leading processes of migrating neurons are misoriented and do not reach the marginal zone (Haas & Frotscher, 2010).

**Reelin deficiency:**

Our results have shown that there is deficiency of Reelin in hippocampal tissue, in chronic epilepsy patients in comparison with the age matched non epileptic controls. There was statistically significant decrease in mRNA expression of Reelin in 14 cases (93%) out of 15. All the patients demonstrated significant reduction of Reelin protein in the hippocampus. We did not find any statistically significant correlation between the quantum of Reelin deficiency with age or sex of patient, age of epilepsy onset, duration of epilepsy or seizure frequency. Further we divided all the patients into two groups according to the duration of epilepsy; up to 10 years and more than 10 years. We compared the two groups but no correlation was found.

**Reelin receptors:**

Active Reelin molecule attaches to their extracellular domains leading to the clustering of the membrane receptors, VLDLR & ApoER2 and triggers the canonical-signaling pathway (Lussier, Weeber, & Rebeck, 2016). In our results ApoER2 protein was increased and VLDLR was decreased. To some extent, the opposite effects of both the receptors i.e. ApoER2 and VLDLR might be because both receptors compensate for each other (Duit, Mayer, Blake, Schneider, & Nimpf, 2010). Moreover Reelin degrades the ApoER2 receptor but not the VLDLR (D’Arcangelo et al., 1999). Thus the Reelin deficiency might be the cause for the increase found in the ApoER2 protein. ApoER2 up regulation & normal VLDLR have been reported by other authors as well (Duit et al., 2010; Hack et al., 2007). Characterization of individual roles of ApoER2 and VLDLR for the development of cortical layers was examined using knockout mice models. It was evidenced that ApoER2 is important for the proper migration of late generated neurons whereas VLDLR acts as a stop signal for Reelin which prevents migrating neurons from entering the marginal zone (Hack et al., 2007). It has been investigated in mice model that both the receptors contribute to signalling pathway, showing comparable affinity. Loss of either receptor results in developmental defect in hippocampus.
Signal transduction:

Disabled1 or Dab1 is a cytoplasmic molecule which does not interact with Reelin directly. As reelin binds to the receptors ApoER2 & VLDLR, they cluster together and results in tyrosine phosphorylation of Dab1 attached to the intracellular ligand binding regions of these receptors (Lussier et al., 2016). Dab1 acts as a signal transducer to Reelin signal (Bock & May, 2016). In our study, we observed decreased Dab1 gene in majority of patients and decreased expression Dab1 protein in all patients, as compared to controls. Thrombospondin is another functional ligand for these receptors which promotes Dab1 phosphorylation without eliciting the canonical Reelin signaling pathway (Blake et al., 2008).

Cytoskeletal reorganization involves the assembly and disassembly of filamentous actin proteins. Cofilin is an actin-depolymerizing protein. Actual migration of neurons depends upon cofilin (Ducharme, Zarruk, David, & Paquin, 2018) as cofilin provides actin monomers to fibrous skeleton for neuronal movement. Thus, Reelin induced inhibition of Cofilin stabilizes F-actin cytoskeleton and reduces the neuronal migration (Frotscher et al., 2017). In MTLE Reelin deficiency, causes decreased Dab1 phosphorylation leading to Cofilin disinhibition. This results in increased neuronal migration, and resultant GCD (Wasser & Herz, 2017). In our results, the gene expression of Cofilin was increased in majority of cases while its protein expression was significantly increased in all the cases.

Reelin processing:

Matrix metalloproteinases (MMPs) are the extracellular enzymes which conduct remodeling of extracellular matrix. MMP-9 is involved in processing of Reelin; it cleaves Reelin into active fragments & is itself inhibited by TIMP-1 (Quirico-Santos et al., 2013). Animal studies have found increased TIMP-1 & decreased MMP-9 (Tinnes, Ringwald, & Haas, 2013; Tinnes et al., 2011) in MTLE; the increased levels of TIMP-1 causes inhibition of MMP-9 activity in the MTLE cases, leading to the deficiency of active Reelin. It has been observed in an animal study that application of recombinant TIMP-1 alone was sufficient to impair the Reelin cleavage and induce GCD in the hippocampal slice culture under epileptic conditions (Tinnes et al., 2013). We found decreased expression of MMP-9 suggesting that Reelin processing has been disturbed; this was validated by protein analysis also. Contrary to the results of the animal study, we found that the mRNAexpression of TIMP-1 was decreased in 85% of the patients while TIMP-1 protein expression was decreased in all the patients. TIMP-1 is induced as an immediate early gene (Gardner & Ghorpade,
and it reduces with the disease progression, thus studies on human tissue from the refractory epilepsy cases (long duration epilepsy) have found diminished TIMP-1 expression (Acar et al., 2014). In our study, the samples were collected from refractory epileptic patients.

**Conclusions**

The gene and protein expression of the components of Reelin signaling pathway was studied. For all the molecules studied, we found that the protein expression pattern, whether increased or decreased in comparison to the normal control, was uniform in all the patients. But the gene expression was variable among different patients, although majority of the cases were either up regulated or down regulated in concordance with their protein expression. Thus it can be postulated that apart from the modification of gene expression which appears to be the chief cause, causative factors governing the MTLE are also influencing post-transcriptional, translational and/or post-translational changes, leading to lack of correlation between mRNA expression and protein expression.

Reelin signalling pathway seems to play an important role in the pathophysiology of mesial temporal lobe epilepsy. Our results have conclusively proven that Reelin deficiency exist in MTLE patients. Moreover the complete Reelin cascade was deranged at multiple levels. Reelin, its receptor VLDLR and downstream protein Dab-1 were decreased with consequent increase in the Cofilin. Both MMP-9 and TIMP-1 involved in Reelin processing were also reduced. Pharmacological manipulations targeting these anomalies at specific steps, may lead to development of novel treatment options for MTLE patients.

**Limitation of the study**

The study has been conducted on tissue samples obtained from patients with long-standing refractory cases. Hence, early stage changes cannot be established.
References:


lipoprotein receptors. *Neuron, 24*(2), 471–479. https://doi.org/10.1016/S0896-6273(00)80860-0


Legends

Figure 1: Histology of Dentate gyrus

(a) Dentate gyrus with three layers – molecular layer (ML), granule cell layer (GCL) and polymorphic layer (PML) in control case. (b) Granule cell dispersion is evident as increased in the width of the GCL in MTLE patient. At 10X magnification. MTLE: Mesial temporal lobe epilepsy.

Figure 2: Gene and protein expression of Reelin

(a) A graphical representation of the fold change in Reelin mRNA in each patient by 2-ΔΔCt method (b) Relative expression of Reelin mRNA in patient group and control group. Data has been expressed as Mean±Standard deviation (c) Representative western blot images of Reelin and β-actin (d) A graphical representation of the relative protein expression of Reelin in each patient with β-actin as control, after densitometric analysis using Image J software. ***statistically significant p-value

Figure 3: Gene and protein expression of ApoER2 and VLDLR

(a, b) A graphical representation of the relative mRNA expression of ApoER2 and VLDLR in each patient by 2-ΔΔCt method (c, d) Relative mRNA expression of ApoER2 and VLDLR in patient group and control group. Data has been expressed as Mean±Standard deviation. ns - not significant

Figure 4: Gene and protein expression of Dab1 and Cofilin

(a, b) A graphical representation of the relative mRNA expression of Dab1 and Cofilin in each patient by 2-ΔΔCt method (c, d) Relative mRNA expression of Dab1 and Cofilin in patient group and control group. Data has been expressed as Mean±Standard deviation. ns - not significant

Figure 5: Gene and protein expression of MMP-9 and TIMP-1

(a, b) A graphical representation of the relative mRNA expression of MMP-9 and TIMP-1 in each patient by 2-ΔΔCt method (c, d) Relative mRNA expression of MMP-9 and TIMP-1 in patient group and control group. Data has been expressed as Mean±Standard deviation. ns - not significant; * statistically significant p-value
**Figure 6:** Representative western blot images of ApoER2, VLDLR, Dab1, Cofilin, MMP-9 and TIMP-1 & β-actin.

**Figure 7: Relative protein expression**

(a-f) A graphical representation of the relative protein expression of ApoER2, VLDLR, Dab1, Cofilin, MMP-9 and TIMP-1 in each patient with β-actin as control, after densitometric analysis using Image J software.

**Figure 8: Relative gene expression**

A graphical representation of relative gene expression of various components of Reelin signaling pathway both in patient group and control group with GAPDH as control. The Y axis represents ΔCt values.

**Figure 9: Graphical representation of protein expression**

A graphical representation of protein expression of various components of Reelin signaling pathway both in patient group and control group with β actin as reference. Densitometric analysis for protein quantification was done using Image J software with β actin as control.

**Figure 10: Correlation coefficient**

Spearman’s rank correlation was calculated. We correlated the levels of Reelin with (a) age of the patients, (b) age at epilepsy onset, (c) duration of epilepsy, and (d) frequency of seizures. (e) The duration of epilepsy was divided into two groups – below 10 years and more than 10 years. The results were not statistically significant in any of the parameters.
Fig. 1

(a) 

(b) 

GCL, ML, PML
Fig. 2
Fig. 3
Fig. 4
Fig. 5

(a) Relative mRNA expression of MMP-9.
(b) Relative mRNA expression of TIMP-1.
(c) Relative expression comparison between Patient Control and Groups.
(d) Relative expression comparison between Patient Control and Groups.

ns p 0.31

* p 0.04
Fig. 6

- ApoER2 180 kDa
- VLDLR 172 kDa
- Dab1 72 kDa
- β-actin 45 kDa
- Cofilin 58 kDa
- MMP-9 82 kDa
- TIMP-1 72 kDa
- β-actin 45 kDa

Fig. 6
Fig. 7

(a) ApoER2
(b) VLDLR
(c) Dab1
(d) Cofilin
(e) MMP-9
(f) TIMP-1
Fig. 8

Fig. 9
Fig. 10

Correlation of Reelin vs age of patients

Correlation of Reelin vs epilepsy onset age

Correlation of Reelin vs duration of epilepsy

Correlation of Reelin vs frequency of seizures

Correlation of Group 1 vs Group 2
## Table 1: Clinical data of MTLE patients and controls

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Age/Sex</th>
<th>Onset age (yrs)</th>
<th>Surgery age (yrs)</th>
<th>Epilepsy duration (yrs)</th>
<th>Seizure frequency (episodes/month)</th>
<th>Age/Sex</th>
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