ORIGINAL ARTICLE

Association of a Polymorphism Near *CREB1* With Differential Aversion Processing in the Insula of Healthy Participants

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Context: Previous functional neuroimaging studies have identified a network of brain regions that process aversive stimuli, including anger. A polymorphism near the cyclic adenosine monophosphate response element binding protein gene (*CREB1*) has recently been associated with greater self-reported effort at anger control as well as risk for antidepressant treatment–emergent suicidality in men with major depressive disorder, but its functional effects have not been studied.

Objective: To determine whether this genetic variant is associated with altered brain processing of and behavioral avoidance responses to angry facial expressions.

Design and Participants: A total of 28 white participants (mean age, 29.2 years; 13 women) were screened using the Structured Clinical Interview for *DSM-IV* to exclude any lifetime Axis I psychiatric disorder and were genotyped for rs4675690, a single-nucleotide polymorphism near *CREB1*.

Main Outcome Measures: Blood oxygenation level–dependent signal by functional magnetic resonance imaging in the amygdala, insula, anterior cingulate, and orbitofrontal cortex during passive viewing of photographs of faces with emotional expressions. To measure approach and avoidance responses to anger, an off-line key-press task that traded effort for viewing time assessed valuation of angry faces compared with other expressions.

Results: The *CREB1*-linked single-nucleotide polymorphism was associated with significant differential activation in an extended neural network responding to angry and other facial expressions. The *CREB1*-associated insular activation was coincident with activation associated with behavioral avoidance of angry faces.

Conclusions: A polymorphism near *CREB1* is associated with responsiveness to angry faces in a brain network implicated in processing aversion. Coincident activation in the left insula is further associated with behavioral avoidance of these stimuli.

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A DISTRIBUTED SET OF BRAIN structures has been implicated by functional neuroimaging studies[1-4] in the processing of negative environmental stimuli,[5] including facial expressions of anger, which healthy individuals exert significant efforts to avoid.[5] Brain regions active during the visual processing of aversive stimuli include the amygdala,[6-11] orbitofrontal cortex (FOC),[1,2,5,8,11] insula,[12] and anterior cingulate cortex.[2,5,8,11] Amygdala involvement in the assessment of threat and stimulus relevance to survival is important for automatic unconscious processing.[13] Densely interconnected with the amygdala and FOC,[14,15] the insula has also been proposed to play a crucial role in processing and responding to aversive states.[12]

How activity across this network of brain structures that process aversion might be modulated is unknown. Converging lines of evidence suggest a role for the transcription factor cyclic adenosine monophosphate response element binding protein (CREB) in structures related to reward and aversion. The CREB gene product was initially shown to be central to the rewarding effects of cocaine and other drugs of abuse,[16-21] including alcohol.[22] More recently, a role in response to aversive stimuli has been proposed. For example, animal studies show that CREB-mediated transcription is increased in response to stressful stimuli.[23,24] In humans, decreased CREB expression and

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activity has been reported in the prefrontal cortex of suicide victims.[25,26]

Consistent with a possible role in aversion, we have previously reported an association between a variation near the gene coding for CREB (*CREB1*; OMIM 123831) and greater effort at control of internal and external anger among men with major depressive disorder.[27] A subsequent study found the allele associated with greater effort to control anger to be associated with a 3-fold increase in risk of suicidality during acute antidepressant treatment in patients with major depressive disorder. This single-nucleotide polymorphism (SNP) and those within *CREB1* that are in linkage disequilibrium with it do not have obvious effects on transcription of the CREB protein.[28]

Examining intermediate phenotypes derived from neuroimaging has been proposed as a means of understanding the way in which a genetic variation mediates neural functioning.[29] Therefore, we sought to examine the functional implication of this common variation in vivo. If participants with the T allele have reported greater effort to control their internal experience of anger, would this manifest as increased or decreased effort to avoid perceived anger during a behavioral task? In parallel, would perception of anger be associated with an increased or decreased blood oxygenation level-dependent (BOLD) signal by functional magnetic resonance imaging (fMRI) in those brain regions that have been shown to influence response to negative or aversive stimuli? While changes in CREB expression have been most directly demonstrated in the shell of the nucleus accumbens (NAc), amygdala, and (in humans) the prefrontal cortex, we hypothesized that the functional effects of these changes would not necessarily be limited to those structures, but via their projections be observed in other regions that process aversion. To compare these postulated effects with results from prior studies, we also examined the differential activation associated with a serotonin transporter (*SLC6A4*) promoter polymorphism (5HTTLPR) that has previously been shown to modulate amygdala activation to aversive faces.[10]

METHODS

PARTICIPANTS

The participants were 28 white adults (mean [SD] age, 29.2 [9.6] years; mean [SD] educational history, 15.3 (0.43) years; 13 of 28 [46%] women; 27 of 28 [96%] right-handed) who were recruited by advertisements. Key-press data were available for a subgroup of these participants (n = 16) with similar demographics (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 1). All participants underwent a clinical interview with a doctoral-level clinician that included the Structured Clinical Interview for Diagnosis for *DSM-IV* Axis I.[30] Race was determined by self-identification on a standardized form.[31] Participants also completed the Inventory of Depressive Symptoms–Self-Rated,[32,33] the Edinburgh Handedness Inventory,[34] and the Symptom Questionnaire (SQ),[35] which includes a scale for hostile/angry symptoms. Eligible participants were aged 18 to 55 years, without any current or lifetime *DSM-IV* Axis I disorder or major medical illness known to influence brain structure or function, including neurologic disease, human immunodeficiency virus, or hepatitis C. All participants gave written informed consent prior to participation, and the protocol was approved by the institutional review board of Massachusetts General Hospital. Female participants underwent scanning during their midfollicular phase based on self-reported menstrual history, with confirmation at the time of scan based on an absence of progesterone surge on a urine assay. All participants were scanned at normal or corrected normal vision.

DATA ACQUISITION

Experimental Paradigm and Off-line Behavioral Testing

Two fMRI scans were acquired (8 minutes, 40 seconds each), consisting of 20-second blocks for 7 experimental conditions: angry, fearful, sad, neutral, and happy expressions, along with phase-scrambled stimuli and fixation. During each scan, the 7 conditions (blocks) were presented in a counterbalanced order (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 2). Each facial expression block included standardized images of the faces of 8 individuals (4 men)[36] in pseudorandom order, displayed using parameters reported previously.[5,6] Face stimuli were previously normalized at the Massachusetts Institute of Technology Media Laboratory[6] (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 3).

Face stimuli were projected into the magnet bore as described previously.[5,6] Participants were instructed to look at the faces, focusing on the center of the picture at the location of the crosshair. After scanning, participants performed 3 memory tasks to identify faces and facial expressions they had or had not seen (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 4). To be included in the cohort for fMRI analysis, participants had to score better than chance on all tests.

An off-line key-press task used the same set of faces as during imaging (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 5). This task examined the positive and negative valuation of emotionally expressive faces, which defined a participant's relative preferences[4] for these stimuli (ie, his or her utility for the set of faces[3]). It followed procedures resembling those reported previously with model and nonmodel faces[4] and with angry and other facial expressions,[5] with the key-press procedures implemented using MatLab software (The MathWorks Inc, Natick, Massachusetts) on a PC device.

The dependent measure of interest was the amount of work in number of key presses, which participants traded for face-viewing time. Participants had a choice to do nothing (default condition), increase viewing time, decrease viewing time, or a combination of the 2 responses.[3] Key-press results could be amalgamated as total viewing time relative to the default baseline, then used for regression analysis with fMRI data or for an analysis of variance with *CREB1* genotype.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) was performed on a 3-T MAGNETOM Trio (Siemens, Malvern, Pennsylvania) system using an 8-channel phased-array receive-only radiofrequency coil. Participants' heads were stabilized using foam pads and adjustable paddles fixed to the radiofrequency coil assembly. The BOLD functional images were acquired using gradient-echo echoplanar imaging (time to repetition/echo time/α, 2.5 seconds/30 milliseconds/90°; resolution, 3.125 mm × 3.125 mm × 3 mm) with slices situated parallel to the intercommissural line and parallel to the inside curve of the FOC to minimize signal distortion in this region.[37] Structural images were

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acquired using a high-resolution T1-weighted magnetization-prepared rapid acquisition gradient echo sequence (192 sagittal slices over the full head volume; matrix, 224 × 256; field of view, 224 × 256 mm^2; thickness, 1 mm; no gap) before functional scanning.

Genotyping

Genomic DNA was extracted from blood samples through use of a Nucleon II DNA extraction kit (Amersham Pharmacia Biotech, Piscataway, New Jersey), quantified using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc, Wilmington, Delaware), and diluted to 3.0 ng/μL before creating 96-well polymerase chain reaction source plates.

Genotyping of the rs4675690 SNP was performed using the methods previously described.[38] Primers were designed using SpectroDESIGNER software (Sequenom, San Diego, California). Polymerase chain reaction amplification was performed, followed by homogeneous MassEXTEND reaction (Sequenom). Samples were analyzed in automated mode by a MassARRAY RT mass spectrometer (Sequenom).[39] The resulting spectra were analyzed by SPECTROTYPER software (Sequenom) after baseline correction and peak identification. The degree of linkage disequilibrium between this SNP and additional SNPs within *CREB1* have been previously described.[28]

Genotyping of 5HTTLPR was performed by polymerase chain reaction at the Center For Human Genetic Research Genotyping Resource (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 6). Data were analyzed with GeneMapper version 3.7 software (Applied Biosystems, Foster City, California). Genotyping of HTTLPR yielded fragments of 483 and 529 base pairs, representing short (*s*) and long (*l*) alleles, respectively. All participants were genotyped successfully at both 5HTTLPR and rs4675690, and allele frequencies did not deviate significantly from Hardy-Weinberg equilibrium. Concordance for duplicate DNA was 100%; previous work in our laboratory using intraplate and interplate control samples yielded error rates of less than 0.1%.

DATA ANALYSIS

Primary analyses used 2-group contrasts based on rs4675690 genotype groups (TT vs C carriers) (1) to ensure balanced groups, given known allele frequencies in white Europeans, (2) to be consistent with multiple possible models of effect, and (3) to maximize comparability with prior work and with our *SLC6A4* analyses.

Sociodemographic, Clinical, and Memory Measures

The genotype groups were compared in terms of sociodemographic or clinical features and performance on the face-recall task using the Fisher exact test for dichotomous measures or the Mann-Whitney *U* test for continuous measures.

Magnetic Resonance Imaging

We first performed 3 2-way associations between (1) *CREB1* (rs4675690) or 5HTTLPR genotype with fMRI data (*CREB1*-fMRI), (2) key-press behavior of relative preference with fMRI data (RP-fMRI), and (3) *CREB1* or 5HTTLPR genotype with key-press behavior. The MRI associations from the *CREB1*-fMRI and RP-fMRI analyses were then assessed to determine if these 2-way associations were related (ie, a 3-way association between genotype, fMRI, and key-press data [http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 7]).

MRI Signal Processing. After automated tissue stripping and discarding of dummy volumes, estimated residual motion artifacts in the form of voxel unalignment were assessed for all runs using the Analysis of Functional NeuroImages motion-correction module. Residual misalignment had to meet 2 conservative criteria, and remaining participants then had to meet criteria, ruling out susceptibility artifacts in the FOC, amygdala, and NAc (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 8).

The remaining participants were entered into a set of signal-processing steps using a workflow-control system (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 9) wrapping the platform of the Functional Magnetic Resonance Imaging of the Brain (FMRIB) software library (FSL version 3.3; University of Oxford FMRIB Analysis Group, Oxford, England). Data from these participants underwent 3 steps: (1) image realignment through the motion correction using FBRIB's Linear Image Registration Tool within FSL, (2) spatial smoothing with a 3-dimenisonal Gaussian kernel (full-width at half maximum, 7 mm^3), and (3) temporal smoothing using a high-pass filter. The FMRI Expert Analysis Tool within the FSL platform was used for steps 2 and 3.

MRI Statistical Analysis. Per participant, each image series was fit using a linear signal model with regressors of interest for which intervals of face presentation were represented by square pulses (blocks) relative to fixation (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 10). For RP-fMRI, the height and valence of each block was scaled for each participant to be proportional to his or her deviation from default viewing time (which could be positive or negative, depending on the key used) for that face expression category. Customized square waveforms were then convolved with a double-γ hemodynamic response function and used in FMRIB's Improved Linear Model with local autocorrelation correction to estimate hemodyamic parameters for 1 explanatory variable (for the angry vs fixation conditions) along with regressors of no interest (ie, other emotional expressions vs fixation).

For *CREB1*-fMRI, each image series was fit per participant, with regressors of interest representing the intervals of face presentation by square pulses (blocks) relative to fixation (independent of the participant's key-press results). These invariant square waveforms were then convolved with a double-γ hemodynamic response function and used in FMRIB's Improved Linear Model to estimate hemodyamic parameters for 1 explanatory variable and regressors of no interest (other emotional expressions).

Each participant's echoplanar imaging scans were spatially normalized using his or her T1-weighted structural scans, the International Consortium for Brain Mapping (ICBM)–152 T1 template and FMRIB's Linear Image Registration Tool. After transformation to ICBM152 space, the effect standard error pairs for both image series were pooled using a fixed-effects model to produce a single pair of statistical images. For the RP-fMRI analysis, the summary statistical results from all participants in the cohort were then pooled using a mixed-effects model by FMRIB's Local Analysis of Mixed Effects Stage 1 module. For the *CREB1*-fMRI analysis, this process involved a level 2 contrast of the TT vs CT or CC genotype groups for *CREB1* and contrast of the "long/long" vs "long/short" or "short/short" groups for 5HTTLPR.

MRI Analysis of A Priori Anatomic Regions of Interest. Within 4 a priori regions of interest (amygdala, insula, anterior cingulate, and FOC), foci of BOLD response were identified from *CREB1*-fMRI and RP-fMRI analyses with the angry face condition as an a priori target and 4 other face expressions as post hoc concerns (http://pgp.mgh.harvard.edu/PGP/Perlisetal

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Table 1. Clinical and Sociodemographic Features of Participants by rs4675690 Genotype

Feature	Mean (SD) CC or CT (n=18)	Mean (SD) TT (n=10)	Mann-Whitney *U*	*P* Value
Female, No. (%)	7 (39)	6 (60)	Exact	.43
Right-handed, No. (%)	17 (94)	10 (100)	Exact	.99
Age, y	30.1 (10.9)	27.6 (6.9)	88.5	.94
Education, y	14.8 (1.9)	16.6 (2.4)	45.0	.07
QIDS-SR score	3.1 (3.1)	4.1 (4.4)	77.0	.71
SQ hostility score	0.5 (1.0)	2.5 (2.5)	36.5	.01
SQ anxiety score	1.1 (1.2)	2.4 (2.4)	61.5	.18

Abbreviations: QIDS-SR, Quick Inventory of Depression Symptoms; SQ, symptom questionnaire.

_Aug2008.html, appendices, appendix 11). Gaussian random field theory[40] was used to estimate the cluster volume and voxelwise thresholding needed to survive a clusterwise $P<.05$ (corrected); namely, 40 voxels with an effective threshold of $P<.003$ per voxel (0.05/16 [4 a priori regions × 2 hemispheres × 2-tailed comparisons]). For post hoc analysis of the 4 other face expressions, cluster volume and thresholding needed to survive a more conservative clusterwise $P<.05/4=1.25\times10^{-2}$ (corrected).

To determine whether clusters with 2-way interactions found for *CREB1*-fMRI and RP-fMRI were independent or related, 4 further analyses were performed. First, colocalization of *CREB1*-fMRI foci and RP-fMRI foci was quantified in a priori regions. Second, the effect size of the *CREB1*-fMRI association at foci colocalizing between *CREB1*-fMRI and RP-fMRI was quantified to verify the *CREB1*-fMRI interaction using nomial-by-interval association η.[41] The third procedure estimated effect size between *CREB1*-fMRI signal and relative preference behavior using Pearson product moment correlations. If an effect size supporting a 3-way association was observed, a fourth procedure was performed using the output of the RP-fMRI analysis as an input to a contrast of the TT vs CT or CC genotype groups for *CREB1*. Combined, these criteria represent a conservative extension of association procedures in imaging genetics.[10,42-44] An analogous approach was applied to examine the association between fMRI of emotional expressions and 5HTTLPR and its overlap with RP-fMRI.

The probability range affiliated with colocalization of distinct statistical maps was estimated as described in appendix 11 (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices). If 2 colocalizing regions share 5 contiguous voxels, this amounts to a probability range of $(1.3\times10^{-4})^5<P<1.3\times10^{-4}$ regarding the likelihood of this being a false-positive result.

Anatomic Localization of A Priori Anatomic Regions of Interest. Foci of significant change in the RP-fMRI and the *CREB1*-fMRI analyses were identified within semiautomated segmentation volumes of the ICBM152 T1 brain (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 12). A priori and post hoc brain regions were segmented and parcellated as individual structures,[45-50] producing core files that could be overlaid on statistical volumes for localization purposes.

RESULTS

Data are organized in 7 sections for 2-way associations (first through fourth), 3-way associations (fifth), and follow-up analyses with clinical indices or 5HTTLPR.

ASSOCIATION OF *CREB1* WITH CLINICAL MEASURES

Distribution of genotypes at rs4675690 among participants was 10 of 28 (36%) TT, 12 of 28 (43%) TC, and 6 of 28 (21%) CC; minor allele frequency (for T) was 46%, comparable with that of the entire neuroimaging cohort (45%). The TT vs C carriers (TC and CC) were not statistically different in age, sex, handedness, years of education, or severity of depressive symptoms by Quick Inventory of Depressive Symptomatology (Self-Reported) (all $P>.05$). The TT group had significantly greater scores on the anger/hostility subscale of the SQ (**Table 1**).

ASSOCIATION BETWEEN *CREB1* AND fMRI (*CREB1*-fMRI)

Contrast between genotypes for activation to angry faces revealed significant BOLD signal differences in the bilateral insula (**Figure 1**A; **Table 2**) but not in other regions of interest. These foci reflected lower BOLD signal in the C-carrier group vs the TT group. Post hoc analysis of the NAc showed no significant effects in right ($z=-1.92$; uncorrected $P=.05$) or left ($z=-1.70$; uncorrected $P=.09$) nuclei.

Meeting the post hoc threshold, the rs4675690 genotype was associated with lower BOLD signal for C-carrier participants than for those with the TT genotype for fearful expressions in the bilateral insula (**Figure 2**). Similar effects that only met the a priori correction were noted in response to sad faces in the left insula, neutral faces in the right insula, and happy faces in the left amygdala (Table 2).

No significant differences in recall accuracy (all $P>.1$) were detected between genotype groups for face identity, identity and expression, or expression alone (http://pgp.mgh.harvard.edu/PGP/Perlisetal_Aug2008.html, appendices, appendix 4).

ASSOCIATION BETWEEN RELATIVE PREFERENCE KEY PRESSING AND fMRI (RP-fMRI)

Regression of key-press responses to angry faces (**Figure 3**) with fMRI BOLD signal revealed significant effects in the

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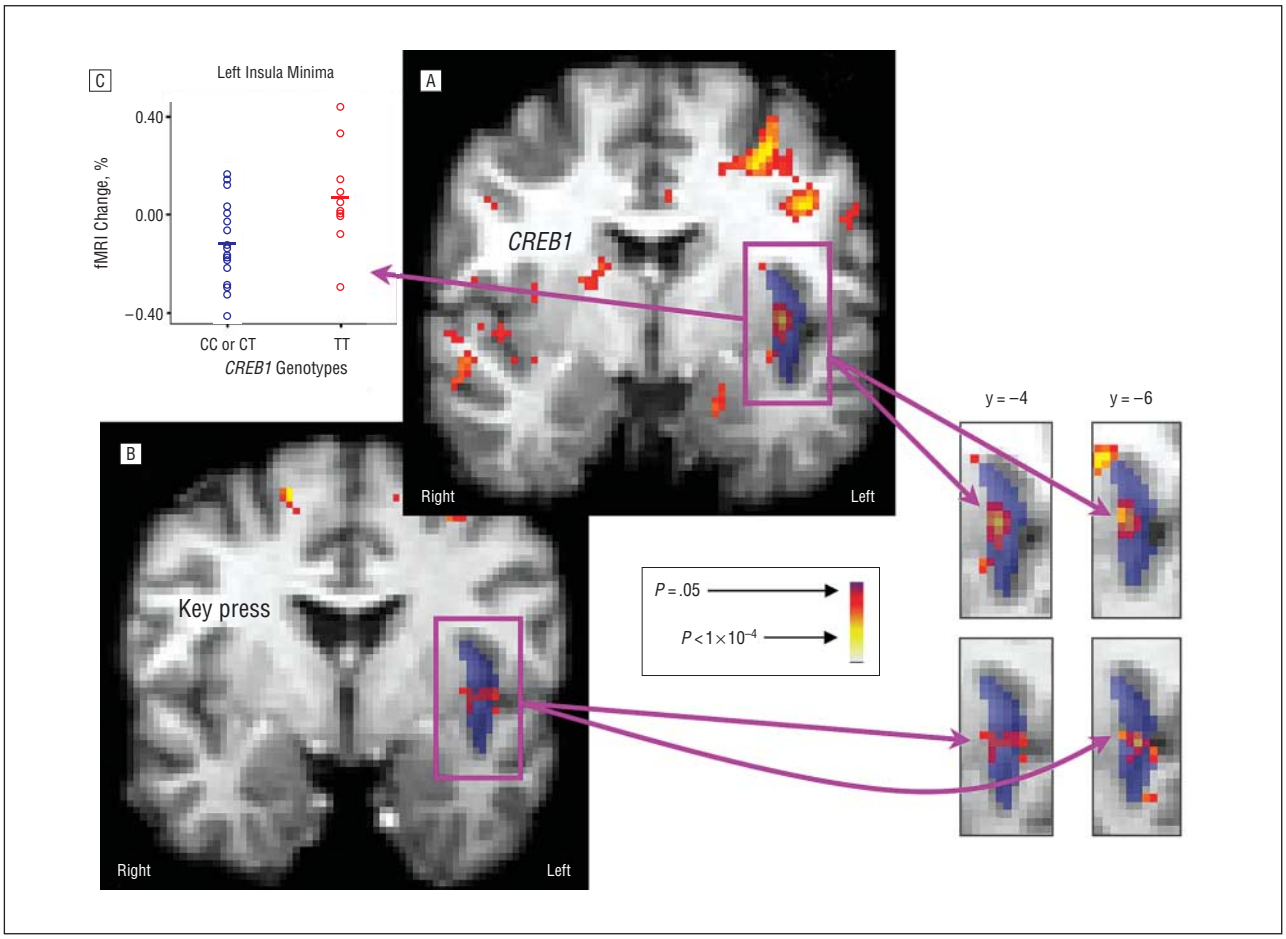

Figure 1. Results of relative preference–functional magnetic resonance imaging (RP-fMRI) and *CREB1*-fMRI analyses. A, Contrast by rs4675690 genotype for fMRI data (threshold uncorrected voxelwise $P<.01$). B, Regression of key-press data to fMRI data (threshold uncorrected voxelwise $P<.01$). Averaged T1 structural images that have been normalized into the International Consortium for Brain Mapping–152 space are shown in gray tone beneath segmentation templates for the left insula (dark blue), as coronal slices with the anterior-posterior y-coordinate show. Superimposed on these segmentation templates are pseudocolor statistical maps (red and yellow). Explicit overlap in these pseudocolor statistical maps across multiple slices can be observed in the insula snapshots on the lower right, indicating a 3-way association. C, Percentage of signal change in the left insula by rs4675690 genotype.

left insula (Table 2; Figure 1B). Effects in the left insula reflected increasing negative key presses (to avoid viewing a stimulus) in association with more negative BOLD signal change. Post hoc analysis of the NAc showed no significant effects in right ($z=-2.05$; uncorrected $P=.04$) or left ($z=-1.56$; uncorrected $P=.12$) nuclei.

Eight other foci of association were noted in the 4 regions, although these foci only met the a priori and not the post hoc correction (Table 2). Seven of these reflected increasing negative key-press responses in association with a more negative BOLD signal.

ASSOCIATION BETWEEN *CREB1* AND RELATIVE PREFERENCE KEY PRESSING

No significant association was observed between rs4675690 genotype and measures from the relative preference task for angry faces (total viewing time, key-press increases, or key-press decreases [all $F<1.5$; $P>.05$]). Estimation of effect size between rs4675690 genotype and anger viewing time was less than 1% ($\eta_{2,16}=0.1$; $P>0.1$). Although qualitative differences were apparent (**Figure 4**), no significant relationships, after correction for multiple comparisons, were noted between *CREB1* genotype and viewing time or key-press responses for fearful, sad, neutral, or happy faces (all $F<1.5$; $P>.05$, excepting happy viewing time where $F_{2,16}=2.23$; $P=.02$).

THREE-WAY ASSOCIATION BETWEEN *CREB1* GENOTYPE, fMRI, AND RELATIVE PREFERENCE KEY PRESSING

Two foci demonstrated significant colocalization of *CREB1*-fMRI and RP-fMRI outcomes. In the left insula, 8 coinciding voxels displayed significant association with both genotype and key-press efforts to reduce the viewing of angry faces (Figure 1A and B). The probability of random overlap of this size across a priori regions is $(1.3\times10^{-4})^8<P<1.3\times10^{-4}$. Inspection of the data revealed that C carriers have more negative fMRI signals to angry faces relative to fixation in the left insula than participants with the TT genotype (Figure 1C). There is a mean negative signal shift from baseline for angry faces across the total cohort of 28 participants. The regression of key-press to angry-face fMRI response shows that increasing negative key pressing (to reduce viewing of angry faces) is associated with decreasing BOLD signal from baseline.

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Table 2. Peak Differential Activation to Emotional Faces in Regions of Interest

	fMRI and *CREB1*								fMRI and RP							
ROI	Condition	x[a]	y[a]	z[a]	z Score[b]	P Value	Valence of Subtraction	AP[c] vs PH[d]	Condition	x[a]	y[a]	z[a]	z Score[b]	P Value	Valence of Regression	AP[c] vs PH[d]
Left amygdala	Happy	−22	−6	−20	4.41	1×10^{-5}	–	AP					...			
Left FOC					...				Fearful	−32	18	−16	4.55	5×10^{-6}	+	AP
Left INS	Angry	−38	−4	0	3.97	7.1×10^{-5}[e]	–	PH	Angry	−40	−6	−2	3.86	1.13×10^{-4}[e]	+	AP
	Fearful	−36	−10	16	3.84	1.22×10^{-4}	–	PH	Fearful	−28	12	−14	4.00	6.2×10^{-5}	+	AP
	Sad	−34	−10	16	4.40	1.0×10^{-5}	–	AP					...			
Left anterior cingulate					...				Happy	−8	12	34	3.91	1.0×10^{-4}	+	AP
Right amygdala					...								...			
Right FOC					...				Fearful	26	16	−16	4.29	1.8×10^{-5}	+	AP
									Sad	26	16	−16	3.56	3.7×10^{-4}	+	AP
									Neutral	48	32	−16	3.55	3.9×10^{-4}	+	AP
Right INS	Angry	38	0	2	3.39	7.02×10^{-4}	–	AP					...			
	Fearful	38	2	2	3.79	1.5×10^{-4}[e]	–	PH	Fearful	30	24	−2	3.59	3.32×10^{-4}[e]	–	AP
	Neutral	38	0	2	3.65	2.65×10^{-4}	–	AP					...			
Right anterior cingulate					...				Sad	8	38	20	3.96	1.0×10^{-4}	+	AP

Abbreviations: AP, a priori; *CREB1*, cyclic adenosine monophosphate response element binding protein gene; ellipses, no data; fMRI, functional magnetic resonance imaging; FOC, orbitofrontal cortex; ICBM, International Consortium for Brain Mapping; INS, insula; PH, post hoc; ROI, region of interest; RP, relative preference; –, negative valence to the genotype-based subtraction or key press–based regression; +, positive valence to the genotype-based subtraction or key press–based regression.

[a] Variables x, y, and z refer to coordinates of peak differential activation in ICBM152 space.

[b] Absolute z score.

[c] Activation survives clusterwise AP correction, $P<.05$.

[d] Activation survives clusterwise PH correction, $P<.05/4$.

[e] These indicate colocalizing activations beyond 5 voxels.

In the right insula, 11 coinciding voxels (broken into 1 group of 5 voxels and a second group of 6 voxels) displayed significant association with both genotype and effort to reduce the viewing of fearful faces. The probability of a random overlap of this size across a priori regions is $(1.3\times10^{-4})^6<P<1.3\times10^{-4}$. For this region of overlap, C carriers have a lower fMRI signal to fearful faces than participants with the TT genotype, producing a negative subtraction result (ie, $-z$). The regression of key pressing to fearful-face fMRI response indicates that key pressing to reduce the viewing of fearful faces is associated with increasing BOLD signal from baseline.

The effect size for the left insula (angry) focus was 21.2% ($\eta_{2,26}=0.460$; $P<.014$), whereas for the right insula (fearful) focus, it was 36.5% ($\eta_{2,26}=0.604$; $P<.001$). Estimation of the effect size of interaction between *CREB1*-fMRI signal and key-press behavior (ie, as between data in the graph in Figure 1 and key-press data in Figure 4) was approximated by Pearson product moment correlations. These showed an effect size of 21.9% for the left insula (angry) focus ($r_{2,16}=0.47$; $P<.068$) and less than 1% for the right insula (fearful) focus ($r_{2,16}<0.1$; $P>0.1$). Thus, the *CREB1*-fMRI and RP-fMRI colocalization was supported for the left insula (angry) site but not for the right insula (fearful) site.

As a follow-up analysis of 3-way association, we used the output of the RP-fMRI analysis as an input to a contrast of the TT vs CT or CC genotype groups for *CREB1*, and found 1 significant focus ($z=3.56$; uncorrected $P<3.7\times10^{-4}$), which colocalized with the left insula (anger) results from the *CREB1*-fMRI and RP-fMRI analyses.

To assess potential sensitization of fMRI signals in the insula[5] (as opposed to habituation in the amygdala[6,51]), we assessed fMRI adaptation dynamics to angry faces in the left insula. Both C-carriers and those with a TT genotype evidenced sensitization of signal in the left insula to angry faces over the course of the experiment (both <1%), and there was not a significant difference between groups ($t_{2,26}<1.0$; $P>.1$).

RELATIONSHIP BETWEEN COLOCALIZING REGIONS OF *CREB1*-fMRI AND RP-fMRI, WITH CLINICAL VARIABLES

Across 3 clinical variables (Inventory of Depressive Symptoms–Self-rated, SQ depression, and SQ anger), activation in the left insula (angry) focus was associated only with SQ depression ($r_{2,26}=0.495$; $P<.007$) after correction for multiple comparisons ($P<.05/6$ tests [.008]). In the right insula (fearful) focus, correlations were observed for the SQ depression ($r_{2,26}=0.571$; $P<.002$) and SQ anger ($r_{2,26}=0.549$; $P<.003$) measures. Independent correlation of SQ anger with anger key pressing across participants was not significant ($r_{2,16}=-0.127$; $P>.05$).

COMPARATIVE ANALYSES WITH *SLC6A4*

In the comparison of individuals with 2 copies of the "long" allele (n=13) to all others (n=15), consistent with prior studies,[10] no subtraction foci for *SLC6A4* met the clusterwise threshold used with *CREB1* data, although trends toward significant effects were noted in the left

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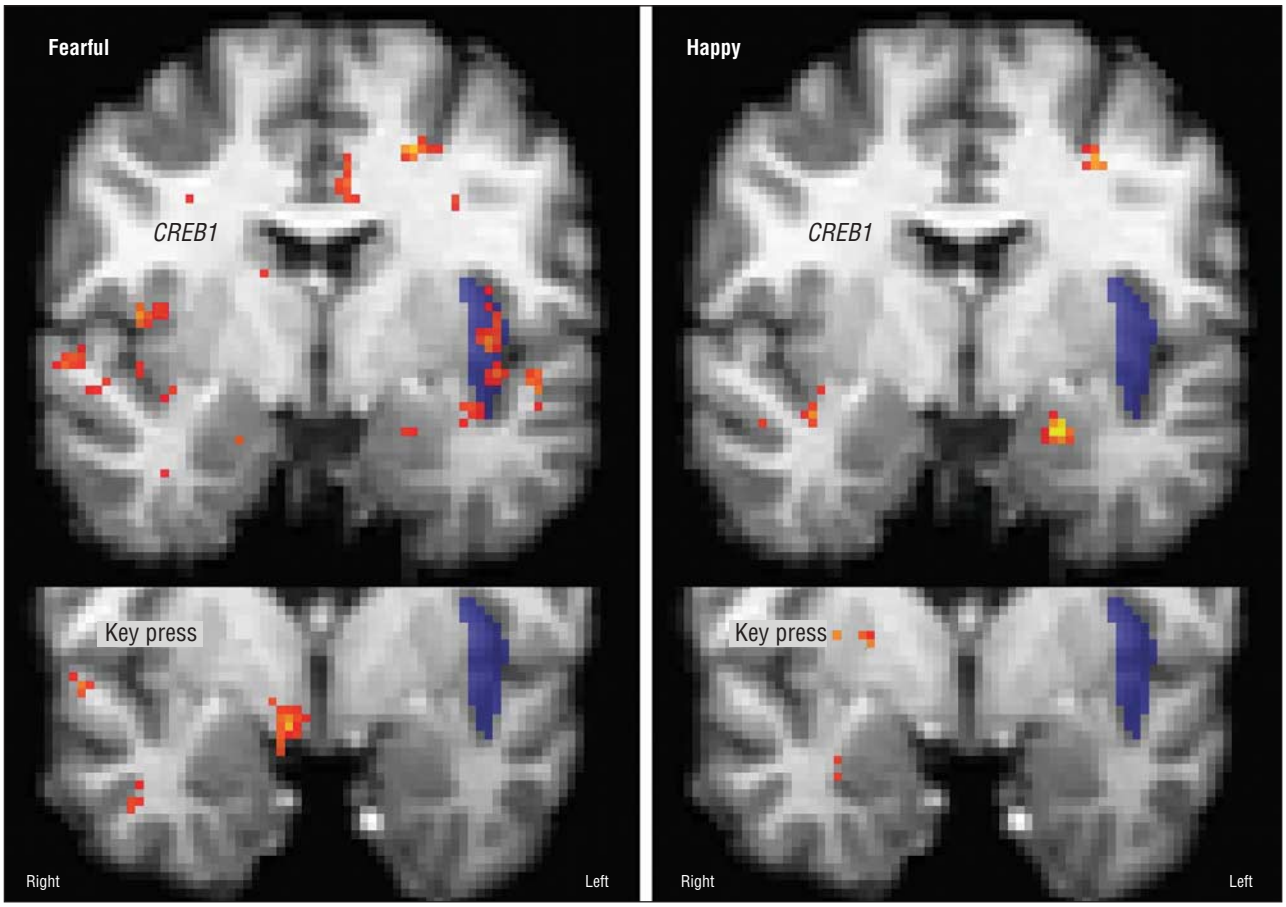

Figure 2. Key-press regression and genotype subtraction with functional magnetic resonance imaging data for fearful and happy faces. Note the lack of colocalization in the left insula.

amygdala (z=3.49; $P=5\times10^{-4}$ [uncorrected]; −20, −4, −24 [x, y, z]; 13 voxels at $P<.003$) and right insula (z=3.31; $P=9\times10^{-4}$ [uncorrected]; 36, −6, 2 [x, y, z]; 13 voxels at $P<.003$). These foci suggested a higher BOLD signal for the participants with at least 1 "short" allele. Also, as expected, there was no association between genotype at *SLC6A4* and *CREB1* (Fisher exact P=.42).

COMMENT

Our results suggest that a *CREB1*-linked polymorphism is associated with the responsiveness of a network of brain regions that have been implicated in detection of and response to aversive stimuli. Individuals who are homozygous for the T allele of rs4675690 display less negative BOLD signals than C carriers in the insula bilaterally for angry and fearful faces. Although not surviving post hoc correction, similar effects between TT and C carriers were observed for happy expressions in the amygdala and sad and neutral ones in the insula. Likewise, an association between more negative BOLD signal and stronger key-press responses to avoid angry faces was observed in a left insular focus that colocalized with a focus of BOLD signal difference by *CREB1* genotype. The interaction of these colocalizing foci was confirmed (1) by a correlation analysis showing an effect size of 21.9% between (a) key-press behavior and (b) fMRI signal from the contrast activation associated with *CREB1* genotype, and (2) by a significant 3-way association between anger key-press data, left insula BOLD signal, and *CREB1* genotype. Together these observations point to a finding that is specific to anger and not to other facial expressions.

The insula has been shown to exhibit differential activation in response to emotionally expressive faces[52] and angry hand gestures.[53] A recent review proposes that the insula and connected regions (ie, amygdala and FOC) are central to a network that detects and responds to aversive stimuli.[12] This network is associated with anticipation of aversive states related to pain[54] or the viewing of aversive pictures.[11] It is also implicated in the neuroanatomy of anxiety.[55-57]

In a post hoc analysis, we did not identify significant *CREB1* interaction with NAc activation. A number of recent studies connect preference assessments with both NAc and insula activation[58,59] during human decision making. Changes in CREB expression or phosphorylation in the NAc have been consistently associated with models of dysphoria or aversive states in animals.[60-62] However, our results are still consistent with a model in which changes in CREB in the NAc may be reflected in the activity of other structures within the reward/aversion network.[1-4]

The present findings build on 2 previous association studies that reported consistent effects for rs4675690 near *CREB1*. The T allele in rs4675690 was associated with

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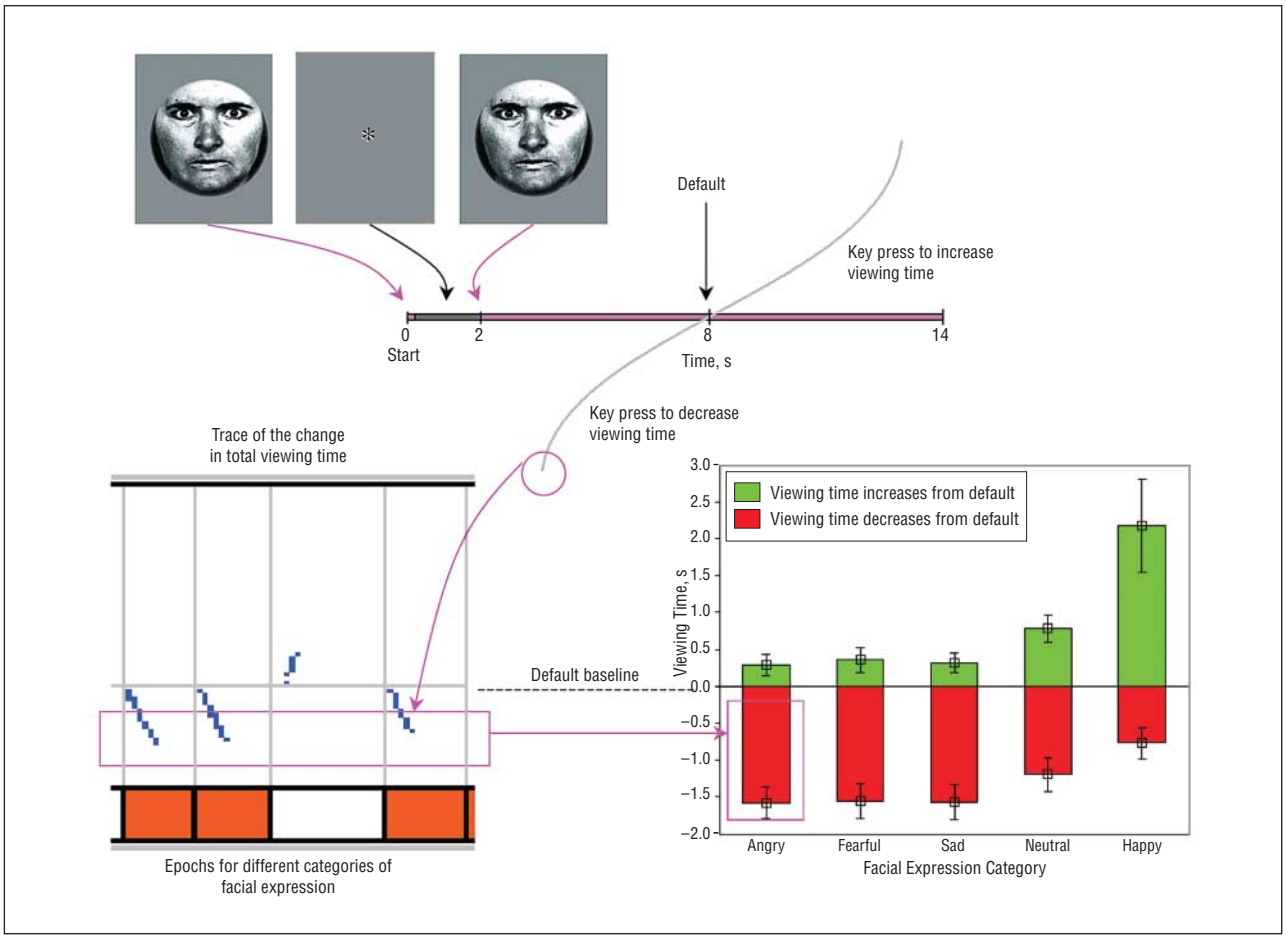

Figure 3. Experimental design of key-press procedure. Drawing at top and plots below schematize the experimental design, its output data, and 1 type of data summary. The drawing illustrates the 4 potential responses to increase, decrease, variably increase or decrease, or accept the default viewing time of 8 seconds. The default condition controls for those who intended to press the key to increase or decrease viewing time, but who did not act on this intention. This model integrates the stimulus presentation and the time course of potential responses by participants. The traces of individual responses to each picture are shown with raster plots from an anonymous participant in the lower left. Each time interval during which this subject viewed a particular stimulus is indicated by a pink color bar, above which is a blue trace of actual key-press data set to the default baseline for viewing. The bar graph shows summary data (location and standard errors) for the different facial expressions. All responses in the graph are coded as time alterations from the default. * Indicates fixation point.

greater internal effort at anger control,[27] greater risk of treatment-emergent suicidality in major depressive disorder,[28] and in the present study, reduced negative BOLD signal in the left insula when viewing angry faces, with reduced key pressing to avoid exposure to them. In general, TT homozygotes produced fewer approach or avoidance key-press responses for any emotion. Together, these findings suggest a hypothesis of increased suppression of neural and behavioral responses to anger with the T allele.

In this study, a genetic variation also implicated in stress response and mood reactivity, 5HTTLPR,[10] was modestly associated with effects that were unrelated to *CREB1* effects. Increased right amygdala activation has been reported in individuals with the "short" allele when viewing aversive faces.[10] Our results are qualitatively similar though of lesser magnitude.

The insula and amygdala have been implicated with both reward and aversion processing.[63,64] The interaction of *CREB1* genotype with BOLD signal in the insula, overlapping and correlating with the association of key-press responses to angry faces and insula BOLD signal, represents a genetic association with a microeconomic

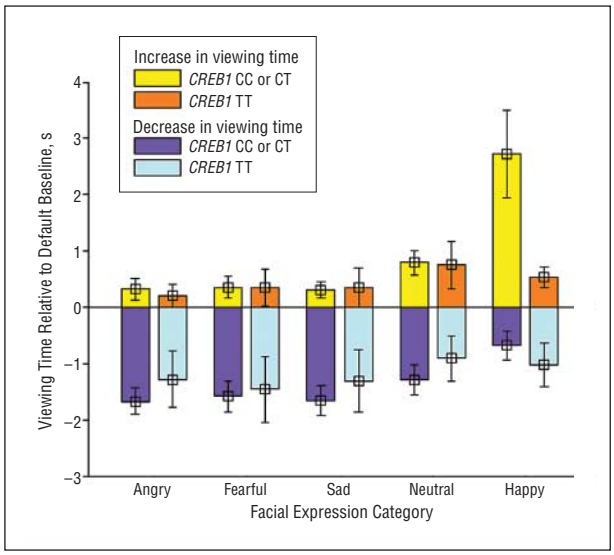

Figure 4. Viewing time changes in seconds from the default condition are shown for each of 5 facial expressions. The effects of *CREB1* genotype are juxtaposed for each category of facial expression. Qualitatively, C-carrying participants tend to key press more to avoid aversive expressions (angry, fearful, sad) and to approach positive expressions (happy).

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task.[63,64] In general terms, this means that variation in *CREB1* is associated with judgment and decision making[2,3,58,63,64] during a paradigm that requires normal controls to weigh different options and express specific preferences based on relative valuation of stimuli.[4]

A limitation in the present study is that the function at a molecular level of rs4675690 has not been established. We hypothesize that, because *CREB1* is such a central gene in signal transduction, common polymorphisms with large effects on expression would likely have diverse effects not limited to phenotypes such as response to aversive faces. We know that rs4675690 is in linkage disequilibrium with multiple other SNPs in and near *CREB1*, which may exert more subtle effects on regulation, regional expression, or translation.[28,65] The importance of a network involving CREB, the responsive element silencing transcription factor, and microRNA in regulating neuronal gene expression has recently been described,[66] highlighting the complex regulation of this system and thus the many ways in which *CREB1* polymorphisms could influence it. To examine *cis*-acting effects of rs4675690, we queried 2 lymphoblastoid cell line data sets for participants from the Centre Etude Polymorphisme Humaine families, genotyped in the International Haplotype Map Project.[67,68] No significant association with *CREB1* expression or the adjacent gene *LOC151194* was detected (results not shown). As the correlation between expression in transformed lymphoblastoid cell lines and neurons is imperfect, we cannot rule out neuronal effects on expression. Finally, we note other recent studies of apparently nonfunctional SNPs with a significant effect on gene products.[69] In the face of this complexity, we elected to pursue in vivo function in parallel with in vitro function, for which fMRI is particularly well suited.

Given the limited sample size of this study, replication with a larger cohort will be required to examine possible sex differences. Men and women may differ in response to or expression of anger in some paradigms, including in studies with *CREB1*.[27,28] While power considerations precluded formal statistical testing for gene-by-sex interaction, after stratifying by sex, peak bilateral insula activation remained salient (results not shown) in both groups.

We find that a polymorphism in linkage disequilibrium with *CREB1*, previously implicated in self-report–based phenotypes related to negative affect in 2 independent cohorts,[27,28] is associated with differential BOLD signal in a network of structures related to the processing of aversive information. Activation of adjacent or overlapping areas within that network is independently associated with key-press behavior in a task involving angry faces. These results suggest that *CREB1* plays a role in moderating neural responsiveness to aversive stimuli in the environment, with detectable behavioral consequences.

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pirones and bupropion in major depressive disorder, and receiving copyright royalties for rating scales not used in this investigation, MGH CPFQ, DESS, and SAFER. Dr Rosenbaum reported having been on the advisory boards of Forest Pharmaceuticals Inc, Eli Lilly & Company, MedAvante Inc, Novartis, Sanofi-Aventis, and Wyeth-Ayerst Laboratories. He has consulted for Neuronetics, Organon Inc, Somaxon, and Supernus. He reported being a speaker for Boehringer Ingelheim Italia and regarding educational content development for Primedia Health Care. He reported having equity holdings in Compellis, Medavante, Somaxon.

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