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SNAT1 AND A FAMILY WITH HIGH RATES OF SUICIDAL BEHAVIOR

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Abstract—Several lines of evidence suggest that suicide may have, in part, a genetic predisposition. In this study, we identified a family with high rates of suicidal behavior and assessed brain gene expression levels in the proband. A neuronally-expressed solute carrier for glutamine (Sodium-coupled neutral amino acid transporter 1 (SNAT1), also known as solute carrier family 38, member 1 (SLC38A1)) was identified as severely decreased across all brain regions. Follow-up analysis by semi-quantitative polymerase chain reaction (qPCR) and Western blot confirmed the reduction of SNAT1. We categorized the SNAT1 gene in human brain, cloned the gene promoter and assessed in silico the expression pattern of SNAT1 in >25 tissues from human. Complete DNA sequencing of the SNAT1 gene was performed in the family and 276 controls. The family was homozygous for rare alleles which suggests a possible association between low expression of SNAT1 and suicidal behavior. Crown Copyright © 2009 Published by Elsevier Ltd on behalf of IBRO. All rights reserved.

Key words: suicide, SNAT1, glutamate metabolism, neurogenetics.

Psychiatric phenotypes are etiologically heterogeneous (Gottesman and Gould, 2003; Cannon and Keller, 2006). While many lines of evidence support the role of genetic factors in the predisposition to most psychiatric conditions (Brunner et al., 1993; Brent et al., 1996), to date, the majority of studies attempting to find specific molecular factors increasing phenotypic predisposition have failed (Kanazawa et al., 2007; Barnett et al., 2008; Gaysina et al., 2008; Kawashima et al., 2008; Szczepankiewicz et al., 2008; Zalsman et al., 2008). It is widely recognized that significant etiological heterogeneity is in large part associated to this failure (Fanous et al., 2004; Abdolmaleky et al., 2005). As such, there has been growing interest in the identification of subgroups that could help define more homogeneous groups. To this end, we have recently proposed a method to identify extreme expression values from microarray data that may homogenize a psychiatric population based on the expression level of a given gene (Ernst et al., 2008, 2009). Another approach to counter the heterogeneity observed in psychiatric genetics is to identify families that have a number of affected members, which may be suggestive of a genetic cause for disorder in a particular family. This approach has proven fruitful in aggressive behaviors (Brunner et al., 1993) schizophrenia (Millar et al., 2005), and mental retardation (Gleeson et al., 1998).

Suicide is a complex condition with clear genetic-epidemiological evidence of a genetic component modifying risk (Brent et al., 1996; Brezo et al., 2008). We identified through a proband who died by suicide, a family with important loading of suicidal behavior. Here we report on gene expression patterns in the proband using the extreme values analysis (EVA) that was followed with a series of complementary techniques. Our results provide evidence that Sodium-coupled neutral amino acid transporter 1 (SNAT 1) may be involved in suicide predisposition, further implicating the glutamate system in suicide and major depression, consistent with results from previous studies (Choudary et al., 2005; Ploski et al., 2006; Klempan et al., 2009).

EXPERIMENTAL PROCEDURES

Family recruitment

We identified a family that had significant loading of suicidal behaviors and where the brain of the proband was available (Fig. 1). This family was of interest because of the extent of suicide and depression in the family, and because of its size (11 siblings).

We met seven of a possible 10 siblings and performed brief psychological interviews with each (questions related to depression and suicidal behavior) and took a DNA sample. We later interviewed the mother and collected her DNA. The proband in this family attempted suicide multiple times, each of which required hospitalization, before a completing suicide at age 39.

Every interviewee had clinical depressive episodes, suicidal thoughts or attempts. Three siblings declined to be interviewed for this study; however, the other siblings reported that one of those that declined to be interviewed had attempted suicide multiple times, each requiring hospitalization. No other information was collected on the three absent siblings.

The mother of the siblings was first hospitalized at 25 for depression and now lives at a Douglas Hospital psychiatric facility (age 82). She reported no psychotic symptoms, only severe depression.

Information from only two of the 20 nephews and nieces of proband was available. Of these individuals, one completed suicide.

Group characteristics (microarray study)

All subjects were recruited at the Montreal Morgue as part of ongoing recruitment procedures through the McGill Group for Suicide Studies. Briefly, once a case is identified a family member is contacted by a trained clinician who asks for consent for organ harvesting and access to medical records. After consent from next

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Abbreviations: CA, cardiac arrest; EVA, extreme values analysis; MVA, motor vehicle accident; PAINT, promoter analysis and interaction network generation tool; qPCR, quantitative polymerase chain reaction; SNAT1, Sodium-coupled neutral amino acid transporter 1; TBS, Tris-buffered saline.

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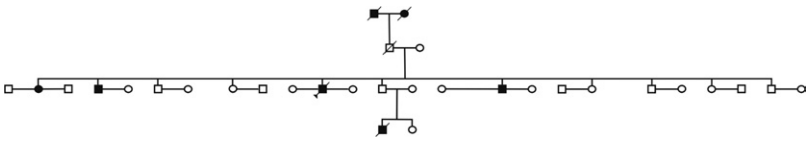

Fig. 1. A family with high levels of suicide behaviors. Indicated in the pedigree (blackened) are people with severe suicide attempts or completions (n=2 completers). Proband is indicated by the black triangle.

of kin, interviews are scheduled with a best possible informant (the person who can best answer questions about a given case's clinical history) to undertake a psychological reconstruction of the life of the deceased. Psychological reconstructions involve structured clinical interviews for Axis 1 and Axis 2 disorders and other validated questionnaires that have been described elsewhere (Dumais et al., 2005).

All subjects used in microarray expression and follow-up experiments are male Caucasians. Twenty-eight suicide completers and 11 controls were recruited. Brodmann areas 4, 6, 8/9, 10, 11, 20, 21, 38, 44, 46, 47, and the hippocampus and the nucleus accumbens from the left hemisphere (gray matter only), were dissected. The mean age of all subjects, both suicide and controls, was 35±11 years with an average PMI of 24±5.5 h.

Microarray analysis

We have designed an analysis algorithm to assess for extreme expression values in microarray data (Ernst et al., 2008), termed EVA. Briefly, individual data points were assessed across control and suicide groups for any subject that may lie outside threefold from a mean and outside of 1.5 SDs in all brain regions. This analysis method is similar to other described protocols (Lyons-Weiler et al., 2004; Tomlins et al., 2005). The basic premise is that psychiatric traits are heterogeneous and complex, therefore not all affected subjects will share quantifiable traits (i.e. gene expression level). Studying group mean effects may miss biologically important factors in select subjects or subgroups. Any probe set/subject pair that meets EVA criteria (threefold change and 1.5 SDs from the mean) in all brain regions likely reflects a very real biological phenomenon. Affymetrix HG-U133A, B chips were used in all analyses. RMA normalized values were used for all comparisons. RNA quality was classified through a minimum 1.8 A260/A280 ratio.

Semi-quantitative polymerase chain reaction (qPCR)

cDNA preparations using oligodTs were completed using 100 ng/μl RNA samples extracted from various Brodmann areas. Each qPCR used only samples from one cDNA preparation stock plate. β-Actin was used as a loading control across all subjects. β-Actin was used as a control across all subjects. Densitometry was performed using Gene Tools (SynGene, Frederick, MD, USA) for quantification of bands to assess gene to β-actin ratios.

Western blots

Twenty-five micrograms of brain homogenate and sample buffer (0.25 M Tris–HCl, 20% glycerol, 4% SDS, 0.005% Bromophenol Blue, and 1% 2 M DTT) were boiled at 95 °C for 2 min. Samples were loaded on a Novex sure-lock electrophoresis system (Invitrogen), using 4%–20% precast gels (Invitrogen). After gel transfer membranes were blocked with a 5% milk solution (Carnation fat free milk, in Tris-buffered saline; TBS) shaking for 1 h. Membranes were submerged in a 5% albumin solution (albumin in TBS with 0.2% Tween) with primary antibody, 1:1000, rabbit anti-SNAT1 (Weiss et al., 2003) and left shaking overnight at 4 °C. Membranes were thoroughly washed and left shaking for 1 h with secondary antibody (1:2000, donkey-HRP anti-rabbit, Santa Cruz). Membranes were then exposed using an ECL kit (Amersham) and apposed to film. To verify the accuracy of loading, membranes were stripped and re-probed with an antibody to β-actin (Santa Cruz).

DNA sequencing

Sequencing of non-purified PCR products was completed using the 3730xl DNA Analyzer system from Applied Biosystems at Genome Quebec. Sequencing was carried out on genomic samples to account for the possibility of intronic variations affecting proper splicing. Both forward and reverse sequences were analyzed. Trimmed sequences were aligned using ClustalW (Higgins et al., 1996). Raw data from the chromatogram were used to detect heterozygous SNPs in the sequences. All PCR reactions were completed using Applied Biosystems GeneAmp PCR System 9700 thermal cyclers. The PAINT (promoter analysis and interaction network generation tool) algorithm was used to identify 5′ genomic regulatory regions (Vadigepalli et al., 2003).

Luciferase experiments

A 1557 bp PCR product was cloned into the pGL3 vector (Promega, Madison, WI, USA), verified for orientation, and sequenced. Plasmid propagation was done by transforming competent *E. coli* cells (Qiagen PCR CloningPlus Kit). Plasmids were then co-transfected into HEK293 cells (ATCC) with a loading control plasmid. Transfections were done using lipofectamine 2000 and left overnight in DMEM media (GIBCO). Luciferase experiments were performed using a dual injection luminometer (Berthold, Oak Ridge, TN, USA) and the Dual-Luciferase Reporter Assay system (Promega). Firefly luciferase (pGL3 vector) activity was then measured. Experiments were run in triplicate and were compared to the pGL3_basic plasmid in all experiments.

Global expression of SNAT1

Using genome expression omnibus information a whole body expression map was generated for SNAT1 expression using RMA values for the 218237_s_at probe set (Roth et al., 2006). This pattern of expression was generated from 352 tissue types on Affymetrix Human Genome U133 Plus 2.0 Arrays. Samples were taken from 10 donors both male and female, varying in age from 23 to 53 years old (Roth et al., 2006).

RESULTS

Identification of an extended pedigree with high rates of suicidal behaviors

Fig. 1 shows details of the family recruited for this study (n=17 blood relatives). Briefly, 41% (n=7) of the individuals manifested suicidal behavior and two died by suicide. Of the five suicide attempters, four were hospitalized as a result of suicide attempts, and attempted multiple times. Important psychopathology segregated in this family. First, nine of 10 siblings of the proband are being treated with antidepressants. The mother of the children has been hospitalized for the past 57 years for severe depression