

Increased expression of retinoic acid-induced gene 1 in the dorsolateral prefrontal cortex in schizophrenia, bipolar disorder, and major depression

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Background: Retinoids regulate gene expression in different cells and tissues at the transcriptional level. Retinoic acid transcriptionally regulates downstream regulatory molecules, including enzymes, transcription factors, cytokines, and cytokine receptors. Animal models indicate an involvement of retinoid signaling pathways in the regulation of synaptic plasticity and learning, especially in the hippocampus. *Retinoic acid-inducible or induced gene 1 (RAI-1)* is induced during neuronal differentiation, and was associated with the severity of the phenotype and response to medication in schizophrenic patients.

Methods: In the present study, we used immunohistochemistry to investigate the expression of RAI-1 in 60 brains from the Stanley Neuropathology Consortium (15 cases each from controls and from patients with schizophrenia, bipolar disorder, and major depression). Rating scores for density and intensity were determined in the dorsolateral prefrontal cortex.

Results: All four groups showed high interindividual variation. RAI-1-positive cells were identified as neurons and astrocytes. Significantly increased intensities in cortical neurons were noted in all three major psychiatric groups compared with controls. The density of RAI-1-positive neurons was increased ($P=0.06$) in schizophrenia and bipolar disorder. In bipolar disorder, RAI-1-positive astrocytes in gray matter showed a significantly increased intensity and compound value. Thus, a significant increase in the parameters measured was found in schizophrenia, bipolar disorder, and major depression.

Conclusion: Our study shows a significant increase in expression of RAI-1 in the brains from patients with schizophrenia, bipolar disorder, or major depression. The increased expression might reflect altered signaling pathways, like that for retinoic acid. The underlying mechanisms leading to the increased expression and its functional consequences are so far unknown, and remain to be investigated in future studies.

Keywords: retinoic acid-inducible gene 1, dorsolateral prefrontal cortex, schizophrenia, bipolar disorder, major depression

Introduction

Schizophrenia, bipolar disorder, and major depression are complex, multigenetic, multifactorial diseases. They lead to disruptive psychopathologies involving thought, perception, behavior, emotion, cognition, movement, and mood.^{1,2} The possible underlying pathogenic mechanisms are not fully understood. The morphological changes seen in the brains of these patients constitute the platform upon which various pathogenic factors may act at different time points.³ The morphological changes include reduced volume in different brain regions, focally disturbed cytoarchitecture

in various cortical areas, changed synaptic numbers and proteins, loss of myelin proteins, and a not yet fully understood reaction pattern of astrocytes and microglia (for review, see Soares and Gershon¹). Large-scale microarray studies showed that a multitude of genes representing various pathways are upregulated or downregulated in these disorders.^{4,5} The etiology of these multifaceted changes remains obscure.

Retinoic acid-inducible gene 1 (RAI-1) is a protein, expression of which is modulated by retinoic acid (RA). Very little is known about its role in the human body; however, it occurs intracellularly and functions as a pattern recognition receptor by sensing viral double-stranded RNA, like in measles,⁶ influenza,⁷ or hepatitis B or C infection.^{8,9}

Besides its roles as a virus sensor, RAI-1 plays an important role in tumor cell proliferation, where it is able to inhibit the proliferation of leukemia cells. It can indirectly lead to apoptosis in hepatoma and melanoma cells through pathways that are not yet fully understood. One mechanism could be the induction of interferon- β which has proapoptotic properties. Further, RAI-1 plays a crucial role in stress-related pathways, such as DNA damage.¹⁰

Joober et al demonstrated that the number of CAG repeats in STS GCT10D04 (Accession number G09710) was associated with the severity of the phenotype and the response to medication in schizophrenic patients.¹¹ Database searches revealed this STS to be homologous to a part of the mouse *retinoic acid inducible-1 gene* (*Rai1*, Accession number D29801), which is induced by RA during neuronal differentiation of P19 embryonal carcinoma cells.¹²

A congenital disorder in which RAI-1 plays a crucial role is the Smith-Magenis syndrome (SMS). Patients show craniofacial and behavioral abnormalities such as brachydactyly, mental retardation, self-injurious behavior, and behavioral stereotypes. The underlying etiology in 70% of cases is a de novo deletion on chromosome 17 in 17p11.2, which encompasses about 3.7 Mb.¹³ Another factor that leads to an SMS-like phenotype is a haploinsufficiency of the *RAI-1* gene. Vilboux et al found that patients with SMS-like features who show either the deletion on chromosome 17 or a de novo *RAI-1* mutation have a lower mRNA level of *RAI-1*.¹⁴ In their study, decreased expression of RAI-1 led to ocular and behavioral abnormalities like polyembolokoiomania (insertion of foreign objects, usually into the ears). These findings match with other studies that showed *RAI-1* mutations occurring in patients with SMS-features.^{15,16} *Rai-1* knockout mouse models showed deficits in locomotor and learning abilities if the animals survived.¹⁷ Most of the

knockout mice died in utero, indicating an important role of RAI-1 during fetal development.¹⁸

Carmona-Mora et al found that a mutation on *RAI-1* can impair its function by prohibiting its translocation into the nucleus and therefore impairing its action as a transcription factor.¹⁹ As RAI-1 under normal circumstances induces expression of brain-derived neurotrophic factor,²⁰ which plays a role in neurogenesis and neuroprotective actions, it could counteract the mental retardation in SMS.

RA is a metabolic product of vitamin A which is taken up through food and is stored in the liver as retinyl esters.²¹ RA leaves the cell nucleus towards the cytoplasm where it is catabolized by cytochrome P450 enzymes.²¹ The data suggest different physiological roles of RA during the life cycle in an individual as well as in various brain regions, particularly in areas with a high turnover of cell connections.²²

RA signaling levels are highest in areas with high synaptic plasticity. Examples are the olfactory system and the limbic area, in particular the hippocampus. The receptors RAR α , RAR β , and RAR γ were reported to be present at different subcellular localizations in the hippocampus.²³ Here, RA influences neurogenesis in the granular layer of the dentate gyrus, and can enhance synaptic strength by increasing post-synaptic glutamate receptor 1 expression. The RA-triggered increase in synaptic strength was reported to be inactivated by activity blockade-driven synaptic scaling.²⁴ Interestingly, in this situation, RA acts at the translational but not at the transcriptional level.²⁴

Moreover, RA is involved in the maintenance of the differentiated state of adult neurons. Destruction of RA signaling pathways in the adult leads to degeneration of motor neurons, development of Alzheimer's disease,²⁵⁻²⁹ and perhaps to a certain extent, development of Parkinson's disease.^{30,31}

The data published so far regarding the possible functions of RA and consequently RAI-1 make the RA pathway a potential pathogenic candidate for severe mental disorders. In this work, we aimed to elucidate the immunohistochemical expression level and pattern of RAI-1 in the brains of patients with schizophrenia, bipolar disorder, or major depression.

Materials and methods

Materials

In the present study, the dorsolateral prefrontal cortex was investigated in 15 brains each from a control group and from patients with schizophrenia, bipolar disorder, or major depression. The brains are part of the Stanley Neuropathology Consortium Collection, details of which were reported elsewhere² (Table 1). A public statement about the Stanley

Table 1 Demographics of the study cases

	Control	Schizophrenia	Bipolar disorder	Depression
Number of cases	15	15	15	15
Age, mean (years)	48.1	44.2	42.3	46.4
Age, range (years)	29–68	25–62	25–61	30–65
Sex (male/female)	9/6	9/6	9/6	9/6
Hemisphere (right/left)	8/7	9/6	8/7	9/6
Race	14 C, 1 AA	13 C, 2 AS	14 C, 1 AA	15 C
Post mortem interval, mean (hours)	23.7	33.7	32.5	27.5
Post mortem interval, range (hours)	8–42	12–61	13–62	7–47
Tissue pH, mean	6.3	6.1	6.2	6.2
Tissue pH, range	5.8–6.6	5.8–6.6	5.8–6.5	5.8–6.5
Cerebellar granular necrosis	1	1	1	2
Fixation, mean (months)	4.40	11.20	9.67	8.40
Fixation, range (months)	1–13	3–31	2–16	1–19
Alcohol abuse (yes)	10	10	13	10
Drug abuse (yes)	1	5	8	4
Smoking	3 [8]	7 [3]	7 [5]	5 [5]
Suicide	0	3 [2]	8 [1]	7 [1]
Psychosis	0	15	11	0
On antipsychotic medication at time of death	0	12	10 [12]	1 [12]
Antipsychotics, first-generation	0	8	5	0
Antipsychotics, second-generation	0	8	3	0
Lithium	0	2	5	2
Mood stabilizers	0	1	7	0
Antidepressant	0	5	8	13
Anticholinergic	0	3	3	1
HSV-1 IgG positive	4	6	7	9
HSV-2 IgG positive	4	1	3	1
CMV IgG positive	8	3	1	6
Toxoplasmosis IgG positive	1	2	3	1

Note: Numbers shown in square brackets represent the number of cases with available data.

Abbreviations: AA, African-American; AS, Asian; C, Caucasian; CMV, cytomegalovirus; HSV, herpes simplex virus; IgG, immunoglobulin G.

Brain Collection from January 2007 (updated August 2009) is available online (www.stanleyresearch.org).

Immunohistochemistry

Immunohistochemistry was performed on 10 µm thick frozen sections on Superfrost Plus slides (M6146-Plus, Allegiance, McGraw Park, IL, USA). Rehydrated sections underwent antigen retrieval using 2 mmol/L HCl for 20 minutes in a water bath at 95°–100°. All subsequent steps were carried out using the S3400 autostainer immunostaining system and EnVison™ + kit (code K4007, DakoCytomation, Carpinteria, CA, USA). Sections were treated with 3% H₂O₂ for 5 minutes to block endogenous peroxidase followed by a protein block for 5 minutes.^{3,32}

Antibody generation

Polyclonal antibodies were generated to regions of the RAI-1. The program Protean (DNASTAR) was used to select optimal peptide epitopes 15–20 residues in size. Freeware NetPhos was employed to check for probability

of phosphorylation and freeware COILS was employed to avoid coiled-coil regions. Three different antibodies were generated as follows.

A RAI-1 antibody (AB)-1 was generated by injecting rabbits with a peptide composed of a sequence found near epitope A near the N-terminus of RAI-1, ie, KQQNY-QQTSQETSRLLEC, with a C residue added at the carboxy terminus for adjuvant purposes. The region selected was predicted to be without phosphorylation and with a high probability of surface exposure.

A RAI-1 AB-2 was generated by injecting rabbits with a peptide composed of a sequence found in a central region with a low probability of phosphorylation, ie, TRAQKQPGHT-NYSSYSK, with a C residue added at the carboxy terminus to increase antigenicity.

A RAI-1 AB-3 was generated by injecting rabbits with a peptide composed of a sequence found in a central region of RAI-1 with a low probability of phosphorylation, ie, GKEERPEGSPTLFKRM, with a C residue added at the carboxy terminus to increase antigenicity.

Polyclonal antibody generation and enzyme-linked immunosorbent assay measurements were carried out by GeneMed Synthesis (San Francisco, CA, USA). The enzyme-linked immunosorbent assay results showed significant peptide-specific reactions at serum dilutions of 1:1,000 and 1:10,000.

None of the antibodies worked on formalin-fixed, paraffin-embedded tissue; AB3 gave reliable results when used on frozen tissue. The AB-3 was used at a concentration of 1:25 for 60 minutes. Sections were incubated with the secondary anti-rabbit antibody for 30 minutes. The reaction product was visualized using diaminobenzidine chromogen (liquid DAB+, K3468, DakoCytomation) for 5 minutes. The sections were then counterstained with Gill 2 hematoxylin (Richard-Allan Scientific, Kalamazoo, MI, USA). As a negative control, the primary antibody was omitted and replaced by normal rabbit serum (code X0903, DakoCytomation).

Evaluation of immunohistochemical stains

On each immunohistochemically stained section, neurons and glial cells were analyzed separately for gray and white matter.^{3,32} For each cell type, the staining intensity was scored as follows: (0) no staining, (1) weak staining, (2) moderate staining, and (3) strong staining. The density (% of positive cells relative to the density of the total population of that specific cell type) of stained cells was rated as follows: (0) no cell stained, (1) low density (<25%), (2) moderate density (25%–50%), and (3) high density (>50%). The histological analysis was performed by an experienced neuropathologist blinded to the diagnosis. For each single analyzed cell type (ie, neurons and glial cells), compound values were calculated by multiplying the staining intensity score by the density score. Finally, sums were calculated for gray matter by adding the compound values of neurons and glial cells, and for the total brain by adding the compound values of neurons and glial cells in gray matter and of glial cells in white matter.^{3,32}

Statistical analysis

Differences between the control and diagnostic groups were calculated using the non-parametric Kolmogorov-Smirnov test for overall differences and the Mann–Whitney *U*-test for post hoc testing between the various groups.

Confounding variables, grouped according to their scaling properties, were used to assess their influence on the dependent variables. These included age, sex, side of the hemisphere examined, suicide status, smoking at time of death, post mortem interval, brain pH, cerebellar granular cell layer necrosis, rapidity of death, lifetime antipsychotic

intake (in fluphenazine mg equivalents), data on intake of first-generation and second-generation generic antipsychotic drugs, generic mood stabilizer drugs, lithium, generic antidepressant drugs, and generic anticholinergic drugs.

At first, we assessed if there was a significant difference for the confounding variables between the various diagnostic groups either by analysis of variance (ANOVA) or logistic regression. As a second step, to determine if the effect of a confounding variable could account for the differences we obtained in our immunohistochemistry, significant differences between disease groups and measured dependent variables as well as confounders and measured dependent variables were compared. In the event that a confounding variable was significant in step one, it was checked if this variable also showed a significant change in step two. Only confounding variables that significantly influenced all measured variables were added to the ANOVA/regression model to remove the confounding effect on the disease/outcome relationship. For example, if fixation time was different between the studied groups, fixation time would only be added to the ANOVA/regression model if we observed a significant effect on staining density and intensity in neurons and glial cells. In other words, we did not include confounding variables in our ANOVA/regression model if they did not contribute to the observed differences. A critical value of $\alpha=0.05$ was used for all analyses.

Results

Immunohistochemistry

RAI-1 immunoreactivities were seen in neurons and astroglial cells (Figures 1 and 2). Astroglial cells were identified based on their histological characteristics, ie, a large, pale nucleus and scant cytoplasm. Double-labeling immunohistochemistry was not done as only a minor percentage of astrocytes stain with glial fibrillary acidic protein.³³ Neurons cannot be reliably stained with antibodies against NeuN in the autopsy setting.

Confounding variables

The following confounding variables differed significantly compared with controls: post mortem time in schizophrenia; time between death and body refrigeration, suicide, and heavy drug abuse in schizophrenia, bipolar disorder and depression; rapidity of death, being on drugs of abuse, cocaine abuse, and heavy alcohol abuse in bipolar disorder.

Rating scales

Using rating scores and compound values, the following results were obtained. All four groups showed a high

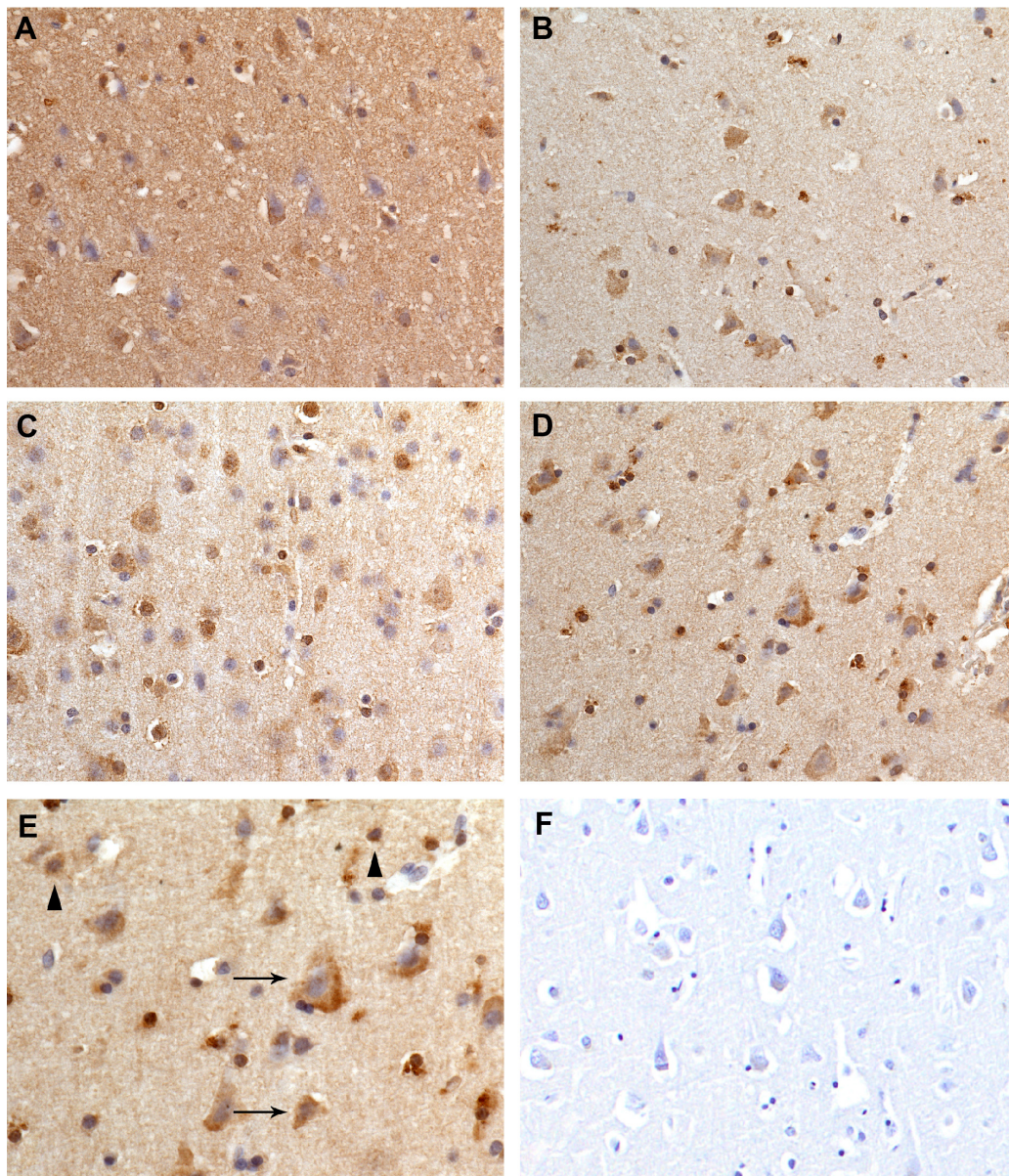


Figure 1 Immunohistochemical demonstration of RAI-1 positive neurons and glial cells from the brains of patients with (A) schizophrenia, (B) bipolar disorder, and (C) major depression in comparison with (D) a normal healthy control. (E) Arrows indicate neurons, arrowheads indicate glial cells. The secondary antibody only control is shown in (F). (A–D, F) Gray matter, objective magnification 40 \times . (E) Gray matter, objective magnification 60 \times .

Abbreviation: RAI-1, retinoic acid induced gene 1.

interindividual variation (Table 2). Significantly increased intensities of RAI-1 protein expression were noted in cortical neurons from all three major psychiatric groups compared with controls. The density of RAI-1-positive neurons was increased ($P=0.06$) in schizophrenia and bipolar disorder ($P=0.06$). RAI-1-positive glial cells in gray matter showed a significantly increased intensity in bipolar disorder. The compound values for the cortex were significantly increased in schizophrenia, bipolar disorder, and major depression ($P=0.01$, $P=0.00$, and $P=0.04$ respectively). No significant changes were noted for the white matter.

Effect of confounding variables

Post mortem time as well as time between death and body refrigeration had no effect on any variable assessed in RAI-positive cells from each diagnostic group. The effect of other confounding variables is shown in Table 3. For the whole group, granular cell layer necrosis of the cerebellum (assessed on the formalin-fixed, paraffin-embedded cerebellar tissue of the contralateral side) was associated with a significant increase in gray matter glial density ($P=0.03$), gray matter glial compound ($P=0.05$), and glia total ($P=0.02$); in brains of persons with alcohol intake, white matter glial density was

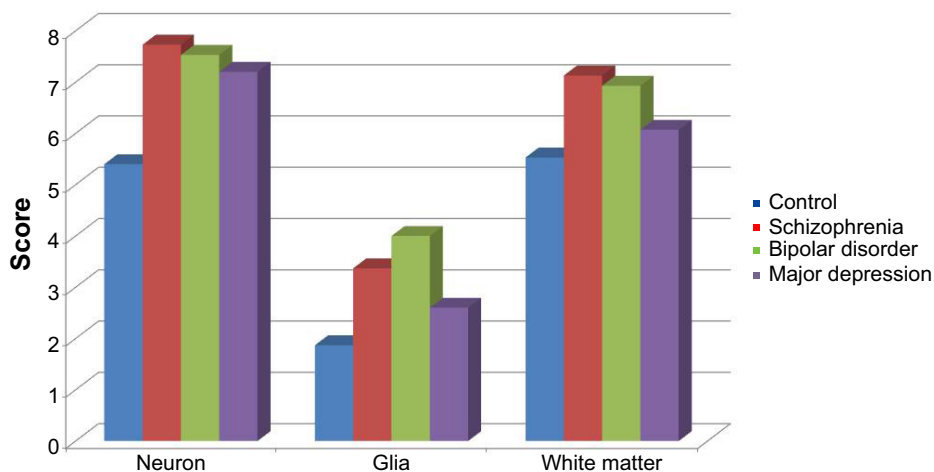


Figure 2 Histogram showing the compound values for neurons, glial cells, and White matter.

Notes: Histogram shows the compound values for neurons and glial cells of the Gray matter, and glial cells of the White matter in control subjects, and patients with schizophrenia, bipolar disorder, and major depression.

significantly increased ($P=0.02$). However, when analyzing these variables for each diagnostic group separately, there were no significant changes.

Effect of medication

The effect of medication is shown in Table 3. The intake of lithium in the bipolar group led to a significant decrease in gray

matter glial RAI-1 density and gray matter glial compound value. Within the same diagnostic group of bipolar patients, intake of mood stabilizers induced an increased expression of RAI-1, reflected as an increased intensity in white matter glial cells and white matter compound value. In bipolar patients, being on antipsychotic medication at the time of death resulted in an increased intensity of RAI-1 in white matter glial cells.

Table 2 Results using rating scales to assess RAI-1-immunostained cells in the dorsolateral prefrontal cortex

RAI-1	Controls		Schizophrenia		Bipolar disorder		Depression	
	MV	SEM	MV	SEM	MV	SEM	MV	SEM
GM neuron density	2.33	0.23	2.80	0.14	2.80	0.14	2.60	0.19
GM neuron intensity	2.07	0.21	2.67	0.16	2.60	0.16	2.60	0.19
GM neuron compound	5.40	0.73	7.73	0.63	7.53	0.63	7.20	0.76
GM glia density	1.40	0.16	1.80	0.17	1.93	0.23	1.40	0.19
GM glia intensity	1.27	0.15	1.73	0.23	1.87	0.19	1.60	0.19
GM glia compound	1.87	0.27	3.47	0.72	4.00	0.79	2.60	0.57
WM density	2.27	0.25	2.73	0.12	2.73	0.15	2.47	0.19
WM intensity	2.07	0.28	2.53	0.17	2.47	0.17	2.27	0.23
WM compound	5.53	0.96	7.13	0.65	6.93	0.61	6.07	0.84
Glia total	7.40	1.09	10.60	1.13	10.93	1.04	8.67	1.12
Cortex	7.27	0.82	11.20	1.09	11.53	1.16	9.80	1.12
Total	12.80	1.32	18.33	1.40	18.47	1.50	15.87	1.56
P-values	CON-SZ	CON-BP	CON-DEP	SZ-BP	SZ-DEP	BP-DEP		
GM neuron density	0.06	0.06	0.32	1.00	0.37	0.37		
GM neuron intensity	0.02	0.04	0.03	0.72	0.91	0.82		
GM neuron compound	0.01	0.03	0.06	0.74	0.64	0.88		
GM glia density	0.14	0.11	0.87	0.71	0.13	0.11		
GM glia intensity	0.18	0.03	0.16	0.56	0.88	0.40		
GM glia compound	0.15	0.05	0.48	0.61	0.44	0.21		
WM density	0.17	0.12	0.63	0.76	0.34	0.25		
WM intensity	0.30	0.41	0.68	0.74	0.46	0.63		
WM compound	0.21	0.43	0.72	0.77	0.35	0.58		
Glia total	0.08	0.07	0.62	0.90	0.18	0.19		
Cortex	0.01	0.00	0.04	0.77	0.57	0.40		
Total	0.01	0.01	0.13	0.92	0.23	0.23		

Abbreviations: BP, bipolar disorder; CON, control; DEP, major depression; GM, gray matter; MV, mean value; RAI-1, retinoic acid induced gene 1; SEM, standard error of the mean; SZ, schizophrenia; WM, white matter.

Table 3 Effect of confounding variables and medication on RAI-1-positive neurons and glial cells

Variable	All	CON	SZ	BP	DEP
Sex	–	–	–	–	–
Hemisphere side	–	–	–	–	–
Suicide	–	No suicide	–	–	–
Death rate	–	–	–	–	–
Granular cell layer	GMGL dens 0.05 inc Glia tot 0.02 inc	–	–	–	–
Alcohol intake	WM dens 0.02 inc WM comp 0.05 inc	–	–	–	–
Drug intake binary	–	–	–	–	–
Smoking	–	–	–	–	–
Problems during pregnancy	–	NA	–	–	–
Problems during childhood	–	NA	–	–	–
On antipsychotic medication at time of death	NA	NA	–	WM int 0.02 inc	–
Antipsychotics, first-generation	NA	NA	–	–	–
Antipsychotics, second-generation	NA	NA	–	–	–
Lithium	NA	NA	–	GMGL dens 0.03 dec, GMGL comp 0.03 dec	–
Mood stabilizers	NA	NA	–	WM int 0.04 inc WM comp 0.009 inc	–
Anticholinergics	NA	–	–	–	–
HSV1-IgG positive	–	–	–	–	–
HSV2-IgG positive	–	–	–	–	–
CMV-IgG positive	–	–	–	–	–
Toxoplasmosis-IgG positive	–	–	–	–	–

Note: –Represents no change.

Abbreviations: BP, bipolar disorder; CMV, cytomegalovirus; comp, compound value; CON, control; dens, density; dec, decreased; DEP, major depression; GM, gray matter; GMGL, gray matter glia; HSV, herpes simplex virus; IgG, immunoglobulin G; inc, increased; int, intensity; NA, not available; RAI-1, retinoic acid induced gene 1; SZ, schizophrenia; tot, total; WM, white matter.

Discussion

In the present study, immunoreactivity for RAI-1 was seen in neurons and astroglial cells from brains of patients with schizophrenia, bipolar disorder, or major depression. The major changes noted were a significantly increased intensity, ie, increased expression, of RAI-1 in cortical neurons in the three disorders examined and in cortical astroglial cells in bipolar disorder, as well as an increased density of RAI-1-positive neurons in schizophrenia and bipolar disorder. A high interindividual variability within each group and a high intergroup variability of RAI-1-immunoreactive neurons and astroglial cells were noted. Although part of this variability can be explained by confounding variables (ie, medication), the factors regulating low and high expression of RAI-1 in brain cells have still to be elucidated.

RAI-1 in schizophrenia and bipolar disorder

The increased expression of RAI-1 protein reported here is likely a reflection of an increased tone of retinoic acid in schizophrenia and bipolar disorder and, therefore, is likely associated with concomitant effects on the development of dopaminergic neurons. Although expression in the adult brain

is not necessarily illustrative of the retinoid status during development, adult RAI-1 expression does have relevance to any ongoing commitment of stem cells to a dopaminergic path and is certainly of relevance to dopaminergic cell maintenance potentially leading to a build-up of cytotoxic compounds.

The human *RAI-1* gene is very similar to its mouse ortholog, both in DNA and protein sequences and in expression patterns. If we take into consideration that a polyglutamine polymorphism of a homolog of *RAI-1* is associated with schizophrenia and that it is highly expressed in neuronal brain regions, RAI-1 protein might be an important modulator of susceptibility to schizophrenia by influencing some aspects of neuron differentiation or function. RAI-1 is likely to function as a transcription factor.¹⁵ It is about 50% homologous to TCF20 (another transcription cofactor), and like many other proteins that can induce gene expression, it has a polyglutamine stretch that modulates activation of transcription.³⁴ In 2005, a study showed that mouse RAI-1 protein has moderate transcription activity in HeLa cells.¹⁸ In 2010, a study assessed the activity of RAI-1 in Neuro-2a cells and found that it has much stronger transactivation activity than that in HeLa cells.³⁵

Another study reported that particular genes are dysregulated in haploinsufficient HEK293T (human embryonic

kidney) cells. The genes that were most significantly upregulated included *SCN12A*, *PSEN2*, *ZIC1*, *RXR β* , and *CLN8*. These genes are involved in sensory transmission, nociceptive behavior, Alzheimer's disease, neurogenesis, neurological function, epilepsy, and mental retardation. The genes downregulated were *NF1*, *MLL*, and *NRID2*, which are involved in neurofibromatosis type I, schizophrenia, circadian activity, and transcription.³⁶ Haploinsufficiency of *Rai-1* in mice (*Rai-1*) and in humans (*RAI-1*) leads to downregulation of brain-derived neurotrophic factor,²⁰ which is involved in neuronal maintenance and regulation of cell survival, differentiation, and growth during development.¹⁶ Since haploinsufficiency may trigger schizophrenia-like conditions and *RAI-1* represents a growth-promoting factor through induction of transcription of brain-derived neurotrophic factor, a clinical potential for *RAI-1* might be foreseen.²⁰ Taking into consideration the reported increase in *RAI-1* expression, one might assume that *RAI* is involved in the pathogenesis of major psychiatric disorders and could also possibly serve as potential therapeutic target.

RA induces the differentiation of various types of neurons and glia by activating the transcription of different families of genes.³⁷⁻³⁹ A role for all-trans retinoic acids (ATRA) in the physiological function of the hippocampus is suggested by the presence of components of retinoid signaling pathways.⁴⁰ *RAR α* and *RXR- α* , *RXR- β* , and *RXR- γ* mRNA transcripts were seen in the hippocampus.^{23,41} As the hippocampus is a center for memory and learning, retinoid signaling might show an effect on these coordinated processes. Obviously, retinoid signaling has a physiological role in synaptic plasticity, learning, and memory. Vitamin A deprivation in adult mice and rats highlights the importance of adequate vitamin A status for such cognitive functions; however, the precise targets of the retinoid signaling pathways underlying these altered behaviors remain to be identified.

RA pathway in schizophrenia and bipolar disorder

Schizophrenia and bipolar disorder are complex diseases, with multiple neurotransmitter systems being involved. There is evidence that altered dopaminergic function underlies the psychotic aspects of these disorders, as drugs that increase dopamine at the synapse exacerbate positive symptoms (reviewed by Seeman⁴² and Davis et al⁴³). Although retinoid analogs have been proposed as possible therapeutic drugs for schizophrenia,^{44,45} it is not yet clear that the multifaceted effects of retinoic acid agonists or antagonists on the dopaminergic system could reliably be expected to be

therapeutic. Thus, retinoic acid is involved in directing stem cells to form dopaminergic neurons⁴⁶⁻⁴⁸ and increases the expression of dopaminergic D2R receptors;⁴⁹ however, it can also exert negative effects on the integrity of dopaminergic neurons in the substantia nigra and ventral tegmental areas.

Although the mechanism of action of antipsychotics is not yet clearly understood, both the typical and atypical antipsychotic drugs antagonize dopamine D2 receptors (reviewed by Davis et al⁴³), an effect which at a minimum contributes to side effects such as blunting of affect. Retinoid therapy that would increase expression of D2 receptors might therefore be expected to help minimize the D2-related effects of antipsychotics. Of note, chronic treatment of adult rats with haloperidol, but not clozapine, resulted in a small but significant increase in abundance of mRNA for *RAR β* and *RXR γ* , the predominant isoforms expressed in the striatum; however, increased mRNA abundance does not always translate into increased protein expression.^{28,29} The fact that the effect was limited in degree and restricted to haloperidol is consistent with antipsychotics not being a major confounder of *RAI-1* expression data.

RA in depression

An increase in depressive episodes through hypervitaminosis A is well documented.^{50,51} Potential targets for retinoid signaling in depression include dopaminergic, serotonergic, and noradrenergic pathways or a complex interaction between these neurotransmitter systems. ATRA have been reported to regulate the differentiation of serotonergic neurons in mouse neural crest cells, to promote the proliferation of these neurons,⁵² and to induce the expression of 5-HT_{1A} receptors in neuronal cells.⁵³ Interestingly, whereas ATRA have been shown to promote adult neurogenesis in vitro,^{54,55} chronic administration of 13-cis-RA in vivo results in a significant decrease in hippocampal neurogenesis in adults.⁵⁶

Maret et al⁵⁷ showed that retinoids play a role in synchronizing cortical activity, whereby *RAR β* determines the contribution of delta oscillations to the sleep electroencephalogram. Desynchronization of cortical activity is a conspicuous feature in schizophrenia⁵⁸ and autism.⁵⁹ In Spencer et al, patients described the following changes: absence of the posterior component of the early visual gamma band response to Gestalt stimuli; abnormalities in the topography, latency, and frequency of the anterior component of this response; delayed onset of phase coherence changes; and decreased pattern of interhemispheric coherence.⁵⁸ Thus, a failure of gamma band synchronization, especially in the 40 Hz range, is present in schizophrenia. RA signaling, which is involved

in the patterning of the brain and dopaminergic pathways, regulates cortical synchrony in the adult.

Given the large number of neuronal genes that could potentially be transcriptionally regulated by retinoids in the adult brain, surprisingly little is known about the impact of ATRA signaling on other brain functions. The use of transgenic mouse models has indicated a role for retinoids in the regulation of striatal dopaminergic function and the control of locomotor activity. However, such models do not clearly delineate between the well described developmental effects of retinoids and any novel adult-specific effects.

Relevance of RA to other CNS disorders RA pathway in Alzheimer's disease

Retinoid signaling has been implicated in the pathology of Alzheimer's disease.⁶⁰ Application of ATRA to cells upregulates amyloid precursor protein (*APP*) mRNA^{61,62} which is likely mediated by a direct effect on a RARE in the APP promoter. Amyloid is the central component of senile plaques, and these plaques may be generated as a result of overproduction or disturbed metabolism of APP,⁶³ or due to differential expression of APP mRNA transcripts.⁶⁴ Ono et al⁶⁵ showed that vitamin A had anti-amyloidogenic and destabilizing effects on fibril formation in vitro.⁶⁵ This effect was independent of any changes in gene expression and most likely resulted from the antioxidant properties of retinoids.⁶⁵ Divergent results by Rinaldi et al⁶⁶ reported that plasma vitamin A levels are reduced in patients suffering from Alzheimer's disease, whereas Connor and Sidell⁶⁷ have shown that hippocampal retinoid content is similar in Alzheimer's disease and control groups. Corocan et al⁶⁸ reported a decreased abundance of RAR α and RALDH2 in brains with Alzheimer's disease, suggesting that RA signaling is likely to be compromised in patients.

RA pathway in Parkinson's disease

Parkinson's disease is characterized by loss of dopaminergic neurons in the substantia nigra and intracytoplasmic accumulation of Lewy bodies, with alpha synuclein being the most abundant component of Lewy bodies. The RA pathway might play a role in the pathogenesis of Parkinson's disease, but also provides a platform for possible treatment approaches.

RA is required for the establishment of proper mesodiencephalic dopaminergic neuronal identity.⁴⁸ Early and specific exposure to retinoic acid improved the regional identity of neural progenitor cells derived from human embryonic stem cells, Parkinson's disease, or healthy subject-specific induced

pluripotent stem cells.⁶⁹ ATRA induce differentiation of cord blood-derived multipotent stem cells into dopaminergic neurons, thus opening up new ways for alternative therapy for Parkinson's disease.⁷⁰

Embryonic stem cells treated by retinoic acid and transplanted into Parkinsonian rats relieved apomorphine-induced asymmetric motor behavior; the transplanted cells showed tyrosine hydroxylase immunoreactivity and displayed neuronal and glial features.⁷¹ Ulusoy et al⁷² showed that pioglitazone and retinoic acid have some beneficial effects in a rotenone-induced model of Parkinson's disease in rats whereby pioglitazone seemed to be more effective than retinoic acid. Pioglitazone, but not retinoic acid, significantly reversed the reduced striatal dopamine level. Data from Yin et al⁷³ suggested that early post-treatment with 9-cis-retinoic acid has a protective effect against neurodegeneration in nigrostriatal dopaminergic neurons in an animal model of Parkinson's disease.

RA promotes differentiation and α -synuclein oligomer formation in neuroblastoma cells.⁷⁴ Addition of retinoic acid to SHSY-5Y cells⁷⁵ and 3D5-transfected cells⁷⁶ leads to formation of alpha-synuclein oligomers and promotes assembly of alpha-synuclein aggregates, respectively.

Conclusion

Retinoids, retinoid agonists and antagonists offer some potential as therapeutics in schizophrenia, depression, Alzheimer's disease, and possibly even Parkinson's disease. In the present study, we observed by immunohistochemistry a significant increase in expression of RAI-1 in the brains of patients with schizophrenia, bipolar disorder, or major depression. One might assume that RAI-1 is increased due to its modulatory function in neuronal maintenance, altered signaling pathways, and cell survival. The factors leading to increased RAI-1 expression are still not known.

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Disclosure

The authors report no conflicts of interest in this work.

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