Dysregulation of the fibroblast growth factor system in major depression

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In this report we describe findings that imply dysregulation of several fibroblast growth factor (FGF) system transcripts in frontal cortical regions of brains from human subjects with major depressive disorder (MDD). This altered gene expression was discovered by microarray analysis of frontal cortical tissue from MDD, bipolar, and nonpsychiatric control subjects and was verified by quantitative real-time PCR analysis and, importantly, in a separate cohort of MDD subjects. Furthermore, we show, through a separate analysis of specific serotonin reuptake inhibitor (SSRI)-treated and non-SSRI-treated MDD subjects that the observed changes in expression of FGF transcripts are not secondary to drug treatment. Rather, changes in specific FGF transcripts are attenuated by SSRIs and may thus be partially responsible for the mechanism of action of these drugs. We also make available the gene-expression profile of all of the other growth factors and growth factor receptors detected in these postmortem samples.

Major depressive disorder (MDD) and bipolar disorder (BPD) are mood disorders that strike a large proportion of the population. They are complex disorders with unknown etiology, likely the result of the interplay between vulnerability genes and environmental stressors (1). These affective diseases depend on alterations in the emotional circuitry in the brain and are hypothesized to involve disturbed activity of the cerebral cortex. Imaging techniques have implicated the dorsolateral prefrontal cortex (DLPFC) and the anterior cingulate cortex (AnCg) in these disorders, because brains from affected subjects can display alterations in volumetric measurements (2, 3) and because living subjects show altered activity in response to a cognitive challenge (4, 5). By using a candidate approach, several studies have demonstrated altered cortical expression of specific neurotransmitter- and stress-related genes in affective illness (6–8). The demonstrated changes suggest that alterations in cortical activity in affected subjects may be correlated with gene-expression changes, particularly those that affect neuronal signaling and plasticity. The mood disorders may represent the clinical manifestation of an altered "neural phenotype," and gene-expression changes might provide signatures of these clinical states. However, the full extent of the alteration in cortical activity has not been fully described in these diseases, nor has an unbiased "discovery" approach been applied to characterize the extent of the cortical alterations associated with severe depression.

Microarray technology is currently the most powerful technique available to evaluate the global transcriptional profile of a large number of biological samples. Given the likely involvement of both the AnCg and DLPFC in mood disorders, we have applied microarray technology to the study of these regions in postmortem samples from MDD and BPD subjects and nonpsychiatric controls. The sample of subjects was carefully selected to avoid factors that could systematically confound transcriptional profiling in postmortem human brains, such as agonal factors and tissue pH (9, 10). This transcriptional profiling study reports significant alterations of gene expression in major depression and contrasts the two major mood disorders (MDD and BPD) with the same group of controls.

These results have revealed several hundred transcripts that are differentially expressed among controls and each of the psychiatric diseases across both the AnCg and DLPFC. Some of the observed changes in expression are common across both diseases, and others are disease-specific. The most striking results are changes in ensembles of genes in which multiple members of a given family are systematically altered in one of the conditions, lending validation to the involvement of a given signaling pathway in that disorder. Among these is dysregulation of the fibroblast growth factor (FGF) system in MDD subjects but not in BPD subjects.

Here, we report that dysregulation of the FGF system in MDD involves two specific FGF receptors, FGFR2 and FGFR3, as well as several FGF ligands, including FGF1, FGF2, FGF9, and FGF12. We also demonstrate that the changes are unlikely to be secondary to treatment with antidepressants.

Methods

Human Tissue Acquisition and Preparation. Acquisition, preparation, and GeneChip analysis of human brain tissue by this research group is described in detail in ref. 11. Tables 1 and 2 list all subjects used in the current study and include subject age, gender, postmortem interval (PMI) (from time of death to freezing of the brain), and brain pH. Previous work from our group has shown that agonal factors and low pH can have significant and systematic effects on gene expression (9, 10). Thus, all subjects whose brains were used in the current study had no associated agonal factors, such as coma, pyrexia, skull fracture, hypoxia, dehydration, hypoglycemia, multiorgan failure, seizures, ingestion of neurotoxic substances, or prolonged death as determined by the Hardy scale (12).

Total RNA was extracted from microdissected brain regions from each brain, and each RNA sample was processed and hybridized to Affymetrix HG-U133A arrays (Affymetrix, Santa Clara, CA) per the manufacturer's instructions. RNA processing and array hybridizations were performed independently at the

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Abbreviations: AnCg, anterior cingulate cortex; BDNF, brain-derived neurotrophic factor; BPD, bipolar disorder; CB, cerebellum; DLPFC, dorsolateral prefrontal cortex; FGF, fibroblast growth factor; FGFR, FGF receptor; ISH, *in situ* hybridization; MDD, major depressive disorder; SSRI, serotonin reuptake inhibitor.

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Table 1. Subject data for cohort A

Subject ID	Gender	Age at time of death*	Diagnosis	Brain pH†	Postmortem interval‡	Cause of death	SSRIs at time of death
1881	M	69	BPD	6.9	11	Accident	No
2311	M	23	BPD	7.1	9	Suicide	No
2466	M	26	BPD	6.9	19	Suicide	No
2566	F	56	BPD	6.8	29	Suicide	Yes
3038	M	52	BPD	7.1	28	Suicide	No
3241	M	59	BPD	7.0	16	s.m.c.	No
2861	F	60	Ct	7.0	24	s.m.c.	No
3018	M	70	Ct	7.0	27	s.m.c.	No
2169	M	18	Ct	7.0	22	Accident	No
2316	M	58	Ct	7.0	26	s.m.c.	No
2292	M	55	Ct	6.9	15	s.m.c.	No
2805	M	45	Ct	6.9	21	s.m.c.	No
3196	M	44	Ct	6.9	23	s.m.c.	No
2208	F	72	MDD	7.1	21	Suicide	No
2267	M	19	MDD	7.1	18	Suicide	No
2315	M	58	MDD	6.9	24	Suicide	Yes
3071	M	49	MDD	7.0	31	Unknown	Yes
3064	M	46	MDD	6.9	27	s.m.c.	No
3031	M	49	MDD	7.2	27	Suicide	No
2944	M	52	MDD	6.8	16	s.m.c.	Yes
3004	F	48	MDD	7.0	37	Suicide	Yes
3168	M	39	MDD	6.8	28	Suicide	Yes

s.m.c., sudden medical condition; Ct, control.
*Averages: BPD, 47.5 ± 18.7; control, 50 ± 16.7; MDD, 48.0 ± 14.2.
†Averages: BPD, 6.97 ± 0.1; control, 7.0 ± 0.1; MDD, 6.98 ± 0.1.
‡Averages: BPD, 18.6 ± 8.4; control, 22.6 ± 4.0; MDD, 25.4 ± 6.5.

collaborating institutions. DLPFC samples were processed at the University of California, Davis, and the University of Michigan. AnCg samples were processed at the University of California, Irvine, and the University of Michigan.

Microarray Data Analysis. Affymetrix CEL files generated after hybridization of samples to HG-133A arrays were batched within a brain region for analysis. All same-region samples were normalized together by using the robust multiarray average (RMA) (available at www.bioconductor.org), employing a custom probe mapping file (replacing the Affymetrix .cdf file) based on UniGene build no. 170. The custom mapping file is generated by reassigning each Affymetrix probe independently to an updated UniGene cluster before condensation of signal into probe sets. This alleviates contamination of signal by incorrect probe sequences and removes redundancies from the data output. The current .cdf file is available at http://brainarray.mhri.med.umich.edu/Brainarray/Database/CustomCDF/genomic_curated_CDF.asp, and the associated methodology of its creation is described at the same web site. Output from RMA normalization was imported into PARTEK PRO 5.1 (Partek, St. Charles, MO) for statistical analysis with a mixed-model multivariate ANOVA. At this stage, cohorts A and B were analyzed independently. Factors included "site," "batch," "subject," and "gender" as categorical independent variables and "diagnosis" as the class variable. Post hoc tests (least-squares difference) were run simultaneously to generate *P* values for the difference between MDD, BPD, and control means. A false discovery-rate

Table 2. Subject data for cohort B

Subject ID	Gender	Age at time of death*	Diagnosis	Brain pH†	Postmortem interval‡	Cause of death	SSRIs at time of death
3145	M	77	Ct	6.6	7	s.m.c.	No
3281	F	70	Ct	6.9	21	Accident	No
3516	M	41	Ct	7.0	23	s.m.c.	No
3519	M	65	Ct	6.9	14	s.m.c.	No
3523	M	40	Ct	7.1	37	s.m.c.	No
3572	M	49	Ct	6.7	28	s.m.c.	No
Averages for controls		57.0 ± 15.8		6.9 ± 0.2	21.7 ± 10.5		
3169	M	35	MDD	7.0	25	Accident	No
3365	M	47	MDD	7.3	29	Suicide	No
3398	F	80	MDD	6.7	15	s.m.c.	No
3481	M	66	MDD	7.1	32	s.m.c.	Yes
Averages for MDDs		57.0 ± 19.9		7.0 ± 0.3	25.3 ± 7.4		

s.m.c., sudden medical condition; Ct, control.
*Averages: controls, 57.0 ± 15.8; MDD, 57.0 ± 19.9
†Averages: control, 6.9 ± 0.2; MDD, 7.0 ± 0.3.
‡Averages: control, 21.7 ± 10.5; MDD, 25.3 ± 7.4.

Table 3. Microarray and real-time PCR data for all FGF transcripts reliably detected in either DLPFC or AnCg

		DLPFC *P* values				AnCg *P* values			
UniGene ID	Transcript	Cohort A	Cohort B	Real-time PCR	DLPFC direction	Cohort A	Cohort B	Real-time PCR	AnCg direction
Hs.278954	FGF1	<0.01*	<0.01	<0.01	Down	0.01	NS	NS	Down
Hs.284244	FGF2	NS	0.01	NS	Down	<0.01*	<0.01	NS	Down
Hs.433252	FGF7	NS	NS	ND	NC	NS	NS	ND	NC
Hs.111	FGF9	<0.01	NS	NS	Up	<0.01*	NS	NS	Up
Hs.343809	FGF12	NS	NS	NS	NC	<0.01*	NS	NS	Up
Hs.6540	FGF13	NS	NS	ND	NC	NS	NS	ND	NC
Hs.223851	FGF14	NS	NS	ND	NC	NS	NS	ND	NC
Hs.748	FGFR1	NS	NS	ND	NC	NS	NS	ND	NC
Hs.404081	FGFR2	<0.01*	0.03	0.01	Down	<0.01*	0.02	NS	Down
Hs.1420	FGFR3	<0.01*	<0.01	<0.01	Down	<0.01*	NS	NS	Down

NS, not significant; NC, no change; ND, not done.
*Met FDR multiple-testing correction at the level of accepting 5% false positives.

correction (13) was applied to each ANOVA result, and those genes that passed correction are indicated with an asterisk in Table 3.

Real-Time PCR. Real-time PCR methodology is described by Li *et al.* in ref. 9. Briefly, all amplicons were 70–130 bp in length within the 3′-most 600 base pairs of the target mRNA. Real-time PCR amplification reactions used SYBR-green detection and were performed on the iCycler system (Bio-Rad). Each sample was measured in duplicate and normalized to reference gene expression, including GAPDH, ACTB, ACTG1, and PPIA. All subjects from cohort A were analyzed independently in the real-time PCR studies.

***In Situ* Hybridization (ISH).** The ISH method used in this study is described in detail for human tissue use in refs. 6, 14, and 15. Briefly, tissue was sectioned at −20°C at a thickness of 12 μM, mounted onto poly(L-lysine)-coated slides, and stored at −80°C until use. Before probe hybridization, tissue was fixed in 4% paraformaldehyde at room temperature, rinsed with aqueous buffers, and dehydrated with graded alcohols. Riboprobes were synthesized with incorporation of ^{35}S-UTP and ^{35}S-CTP and hybridized to tissue overnight at 55°C. Sections were washed with increasing stringency, dehydrated with graded alcohols, air-dried, and exposed to film. Exposure time was chosen to maximize signal and was determined empirically by periodic development of films exposed to test slides.

Riboprobes were synthesized from cDNA fragments that were cloned by our research group. Probe sequences that were generated are complementary to RefSeq database nos. NM_022975.2, position 3704-4100 (human FGFR2); NM_022965.1, position 3086-3522 (human FGFR3); and NM_002010.1, position 583-906 (human FGF9). All probes were complementary to a single exon.

Results

Microarray Studies. The Affymetrix HG-133A array contains probe sets for 21 FGF system transcripts, including all 4 receptors (FGFR 1–4) and 17 FGF peptide ligands (FGF 1–3, 5–9, 12–14, 17, 18, and 20–23). Of these, only 10 were reliably detected in the regions assayed: 3 FGF receptors (FGFR 1–3) and 7 FGF ligands (FGF 1, 2, 7, 9, and 12–14). Of the 10 FGF transcripts reliably detected, 6 were significantly altered in at least one of the two regions studied; 4 were significantly differentially expressed in the DLPFC of MDD subjects, including 2 FGF receptors (FGFR 2 and 3) and 2 FGF ligands (FGF 1 and 9); and six were significantly differentially expressed in the AnCg, including two receptors (FGFR 2 and 3) and four ligands (FGF 1, 2, 9, and 12). The probability that this family of molecules would have emerged in the data set by chance, based on a hypergeometric distribution, is $P < 0.001$. These data are summarized in Table 3, which also reports the transcripts confirmed by real-time PCR analysis and/or those observations replicated in a second independent cohort of MDD and control subjects. In all cases, the direction of change found by real-time PCR was in agreement with that found by microarray; however, we only report confirmation for those that reached significance through both techniques. Importantly, none of the above transcripts was observed to be significantly differentially expressed in BPD by microarray or by real-time PCR analysis, demonstrating the specificity of this change for MDD.

Given our data implying dysregulation of FGF system transcripts in MDD, we asked whether changes might be secondary to antidepressant therapy, because a subset of the patients was on antidepressants. Although subjects were prescribed a variety of psychotropic drugs, the majority of depressed subjects in cohort A ($n = 5$) were taking specific serotonin reuptake inhibitors (SSRIs), whereas the remaining depressed subjects were either taking no medication ($n = 2$) or anxiolytics ($n = 2$). Thus, we separated the analysis of microarray data into controls, MDD subjects who were prescribed SSRIs, and MDD subjects who were not prescribed SSRIs. These data (Fig. 1) show that SSRIs tend to attenuate the altered expression of FGF system transcripts toward levels observed in controls. ANOVA analysis of the three groups reveals a significant group difference for FGF2, FGFR2, and FGFR3 in DLPFC (0.04, 0.04, and 0.05, respectively). Post hoc analysis reveals that, in the case of FGF2 and FGFR3, MDD subjects who were prescribed SSRIs showed significantly higher expression than MDD subjects who were not prescribed SSRIs (both <0.01, least-squares difference). These data strongly suggest that the decreased expression of the transcripts observed in depressed subjects relative to controls is not secondary to drug treatment.

Anatomical Studies. Because the distribution of many of the products of the affected genes has not been described in human brains, we explored the expression profiles of FGFR2, FGFR3, and FGF9 in cortical tissue from a subset of subjects used for the microarray studies (Figs. 2-4). We also examined the cerebellar cortex as a means of validating our probes, because these transcripts have been reported to have high expression in the cerebellum (CB) in rodent studies (16, 17). These studies were designed not to be quantitative comparisons between cases and controls but to describe the potential specificity of expression in the human cortex. In Fig. 2, intense expression of FGFR2 is observed in the Purkinje cell layer of the cerebellum. Diffuse FGFR2 mRNA expression is observed in the deep cortical layers

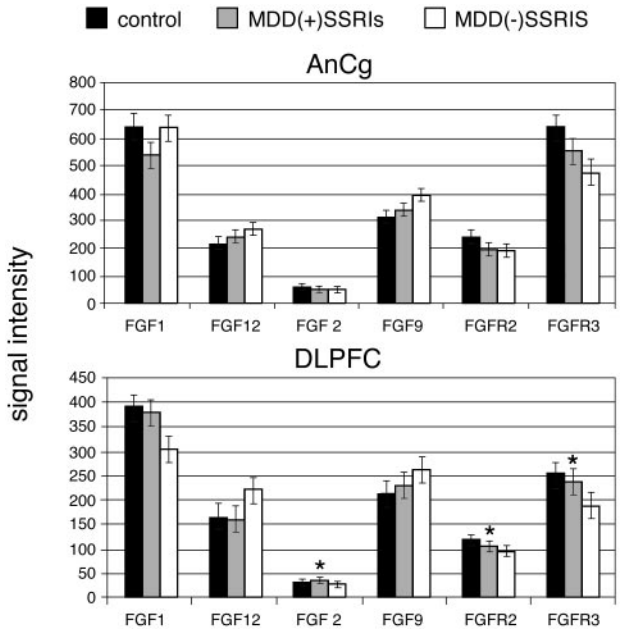

Fig. 1. Effect of SSRI treatment on FGF system transcript expression in MDD subjects. The graphs show normalized mean expression values for control subjects, MDD subjects who were prescribed SSRIs, and MDD subjects who were not prescribed SSRIs, as indicated, for all FGF transcripts that were significantly differentially expressed in either AnCg or DLPFC. Error bars, standard error; *, transcripts that showed significant differential expression ($P < 0.05$, least-squares difference) between MDD(+)SSRI and MDD(−)SSRI groups.

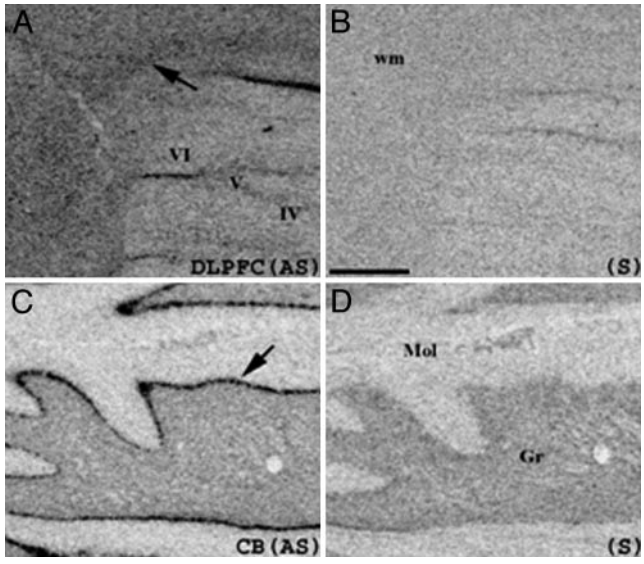

Fig. 2. Human FGFR2 ISH. Images depict FGFR2 mRNA expression in DLPFC (*A*) and CB (*C*), with respective sense *in situ* controls (*B* and *D*). The arrow in *A* points to FGFR2 mRNA expression in the DLPFC, which generates a diffuse signal intensity in the superficial subcortical white matter. This signal is not present in an adjacent section under sense strand control (*B*). The arrow in *C* points to FGFR2 mRNA expression adjacent to the granule cell layer of the cerebellum. This intense signal appears to reside in the Purkinje cell layer and is lost in an adjacent section under sense strand control (*D*). IV–VI, cortical layers IV–VI; AS, antisense strand; Gr, granule cell layer of the cerebellar cortex; Mol, molecular layer of the cerebellar cortex; S, sense strand; wm, superficial subcortical white matter.

of DLPFC. FGFR3 mRNA expression (Fig. 3) in the CB is less intense but similar to that observed for FGFR2. In contrast, FGFR3 mRNA expression in DLPFC, although diffuse, is less intense than that of FGFR2 and is concentrated in cortical layers II and III. In AnCg, FGFR3 mRNA expression is observed in the deep cortical layers (Fig. 3). Interestingly, previous studies in rodents have shown that FGFR2 is predominately expressed in neuroglia, whereas FGFR3 expression is potentially neuronal (16). In sharp contrast to the FGF receptors, FGF9 demonstrates intense mRNA expression in the cerebellar granule cell layer and moderate expression in layer III of the DLPFC (Fig. 4). This latter observation is consistent with a previous report of FGF9 immunoreactivity in layer III of the human cerebral cortex (18). To our knowledge, the present data are the first anatomical description of FGFR2, FGFR3, or FGF9 mRNA expression in the human cortex.

Discussion

In this study, we focused on the altered gene-expression profile of the FGF family in two frontal cortical areas in which the FGF family emerged as the most significantly altered ensemble of genes in MDD across the entire transcriptome. To put this information in context and to share a broader range of results, we are making available the expression profile of all of the genes within the two brain regions that have been detected on the Affymetrix chips and that can be classified as coding for either a growth factor or a growth factor receptor (see Tables 4 and 5, which are published as supporting information on the PNAS web site). The list shows altered expression in other genes, most notably the brain-derived neurotrophic factor (BDNF) receptor Ntrk2, which exhibited a significant decrease in the MDD subjects.

In the context of the FGF family, we showed that in the DLPFC and/or AnCg, several FGF system transcripts, including FGF1, FGF2, FGF9, FGF12, FGFR2, and FGFR3, are differentially expressed in subjects with MDD relative to controls. The most salient of these findings, because they have been confirmed by either real-time PCR studies or in an independent cohort of depressed subjects, are the down-regulation of FGF1, FGF2, FGFR2, and FGFR3. We have also described the localization of

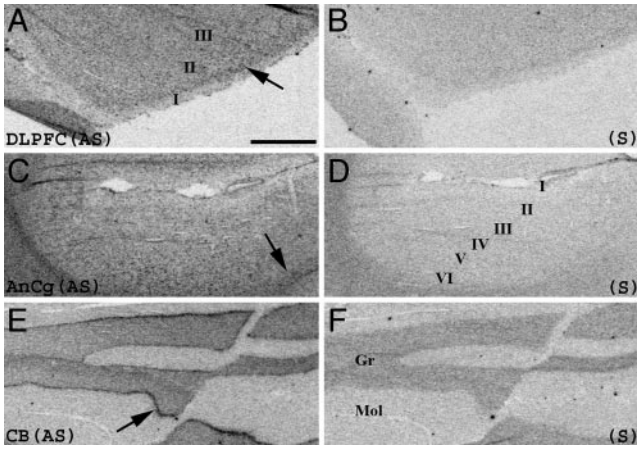

Fig. 3. Human FGFR3 ISH. Images depict FGFR3 mRNA expression in DLPFC (*A*), AnCg (*C*), and CB (*E*), with respective sense strand technical controls (*B*, *D*, and *F*). The arrow in *A* points to diffuse FGFR3 mRNA expression in layers II and III of the DLPFC. This signal is not present in an adjacent section under sense strand control (*B*). The arrow in *C* points to FGFR3 mRNA expression in layer VI of the AnCg. This diffuse signal is lost in an adjacent section under sense strand control (*D*). The arrow in *E* points to FGFR3 mRNA expression adjacent to the granule cell layer of the cerebellum. Similar to what was observed with FGFR2 mRNA expression, the FGFR3 signal appears to reside in the Purkinje cell layer and is not present in an adjacent section under sense strand control (*F*). (Scale bar: 2,200 μm.) I–V, cortical layers I–V; Gr, granule cell layer of the cerebellar cortex; Mol, molecular layer of the cerebellar cortex.

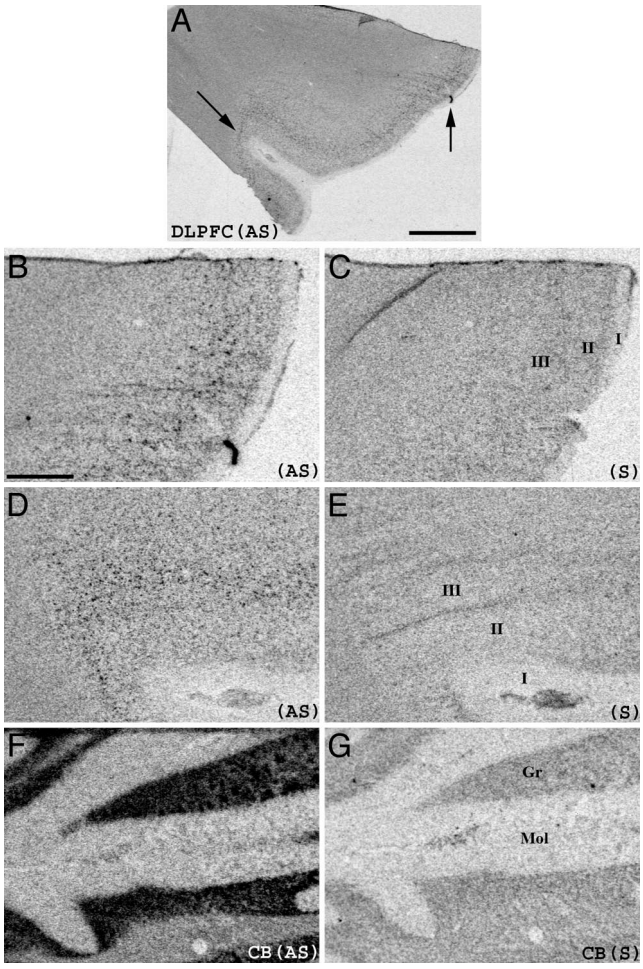

Fig. 4. Human FGF9 ISH. Images depict FGF9 mRNA expression in DLPFC (*A–E*) and cerebellum (*F* and *G*), with respective sense strand technical controls. (*A*) Lower-power view of a section of human DLPFC showing FGF9 mRNA expression. The arrows point to regions represented by higher-power images in *B–E*. (*B* and *D*) Moderate FGF9 mRNA expression in layer III. This signal is not present in adjacent sections under sense strand control (*C* and *E*). (*F*) Intense FGF9 mRNA expression within the granule cell layer of the cerebellum, with no signal detected within the molecular layer. There is no granule cell signal detected in the adjacent sense control (*G*). (Scale bars: *A*, 5,000 μm; *B*, 2,500 μm. Scale bar for *B* applies to *B–G*.) I–III, cortical layers I–III; Gr, granule cell layer of the cerebellar cortex; Mol, molecular layer of the cerebellar cortex.

FGFR2, FGFR3, and FGF9 mRNA in frontal cortical regions of the human brain. Furthermore, we have shown that our observations are not an effect of antidepressant therapy; rather, the FGF system might be involved in the SSRI mechanism of action, because MDD subjects on SSRIs had a significantly attenuated decrease in some FGF transcripts. Admittedly, the variety of drug histories of the depressed subjects and the difficulty in establishing the compliance rate of taking the prescribed medications are not ideal for this latter conclusion. However, our data strongly suggest that the decreases observed in several FGF transcripts in depressed subjects are not secondary to drug therapy. Thus, several FGF transcripts show altered expression in severe depression and are partially reversed by SSRI therapy.

The pattern of change in the FGF system seen in MDD is complex. The expression of some genes is up-regulated, but that of others is down-regulated. It can be argued that the overall "tone" of the system depends on the final step—the level of expression of the receptors, which are significantly and consistently decreased. It is unclear whether changes in mRNA for FGF ligands or receptors are due to structural differences in the FGF genes (e.g., allelic variations that impact the level of expression) or from being downstream from some gene that modifies their expression. Finally, the multiple splice variants of these receptors may exhibit differential patterns of expression and regulation. These issues deserve careful analysis in future studies.

The involvement of the FGF system in MDD that is implied by our studies using microarray technology is previously unrecognized. However, other growth factors previously have been hypothesized to contribute to the etiology and maintenance of mental illnesses. Most notably, BDNF has been implicated repeatedly in MDD, BPD, and schizophrenia. BDNF mRNA levels reportedly are decreased in the DLPFC (19) of schizophrenics, and BDNF protein levels are decreased in the serum of MDD (20) and schizophrenia (21) patients. Other studies implicate nerve growth factor (22), epidermal growth factor (23), and neurotrophin-3 (24–26) in psychiatric illness.

Although our current findings relate to alterations of FGF expression in the cortex, a larger body of literature focused on growth factor activity in the hippocampus provides a framework in which to place these results. One theory proposes that growth factor levels positively correlate with hippocampal volume and that hippocampal volume negatively correlates with susceptibility to stress-induced illness. Thus, increased BDNF (27) and FGF2 levels (28, 29) have been shown to attenuate the loss in hippocampal volume and increase hippocampal neurogenesis after various stressors. Hippocampal volume, in turn, has been negatively correlated with predisposition to posttraumatic stress disorder (PTSD) (30). Furthermore, BDNF expression is increased in the hippocampus (31) and cortex (32) by antidepressant therapies, and our current report suggests that this latter observation is also true for FGF system transcripts. Together, this body of evidence has led to the hypothesis that BDNF signaling is important in depression (33), and the present findings lead to the hypothesis that the FGF system may be equally important. Efforts are under way in our laboratories to examine the expression of the FGF system in the hippocampi from the same subjects used for the present study.

Growth factors play significant roles in development and maintenance of the CNS. In the developing brain, growth factors are involved in specific neuronal terminal differentiation and migration of neurons to appropriate regions. In the adult brain, growth factors continue to play a critical role in neuronal survival, axonal branching, and synaptic plasticity. Specifically, FGFs have been shown to play an important role in development of the neocortex. FGF2 (34) and FGF8 (35) recently have been shown to interact with Wnt in development of the cortex in mouse and chick embryos, respectively. In the adult brain, FGF2 promotes neuronal survival and axonal branching (36), and its mRNA expression is increased by various stressors in multiple brain regions (37). Recent reviews have been published on the roles of FGFs in the CNS (38, 39).

Gene-expression profiling, although strongly implicating the FGF family in MDD, does not address whether the observed dysregulation represents a predisposing factor to the illness or a consequence of the disease process. Determining the exact role of the FGF system in mood disorders would require genomic analysis to ascertain the presence of allelic variations in genes that might represent vulnerability genes for severe depression. The present findings suggest that FGF family members, especially FGF1, FGF2, FGFR2, and FGFR3, are candidate genes for such a genomic analysis. Developmental events, stress and other environmental events, and the use of antidepressants could impact the FGF system and the initial expression and course of severe depression.

In sum, growth factor activity in the brain is hypothesized to be critical in mood disorders. The present findings focus attention on the complex and powerful FGF family as a potential key player in

either the etiology or the expression of severe depression and suggest new strategies for developing treatments for this disease.

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