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Eur J Hum Genet. Author manuscript; available in PMC 2015 August 01.

Published in final edited form as:
Eur J Hum Genet. 2015 February ; 23(2): 195–201. doi:10.1038/ejhg.2014.81.

GluD1 is a common altered player in neuronal differentiation from both *MECP2*-mutated and *CDKL5*-mutated iPS

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Abstract

Rett syndrome is a monogenic disease due to de novo mutations in either *MECP2* or *CDKL5* genes. In spite of their involvement in the same disease, a functional interaction between the two genes has not been proven. MeCP2 is a transcriptional regulator; *CDKL5* encodes for a kinase protein that might be involved in the regulation of gene expression. Therefore, we hypothesized that mutations affecting the two genes may lead to similar phenotypes by dys-regulating the expression of common genes. To test this hypothesis we used induced pluripotent stem (iPS) cells derived from fibroblasts of one Rett patient with a *MECP2* mutation (p.Arg306C) and 2 patients with mutations in *CDKL5* (p.Gln347Ter and p.Thr288Ile). Expression profiling was performed in *CDKL5*-mutated cells and genes of interest were confirmed by real-time RT-PCR in both *CDKL5* and *MECP2* mutated cells. The only major change in gene expression common to *MECP2*-and *CDKL5*-mutated cells was for *GRID1*, encoding for glutamate D1 receptor (GluD1), a member of the delta family of ionotropic glutamate receptors. GluD1 does not form AMPA or NMDA - glutamate receptors. It acts like an adhesion molecule by linking the postsynaptic and presynaptic compartments, preferentially inducing the inhibitory presynaptic differentiation of cortical neurons. Our results demonstrate that *GRID1* expression is down-regulated in both *MECP2* and *CDKL5*-mutated iPS cells and up-regulated in neuronal precursors and mature neurons. These data

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Disclosure Statement: The authors declare no conflict of interest.

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provide novel insights into disease pathophysiology and identify possible new targets for therapeutic treatment of Rett syndrome.

Keywords

Rett syndrome; IPSCs; *GRID1*

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Introduction

Rett syndrome (RTT, OMIM#312750) is a monogenic disorder that affects normal brain development during early childhood with an incidence of 1 in 10,000–15,000 live female births [1,2]. Patients with the classic form of RTT show apparently normal development until 6-18 months after birth when clinical signs of a mental regression start to become apparent. These include loss of speech and purposeful hand use, stereotypic hand movements, appearance of postnatal microcephaly, autistic features, ataxia, and hand apraxia [2,3]. Other clinical hallmarks include spasticity, respiratory abnormalities, autonomic dysfunctions [4]. Depending on disease severity some variants such as Z-RTT (previously known as Preserved Speech Variant - PSV), '*forme fruste*', congenital form, and early-onset seizures variant have been described in addition to classic RTT [2,5].

Mutations in *MECP2* gene, located in Xq28, are responsible for ~ 90% of classical RTT cases and a lower percentage of variant patients [6,7]. *MECP2* encodes for a transcriptional regulator and epigenetic modifier. MeCP2 protein is widely expressed and its relatively higher expression in brain is spatially and developmentally regulated: its levels are low during embryogenesis and increase during postnatal neuronal maturation [8,9]. A very small number of MeCP2 target genes have been identified so far and the several transcriptional profiling studies performed on various tissues (brain from mouse models, fibroblasts and lymphocytes derived from mutated patients and autoptic brain tissue) resulted in sets of de-regulated genes that differed for each study [10-12]. Notably, analysis of postmortem brain tissue from patients carrying *MECP2* mutations showed reduction in the mRNA and protein levels of many synaptic markers and a decreased number of dendritic spines in distinct cortical areas [13,14]. A significant alteration of NMDA, AMPA, and GABA receptors was demonstrated in the cortex and basal ganglia of patients, indicating that RTT is associated with abnormalities in the expression of molecules crucial for both excitatory and inhibitory synaptic transmission [15,16].

Mutations in another X-linked gene, *CDKL5* (Cyclin Dependent Kinase Like 5), are responsible for the early-onset seizures variant of RTT [17,18]. Cdkl5 is a ubiquitous protein kinase particularly expressed in the brain [19,20]. It shuttles between the cytoplasm and the nucleus [21]; in neurons, the shuttling is regulated by the activation of extra-synaptic NMDA receptors that induce protein re-localization from nucleus to cytoplasm but also regulate its degradation by the proteasome [22]. Moreover, neuronal Cdkl5 is enriched on the post-synaptic site of excitatory synapses where it seems to be involved in the establishment of correct spine morphology and function [23]. A direct interaction of Cdkl5 with Dnmt1 has been reported, suggesting that it might also be involved in the regulation of DNA

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methylation and thus gene expression [24]. Transcriptome analysis has been recently performed on fibroblasts from *CDKL5* mutant patients revealing alterations in a few genes involved in differentiation and oxidative stress [25].

The overlapping phenotype of *MECP2* and *CDKL5*-mutated patients, the similar expression pattern during postnatal mouse brain development and the interaction of both proteins with Dnmt1 suggest that Cdkl5 and MeCP2 might participate in a common neuronal pathway [24]. Studies investigating the possibility of a direct functional interaction between the two proteins gave conflicting results. Mari et al suggested that: 1, CDKL5 binds and phosphorylates MeCP2 i*n vitro* and *in vi*vo [26]; 2, CDKL5 is able to auto-phosphorylate itself and to phosphorylate MeCP2 *in vitro;* and 3, this capability is lost in mutant MeCP2 proteins [27]. Opposite results were obtained by Lin who showed that MeCP2 binds CDKL5 but is not a direct target of its phosphorylation [20]. More recently, a role for MeCP2 in the regulation of CDKL5 expression levels in brain has been demonstrated [28].

A revolutionary approach for the creation of patient-specific human cellular models for neurologic and neurodevelopmental disorders has been developed in 2007 when Yamanaka and colleagues demonstrated that adult somatic cells can be reverted to induced Pluripotent Stem (iPS) cells, an immature status analogous to embryonic stem cells [29]. iPS cells are similar to human Embryonic Stem Cells (hESC) for gene expression, morphology, proliferation and pluripotency [29,30]. Thanks to the capacity of these cells to differentiate into neurons, many neurodegenerative (Amyotrophic Lateral Sclerosis-ALS, Spinal Muscular Atrophy-SMA, Huntington Disease- HD, Familial Dysautonomia-FD, Parkinson Disease-PD, Alzheimer Disease-AD) and neurodevelopmental diseases (Fragile X Syndrome- FXS, Schizophrenia-SCZD, Prader Willi-Angelman Syndrome-PW-AS) have been modeled with patient-specific iPS [31-33]. This technology has also been employed for the study of RTT. In particular, different groups generated *MECP2*-mutated iPS lines and characterized the resulting neurons reporting morphological and electrophysiological defects corresponding to those found in both mouse brain and patient autoptic material [34-36]. In 2010 we generated iPS lines from two patients with *CDKL5* mutations, one female and one male [37]. For the female patient, isogenic clones expressing either the wild-type or the mutant *CDKL5* allele were obtained. In order to test whether *CDKL5* could be involved in the regulation of gene expression and to define the molecular mechanisms involved in *CDKL5*-associated disease, we now searched for alterations in global gene expression profiles in *CDKL5*- mutant iPS cells. This analysis identified 6 dysregulated genes of which *GRID1* was dysregulated in both *MECP2*-mutated and *CDKL5*-mutated iPS cells.

Methods

See supplemental online data for full methods

iPS clones

Previously characterized fully reprogrammed iPS clones derived from one male and one female *CDKL5*-mutated patient were used [37] The female patient harbors a truncating mutation c.1039C>T (p.(Gln347Ter)) while the male patient has a missense change c. 863C>T (p.(Thr288Ile)). For the female patient, three iPS clones differing for *CDKL5* allelic

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expression were used: clone#20 that exclusively expresses the wild type *CDKL5* allele and clone#46 and clone#33 with exclusive expression of the mutated *CDKL5* allele. For the male patient one clone was used (clone#58) and a clone derived from normal male fibroblasts (BJ) was used as control. One *MECP2* mutant clone c.916C>T (p.(Arg306Cys)) and BJ control clone were obtained from James Ellis laboratory [38]. Mutations numbering is based on the following reference sequences: NM_004992.3 for *MECP2* and NM_003159.2 for *CDKL5*.

Clones maintenance and neuronal differentiation

iPS cells were maintained and differentiated as previously described (see Supplementary data) [37]. At day 18 neural precursor cells (NPCs) were isolated using Anti-PSA-NCAM MicroBeads (30-092-966¸ Miltenyi Biotec) according to the manufacturer's protocol. Sorted cells were fluorescently stained with an Anti-IgM antibody conjugated with PE dye (130-095-908; Miltenyi Biotec) and analysed by flow cytometry using a GUAVA EasyCyte 6-2L instrument to assess purity of the recovered population. Unlabelled cells were used as negative control. Cultures were harvested at day 70 for analysis of mature neurons. To obtain a more pure population of neurons for qRT-PCR, mature cultures were sorted using a CD24 MicroBead Kit (130-095-951, Miltenyi Biotec)[39]. Sorted neurons were plated on poly-ornithine and laminin coated slides and allowed to attach for 48 hours before being stained for β-III-Tubulin (Chemicon) to confirm their identity.

RNA isolation

Total RNA was extracted from iPS clones with RNeasy mini kit (Qiagen). For clones derived from the female patient, the exclusive expression of only one *CDKL5* allele was confirmed as previously reported [37].

Microarray analysis

Agilent Two-Color Microarray-Based gene expression Analysis (Quick Amp Labeling) Protocol was used for global gene expression analysis. For each clone, four technical replicates were performed to control technical bias. Microarray raw data were analysed by Agilent Feature Extraction Software v9.5 for quality assessment. Gene Spring GX software v11.5 was used for data processing and analysis. Data for each mutant/control pair were analyzed independently. Significantly modulated genes were defined as those with absolute fold change (FC) > 1.5. The lists of deregulated genes obtained for the two mutant/control pairs were compared in order to identify common entries. Microarray data are available in the ArrayExpress database (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-2223.

Real Time qRT-PCR

Quantitation was performed using commercial TaqMan probes from Applied Biosystem (*GRID1* assay id: Hs00324946_m1; *LST1* assay id: Hs00938298_g1; *RESP18* assay id: Hs01563187_m1; *ODF4* assay id: Hs00537806_m1). The Cyclophilin (*CYCLO*) gene was used as a reference (assay id: Hs01565700_g1). The Student's *t*-test with a significance level

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of 95% was used for the identification of statistically significant differences in expression levels among different clones.

Immunoblotting

Proteins from neuronal cell pellets were extracted with a tenfold excess of RIPA buffer, cleared with an ultracentrifugation step and analyzed by Western Blot.

Immunoprecipitation

Proteins were immunoprecipitated from mouse brain lysate by incubation with Protein A-covered beads and the specific antibody, before being washed three times and being analyzed in Western Blot[40].

Chromatin immunoprecipitation (ChIP)

Chromatin was prepared from human SHSY5Y neuroblastoma cells as described previously [41]. ChIP isolated chromatin was subjected to quantitative PCR with primers targeting the *GRID1* promoter region (*GRID1*_For: ATGAGCCAGCAAGGTGACTT; *GRID1*_Rev: GGGTGGGTCAGGTTTCACTA). PCR was performed in triplicate. Statistical evaluation was performed using a two tailed t-test, assuming unequal variance.

Results

Microarray analysis of iPS cells

Expression profiling was performed on iPS clones from two patients (one female and one male) with *CDKL5* mutations [37]. For the female patient (*CDKL5* mutation c.1039C>T, p.(Gln347Ter)) we compared one clone expressing the mutant *CDKL5* allele (#46) to another clone from the same patient that expresses the wild-type allele (#20). Maintenance of X chromosome inactivation and the resulting mono-allelic expression of *CDKL5* were confirmed by androgen receptor assay and direct sequencing of *CDKL5* mRNA. The clone derived from the male patient (#58; *CDKL5* mutation c.863C>T, p.(Thr288Ile)) was compared to a clone derived from a normal newborn male (BJ) [38]. For each mutant/control pair four technical replicates were performed for a total of eight chip hybridizations. Differences in gene expression between wild-type and mutant clones for each patient were calculated using a Fold Change (FC) cut-off of 1.5 and a $p<0.05$. From the first comparison (#46 versus #20) we obtained 137 dysregulated genes while for the second comparison (#58 versus BJ) 190 genes with altered expression were identified (Supplementary tables 1 and 2). None of these genes passed the Benjamini and Hochberg correction for FDR, probably due to the very low number of genes with altered expression. We decided, however, to compare the two gene lists to check whether common genes were present and we identified six common genes; four genes were consistently dysregulated in the same direction in both cases, one with decreased levels and the others with increased expression (Table 1). To confirm the dysregulation, real time RT-PCR analysis was performed on an independent preparation of RNA from the same clones. RNA derived from a second clone from the female patient expressing mutant *CDKL5* allele (clone#33) and from an *MECP2*-mutated iPS clone (#15; MeCP2 mutation c.916C>T, p.(Arg306Cys)) were also included. A down regulation (about 50%) of *GRID1* gene was confirmed in the *CDKL5*-mutated clones;

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intriguingly a similar reduction was observed also in the clone with *MECP2* mutation (Figure 1). *GRID1* expression in Clone#20 (expressing wild-type *CDKL5* allele) was comparable to that observed in BJ control (Figure 1), strongly suggesting that the reduction in expression is a specific consequence of *CDKL5* or *MECP2* mutation.

GRID1 expression in neuronal precursor cells and neurons

GRID1 encodes GluD1, a member of the orphan delta glutamate ionotropic receptor family. We thus decided to validate our results on neuronal precursor cells (NPCs) and mature neurons. To reduce the potentially confounding effect of genetic background, for *CDKL5* mutations only the clones derived from the female patient were used. iPSCs were differentiated toward a neuronal fate and NPCs were isolated from differentiating cultures using a MicroBeads-conjugated antibody that recognizes PSA-NCAM [42] (Figure 2A). Flow cytometry analysis on purified NPCs confirmed the efficiency of enrichment (Figure 2B). *GRID1* expression in NPCs appears more variable with significantly different expression between BJ control and clone #20 (Figure 2C). Surprisingly, a significant over-expression was observed in NPCs from both clone#15 (*MECP2* mutation) and clones #46 and #33 (*CDKL5* mutation c.1039C>T, p.(Gln347Ter)). Neurons were isolated from mature cultures using a MicroBeadsconjugated anti-CD24 antibody [39](Figure 2A). Immunofluorescence staining with β-IIITubulin confirmed the neuronal identity of the sorted cells (Supplementary figure). Analysis of mature neurons confirmed *GRID1* overexpression, although the difference did not reach statistical significance for the *MECP2*-mutated clone (Figure 2D). To verify whether the increased mRNA levels were accompanied by alterations in protein levels, GluD1 protein was quantitated in extracts from mature neurons differentiated with the same protocol. Due to the difficulty of isolating sufficient amounts of protein from sorted neurons, the analysis was performed on non-sorted cultures. The analysis confirmed a significant increase in protein levels in extracts obtained from both *CDKL5*-mutated and *MECP2*-mutated clones (Figure 3).

Considering the role of MeCP2 as a transcriptional regulator, we wondered whether the alteration in *GRID1* expression in the *MECP2*-mutant clone might indicate that MeCP2 protein directly binds to the *GRID1* promoter to regulate its expression. To answer this question, chromatin-immunoprecipitation (ChIP) experiments with an anti-MeCP2 antibody were performed in SHSY-5Y neurons. The analysis indeed demonstrated a modest but statistically significant binding of MeCP2 protein to the *GRID1* promoter (Figure 4).

Discussion

Rett syndrome is a monogenic disease due to de novo mutations in either *MECP2* (classic and Zappella variant) or *CDKL5* (early-onset seizures variant) genes on the X chromosome. The overlapping phenotype observed in patients with mutations in the two genes suggests that they might act, at least in part, on common pathways. MeCP2 is a transcriptional regulator involved in both activation and repression of gene expression [43]. *CDKL5* encodes for a kinase protein that exerts multiple functions inside the cell [23,24,44,45]. It directly interacts with Dnmt1 and mediates its phosphorylation and it is involved in the structural organization and dynamics of nuclear speckles, suggesting that, like MeCP2, it might be

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implicated in the regulation of gene expression [24,45]. We thus hypothesized that mutations in the two genes may lead to similar phenotypes by dysregulating the expression of common genes. To test this hypothesis we used iPS cells derived from patients mutated in *MECP2* and *CDKL5*. Expression profiling in *CDKL5*-mutated iPS cells from two patients with different mutations resulted in six common genes and a larger number of clone-specific alterations. None of these genes is present among those recently reported by Nectoux and colleagues in fibroblasts from three patients with frameshift *CDKL5* mutations [46]. This is not an unexpected finding, considering the different cell types analyzed (iPS versus fibroblasts). Moreover, very limited overlap is usually present among dysregulated genes resulting from different expression profiling analyses, as widely documented from studies on *MECP2* [11,14,47]. Validation of the six common genes demonstrated that *GRID1* was consistently down-regulated in both *CDKL5*-mutated and *MECP2*-mutated iPS cells (Figure 1). Analysis on neuronal precursors derived from these clones demonstrated that the absence of a functional MeCP2 or CDKL5 protein resulted in a significant up-regulation of *GRID1* mRNA in these cells (Figure 2). The up-regulation persists in mature neurons.

GRID1 encodes the orphan delta glutamate receptor subunit-1 (GluD1) and, together with *GRID2*, constitutes the delta family of ionotropic glutamate receptors [48]. However, no channel function as been presently demonstrated either alone or in combination with other receptor subunits and available data point to a role in synapse development rather than functioning [49-51]. In particular, the postsynaptic GluD1 binds to presynaptic Neurexin through Cerebellin (Cbln) to induce pre-synaptic differentiation. Depending on the specific brain region, this can result in the formation of glutamatergic (cerebellum and hippocampus) or GABAergic (cortex and hippocampus) synapses, although GABAergic differentiation seems to be the prevalent outcome [51-53]. Our experiments show that GluD1 is over-expressed in mature neurons derived from both *MECP2*- and *CDKL5*-mutated iPS. ChIP analysis in SHSY-5Y neurons indicate that MeCP2 binds to the *GRID1* promoter, suggesting that the increased expression might arise from the absence of a direct repression by MeCP2. In *Mecp2* mutant mice a significant reduction in dendritic spine number has been reported, suggesting decreased excitatory synapse capacity [54,55]. Accordingly, *MECP2* null neurons have a significant reduction in VGLUT/PSD-95 puncta and Excitatory Post-synaptic Currents (EPSC) amplitude, whereas MeCP2 over-expression results in the opposite effects, suggesting that MeCP2 might regulate the excitation/inhibition balance in the CNS by controlling the number of excitatory synapses [56,57]. A similar impairment has been reported in both *CDKL5*-silenced mouse hippocampal neurons and patient iPS-derived neurons [23]. Considering the ability of GluD1 to induce the maturation of GABAergic synapses in telencephalic neurons, it can be hypothesized that its overexpression in the absence of both MeCP2 and CDKL5 might contribute to the reduction in excitatory synapses number by causing a shift toward differentiation of inhibitory synapses. Although no such alteration has been evidenced in the above mentioned studies, analyses on *Mecp2* mouse models have demonstrated extensive region-specific variation in synaptic defects [58,59,60,51]. Additional studies will thus be necessary to verify whether an increased GABAergic differentiation is indeed present in our culture conditions and to possibly characterize the identity of the neuronal population involved.

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Although available data point to a role for *GRID1* in neuronal maturation, we already detected alteration of *GRID1* expression neuronal precursor cells. No data are presently available on which could be the function of GluD1 in these cells. However, both Glutamate and GABA ionotropic receptor subunits and functional receptor channels are expressed very early during brain development in proliferating neuroepitelial cells and are considered important for events such as precursors proliferation, migration, differentiation and survival [61-63]. It is thus possible that an alteration of GluD1 levels in precursor cells might influence one of these processes and result in alteration of subsequent brain development.

Apart from the six common dysregulated genes, independent analysis of expression profiling results for the two *CDKL5*-mutated clones identified more than 300 clone-specific genes. The easiest explanation for this very limited overlap is the presence of different *CDKL5* mutations that might have different consequences on protein functionality: the female patient has a truncating mutation (p.Gln347Ter) that leads to a protein lacking the domains responsible for the interaction with MeCP2 and DNMT1 and the nuclear export signal while the male patient has a missense change (p.Thr288Ile) in an highly conserved amino acid inside the catalytic kinase domain that might result in a protein with altered kinase activity [37]. This scenario is consistent with the phenotypic differences observed between the two patients. In fact, the female patient has been diagnosed as having an early-onset seizures variant of RTT, while the male has X-linked epileptic encephalopathy. While we are conscious that comparing cells from patients with different phenotypes might reduce the number of common genes identified, we selected this strategy since it might lead to the identification of those genes that represent the basal targets of *CDKL5*. In support of our hypothesis, a similar mutation-specific effect has been reported also from Nectoux and colleagues in *CDKL5*-mutated fibroblasts [46]. Finally, we cannot exclude that some of the clone-specific alterations might be a non-specific consequence of the integration site of reprogramming virus rather than a consequence of *CDKL5* absence.

The finding that GluD1 expression is altered in the presence of a mutation in *MECP2* or *CDKL5* appears particularly intriguing since mutations in GluD1 pre-synaptic partner are associated to Pitt-Hopkins-like syndrome 1 (OMIM#610042), that shares phenotypic features with RTT [64,65]. The other gene mutated in this syndrome is *CNTNAP2* (also known as *NRXN4*), another member of Neurexin gene family. Data recently obtained from our group indicate that *CNTNAP2* might be involved in the modulation of the phenotype arising from mutations in *MECP2* [66]. In light of these results, it seems likely that all these genes act on the regulation of common processes and thus the alteration of one or more of them result in overlapping phenotypic outcomes. If this is the case, we would expect that alterations in *GRID1* might also cause a neurological phenotype. Indeed, linkage and association studies have established *GRID1* as a strong candidate gene for Schizophrenia, major depressive disorder and Autism Spectrum Disorders (ASD) [67-70]. Moreover, *GRID1* null mice present aberrant social and emotional behaviours, further stressing the importance of this gene for normal cognition [71].

In conclusion, our results demonstrate that *GRID1* gene expression is down-regulated in both *MECP2* and *CDKL5*-mutant iPS cells and up-regulated in neuronal precursors and mature neurons. Our data provide the first potential functional link between *MECP2* and

CDKL5 genes. These data provide novel insights into disease pathophysiology and identify possible new targets for therapeutic treatments of Rett syndrome.

Supplementary Material

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Acknowledgements

We are grateful to A. Bartolini for help with culture maintenance and differentiation. The work was partially funded by Telethon grant (GGP11110A), by Italian Health Ministry “Ricerca finalizzata 2008” (RF-TOS-2008_1225570) and “Ricerca finalizzata 2010” (RF-2010-2317597) grants and by and AIRETT (Associazione Italiana Rett) 2011 grant to AR. The “Cell lines and DNA bank of Rett Syndrome, X-linked mental retardation and other genetic diseases”, member of the Telethon Network of Genetic Biobanks (project no. GTB12001), funded by Telethon Italy, and of the EuroBioBank network, provided us with specimens.

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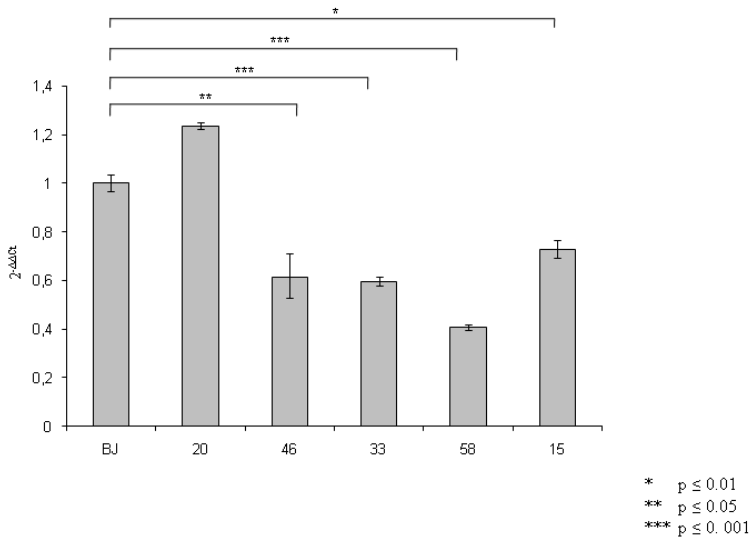

Figure 1.

Real time RT-PCR analysis of *GRID1* mRNA levels in iPS clones. *CDKL5* (#33, #46, #58) and *MECP2* (#15) mutated clones were analysed. BJ is a control clone derived from a healthy newborn male and #20 is a clone harbouring the *CDKL5* mutation in one X chromosome but expressing the wild type allele from the other X chromosome due to X chromosome inactivation. The y axis shows$2^{-\Delta\Delta Ct}$ values (+/− standard deviation). * p<0.01; **p<0.05; ***p<0.001

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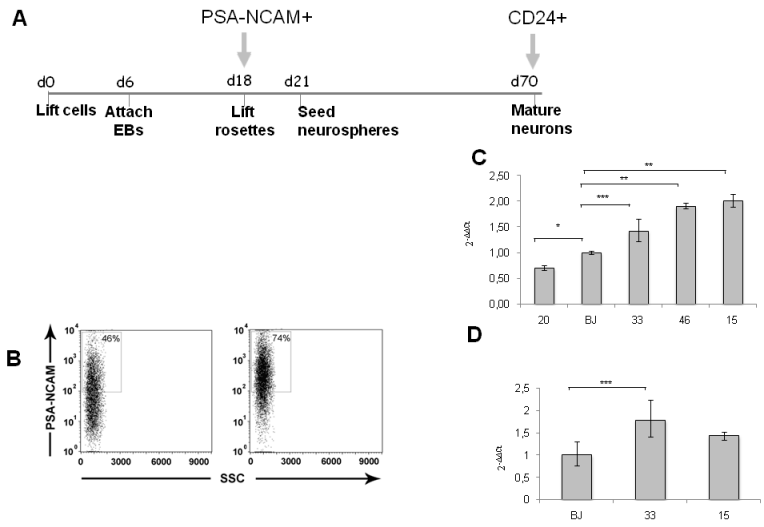

Figure 2.

(**A**) Overview of the neuronal differentiation protocol showing the timing of specific passages expressed in days respect to day 0 defined as the first day of the protocol. The red arrows indicate the day at which neuronal precursors (PSA-NCAM+) or neurons (CD24+) were sorted for expression analysis. (**B**) FACS analysis of differentiating cultures before sorting (left) and after sorting with anti-PSA-NCAM micro-beads (right) showing a significant enrichment in positive cells. The x axis shows SSC (Side Scatter), the y axis shows fluorescence intensity. (**C**) *GRID1* expression analysis in PSANCAM^{+} NPCs (day 18) mRNA derived from neuronal differentiation of *CDKL5* (#20, #46 and #33) and *MECP2* (#15) mutated clones (for clone identification see Fig. 1 legend). (**D**) Expression analysis in mature neurons at day 70 of differentiation protocol sorted with anti-CD24 micro-beads. The y axis in (C) and (D) shows $2^{-\Delta\Delta Ct}$ values (+/-standard deviation). * p<0.01; **p<0.0001; ***p<0.05

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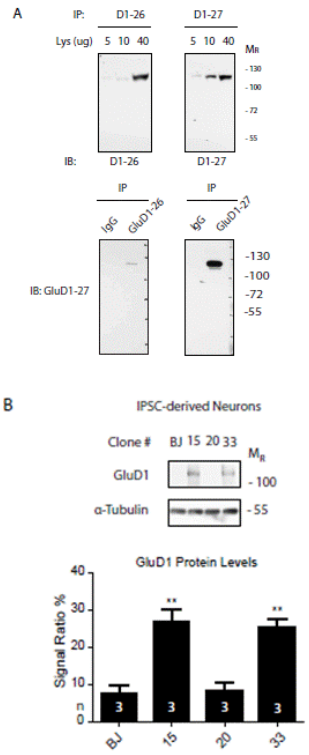

Figure 3.

(**A**). Anti-GluD1 antibody characterization. Different amounts of mouse brain lysate as well as immunoprecipitates (with mouse non-specific IgG as control) were loaded and probed for two different antibody batches (#26 and #27) to double-proof the identity of the recognized epitope. (**B**). GluD1 expression in iPSC-derived neurons. Each lane was loaded with 30 µg of total protein. Experiments were performed in triplicate (n=3). Immunosignals were detected using autoradiographic films, Glutamate-Delta-1 receptor levels were quantified using Photoshop CS3 software and normalized against alpha-tubulin levels (**p<0,01, student t test).

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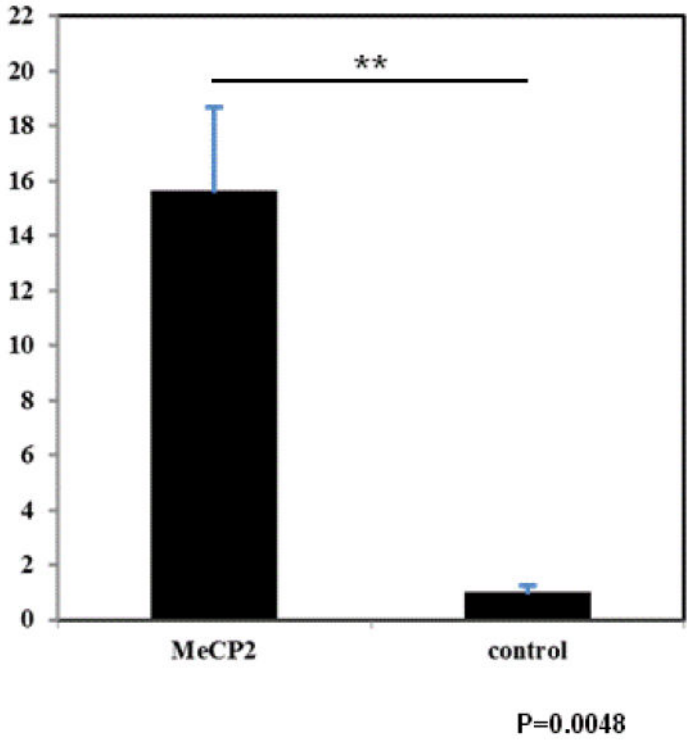

Figure 4.

Chromatin immunoprecipitation with anti-MeCP2 and control antibodies shows MeCP2 binding to the *GRID1* promoter in SHSY-5Y neuroblastoma cells. The graph shows fold enrichment of target DNA isolated by ChIP using MeCP2 NH2-antibody normalized to a control pre-immune antibody. Error bars show the standard error of the mean (SEM). P=0.0048 by two tailed t-test assuming unequal variance.

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Table 1

Common deregulated genes

Gene symbol	Gene Name	Accession number	Fold Change #58/BJ	Fold Change #46/#20
GRID1	glutamate receptor, ionotropic, delta 1	NM_017551	−2,1036077	−1,6889609
LST1	leukocyte specific transcript 1	NM_007161	3,537233	1,5218107
SLC23A3	solute carrier family 23 (nucleobase transporters), member 3	AK055730	2,0275977	3,1108685
RESP18	regulated endocrine-specific protein 18	NM_001007089	2,1038992	2,5666316
HLA-DQA2*	major histocompatibility complex, class II, DQ alpha 2	NM_020056	−1,5860842	1,5345135
GUSBP3*	glucuronidase, beta pseudogene 3	NR_027386	−2,231126	1,6598059

*these genes have been excluded from RT-PCR validation due to the opposite values of fold change observed in the two sample pairs.