Published in final edited form as:
Biol Psychiatry. 2011 October 1; 70(7): 626–635. doi:10.1016/j.biopsych.2011.06.016.

Loss of function studies in mice and genetic association link receptor protein tyrosine phosphatase α to schizophrenia

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FINANCIAL DISCLOSURES

All authors reported no biomedical financial interests or potential conflicts of interest.

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Abstract

Background—Solid evidence links schizophrenia (SZ) susceptibility to neurodevelopmental processes involving tyrosine phosphorylation-mediated signaling. Mouse studies implicate the *Ptpra* gene, encoding protein tyrosine phosphatase RPTPα, in the control of radial neuronal migration, cortical cytoarchitecture, and oligodendrocyte differentiation. The human gene encoding RPTPα, *PTPRA*, maps to a chromosomal region (20p13) associated with susceptibility to psychotic illness.

Methods—We characterized neurobehavioral parameters, as well as gene expression in the central nervous system, of mice with a null mutation in the *Ptpra* gene. We searched for genetic association between polymorphisms in *PTPRA* and schizophrenia risk (2 independent cohorts; total of 1420 cases and 1377 controls), and we monitored *PTPRA* expression in prefrontal dorsolateral cortex of SZ patients (35 cases, 2 control groups of 35 cases)

Results—We find that $Ptpra^{-/-}$ mice reproduce neurobehavioral endophenotypes of human SZ: sensitization to metamphetamine-induced hyperactivity, defective sensorimotor gating, and defective habituation to a startle response. *Ptpra* loss of function also leads to reduced expression of multiple myelination genes, mimicking the hypomyelination-associated changes in gene expression observed in *post mortem* patient brains. We further report that a polymorphism at the *PTPRA* locus is genetically associated with SZ, and that *PTPRA* mRNA levels are reduced in *post mortem* dorsolateral prefrontal cortex of subjects with SZ.

Conclusion—The implication of this well-studied signaling protein in SZ risk and endophenotype manifestation provides novel entry points into the etiopathology of this disease.

Keywords

schizophrenia; tyrosine phosphatase; myelination; mouse model; RPTPα; *PTPRA*

Introduction

Schizophrenia (SZ, MIM #181500) is diagnosed by the joint appearance of positive (hallucinations, delusions), negative (disturbed affective and social functioning), and cognitive symptoms. Initial hypotheses about the pathophysiological mechanism derive from pharmacological observations: blocking D2 receptors can alleviate positive symptoms, and NMDA-R antagonists can mimic disease symptoms, which provides support for the dopaminergic and glutamatergic hypotheses. Imaging and *post mortem* analyses reveal that SZ is accompanied by neuropathological abnormalities, including decreased myelin content, atypical neuronal cytoarchitecture, and altered laminar organization, suggesting abnormalities in neural development (1). Gene expression studies indicate abnormalities in myelination (2).

The disease has a significant genetic basis (3). Non-affected kin can display quantifiable neurobehavioral abnormalities, perhaps reflecting manifestation of a subset of genetic predispositions. The identification of SZ-associated genes is starting to provide insights into disease etiology, by implicating molecular signaling pathways. One of the first and most reproducible instances of genetic association with SZ is *neuregulin 1* (*NRG1*), whose product, signaling via ERBB receptor tyrosine kinases, modulates oligodendrocyte development, neuronal migration and differentiation, and glutamatergic and GABA-ergic neurotransmission (4–8). Two other genes in the NRG1 pathway, *ERBB4* encoding a

tyrosine kinase receptor for NRG1, and *PTPRZ1* encoding an ERBB4-associated protein tyrosine phosphatase in the oligodendrocyte lineage, are also genetically associated with SZ (9–11). NRG1 signaling may be functionally linked to NMDA-R modulation (11, 12), perhaps via phosphorylation of the latter by the Src-family tyrosine kinases Fyn (13) and c-Src (14). Among other predisposition genes for SZ are cell adhesion molecules such as CHL1 and NCAM (15–21), the signaling activities of which also rely on Src-family kinases (SFKs).

Receptor protein tyrosine phosphatase RPTPα (encoded by human *PTPRA* and mouse *Ptpra*) is a physically associated signaling subunit of CHL1 and NCAM, through its well-documented role in regulating Fyn (22–24). RPTPα can also modulate SFKs downstream of EGF-receptor (*ERBB1*) activation (25). *Ptpra* is abundantly expressed in the developing central nervous system (CNS), and remains highly expressed in the adult (26, 27). In mice, loss of *Ptpra* function is associated with neurodevelopmental defects in peripheral myelination (28), oligodendrocyte differentiation and myelin basic protein (MBP) expression (29), radial cortical migration (27), mis-orientation of apical dendrites of deep layer pyramidal neurons (24), reduced NMDA-R phosphorylation, and impairments in synaptic plasticity and short-term memory (27, 30, 31); many of these effects reflect a function of RPTPα in regulating SFKs (24, 25, 29, 31–33). Interestingly, *PTPRA* maps to a chromosomal region (20p13) that has been linked to SZ (34, 35).

Given the multifold involvement of RPTPα in neurodevelopmental and signaling pathways associated with SZ, we set out to explore whether loss of *Ptpra* function in mice engendered neurobehavioral abnormalities or gene expression signatures relevant to SZ. Positive findings led us to pursue association between polymorphisms at the *PTPRA* locus and disease risk, and changes in *PTPRA* expression in dorsolateral prefrontal cortex of patients.

MATERIALS AND METHODS

Full details of all procedures can be found in the Supplement.

Mice

Generation of RPTPα-deficient ($Ptpra^{-/-}$) mice has been described (33). The allele was backcrossed 10 times (N=10) with C57Bl/6J mice. Control (wt) mice were generated by intercross of $Ptpra^{+/-}$ heterozygotes.

Mouse motor and neurobehavioral testing

Spontaneous exploratory locomotor activity and drug-induced hyperactivity were generally assessed as in (36), and prepulse inhibition and acute startle responses as in (37).

Gene expression analysis

RNA was extracted from mouse whole brain and human dorsolateral prefrontal cortex and subject to qPCR analysis.

Genetic association

This was performed essentially as in (38), followed by inclusion of a second independent cohort.

RESULTS

Ptpra$^{-/-}$ mice display enhanced psychostimulant-induced hyperactivity, deficient sensorimotor gating, and failure to habituate to a startle response

Dissection of multifactorial diseases is helped by the identification of genetically-based quantitative non-apparent "endophenotypes" that are proximal consequences of genetic predisposition, but precede or are not necessarily accompanied by manifestation of the disease itself. This reductionist approach is particularly relevant to the dissection of psychiatric disease, and to its animal modeling (39).

RPTPα participates in several processes implicated in pharmacological and neurodevelopmental descriptions of schizophrenia, and *Ptpra*$^{-/-}$ mice manifest neuropathological abnormalities reminiscent of those reported in patients. To determine whether loss-of-function of mouse *Ptpra* results in behavioral and neuropsychological abnormalities associated with SZ, we focused on three models: Locomotor response to psychostimulants, pre-pulse inhibition (PPI) as a measure of sensorimotor gating, and the watermaze test for spatial memory.

The studies were performed on a previously described *Ptpra* null allele (27). We first assessed sensorimotor capabilities to exclude the possibility of compounding effects (Table S1 in the Supplement). Latency to fall off a beam or from an accelerating rotarod revealed no obvious abnormality in general sensorimotor capability of *Ptpra*$^{-/-}$ mice (beamwalk: $F(1,33)=0$, $p=1$ and $F(1,33) = 0.298$, $p=0.589$ respectively). Spontaneous exploratory locomotor activity was also unaffected by *Ptpra* allelic status ($F(1,33)=1.983$, $p=0.169$). We concluded that *Ptpra* loss of function (LOF) does not engender sensorimotor abnormalities that would affect the subsequent analyses.

We subsequently asked whether *Ptpra* LOF altered the locomotor response to the psychostimulant methamphetamine (MAMPH), a pharmacological model inspired by the dopaminergic hypothesis of SZ (40). We found locomotor activity after acute MAMPH administration (2 mg/kg) to be significantly higher in *Ptpra*$^{-/-}$ mice than in controls (main effect of genotype $F(1,31)=5.753$, $p=0.023$; drug treatment: $F(1,31)=72.386$, $p<0.001$; genotype × drug treatment: $F(1,31)=8.797$, $p=0.006$; *post hoc* multiple comparisons: MAMPH (WT) *vs*. MAMPH (*Ptpra*$^{-/-}$): $p<0.001$) (Fig. 1A).

Given the functional association of RPTPα with NMDA-R (27, 30, 31), we also investigated the effect of administration of the non-competitive NMDA-R antagonist MK-801, known for its ability to induce psychotic symptoms in healthy humans. However, at the dose used (0.2 mg/kg), MK-801 did not significantly increase locomotor activity in WT mice (not shown).

Secondly, we assessed prepulse inhibition (PPI) of the startle response, a suggested SZ endophenotype. PPI denotes attenuation of a startle motor response to a sensory (acoustic) stimulus when the latter is immediately (<500 msec) preceded by a milder stimulus. Used as an operational measure of sensorimotor gating, PPI is impaired in SZ individuals and their unaffected relatives, and antipsychotics can reverse impairment of PPI in experimental models (41). It constitutes a pre-attentive process akin to a reflex response. We tested the mice on two occasions (2.5 months apart) to determine: (i) if any PPI abnormality persisted; and (ii) whether normal habituation to the startle response occurred, as impaired habituation is a hallmark of schizophrenia (42). Such a longitudinal design is rarely applied to KO mice, partly due to difficulties in the use of test batteries in rodents (43, 44).

At the initial age of analysis (2.5 months), we observed no difference in startle response between *Ptpra*$^{-/-}$ and WT mice (Fig. 1C) (F(1,14)= 0.332, p=0.576). By contrast, *Ptpra*$^{-/-}$ mice manifested a significant reduction in PPI (Fig. 1B) (F(1,14)=6.006, p=0.032). This genotype-dependent difference in PPI disappeared at a more advanced age (F(1,13)=0.034, p=0.857) (5 months; Fig. 1D), suggesting a critical time-period for manifestation of this abnormal phenotype. Strikingly however, at the more advanced age, the typical habituation (reduced response) to the acoustic startle stimulus alone observed in WT animals (startle at 2.5 months as compared to 5 months: F(1,14)=11.797, p=0.014) did not occur in *Ptpra*$^{-/-}$ mice (startle at 2.5 months in comparison to 5 months: F(1,13)=0.013, p=0.914), leading to a significant difference in acoustic startle response during this re-testing at 5 months (Fig. 1E).

Finally, we subjected *Ptpra*$^{-/-}$ mice to a water maze test, a hippocampal-dependent model of spatial memory. Impaired hippocampal-based function in SZ is well-documented (45). Detailed analysis revealed no genotype differences in place finding (swim distance and latency to target; Fig 2A), nor in the probe test (recall of spatial position of the platform; Fig 2B), or reversal learning (Fig. 2C).

Loss of *Ptpra* function leads to reduced CNS levels of myelin markers and SZ-associated genes

Increased metamphetamine sensitivity, impaired PPI, and failure to habituate to a startle response are commonly accepted indicators for modeling SZ-associated states in mice. To assess whether the relevance of *Ptpra*$^{-/-}$ mice as a model for SZ-associated abnormalities extends beyond neuropsychological parameters, we started assessing SZ-associated gene expression markers. Imaging analysis, post *mortem brain* studies, genetic association studies, and gene expression studies reveal that abnormal oligodendroglial function and myelination are commonly associated with schizophrenia (2, 46–49). One of the major targets of RPTPα, the Src family kinase Fyn, plays important roles in myelination (50–53). A transient defect in peripheral myelination has been documented in the strain of *Ptpra*$^{-/-}$ mice studied here (28); an independently generated *Ptpra*$^{-/-}$ strain was recently reported to display impaired oligodendrocyte differentiation *in vitro*, and reduced MBP immunostaining *in vivo* (29). We therefore investigated the expression of myelin related genes in the brains of our strain of *Ptpra*$^{-/-}$ mice.

We found that mRNA levels of 8/9 tested oligodendrocyte lineage marker genes were significantly ($p<0.05$ to $p<0.001$) reduced (range 53–67 %) in *Ptpra*$^{-/-}$ mice (Fig. 3). This phenomenon applied not only to an oligodendrocyte marker (myelin basic protein, MBP), but also to genes that are functionally involved in oligodendrocyte differentiation (e.g. *Sox10, Qk*), and oligodendrocyte lineage genes whose reduced expression in human SZ brain is well-documented (e.g. *Cnp1, Cldn11, Qk*) (48), or that are genetically associated with SZ (e.g. *Erbb4* (9) and *Qk* (54)).

A polymorphism in human *PTPRA* demonstrates close genetic association with schizophrenia susceptibility

Our finding that ablation of mouse *Ptpra* mimics neuropsychological and gene expression abnormalities associated with SZ prompted us to pursue a genetic link between human *PTPRA* and SZ risk. *PTPRA* maps to 20p13, identified as a susceptibility locus by low-resolution linkage studies in two different human groups (34, 35). We pursued more detailed SNP fine-mapping analysis on a third population to search for evidence for closer association between SZ and *PTPRA*.

In the first stage, 560 cases and 548 controls were genotyped using the GeneChip® Human Mapping 5.0 Array (Affymetrix). Out of 21 SNPs genotyped across the *PTPRA* locus, six

yielded nominally significant association with SZ (rs6132976, rs6132977, rs6132978, rs1016753, rs1178032 and rs16988201) (best uncorrected p=0.002). To confirm this association, we performed a replication using an independent sample comprised of 850 cases and 829 controls. Based on the linkage disequilibrium (LD) pattern from the first stage analysis, three SNPs (rs1016753, rs1178032, and rs16988201) were selected (rs6132976, rs6132977 and rs6132978 were represented by rs1016753; Figure 4). Analysis of imputation (Table S2 in the Supplement) and LD pattern within the *PTPRA* locus suggested that the SNPs selected for follow up capture all ungenotyped SNPs which increase the risk of developing schizophrenia. In the replication, only rs1016753 showed significant association (p=0.04), with the same direction of association (Breslow-Day P=0.218). Pooled analysis of 1st and 2nd (1420 cases, 1377 controls) showed highly significant association of this SNP with schizophrenia (p = 0.0008) (Table S3 in the Supplement).

Reduced *PTPRA* expression levels in dorsolateral and prefrontal cortex from schizophrenia patients

As an independent approach to explore a possible involvement of *PTPRA* in schizophrenia, we examined its expression level in *post mortem* samples from patients, as compared to healthy controls and to sufferers from bipolar disorder (35 each). qPCR analysis (Fig. 5) showed that *PTPRA* expression was significantly reduced in dorsolateral prefrontal cortex from SZ patients (13 % decrease; p=0.018), with trend level reductions in samples from patients with bipolar disorder (p=0.078).

DISCUSSION

This study was prompted by implications of *Ptpra* in developmental processes linked to schizophrenia (neuronal migration, myelination); by RPTPα acting as a signaling subunit for cell adhesion molecules (NCAM and CHL1) whose genes have been related to SZ risk; and by the mapping of a schizophrenia locus close to *PTPRA*. The avenues we explored provide independent lines of convergent evidence linking RPTPα to SZ: typical changes in neuropsychological parameters in RPTPα-deficient mice; association of the human gene with disease risk; and reduced cortical *PTPRA* expression in SZ patients.

Behavioral characteristics of *Ptpra*$^{-/-}$ mice relevant to SZ

We demonstrate that *Ptpra* LOF is associated with enhanced methamphetamine responsiveness (Fig. 1A), defective sensorimotor gating as measured by PPI (Fig. 1B), and failure to habituate to a startle response (Fig. 1E). All these endpoints implicate a schizophrenia-like profile based on current clinical knowledge. The deficits could not be accounted for by obvious sensorimotor deficits, since *Ptpra*$^{-/-}$ mice did not display differences in motility, rotarod and beam walk tests, or initial startle response.

The enhanced response of *Ptpra*$^{-/-}$ mice to MAMPH suggests an augmented dopaminergic system (40). In a previous study (55), Skelton *et al*. failed to detect an altered amphetamine response in a different *Ptpra*$^{-/-}$ strain; this negative result may reflect a different dosing regime (we used 2 mg/kg whereas Skelton *et al*. used 1 mg/kg), or, more likely, a less uniform genetic background. We backcrossed our *Ptpra*$^{-/-}$ allele ten times into inbred C57Bl/6J. By contrast, the founder animals of Skelton *et al*. were crossed into outbred Black Swiss mice (32, 55); the ensuing higher genetic heterogeneity may have made the change in MAMPH responsiveness associated with loss of *Ptpra* function difficult to detect. In the absence of studies on the effect of *Ptpra* ablation on dopamine receptor expression, agonist binding, or activity, it seems premature to speculate about the mechanism of the MAMPH effect. Strikingly, haloperidol-induced catalepsy requires the *Fyn* gene, and this drug activates the FYN kinase in striatum (56). As Fyn is a well-established RPTPα target (25,

29, 32, 33), defective Fyn activation in absence of RPTPα may fail to inhibit striatal dopamine signaling.

Contrasting with the increased MAMPH responsiveness in *Ptpra*$^{-/-}$ mice, we observed no effect of *Ptpra* status on sensitivity to the glutamate antagonist MK-801. Whilst somewhat surprising given links between RPTPα and NMDA-R (30, 31), our negative finding may merely be a function of the selected dose (0.2 mg/kg), since no hyperactive response was seen in the control mice either. In our hands, 0.2 mg/kg MK-801 reproducibly induces hyperactivity in outbred NMRI mice (not shown). Therefore, pending further dose exploration in the *Ptpra*$^{-/-}$ mice, we suggest the failure to detect changes in MK-801 responsiveness is equivocal.

2.5 months old *Ptpra*$^{-/-}$ mice show a pronounced PPI deficit (Fig. 1B). “Inhibitory failure” revealed by defective PPI is considered a correlate of defects in acute attention and gating associated with psychiatric diseases, including schizophrenia. As an endophenotype, PPI is decreased in non-affected relatives of SZ patients, suggesting it may be a proximal indicator of genetic susceptibility (39). Interestingly, the PPI deficit in *Ptpra*$^{-/-}$ mice did not persist when the same mice were re-tested at 5 months of age (Fig. 1D). This restriction of the deficit to early adulthood suggests involvement of compensatory changes with aging. Since knockout of *Ptpra* alone cannot sustain this phenotype, *Ptpra*$^{-/-}$ mice may provide a point-of-entry to identify genetic or environmental parameters that will specify PPI extinction or exacerbation. Such studies may provide insights into factors and processes that determine SZ prognosis.

The startle response in mice displays plasticity not only in terms of gating, but also in terms of habituation. Whereas *Ptpra*$^{-/-}$ and WT mice had an equivalent startle response at 2.5 months (Fig. 1C), we observed habituation by 5 months in WT but not *Ptpra*$^{-/-}$ mice (Fig. 1E). This finding is interesting since schizophrenics also have a deficit in habituation, for example, the eye blink reflex in response to auditory stimuli (57). A deficit in pre-attentive inhibitory mechanisms to extraneous information is thought to underlie an altered habituation response in schizophrenia (42).

We found no deficit in Morris water maze acquisition or memory in *Ptpra*$^{-/-}$ mice. Here again, our findings seem to differ from Skelton *et al*. (55) who did report such a deficit. The different genetic background may again constitute a first possible explanation. In addition, Skelton *et al.* report water maze defects only under very particular conditions (platform in the NE, but not in the SW quadrant); this interaction between spatial environment and genotype may primarily reflect subtle effects of *Ptpra* status on sensitivity to spatial cues or on orientation skills. Interestingly, *Ptpra*$^{-/-}$ mice display defective learning in a radial arm water maze test (27), which may reflect the higher sensitivity, and/or increased requirement for short-term and working memory of this assay. Taken together, the present findings and those of Skelton *et al*. (55) and Petrone *et al*. (27) indicate that the hippocampal system is not overly impacted by *Ptpra* LOF, in spite of the architectural abnormalities in hippocampus resulting from radial migratory dysfunction in *Ptpra*$^{-/-}$ mice (27).

Genetic association of *PTPRA* with SZ

KO studies can be confounded by flanking markers from back- or outcrossing, or by inadvertent consequences of genome manipulation unrelated to changes in *Ptpra* function (e.g. altered expression of known or unknown flanking genes). A strong case can be made for a direct link between *Ptpra* and the observed phenotypes. Two independent *Ptpra*$^{-/-}$ mice both reveal effects of *Ptpra* knockdown on NMDA-R phosphorylation (27, 31); an electrophysiological study shows rescue of the NMDA-R gating defect in *Ptpra*$^{-/-}$ cells by RPTPα expression, and mimicking of the defect by antibodies against RPTPα (30). The two

lines also show similar effects on SFK-dependent pathways, which can also be rescued by RPTPα expression (33, 58, 59), or mimicked by RPTPα knockdown (29, 60, 61). Indeed, many *Ptpra*$^{-/-}$ phenotypes can be clearly linked to deregulation of the 2 best-established RPTPα substrates, the tyrosine kinases c-Src and Fyn (e.g. (22, 62)). Our finding impaired oligodendrocyte marker expression in *Ptpra*$^{-/-}$ mice is again consistent with studies using an independent *Ptpra* LOF allele and different assays (29), and with Fyn dysfunction (63).

The collective mouse evidence thus makes *PTPRA* a valid candidate for follow-up study in humans. Accordingly, we report highly significant association of a SNP in *PTPRA* with schizophrenia in a Japanese population. The sample size (~ 2600) is enough to detect mild to moderate effects of SNPs, and the evidence of *PTPRA* association is robust, since the two-stage analysis reduces the potential for type I error. Based on LD analysis in the 1st stage, we selected rs1016753 as a representative SNP for rs6132976, rs6132977 and rs6132978. Therefore, the association of rs1016753 might reflect possible association of these or other linked SNPs. Since the LD structure of *PTPRA* is relatively loose, we cannot narrow down the associated region to identify the "true" SNPs. Unbiased genome-wide association studies searching for genetic SZ risk determinants have failed to implicate the *PTPRA* locus; our focus on a particular population may have lowered the detection threshold, for environmental or genetic reasons.

It remains premature to speculate about the relevance of rs1016753 or linked SNPs for *PTPRA* function. *PTPRA* expression and the choice among alternative splicing events can be surveyed. Based on exon array data (not shown), we performed QPCR on immortalized lymphoblastoid cell lines derived from 48 participants in the association study (43 CC carriers, 5 CG carriers), using primers directed against exons specific to each of the 3 *PTPRA* transcripts described by NCBI. This revealed significantly increased expression of the NM_080840.2 transcript (as defined by its "exon 1" with physical position Chr20: 2,802,142–2,802,406 based on NCBI B36 assembly), but not of the NM_002836.3 and NM_080841.2 transcripts, in CG as compared to CC carriers (Table S4 in the Supplement). Unfortunately, we were unable to perform a similar analysis on the human brain samples used for figure 5, due to the low minor allele frequency in this cohort. At the protein level, the effect of rs1016753 allelic status and altered NM_080840.2 expression on the balance between two RPTPα isoforms with known differences in biological activity (64, 65) can also be a subject for further inquiry.

Molecular pathways involving *PTPRA* relevant to SZ

RPTPα is a signaling mediator for surface molecules that are themselves devoid of catalytic activity, most prominently integrins and canonical CAMs. Among the latter, there are reports for *cis* association and signaling functions of RPTPα in the context of TAG-1 (50), contactin (66), NCAM (22), and the CHL1/NB-3 complex (24).

Adult *Ptpra*$^{-/-}$ mice show a decrease of oligodendrocyte lineage gene expression. Further *in vivo* studies will be needed to establish whether this effect is primary or degenerative, and whether it is autonomous to the OLG lineage. The Pallen group recently reported decreased MBP protein levels in the brain of P18 *Ptpra*$^{-/-}$ mice, and provided strong evidence for a lineage-autonomous role for RPTPα in oligodendrocyte differentiation *in vitro*, with deficient Fyn activation as a plausible mechanism (29, 53). Interestingly, oligodendrocyte Fyn integrates signaling in a complex between contactin-1 and integrins (67), i.e. between members of 2 classes of cell surface molecules that rely on RPTPα for signal transduction (58, 66). Abnormalities in oligodendrocyte function are a robust biological marker of human schizophrenia (48, 49), but elucidation of links between myelination and the disease still remains more a matter of speculation than of hypothesis testing. A broad question is how to link the white matter abnormalities in patients to the as yet more clinically relevant

pharmacological evidence of neurotransmitter pathway dysfunction. The *Ptpra*$^{-/-}$ model may play a valuable role in exploring this issue. More specifically, manipulation of the *Ptpra gene* will be useful to explore to what extent the neurobehavioral abnormalities result from loss of *Ptpra* function in the neuronal or oligodendroglial lineage, and whether or how *Ptpra* dysfunction in one lineage may impact other lineages, and neurotransmitter systems.

CAMs are linked to NMDA neurotransmission, dysfunction of which is also linked to SZ. Long-term potentiation (LTP) at CA3-CA1 excitatory synapses is reduced in *Chl1*$^{-/-}$ mice (68) and in a hippocampal-specific NCAM knockout (69); NMDA-mediated behavioral alterations have also been observed in these mice (69, 70). A recent study links NCAM poly-sialylation to NMDA-R signaling (71). Absence of the NCAM isoform NCAM180 leads to increased lateral ventricle size, one of the most reliable morphological features in brains of schizophrenics, and is often accompanied by cognitive impairments (70). Association studies implicate *NCAM* and *CHL1* in human SZ risk, and LOF of the corresponding genes in mice engenders intriguing phenotypic overlaps with *Ptpra* LOF, in terms of cortical radial migration (72), dendrite orientation (24), impaired LTP (69), and impaired sensorimotor gating/PPI (73). Thus, phenotypes observed in *Ptpra*$^{-/-}$ mice could be mediated by the effect of RPTPα on these molecules.

The best characterized substrate and effector for RPTPα in NCAM- and CHL1/NB-3 signaling complexes is the SFK Fyn. That RPTPα is a net activator of Fyn kinase activity (32, 33) would be consistent with phenotypic overlap between LOF in either gene. Like *Ptpra*$^{-/-}$ mice (27, 29), *Fyn*$^{-/-}$ mice exhibit abnormal long-term potentiation, spatial learning, radial migration, myelination (62, 74), and myelin gene expression (51). Genetic association of *FYN* with SZ was reported as absent (75), although there are positive data about prefrontal function in patients (76). Interestingly, *Fyn* is required for haloperidol signaling in striatal neurons (56), and platelets from SZ patients show decreased expression and altered *FYN* splicing (77). Fyn also phosphorylates NMDA-R subunits (52). Phosphorylation of NMDA-R subunits is reduced in *Ptpra*$^{-/-}$ mice, and RPTPα associates with and controls gating of NMDA-R (27, 30, 31). Thus, reduced RPTPα function could contribute to a schizophrenic phenotype through impairment of Fyn activity.

Taken together, one can envision a SZ-relevant pathway as NCAM/CHL1-NB3 → RPTPα → Fyn → NMDA-R. However, this is mainly a highly speculative working hypothesis. Not only are the links between Fyn and SZ relatively tenuous, there are also important phenotypic differences between *Fyn*$^{-/-}$ *and Ptpra*$^{-/-}$ mice (e.g. in hippocampal structure), RPTPα can act on other SZ-relevant SFKs (including c-Src (14)), and RPTPα directs SFKs towards only a subset of their substrates (25).

Our findings also warrant consideration of cross-talk of RPTPα with the NRG1 - ERBB4 pathway. RPTPα can affect ERBB1 signaling (25, 78), and we find that *Ptpra* ablation results in reduced *Erbb4* expression (Figure 3). NRG1/ERBB4 signaling suppresses upregulation of NMDA-R by c-Src (14). *Nrg1*$^{+/-}$ mice show reduced Fyn/Pyk2-mediated phosphorylation of Y1472 in the NR2B subunit of NMDA-R, that can be rescued by the antipsychotic clozapine (13); it remains to be seen whether clozapine can reverse the reduced phosphorylation of Fyn and NR2B and the abnormal behavior in *Ptpra*$^{-/-}$ mice.

The convergent evidence reported here linking RPTPα to schizophrenia can allow enunciation of novel hypotheses and open up avenues for modeling and dissection of the disease mechanism that may yield clues for therapeutic exploration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Research funded by the Lundbeck Foundation and by *Vera og Carl Johan Michaelsens legat* (JS). TS and JDB acknowledge the support of the NIMH through a Conte Center grant MH066392 and the Stanley Foundation. TS was supported by the New York State spinal cord injury research program. NT appreciates the Mitsubishi Pharma Research Foundation for their support. BA and NO acknowledge the support of the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Core Research for Evolutional Science and Technology.

JS gratefully acknowledges Kjeld Møllgård and Niels-Henrik Holstein-Rathlou at the University of Copenhagen for support. We thank Bente Møller and Tanya Yankelevich for genotyping. The invaluable support of Lisa Gasperini, Giuseppe Legname, Marco Stebel, Yotis Senis, and Mauro Torti with mouse colony management is also greatly appreciated.

Abbreviations

CAM	cell adhesion molecule
GWAS	Genome-wide association study
LD	linkage disequilibrium
LOF	loss of function
MAMPH	Metamphetamine
MBP	myelin basic protein
NMDA-R	NMDA-receptor
OMR	oligodendrocyte- and myelin-related
PPI	pre-pulse inhibition
PTP	protein tyrosine phosphatase
RPTP	receptor protein tyrosine phosphatase
SFK	Src-family kinase
SNP	single nucleotide polymorphism
SZ	schizophrenia
veh	vehicle

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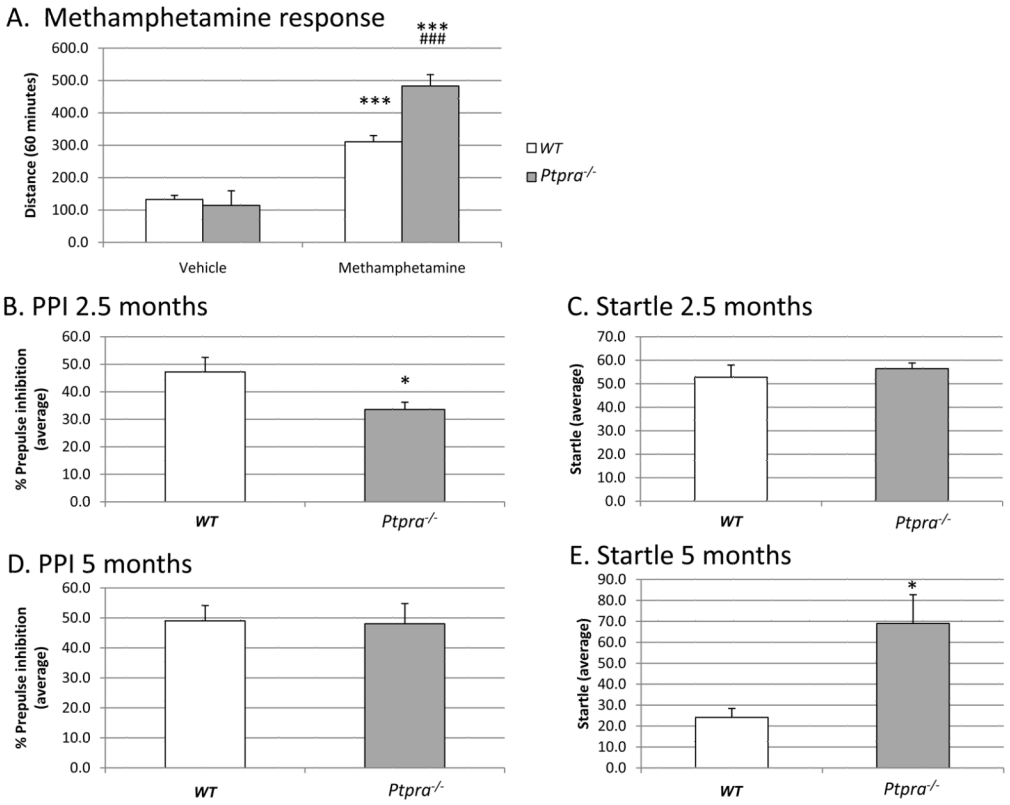

Figure 1. Metamphetamine sensitivity, pre-pulse inhibition (PPI), and startle response in Wt and *Ptpra*$^{-/-}$ mice

1A. *Influence of genotype on locomotor response to metamphetamine challenge.* Methamphetamine resulted in pronounced hyperactivity in both genotypes ($p<0.001$ *vs.* veh (wt) and veh (*Ptpra*$^{-/-}$) respectively). However, the locomotor response to methamphetamine was exaggerated in *Ptpra*$^{-/-}$ as compared to WT mice ($p<0.001$, MAMPH (wt) *vs*. MAMPH (*Ptpra*$^{-/-}$)). The study was run as a within-subject design, where each individual mouse served as its own control by injecting them with either vehicle, MAMPH (2 mg/kg) or MK801 (0.2 mg/kg, data not shown, see main text) in a semi-randomized order ensuring representation of all treatment groups on each test day over 3 days. Compounds were dosed i.p. immediately before test start, n=7–8 (*Ptpra*$^{-/-}$) and 8–9 (WT). The animals were 3.5 months old at time of testing. Data are represented as mean distance travelled (± S.E.M) over 60 minutes. Statistical evaluation was performed by applying two-way ANOVA with genotype and drug as factors followed by Fishers LSD test for multiple comparisons. (***: $p<0.001$ *vs*. Veh (*Ptpra*$^{-/-}$) and veh (WT) respectively) (###: $p<0.001$ *vs*. WT-methamphetamine).

1B. *Effect of genotype on prepulse inhibition of the acoustic startle response at 2.5 months of age. Ptpra* gene disruption leads to reduced prepulse inhibition of the acoustic startle response as compared to wt mice at the age of 2.5 months ($p<0.05$). Data from 4 different prepulse intensities (pp. 4, pp 8, pp 16 and pp 24) are collapsed and expressed as mean ± S.E.M, n=7 (*Ptpra*$^{-/-}$) and 8 (WT). Statistical evaluation was performed by applying two-way ANOVA with genotype and sex as factors (*: $p<0.05$ *vs*. WT).

1C. *Effect of genotype on acoustic startle response at 2.5 months of age.* No effect of *Ptpra* gene disruption is seen on the startle response to a 120 dB noise burst at the age of 2.5 months of age as compared to wt mice ($p>0.05$). Data are expressed as mean ± S.E.M, n=7 (*Ptpra*$^{-/-}$) and 8 (WT). Statistical evaluation was performed by applying two-way ANOVA with genotype and sex as factors.

1D. *Effect of genotype on prepulse inhibition of the acoustic startle response at 5 months of age.* At 5 months of age the reduction of prepulse inhibition noted at 2.5 months of age was no longer evident in *Ptpra*$^{-/-}$ mice ($p>0.05$ *vs*. WT) indicating a critical time period for manifestation of this phenotype. Data from 4 different prepulse intensities (pp. 4, pp 8, pp 16 and pp 24) are collapsed and expressed as mean ± S.E.M, n=7 for both genotypes. Statistical evaluation was performed by applying two-way ANOVA with genotype and sex as factors.
1E. *Effect of genotype on acoustic startle response at 5 months of age.* At 5 months of age, *Ptpra*$^{-/-}$ mice displayed an increased startle response to a 120 dB noise burst as compared to wildtype mice ($p=0.013$). This difference is due to a significantly reduced startle response in wildtype mice at the age of 5 months as compared to 2.5 months ($p=0.014$). This habituated response to a startle inducing stimulus is not evident in *Ptpra*$^{-/-}$ mice, since the startle response at 5 months of age is similar to that at 2.5 months of age ($p=0.914$). Data are expressed as mean ± S.E.M, n=7 (*Ptpra*$^{-/-}$ and WT). Intergroup comparisons were performed by applying two-way ANOVA with genotype and sex as factors. Intragroup comparisons were performed by applying one way repeated measure ANOVA with age and genotype as factors. (*: $p<0.05$ *vs*. WT)

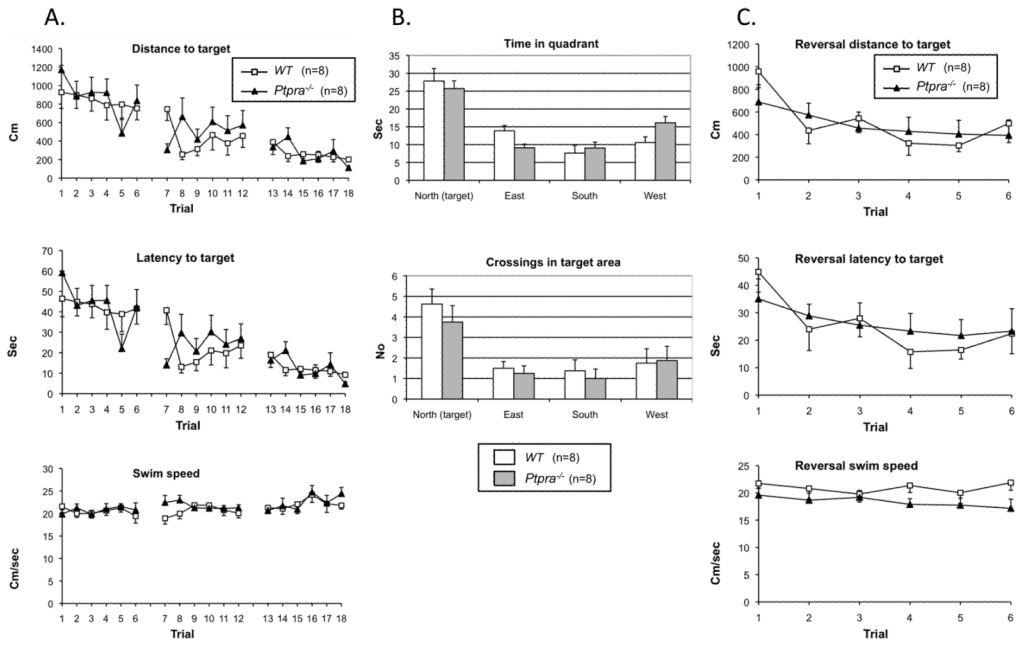

Figure 2. Morris water maze analysis of Wt and *Ptpra*$^{-/-}$ mice

2A. *Training: swim distance to target, escape latency and swim speed.* Both wildtype (n=8) and *Ptpra*$^{-/-}$ (n=8) used shorter distances to locate the hidden platform over trials. No effect of genotype or genotype × trial interaction on distance to platform, escape latency or swim speed was found. One-way RM ANOVA wildtype: $F(17,119)=4,982$; $p<0.001$ and *Ptpra*$^{-/-}$: $F(17,119)=5.477$; $p<0.001$). Two-way RM ANOVA found no effect of genotype for distance ($F(1,238)=0.803$; $p=0.385$) and no genotype × trial interaction ($F(17,238)=1.231$; $p=0.241$).

2B. *Probe test: time in quadrants and crossings in platform area*. Both genotypes spent significantly longer time in the northern quadrant where the platform used to be located than in other quadrants, and had more crossings in the area previously occupied by the platform than in areas of identical size and position in the other quadrants.

Time spent in northern quadrant compared with other quadrants ($P=0.006$ or less two-way RM ANOVA, Fisher LSD *post hoc*); no effect of genotype ($F(1,42)=1.340$; $p=0.266$) or genotype × quadrant interaction ($F(3,42)=1.801$; $p=0.162$).

Number of crossings in the area where the platform was during training (D=9 cm) compared with crossings in areas of equal size and position in the other quadrants ($F(3,42)=9.199$; $p<0.001$); no effect of genotype ($F(1,42)=1.317$; $p=0.27$) or genotype × quadrant interaction ($F(3,42)=0.203$; $p=0.894$). *Post hoc* analysis revealed significantly more crossings in the target area for both groups ($p=0.047$ or less) compared to crossings in areas equal in size and position in the other quadrants.

2C. *Reversal learning: swim distance to target, escape latency and swim speed during reversal learning*. Two-way RM ANOVA on distance to target showed significant effect of trial ($F(5,70)=3.383$; $p=0.009$) but no effect of genotype ($F(1,70)=0.0591$; $p=0.811$) or genotype × trial interaction ($F(5,70)=0.757$; $p=0.584$). No effect of genotype or genotype × trial interaction was found on escape latency or swim speed.

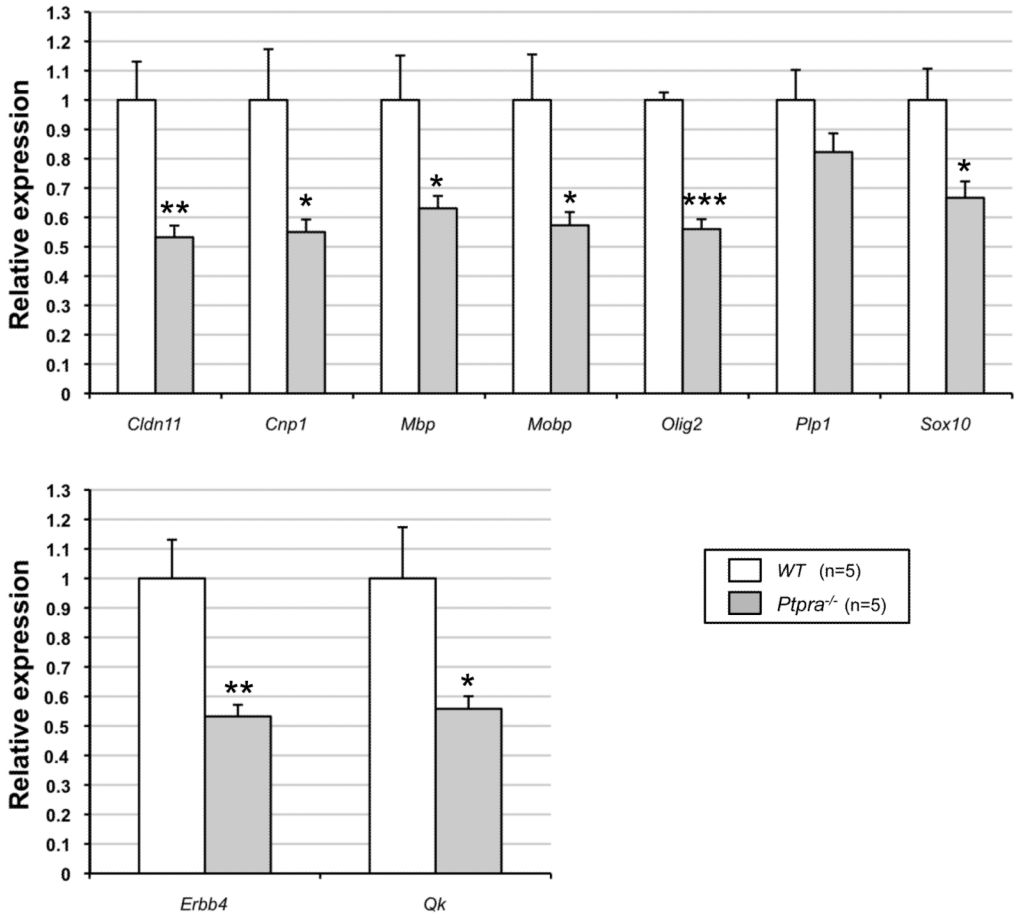

Figure 3. Reduced expression of oligodendrocyte- and myelin-related (OMR) gene expression in total brain of *Ptpra*$^{-/-}$ mice

5 month old animals (5 males/genotype) were analyzed by qPCR. Four endogenous control genes (*Actb*, *B2m*, *Gusb*, and *Ppia*) were used for normalization.
(*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ *vs.* wt)

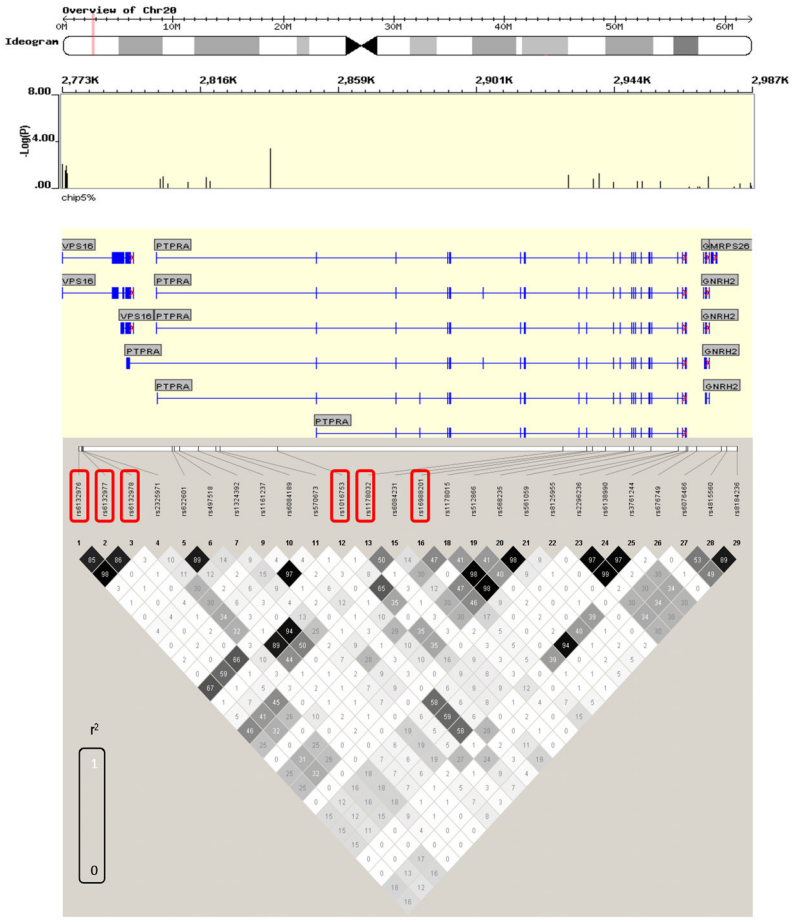

Figure 4. Genetic association of SNPs around and in the *PTPRA* gene with schizophrenia

Red boxes indicate nominally associated SNPs in the 1st stage analysis (GWAS screening sample). r^2 is the correlation coefficient between the two loci. The numbers are correlation coefficients calculated based on the GWAS sample.

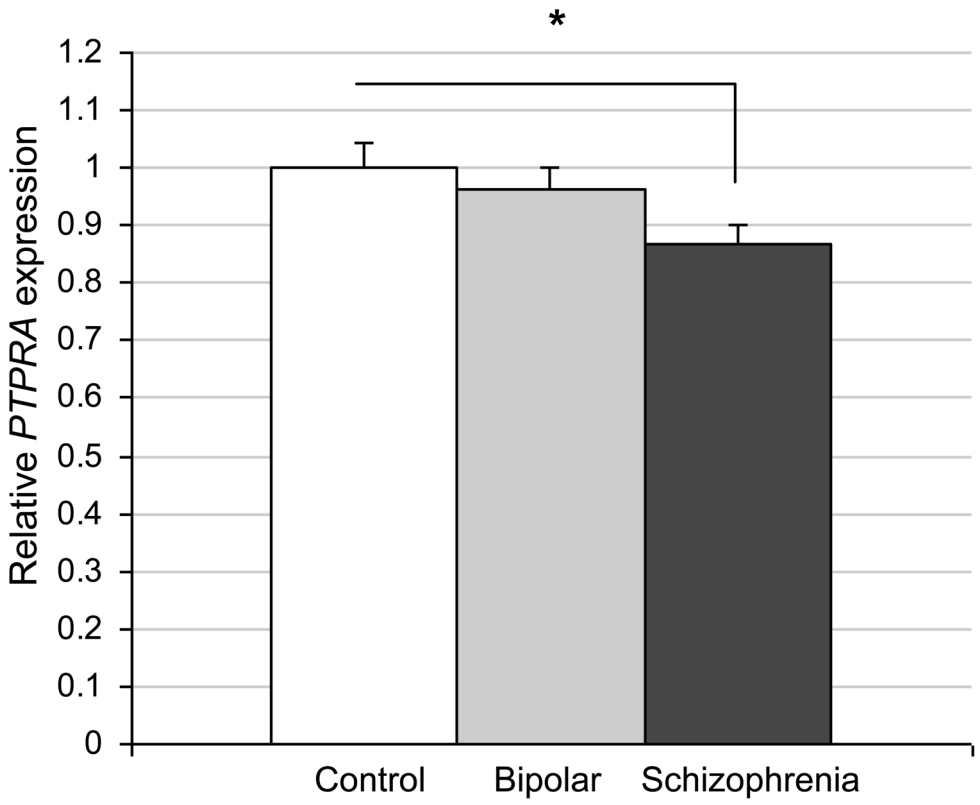

Figure 5. Reduced *PTPRA* expression levels in dorsolateral prefrontal cortex specimens from schizophrenia patients

35 patient samples for each category (healthy controls, patients with schizophrenia, and patients with bipolar disorder) were analyzed by qPCR. Four endogenous control genes (*ACTB*, *GAPD*, *GUSB*, and *PPIA*) were used for normalization (*: $p < 0.05$ *vs*. control).