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ORIGINAL INVESTIGATION

Transcriptome proÞle reveals AMPA receptor dysfunction in the hippocampus of theRsk2-knockout mice, an animal model of CofÞnÐLowry syndrome

Tahir Mehmood € Anne Schneider € Jérémie Sibillec € Patricia Marques Pereira €
Solange Pannetier € Mohamed Raafet Ammar € Doulaye Dembele €
Christelle Thibault-Carpentier € Nathalie Rouach € André Hanauer

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Abstract CofÞnÐLowry syndrome (CLS) is a syndromic form of mental retardation caused by loss of function mutations in the X-linkedRPS6KA3gene, which encodes RSK2, a serine/threonine kinase acting in the MAPK/ERK pathway. The mouse invalidated for theRps6ka3(Rsk2 KO) gene displays learning and long-term spatial memory deÞcits. In the current study, we compared hippocampal gene expression proÞles fromRsk2KO and normal littermate mice to identify changes in molecular pathways. Differential expression was observed for 100 genes encoding proteins acting in various biological pathways, including cell growth and proliferation, cell death and higher brain function. The twofold up-regulated gene (Gria2) was of particular interest because it encodes the subunit GLUR2 of the AMPA glutamate receptor. AMPA receptors mediate most fast excitatory synaptic transmission in the central nervous system. We provide evidence that in the hippocampus ofRsk2KO mice, expression of GLUR2 at the mRNA and at the protein levels is signiÞcantly increased, whereas basal AMPA receptor-mediated transmission in the hippocampus ofRsk2KO mice is significantly decreased. This is the Þrst time that such deregulations have been demonstrated in the mouse model of the CofÞnÐLowry syndrome. Our Þndings suggest that a defect in AMPA neurotransmission and plasticity contribute to mental retardation in CLS patients.

T. Mehmood and A. Schneider contributed equally to this work.

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T. Mehmood · A. Schneider · P. Marques Pereira · S. Pannetier · M. R. Ammar · A. Hanauer (✉)
Department of Translational Medicine and Neurogenetics, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Institut National de Santé et de Recherche Médicale (INSERM) U964/Centre National de Recherche Scientifique (CNRS) UMR 7104/Université de Strasbourg, 67404 Illkirch, France
e-mail: Andre.HANAUER@igbmc.fr

T. Mehmood
Department of Chemistry, University of Sargodha, Sargodha 40100, Pakistan

D. Dembele · C. Thibault-Carpentier
Microarray and Sequencing Platform, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Institut National de Santé et de Recherche Médicale (INSERM) U964/Centre National de Recherche Scientifique (CNRS) UMR 7104/Université de Strasbourg, 67404 Illkirch, France

J. Sibillec · N. Rouach
Collège de France, 75005 Paris, France

Introduction

CofÞnÐLowry syndrome (CLS; MIM#303600) is a rare syndromic form of mental retardation that is characterized by moderate to severe psychomotor retardation, growth retardation, facial and digital dysmorphisms, as well as progressive skeletal deformations (Hanauer and Young 2002). The gene mutated in CLS patients (*RPS6KA3*) encodes a protein of 740 amino acids, RSK2 (alternative names: $p90^{RSK2}$, MAPKAPK1B), which belongs to a family of four highly homologous proteins (RSK1Ð4), encoded by distinct genes. RSKs are Ser/Thr protein kinases that act at the distal end of the mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK) signaling pathway. RSKs are directly phosphorylated and activated by ERK1/2 in response to many growth factors and neurotransmitters (Frodin and

Gammeltoft 1999). RSK2 phosphorylates a wide range of cytosolic substrates, such as GSK3 and IκBα, and nuclear substrates including ATF4, c-FOS and NUR77, CREB and histone H3 (De Cesare et al. 1998; Sassone-Corsi et al. 1999). Activation of RSK2 is, therefore, thought to influence gene expression and to be involved in cell proliferation and survival. Numerous studies implicate the MAPK/ERK signaling cascade and CREB-mediated gene transcription in synaptic plasticity and memory (Davis and Laroche 2006). In human and mouse brain, RSK2 is highly expressed in the hippocampus, that is, an essential brain structure in cognitive function and learning (Zeniou et al. 2002; Guimiot et al. 2004). RSK2-deficient mice show delayed acquisition of a spatial memory reference task and long-term spatial memory deficits (Poirier et al. 2007). Thus, together the data suggest that RSK2 plays an important role in cognitive function in human and in mice.

To gain greater insight into the molecular mechanisms leading to learning and memory impairments in Rsk2 KO mice and to mental retardation in CLS, we examined in the present study global gene expression profiles in hippocampus from KO mice. The data revealed significant alteration of 100 genes acting in a great variety of biological pathway in Rsk2KO hippocampi. We further investigated the function of one of these genes, Gria2, which showed a twofold up-regulation in mutant mice. Gria2 encodes the subunit GLUR2 of the AMPA receptor (AMPAR). AMPARs are ligand-activated cation channels that mediate the fast component of excitatory postsynaptic currents in neurons of the central nervous system. The GLUR2 subunit controls AMPAR Ca^{++} permeability, and is involved in several forms of long-term synaptic plasticity. Our results show that the expression of GLUR2 is increased at the mRNA and at the protein level in the hippocampus, as well as at the surface of synapses in hippocampal primary cell cultures. Furthermore, basal excitatory synaptic transmission through AMPARs is impaired in the hippocampus of Rsk2mutants.

Materials and methods

Animals and tissue dissection

Male Rsk2KO and WT mice with a C57Bl/6x genetic background were killed by cervical dislocation. Brains were rapidly dissected and the hippocampus was isolated using a standard dissection procedure. Tissue samples were immediately frozen in liquid nitrogen and kept at −80 C until use. All experiments were carried out in accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC). Every effort was made to minimize the number of animals used and their suffering.

Microarray hybridization

To reduce variability, but also to obtain enough RNA for the hybridization of each DNA chip, all probes for the gene array experiments consisted of pooled RNA samples from either two WT or two KO animals. In brief, total RNA was extracted from hippocampi of six KO and six WT 5-month-old male mice and purified using the TRIzol reagent (Invitrogen, Cergy Pontoise, France) according to the manufacturerÕs instructions. The quality of total RNA was monitored by Agilent 2100 Bioanalyzer (LabChip, Agilent technologies, Massy, France). Two RNA samples for each genotype were then pooled in equal quantities (thus, resulting in a total of three arrays for each genotype). Generation of double-stranded cDNA from 2.5 μg of total RNA of each pooled RNA sample, preparation and labeling of cRNA, hybridization to 430A 2.0 mouse genome arrays (Affymetrix, Santa Clara, CA), washing, and scanning were performed according to the protocols recommended by Affymetrix in their GeneChip Expression Analysis Technical Manual (Affymetrix). The data of the expression arrays produced for this report have been deposited in the Gene Expression Omnibus (GEO) databank.

Microarray data analysis

Data were processed using the Affymetrix GeneChip Operating Software [GCOS v1.4; Microarray Suite (MAS 5.0) algorithm]. Genes differentially expressed were selected using the following steps: (1) selection of probesets having a signal value above 15 (35th percentile of all expression values) in at least one array out the 6, (2) selection of probesets called present in at least two out of three arrays for one of the two genotypes, (3) selection of probesets with a t test $p < 0.03$, (4) selection of probesets having a fold change greater than 1.5. We finally verified that selected probes have acceptable false discovery rate (10%) (Benjamini and Hochberg 1995).

Ingenuity pathways analysis

Biologically relevant networks were created using the ingenuity pathways analysis program (http://www.Ingenuity.com), using the default parameters. Based on the algorithmically generated connectivity between gene–gene, gene–protein, and protein–protein interactions, the program develops functional molecular networks that overlay genes in the dataset. This program calculates a p value for each network by comparing the number of focus genes that were mapped in a given network, relative to the total number of occurrences of those genes in all networks. The score for each network is shown as the negative log of the p value, which indicates the likelihood of finding a set of genes in the

network by random chance. A score of 20 indicates that there is a 10^{-20} chance that the focus genes would be in a network because of random chance.

Real-time QRT-PCR analysis

QRT-PCR assays were performed on hippocampal RNA samples obtained from WT and KO mice different from those used for transcriptional proÞling. RNA extraction and QRT-PCR was performed as previously described (Marques Pereira et al. 2008). A probe set for detection of mouse *Gapdh* was used as an endogenous control gene. The sequences of primers of the tested genes are listed in Supplemental Table 1.

Western blot analysis

Protein extractions and Western blot analyses were performed as previously described (Marques Pereira et al. 2008). QuantiÞcations were carried out with the GeneTools software of the Chemigenius apparatus (Syngene, Frederick, MD, USA). Data were normalized either to GAPDH or to β-TUBULIN. StudentÕs *t* test (two-tailed) was used to determine the signiÞcance between the control and *Rsk2*-KO samples, and $p \leq 0.05$ was considered signiÞcant. Antibodies against GLUR2 (Millipore Corporation), CACNG8 and VAMP4 (Abcam), EIF3A (Cell Signaling Technology), DIABLO (Calbiochem), GAPDH (Chemicon) and β TUBULIN (Millipore) were used.

Immunohistochemistry

Frozen brain section was left 10 min at room temperature, Þxed for 10 min with 4% PFA and washed twice ×1 PBS). Endogenous peroxidase was inhibited by a treatment with 0.3% H_2O_2 solution. After washing, slides were incubated in 10% normal goat serum in ×1 PBS for 1 h. Primary antibodies were added to the sections in 10% normal goat serum and incubated overnight at 4°C. Antibody dilutions were as follows: rabbit anti-GLUR2 (1:1000, Millipore Corporation), rabbit anti-VAMP4 (1:1,000, Abcam), and rabbit anti-IGF-1 (1:100, Abcam). Slides were subsequently washed four times for 10 min in ×1 PBS and incubated with biotinylated secondary antibody for 2 h. After washing, sections were incubated for 30 min in Vectastain elite ABC reagent and treated with peroxidase substrate solution until desired stain intensity. After washing, samples were mounted with KAISERÕs glycerol gelatin (Merck).

In situ hybridization

Plasmids containing 3′UTR regions of mouse *Gria1* (encoding GLUR1) or *Gria2* (encoding GLUR2) cDNAs were ampliÞed by PCR using vector-speciÞc primers and PCR reactions were puriÞed using Montage 96 (Millipore Corporation, Bedford, USA). Amplicons were then used as template for in vitro transcription of sense and anti-sense Dig-labeled riboprobes. To this aim, 1 µg linearized DNA was transcribed using T7, T3 or Sp6 polymerases and the ×10 DIG RNA labeling mix (Roche Diagnostics, Meylan, France) according to the manufacturerÕs instructions. Brain sections (25-µm thick were processed for ISH using GenePaint robotic equipment and procedures (http://www.genepaint.org) as previously described (Nakamoto et al. 2007).

Primary hippocampal cultures

Hippocampi dissected from WT and *Rsk2*-KO male mice at embryonic day 17 were triturated and plated into wells of 24-well plates containing poly-L-lysine-coated coverslips (Sigma), at a density of ~ 1,00,000 neurons/well. Growth media consisted of NeuroBasal (GIBCO, Invitrogen) supplemented with 1× B27 (GIBCO, Invitrogen), 0.5 mM L-glutamine and 1× penicillin/streptomycin. The cultures were maintained at 37°C in a humidiÞed atmosphere containing 5% CO_2 and cultivated for 14 DIV prior to experimentation.

Immunocytochemistry

To label surface GLUR1 (sGLUR1) and GLUR2 (sGLUR2)-containing AMPARs, 14 DIV live neurons were treated as previously described (Ghate et al. 2007) with minor modiÞcations. Cells were incubated with rabbit anti-N-terminal GLUR1 (Calbiochem) or mouse anti-N-terminal GLUR2 (Millipore) and mouse anti-PSD95 (NeuroMab).

Microscopy and data analysis

All images acquisitions and quantiÞcations were performed using standardized settings on a microscope (model DM4000 B, Leica) equipped with CCD camera (CoolSnap CF, color) with a ×63 objective. Obtained Tiff Þles were subjected to quantiÞcation with ImageJ software (http://www.rsb.info.nih.gov/ij/). For sGLURs quantiÞcation, the three thickest dendrites per pyramidal neuron and Þve neurons per sample were blindly chosen and the dendritic branches were manually traced and measured. AMPA receptors clusters were counted and the number of clusters was normalized with the dendritic length. StudentÕs *t* tests were used for comparison between WT and *Rsk2*-KO cultures.

Determination of relative *Gria2* Q/R and R/G editing, and ßip/ßop splice levels

Total RNA was extracted (as above) from Þve *Rsk2*-KO and Þve WT hippocampi, the *Gria2* mRNA ampliÞed by

RT–PCR (three times each from independent RNA preparations) and the product sequenced to determine the relative levels of editing and splicing (Lee et al. 1998).

Electrophysiology

Standard techniques were used to prepare transverse acute hippocampal slices (400 μm thick) from 4-week-old mice. Slices were maintained at room temperature in a storage chamber that was perfused with an artificial cerebrospinal fluid (ACSF) (mM: 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1 NaH_2PO_4, 26 $NaHCO_3$ and 11 glucose and equilibrated with 95% O_2 and 5% CO_2) for at least 1 h prior to recording. For synaptic recordings, a cut was made between the CA3 and CA1 region to prevent bursting, and the slices were bathed in a modified ACSF containing 100-μm picrotoxin to block GABA$_A$receptor- mediated inhibitory postsynaptic currents. Field excitatory postsynaptic potentials (fEPSPs) were recorded with glass pipettes (2–5 MΩ) filled with ACSF, by stimulating Schaffer collaterals in stratum radiatum (0.1 Hz) with a monopolar stimulating electrode. Responses were collected with Axopatch-1D amplifier (Axon Instruments), filtered at 2 kHz, digitized at 10 kHz, and analyzed online using ClampFit software (Molecular Devices).

Results

Expression profiling of wild-type and Rsk2KO mice

To identify molecular changes potentially responsible for the phenotype associated with RSPS6KA3 gene mutation in the hippocampus, we performed a detailed comparison of the transcriptional profiles of hippocampi isolated from six KO and six WT 5-month-old male mice. To reduce variability, equivalent amounts of RNA from two mice with the same genotype were pooled and processed for hybridization to the genome wide oligonucleotide microarray (thus, three arrays per genotype). Out of the 22,690 probesets represented on the microarray, filter A selected 16,865 probesets that were restricted to 14,348 probesets by filter b and to 635 probesets by filter c. Filter d, selected a final number of 109 probesets. Eight of these 109 differentially expressed genes were verified by two or, in one case, three distinct probe sets. These multiple probe sets of eight genes displayed consistent direction and similar extent of changes in abundances of corresponding mRNAs. The final list of 100 significant non-redundant genes is shown in Table 1. Genes are tabulated according to functional category and degree of over-expression/repression.

Most of them have a recognized function and can be assigned to functional categories and subcategories. Among them, 75 genes were transcriptionally up-regulated, whereas 25 genes were down-regulated. The most upregulated genes were thymosin beta 10 (Tmsb10, fold change of 3.51), followed by establishment of cohesion 1 homolog A (Esco 1, fold change of 2.91) and thyroid hormone receptor interactor 11 (Trip 11, fold change of 2.27). The most downregulated genes were erythroid differentiation regulator 1 (Erdr1) and serpin peptidase inhibitor, clade C, member 1 (Serpin1) (−2.9 and −2.4 fold downregulations, respectively). Major functional categories include enzymes with various activities (17 up-regulated/2 down-regulated), growth factors (2/0), ion channels (3/0), kinases (1/3), peptidases (2/1), transcription regulators (6/1), transmembrane receptors (1/0), transporters (7/2) and molecules with other functions (33/14).

Validation of microarray data by QRT-PCR

Twenty-four of these differentially expressed genes were selected for validation, by QRT-PCR, based on the known or putative neuronal functional roles (Table 1). These genes represent different categories: genes implicated in exocytosis (Stxbp3 and Vamp4), in mental retardation (Cul4b, Lamp2, Vldlr, Igf1), in apoptosis (Stk3, Rasl10a, Diablo), in cell differentiation and cytoskeleton organization (Phkg1, Pdlim5, Timsb10, Enc1, Nptxr, Ptpn2, Carhsp1, Phip, Plek, Arhgap12, Cfl1), in translation regulation (Eif3A) and finally genes encoding ion channel sub-units (Cacnb4, Cacng8, Gria2). The results of the microarray findings were validated in all the genes tested by real-time PCR, although the fold change was not always accurately replicated. We also confirmed by QRT-PCR unaltered expression of some genes (including cFos, CREB) that had similar levels of expression in KO and WT animals on microarrays (results not shown).

Identification of biologically relevant networks

To gain insight into interactions among the differentially expressed genes, we constructed biologically relevant networks using the ingenuity pathway analysis software. From the 100 differentially expressed genes, 78 genes were mapped and assembled into five biological networks with a score of ≥20. The network with the most significant score (of 45) contains 23 of the differentially expressed genes. This network contains genes involved in cell cycle, cellular development, growth and proliferation and centers on the NFκB complex. This network contains also Rb1 and Sod2, both important actors in cellular growth and apoptosis, and over expressed in Rsk2KO neurons. The network with the second highest score (of 34) is centered on TGFβ1, which controls proliferation, cellular differentiation, but also various other functions. Fourteen genes with altered

Table 1 List of genes differentially expressed in the hippocampus of Rsk2-KO mice

Functional category	Probe set	Genbank	Gene symbol	Gene name	Microarray		qRT-PCR			
					FC KO/WT	p	WT mean Ct ± SD	KO mean Ct ± SD	FC	p
Genes showing decreased expression										
Kinases	1422315_x_at	NM_011079	Phkg1	Phosphorylase kinase gamma 1	0.54	0.0162	± 0.03	0.68 ± 0.11	0.68	0.003
	1418052_at	NM_023556	Mvk	Mevalonate kinase	0.55	0.0190				
	1427282_a_at	NM_008044	Fxn	Frataxin	0.61	0.0074				
Enzymes	1448330_at	NM_010358	Gstm5	Glutathione S-transferase M5	0.61	0.023				
	1427975_at	NM_145216	Rasl10a	Ras-like, family 10, member A	0.62	0.015	± 0.05	0.70 ± 0.059	0.70	0.004
Peptidase	1433918_at	NM_034941	Atg4d		0.59	0.0057				
Cytokine	1418345_at	NM_023517	Tnfsf13	Tumor necrosis factor (ligand) superfamily, member 13	0.60	0.0057				
Transcription regulator	1418636_at	NM_001083318	Etv3	Ets variant gene 3	0.55	0.026				
Transporters	1423927_at	NM_028662	Slc35b2	Solute carrier family 35, member B2	0.59	0.011				
	1450073_at	NM_008444	Kif3b	Kinesin family, member 3B	0.64	0.00093				
Transmembrane receptor	1450147_at	NM_030689	Nptxr	Neuronal pentraxin receptor	0.62	0.013	± 0.008	0.87 ± 0.35	0.87	0.035
Other functions	1452406_x_at	NM_133362	Erdr1	Erythroid differentiation regulator 1	0.35	0.029				
	1417909_at	NM_080844	Serpinc1	Serpin peptidase inhibitor, clade C, member 1	0.41	0.023				
	1415976_a_at	NM_025821	Carhsp1	Calcium regulated head stable protein 1,24 kDa	0.50	0.00036	± 0.07	0.63 ± 0.07	0.63	0.034
	1452652_a_at	NM_001002267	Tmem158	Transmembrane protein 158	0.55	0.015				
	1448577_x_at	NM_009304	Syngr2	Synaptogyrin 2	0.56	0.021				
	1455098_a_at	NM_011707	Vtn	Vitronectin	0.57	0.0022				
	1453111_a_at	NM_026542	Slc25a39	Solute carrier family 25, member 39	0.61	0.011				
	1418396_at	NM_134116	Gpsm3	G-protein signaling modulator 3	0.63	0.016				
	1423940_at	NM_026553	Yif1A	Yip1 interacting factor homolog A (*S. cerevisiae*)	0.63	0.016				
	1418123_at	NM_011676	Unc119	Unc-119 homolog (*C. elegans*)	0.64	0.0016				
	1450468_at	NM_010865	Myoc	Myocilin	0.64	0.0078				
	1415886_at	NM_013781	Sh2d3c	SH2 domain containing 3C	0.64	0.001				
	1422596_at	NM_021426	Nkain4	Na+/K+ transporting ATPase interacting 4	0.65	0.022				
	1450255_at	NM_020260	Cdgap	Cdc42 GTPase-activating protein	0.65	0.009				
Genes showing increased expression										
Kinases	1418513_at	NM_019635	Stk3	Serine/threonine kinase 3	1.76	0.023	± 0.05	1.45 ± 0.08	1.45	0.042
Phosphatase	1438562_a_at	NM_001127177	Ptpn2	Protein tyrosine phosphatase, non-receptor type 2	1.95	0.016	± 0.091	1.85 ± 0.28	1.83	0.045
Growth factors	1437401_at	NM_010512	Igf1	Insulin-like growth factor 1	1.65	0.0093	± 0.09	2.24 ± 0.32	2.24	0.042
	1417069_a_at	NM_022023	Gmfb	Maturation factor, beta	1.54	0.0055				

Table 1 continued

Functional category	Probe set	Genbank	Gene symbol	Gene name	Microarray		qRT-PCR			
					FC KO/WT	p	WT mean Ct ± SD	KO mean Ct ± SD	FC	p
Enzymes	1448734_at	NM_001042611	Cp	Ceruloplasmin	2.29	0.029				
	1417262_at	NM_011198	Ptgs2	Prostaglandin-endoperoxidase synthase 2	2.28	0.0047				
	1452484_at	NM_053070	Ca7	Carbonic anhydrase VII	2.23	0.028				
	1417194_at	NM_013671	Sod2	Superoxide dismutase 2	1.98	0.0089				
	1452158_at	NM_029735	Eprs	Glutamyl-prolyl-tRNA synthetase	1.95	0.015				
	1453928_a_at	NM_009278	Ssb	Sjogren syndrome antigen B	1.86	0.020				
	1451436_at	NM_001081203	Sbno1	sno, strawberry notch homolog 1 (Drosophila)	1.78	0.022				
	1454696_at	NM_008142	Gnb1	Guanine nucleotide binding protein, beta polypeptide 1	1.70	0.0016	± 0.1033	1.36 ± 0.061	1.36	0.0005
	1419064_a_at	NM_011674	Ugt8	UDP-glycosyltransferase 8	1.69	0.0059				
	1416497_at	NM_009787	Pdia4	Protein disulfide isomerase family A, member 4	1.68	0.0061				
	1423033_at	NM_008408	Stt3a	Subunit of the oligosacchayltransferase complex, homolog A	1.66	0.028				
	1435164_s_at	NM_011666	Ube1c	Ubiquitin-activating enzyme E1C	1.65	0.0062				
	145388_at	NM_183028	Pcmtd1	protein-L-isoaspartate O-methyltransferase domain containing 1	1.60	0.026				
	1416343_a_at	NM_010685	Lamp2	Lysosomal-associated membrane protein 2	1.58	0.023	± 0.105	1.65 ± 0.19	1.65	0.038
	1418908_at	NM_013626	Pam	Peptidylglycine alpha-amidating monooxygenase	1.56	0.021				
	1451828_a_at	NM_207625	Acsl4	Acyl-CoA synthetase long chain family member 4	1.54	0.0035				
	1417697_at	NM_009230	Soat1	Sterol O-acyltransferase	1.51	0.015				
Peptidase	1420964_at	NM_007930	Enc1	Ectodermal-neural cortex	2.07	0.015	± 0.05	1.77 ± 0.08	1.77	0.002
	1449718_s_at	NM_026273	C3orf38	Chromosome 3 open reading frame 38 homolog (human)	1.54	0.019				
Transcription regulators	1427406_at	NM_028446	Trip11	Thyroid hormone receptor interactor 11	2.27	0.0045				
	1423501_at	NM_008558	Max	MYC-associated factor X	1.91	0.023				
	1417187_at	NM_016786	Hip2	Huntington interacting protein 2	1.76	0.0071				
	1424704_at	NM_001145920	Runx2	Runt-related transcription factor 2	1.67	0.0068				
	1418265_s_at	NM_008391	Irf2	Interferon regulatory factor	1.60	0.023				
	1417850_at	NM_009029	Rb1	Retinoblastoma 1	1.58	0.0053				
Translation regulator	1416661_at	NM_010123	Eif3a	Eukaryotic translation initiation factor 3, subunit A	1.74	0.0087	± 0.05	1.84 ± 0.11	1.84	0.005
Nuclear receptors	1422631_at	NM_013464	Ahr	Aryl hydrocarbon receptor	1.61	0.028				
	1416958_at	NM_011584	NriD2	Nuclear receptor subfamily 1, group D, member 2	1.54	0.0064				
Transporters	1422966_a_at	NM_011638	Tfrc	Transferrin receptor	2.10	0.0010				
	1416653_at	NM_011504	Stxbp3	Syntaxin-binding protein 3	1.92	0.013	± 0.03	2.19 ± 0.6	2.19	0.038
	1442169_at	NM_013703	Vldlr	Very low density lipoprotein receptor	1.79	0.0068	± 0.05	1.44 ± 0.055	1.44	0.0007
	1424924_at	NM_153055	Sec63	SEC63 homolog (S. cerevisiae)	1.74	0.015				
	1419975_at	NM_011327	Scp2	Sterol carrier protein 2	1.66	0.023				
	1416374_at	NM_018829	Ap3m1	Adaptor-related protein complex 3 mu 1 subunit	1.59	0.0032				
	1434513_at	NM_001128096	Atp13a3	ATPase type 13A3	1.52	0.0054				
Ion channels	1453098_at	NM_013540	Gria2	Glutamate receptor, ionotropic, AMPA2	2.01	0.024	± 0.032	1.87 ± 0.09	1.87	0.0003
	1452089_at	NM_001037099	Cacnb4	Calcium channel, voltage-dependent, beta 4 subunit	1.85	0.0031	± 0.022	1.34 ± 0.02	1.34	0.022
	1451864_at	NM_133190	Cacng8	Calcium channel, voltage-dependent, gamma subunit 8	1.79	0.012	± 0.08	1.84 ± 0.26	1.84	0.005

Table 1 continued

Functional category	Probe set	Genbank	Gene symbol	Gene name	Microarray		qRT-PCR			
					FC KO/WT	p	WT mean Ct ± SD	KO mean Ct ± SD	FC	p
Other functions	1455946_x_at	NM_001039392	Tmsb10	Thymosin, beta 10	3.51	0.0052	± 1.04	2.56 ± 0.05	2.56	0.028
	1424325_at	NM_001081222	Esco1	Establishment of cohesion 1 homolog 1 (S. cerevisiae)	2.91	0.024				
	1425768_at	NM_023232	Diablo	Diablo homolog (Drosophila)	2.05	0.023	1 ± 0.032	2.16 ± 0.05	2.16	0.0002
	1455475_at	NM_026622	C4orf29	Chromosome 4 open reading frame 29 homolog (human)	2.00	0.023				
	1423198_a_at	NM_134034	Smek2	SMEK homolog 2, suppressor of mek1 (Dictyostelium)	1.93	0.014				
	1424074_at	NM_027453	Btf3l4	Basic transcription factor 3-like 4	1.88	0.018				
	1420485_at	NM_023554	Nol7	Nucleolar protein 7	1.78	0.016				
	1438418_at	NM_144535	Mudeng	MU-2/AP1M2 domain containing, death-inducing	1.77	0.014				
	1421945_a_at	NM_001042556	Bxdc1	Brix domain containing 1	1.77	0.0077				
	1450418_a_at	NM_026553	Yif1a	Yip1-interacting factor homolog A (S. cerevisiae)	1.74	0.029				
	1449884_at	NM_026626	Efcab2	EF-hand calcium binding domain 2	1.71	0.020				
	1418008_at	NM_026110	C21orf66	Chromosome 21 open reading frame 66 homolog (human)	1.68	0.023				
	1438535_at	NM_001081216	Phip	Pleckstrin homology domain interacting protein	1.65	0.011	± 0.19	2.08 ± 0.09	2.08	0.03
	1451525_at	NM_001039692	Arhgap12	Rho GTPase-activating protein 12	1.64	0.020	± 0.01	1.54 ± 0.09	1.54	0.033
	1426271_at	NM_153808	Smc5	Structural maintenance of chromosome 5	1.63	0.015				
	1417170_at	NM_033322	Lztß1	Leucine zipper transcription factor-like 1	1.62	0.029				
	1452247_at	NM_001113188	Fxr1	Fragile X mental retardation, autosomal homolog 1	1.62	0.0047				
	1451325_at	NM_027226	Fyttd1	Forty-two-three domain containing 1	1.61	0.0050				
	1425913_a_at	NM_144882	Dnaptp6	Viral DNA polymerase-trans-activated protein 6	1.60	0.012				
	1421940_at	NM_009282	Stag1	Stromal antigen 1	1.59	0.011				
	1448748_at	NM_019549	Plek	Pleckstrin	1.58	0.021	± 0.05	1.12 ± 0.09	1.12	0.004
	1451830_a_at	NM_009260	Spnb2	Spectrin, beta, non-erythrocytic 1	1.58	0.019				
	1437463_x_at	NM_011577	Tgfbl	Transforming growth factor, beta-induced	1.57	0.016				
	1423841_at	NM_026396	Bxdc2	Brix domain containing 2	1.57	0.026				
	1427104_at	NM_175480	Znf23	Zinc finger protein 612	1.56	0.015				
	1426806_at	NM_028696	Obfc2a	Oligonucleotide/oligosaccharide-binding fold containing 2A	1.55	0.023				
	1422896_at	NM_016796	Vamp4	Vesicle-associated membrane, protein 4	1.55	0.016	± 0.07	1.48 ± 0.16	1.48	0.034
	1437790_at	NM_001081191	Eml5	Echinoderm microtubule associated protein like 5	1.54	0.020				
	1417453_at	NM_001110142	Cul4b	Cullin 4B	1.53	0.028	± 0.03	1.12 ± 0.09	1.12	0.018
	1418066_at	NM_007688	Cß2	Coflin 2	1.53	0.019				
	1450407_a_at	NM_009672	Anp32a	Acidic nuclear phosphoprotein 32 family, member A	1.53	0.013				
	1450786_at	NM_019808	Pdlim5	PDZ and LIM domain 5	1.53	0.0057	± 0.11	1.44 ± 0.059	1.44	0.047
	1425597_a_at	NM_021881	Qk	Quaking homolog, KH domain RNA binding	1.52	0.029				

Real-time quantitative RT-PCR was performed for 24 genes in hippocampus of Rsk2-KO (n = 6) and WT (n = 6) mice, using the primer pairs shown in Table S1. Relative expression levels are shown with the average expression value of the WT group set to 1

Ct cycle threshold values, SD standard deviation, FC fold change, p p value

expression in Rsk2KO mice are associated with this second network, among which Nptxr, Cacng8, Pdlim5 and Soat1. The third network centered on MYC is mainly implicated in lipid metabolism and cell death. Thirteen focus genes are associated with this network, including Acsl4, Eif3a, Ca7, Cß2 and Fxr1. The last two networks are mainly implicated in molecular transport and lipid metabolism (4th network) and amino acid metabolism and protein synthesis (5th network). The fourth network is centered on Ptgs2 and includes 12 focus genes, among which Igf1, Vamp4, Mvk, Stxbp3 and Gnb1. The Þfth network is centered on retinoic acid and contains 13 focus genes, among which Cacnb4 and Carhsp1. Fig. 1 shows the two most signiÞcant networks (the others are available on request to AH). Supplemental Table 2 lists the top related biological functions and diseases. Top biological functions include organismal injury and abnormalities (9 genes, including Fxn, Sod2, Igf1, Ahr and Ptgs2), cell cycle (12 genes including Rb1, Igf1 and Runx2), nervous system development and function (10 genes including Igf1, Ptgs2, Sod2, Gria2, Vldlr, Vtn and Rb1), organismal development and free radical scavenging (9 and 4 genes, respectively). Interestingly, Þve genes, including Gria2, Igf1, Ptgs2, Sod2 and Vldlr have been implicated in behavior, with the Þrst four genes speciÞcally in spatial memory formation. These Þve genes are all up-regulated in the hippocampus of Rsk2KO mice. The p values in the range of 2.52×10^{-5} to 1.10×10^{-2} indicate statistical signiÞcance.

ConÞrmation of altered expression at the protein level

We conÞrmed increased expression at the protein level of GLUR2 (Gria2 gene), CACNG8, VAMP4, EIF3A and DIABLO using quantitative western blot analysis (Fig. 2). These Þndings are in line with the changes detected by microarray-based analysis. We also conÞrmed differential expression of GLUR2, VAMP4 and IGF1 using immunohistochemical analysis. As shown in Fig. 3, we found increased expression of GLUR2 in the CA1 and CA3 regions and in the dentate gyrus of Rsk2KO hippocampus. VAMP4 and IGF1 were increased in the whole hippocampus. Although the lack of speciÞc antibodies precluded similar experimental validations for many other genes listed in Table 1, these observations suggested that the transcriptional changes observed in mutant mice may be generally reßected by matching changes in the levels of expression of their corresponding protein products.

ConÞrmation by in situ hybridization of Gria2 up-regulation

The expression of a number of genes involved in neurotransmission, including vesicle and receptor traffÞcking proteins, neurotransmitter receptors and ion channels, were altered in Rsk2KO mice (Table 1). Of particular interest was the twofold increased level of expression of Gria2, encoding the subunit GLUR2 of the AMPAR. Since AMPARs mediate fast synaptic transmission at excitatory synapses in the brain and are thought to play key roles in synaptic plasticity, learning and memory (Seidenman et al. 2003) we wanted to further conÞrm up-regulation of the Gria2 gene by in situ hybridization of WT and KO mice hippocampi. The expression of Gria2 was signiÞcantly increased in all areas of the anterior hippocampus of mutant mice (Fig. 4a), whereas in the posterior hippocampus, the level of Gria2-mRNA was mainly increased in the dentate gyrus, in CA1 and in the CA3 region (Fig. 4b). We did not Þnd any change in the level of expression of GLUR1 (not shown).

Increase in GLUR2 surface expression

We next wondered whether expression of GLUR2 at surface of synapses was also up-regulated since AMPARs in hippocampal neurons are mainly expressed as heteromers of GLUR1/2 as well as GLUR2/3. We compared the amount of GLUR2 at synapses in KO and WT cultured primary hippocampal neurons by staining surface GLUR2 (or GLUR1) clusters and counterstaining for PSD95. GLUR2 surface staining was punctate (Fig. 5a, b), and the number of GLUR2 puncta that were synaptic did significantly differ among WT and Rsk2KO cultures (WT 8.1 ± 1.4, $n = 12$ embryos, KO 14.9 ± 2.2, $n = 12$, $p = 0.015$). This provided evidence that increase in total GLUR2 was correlated with increased surface-expressed GLUR2. No signiÞcant difference of surface GLUR1 was detected (WT 12.4 ± 7.4, $n = 4$, KO 10.7 ± 5, $n = 6$, $p = 0.8$) (Supplemental Fig. 1).

Determination of relative Gria2 R/G editing and ßip/ßop splice levels

The great majority of native AMPA receptors are impermeable to calcium ions, due to the presence of the GLUR2 subunit. This subunit confers calcium impermeability on the channel due to RNA editing of a glutamine (Q) to an arginine (R) at codon 607. In addition to the Q/R site in GLUR2, the GLUR2, 3 and 4 subunits undergo RNA editing (arginine to glycine) at codon 764 (in GLUR2) (Lomeli et al. 1994). The pre-messenger RNA transcripts of all the four GLUR subunits can Þnally be alternatively spliced to produce either the ßip or ßop isoforms. Because the level of expression of GLUR2 was increased in Rsk2KO mice, we wondered whether Gria2 RNA editing and splicing were altered.

To determine whether there are changes in the Q/R, R/G site editing and ßip/ßop splice levels of the Gria2

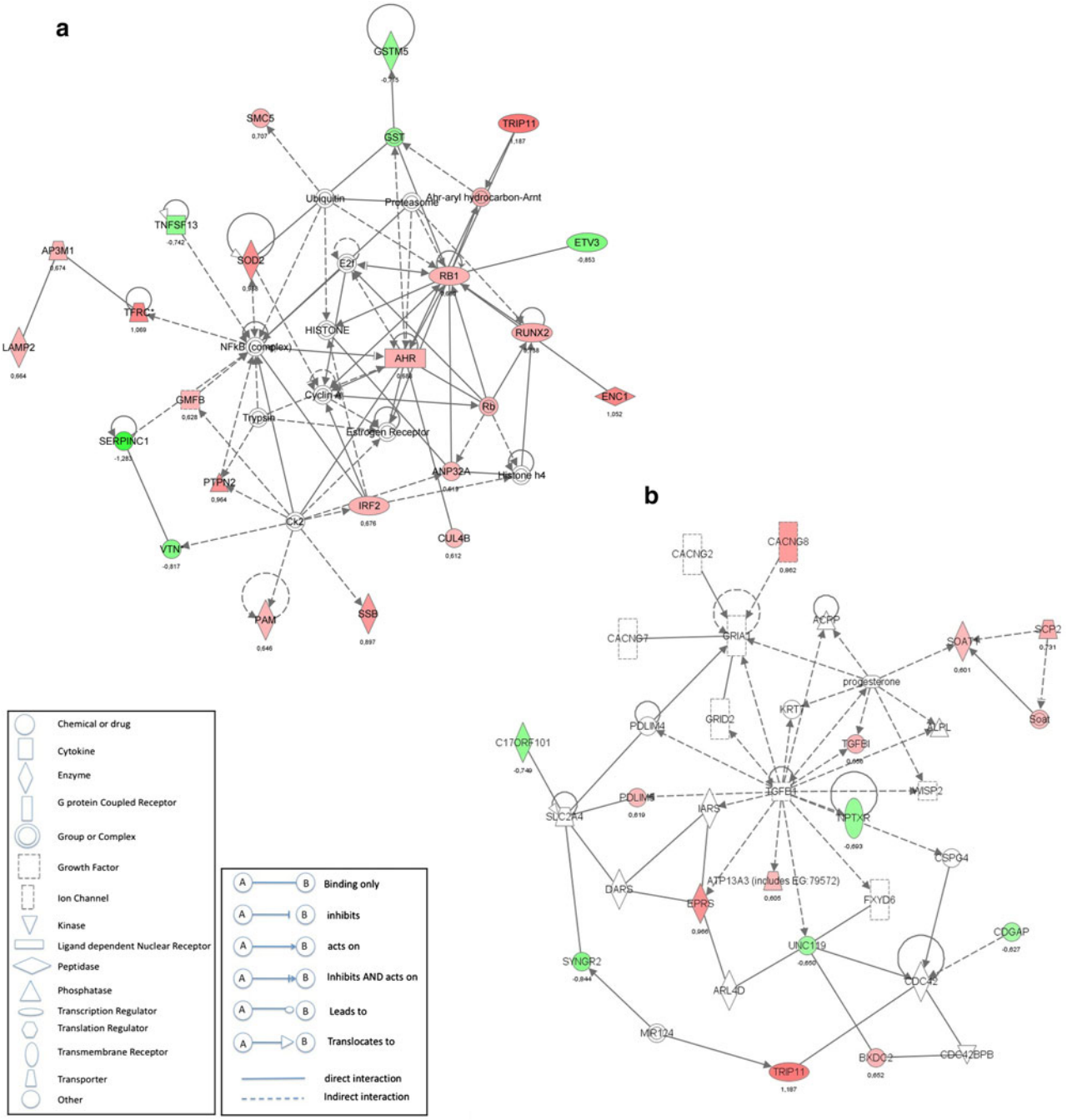

Fig. 1 Top integrated networks dysregulated in the hippocampus of Rsk2KO. Networks were created by the Ingenuity Pathway Analysis Software. Up-regulated genes are listed in red and down-regulated in green a This network is centered on NFkB. Twenty-five differentially expressed focus genes were brought into this network with a score of 45. b This network is centered on TGFβ. Fifteen differentially expressed focus genes were brought into this network with a score of 26. Nodes and edges are described below the networks

messenger in 5-month-old Rsk2KO hippocampi, the Gria2 mRNA from five Rsk2KO and five WT hippocampi was amplified by RT-PCR and the products sequenced to determine the relative levels of editing and splicing (Lee et al. 1998). No unedited form of the Gria2 transcript at the Q/R site (codon 607) was detectable (not shown) neither in KO nor in WT mice, suggesting that the amount is very low. These data are in accordance with the previously reported results indicating that editing of Q/R site is 99% complete in postnatal brain (Carlson et al. 2000). At the R/G editing site (codon 764) edited (codon AGA) and unedited (codon AGA) forms were detectable in both WT and mutant mice.

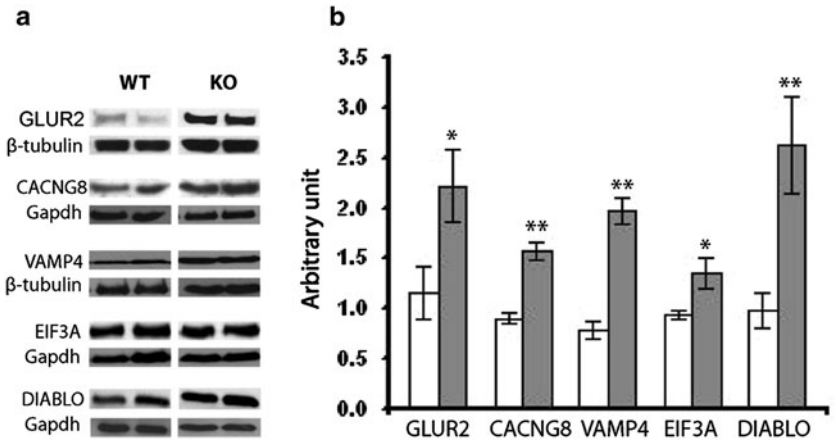

Fig. 2 Quantitative Western blot analyses. Levels of proteins expressed by the five up-regulated genes assayed are significantly increased in the hippocampus of Rsk2KO mice. a Proteins detected in two Rsk2KO and two WT mice are shown. b Data normalized either to GAPDH or to β-TUBULIN are represented as the mean ± SEM for six mice of each genotype for GLUR2 and CACNG8 and four mice for EIF3A, VAMP4 and DIABLO. WT white bar, KO gray bar. *$p < 0.05$ and **$p < 0.01$

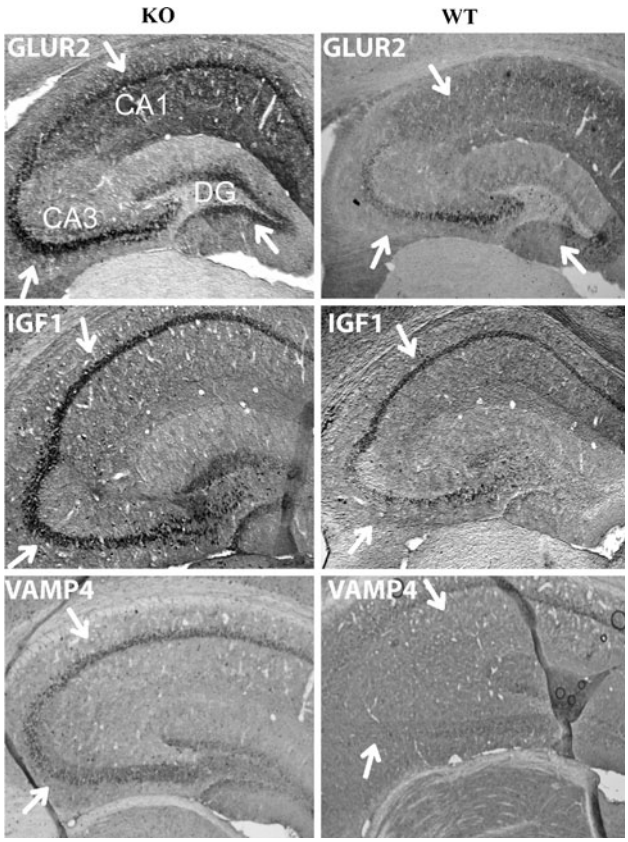

Fig. 3 Immunohistochemical analysis. Proteins expressed by three up-regulated genes show significantly higher expression in Rsk2KO mice hippocampus. Three mice for each genotype were analyzed. Each picture represents one of the triplicates. Arrows point to hippocampus sub-regions showing increased expression in KO versus WT mice. CA1, CA3 and DG (dentate gyrus): hippocampus sub-regions

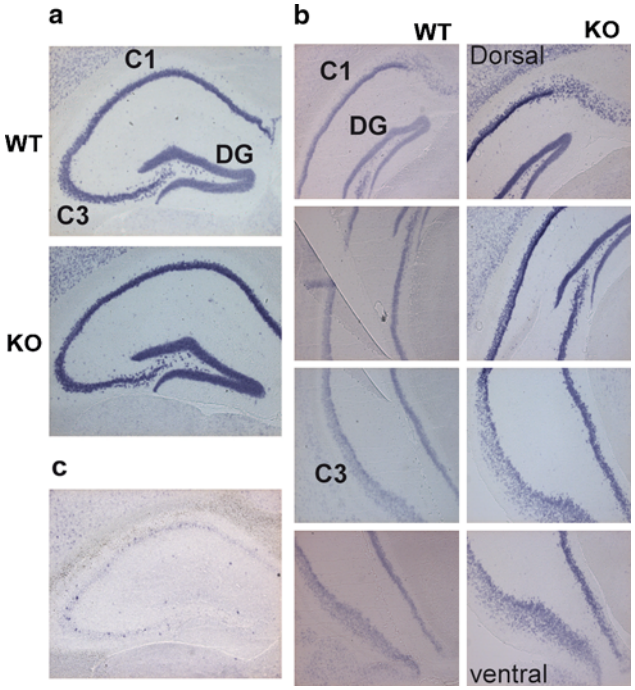

Fig. 4 In situ hybridization. Dig-labeled Gria2 sense and anti-sense RNAs were hybridized to 25-μm coronal sections of three Rsk2KO and three WT adult mouse brains. One picture from each genotype is shown. a Significantly increased expression in all areas of the anterior hippocampus of Rsk2KO mice. b In the posterior hippocampus the level of Gria2-mRNA was mainly increased in the dentate gyrus, in CA1 and in the ventral CA3 region. c No staining was observed with sense RNA

The peak intensity of the G nucleotide signal at the edited position was measured and reported as a percentage of the total signal (A and G). Representative chromatograms from one KO and one WT littermate are shown in Supplementary Fig. 2. In WT hippocampi, the Gria2 mRNA was approximately 61 ± 4% edited, whereas there was less editing in Rsk2KO hippocampi (43 ± 3%, $p = 0.005$). Our data for WT mice are in accordance with the previous reports

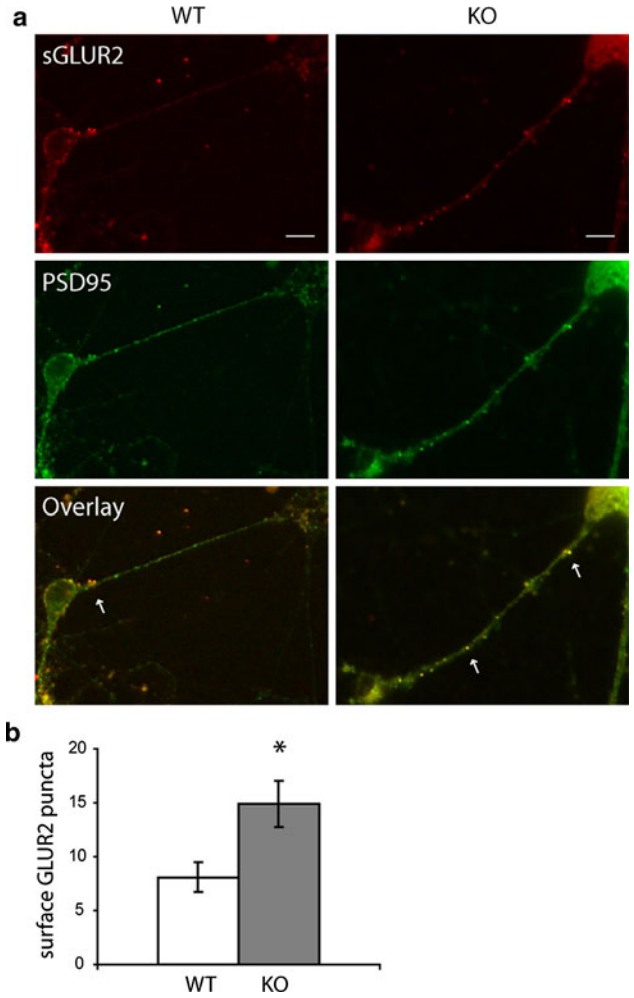

Fig. 5 Surface expression of AMPARs. a WT and Rsk2KO hippocampal neurons were labeled with N-terminal GLUR2 antibody under non-permeabilized condition to stain surface GLUR2 (sGLUR2), followed by PSD95 staining (a post-synaptic marker). Arrows point to post-synaptic surface-expressed GLUR2. Scale bar 10 μm. b Quantification of sGLUR2 puncta. Data represent mean ± SEM of detected sGLUR2 clusters per unit dendrite length, from n = 12 (WT) and 12 (KO) embryos. *$p < 0.05$

demonstrating an editing status at the R/G site of approximately 64% (Lai et al. 1997). Thus, in Rsk2KO mice the extent of R/G editing was significantly decreased (relative decrease: 18%, $p = 0.003$) in the hippocampal tissue.

To determine the ratio of transcripts in the flip/flop alternative splice form, the peak intensity of the first nucleotide difference (C vs. A) between the two variants was measured (Lee et al. 1998). In the WT hippocampi, there were approximately 55 ± 5% of Glur2 transcript in the flip form, whereas the percentage was significantly lower (43 ± 3%, $p = 0.002$) in the Rsk2KO hippocampi (Supplemental Fig. 2c, d). No significant difference for the Gria1 mRNA (encoding GLUR1) for the R/G editing site or flip/flop splice levels was found between WT and mutant mice (Supplemental Fig. 2a, b).

Reduced AMPA synaptic transmission

We then investigated whether changes in Gria2 expression, editing and splicing affect basal AMPAR-mediated synaptic transmission in hippocampal slices from 4-week-old Rsk2KO mice. To assess the strength of synaptic transmission, we compared the size of the presynaptic fiber volley (input) to the slope of the EPSP (output) in striatum radiatum and found a ~ 25% significant reduction in Rsk2 KO ($n = 9$) mice when compared with WT littermates ($n = 7$) (Fig. 6a). We evaluated also paired-pulse facilitation (PPF), a measure of release probability from presynaptic terminals. The PPF curves were essentially identical in slices from control and Rsk2KO mice (Fig. 6b) indicating that RSK2 most likely modulates AMPA neurotransmission postsynaptically with no effect on presynaptic function.

Discussion

The absence of gross structural alterations in the brain of Rsk2KO mice strongly indicates that their defective cognitive phenotype should be linked to subtler molecular or cellular alterations. In an effort to identify such alterations, we carried out a detailed characterization of the differences existing between the transcriptional profiles of the hippocampus of WT and KO animals. Our analysis by oligonucleotide microarrays yielded a list of 100 differentially expressed genes with high degree of statistical significance. These results were further confirmed for 24 genes by quantitative RT-PCR demonstrating their robustness.

Our study revealed a great variety of RSK2-influenced genes acting in various biological pathways. Indeed, the network with the highest score (as determined by ingenuity pathway analysis) centers on the NF-κB complex, which plays a prominent role in cell differentiation and proliferation and apoptosis (Brand et al. 1997). Twelve genes are implicated at various stages of the cell cycle in Rsk2KO neurons (including Igf1, Rb1, Max, Sod2, and, Ptgs2). This result suggests strongly that abnormal cell proliferation contributes to the CLS phenotype. In addition, 34 of the altered genes have been implicated in cell death or survival, out of which 7 (Acng8, Diablo, Gria2, Igf1, Ptgs2, Rb1, Sod2) have been specifically associated with neuronal cell death. Interestingly, previous studies suggested that apoptotic and antiapoptotic cascades are tightly associated with cognitive dysfunctions and neurological disorders (Lutz 2007). Further studies are, therefore, necessary to investigate cell proliferation and death in Rsk2KO mice. Four genes, including Sod2, Fxn, Gmfb and Cp are implicated in free radical scavenging, suggesting also a possible involvement of free radicals in the

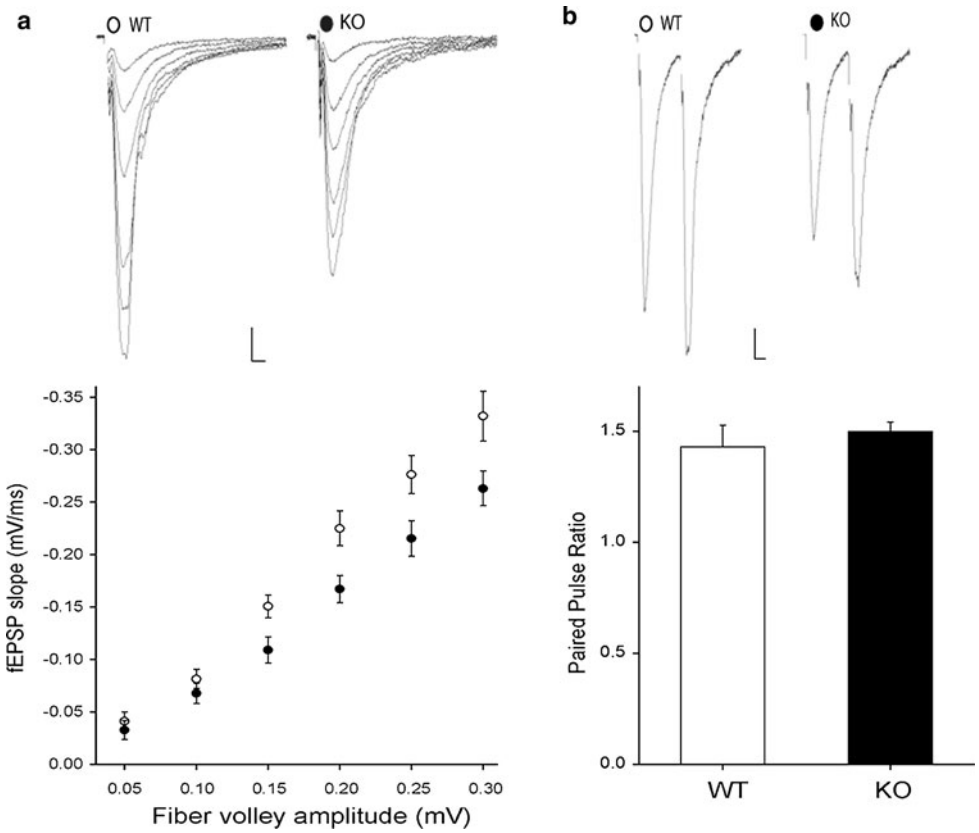

Fig. 6 Patch-clamp analysis. **a** Input–output curves for basal synaptic transmission in hippocampal slices. As illustrated in the sample traces and the graph, for each input (fiber volley ≥ 0.15 mV), the output (fEPSP) is reduced by 25% in Rsk2-KO slices ($p \leq 0.05$, WT $n = 10$; KO $n = 9$). Scale bar 0.1 mV, 5 ms. **b** Paired-pulse facilitation (PPF) does not differ between Rsk2-KO ($n = 9$) and WT ($n = 6$) cells. Sample traces are illustrated above the bar graph. Scale bar 0.05 mV, 10 ms

CLS phenotype. Ten genes (among which *Gria2*, *Igf1*, *Ptgs2*, *Sod2*, *Nptxr*, *Ahr* and *Vtn*) play a role in nervous system development and function. IGF1 for instance is essential for normal dendritic growth (Cheng et al. 2003). NPTXR is thought to be involved in activity-dependent synaptic plasticity (Xu et al. 2003). The AHR homologs in Drosophila, Spineless (Ss), and in Caenorhabditis elegans, ahr-1, regulate dendrite morphology (Kim et al. 2006) and neuronal differentiation (Qin and Powell-Coffman 2004). The expression of a number of genes involved in neurotransmission was also found affected. Up-regulation of the *Vamp4* and *Stxbp3* genes in mutant mice points to a role of RSK2 in pre-synaptic vesicle trafficking (Wang and Tang 2006). Up-regulation of *Cacnb4*, encoding a β-subunit of Voltage-gated Ca^{++} channels, suggests that influx of Ca^{++} into the cell upon membrane polarization is regulated via RSK2 (Birnbaumer et al. 1998). Moreover, alteration of *Gnb1* and *Gria2* expression suggests that RSK2 is involved in glutamate receptor signaling. GNB1 mediates the fast voltage-dependent inhibition of N-type Ca^{++} channels (Fu and Cheung 1999). The function of Gria2 will be discussed below. Finally, the expression of several genes encoding proteins implicated in gene expression was altered in Rsk2-KO hippocampi (including the transcription regulators Etv3, Hip2, Rb1, Irf2, Max, Runx2 and Trip11) suggesting that some of the RSK2 effects may be direct and others indirect. Strikingly, two of the genes with altered expression in Rsk2-KO hippocampi, *Lamp2* and *Cul4b*, have previously been associated with syndromic forms of X-linked mental retardation (Nishino et al. 2000; Zou et al. 2007).

Among the genes associated with a specific neuronal function, *Gria2* was of particular interest because GLUR2 controls the key biophysical properties of AMPA receptors, which are implicated in learning and memory (Kessels and Malinow 2009). Most excitatory synaptic transmission in the brain being mediated through AMPAR, changes in the properties of these receptors are likely to have a major impact on brain function. Furthermore, GLUR2 was shown to bind directly to RSK2 in murine neurons suggesting a direct influence of RSK2 on GLUR2 function (Thomas et al. 2005). The GLUR family contains four closely related members (GLUR1-4). AMPARs are tetrameric, composed of various combinations of GLUR1-4 subunits, and the conductance properties of the receptors are highly dependent on their subunit composition (Kuner et al. 2001). GLUR2-lacking receptors have a higher Ca^{++} permeability, channel conductance, open probability and rectification than GLUR2-containing receptors (Isaac et al. 2007). Therefore, the presence or absence of the GLUR2 subunit can dramatically alter AMPAR properties and thereby synaptic transmission.

Our results provide evidence that in the hippocampus of Rsk2KO mice total expression of GLUR2 is increased, and that the expression is also increased at the surface of synapses in cultured primary hippocampal cells. It was reported previously that surface insertion of GLUR2 occurs constitutively under basal conditions (Passafaro et al. 2001). Our results are compatible with these data. It may be speculated that over-representation of the GLUR2 subunit in synaptic AMPARs results in decreasing of Ca^{++} permeability and channel conductance. We show that there is, indeed, a 25% reduction in basal AMPAR-mediated transmission in the hippocampus of Rsk2KO mice.

The Q/R site was completely edited in both WT and Rsk2KO hippocampi, whereas the extent of R/G editing was signiÞcantly decreased. The GLUR2 subunit confers Ca^{++} impermeability on the channel due to a single arginine (R) residue located at amino acid position 607, which is a glutamine (Q) in the other AMPA receptor subunits. RNA editing of the Q/R site is speciÞc to GLUR2 and is complete in postnatal brain. Our results in WT mice conÞrm further these latter data and show that Q/R editing is unaltered in Rsk2KO mice. GLUR2-4 undergo also RNA editing [arginine (R) to glycine (G)] at amino acid position 743 (Lomeli et al. 1994). The presence of edited GLUR subunits at position 743 yields channels with faster kinetics. The R/G editing state of GLUR2ÐD4 inßuences also the assembly and surface expression of AMPAR complexes. We show that R/G editing is signiÞcantly altered in Rsk2 KO mice. Finally, alternative splicing in the extracellular ligand binding domain of the AMPARs generates two variants, i.e., ßip and ßop. Native AMPAR are heteromeric assemblies of different subunits that may have different ßip and ßop isoforms. We show that in Rsk2KO mice the proportion of GLUR2 molecules with a ßop exon is significantly higher than in WT littermates. The ßop variants desensitize at least 3 times faster but recover more slowly from desensitization than the ßip counterparts (Pei et al. 2009). It was also previously shown that the ßip/ßop splicing has an effect on the maturation and cellular traÞcking of AMPARs (Brorson et al. 2004). Alteration of R/G editing and splicing of GLUR2 in Rsk2KO mice are therefore expected to alter AMPARs channel kinetic, desensitization and traÞcking. Further functional studies are required to address the precise functional consequences of these editing and splicing changes in Rsk2KO neurons. Furthermore, it was shown that proteins binding to GLUR2 are necessary for constitutive replacement of newly inserted GLUR1-containing receptors to maintain synaptic strength during LTP (Malinow and Malenka 2002). It has also been proposed that the expression of hippocampal LTD is critically dependent on GLUR2 (Malinow and Malenka 2002). Further studies will address the consequences of up-regulation of GLUR2 in Rsk2KO mice for LTP and LTD. The signaling mechanisms involved in the increased levels of transcription of Gria2 in Rsk2KO hippocampal neurons are not yet known. The regulation of Gria2 splicing and editing events is poorly understood as well. Gria2 expression is inßuenced strongly at the transcriptional level by at least three regulatory elements in the 5′ proximal region of the promoter (Borges and Dingledine 2001). RNA editing is mediated by adenosine deaminase acting on RNA (ADAR) enzymes. Three structurally related ADARs (ADAR1 to ADAR3) have been identiÞed in mammals. ADAR2 predominantly catalyzes RNA editing at the Q/R site of GLUR2 (Peng et al. 2006), whereas it is not yet clear how the R/G site is edited. The underlying mechanism of the R/G editing dysregulation may be caused by altered function or expression of one or several ADAR enzymes. However, the fact that editing of the Q/R site in GLUR2 is not affected in Rsk2KO mice suggesting that ADAR2 is excluded. Further investigations are necessary to determine precisely the molecular events leading to up-regulation of the Gria2 gene and alteration of RNA editing, and the contribution of each of these dysregulations to the cognitive dysfunction. The contribution of other pathways remains also to be investigated. Among the deregulated genes at least one other participates in regulation of AMPAR function: Cacng8 This gene encodes a synaptic protein, TARP-γ8 that participates in consolidation phase of memory and is involved in modulating neurotransmitter release. Evidence was provided that TARP-γ8 is critical for basal AMPAR expression and localization at extrasynaptic sites in the hippocampus (Rouach et al. 2005). Up-regulation of Cacng8 may thus contribute to AMPAR dysfunction.

The data in this study provide a Þrst glimpse of the gene expression proÞle of adult hippocampi in the absence of RSK2 expression. However, the Rsk2KO animals represent a value model to study human CoffÞn-Lowry syndrome, it has signiÞcant limitations due to potential compensatory adaptation mechanisms in the developing nervous system (in particular through other RSK family members). Thus, it would be interesting to perform expression proÞling following Rsk2 gene silencing by RNA interference technology. Indeed, cellular or animal models based on this technology could offer further clues about the function of RSK2.

In conclusion, functional impairment of neurotransmission and plasticity due to AMPAR dysfunction may, indeed, contribute to the cognitive deÞcit of Rsk2KO mice. However, further investigations are necessary to determine precisely the molecular events leading to alteration of GLUR2 expression and the contribution of this dysregulation to the cognitive dysfunction. The involvement of other pathways, including in particular cellular proliferation and apoptosis, remains also to be investigated.

Finally, the genes identiÞed by our microarray analysis will help in further unravel the various functions of RSK2 in the hippocampus can be speculated to play a role in the pathogenesis of mental retardation in CofÞnÐLowry syndrome and may provide targets for pharmaceutical intervention.

Accession number

The data of the expression arrays produced for this report have been submitted to NCBIs Gene Expression Omnibus (GEO: http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE22137.

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Conßict of interest The authors declare that they have no conßict of interest.

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