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SHORT COMMUNICATION

Differential expression of HINT1 in schizophrenia brain tissue

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Abstract Recent findings in the literature suggest a relation between histidine triad nucleotide-binding protein-1 (HINT1) and psychiatric disorders such as major depression, anxiety, and schizophrenia, although its physiological roles are not completely comprehended. Using Western blot, we compared HINT1 protein expression in the postmortem dorsolateral prefrontal cortex and thalamus of schizophrenia patients and healthy controls for contributing to elucidate the role of HINT1 in schizophrenia pathophysiology. HINT1 was found to be downregulated in the dorsolateral prefrontal cortex and upregulated in the thalamus. Our results combined to previous studies in human samples and preclinical models support the notion that HINT1 must be more explored as a potential target for psychiatric disorders.

Keywords Schizophrenia · HINT1 · Histidine triad nucleotide-binding protein-1 · Western blot · Proteomics · Brain

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Histidine triad nucleotide-binding protein-1 (HINT1) is a member of the histidine triad protein (Hit) family that by definition presents the conserved sequence motif HisX-HisXHis ('X' = hydrophobic amino acid). HINT1 has nucleotidyl hydrolase and transferase activities and is widely expressed in a number of tissues including liver, kidney, and brain [1]. Although HINT1 was originally described as a protein kinase C (PRKC) inhibitor, this function was not confirmed in later studies. Other roles that have been attributed to HINT1 are inhibition of several pathways that control transcriptional processes and tumor susceptibility and suppression [2].

There is also evidence that HINT1 plays a role in psychiatric disorders such as schizophrenia, bipolar disorder, major depression, and anxiety. Recently, anxiety-like behavior has been observed in Hint1 knockout (KO) mice [3–5], and Hint1 expression in brain areas related to mood disorders has been reported [6]. Moreover, HINT1 protein has been found to be downregulated in fetal brains of Down syndrome patients [7].

The HINT1 gene is part of the 5q31.2 genetic locus, which has been associated with schizophrenia. In addition to sex-specific genetic association between HINT1 and schizophrenia [8], gene variants of HINT1 were recently found to be associated with nicotine dependence, which is frequently comorbid with schizophrenia [9]. Interestingly, lower levels of HINT1 mRNA were found in the dorsolateral prefrontal cortex (DLPFC) of three pools of 15 patients with schizophrenia when compared to three pools of 15 matched controls confirming their own previous findings [10]. Authors have even discussed the potential role of HINT1 in cellular stress and apoptosis along with other proteins such as MAPK14, TNFRSF6, NAIP, and UBE2N, which were also found differentially expressed [10].

In the present study, we have analyzed HINT1 protein expression in the mediodorsal thalamus and the DLPFC from schizophrenia patients lending further support to the protein's role in schizophrenia pathophysiology.

Human brain samples were collected postmortem from the DLPFC (BA46) of 9 chronically treated schizophrenia patients and 7 controls and from the mediodorsal thalamus of 11 patients and 8 controls (Table 1). The thalamus acts as a relay center organizing and redistributing stimuli from several brain regions. Moreover, it is involved with auditory, somatic, visceral, gustatory, and visual systems as well as regulation of sleep states, wakefulness, and consciousness [11]. Considering the significant alterations found in cerebral metabolic activity and related circuitry by functional magnetic resonance imaging and positron emission tomography, the thalamus is hypothesized to be a key structure in SCZ [12]. The mediodorsal thalamus specifically acts as a central relay station by transferring peripheral sensory inputs to the cortex. Therefore, it is interesting to analyze the DLPFC as a complement. In addition, cognitive deficits and anomalous cytoarchitecture of the PFC are associated with schizophrenia [13]. Patients' samples were from the Mental State Hospital, Wiesloch, Germany, where patients had been receiving treatment. SCZ diagnosis according to the DSM-IV criteria (American Psychiatric Association [14]) had been made antemortem by a psychiatrist. All patients were residual schizophrenics with chronic paranoid episodes. The antipsychotic treatment history of each patient was assessed by examining the medical charts and calculated as chlorpromazine equivalents (CPE) using the algorithm developed by Jahn and Mussgay [15] for typical neuroleptics and clozapine, while CPE for olanzapine was calculated using the mean doses [16]. Controls' samples were from the Institute of Neuropathology, Heidelberg University, Heidelberg, Germany. The controls had not suffered from psychiatric or neurological disorders, somatic diseases, or brain tumors and had never been treated with antidepressant or antipsychotic medications. Clinical records were collected from relatives and general practitioners. Brain samples from patients and controls were dissected by an experienced neuropathologist and deep-frozen immediately after collection. All patients and controls were German Caucasians and had no history of alcohol or drug abuse or any severe illness. The samples were submitted for neuropathologic characterization to rule out associated neurovascular or neurodegenerative disorders. The classification according to Braak was stage II or less for all subjects [17, 18]. There were no significant differences between patients and controls for age, PMI, or pH as previously shown [19, 20]. All assessments and postmortem evaluations and procedures were approved by the ethics committee of the Faculty of Medicine of Heidelberg University, Heidelberg, Germany.

Fifty milligram of DLPFC and thalamus was homogenized separately in 200 µl of 7 M urea, 2 M thiourea, 4% CHAPS, 2% ASB-14, and 70 mM DTT buffer and quantified by Bradford assay as previously described in detail [21]. For Western blot, 15 µg of total protein from DLPFC and thalamus samples was run on a 12% SDS minigel and subsequently transferred to PVDF membranes (100 V for 1 h). Membranes were treated with 5% Carnation non-fat dry milk in TBS-T for 4 h, rinsed in TBS-T 3 times for 20 min, and incubated with a rabbit anti-Hint1 antibody (ABCam) at a 1:1,000 dilution in TBS-T overnight at 4°C. After incubation, membranes were washed thrice with TBS-T for 15 min. Membranes were incubated with anti-c-MYC-peroxidase antibody for 40 min at RT, then washed with water, incubated with ECL mixture for 1 min, and exposed to ECL film. Films were developed and subsequently scanned, and the band signals (optical densities) were assessed using *QuantityOne* software. The significant changes of individual samples between schizophrenia patients and controls were determined using Mann–Whitney tests. Even with the limited number of samples analyzed, we used SPSS 15 for Windows to analyze correlations between the patients' and healthy controls' Western blots densitometry data and their respective sociodemographic data and antipsychotic medication in chlorpromazine equivalents as described previously [20] aiming to detect whether external interferences might have led to protein expression differences not related to schizophrenia.

Figure 1 shows that schizophrenia patients have lower HINT1 protein levels in the DLPFC compared with controls, thus confirming earlier mRNA analyses [10] and lending support to the notion of potential biomarker candidacy [22] for HINT1 in schizophrenia. Contrary to the DLPFC results, we observed higher HINT1 protein levels in the thalamus of schizophrenia patients compared with controls (Fig. 1). We correlated the two measurements from different brain regions from the same subjects using Spearman's rank correlation (nonparametric) coefficient. As shown in Fig. 2, there is a significant positive correlation of HINT1 expression between both brain regions (Spearman $r = 0.9286$, $p = 0.0022$). No correlations were found between HINT1 expression and antipsychotic administration, duration of disease, and age at onset. Apparently, HINT1 protein expression shows distinct regulations in different brain regions, a finding that is not unexpected for a tissue of this complexity where the distribution of excitatory or inhibitory interneurons differs depending on the region. While studying the proteome of schizophrenia brain tissue, we found an upregulation of PRKC gamma (PRKCG) in DLPFC and thalamus [19, 20], which might support HINT1–PRKC interaction, although this cannot be confirmed without further experiments.

Table 1 Clinical data of patients and controls used for dorsolateral prefrontal cortex and mediodorsal thalamus analyses

Sample ID	Analyzed in	Case	Age (years)	Gender	PMI (h)	pH values	Duration of disease (years)	Duration of medication (years)	atyptyp	CPE last dose	CPE last 10 years	Cause of death	DSM-IV	Age at Onset	Last medication	Cigarettes	Alcohol	Hosp (years)	ECT
13/00	DLPFC and thalamus	SCZ	64	F	11	6.7	48	45	3	1,536	7.7	Pulmonary insufficiency	295.6	16	Clozapine 500 mg, Haloperidol 40 mg, Ciatyl 40 mg	0	No	21	Yes
36/02	DLPFC and thalamus	SCZ	73	M	20	6.6	43	40	1	507.4	1.7	Heart infarction	295.6	30	Perphenazine 32 mg, Promethazine 150 mg	30/day	No	33	No
39/02	DLPFC and thalamus	SCZ	43	M	18	6.9	22	20	2	464	2.6	Heart infarction	295.6	20	Zuclopenthixol 40 mg, Valproate 1,200 mg, Tiapride 300 mg	0	No	13	No
39/03	DLPFC and thalamus	SCZ	77	F	32	6.5	49	48	2	2,555	8.3	Lung embolism	295.6	28	Clozapine 400 mg, Benperidol 25 mg, Chlorprothixen 150 mg	0	No	48	Yes
43/03	DLPFC and thalamus	SCZ	76	F	17	6.8	49	47	1	300	4.9	Cardio-pulmonary insufficiency	295.6	27	Perazine 300 mg	0	No	30	Yes
46/00	DLPFC and thalamus	SCZ	63	F	31	6.8	40	30	3	75	1.8	Heart infarction	295.6	24	Olanzapine 15 mg	30/day	No	30	Yes
75/02	DLPFC and thalamus	SCZ	92	F	37	6.9	51	48	1	100	3.4	Cardio-pulmonary insufficiency	295.6	41	Prothipendyl 160 mg, Perazine 100 mg	0	No	51	No
83/01	DLPFC and thalamus	SCZ	71	M	28	6.4	40	35	1	782.4	10	Heart infarction	295.6	30	Haloperidol 32 mg, Pipamperone 40 mg	40/day	No	12	No
48/00	Thalamus	SCZ	51	M	7	6.1	25	25	1	147	0.6	Heart infarction	295.6	19	Flupenthixol 15 mg	30/day	No	20	No
01/00	Thalamus	SCZ	51	M	12	6.7	28	25	2	450	1.8	Heart infarction	295.6	23	Clozapine 500 mg	30/day	No	17	No

Table 1 continued

Sample ID	Analyzed in	Case	Age (years)	Gender	PMI (h)	pH values	Duration of disease (years)	Duration of medication (years)	atyptyp	CPE last dose	CPE last 10 years	Cause of death	DSM-IV	Age at Onset	Last medication	Cigarettes	Alcohol	Hosp (years)	ECT
35/00	Thalamus	SCZ	64	F	23	6.6	41	40	2	54.5	4.6	Heart infarction	295.6	24	Zotepine 150 mg, Olanzapine 10 mg	20/day	No	5	Yes
50/01	DLPFC	SCZ	81	M	4	7.1	62	50	1	92.8	1.4	Cor pulmonale, heart insufficiency	295.6	19	Haloperidol 4 mg Prothypendyl 80 mg	20/day	No	48	No
02/02	DLPFC and thalamus	Control	41	M	7	6.5						Heart infarction				0	No		
43/01	DLPFC and thalamus	Control	91	F	16	6.7						Cardio-pulmonary insuffiency				0	No		
50/02	DLPFC and thalamus	Control	69	F	96	6.4						Lung embolism				0	No		
51/02	DLPFC and thalamus	Control	57	M	24	6.9						Heart infarction				0	No		
57/02	DLPFC and thalamus	Control	53	M	18	7.0						Heart infarction				0	No		
59/02	DLPFC and thalamus	Control	63	M	13	6.5						Heart infarction				0	No		
61/01	DLPFC and thalamus	Control	66	M	16	6.8						Heart infarction				0	No		
73/02	Thalamus	Control	79	M	24	6.4						Heart infarction				0	No		

atyptyp Duration of atypical treatment/duration of treatment with typical neuroleptics during lifetime, *CPE* medication calculated in chlorpromazine equivalents (mg), *CPE last 10 years* the sum of medications during the last 10 years in kg, *Hosp* Hospitalization time in years, *ECT* electroconvulsive therapy

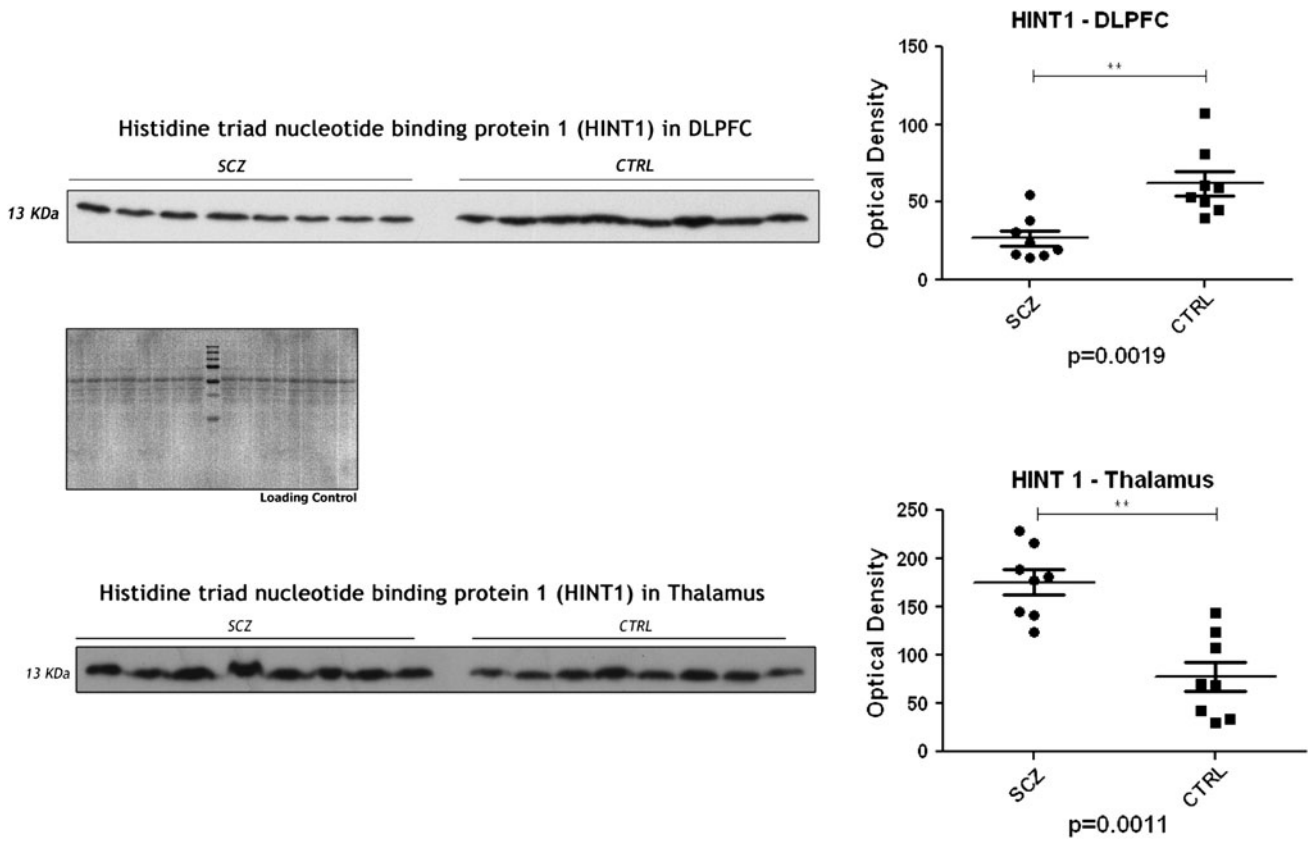

Fig. 1 Western blot analyses of HINT1 in thalamus and dorsolateral prefrontal cortex from schizophrenia (SCZ) patients and controls (CTRL). The significant changes of individual samples between schizophrenia patients and controls were determined using Mann–Whitney test

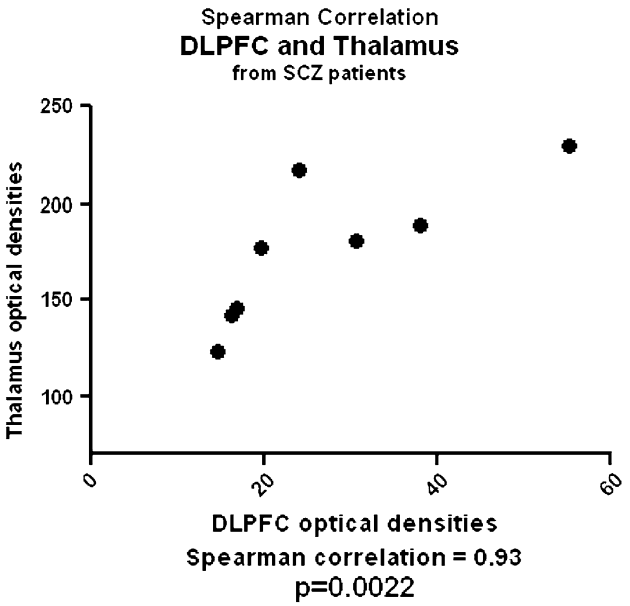

Fig. 2 Spearman Correlation between DLPFC and thalamus from SCZ patients

The importance of HINT1 for psychiatric traits has also been explored in HINT1 KO mice. Through amphetamine-sensitivity tests in HINT1 KO mice and wild-type (WT) animals, Barbier et al. found that the absence of HINT1 disturbs postsynaptic dopamine transmission rather then presynaptic dopamine transmission in striatum and nucleus accumbens. The same group also found elevated corticosterone levels in plasma of HINT1 KO mice indicating alterations in hypothalamic–pituitary–adrenal axis [4], while our group found no significant differences [5]. These findings and our data warrant further studies on HINT1–for instance including a group of drug-naïve schizophrenia patients and other tissues–to unravel its roles and related biochemical pathways in schizophrenia and psychiatric disorders.

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Conflict of interest All authors declare that they have no conflicts of interest.

References

1. Brzoska PM, Chen H, Levin NA, Kuo WL, Collins C, Fu KK, Gray JW, Christman MF (1996) Cloning, mapping, and *in vivo* localization of a human member of the PKCI-1 protein family (PRKCNH1). Genomics 36(1):151–156
2. Su T, Suzui M, Wang L, Lin CS, Xing WQ, Weinstein IB (2003) Deletion of histidine triad nucleotide-binding protein 1/PKC-interacting protein in mice enhances cell growth and carcinogenesis. Proc Natl Acad Sci USA 100(13):7824–7829
3. Barbier E, Zapata A, Oh E, Liu Q, Zhu F, Undie A, Shippenberg T, Wang JB (2007) Supersensitivity to amphetamine in protein kinase-C interacting protein/HINT1 knockout mice. Neuropsychopharmacology 32(8):1774–1782
4. Barbier E, Wang JB (2009) Anti-depressant and anxiolytic like behaviors in PKCI/HINT1 knockout mice associated with elevated plasma corticosterone level. BMC Neurosci 10:132
5. Varadarajulu J, Lebar M, Krishnamoorthy G, Habelt S, Lu J, Bernard Weinstein I, Li H, Holsboer F, Turck CW, Touma C (2011) Increased anxiety-related behaviour in Hint1 knockout mice. Behav Brain Res. [Epub ahead of print]
6. Liu Q, Puche AC, Wang JB (2008) Distribution and expression of protein kinase C interactive protein (PKCI/HINT1) in mouse central nervous system (CNS). Neurochem Res 33(7):1263–1276
7. Weitzdoerfer R, Stolzlechner D, Dierssen M, Ferreres J, Fountoulakis M, Lubec G (2001) Reduction of nucleoside diphosphate kinase B, Rab GDP-dissociation inhibitor beta and histidine triad nucleotide-binding protein in fetal Down syndrome brain. J Neural Transm Suppl 61:347–359
8. Chen Q, Wang X, O'Neill FA, Walsh D, Kendler KS, Chen X (2008) Is the histidine triad nucleotide-binding protein (HINT1) gene a candidate for schizophrenia? Schizophr Res 106:200–207
9. Jackson KJ, Chen Q, Chen J, Aggen SH, Kendler KS, Chen X (2011) Association of the histidine-triad nucleotide-binding protein-1 (HINT1) gene variants with nicotine dependence. Pharmacogenomics J. [Epub ahead of print]
10. Vawter MP, Crook JM, Hyde TM, Kleinman JE, Weinberger DR, Becker KG, Freed WJ (2002) Microarray analysis of gene expression in the prefrontal cortex in schizophrenia: a preliminary study. Schizophr Res 58:11–20
11. Steriade M, Llinás RR (1988) The functional states of the thalamus and the associated neuronal interplay. Physiol Rev 68(3):649–742
12. Andreasen NC, Arndtm S, Swayze V 2nd, Cizadlo T, Flaum M, O'Leary D, Ehrhardt JC, Yuh WT (1994) Thalamic abnormalities in schizophrenia visualized through magnetic resonance image averaging. Science 266:294–298
13. Barch DM (2005) The cognitive neuroscience of schizophrenia. Annu Rev Clin Psychol 1:321–353
14. American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Association, Washington
15. Jahn T, Mussgay L (1989) Die statistische Kontrolle möglicher Medikamenteneinflüsse in experimentalpsychologischen Schizophreniestudien: Ein Vorschlag zur Berechnung von Chlorprom-azinäquivalenten. Zeitschrift für klinische Psychologie und Psychotherapie 18:257e67
16. Meltzer HY, Fatemi SH (1998) Treatment of schizophrenia. In: Schatzberg AF, Nemeroff CB (eds) The American psychiatric text book of psychopharmacology. American Psychiatric Press, Washington, pp 127–135
17. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K (2006) Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 112(4):389–404
18. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82(4):239–259
19. Martins-de-Souza D, Gattaz WF, Schmitt A, Rewerts C, Maccarrone G, Dias-Neto E, Turck CW (2009) Prefrontal cortex shotgun proteome analysis reveals altered calcium homeostasis and immune system imbalance in schizophrenia. Eur Arch Psychiatry Clin Neurosci 259(3):151–163
20. Martins-de-Souza D, Maccarrone G, Wobrock T, Zerr I, Gormanns P, Reckow S, Falkai P, Schmitt A, Turck CW (2010) Proteome analysis of the thalamus and cerebrospinal fluid reveals glycolysis dysfunction and potential biomarkers candidates for schizophrenia. J Psychiatr Res 44(16):1176–1189
21. Martins-de-Souza D, Menezes de Oliveira B, dos Santos Farias A, Horiuchi RS, Crepaldi Domingues C, de Paula E, Marangoni S, Gattaz WF, Dias-Neto E, Camillo Novello J (2007) The use of ASB-14 in combination with CHAPS is the best for solubilization of human brain proteins for two-dimensional gel electrophoresis. Brief Funct Genomic Proteomic 6(1):70–75
22. Martins-de-Souza D (2010) Is the word 'biomarker' being properly used by proteomics research in neuroscience? Eur Arch Psychiatry Clin Neurosci 260(7):561–562