ORIGINAL ARTICLE

Decreased Thalamic Expression of the Homeobox Gene *DLX1* in Psychosis

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Context: A shared vulnerability to develop psychosis can be related to abnormalities in thalamic circuits in schizophrenia and bipolar disorder and could be a genetic link between these disorders. Homeobox genes involved in development and differentiation of the brain could play an important role in these disorders.

Objective: To determine whether patients with schizophrenia and bipolar disorder have different thalamic expression patterns of 2 homeobox genes, *DLX1* and *SHOX2* (alias *OG12X* or *SHOT*) compared with psychiatric and nonpsychiatric control subjects.

Design: Postmortem sections containing the thalamic mediodorsal nucleus were subjected to in situ hybridization with mouse *Dlx1* and human *SHOX2* RNA probes. The number of both *DLX1*- and *SHOX2*-positive neurons relative to Nissl-stained neurons was estimated in systematic randomly sampled volume probes.

Patients: Fifteen patients with schizophrenia, 15 with bipolar disorder with or without history of psychosis, 15 with major depressive disorder, and 15 nonpsychiatric controls from the Stanley Foundation Brain Bank.

Main Outcome Measure: Relative numbers of *DLX1*- and *SHOX2*-positive neurons in patients with schizophrenia and bipolar disorder with history of psychosis compared with psychiatric and nonpsychiatric controls.

Results: Patients with a history of psychosis showed significantly decreased relative numbers of *DLX1*-positive neurons compared with patients without history of psychosis and nonpsychiatric controls ($P=.02$), whereas no differences could be found in relative numbers of *SHOX2*-positive neurons ($P>.15$). Results were obtained blind to diagnosis, symptoms, or any other variable except hemisphere.

Conclusion: Decreased thalamic expression of *DLX1* in schizophrenia and bipolar disorder with psychosis suggests shared genetic deficits in expression of this homeobox gene.

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THE THALAMUS has been implicated in the pathogenesis of schizophrenia (SZ) as it plays an important role in sensory gating, a process disturbed in psychosis.[1] Thalamic abnormalities found in SZ consist of decreased volume and total number of neurons of the thalamus, specifically the mediodorsal and anteroventral nucleus, in the absence of gliosis, as shown in several postmortem studies.[2-5] This decrease in thalamic volume in SZ has been corroborated by neuroimaging studies, with an accompanying decrease in metabolism and *N*-acetylaspartate levels.[6,7] Decreased thalamic volume has also been found in imaging studies in siblings of schizophrenia patients,[8-10] suggesting that this anomaly is possibly related to genetic defects that could induce a susceptibility to SZ.

However, it is not clear whether the thalamic abnormalities found in SZ are specific to this disorder. Thalamic abnormalities have also been reported in neuroimaging studies in patients with chronic bipolar disorder (BPD), albeit not consistently.[11-18] Only 1 postmortem study has investigated volume and cell numbers of the thalamus in patients with BPD, reporting no significant differences in comparison with the schizophrenic control subjects.[19] Other postmortem studies in BPD show no increase in gliosis, suggesting that brain abnormalities present in BPD are, like those in SZ, not the result of a neurodegenerative process but of a possible neurodevelopmental etiology.[11]

Although not all brain abnormalities in SZ can be found in BPD and vice versa,[20,21] similar findings in some postmortem and imaging studies occur in both

SZ and BPD and are accompanied by an overlap in epidemiology,[22] symptomatology,[23] and biology, especially when psychotic BPD is included.[23-26] Thus, a genetic link between both disorders could be the shared vulnerability to develop psychosis. This could be the result of shared gene polymorphisms or mutations in both disorders, contributing to the molecular basis of psychosis by affecting brain growth and development (eg, of the thalamus, in both patient populations).[11,27]

The specification of neuronal phenotypes and neuronal connectivity relies largely on gene expression programs in progenitor cells. The homeobox (or homeodomain) family comprises one of the largest classes of transcription factors and is instrumental in cell-specific gene expression.[28,29] Homeobox genes have restricted expression patterns during development of the embryonic brain, and some persist during adult life.[30] Genetic studies in mice and humans have demonstrated that homeobox genes can initiate and modulate cascades of gene expression that define embryonic development and differentiation.[31]

One of the few homeobox genes that is expressed mainly in the thalamus in the adult rat is *Prx3*.[32] The human homologue, *SHOX2* (also named *OG12X* or *SHOT*), is situated on human chromosome 3q22-26.[33] In the adult rat the expression is restricted to a number of thalamic nuclei, including the mediodorsal nucleus (MD), the superior and inferior colliculus, and pontine reticular formation.[32] It is not known which gene or genes are targeted by *SHOX2* or *Prx3*, respectively. We have shown that, in accordance with rodent data, *SHOX2* is also expressed in postmortem human thalamic tissue (M.K., A.J.C.G.M.H., R.S.K., M.P.S., and J.P.H.B., unpublished data, 2001).

The homeobox gene *Dlx1* has been extensively investigated in rodents.[34,35] *Dlx1* in embryo mouse brain is expressed in cells that co-express *Dlx2*, which are primarily late-born neuronal precursors and subsets of postmitotic cells,[35] mainly in the primordia of the basal ganglia (the telencephalic medial and lateral ganglionic eminences) and ventral thalamus.[34,35] Expression of *Dlx1* and *Dlx2* in mice seems to be required for the production, migration, and differentiation of (most of) the neocortical, hippocampal, and olfactory bulb γ-aminobutyric acid (GABA)-ergic interneurons, which are also derived from the ganglionic eminences.[36,37] In humans, *DLX1* and *DLX2* are closely linked at chromosome 2q32.[38] Although *Dlx1* is not expressed in the adult mouse mediodorsal thalamic nucleus, in humans we did find expression of *DLX1* messenger RNA (mRNA) in this brain structure (M.K., Ceriel H.J. Asbreuk, R.S.K., M.P.S., and J.P.H.B., unpublished data, 2001).

Both *SHOX2* and *Dlx1* are expressed during brain development in the primordia of the thalamus in rodents and in the human thalamus.[32,35] As thalamic abnormalities found in SZ and BPD could be of possible neurodevelopmental origin with a genetic etiology, we hypothesized that possible differences in expression patterns of these genes in the MD in SZ and BPD compared with nonpsychiatric controls (NCs) might be related to these psychiatric disorders. Alternatively, we hypothesized that possible abnormalities in gene expression could be related to the presence or absence of psychosis. Therefore, we investigated the expression of *SHOX2* and *DLX1* in the postmortem MD of patients with SZ, BPD with and without history of psychosis, major depressive disorder (MDD) without history of psychosis, and NCs.

METHODS

SUBJECTS

Five consecutive frozen 14-µm-thick coronal sections of the thalamus containing the rostral part of the MD from 15 patients with SZ, 15 with BPD with (n=11) and without (n=4) history of psychosis, 15 with MDD without history of psychosis, and 15 NCs were obtained from the Stanley Foundation (Bethesda, Md) Neuropathology Consortium (**Table**).[39] The sections used in this study contained excellent- to good-quality mRNA, as established by the Stanley Foundation.[39] All analyses were performed blind to diagnosis or any other variable, except for hemisphere. Ten subjects (1 with SZ, 3 with BPD, 1 with MDD, and 5 NCs) were excluded from analysis because of absence of the MD (n=7), low quality of staining (n=1), or severe damage to the tissue (n=2).

IN SITU HYBRIDIZATION AND NISSL STAINING

Digoxigenin-labeled (anti)sense RNA probes were generated according to the manufacturer's instructions (Roche Diagnostics, Basel, Switzerland). *SHOX2* RNA probes were synthesized from a *Hind*III fragment (base pairs [bp] 1-454) of an approximately 1200-bp human cDNA clone containing part of the coding region (Semina et al,[33] GenBank accession No. AF022654). As no human *DLX1* cDNA clone was available, we used mouse *Dlx1* cDNA, which was overall 93% identical to human *DLX1* in the coding region with a stretch of 100% homology. *Dlx1* RNA probes were generated from a full-length 2.8-kb mouse cDNA clone (McGuinness et al,[40] GenBank accession No. NM010053). The identity of the *SHOX2* and *Dlx1* cDNAs was confirmed by sequencing with a DNA sequence analysis instrument (Beckman CEQ 2000; Beckman Coulter, Fullerton, Calif).

From each subject, 1 section for each probe was used in the in situ hybridization (ISH), according to Asbreuk et al,[41] with a hybridization temperature of 65°C. Test sections without probe added to the hybridization mix showed no staining after completion of the ISH. Consecutive sections were Nissl stained according to standard methods.

COUNTING PROCEDURE

With the use of a microscope (Zeiss Axioskop 2) attached to a camera system (Sony PowerHAD 3 CCD color video camera; Sony Electronics Inc, Park Ridge, NJ) and image analysis software (MCID-M5; Imaging Research Inc, St Catharines, Ontario), pictures of the Nissl-stained, *SHOX2*- and *DLX1*-positive sections were taken. The MD was outlined according to the method of Morel et al.[42] In systematic randomly sampled volume probes (616×616×3 µm), neurons were counted in the MD at ×200 by means of an ocular grid.[43] Both Nissl and ISH sections were equally shrunk to a 3-µm thickness. In all volume probes, only neurons containing a nucleus within or in contact with the upper or right-hand border of the ocular grid were examined. Further inclusion criteria were as follows: for Nissl staining: visible single nucleolus and presence of cresyl violet–stained cytoplasm, with shape and texture typical of neurons[21]; for the ISH-processed sections, positive ISH staining of the cytoplasm, presence of a clear nucleus, and clear and visible outline of cell borders.

To adjust for possible differences in total number of neurons present in the MD, final estimates of positive neurons were calculated as the ratio of density number of positive neurons

Demographic, Clinical, and Histologic Data*

Variable	SZ (n = 14)	BPD (n = 12)	MDD (n = 14)	NC (n = 10)	Statistical Analysis
Postmortem variables					
Age at death, y	43.6 (13.0)	43.4 (12.2)	47.6 (8.7)	47.9 (10.4)	$F_{3,46} = 0.59$, $P = .63$
Postmortem interval, h	34.2 (15.0)	32.5 (16.0)	26.1 (9.6)	22.9 (9.6)	$F_{3,46} = 2.0$, $P = .13$
Brain pH	6.18 (0.26)	6.20 (0.23)	6.19 (0.21)	6.28 (0.28)	$F_{3,46} = 0.38$, $P = .77$
Brain hemisphere, No.	5 R, 9 L	6 R, 6 L	6 R, 8 L	5 R, 5 L	$\chi^2_3 = 0.72$, $P = .87$
Brain weight, g	1476 (111)	1428 (186)	1459 (147)	1489 (170)	$F_{3,46} = 0.35$, $P = .79$
Clinical variables					
Age at onset, y†	21.9 (6.3)	23.6 (8.3)	34.1 (13.8)		$F_{2,36} = 5.9$, $P = .006$
Duration of disease, y	21.7 (11.8)	20.1 (10.7)	13.5 (11)		$F_{2,37} = 2.1$, $P = .14$
Flufenazine eq, mg					
Minimum	0	0			$t_{24} = 1.79$ (unequal variances), $P = .91$
Median	32 500	9750			
Maximum	200 000	60 000			
CNS medication at time of death, No.					
Mood stabilizer‡	3	8	2	0	Fisher exact $P = .01$
Antidepressants	5	7	8	0	$\chi^2_2 = 1.76$, $P = .41$
Antipsychotics§	11	5	0	0	Fisher exact $P = .10$
Drug abuse at time of death, No.	1	2	2	0	Fisher exact $P = .74$
Alcohol abuse at time of death, No.	1	2	3	0	Fisher exact $P = .54$
Family history,‖ No.					
None	4	2	2		
SZ	4	2	0		
BPD	1	4	1		
MDD	2	2	9		
Unknown	3	3	2		
Demographic variables					
Sex, No.	5 F, 9 M	6 F, 6 M	5 F, 9 M	5 F, 5 M	$\chi^2_3 = 1.03$, $P = .79$
Occupation,¶ No.	2	9	14	10	
Smoking, No.					
Yes	7	6	5	1	Fisher exact $P = .43$
Unknown	3	4	4	5	
Cause of death, No.					
Suicide	4	5	6	0	Fisher exact $P = .12$
Accident	3	0	1	1	
Organic disease	7	7	7	9	

Abbreviations: BPD, bipolar disorder; CNS, central nervous system; eq, equivalents; L, left; MDD, major depressive disorder; NC, nonpsychiatric control; R, right; SZ, schizophrenia.

*Values are given as mean (SD) except where indicated.

†Bonferroni-corrected analyses: patients with MDD had an older age at onset compared with SZ and BPD, $P = .008$ and $P = .04$, respectively. For 1 patient with BPD, age at onset was unknown.

‡Lithium carbonate, carbamazepine, valproate sodium.

§Fisher exact test between BPD and SZ only.

‖Family history scored when positive in first- or second-degree relatives; unknown indicates insufficient information available. For BPD, 1 subject had both bipolar disorder and depression in first-degree relatives.

¶Subjects who held a job, including housekeeping.

in the ISH section divided by the density number of neurons in the Nissl section. This resulted in the relative number of *SHOX2*-positive neurons/Nissl-stained neurons (*SHOX2*/Nissl) and of *DLX1*-positive neurons/Nissl-stained neurons (*DLX1*/Nissl), respectively. Because of the criterion of the presence of a nucleus instead of a nucleolus, neurons in the ISH were relatively easier to include, which could result in some cases in a ratio greater than 1.

STATISTICAL ANALYSIS

All sections of every fifth subject were recounted 1 to 3 days later to ensure reliability of the assessments. The intraclass correlation coefficient of these 10 recounts was 0.98 or more for Nissl, *SHOX2*, and *DLX1* (paired-samples *t* test, $P<.001$).

Demographic, postmortem, and clinical variables were evaluated with χ^2 test and 1-way analysis of variance (ANOVA) followed by Bonferroni tests when appropriate. Overall data were normally distributed, and 1-way ANOVA was used to analyze the differences in *SHOX2*/Nissl and *DLX1*/Nissl between diagnostic groups. A 2-tailed *t* test was used to analyze differences in *SHOX2*/Nissl and *DLX1*/Nissl between subjects with and without history of psychosis. Bonferroni-corrected α is indicated when necessary. No overall significant differences were found between hemispheres, and data from both hemispheres were pooled. Pearson correlations were determined between mRNA expression and possible confounding variables. To investigate the effect of psychosis, subjects were divided into psychosis and nonpsychosis groups, resulting in 22 subjects with psychosis (13 with SZ and 9 with BPD) and 24 subjects without psychosis (3 with BPD, 11 with MDD, and 10 NCs) for *SHOX2*/Nissl, and 18 with psychosis (11 with SZ and 7 with BPD) and 22 without psychosis (3 with BPD, 11 with MDD, and 8 NCs) for *DLX1*/Nissl.

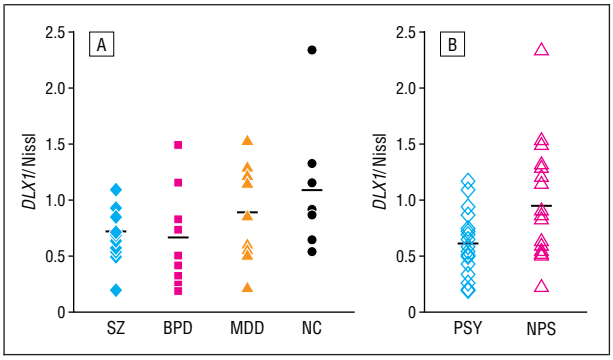

Figure 1. Individual data for relative numbers of *DLX1*-positive neurons by Nissl-stained neurons by diagnosis (schizophrenia [SZ; n=11], bipolar disorder [BPD; n=10], major depressive disorder [MDD; n=11], and nonpsychiatric controls [NC; n=8]) (A) and in subjects with (PSY; n=18) and without (NPS; n=22; includes nonpsychiatric controls) history of psychosis (B). Horizontal lines indicate mean levels of *DLX1*/Nissl.

RESULTS

SHOX2/NISSL

No significant differences in *SHOX2*/Nissl were found between subjects with SZ, BPD, MDD, and NCs ($F_{3,42}=0.56$, $P=.60$) or between psychosis and nonpsychosis groups ($t_{44}=1.3$, $P>.15$, data not shown).

DLX1/NISSL

One-way ANOVA showed no significant difference in *DLX1*/Nissl between SZ, BPD, MDD, and NC groups ($F_{3,36}=2.05$, $P=.12$; **Figure 1**A). However, the psychosis group showed a significant 35% decrease in *DLX1*/Nissl compared with the nonpsychosis group ($t_{38}=2.5$, $P=.02$; Figure 1B). Removing the outlier in the nonpsychosis group, as shown in Figure 1B, did not affect the result ($t_{37}=2.4$, $P=.02$). When the BPD group was divided into patients with (n=7) and without (n=3) a history of psychosis, the patients with psychosis showed a trend for decreased *DLX1*/Nissl ($t_{13}=2.3$, $P=.04$, Bonferroni-corrected $\alpha=.05\div2=.025$), whereas the patients without psychosis showed no significant difference ($t_9=0.4$, $P>.70$) compared with the NCs. **Figure 2** shows an example of *DLX1* expression in a patient with BPD with psychosis and an NC.

Analyses of demographic, clinical, and postmortem data are given in the Table. No significant correlations could be found between any of the demographic, postmortem, or clinical variables and *DLX1*/Nissl. Presence of mood stabilizers, including lithium carbonate, or the use of typical vs atypical antipsychotics could not be related to specific levels of *DLX1*/Nissl.

COMMENT

This study investigated the expression of 2 homeobox genes by ISH in postmortem adult human brain tissue of patients with SZ, BPD, MDD, and NCs. We found decreased expression of *DLX1* but not *SHOX2* in the MD in patients with a history of psychosis compared with patients without history of psychosis and NCs. We were

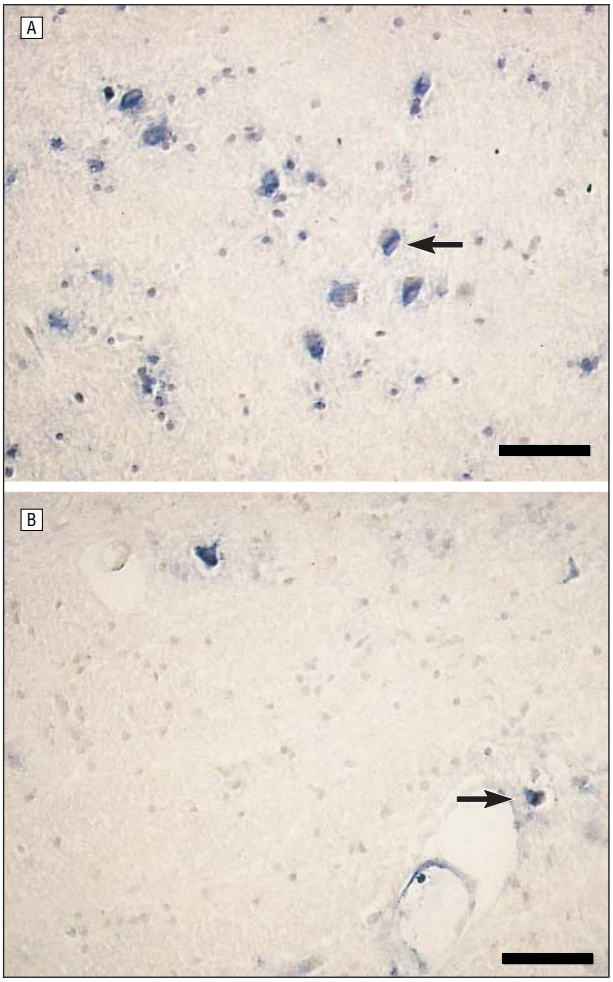

Figure 2. Expression of *DLX1* messenger RNA in the human mediodorsal thalamic nucleus detected by digoxigenin-labeled messenger RNA probe. Arrows show neurons containing blue staining, indicating presence of *DLX1* messenger RNA. Bar indicates 60 µm. A, Nonpsychiatric control. B, Patient with bipolar disorder with a history of psychosis.

unable to identify any known demographic, histologic, or clinical variable that could possibly have induced this change in expression levels. These results are therefore taken to indicate that *DLX1* expression in the human MD may play a role in the pathogenesis of psychosis.

Our results could suggest that part of the etiology in both SZ and BPD is developmental in origin, at least regarding the vulnerability to develop psychosis, or that a common degenerative process could induce loss of neuronal *DLX1* expression in both SZ and BPD with psychosis. Psychotic BPD therefore may delineate a different subtype of BPD, more resembling SZ in the developmental,[44] epidemiologic,[22] anatomic,[11,26] symptomatologic,[45] and genetic[23,27] aspects of psychosis.

Our study does not elucidate the type of cells that express *DLX1* in the MD of the thalamus in humans, although the size and shape of the *DLX1*-positive cells define them as neurons. However, recent studies indicate that *DLX1*-positive neurons are GABAergic interneurons.[37,46-48] In rodents, it has been shown that *Dlx1/Dlx2*-positive GABAergic neurons migrate from the ganglionic eminences to the cortex, hippocampus, and olfactory bulb, but not to the thalamus during brain development.[36,46] Loss

of both *Dlx1* and *Dlx2* induces a severe reduction in cortical interneurons and lack of normal GABAergic interneurons in the hippocampus and olfactory bulb in mice.[28,36,49] In the human embryonic brain, *DLX1/DLX2*-positive GABAergic interneurons have been shown to migrate from the ganglionic eminences to the cortex and, in contrast to the rodent and macaque, also to the MD and pulvinar of the thalamus. Thus, this thalamic stream of *DLX1/DLX2*-positive GABAergic neurons seems human-specific. In this study, deficits in *DLX1*-expressing neurons of the human MD, which are presumably derived from the ganglionic eminences, could therefore be related to deficits in cortical interneurons derived from the same progenitor cells in SZ and BPD. Several postmortem studies have shown decreased expression of markers for and displacement of cortical GABAergic interneurons in psychosis, suggesting an abnormal development of GABAergic neurons related and etiologically linked to psychosis or the vulnerability to develop it. For instance, in the prefrontal cortex, Guidotti et al[25] found a decrease in reelin and glutamic acid decarboxylase 67 (GAD_{67}) mRNA- and protein-positive neurons in patients with SZ and BPD with history of psychosis, in the same set of postmortem brains that we have used. Other studies have also shown a loss of GAD_{67} or the GABA membrane transporter 1 (GAT-1) mRNA and protein in the prefrontal cortex in SZ.[50-54] In addition, disturbed migratory processes of GABAergic interneurons in SZ have been shown with the use of nicotinamide–adenine dinucleotide phosphate–diaphorase (NADPH-d) as a marker. A significant decrease in interneurons of the dorsolateral prefrontal cortex containing NADPH-d has been found, with an accompanying significant increase of these neurons in the underlying white matter.[55,56] Preliminary data suggest that this deficit is also present in BPD.[57] Volk et al[52(p264)] already suggested that "the site of embryonic origin [of the cortical interneurons] may be associated with a greater susceptibility to altered gene expression in schizophrenia."

Another speculative explanation for the decrease in *DLX1* expression but not *SHOX2* expression in our study is a common degenerative pathway in psychosis. The exact function of homeobox genes that are expressed during adult life is not known, but it is thought that these genes play a role in maintenance and regulation of neuronal systems. It cannot be excluded that a yet unidentified factor affects neurons expressing *DLX1* during adult life, leading to a relative progressive decrease in *DLX1* expression in psychosis, although no correlation between *DLX1* expression and age at onset or duration of disease could be found in our study.

In conclusion, we found decreased thalamic expression of *DLX1* mRNA in patients with history of psychosis compared with patients without history of psychosis and NCs. A speculative explanation for the down-regulation of *DLX1* mRNA expression in the MD of the thalamus in psychosis could be a defect in differentiation of ganglionic eminence progenitor cells or GABAergic interneurons, or a defect in migratory pathways during brain development. Down-regulation of *DLX1* in patients with SZ and BPD with a history of psychosis suggests shared genetic deficits in both disorders in relation to a vulnerability to psychosis.

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