*Review*

# The *Reeler* Mouse: A Translational Model of Human Neurological Conditions or Simply a Good Tool for Better Understanding Neurodevelopment?

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**Abstract:**

The *Reeler* mutation was described in mouse more than fifty years ago. Later, its causative gene (*reln*) was discovered in mouse, and its human orthologue (*RELN*) was demonstrated to be causative of lissencephaly 2 (LIS2) and about 20% of the cases of autosomal-dominant lateral temporal epilepsy (ADLTE). In both human and mice the gene encodes for a glycoprotein referred to as Reelin (Reln) that plays a primary role in neuronal migration during development and synaptic stabilization in adulthood. Besides LIS2 and ADLTE, *RELN* and/or other genes coding for the proteins of the Reln intracellular cascade have been associated more or less substantially to other conditions such as spinocerebellar ataxia type 7 and 37, *VLDLR*-associated cerebellar hypoplasia, *PAFAH1B1*-associated lissencephaly, autism and schizophrenia. According to their modalities of inheritances and with substantial differences among each other, these neuropsychiatric disorders can be modeled in the homozygous ($reln^{-/-}$) or heterozygous ($reln^{+/-}$) mouse. The usefulness of these mice as translational models is discussed, with focus on their construct and face validity. Face validity, i.e. the resemblance of phenotypes between the two species is focused onto the histological, neurochemical and functional observations in the cerebral cortex, hippocampus and cerebellum of *Reeler* mice and their human counterparts.

**Keywords:** Reelin; LIS2; ADLTE; Autism; Schizophrenia; Translational models; GABAergic interneurons; Dendritic spines; Forebrain; Cerebellum

## 1. Introduction

Reelin (Reln), a large glycoprotein of the extracellular matrix, has a fundamental role in neuronal migration and correct positioning during the course of neurogenesis [1,2]. The name was given to the protein after the discovery of its coding gene, and the recognition that its absence was causative of the *Reeler* mutation in mouse [3], which was described several decades before as being characterized by an ataxic walk [4]. The mutation is autosomic and displays recessive transmission. Thus, only homozygous recessive *Reeler* mice ($reln^{-/-}$) completely lack Reln and have a well-defined phenotype. Behaviorally, the latter consists of dystonia, ataxia, and tremor; structurally it primarily impacts upon the architecture of the cerebral and cerebellar cortices and hippocampus [5,6]. Differently from the mutants, heterozygous *Reeler* mice ($reln^{+/-}$) are phenotypically normal but have been proposed as putative translational models for certain human neuropsychiatric disorders [7].

Soon after the initial discovery, it was demonstrated that the mouse gene (*reln*) had a very high homology to that in humans (*RELN*) [8]. A few years later, it was shown that autosomic recessive mutations of the *RELN* gene were linked to a form of lissencephaly with cerebellar hypoplasia (LCH) [9], association studies indicated that *RELN* was linked to some neuropsychiatric conditions [10], and RELN was demonstrated to be down-regulated in the autistic cerebellum after Western blotting and immunodetection [11].

Establishing a good translational mouse model for a neuropsychiatric disorder requires construct, predictive, and face validity [12]. Strictly speaking, *construct validity* only applies to transgenic mice but, in a broader sense, it also comprehends the syndromic models and the spontaneous mutations affecting the DNA that may be linked to the phenotype under study. In other words, this parameter defines the resemblance of the pathology between the mouse and the human condition in terms of the causative gene(s) as *e.g.* inferred from gene association and linkage studies. As mentioned above, LCH is a human monogenic condition caused by a mutation in *RELN*. Therefore, the *Reeler* mouse meets the criterion of construct validity for the condition. There is also evidence for genetics to be implicated in the etiology of several neuropsychiatric conditions, such as autism and schizophrenia, but, as a consequence of their multifaceted clinical symptoms, causative gene(s), if any, remain to be discovered [13,14]. Nonetheless, there are numerous genes associated with the human autistic pathology after analysis of Mendelian disorders (syndromes), rare mutations or association studies – see e.g. [15].

*Predictive validity*, *i.e.* the resemblance of the response to treatment in humans and mice can hardly be assessed, in the absence of an established therapy in humans [14]. Thus, in the context of this discussion, *face validity*, *i.e.* the resemblance of the model phenotype to that of the human disorder, is the most important parameter to be taken into consideration. There are two main streams along which face validity of murine models in neuroscience translational studies can be addressed properly, *i.e.* the validity of the behavioral phenotype and that of the structural phenotype. Broadly speaking, there are conflicting views as regarding the recapitulation in mouse of the human behavioral neuropsychiatric alterations. This is somewhat not surprising as only a few tests, such as *e.g.* pre-pulse inhibition (PPI), which measures sensory-motor responses, can be performed with minimal modifications in the two species [16]. The issue has been very recently and authoritatively reviewed for the animal models of autism [17] to conclude that, although most of the rodent models that have been used in drug discovery display behaviors with face validity for the human symptoms (*i.e.* deficits in social communication and restricted interests/repetitive behaviors), many drugs that were found to be effective in improving these autism-related behaviors in mice were ineffective in humans.

Therefore, it becomes very important to properly compare the *structural alterations* of the brains in the two species to validate or invalidate the models. We here summarize the state-of-art knowledge on the translational validity of homozygous (*reln*$^{-/-}$) and heterozygous (*reln*$^{+/-}$) *Reeler* mice with reference to the most common neuropsychiatric conditions directly or indirectly related to *RELN*. As a consequence of its importance, we will primarily focus onto the brain structural modifications in the two species.

## 2. The Reelin Gene and Protein

In humans, *RELN*, which has 94.2% homology with the mouse orthologue [8], is located in chromosome 7q22 [18] and encodes for Reelin (RELN), a large glycoprotein of the extracellular matrix. The murine gene (*reln*) that also encodes for Reln was originally cloned as the mutated gene in the *Reeler* mouse, which displays, among others, abnormal lamination of the cerebral and cerebellar cortices, with an inversion of the normal 'inside-out' pattern found in mammals [3,19]. The mouse and the human proteins have a similar size of 388 kD. The structure of the protein recalls that of certain cell adhesion molecules that are produced by specific cell types during brain and spinal cord development.

In the neocortex, after being synthesized by the Cajal–Retzius cells [20], the protein is secreted into the extracellular space and, in post mitotic migrating neurons, activates a specific signaling pathway that is required for proper positioning of these neurons. Northern blot hybridization showed that Reln is also expressed in other areas of the fetal and postnatal brain, with levels particularly high in cerebellum.

The protein is part of a signal transduction pathway that includes the apolipoprotein E2 (ApoER2), the very low-density lipoprotein receptors (VLDLR) and the cytoplasmic protein Dab1 [21]. Notably, the brain phenotype of mice with disruptions of *mDab1* or of both *apoER1* and *vldlr* closely resemble the brain of the *Reeler* mouse [22]. Another gene that interacts with the components of the Reelin signaling pathways is platelet-activating factor acetyl hydrolase IB subunit α *(PAFAH1B1)* [23].

### 3. *RELN*-Related Human Conditions

A number of human neurological conditions can be directly or indirectly related to *RELN* and its encoded protein, as well as to components of the RELN signaling pathway (Fig. 1 and Table 1). These conditions will be briefly described below aiming to put in the better perspective those features that may be useful for better focusing the translational relevance of the *Reeler* mouse.

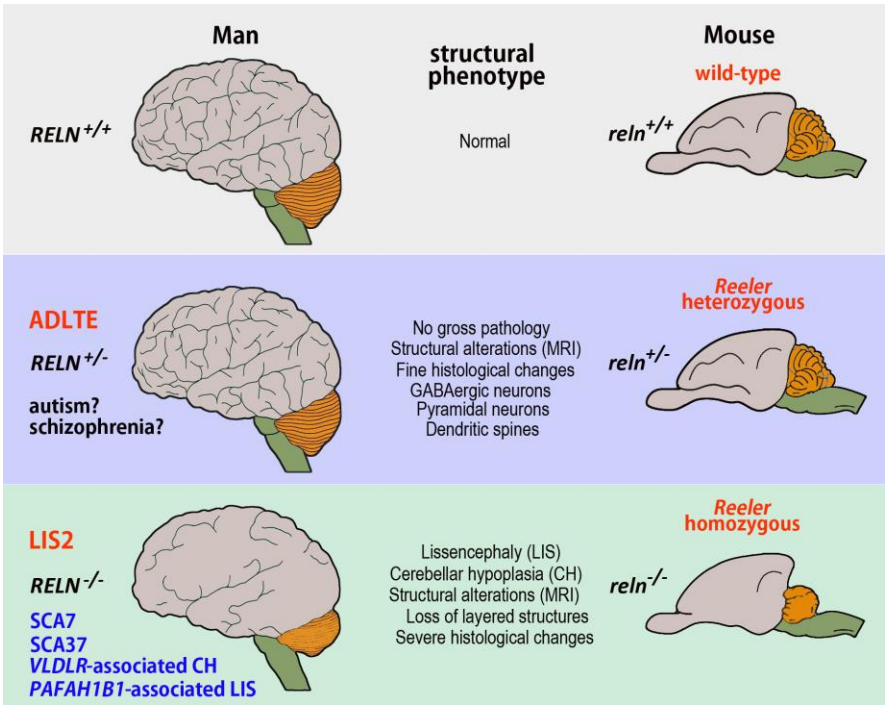


**Figure 1: Summary of the most relevant human pathologies that can be modeled in the Reeler mouse.** The monogenic conditions provoked by the *RELN* gene, i.e. ADLTE and LIS2, are indicated in red, those related to genes encoding for the proteins of the Reln intracellular cascade or only tentatively related to RELN are indicated in blue. Autism and schizophrenia, which have a complex multifactorial etiology, are indicated in black with an interrogative mark to underline the still tentative association of the two disorders with *RELN*. *Abbreviations:* LIS2 lissencephaly 2, PAFAH1B1 platelet-activating factor acetyl hydrolase IB subunit α, *RELN* Reelin gene (human), *reln* Reelin gene (mouse), SCA37 spinocerebellar ataxia type 37, SCA7 spinocerebellar ataxia type 7, VLDLR Very low density lipoprotein receptor.

### 3.1 Conditions Caused by *RELN* Mutations

#### *3.1.1. Lissencephaly 2*

Lissencephalies are a group of cortical malformations that are consequent to neuronal migration disorders. The structural phenotype in lissencephalies ranges from a thickened cortex and complete absence of sulci (agyria) to a thickened cortex and a few, shallow sulci (pachygyria) [24].

| Disease | Transmission | Causative gene(s) | *Reeler* mutants of translational interest | Other mouse models |
|---|---|---|---|---|
| LIS 2 | Autosomal recessive | *RELN* | Homozygous | may be relevant for LIS1 (see text) |
| ADLTE | Autosomal dominant | *RELN* (in 17.5% of cases) | Heterozygous | *LG11*-mutated |
| *VLDLR*-associated cerebellar hypoplasia | Autosomal recessive | *VLDLR* | Homozygous | *VLDLR* knock-out |
| SCA37 | Autosomal dominant | *DAB1* | Homozygous | *DAB1* knock-out *apoER1* knock-out |
| *PAFAH1B1*-associated lissencephaly | Autosomal dominant | *PAFAH1B1* | Homozygous | *Lis1*$^{+/-}$ |
| SCA7 | Autosomal dominant | *ATXN7* | Homozygous | SCA7 knock-in |
| Autism | Isolated cases Multifactorial | see https://omim.org # 209850 | Heterozygous | see text |
| Schizophrenia | Autosomal dominant | see https://omim.org # 181500 | Heterozygous | see text |

**Table 1: Summary list of the human neurological conditions related to the *RELN* gene.** Note that only LIS2 and ADLTE have a demonstrated link with *RELN*.

Classic lissencephaly, formerly referred to as type I lissencephaly but today named lissencephaly 1 (LIS1), is characterized by a marked thickening of the cerebral cortex with a posterior to anterior gradient of severity. LIS1 is caused by an abnormal neuronal migration in the interval between the 9$^{th}$ to the 13$^{th}$ week of gestation, resulting in a spectrum of agyria, mixed agyria/pachygyria, and pachygyria. LIS1 is characterized by an unusually thick and poorly organized cortex with four primitive layers, diffuse neuronal heterotopia, enlarged and dysmorphic ventricles, and, often, hypoplasia of the corpus callosum [25]; the basal ganglia are normal, except that the anterior limb of the internal capsule is usually not visible, and, most often, the cerebellum is normal as well. Some rare forms of lissencephaly are associated with a disproportionately small cerebellum and are often referred to as LCH. Lissencephalies are now classified based on brain imaging findings and molecular analysis [26], as they have been associated with mutations in several genes such as *LIS1* (*PAFAH1B1*; MIM#601545), *DCX* (Doublecortin; MIM#300121), *ARX* (Aristaless-related homeobox gene; MIM#300382), *RELN* (Reelin; MIM#600514), *VLDLR* (MIM#224050) and *TUBA1A* (αtubulin 1a) [27].

Lissencephaly 2 (LIS2) also referred to lissencephaly syndrome, Norman-Roberts type or Norman-Roberts syndrome (OMIM #257320), is associated with LIS1 but displays several specific clinical features. In 2000, Hong and colleagues were the first to describe an autosomal recessive form of lissencephaly that also displayed severe abnormalities of the cerebellum, hippocampus, and brainstem. They showed that the responsible gene mapped to chromosome 7q22 and that the condition was associated with two independent mutations in *RELN*, resulting in low or undetectable amounts of RELN [9]. They also noticed that the brain phenotype was similar to that of the *Reeler* mouse mutant, in which *reln* mutations cause cerebellar hypoplasia, abnormal cerebral cortical neuronal migration and abnormal axonal connectivity (see 4. The homozygous *Reeler* mouse). The same type of LIS2 was subsequently observed in two other unrelated groups of patients [28]. These were children that displayed a 5-10 mm thick cerebral cortex, a malformed hippocampus and a very hypoplastic cerebellum, almost completely devoid of folia. A similar phenotype was described in

mice and patients with lissencephaly 3 (LIS3), which is caused by *TUBA1A* mutations [29,30]-. TUBA1A is predominantly expressed in post-mitotic neurons of the cerebral cortex, hippocampus, cerebellum and brainstem, with expression reducing soon after birth but still persisting through adulthood [31]. The mouse phenotype consists, among others, in a failure of the cerebellar Purkinje neurons to migrate, so that they remain entrapped into the medullary body, arranged in streaks and intermingled with the neurons of the cerebellar nuclei [32]. Several other mutations of *TUBA1A* were subsequently discovered, giving rise to a predominant phenotype of LCH, plus abnormalities of the corpus callosum and the basal ganglia/internal capsule [33].

#### *3.1.2. Autosomal-Dominant Lateral Temporal Epilepsy*

Autosomal-dominant lateral temporal epilepsy (ADLTE) is a genetic epileptic syndrome, clinically characterized by focal seizures with prominent auditory symptoms. ADLTE is genetically heterogeneous, and mutations in the leucine-rich, glioma inactivated 1 gene (*LGI1*) account for fewer than 50% of affected families. Very recent observations demonstrated that heterozygous *RELN* mutations cause a typical ADLTE syndrome, indistinguishable from that associated with mutations of *LGI1*. Seven different heterozygous missense mutations in *RELN* were, in fact, described in some unrelated families of Italian descent with familial temporal lobe epilepsy-7 (ETL7 – OMIM #616436) with an incidence of 17.5% over the total number of families studied, specifically affected by lateral temporal lobe epilepsy [34]. By three-dimensional modeling, the same authors predicted that the mutations would result in structural defects and protein misfolding. Some of the affected individuals displayed a reduction up to 50% of their serum levels of the 310 kD RELN isoform compared to controls, suggesting that the mutations resulted in a loss of function. In a subsequent study on the same patients, 1.5 T MRI scans were not useful in detecting structural anomalies of the brain [35], as it was the case of a very recent study on a 18-year old ADLTE patient, where 3 T MRI brain scans could not provide relevant information on blurred grey-white matter junctions, voxel-based morphometry, and cortical thickness [36]. However, functional connectivity analysis revealed higher local synchrony in the left temporal (middle temporal gyrus), left frontal (supplementary motor area, superior frontal gyrus), and left parietal (gyrus angularis, gyrus supramarginalis) regions of the cerebral cortex and the cingulate cortex (middle cingulate gyrus) as compared to healthy controls [36].

### 3.2. Conditions Caused by Mutations of Genes of the Reln Intracellular Pathway

#### *3.2.1. VLDLR-Associated Cerebellar Hypoplasia*

*VLDLR*-associated cerebellar hypoplasia is an autosomal recessive genetic form of non-progressive congenital ataxia [37]. The main clinical symptom of the condition is a predominantly truncal ataxia with retarded ambulation, so that children either learn to walk after six years of age or never succeed to independently walk. Dysarthria, strabismus, moderate-to-profound intellectual disability, and seizures are other features of the disorder. MRI findings include hypoplasia of the inferior portion of the cerebellar vermis and hemispheres; pachygyria of the cerebral hemispheres with minimally thickened but uniform cortex in the absence of a clear anteroposterior gradient; reduction is size of the brainstem, particularly the pons. The condition is monogenic, and due to mutations in *VLDLR*.

#### *3.2.2. Spinocerebellar Ataxia Type 37*

Spinocerebellar ataxia type 37 (SCA37) is a late onset syndrome that affects adults, with dysarthria, slowly progressive gait and limb ataxia, severe dysmetria in the lower extremities, mild dysmetria in the upper extremities, dysphagia, and abnormal ocular movements. In most cases, the first clinical signs encompass falls, dysarthria, or stiffness followed by a complete cerebellar syndrome. The early

presence of altered vertical eye movements is a characteristic clinical feature of SCA37 that foregoes the symptoms of ataxia. The progression is slow and affected individuals usually become wheelchair bound between ten and thirty-three years after the onset of the disease [38]. At MRI there is an initial atrophy of the vermis that rapidly diffuses to the entire cerebellum, without alterations of the brainstem [39]. Molecular analysis has shown that an unstable repeat insertion in *DAB1* is the cause of the cerebellar degeneration and, on the basis of the genetic and phenotypic evidence, the mutation has been proposed as the molecular basis for SCA37 [40].

#### *3.2.3. PAFAH1B1-Associated Lissencephaly/Subcortical Band Heterotopia*

*PAFAH1B1*-associated lissencephaly/subcortical band heterotopia, also referred to as *LIS1*-associated lissencephaly/subcortical band heterotopia, includes Miller-Dieker syndrome (MDS), isolated lissencephaly sequence (ILS) and, infrequently, subcortical band heterotopia (SBH) [41]. MRI findings for lissencephaly are the absence or the abnormal broadening of cerebral gyri, and the aberrant thickness of the cerebral cortex. Less frequently, it may be possible to observe an enlargement of the lateral ventricles, mild hypoplasia of the corpus callosum and of the cerebellar vermis. In *PAFAH1B1*-associated SBH, just beneath the cortex of the parietal and occipital lobes there are subcortical bands of heterotopic gray matter separated from the superficial cerebral cortex by a thin layer of white matter. Histologically, the cerebral cortex in LIS1-associated lissencephaly consists of four layers: a marginal zone, which is poorly defined but has a very high cell density; a superficial neuronal layer with diffusely scattered neurons; a deeper neuronal layer with relatively sparse neurons; and a deepest neuronal layer with neurons arranged in columns.

### 3.3. Conditions Possibly Related to *RELN* Mutations

#### *3.3.1. Spinocerebellar Ataxia Type 7*

Spinocerebellar ataxia type 7 (SCA7) is an autosomal-dominant neurodegenerative disorder that results from polyglutamine expansion of ataxin 7 (ATXN7). Remarkably, although ATXN7 is expressed throughout the body in SCA7 patients, the pathology primarily hits the cerebellum and the retina [42]. A recently published paper suggested that RELN could be a previously unknown factor involved in the tissue specificity of SCA7 [43].

#### *3.3.1. Autism*

The disorders of the autistic spectrum (ASD), which are characterized by social, behavioral, and language deficits, comprise Asperger syndrome, autism, and pervasive developmental disorder-not otherwise specified (PDD-NOS). Less than 20% of these disorders, known as ''syndromic autism'', is attributable to monogenetic diseases, most commonly fragile X syndrome and tuberous sclerosis. The remaining 80% of ASD cases are considered ''non-syndromic autism'' and are widely investigated to find candidate genes that may contribute to pathology [44].

##### *3.3.1.1 Genetics*

At present autism cannot be considered, strictly speaking, a genetic disease, as one or more causative gene(s) has (have) not been found yet. The first gene association study implicating *RELN* in autism dates back to 2001 [45]. However, subsequent gene population surveys yielded contrasting results [46-49]. Nonetheless, a more recent meta-analysis showed that at least one single nucleotide polymorphism (SNP) in *RELN* was significantly associated with the risk of autism [50]. Therefore, results of SNP analysis appear to be compatible with the idea that heterozygous mutations in *RELN* may contribute to the onset of the disorder. Genetic studies on autism led to two main outcomes: 1. the more predominant existence of rare or *de novo* inherited mutations of a number of genes in autistic patients; or 2. the discovery of certain common gene variants that contribute to the risk of autism but are also present, albeit at lower frequency, in the normal population [51]. As far as a given condition is considered, it is currently held that when more than two *de novo* mutations occur

in a gene, the latter becomes a very likely causative candidate of the disorder. There are four unique documented *de novo* mutations of *RELN* associated with autism [52-54] thus implicating *RELN* as a possible cause of autism. However, although by whole-exome sequencing nonsense mutations were found to be more frequent in autistic patients than in controls, there is not a striking gross increase of *de novo* mutations in the former [53]. Using a different approach that distinguishes total narrow-sense hereditability from that due to common gene variants, it was more recently concluded that narrow-sense heritability of autism is ~52.4%, and that the main contribution hereditability was due to common gene variants, whereas rare *de novo* mutations contribute only for about 2.6% of cases, but substantially influence individual liability [55]. Thus, *RELN* may primarily have a role in the individual *predisposition* to manifest autism rather than being one of the contributory causes of the disorder.

Further support for a RELN involvement in autism derived from the observation of decreased expression of the *RELN* transcript and encoded protein in autistic patients. Decreased RELN levels were detected in the superior frontal cortex [10] and cerebellum of autistic subjects as compared to controls [10,11,56]. In these areas the *RELN* mRNA was also reduced, as was the *DAB1* transcript, whereas *VLDLR* mRNA levels were increased.

*3.3.1.2. Imaging*

Imaging findings in autism have been recently reviewed [57]. Numerous observations converge to demonstrate that there is an atypical development of the brain in autistic children. Early cross-sectional studies show that the brain of these children has a higher volume than that of regularly developing subjects. However, growth curves in the two groups eventually meet at later childhood. More specifically, in the 6-35 year interval, there is an initial period of brain overgrowth, and then growth slows down or even stops during early and late childhood to be eventually followed by a phase of fast reduction of the brain volume [58]. Neuroimaging data also suggest that differences in the brain of autistic individuals start to be detectable within the first two years after birth, *before* clinical symptoms become obvious. There are conflicting views about the possibleness that an accelerated growth rate of the brain in this postnatal window is accompanied by the occurrence of early neurodevelopmental perturbations [57]. In relation to this, it must be stressed out that we still do not know when the initial neuropathological signs of autism occur, also from the paucity of studies on autistic children during the first year of life.

The mechanisms at the basis of the abnormal growth of the autistic brain are also poorly understood. Although most imaging studies have focused onto the gray matter of the cerebral cortex, there are data indicating that the enlargement of the autistic brain is accompanied by an increased amount of cerebrospinal fluid in the subarachnoid space [59] and/or a greater volume of the white matter [60]. As regarding the cerebral cortex, it was reported that surface, but not thickness, is magnified in the autistic brain [61].

To summarize, that early brain overgrowth may be considered a reliable biomarker for autism still remains highly questionable. Thus, it has been proposed to focus onto regional structural differences in the brain in the search for new neuroanatomical observations of clinical relevance [57].

Before entering the description of regional MRI neuroanatomical investigations in autism, it is important to stress that, at present, there are no specific and/or causative objective findings for the condition, but, instead, the very same regions that are altered in autism may be interested in other psychiatric conditions (see below).

The individual components of the neural circuitries underlying ASD are well established and include regions of the fronto-temporal, fronto-parietal and dorsolateral prefrontal cortex; parts of the limbic system; the fronto-striatal circuitry and the cerebellum. Neuroimaging studies on these regions have employed different approaches such as the definition of a region-of-interest (ROI), voxel- or vertex-vise methods. Traditional ROI studies have reported atypical findings in brain regions that are involved in social cognition such as the medial prefrontal cortex, the anterior cingulate cortex, the inferior frontal cortex, the superior temporal sulcus, the amygdala, and the anterior insula.

The cerebellum was found to be larger than in controls in several MRI studies on autistic patients older than 3 years [62]. However, such an enlargement was not confirmed in younger children [61]. Differently from the cerebellum as a whole, the size of the vermis was smaller [63-65] or larger [64] or did not display any relevant difference [65], and such discrepancies have been related to the different clinical presentations of the condition [65,66]. It is also unclear whether there are differences in size of individual vermal lobules, as they have been reported by some authors [63], but not others [65]. Similarly, no differences were described between cerebellar hemispheres in one study [65], whereas another group has found the hemispheric size as the only significant structural dissimilarity between verbal and nonverbal subjects [67].

*3.3.1.3. Histopathology*

A series of histological alterations of the whole brain were described to occur in the autistic brain. Qualitatively, it was initially observed that the only cortical area showing structural abnormalities was the anterior cingulate cortex that, in autistic patients, lacked architectural refinement and had only a coarse lamination [68]. However, in the following decades substantial amounts of data have been collected and the list of cerebral structures displaying histopathological changes in autism has grown substantially to include a series of cortical regions, the amygdala, the cerebellum and the brainstem, see e.g. [69,70]. The most significant histopathological findings in human patients are briefly summarized below. However, it must be well kept in mind that the interpretation of these finding needs often much caution, because not all studies were based on sound quantitative approaches and/or proper stereological procedures.

*3.3.1.3.1. Changes Affecting the Whole Brain*

The diffuse alterations observed in the brains of autistic subjects at post mortem include cortical dysplasia and neuronal heterotopia, with the formation of aggregates of neuronal perikarya in anomalous positions [71]. Other alterations, i.e. differences in size of the neuronal nucleus and perikaryon, can be observed at the cytological level. These difference start being evident in young children and become more evident in adults, but then tend to re-equilibrate with time [72]. It must in fact be noticed that there might be some compensations between different areas, as in some parts of the brain there neurons are bigger, but smaller in others.

In the autistic brain there is also an increase of the neuropil extension in certain but not all cortical areas that have been investigated so far [73]. It is unclear which neuropil component(s) is (are) responsible of these volumetric variations as fewer dendrites were observed after microtubule-associated protein 2 (MAP2) immunostaining in the prefrontal cortex [74] and a reduction of dendritic spines was reported in hippocampus [75], but other studies reached completely opposite conclusions after examination of pyramidal neurons from layers 2 and 5 of the frontal, temporal, and parietal cortex [76]. The issue of dendrite and dendritic spines density is quite important in the general framework of this discussion, because these parameters have been widely investigated, primarily aiming to validate the heterozygous *Reeler* mouse as a translational model of autism and other neurological conditions.

Another issue of interest is related to the possibility that there are alterations in the minicolumnar organization of the cerebral cortex in early age onset autism [73], as this type of pathology may be recapitulated in *Reeler* mice. Specifically, it appears that minicolumns are smaller, more numerous and with lower neuronal density in several cortical areas of autistic (and Asperger's

syndrome) patients, although these observations still have to be confirmed in full. In addition, one has to keep in mind that the idea that minicolumns are indeed the fundamental modular units of neocortical organization is currently still under debate, see e.g. [77] for review.

It is also assumed that alterations in neuronal differentiation and migration occur in the autistic nervous system and thus the consequences of a dysregulation of these processes may be at the basis of whole brain changes in autism [71]. In spite of this, there are only a few investigations on the expression of RELN in the brain of autistic patients and after quantitative analysis there was no alteration in the density of layer 1 RELN+ neurons in the superior temporal lobe of the autistic brain, although these neurons represent about 70% of the total layer 1 population [78].

*3.3.1.3.2. Brain Regional Changes*

3.3.1.3.2.1. Forebrain

Table 2 reports the main histopathological changes observed in the forebrain of autistic patients. The table only reports the most significant results in relation to the present discussion. A point of attention in considering these studies is that, in several cases, brain volume estimates between autistic patients and controls are missing, while they are, instead, necessary to confirm whether differences in cell density reflect true differences in total cell counts. A careful consideration of Table 2 shows that the majority of observations have been focused on cerebral cortex and hippocampus and that the alterations are almost exclusively restricted to neurons. The parameters considered have been size, number, and density of the different neuronal populations, often in relation to the cortical layers or hippocampal subfields. Notably, alterations in hippocampus primarily concern the GABAergic inhibitory interneurons and the excitatory pyramidal neurons. As discussed in section 5. The heterozygous Reeler mouse, the forebrain pathology is compatible with the phenotype of *reln*$^{+/-}$ mutants.

3.3.1.3.2.2. Cerebellum

Analysis of cerebellar alterations in autism has attracted many efforts of the basic researchers and clinicians. The most consistent anatomic findings in autistic patients are a reduction in size of certain lobules of the cerebellar vermis (but see 3.3.1.2. Imaging) and a decrease in the number [79-83] and size [84,85] of the Purkinje neurons. The inhibitory GABAergic basket and stellate interneurons that innervate the Purkinje cells did not show quantitative differences compared to normal cerebella, an observation that is indicative of a late developmental loss of the Purkinje neurons [86], as they are generated well before the interneurons.

In addition to structural observation, a Western blot study has demonstrated a reduction of about 40% in the level of expression of RELN in autistic patients compared to age and sex matched controls [11].

**3.3.2. Schizophrenia**

Schizophrenia is a devastating psychiatric disorder that affects approximately 1% of the population. Its main clinical symptoms are hallucinations, delusions and cognitive disturbances. These symptoms derive from brain dysfunctions that are attributed to genetic and environmental factors [87]. However, schizophrenia is not strictly a genetic disease, although gene deletions, duplications and variations may be risk factors for the disorder. At present, the gene(s) that could be involved in the pathology remain elusive for the most (see OMIM #181500), but a microdeletion in a region of chromosome 22, called 22q11, was recently demonstrated to be involved in a small percentage of cases [88].

| Brain Region | Subdivision | Main function | General Histology | Specific neuronal alterations | Refs |
|---|---|---|---|---|---|
| ***Cerebral cortex*** | Prefrontal cortex | Cognitive control | Overgrowth # neurons ↑ Glia unaffected | • # parvalbumin chandelier neurons ↑<br>• # calbindin and calretinin interneurons unaffected | [70,89-91] |
| | Inferior frontal cortex | | | • size small pyramidal neurons ↓<br>• # small pyramidal neurons unaffected | [91] |
| | Fusiform gyrus | Facial recognition/ social interactions | | • density (layer 3)↓<br>• # (layers 2, 5, 6)↓<br>• size (layers 5-6) ↓ | [92] not [93] |
| | Frontoinsular cortex | Emotional regulation Self and others awareness | | • # von Economo neurons (layer 5)↑ | [94-96] |
| | Anterior cingulate cortex | | Poor lamination | • density (layers I-II of area 24a - left hemisphere)↑<br>• size (all layers area 24b) ↓<br>• density (layers 5-6 area 24c) ↓ | [68,97] |
| | Anterior midcingulate cortex | Decision making | | • # von Economo neurons and pyramidal neurons (layer 5)↑<br>• size pyramidal neurons ↓ | [98] |
| | Entorhinal cortex | Memory, navigation and perception of time | Spheroids (swollen terminals) | | [99] |
| ***Hippocampus*** | | Learning and memory | Spheroids (swollen terminals) in all subfields | • size ↓<br>• density ↑<br>• reduction of dendritic arbors<br>• # pyramidal neurons (CA1) ↑<br>• # pyramidal neurons (adjacent areas) ↓<br>• density GABAergic interneurons ↑<br>• density calbindin+ neurons (DG) ↑<br>• density parvalbumin+ neurons (CA1 and CA3)↑<br>• density of calretinin+ neurons (CA1) ↑ | [68,75,99-101] |
| ***Amygdala*** | | Emotional learning | Size↑ | density (medial, central, and cortical nuclei) ↑ # ↓ (may be age-related) | [68,83,102-104] |

**Table 2: Region-specific histopathological changes in the autistic forebrain.**
Legend: ↑increase; ↓ decrease; DG dentate gyrus of hippocampus; CA1-CA3 cornu Ammonis subfields of hippocampus proper.

Genetic studies have shown that *RELN* is associated with schizophrenia [105] and over the past decade many SNPs in the gene loci were related with the onset and/or severity of the clinical symptoms [106], but results still are under debate and need further verification [107]. It should perhaps stressed out that studies of gene expression have converged to show that the genes implicated in schizophrenia are more highly expressed during fetal than postnatal life [108], thus making more difficult to ascertain their true role in the etiology of the condition.

Structural MRI findings in schizophrenia have been recently reviewed [109]. There is sufficient evidence to suggest that the condition is associated with a progressive development of gray matter abnormalities, particularly during the first stages of the disease. Reduction of the cortex in the

superior temporal and inferior frontal regions was reported in individuals that later became psychotic. In patients with first episode psychosis, there was instead a reduction in the thickness of the superior and inferior frontal cortex, and in the volume of thalamus. In chronic schizophrenia, the gray matter decreased further in the frontal and temporal areas, cingulate cortices, and thalamus, particularly in patients with unfortunate outcomes. Structural modifications of the white matter were only reported in a small number of longitudinal studies.

Although gross structural alterations are lacking, subtle pathological changes in specific populations of neurons and in cell-to-cell communication were reported [110]. Under this perspective, the most widely investigated area has been the prefrontal cortex that displayed increased neuronal density and altered neuroplasticity with age-related modifications [111]. It also appears that there is a particular vulnerability of the inhibitory cortical circuits, with markers of the cortical interneurons (e.g. 67kD glutamate decarboxylase - GAD67 or parvalbumin) showing some of the more consistent alterations [111,112].

## 4. The Homozygous *Reeler* Mouse

As mentioned, alterations in *Reeler* homozygous recessive mice fully recapitulate those in human LIS2 (Fig. 1), which is a monogenetic condition due to the lack of RELN, and are very similar to the human brain phenotype in LIS3 (see 3.1.1. Lissencephaly 2). The brain phenotype of the human monogenetic conditions that are consequent to mutations of the genes coding for proteins of the RELN intracellular signaling pathway is also much similar to that of the *reln*$^{-/-}$ mouse brain, except that, in most cases, differently from mouse, the human cerebellum is spared (Fig. 1 and see 3.2. Conditions caused by mutations of genes of the RELN intracellular pathway).

### *4.1. Imaging*

There is a limited number of imaging studies on the brain of the homozygous *Reeler* mouse. The first of these surveys is a detailed MRI description of the neuroanatomical phenotypes in homozygous and heterozygous mice using morphometry and texture analysis [113]. The *reln*$^{-/-}$mice were observed to have a smaller brain, but larger lateral ventricles compared to wild-type littermates. Specific, shape differences were found between mutants and wild-type mice in cerebellum, olfactory bulbs, dorsomedial frontal and parietal cortex, certain regions of temporal and occipital lobes, as well as in the lateral ventricles and ventral hippocampus. Gadolinium-based active staining demonstrated a general disorganization of the hippocampus as well as differences in thickness of individual hippocampal layers in *reln*$^{-/-}$mice, with particularly clear differences in the ventral hippocampus. On these observations, the authors concluded that the structural features of the *Reeler* brain most closely copy the MRI phenotype of LIS2 patients.

A subsequent study is a methodological paper describing the use of manganese-enhanced MRI (MEMRI) to detect cortical laminar architecture, where the mutant was used to confirm the usefulness of both systemic and tract tracing in the rodent brain [114]. The authors have compared the MEMRI signal intensity in the cerebral cortex of normal and mutant mice and observed that, in the former, signal was low in layer 1, increased in layer 2, decreased in layer 3 until mid-layer 4, and increased again, peaking in layer 5, before decreasing through layer 6. In *Reeler* there were instead no appreciable changes in signal intensity, an observation consistent with the absence of cortical lamination after histological examination.

A more recent study has employed diffusion tractography imaging (DTI) to map the remodeling of the lemniscal thalamo-cortical projections in mutant mice as a consequence of the highly disorganized cortical lamination [115]. By such an elegant approach, the authors have been capable to perform an in vivo origin-to-ending reconstruction of the mouse somatosensory thalamo-cortical projections and to demonstrate the occurrence of an extensive remodeling of these projections in *Reeler* mutants.

### *4.2. Histology and Electrophysiology*

In keeping with MRI studies, the first observation that can be made at gross anatomical examination of the *reln*$^{-/-}$ mouse brain is its atrophy, as the total volume of the brain in mutants is reduced of about 19% when compared to normal mice [113]. Such a reduction is particularly evident in the cerebellum that also displays a very limited degree of foliation. The histological anomalies in mutants have been mainly interpreted as a consequence of an abnormal migration of neurons, rather than the effect of an alteration in cell fate determination or axonal guidance. Among these anomalies the most distinguishing ones are that the cerebral and cerebellar cortices lose their layered structure, in accordance with the aforementioned MEMRI observations [114]; numerous neuronal nuclei disappear or, at least, become hardly recognizable in several brain regions; and neurons are often ectopically localized. Table 3 summarizes the most important findings regarding the structural anomalies of the *reln*$^{-/-}$ CNS without taking into consideration the histological alterations in the cerebral cortex, hippocampus and cerebellum that will be discussed analytically in the following. Detailed descriptions of the morphological phenotype of the *Reeler* mouse CNS can be found e.g. in [113,116].

#### *4.1.1. Cerebral Cortex and Hippocampus*

Very early observations demonstrated the occurrence of dendritic anomalies in cortical and hippocampal neurons of *Reeler* mice [117,118]. After the discovery of *Reln*, it has been then proved that the Reln signaling pathway is required for the correct maturation and differentiation of dendritic branches and spines in hippocampal and neocortical pyramidal neurons [119,120]. Due to the complexity of the phenomena involved in dendritic maturation, one can argue that dendritic anomalies represent a consequence of the deep cytoarchitectonic derangement occurring in *Reeler* mice rather than a primary effect of the lack of Reln. However, Niu et al. [121] observed a reduction of dendritic complexity also in heterozygous mice, which, as discussed in a subsequent section, do not display obvious neuronal ectopias. On the same line is the finding of a reduced density of dendritic spines in pyramidal neurons of the prefrontal cortex and hippocampus of heterozygous mice [122].

Interestingly, the block of the Reln signaling by means of specific antibodies resulted in an increased complexity of branching in the apical dendrites of layer 2/3 cortical pyramidal neurons, whereas their basal arborizations remained unaffected [123].

There are many important issues related to the structure and role of the dendritic tree of neocortical and hippocampal pyramidal neurons that make the *Reeler* mouse an important tool for the study of (forebrain) neurodevelopment. Inputs to layer 5 neurons are processed by separate compartments, with the basal dendrites receiving bottom-up information and the apical dendrite being the recipient of feedback input from higher cortical areas, see e.g. [124]. This framework is made even more complex by the fact that the apical dendrite of these neurons span most cortical layers before reaching layer 1, where the apical tuft is located [125] . Moreover, it is known that the electrophysiological properties of neurons are ultimately determined by the type and distribution of their ion channels. Essential to the function of the long apical dendrite of the pyramidal neurons is the progressively increasing density of hyperpolarization-activated cyclic nucleotide–gated (HCN) channels, proceeding from proximal to distal segments [126]. Such a gradient critically contributes to the functional distinction of dendritic compartments and seems to be specified by Reln signaling [127], but see [128]. The evidence that Reln is involved in the trafficking and targeting of ion channels in cortical and hippocampal neurons suggests that their intrinsic electrophysiological properties might be modified in the *Reeler* mouse. An early study by Bliss and Chung [129]

522 demonstrated that, despite the layering derangement, the basic synaptic organization of the
523 hippocampus is largely preserved in mutants.
524

| Division of CNS | Region/division | Subdivision/Nucleus | Type(s) of alteration | References |
|---|---|---|---|---|
| **Forebrain** | Olfactory bulb | | • Slight disruption of the glomerular layer.<br>• Numerical reduction and clustering of granule cells | [130,131] |
| | Cerebral cortex | | see text | |
| | Hippocampus | | see text | |
| | Diencephalon | | • Misrouting of GnRH neurons to the cerebral cortex | [132] |
| | | Mammilary bodies | • Alteration of projections to hippocampus | [133] |
| **Midbrain** | Rostral colliculus | | • Loss of individual limits in the three more superficial layers<br>• Spread of corticotectal projections<br>• Anomalies of retinotectal projections | [134] |
| | Mesencephalic nucleus of V | | • Spread of neurons along their route of migration | [135] |
| | Substantia nigra | | • Anomalous clustering lateral to the ventral tegmental area | [136] |
| **Hindbrain** | Cerebellum | | • see text | |
| | Medulla oblongata and pons | Dorsal cochlear nucleus | • Partial loss of layered organization | [137] |
| | | Inferior olivary nucleus | • Loss of folding - Swelling | [138] |
| | | Somatic motorneurons (Nucleus ambiguous, facial and trigeminal) | • Slight displacement and loss of somatotopic organization (muscolotopy) | [6,139] |
| | | Pontine nuclei | • Ventral shift | [140] |
| **Spinal cord** | Dorsal horn (laminae I-II) | Nociceptive | | [141] |
| | Lateral horn | Preganglionic sympathetic and parasympathetic neurons | | [142,143] |

525 **Table 3: Main histopathological changes in the homozygous *Reeler* mouse**
526
527 An accurate study dealing with the intrinsic electrophysiological properties of cortical neurons in
528 *Reeler* mice was carried out in more recent times by Silva et al. [144]. These authors showed that the
529 firing pattern and synaptic responses of the pyramidal neurons in these mice was normal, but their
530 radial distribution was inverted, and concluded that, although malpositioned, neurons maintained
531 the membrane properties appropriate to their function.
532 The apparent discrepancy between that data demonstrating the role of Reln in the modulation of ion
533 channels and the relative lack of anomalies in the intrinsic properties of cortical neurons might have
534 several explanations. Other factors, such as neuronal activity [145] could be more effective than Reln
535 for the modulation of membrane channel targeting. Furthermore, the complex machinery of the long
536 apical dendrite is required when layer 5 neurons settle appropriately, but might be useless for the
537 same neurons displaced to more superficial cortical layers. Finally, future investigations based on
538 refined electrophysiological techniques, such as direct dendritic recordings, will help to establish if
539 indeed the cortical neurons in mutant mice display more subtle changes of their firing/intrinsic
540 properties.
541
542 The Reln signaling is also able to modulate key molecules of the cascade leading to synaptic
543 plasticity, such as the NMDA receptors [146,147]. Synaptic plasticity is known to be impaired in
544 several types of mental disorders, including autistic spectrum disorders and schizophrenia [148-150].
545 Therefore, several studies have been focused on the changes of synaptic plasticity in *Reeler* mutants.

Ishida et al. [151] reported that the induction of long term potentiation (LTP) was impaired in the CA1 region of the hippocampus in *Reeler* mice, claiming that the malpositioning of some neuronal populations could account for such an alteration. Later, Weeber et al. [152] observed a defect of LTP in the hippocampus of VLDLR-deficient mice. They also found that the perfusion of hippocampal slices with Reln was able to enhance LTP in CA1. An impairment of both LTP and long term depression (LTD), not accompanied by a change of subunit composition of AMPA and NMDA receptors, has been reported also in the hippocampus of heterozygous mice [153] – see below 5.3. Histology and Electrophysiology.
On the other hand, both the overexpression of Reln in transgenic mice [154] and Reln supplementation strongly increased LTP [155].
As pointed out above, most changes found in *Reeler* mice are characterized by decreased synaptic plasticity. It should be noted that some experimental models of autistic spectrum disorders are rather characterized by hyperplasticity and hyperconnectivity [156], thus arguing against any straight correspondence between Reln deficiency and autism.

The majority of cortical neurons are represented by spiny, glutamatergic pyramidal cells, whose migratory path during prenatal development follows an inside-out radial pattern from the ventricular zone to the final position [157]. Reln signaling is required for the localization of pyramidal neurons to appropriate cortical layers, as reviewed in [158]. As a consequence, the lack of Reln causes a disruption of the layered cortical organization, including abnormal positioning [159,160], as well as an increased percentage of inverted pyramidal cells [161,162].
Inhibitory GABAergic interneurons represent a minority population within the neocortex. Yet, their morphological, neurochemical and functional diversity is thought to play a pivotal role for the cortical function, see e.g. [163]. Furthermore, several anomalies related to the interneuron function are currently considered key features in different types of mental disorders, including schizophrenia, see for a very recent review [112].
Unlike pyramidal neurons, interneurons are generated in the ganglionic eminence of the ventral telencephalon and follow a tangential migratory route to the cortex [157]. While the malpositioning of the principal cells in *Reeler* mice is well documented, it is not clear if the migration of the interneurons is affected by the Reln signaling cascade. Using *Reeler* mutants crossed with mice expressing green fluorescent protein (GFP) in inhibitory neurons, it was shown that cortical interneurons display abnormal laminar position and morphology [164]. However, it remains to be established whether the ectopy of interneurons directly depends from Reln signaling or is rather the consequence of the malpositioning of principal projection neurons. The issue is still debated as contradictory views can be found in the literature. Namely, while some observations [165,166] argue against a direct role of Reln, Hammond et al. [167] showed that only early-generated cortical interneurons are misplaced as a consequence of the ectopy of pyramidal neurons, whereas the correct layering of late-generated interneurons seem to be directly modulated by Reln signaling.

As described above, Reln signaling is essential to correct migration, differentiation, and plastic synaptic remodeling of cortical/hippocampal neurons. Other basic neurodevelopmental features, such as cortical [168] and cerebellar (see below) neurogenesis, seem to be regulated by the glycoprotein as well.
As a consequence, the minicolumnar organization of the cerebral neocortex appeared to be deeply affected by Reln deficiency [169] and some physiological counterparts of cortical connectivity, such as the trans-synaptic signal propagation, were also impaired [170].
However, the outcome of Reln deficiency on the microcircuitry sustaining the cortical machinery is controversial and, surprisingly, the deep architectonic disorganization that follows the lack of the protein may not be paralleled by dramatic functional anomalies. Both early studies and more recent reports point out that the absence of Reln does not prevent the development of functionally appropriate cortical connections and maps [115,171-174]. In addition, when studied at the fine-scale

electron microscopic level, the basic synaptic organization of misplaced cortical neurons is preserved [175].

Therefore, although the laminar organization is thought to be critical for cortical computation [176,177], evidences obtained in *Reeler* mice led Guy and Staiger [178] to challenge the importance of cortical lamination, affirming that "future studies directed toward understanding cortical functions should rather focus on circuits specified by functional cell type composition than mere laminar location".

*4.1.2. Cerebellum*

Macroscopically, the cerebellum of the *Reeler* mouse is smaller than that of age-matched littermates; it is club-shaped with the main axis transverse to the mid plan of the body, and has an almost totally smooth surface, with just a few superficial grooves [179]. Cerebellar folia form as a consequence of the repeated folding of the cortex, the three-layered coating of gray matter covering the entire surface of the organ. Deeply to the cortex the white matter consists of a central mass, the medullary body, with the embedded gray nuclei. The white matter progressively arborizes in a very complex and intriguing fashion and its smallest branches form the central axis of individual folia. The architecture of the *Reeler* cerebellum is profoundly altered from this general pattern, firstly as a consequence of the impairment in the complicated series of migrations made by neurons to reach their final destination in the mature organ. Trajectories of migrating neurons follow both directions from the surface to the depth of the cerebellum, depending from the species, the type(s) of neurons and the developmental stages (for details see e.g. [180]). Eventually, disturbances in the migration of the cerebellar neurons make that *Reeler* mice display a cerebellum that retains several features of immaturity.

Recently, the area of the cerebellar cortex in mutants was analyzed quantitatively during postnatal (P0-P25) development, and resulted to be reduced compared to age-matched controls [181]. Reduction in the extension of the cortex was particularly evident in the molecular layer and the (internal) granular layer. Physiologically, as the cerebellum matures, the molecular layer becomes more and more populated by the parallel fibers: at P25 its increase in size was about seventeen-fold in $reln^{+/+}$ mice, but only six-fold in the mutants [181]. Post-migratory granule cells, which are generated in the temporary subpial external granular layer, progressively populate the (internal) granular layer during normal cerebellar development, and, from P0 to P10, the granular layer of $reln^{+/+}$ mice increased about five-folds in size, but only 2.6-fold in $reln^{-/-}$, where it drastically reduced its size to 0.62-fold after P10 [181]. Differently from the cortex, the medullary body is larger in the mutants than in wild-type animals. Its progressively increasing area mainly reflects the ongoing myelination of the axons of the Purkinje neurons that leave the cortex traveling across the white matter to reach the cerebellar nuclei, as well as the development of the afferent and efferent fiber systems entering or exiting the organ. The size of the medullary body increased in parallel with postnatal age in both *Reeler* and wild-type mice ($reln^{-/-}$ 2.59, $reln^{+/+}$ 1.93-fold), but, at P25, *Reeler* mice had a larger medullary body than their normal counterparts (1.88-fold) [181]. In brief, *Reeler* mice had a smaller cerebellar cortex but a larger medullary body than their normal littermates. The cerebellar hypoplasia was thus demonstrated to be a consequence of a reduction in cortical size and cellularity and the latter, in turn, resulted to be linked to quantitative differences in the degree of cell proliferation and apoptosis, as well as derangements in the timing of postnatal cortical maturation [181]. It was also calculated that the density of proliferating cells was the most important predictive factor to determine the cellularity of the cerebellar cortex in the mutants [181].

Therefore, beside the well-known consequences onto neuronal migration, the lack of Reln also results in a measurable deficit in neuronal proliferation, at least in cerebellum. Ultrastructurally, it was demonstrated that the cerebellar neurons undergo different forms of programmed cell death in the course of postnatal development and that the deficit of Reln affected the type and degree of neuronal death [182].

Perhaps the most striking histological feature of the cortex in mutants is the lack of alignment of the Purkinje neurons to form a discrete layer between the molecular and the granular layer. Thus, in *Reeler*, only about 5% of the Purkinje neurons were normally placed within the cerebellar cortex, 10% are in the granular layer, and the remaining 85% form a central cellular mass intermingled with the white matter [183-185]. Ultrastructurally, in *Reeler* there is a reduction in the density of the contacts between the Purkinje neurons and the parallel and climbing fibers from P5 onward [186]. Functionally, both the normally placed Purkinje neurons and those ectopically dislocated in the granular layer display a 0-1 response to stimulation, indicating that, as in normal mice, they are synaptically contacted by a single climbing fiber. Those in the central cellular mass, instead, show intensity-graded responses to electrical stimulation, as they receive a convergent input from several climbing fibers [183], likely as a failure of physiological pruning to occur [187]. Neurochemically, during postnatal development there are no obvious variations between normal mice and the mutants in the temporal pattern of expression of some widely expressed neuronal and glial markers (NeuN, vimentin, calbindin, GFAP, Smi32, GAD67) [181], but the Bergmann glia was misplaced in *Reeler* [188].

## 5. The Heterozygous *Reeler* Mouse

Heterozygous *reln*$^{+/-}$ mice are haplodeficient in Reln but, differently from *reln*$^{-/-}$ mutants, do dot display an obvious phenotype. These mice have been already for a long time proposed as translational models for autism and schizophrenia. The reasons for this assumption are that their behavioral and structural phenotype is somewhat close to that of the human patients suffering from the two pathologies. Notwithstanding, as discussed previously (see section 3.3. Conditions Possibly Related to RELN Mutations), autism and schizophrenia are only tentatively linked to *RELN* and the behavioral and brain structural modifications in these mice are difficult to ascertain with confidence. Even so, the interest in these mice has been boosted by the discovery of *RELN* heterozygous mutations in human ADLTE (see section 3.1.2. Autosomal-Dominant Lateral Temporal Epilepsy).

### *5.1. Behavior*

The recapitulation of the behavioral modifications typical of human autism, schizophrenia or epilepsy in heterozygous *Reeler* mice still is a subject of debate. The dissimilar outcome of behavioral experiments performed in different laboratories is not surprising, because neuropsychiatric behaviors in humans are primarily related to social interaction, communication, and restricted interest and these behaviors are obviously very difficult to be objectively measured in mice [189]. It is perhaps worth mentioning here that most of our knowledge on the effects of Reln in the cognitive or behavioral field derives from work on hippocampus. This is not surprising as this part of the brain, as discussed previously, has been the primary focus of numerous investigations also in human patients affected by autism, schizophrenia or epilepsy. Several behavioral features similar to those observed in these human conditions were thus described in *reln*$^{+/-}$ mice [190-192], such as deficits in reversal learning after visual discrimination tasks that were hypothesized to follow a diminished visual attention [191]. Also, *reln*$^{+/-}$ mice were tested for anxiety-related behavior, motor impulsivity and morphine-induced analgesia. Their behavioral profile was reported to be different from that of wild type littermates in that they displayed, starting form adolescence, a decreased inhibition and emotionality. To these modifications, a slight increment of impulsive behavior and

altered pain thresholds were associated in adult mice [193]. Heterozygous mice were also tested in a complex series of PPI protocols (unimodal and cross-modal) to conclude that they exhibited a complex pattern of changes in startle reactivity and sensorimotor gating, with both similarities to and differences from schizophrenia [194]. At least partly in line with these latter observations, other investigations failed, partly or in full, to substantiate the behavioral analogies between neuropsychiatric patients and *reln*$^{+/-}$ mice [195-200]. For example, Salinger and co-workers were unsuccessful to find differences between *reln*$^{+/-}$ and *reln*$^{+/+}$ mice after testing gait, emotionality, social aggression, spatial working memory, novel-object detection, fear conditioning, and sensorimotor reflex modulation [195]. In another survey [197], heterozygous *Reeler* mice were assessed for cognitive flexibility in an instrumental reversal learning task, impulsivity in an inhibitory control task, attentional function in a three-choice serial reaction time task, and working memory in a delayed matching-to-position task. No differences were found in comparison to wild-type littermate controls in any prefrontal-related cognitive test. However, *reln*$^{+/-}$ mice showed deficits in the acquisition of two operant tasks. From these observations the authors concluded that heterozygous *Reeler* mice were *not* a good model for the core prefrontal-dependent cognitive deficits observed in schizophrenia, but rather for more general learning deficits in psychiatric disorders.

In another paper it was reported that heterozygous and wild type mice displayed similar levels of overall activity, coordination, thermal nociception, startle responses, anxiety-like behavior, shock threshold; identical cued freezing behavior, and comparable spatial learning in Morris water maze tasks, albeit a significant reduction in contextual fear conditioned learning was observed in *reln*$^{+/-}$ mice only [199]. These authors have then hypothesized that the pharmacological administration of Reln in heterozygous mice could restore the response to PPI. They were unable to find significant differences in acoustic startle reflex between treated and untreated animals, but Reln-treated *reln*$^{+/-}$ mice showed a significant increase in the percent inhibition to 78, 86 and 90 dB pre-pulse [201].

One study has specifically focused onto the *reln*$^{+/-}$ mouse behavioral phenotype in young (P50-70) and fully adult (older than P75) animals to conclude that they are not useful to model schizophrenia [196]. An ample behavioral test battery was employed (Irwin test; rotarod; spontaneous locomotor activity; social behavior; light-dark transition; startle response and pre-pulse inhibition; hot-plate). Heterozygous mice did not differ from their wild-type littermates at either age, although fully adult male *reln*$^{+/-}$ mice were engaged in social investigation for a longer time. In addition, performance on the rotarod deteriorated with age.

Indeed, age appears to be a further issue of complexity. In fact, adult *reln*$^{+/-}$ mice did not display discernible differences in activity, motor coordination, anxiety, or environmental perception when compared with wildtype littermate control mice, but adolescent animals showed lower levels of anxiety- and risk assessment-related behaviors in the elevated plus-maze [153,192].Also, in one of these two studies it was demonstrated that young *reln*$^{+/-}$ mice had a hippocampal-dependent deficit in associative learning and impulsivity–anxiety-related behavior [153]. In addition, one study, starting from the clinical observations that reported the occurrence of vocal and motor anomalies in autistic patients, has described that *reln*$^{+/-}$ mice had a general delay in the development of their repertoire of neonatal vocal and motor behaviors [202].

Finally, it should be taken into consideration that some behaviors appeared to be influenced by gender, although very few studies have focused on this issue. Among these studies, young heterozygous female mice were described to be more active in the light/dark transition test than the heterozygous males that were, instead, more aggressive than females during social interaction [192].

### *5.2. Imaging*

The paper by Badea and co-worker [113], already quoted with reference to homozygous mice, also analyzed the brain of *reln*$^{+/-}$ animals. These authors reported that the total volume of the brain, the ventricular volume and the hippocampal volume were enhanced by approximately 6%, 82%, and 7%, respectively with respect to normal control mice. After statistical analysis, they showed that these volumes were similar to those of *reln*$^{+/+}$ mice, but greater than those of mutant mice. They also measured the areas of different parts of the brain in comparison with wild type mice: no differences

were found in hippocampus and cerebellum, but there was an enlargement of the lateral ventricles. In a more recent paper, the ventricular enlargement was confirmed, but a reduction of the cerebellar volume was described in heterozygous mice, whereas the volume of the motor cortex as well as its thickness was unchanged [198].

*5.3. Histology and Electrophysiology*

5.3.1 Cerebral cortex, hippocampus and striatum

As already mentioned, heterozygous *Reeler* mice only display subtle structural alterations of their brains and perhaps for this reason they have not been investigated thoroughly.

One of the most striking features appears to be the reduction in the levels of GAD67, the key GABA synthetic enzyme, in the fronto-parietal cortex, hippocampus, and striatum [122,203]. Hippocampal levels of the enzyme could be somewhat restored after stereotaxic injections of Reln [155,201]. Therefore, these experiments suggested that the decrease in GAD67 expression was directly related to Reln haplodeficiency and, in keeping with such a possibility, could be partly reversed increased through Reln supplementation. In line with this interpretation, nicotine, which reduces GAD67 promoter methylation and increases its transcription, was shown to be able to restore the normal levels of expression of the enzyme when administered to heterozygous mice [203].

In hippocampus, the GABAergic interneurons target, among others, the CA1 pyramidal neurons. In heterozygous mice these neurons display a reduction in the average length and width of their apical and basal shaft dendritic spines [121], and Reln supplementation was effective in promoting a full (apical) or partial (basal shaft) spine recovery [201]. These morphological observations are in line with a previous report showing that spines were hypertrophic in mice conditionally overexpressing Reln in the forebrain [154]. At electrophysiological recordings CA1 pyramidal neurons in *reln*$^{+/-}$ mice displayed reduced spontaneous inhibitory postsynaptic currents [199], an observation that is fully in line with the reduction of the inhibitory input from the GABAergic interneurons.

Synaptic plasticity is fundamental for hippocampal function. In CA1 of heterozygous mice, LTP is impaired [153] as well as LTD [199], which could be brought back to normal levels by administration of Reln [201]. Additionally, in *reln*$^{+/-}$ and *reln*$^{-/-}$ mice post tetanic potentiation (PTP), a form of short-term plasticity that depends on neurotransmitter release, is reduced in CA1 [201,204] and could also be reversed by Reln [201].

Collectively these data indicate that the morphological, neurochemical and physiological deficits that followed a reduction in brain Reln in heterozygous mice could be reversed by the experimental administration of Reln. In translational terms, this observation is very important because reduced synaptic inhibition in hippocampus and prefrontal cortex is believed to have a role in several neuropsychiatric conditions, among which schizophrenia [205,206].

5.3.3. Cerebellum

Notably most of the histological modifications observed in the human autistic cerebellum are similar to those described in the studies on *reln*$^{+/-}$ mice. In addition, VLDLR-associated cerebellar hypoplasia, SCA7 and SCA37 all display a clear cerebellar phenotype. Thus the cerebellum has been widely investigated to validate the heterozygous *Reeler* mouse as a translational model. Despite of this, few structural observations have focused on the cerebellum of *reln*$^{+/-}$ mice compared to those on the cerebral cortex and hippocampus described in the previous sections. Heterozygous animals have been reported to manifest a progressive loss of Purkinje neurons already during the first weeks of life [207], and lower numbers of these cells were described in adult subjects as well [208]. Although cerebellar lesions are widely described in the human autistic brain, particularly in the vermis (see section 3.3.1.) it remains unclear whether this part of the cerebellum is affected as a whole or rather only specific lobuli may be hit by the pathology. Therefore, our group has at first focused his attention on five different lobules of the vermis - central lobule (II–III), culmen (IV–V), tuber (VIIb), uvula (IX), and nodulus (X), which receive different types of afferent functional inputs, to analyze the number and topological organization of the Purkinje neurons in *reln*$^{+/+}$ and *reln*$^{+/-}$ adult mice (P60)

of both sexes [209]. To specifically visualize these neurons we have generated hybrids of *reln*$^{+/-}$ mice and the L7GFP mice, so that the GFP-tagged Purkinje neurons could be directly identified in cryosections. We have thus shown that the Purkinje neurons: *1.* Displayed a numerical reduction (cells/area) in *reln*$^{+/-}$ males (14.37%) and *reln*$^{+/-}$ females (17.73%) compared to *reln*$^{+/+}$ males; *2.* Were larger in *reln*$^{+/-}$ males than in the three other phenotypes under study, and smaller in females (irrespective of the *reln* genetic background) than *reln*$^{+/+}$ males; *3.* Were more chaotically arranged along the YZ axis of the vermis in *reln*$^{+/-}$ males than in *reln*$^{+/+}$ males and, except in central lobule, *reln*$^{+/-}$ females. These observations offered some additional clues for the validation of the heterozygous *Reeler* mouse as a translational model of autism, but were clearly insufficient to draw a final conclusion. Very recently, as a number of observations have implicated several synapse-related genes in the genesis of autism, epilepsy and some other neuropsychiatric conditions [210,211], we have investigated the expression of synaptophysin 1 (SYP1) and contactin 6 (CNTN6) within the vermis of adult mice of both sexes and different genotypes (*reln*$^{+/-}$ and *reln*$^{+/+}$) [212]. SYP1 is a pre-synaptic marker and CNTN6 is a marker of the synapses made by the parallel fibers onto the Purkinje neurons' dendrites. Notably, there is evidence, although still to be validated in full, that SYP1 is involved in the structural alterations of the autistic synapses [79,213], and very recent observations have shown that copy number variations [214] or a truncating variant [215] of *CNTN6* are found in autistic patients. In addition, *CNTN6* mutations have been proposed as a risk factor for several neurodevelopmental and neuropsychiatric disorders [211,216,217].

In line with these human studies, we have demonstrated that r*eln*$^{+/-}$ mouse males display a statistically significant reduction of 11.89% in the expression of SYP1 compared to sex-matched wild-type animals, whereas no differences were observed between *reln*$^{+/+}$ and *reln*$^{+/-}$ females [212]. In *reln*$^{+/-}$ male mice, reductions are particularly evident in the molecular layer: 10.23% less SYP1 than *reln*$^{+/+}$ males and 5.84% < *reln*$^{+/+}$ females. In *reln*$^{+/-}$ females, decrease is 9.84% versus *reln*$^{+/+}$ males and 5.43% versus *reln*$^{+/+}$ females. Both *reln*$^{+/-}$ males and females show a stronger decrease in CNTN6 expression throughout all the three cortical layers of the vermis: 17–23% in the granular layer, 24-26% in the Purkinje cell layer, and 9–14% in the molecular layer. More specifically, when individual cerebellar lobules are considered, we have shown that alterations in the levels of expression of SYP1 in the molecular layer of male *reln*$^{+/-}$ mice are spread across all vermian lobules except lobule VII, but restricted to lobule II for the granular layer and VII for the Purkinje cell layer.

Thus, the widespread reduction of SYP1 and of CNTN6 in the molecular layer of heterozygous male mice is fully compatible with the human autistic phenotype [217] .

In the vermis (and cerebellum in general), there is evidence for a topographic organization of motor control versus cognitive and affective processing areas, and the different lobules are connected with specific areas of the brain and spinal cord [218]: CNS areas that process sensorimotor information are directly or indirectly linked with the cerebellar anterior lobe (lobules I–V of the vermis), lobule VIII, and, to a smaller degree, with lobule VI; in contrast, cerebral association areas that receive non-motor inputs target vermian lobules VI and VII. Available clinical evidence indicates that the cerebellar vermis is the main target of limbic-related structures, and physiological and behavioral studies support the role of the vermis in the modulation of emotions [219]. Therefore, the neurochemical modifications of the cerebellar cortex in heterozygous mice of both sexes well correlate with idea that the social and communication abnormalities typically found in autism depend on abnormalities in the limbic structures and their connectivity [220,221].

At autopsy, a loss of Purkinje neurons in the posterior cerebellum was long ago described in autistic patients [102,222], but it did not appear to affect the vermis, as then reported by Bauman and Kemper in [79]. Hypoplasia in lobules VI and VII was first observed in vivo after neuroimaging [63], but later work provided evidence of two distinct autistic subtypes associated with vermian hypoplasia or hyperplasia [64]. A systematic review and meta-analysis of structural MRI studies has then demonstrated that the decrease in size of vermian lobules VI–X (*i.e.* the lobules comprised in the posterior cerebellum) displayed a notable heterogeneity that correlated to differences in age and intelligence quotient (IQ) of the study population only in lobules VI–VII [223]. Other studies indicated that the posterior/inferior vermis was more susceptible to pathological changes [224], and

lobules VII, VIIIb (left), and IX were implicated, with a reduction of the gray matter after quantitative imaging of autistic patients [80,225,226]. Therefore, it appears that the cerebellar phenotype of the heterozygous *Reeler* mouse is fully compatible with that in humans and that a depth structural and neurochemical characterization may be useful to direct the discovery of new biomarkers of translational interest.

**6. Usefulness of the Reeler Mouse in Translational Studies: Concluding Remarks**

The analysis of the literature discussed above requires one trying to draw some conclusions about the true usefulness of the Reeler mouse in translational studies.

At first it may perhaps be useful to recall that, as discussed, *RELN* has been recognized to be causative of LIS2 and a small percentage of ADLTE, whereas only tentative associations have been found up to now for the other conditions here considered (see Fig. 1 and Table 1).

Remarkably both LIS2 and ADLTE are rare diseases. Very few cases of LIS2 (around ten) have been described so far (see OMIM #257320). Similarly, patients with lateral temporal epilepsy (LTE) are only about 10% of all temporal epilepsies, and the real prevalence of ADLTE, which has been so far reported in Europe, USA and Japan, is unknown, but it may account for about 19% of familial idiopathic focal epilepsies [227,228]. When one considers the human conditions related to the Reln signaling pathway, still is faced with a group of rare diseases. The actual frequency of *VLDLR*-associated cerebellar hypoplasia is unknown, although the condition has been reported worldwide and more than twenty-five affected individuals were initially reported in Canada and USA [37,229]. *PAFAH1B1*-associated lissencephaly is very rare as the prevalence of classic lissencephaly ranges from 11.7 to 40 per million births [41]. To date, sixty six affected individuals and seven asymptomatic individuals with the ATTTC repeat insertion within *DAB1* have been reported in ten relatives from the south of the Iberian Peninsula, and no individuals with SCA37 from other geographic areas have been reported [38]. SCA7 has a prevalence of less than 1:100,000 and accounts for about 2% of all SCAs [230].

Therefore, one has to deal with the paradox that there is relatively little interest for translational studies for those conditions for which the *Reeler* mouse and/or genetically engineered mice with mutations of the genes of the Reln pathway fully meet the criteria of construct and face validity. Thus, the homozygous Reeler mice appear to be more interesting to the neurobiologist than the clinician and their study will surely be still rewarding in terms of our comprehension of neurodevelopment. For instance, as discussed above, the model helped to establish that many functional and circuit features of cortical neurons are relatively independent from positional cues and cortical lamination, e.g. [178].

Very differently from the above, prevalence of autism in the worldwide population is estimated to be around 1% [231] and that of schizophrenia is just below 1% [110]. As the two conditions are very diffuse in the human population, there is an obvious substantial translational interest for the heterozygous Reeler mouse to model the two disorders. However, such an interest is again paradoxical, as the validity of the model still remains dubious. The first explanation for this uncertainty lies, beyond any doubt, in the substantial lack of construct validity, which is the direct consequence of the complex genetic background of autism and schizophrenia. As regarding face validity, the present survey of the literature clearly points out that there are several similarities but also dissimilarities between the human and the mouse phenotypes. Among dissimilarities one has to consider the heterogeneity of results of the behavioral experiments in mouse. This is further made complex by the vast array of clinical symptoms in human. Structurally, although most of the imaging and post-mortem findings in humans are not specific for each of the two conditions, one has to consider that both the human and mouse phenotype converge to indicate the cerebral cortex, hippocampus and cerebellum as the primary foci of the pathologies and the inhibitory interneurons as major players in the context of the circuitry involved (Fig. 2).

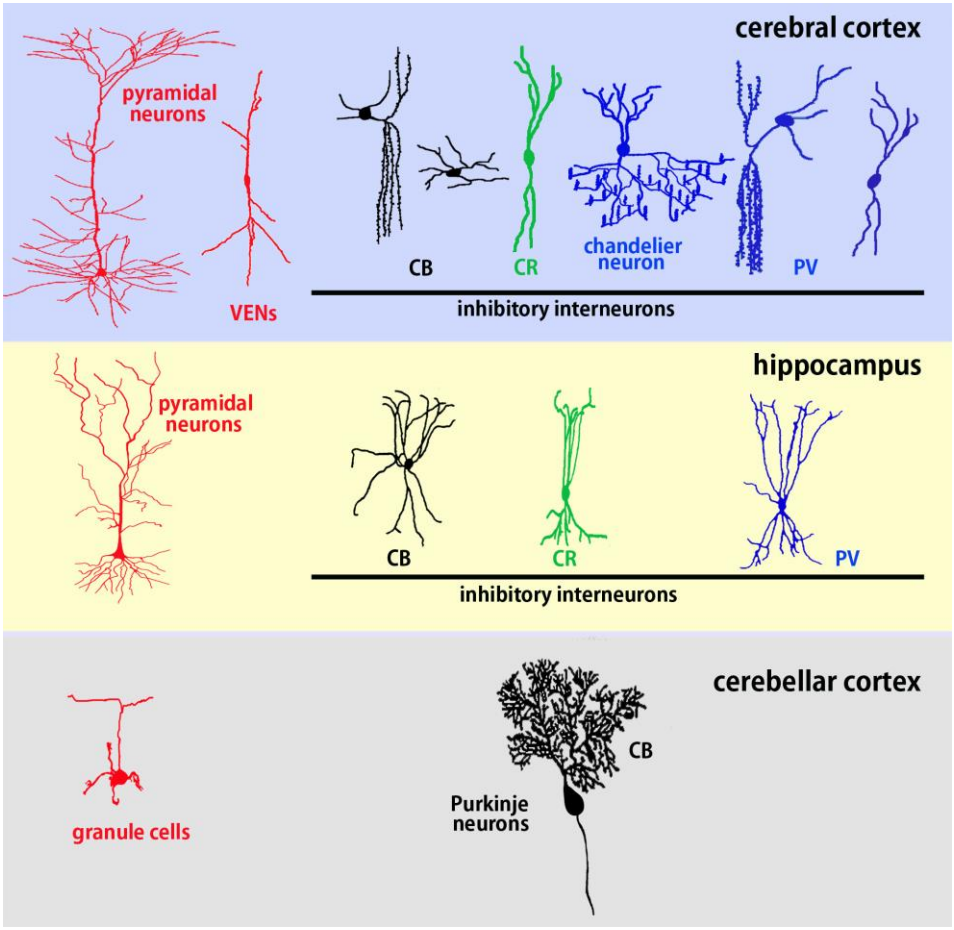


**Figure 2: Main types of cortical, hippocampal and cerebellar neurons showing structural changes in autism and schizophrenia comparable with the structural phenotype of the heterozygous reeler mouse.**
In the cerebral cortex and hippocampus, comparable alterations between the two species can be observed to mainly affect both excitatory (in red) and inhibitory neurons (color coded according to their content of the principal calcium binding proteins[1]). The Purkinje neurons are the cortical cerebellar neurons suspected to be the main histopathological target in both humans and mice; however also the cerebellar granule cells may be hit as a consequence of the Reln haplodeficiency, given their tight developmental link with the Purkinje neurons. See also Table 2 and main text. Abbreviations: CB calbindin; CR calretinin; PV parvalbumin; VENs von Economo neurons.

A serious drawback to a full validation of the heterozygous Reeler mouse as a model of autism and/or schizophrenia lies in the observation that the alterations so far described in mouse are very subtle in both structural, functional and neurochemical terms. Due to the relatively low resolution of current neuroimaging procedures, and the difficulty to obtain post-mortem samples amenable for neurochemical, electrophysiological, and fine (ultra) structural analysis, it remains to be established whether the alterations in heterozygous Reeler mice have a true biological significance, beyond statistics [232,233], and, in the affirmative, if one can take advantage of these alterations to discover novel biomarkers that will be helpful for an earlier and more precise diagnosis.

**Author Contributions:** All authors contributed to write the manuscript

[1] The classification of cortical interneurons has been more recently reconsidered taking into consideration several other neurochemicals beside to the calcium-binding proteins. However, as this type of classification is still widely in use, we made reference to it, also considering that the majority of papers reporting on postmortem human material we based on such a categorization.

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**Conflicts of Interest**

The authors declare no conflict of interest.

**Abbreviations**

ADLTE autosomal-dominant lateral temporal epilepsy
ApoER2 apolipoprotein E2
ARX aristaless-related homeobox gene
ASD autism spectrum disorders
ATXN7 ataxin 7
*AUTS1* autism susceptibility 1 gene
CB calbindin
CNS central nervous system
CNTN6 contactin 6
CR calretinin
DCX doublecortin
DTI diffusion tractography imaging
ETL7 temporal lobe epilepsy-7
GFP green fluorescent protein
HCN hyperpolarization-activated cyclic nucleotide–gated
ILS isolated lissencephaly sequence
IQ intelligence quotient
LCH lissencephaly with cerebellar hypoplasia
*LGI1* leucine-rich, glioma inactivated 1 gene
LIS1 lissencephaly 1
LIS2 lissencephaly 2
LIS3 lissencephaly 3
LTD long term depression
LTE lateral temporal epilepsy
LTP long term potentiation
MAP2 microtubule-associated protein 2
MDS Miller-Dieker syndrome
MEMRI manganese-enhanced MRI
PAFAH1B1 platelet-activating factor acetylhydrolase IB subunit $\alpha$
PDD-NOS pervasive developmental disorder-not otherwise specified
PPI pre-pulse inhibition
PTP post tetanic potentiation
PV parvalbumin
*RELN* Reelin gene (human)
*Reln* Reelin gene (mouse)
RELN Reelin glycoprotein (human)
Reln Reelin glycoprotein (mouse)
ROI region-of-interest
SBH subcortical band heterotopia
SCA37 spinocerebellar ataxia type 37
SCA7 spinocerebellar ataxia type 7
sMRI structural magnetic resonance imaging
SNP single nucleotide polymorphism
SYP1 synaptophysin 1
TUBA1A $\alpha$ tubulin 1A
VENs von Economo neurons

VLDLR very low density lipoprotein receptor

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