

# Reduced DTNBP1 (dysbindin-1) mRNA in the hippocampal formation of schizophrenia patients

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## Abstract

Genetic and molecular studies indicate that dysbindin-1 plays a role in the pathophysiology of schizophrenia. We examined dysbindin-1 mRNA in the hippocampal formation of patients with schizophrenia and found reduced expression in dentate granule and polymorph cells and in hippocampal field CA3, but not in CA1. Furthermore, there were positive correlations between dysbindin-1 mRNA and expression of synaptic markers known to be reduced in schizophrenia. Our results indicate that previously reported dysbindin-1 protein reductions may be due in part to decreased dysbindin-1 mRNA and that reduced dysbindin-1 may contribute to hippocampal formation synaptic pathology in schizophrenia.

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## 1. Introduction

Genetic variation in DTNBP1, which encodes dysbindin-1, is associated with schizophrenia in a number of populations (Riley and Kendler, 2006; Schwab et al., 2003; Straub et al., 2002). One mechanism whereby these alterations may confer susceptibility to schizophrenia is

via reduced dysbindin-1 expression, since risk SNPs and haplotypes appear to influence dysbindin-1 mRNA levels (Bray et al., 2003, 2005; Weickert et al., 2004). In patients with schizophrenia, reduced frontal cortex dysbindin-1 mRNA (Weickert et al., 2004) and lower hippocampal formation dysbindin-1 protein (Talbot et al., 2004) have been observed. We tested whether dysbindin-1 mRNA was reduced in the hippocampal formation of schizophrenia patients.

Abnormalities of glutamate neurotransmission, including reduction in AMPA receptors, have been consistently found in the hippocampal formation of patients with schizophrenia (Harrison and Eastwood, 2001;

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Harrison et al., 2003). We and others have found significant reductions in synaptophysin (Eastwood et al., 1994; Webster et al., 2001) and spinophilin mRNAs (Law et al., 2004) in the DGh (hilar or polymorph region of the dentate gyrus) and CA3 pyramidal cell regions. We hypothesized that synaptophysin and spinophilin mRNAs, markers of the pre- and post-synaptic terminals, respectively, may be related to dysbindin-1 mRNA expression in specific hippocampal formation subregions.

## 2. Materials and methods

### 2.1. Subjects

Mesial temporal lobes were cryostat-sectioned from postmortem brains (Law et al., 2004; Webster et al., 2001). Three sections per case (average) were anatomically matched at the genu and mid-body level. Demographic and diagnostic criteria were previously published

[ $n=10$  schizophrenics and  $n=10$  normal controls (Law et al., 2004; Webster et al., 2001)]. Experimental groups were formed matching for group means in age, tissue pH and PMI [(controls = 55 y, 6.5, 24 h; schizophrenics = 50 y, 6.4, 34 h) (all  $t < 1.73$ , all  $p > 0.10$ )]. Previous studies of cyclophilin, a control mRNA, showed no difference in expression between the schizophrenics and controls in this cohort (Law et al., 2004). Neuroleptic medication for each subject with schizophrenia was recorded after clinical chart review and doses were converted to milligram chlorpromazine (CPZ) equivalents. Neuroleptic exposure was measured as: 1) last recorded neuroleptic dose before death (last CPZ); 2) median daily neuroleptic dose for all medications prescribed during a subject's life (daily CPZ); 3) lifetime neuroleptic exposure as median dose multiplied by treatment duration (lifetime CPZ).

### 2.2. *In situ* hybridization

Riboprobes to measure dysbindin-1 mRNA expression that include transcripts from GenBank Accession nos. BC011912, AF061734, and AL136637, were synthesized, validated, and applied to fresh frozen tissue sections as described (Weickert et al., 2004). Within the hippocampal formation, areas were sampled as regions of interest with the experimenter blind to diagnosis. Measurements were made on the dentate granule cell layer (DGg), the DGh, and the pyramidal cell layer of CA3 and CA1.

### 2.3. Statistical analyses

Diagnostic comparisons were made with ANOVA and Fisher's Least Significant Difference Tests. Specific contrasts for each subregion were planned a priori and were investigated with one-tailed student *t*-tests. Pearson's correlation among dysbindin-1 mRNA levels, demographic variables, spinophilin mRNA levels and synaptophysin mRNA levels were run ( $n=20$ ) with and without a partial correlation for pH and PMI. Spearman correlations between the neuroleptic exposure in patients and dysbindin-1 mRNA were also performed ( $n=10$ ).

## 3. Results

### 3.1. *Dysbindin-1* expression

The antisense dysbindin-1 probe resulted in a robust signal in the neuronal fields of the hippocampus; whereas the dysbindin-1 sense strand control produced no discernible signal when hybridized to adjacent sections from the same case in the same experimental run with the same

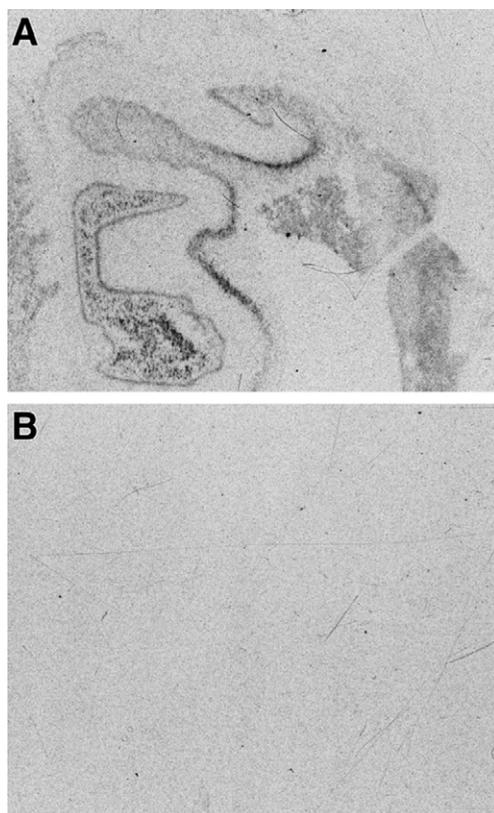


Fig. 1. Autoradiographic film images of the hybridization of dysbindin-1 riboprobe in human hippocampal formation with the adjacent section from the same brain are shown (panels A and B). Panel A shows the image from the antisense strand and panel B shows the image from the sense strand control.

exposure time to film (Fig. 1A and B). Dysbindin-1 mRNA was most abundant in DGg, DGh and CA3 (Figs. 1 and 2). We found 20%–40% reductions in dysbindin-1 mRNA in patients with schizophrenia compared to controls ( $F=4.69$ ,  $df=1$ , 18  $p=0.04$ , Fig. 2C) which were of a similar magnitude as dysbindin-1 protein reductions found previously by Talbot et al. (2004). Dysbindin-1 mRNA levels varied according to cell field ( $F=19.78$ ,  $df=3$ , 54,  $p<0.0001$ ). CA3 had significantly higher dysbindin-1 mRNA levels and CA1 significantly lower dysbindin-1 mRNA levels than all other cell fields studied (all  $p<0.01$ ). An interaction effect between diagnosis and hippocampal region ( $F=3.18$ ,  $df=3$ , 54,  $p=0.03$ ) was detected. Dysbindin-1 expression was significantly reduced in the dentate granule cells (DGg,  $t=-1.90$ ,  $p=0.04$ ), dentate polymorph cells (DGh,  $t=-2.32$ ,  $p=0.02$ ), and in CA3 ( $t=-1.99$ ,  $p=0.03$ ), but not in CA1 ( $t=-1.33$ ,  $p>0.05$ ) (Fig. 2C). In all subfields, dysbindin-1 mRNA correlated

with pH ( $r=0.50$  to  $0.75$ , all  $p<0.05$ ) and PMI ( $r$  range,  $-0.44$  to  $-0.58$ , all  $p<0.05$ ). Dysbindin-1 mRNA did not correlate with age (all  $r<0.30$ , all  $p>0.20$ ) or with last, daily or lifetime CPZ (average  $R=-0.22$ , all  $p>0.29$ ) confirming previous observations showing that dysbindin-1 mRNA was not altered by neuroleptics (Chiba et al., 2006; Weickert et al., 2004).

### 3.2. Dysbindin-1 expression and synaptic markers

In the same cohort of patients studied here, reductions in spinophilin and synaptophysin mRNAs in the hippocampal formation of schizophrenics were found (Law et al., 2004; Webster et al., 2001). Correlations were tested among synaptic markers and dysbindin-1 as follows: 1) between mRNAs of the presynaptic proteins synaptophysin and dysbindin-1 within the same region; 2) between presynaptic and postsynaptic markers within

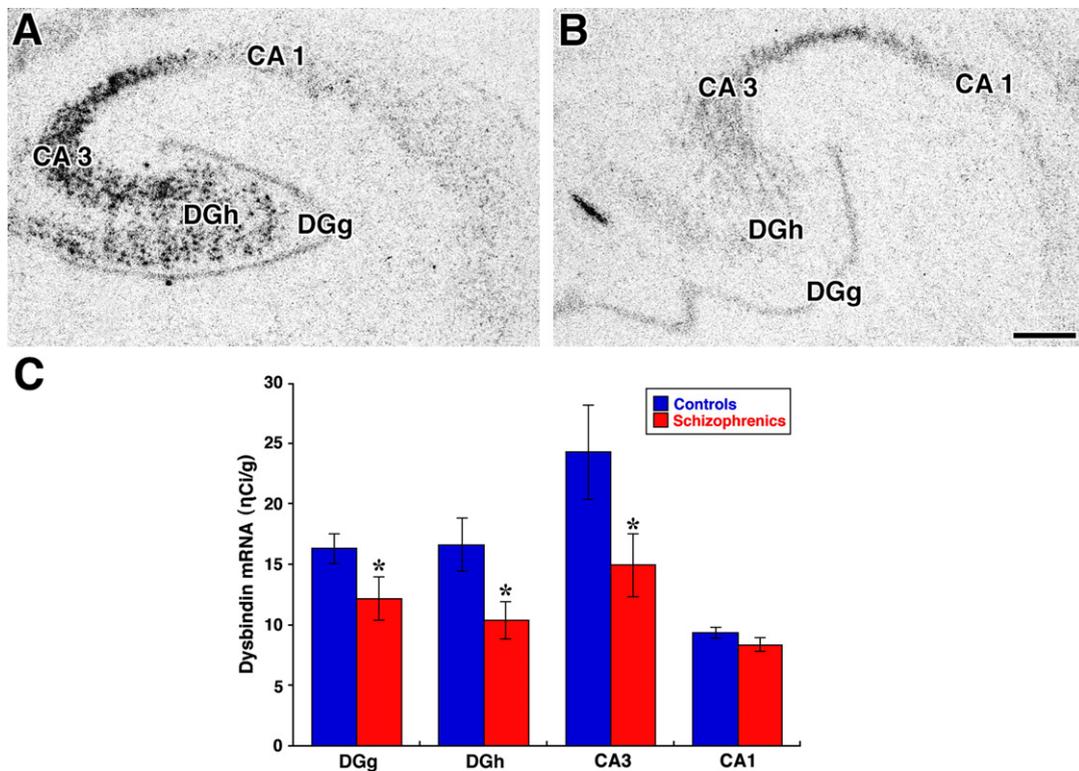


Fig. 2. Autoradiographic film images of the hybridization of dysbindin-1 riboprobe in human hippocampal formation are shown (panels A and B). Panel A is taken from a normal control and panel B from a patient with schizophrenia. The subregions at the midbody level of the hippocampal formation demonstrate different expression levels of dysbindin-1 mRNA. Note the intense hybridization signal in the CA3 and DGh subfields. A distinct but fairly thin line of dysbindin-1 mRNA label is found overlying the DGg (panel A and B). The CA1 region has a modest dysbindin-1 hybridization signal. Scale bar = 1 mm. Panel C: Bar graph shows the mean OD reading from dysbindin-1 mRNA signal expressed as  $\eta$ Ci/g radioactivity. Significant differences between controls (blue bars) and schizophrenia patients (red bars) mRNA are found in the DG (mean (SEM)=16.3(1.2), 12.2(1.8), respectively,  $p=0.04$ ), DGh (mean (SEM)=16.6(2.0), 10.4(1.6), respectively;  $p=0.02$ ), and CA3 (mean (SEM)=24.3(3.9), 14.9(2.6), respectively;  $p=0.03$ ) subregions. There were no significant changes between the groups for CA1 mRNA (mean (SEM)=9.4(0.43), 8.4(0.58), respectively;  $p>0.05$ ).

target subfields; and 3) between dysbindin-1 and the postsynaptic marker (spinophilin) within the same neuronal field. This last analysis was done since recurrent collaterals where neurons synapse back on the originating projection field was identified as abnormal in schizophrenia by Talbot et al. (2004).

Synaptophysin and dysbindin-1 mRNA levels within the DGg ( $r=0.68$ ,  $p=0.002$ ), DGh ( $r=0.82$ ,  $p<0.0001$ ) and CA3 ( $r=0.58$ ,  $p=0.02$ ) were positively correlated. The correlation between dysbindin-1 and synaptophysin mRNA levels within DGh remained significant after partial correlation for pH ( $r=0.76$ ,  $p<0.001$ ), while CA3 showed a trend ( $r=0.46$ ,  $p=0.08$ ), and the DGg was no longer significant.

Expression of dysbindin-1 in the DGg and of spinophilin in the CA3 subfield were correlated ( $r=0.74$ ,  $p=0.0006$ ) and showed a trend in that direction after partial correlation for pH ( $p=0.06$ ). Positive correlations were found for DGh dysbindin-1 and DGg spinophilin ( $r=0.69$ ,  $p=0.002$ ) and a trend was found after partial correlation for pH ( $r=0.47$ ,  $p=0.07$ ). CA3 dysbindin-1 and CA1 spinophilin mRNAs were not correlated ( $r=-0.31$ ,  $p=0.22$ ).

Dysbindin-1 and spinophilin mRNA in CA3 were positively correlated ( $r=0.60$ ,  $p=0.01$ ), but the relationship was not significant when pH was taken into account. We found positive correlations between dysbindin-1 and spinophilin mRNA within the DGg ( $r=0.76$ ,  $p=0.0003$ ), which remained significant after partial correlation for pH ( $p<0.01$ ). The statistical significance of the correlations above were not affected by partial correlation for PMI.

#### 4. Discussion

We detected a reduction of dysbindin-1 mRNA expression in multiple regions of the hippocampal formation in patients with schizophrenia. This observation extends our findings of reduced dysbindin-1 mRNA in the frontal cortex and midbrain of patients with schizophrenia to another brain region (Weickert et al., 2004). The fact that dysbindin-1 mRNA expression was not decreased in CA1 suggests that expression is not ubiquitously altered in schizophrenia. Talbot et al. (2004) likewise found that decreased dysbindin-1 protein in the hippocampal formation of schizophrenia cases was not accompanied by altered levels of dysbindin-1 in the anterior cingulate cortex of the same cases. It should be noted that Talbot et al. (2004) did find significantly reduced dysbindin protein in neuropil areas of CA1, whereas we found no change in dysbindin-1 mRNA in cell bodies areas of CA1. Since the dysbindin-1 protein

in the neuropil areas of CA1 could emanate from terminals from CA3 our lack of ability to find dysbindin mRNA reductions in CA1 are not necessarily inconsistent with the findings of Talbot et al. (2004).

Expression of dysbindin-1 in the human hippocampal formation is robust in DGg, DGh and CA3 subfields as compared to CA1. At the cellular level dysbindin-1 mRNA may be higher per neuron in CA3 as compared to CA1 (Talbot et al., 2004). The distinct neuroanatomical distribution of dysbindin-1 mRNA within the hippocampal formation suggests that it may relate to regional synaptic pathology previously reported within DGh and CA3, but not CA1, in schizophrenia patients (Harrison, 2004).

Our data shows reduced dysbindin-1 mRNA in DGh, which is known to project to the inner molecular of the dentate gyrus, a region where dysbindin-1 protein is reduced in schizophrenia (Talbot et al., 2004). Thus, we suggest that the mechanism of the reduction in hippocampal dysbindin-1 protein involves reduced dysbindin-1 transcript levels, recognizing that increased degradation of the dysbindin-1 protein may also be involved. Reduction in CA3 dysbindin-1 mRNA may relate to protein reductions in CA3 and/or CA1. Our data supports the presumptive deficiency in recurrent collateral connectivity within the hippocampal subregions of patients with schizophrenia, as we find that dysbindin-1 and spinophilin correlate within hippocampal subregions. While we do not know if this correlation represents a direct effect of dysbindin-1, recent detailed anatomical studies have localized dysbindin-1 in postsynaptic sites and along microtubules in the hippocampal formation suggesting a unique function for dysbindin in dendrites (Talbot et al., 2006).

We suggest, as others have (Talbot et al., 2004), that alterations in dysbindin-1 may relate to the observed abnormalities in synaptic connectivity found in schizophrenia. Indeed, in cultured cells, antisense dysbindin-1 RNA impacts synaptic proteins and impairs glutamate release (Numakawa et al., 2004). In our study, the correlation between DGh dysbindin-1 mRNA and DGh synaptophysin mRNA is particularly strong. However, this finding is somewhat surprising as an earlier study of dysbindin-1 and synaptic marker proteins, did not find a reduction in synaptophysin protein in terminal fields of the DGh neurons (Talbot et al., 2004). Since the earlier study measured protein, it is possible that the increased synaptophysin protein in the inner molecular layer in the dentate gyrus originates from terminals other than the DGh. However, since non-DGh inputs to the inner molecular layer (DGiml) are minor in normals, we speculate that a significant deviation from the “normal”

anatomical connectivity could be present in schizophrenia patients to account for this.

Another possible explanation for the apparent difference between the postmortem studies is that synaptophysin mRNA and protein changes are not detectable in all patient groups due to clinical heterogeneity or limits of quantitative techniques. But unlike the somewhat inconsistent findings on synaptic change reported in postmortem studies of patients with schizophrenia, controlled studies on sandy mice show that absence of DGh dysbindin-1 protein could cause reductions in DGiml synaptophysin (Talbot et al., 2005). Furthermore, the loss of dysbindin-1 in homozygous sandy mice is associated with reductions in synaptophysin detected via western blotting of whole brain (Talbot et al., 2005). These results would favor the interpretation that the reductions in dysbindin-1 mRNA may more directly cause reductions in synaptophysin mRNA in the same neuron. In contrast, in patients with schizophrenia, who do not entirely lack dysbindin-1 protein as do the sandy mice, it is possible that the dysbindin mRNA reductions are downstream of a more generalized synaptic pathology.

In summary, we report reductions of dysbindin-1 mRNA in specific regions of the schizophrenic hippocampal formation, particularly those implicated in the synaptic pathology. Our expression results are concordant with reported decreased dysbindin-1 protein levels (Talbot et al., 2004). We provide further evidence that the control of dysbindin-1 gene transcription and/or dysbindin-1 transcript stability is altered in brains of patients with schizophrenia. Moreover, reduced dysbindin-1 may contribute to the changes in synaptic connectivity and glutamate signaling found in schizophrenia. Altered expression of schizophrenia susceptibility genes in patients might be argued to be predominantly upstream, or primary in nature (Bray et al., 2003, 2005; Weickert et al., 2004). To test this hypothesis quantitatively for dysbindin-1, a better understanding of how risk haplotypes influence transcription, splicing, and translation is required that includes a comparison of controls to patients with schizophrenia, bipolar disorder, and other psychiatric conditions.

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The NIMH had no further role in this study or the decision to submit the report for publication.

#### Contributors

*Cynthia Shannon Weickert*: co-designed this study, performed the *in situ* hybridization, and other RNA work not shown, and wrote main body of manuscript.

*Deborah A. Rothmond*: performed image and data analyses, created all figures for the manuscript, co-wrote materials and methods, results and reference sections.

*Thomas M. Hyde*: provided neuroanatomical expertise and the tissue for sectioning.

*Joel E. Kleinman*: designed the protocols for the brain collection and tissue quality, assembled the cohort used in this study.

*Richard E. Straub*: co-designed this study, provided gene transcripts for probe designs, co-wrote discussion section.

#### Conflict of interest

All authors declare they have no conflict of interest.

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