A Deletion in Tropomyosin-Related Kinase B and the Development of Human Anxiety

Carl Ernst, Brigitte Wanner, Jelena Brezo, Frank Vitaro, Richard Tremblay, and Gustavo Turecki

Background: The tropomyosin-related kinase B (TrkB)/brain-derived neurotrophic factor system has been associated with psychiatric disorders, and animal models of defects in this system suggest that it might have a particular role in anxiety.

Methods: DNA sequencing and cloning were used to identify a mutation in *TrkB*, and four different cell lines were used to assess functionality. Clinical samples were from a 22-year longitudinal cohort representative of the Quebec general population ($n = 640$ subjects), randomly selected when they were in kindergarten. Anxiety-related traits were measured with the Social Behaviour Questionnaire, the Diagnostic Assessment of Personality Pathology-Brief Questionnaire, and the Diagnostic Interview Schedule for DSM-IIIR.

Results: An 11 base pair deletion in *TrkB* is significantly associated with increases in anxiety traits during childhood and the development of anxiety disorders in adulthood. We found that this deletion impaired transcription in some human cell lines.

Conclusions: The identification of this deletion provides additional support for the role of TrkB in modulating anxiety-related traits in human.

Key Words: Brain, generalized anxiety disorder, genetics, longitudinal study, panic disorder, TrkB

Tropomyosin-related kinase B (TrkB) (also known as NTRK2) is a neurotrophic factor receptor in the central nervous system that plays a critical role in synaptic modeling, neurodevelopment, and cell signaling (1,2). Tropomyosin-related kinase B is a transmembrane receptor that is bound with high affinity by brain-derived neurotrophic factor (BDNF), and this interaction leads to conformation changes and phosphorylation at intracellular domains of TrkB. These ligand-induced changes generate intracellular signal cascades leading to, among other effects, gene transcription. A number of the genes regulated by TrkB-induced signaling cascades are related to cell growth, cell survival, and cytoskeletal dynamics (3).

Functional studies of TrkB in mice suggest that the gene plays a key role in traits implicated in anxiety. In mouse transgenic studies, overexpression of TrkB reduces anxiety (4), whereas deletion of *TrkB* in forebrain induces impulsive reactions to novel stimuli and inappropriate coping responses when facing stressful paradigms (5). Furthermore, deletion of *TrkB* in adult progenitor cells in the hippocampus increases anxious behavior in mice (6). Studies investigating the effects of BDNF also suggest a role in anxiety—in particular, the finding that reduced neuronal release of BDNF leads to increased anxiety-like traits in mice (7,8). Conceptually, TrkB has been linked to psychiatric illness in humans through the neurotrophin hypothesis of stress-related mood disorders (9,10).

In the current study, we identified an 11 base pair (bp) deletion in a human *TrkB* promoter. We hypothesized that this deletion would be associated with reduced expression of TrkB and that carriers of this deletion would have higher scores on surveys of anxiety-related traits compared with individuals carrying two wild-type TrkB alleles.

From the McGill Group for Suicide Studies (CE, JB, GT), Department of Psychiatry (CE, GT), McGill University; Research Unit on Children's Psychosocial Maladjustment (BW, FV, RT), University of Montréal, Montreal, Canada; and the School of Public Health and Population Sciences (RT), University College Dublin, Dublin, Ireland.

Address correspondence to Gustavo Turecki, M.D., Ph.D., McGill University, McGill Group for Suicide Studies, Department of Psychiatry, Pavilion Frank B Common, 6875 LaSalle Blvd., Montreal, QC, Canada H4H 1R3; E-mail: gustavo.turecki@mcgill.ca.

Received Jun 22, 2010; revised Aug 23, 2010; accepted Oct 7, 2010.

Methods and Materials

Comprehensive descriptions of the community cohort used in this study have been previously described (11). Briefly, 640 (364 female participants, 57%) members of a cohort followed since 1986 were randomly selected from French-speaking public schools in Quebec, Canada when they were in kindergarten. Only subjects whose parents were born in Quebec and whose mother tongue was French were included in this study (12). Included in the present study were subjects that had complete childhood and adult data on behavioral traits through all assessment waves and complete adult psychiatric information and provided a DNA sample ($n = 640$). Subjects were assessed on a series of behavioral and psychiatric questionnaires, a description of which can be found in the Methods and Materials in Supplement 1 along with all statistical analyses. All experiments and data collection were carried out in accordance with the institutional review boards of McGill University and the University of Montréal.

Full description of molecular experiments and cell transfection studies can be found in Methods and Materials in Supplement 1.

Results

While screening *TrkB* in a separate study performed in the French-Canadian population (13), we found two subjects with an 11 bp deletion (Figures 1A and 1B). Although the *TrkB* structure is complex (14,15), this deletion is located in a region thought to be the promoter (14). The wildtype *TrkB* sequence contains a single AluI digestion site within the deleted sequence, allowing for identification of deletion carriers by means of a restriction enzyme assay (Figure 1C). Given the size of the deletion, we could detect deletion carriers by running out the polymerase chain reaction products of DNA amplified through primers flanking the deletion site in agarose gels; in individuals with the deletion, we observed two bands (Figure 1D). Finally, we sequenced both mutant and wildtype bands (Figure 1E) and cloned the deletion (Figure 1F).

We cloned a 1.61 kb fragment that included the deletion region as well as upstream sequences. Consistent with a previous report

0006-3223/$36.00
doi:10.1016/j.biopsych.2010.10.008

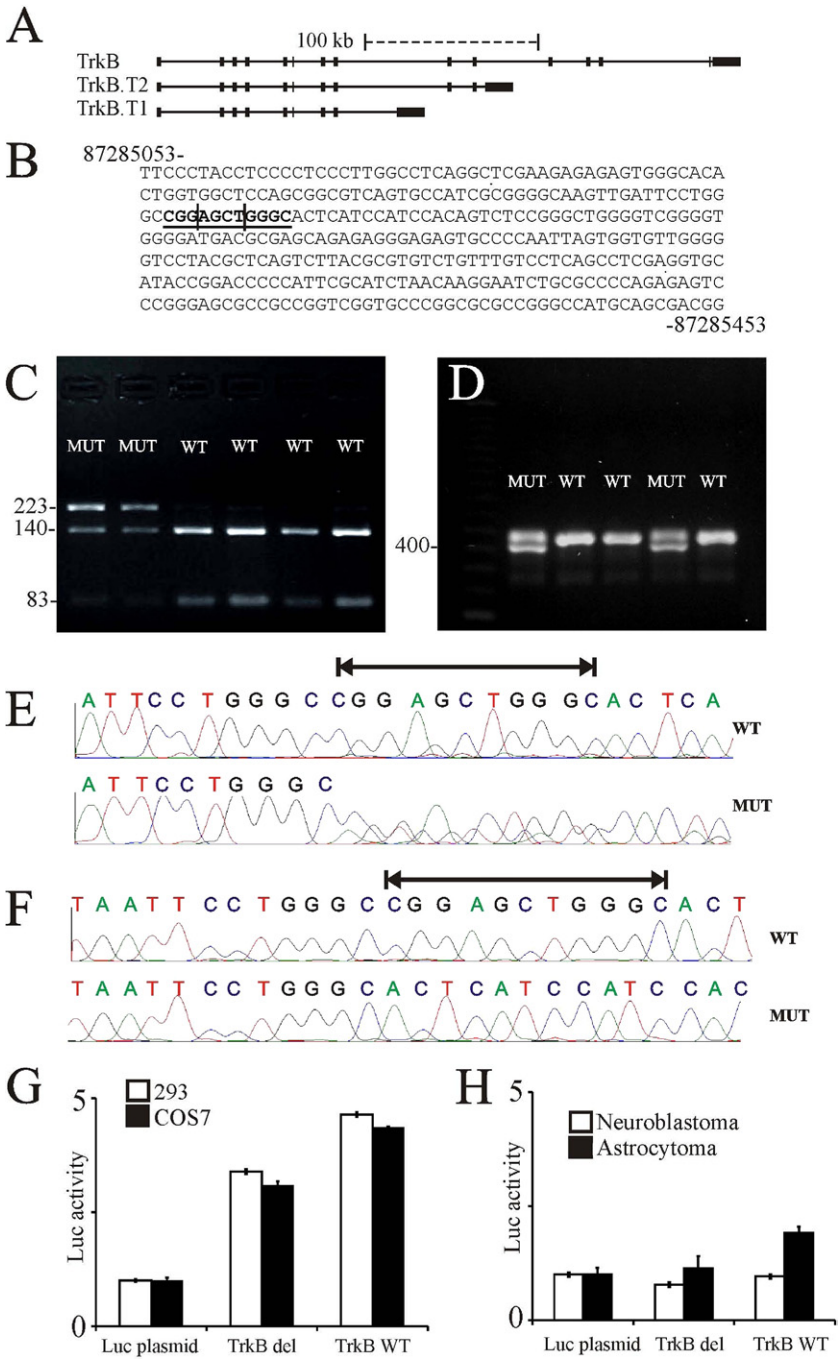

Figure 1. Identification of a deletion in tropomyosin-related kinase B (*TrkB*). **(A)** General structure of *TrkB*, with truncated isoforms (T2 and T1) shown. **(B)** A promoter region of *TrkB*, including the deletion (underlined and emboldened, with AluI cut site, AGCT, emphasized). Numbers represent start and end of sequence region from hg19 genome build. **(C)** Restriction digestion (*AluI*) of the deleted fragment. Subjects with the deletion (MUT) are all heterozygous for the mutation, and therefore one-half of the DNA product is protected from AluI digestion. Note the presence of the full length band in MUT lanes, which are absent in all wildtype (WT) lanes. **(D)** Gel electrophoresis showing MUT and WT subjects. Note the presence of an extra band in MUT lanes. **(E)** Sequencing from MUT and WT bands shown in **D**. Deletion region is depicted with an arrow. **(F)** Sequencing of the cloned bands in MUT and WT subjects from **D**. **(G and H)** Results of luciferase transfection assays with negative control (Luc plasmid), construct with the deletion (TrkB del) and wildtype construct (TrkB WT).

(14), this region showed promoter activity both in COS7 cells and HEK293 cells (Figure 1G), where constructs with the deletion showed lower luciferase activity than wildtype constructs (COS7: $t = 4.33$, $p < .05$; 1.41-fold decrease; HEK293: $t = 4.1$, $p < .01$; 1.37-fold decrease). We also assessed human astrocytoma and neuroblastoma cell lines (Figure 1H). In astrocytes, we found a significant difference between the wildtype and mutant constructs ($t = 2.87$; $p < .05$, 1.68-fold decrease). However, in the neuronal cell line, we observed no significant difference between mutant and wildtype constructs ($t = .79$, $p > .05$; 1.23-fold decrease), although baseline luciferase activity was essentially undetectable with either wildtype or mutant construct in this cell line. We note that a positive control