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Preliminary communication

# Early influence of the rs4675690 on the neural substrates of sadness

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ABSTRACT

*Background:* CREB1 has previously been implicated in mood disorders, suicide, and antidepressant response. There is some evidence that the T allele in rs4675690, a single-nucleotide polymorphism near the CREB1 gene, is involved in the modulation of neural responses to negative stimuli. It is not known whether differential brain activity during negative mood state appears early in life in T allele carriers.
*Methods:* Functional magnetic resonance imaging (fMRI) was used to measure brain activity, during a transient state of sadness, in children homozygous for the T allele or the C allele. This primary emotion was selected given that it is the prevailing mood in major depressive disorder (MDD). Blood-oxygen-level dependent (BOLD) signal changes were measured while subjects viewed blocks of neutral film excerpts and blocks of sad film excerpts.
*Results:* There was significantly greater BOLD activation in the TT group, compared to the CC group, in the right dorsal anterior cingulate cortex (Brodmann area [BA 24]), right putamen, right caudate nucleus and left anterior temporal pole (BA 21), when the brain activity associated with the viewing of the emotionally neutral film excerpts was subtracted from that associated with the viewing of the sad film excerpts.
*Limitations:* A replication study using larger samples may be required for more definitive conclusions.
*Conclusions:* The different pattern of regional brain activation found here during transient sadness – in children carrying the T allele, compared to those carrying the C allele – might increase later in life susceptibility to emotional dysregulation and depressive symptoms.


## 1. Introduction

The cyclic adenosine monophosphate (cAMP) response binding protein (CREB) plays a key role in neuroplastic processes such as differentiation, growth and survival of neurons (Shieh and Ghosh, 1999). Based on the hypothesis that cellular mechanisms involved in synaptic plasticity contribute to the risk of major depressive disorder (MDD), it has been proposed

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that CREB1, the gene encoding CREB, might influence the risk of developing mood disorders (Manier et al., 2001; Zubenko et al., 2003).

Regarding this issue, chronic antidepressant treatment upregulates the cAMP signal transduction cascade, which in turn enhances expression and function of CREB1, resulting in neuronal sprouting and neurogenesis (Burcescu et al., 2005). In other respects, results of postmortem brain studies have revealed reductions of CREB in the temporal cortex (Dowlatshahi et al., 1998) and orbitofrontal cortex (Yamada et al., 2003) of antidepressant-free patients with MDD at the time of death compared to age-matched controls. In addition, another post-mortem investigation has evidenced a reduction of CREB expression in the prefrontal cortex and hippocampus of suicide subjects relative to nonpsychiatric controls (Dwivedi, et al., 2003).

An association between the T allele in rs4675690 – a single-nucleotide polymorphism near the CREB1 gene – and greater effort at anger control has been reported in men with MDD (Perlis et al., 2007a). Furthermore, a recent functional magnetic resonance imaging (fMRI) study (Perlis et al., 2008) has shown that subjects homozygous for the T allele of rs4675690 displayed higher blood-oxygen-level dependent (BOLD) signal in the insula (bilaterally) for angry and fearful faces compared to subjects carrying the C allele. This finding suggests that the rs4675690 is implicated in the modulation of neural responses to negative stimuli (Perlis et al., 2008).

It is not known whether differential brain activity during negative mood state appears early in life in T allele carriers relative to C allele carriers. To address this question, we used fMRI to measure brain activity, during a transient state of sadness, in children homozygous for the T allele and children homozygous for the C allele. This primary emotion was selected given that it is the prevailing mood in MDD. We predicted that the sad state would be correlated with greater activation of brain regions mediating sadness in the T allele carriers relative to the C allele carriers.

## 2. Method

### 2.1. Subjects

Within a prospective twin cohort study (Dubreuil et al., 2003; Ouellet-Morin et al., 2008), 438 children of Caucasian ancestry were scanned when they were 8 years and 4 months of age. The study protocol was approved by the ethics review boards of Sainte-Justine Hospital and Centre hospitalier de l'Université de Montréal (CHUM)-Hôpital Notre-Dame. Written informed consent was obtained from all subjects and their parents. In this cohort, 139 children were genotyped for rs4675690. Distribution of genotype at rs4675690 among children was 27 of 139 (19%) TT and 42 of 139 (30%) CC. Subjects with more than 3 mm (or 3°) movement in the scanner were excluded from this study. Since they were only 12 valid TT subjects, 12 subjects of CC subject were randomly selected. All subjects were unrelated within groups and between groups.

### 2.2. Mental health evaluation

Subjects were assessed using the *Dominic-R Interactive* (Valla et al., 2000). This self-answered, computerized, DSM-IV-based instrument designed to measure mental health in children 6–11 years of age has been used in epidemiologic samples in clinical and research settings (Rousseau et al., 2005; Valla et al., 1994). The two groups scored below the clinical cut-off scores on all mental disorders assessed with the *Dominic-R* (depression, separation anxiety, generalized anxiety, specific phobias, attention-deficit hyperactivity disorder, conduct problems, oppositional-defiant disorder). The depression scores were minimal and not statistically different between groups (CC group: mean = 5.17, SD = 3.64, TT group: mean = 8.64 SD = 5.07) (P = 0.07).

### 2.3. Genotyping

The C_42647845_10 genotyping kit obtained from Applied Biosystems contained the two flanking primers and the C- and T-specific probes labeled with VIC and FAM fluorescent dyes, respectively. All PCR reaction were carried out using the TaqManR Genotyping Master Mix (ABI, Applied Biosystems) with 10 ng of genomic DNA in a final volume of 10 µl. The PCR reaction begins with an initial denaturation and activation at 95 °C for 10 min, followed by 40 cycles of 94 °C for 15 s and 60 °C for 1 min. Genotyping was performed using 7900HT Fast Real-Time PCR System (ABI, Applied Biosystems) and analysis for the allelic discrimination was performed using SDS2.2.2 software, following the instructions of the suppliers.

### 2.4. Experimental procedure

BOLD signal changes were measured while subjects viewed five blocks of neutral film excerpts (Control condition) followed by five blocks of sad-film excerpts (Experimental condition). The sad excerpts were extracted from the film *The Champ* (1979). Each block lasted 39 s and was separated by 15-s resting periods. After scanning, subjects identified the primary emotions they felt during the sad and neutral excerpts using a visual analog scale. Subjects identified sadness as the primary emotion felt. They were asked to rate its degree (sad, very sad, extremely sad, saddest ever).

### 2.5. fMRI acquisition

Echoplanar images (EPI) were acquired on a 1.5-Tesla system (Magnetom Vision, Siemens Electric, Erlangen, Germany). Twenty-eight slices (5 mm thick) were acquired every 2.65 s in an inclined axial plane, aligned with the anterior commissure–posterior axis. These T2*-weighted functional images were acquired using EPI pulse sequence (time repetition [TR] = 0.8 ms, time-echo [TE] = 54 ms, flip = 90°, field of view [FOV] = 215 mm, matrix = 64 × 64, voxel size = 3.36 mm × 3.36 mm × 5 mm). Following functional scanning, high resolution data were acquired via T1-weighted three dimensional volume acquisition obtained using a gradient echo pulse sequence (TR = 9.7 ms, TE = 4 ms, flip = 12°, FOV = 250 mm, matrix = 256 × 256, voxel size = 0.94 $mm^3$).

### 2.6. Image analysis

Data were analyzed using Statistical Parametric Mapping software (SPM5; Wellcome Department of Cognitive Neurology, London, UK). Images for all subjects were realigned to correct for