Journal of Psychiatric Research 43 (2009) 532–537

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Methylation and QTDT analysis of the 5-HT2A receptor 102C allele: Analysis of suicidality in major psychosis

Vincenzo De Luca, Emanuela Viggiano, Ranbir Dhoot, James L. Kennedy, Albert H.C. Wong *

Centre for Addiction and Mental Health, Department of Psychiatry, University of Toronto, 250 College Road, Room 711, Toronto, Ontario, Canada M5T 1R8

ARTICLE INFO

Article history:
Received 15 April 2008
Received in revised form 17 July 2008
Accepted 25 July 2008

Keywords:
Suicide
5-HT2A
Psychoses
Allele ratio
Methylation
Post-mortem brain

ABSTRACT

Suicide is an act deliberately initiated and performed by a person with full knowledge that a fatal outcome is probable. The serotonin 2A (5-HT2A) receptor gene has been implicated in the pathogenesis of suicidal behaviour by a genetic association between the 5-HT2A T102C silent polymorphism and suicidality in patients with mood disorders and schizophrenia. However, a recent meta-analysis failed to confirm this association. We developed an improved quantitative assay for the measurement of allele-specific methylation of the 5-HT2A gene, and found that the methylation of the C allele in the pre-frontal cortex of heterozygous suicide victims ($n = 10$) was not significantly different in comparison with the non-suicide group ($n = 10$) ($p = 0.084$). We also analyzed methylation of the C allele in white blood cell DNA from bipolar and schizophrenic attempters and found a significant difference in the schizophrenic attempters ($p = 0.00013$) but not in the bipolar attempters ($p = 0.616$).

Because the 5-HT2A gene is subject to imprinting, the parent-of-origin may affect inheritance of suicidal behaviour. Thus, we examined the parental origin of specific alleles for genetic association in a genetic family-based sample of major psychoses in which information on suicidal behaviour was available. This result suggests that methylation of the 102C allele does not influence completed suicide.

1. Introduction

Suicide and suicidal behaviour are responsible for considerable morbidity and mortality in schizophrenia (SCZ) and bipolar disorder (BD). The lifetime suicide risk is between 4% and 13% in SCZ (Meltzer, 2005) and 19% in BD (Goodwin, 1990). Between 25% and 60% of bipolar patients make at least one suicide attempt during the course of their illness (Chen and Dilsaver, 1996) and up to 20–50% of schizophrenic patients attempt suicide (Roy et al., 1984) (Radomsky et al., 1999). Genetic contributions to suicidal behaviour are suggested by higher concordance rates for suicidal behaviour among monozygotic twins compared to dizygotic twins or other relatives of suicidal subjects (Baldessarini and Hennen, 2004). The observation that 10/26 monozygotic co-twins of suicide completers also attempted suicide, in comparison to 0/9 dizygotic co-twins, reinforces that suicidal behaviour is influenced by genetic factors (Roy et al., 1995). These data also suggest that a complex inheritance pattern is present and that epigenetic effects may be relevant.

A major form of epigenetic DNA modification is the methylation of cytosine (C) residues within cytosine–guanine dinucleotides (CpG). Although CpG sequences spread throughout the genome are usually heavily methylated, those that cluster in CpG islands in the promoter regions of genes are less methylated (Abdolmaleky et al. 2004). As with most behaviours, genetic factors are not deterministic, but contributory, with environmental factors as potential mediators. Epigenetic mechanisms can mediate the effects of environment on the genome, and represent a potential model to explain gene-environment interactions. For example, neonatal psychological experience can leave lasting effects on adult behaviour through epigenetic regulation of glucocortiod receptor expression (Weaver et al. 2004). However, to date there are no studies that have investigated DNA methylation patterns in suicidal subjects.

The level of methylation at C nucleotides within CpG islands generally reflects levels of gene expression, with hypermethylation associated with gene silencing. In recent years there have been a few reports of changes in DNA methylation patterns associated with specific psychiatric disorders (Abdolmaleky et al., 2005). For example, L-methionine treatment can exacerbate psychosis (Chen et al., 2002); while valproate, a drug producing hypomethylated DNA, reduces such symptoms (Alonso-Aperte et al., 1999). Hypermethylation of the promoter region of the RELN gene correlates with reduced expression of a gene for the protein Reelin, which is necessary for neuronal migration and synaptogenesis, and which is reduced in schizophrenia and bipolar disorder(Chen et al., 2002). Experimental evidence implicates methylation of the promoter regions of the DRD2 and HTR2A genes in schizophrenia and mood disorders as well (Petronis, 2000).

* Corresponding author. Tel.: +1 416 535 8501x4010; fax: +1 416 979 4663.
E-mail address: albert.wong@utoronto.ca (A.H.C. Wong).

0022-3956/$34.00
doi:10.1016/j.jpsychires.2008.07.007

There have been many genetic association studies of polymorphic variants in 5-HT system genes and suicidality. In particular, the HTR2A C102T gene variant has been analyzed extensively (Du et al., 2000). The biological function of the HTR2A C102T polymorphism is unclear because it is synonymous and therefore does not alter amino acid sequence, although it may affect mRNA secondary structure (Arranz et al., 1995). Ertugrul and colleagues (Ertugrul et al., 2004) showed that the Hamilton Depression Scale item for current suicidality was significantly higher in patients heterozygous for the silent polymorphism C102T in HTR2A compared to those with genotype C/C and marginally higher than for the patients with genotype T/T, but a large meta-analysis (including 1599 suicidal patients) did not find a significant association between suicide attempts or suicide completion and the C102T (rs6313) polymorphism (Anguelova et al., 2003). Some studies of expression of HTR2A messenger RNA (mRNA) and protein in postmortem brains suggest that the 102C allele might be less transcriptionally active than the T allele (Polesskaya and Sokolov, 2002). In contrast to the biallelic expression observed in brains (Fukuda et al., 2006), monoallelic expression of HTR2A was common in fibroblasts (Kato et al., 1996). However, transmission pattern analysis revealed that imprinting was not responsible for the monoallelic expression in peripheral blood leukocytes (PBLs) (Fukuda et al., 2006). The 102T/C SNP might directly influence mRNA levels through methylation of the C allele, which is part of a CpG dinucleotide in exon 1 of HTR2A (Petronis, 2000).

In our previous report (De Luca et al., 2007) we investigated polymorphic genomic imprinting, calculating the allelic expression ratio in subjects heterozygous at the HTR2A C102T locus. We found a significant difference in suicide victims compared with non-suicide controls. This analysis of human brain tissues showed that no individuals exhibit mono-allelic expression (polymorphic genomic imprinting), however mono-allelic expression might operate only in a proportion of the cells from each individual. Furthermore, the significant difference we found in suicide victims could suggest different degrees of mono-allelic expression in different diagnostic groups. Genomic imprinting is one explanation for the complex pattern of inheritance observed in most common psychiatric phenotypes. Furthermore, this would have important consequences for the evaluation of genetic association studies of suicidality, since genomic imprinting can be a significant confounding factor.

Another way to uncover genomic imprinting in suicidal behaviour is to track maternal or paternal inheritance (parent-of-origin effect) of the allele or haplotype, so that only the expressed allele would have to be taken into consideration. We were unable to show any parent-of-origin effect (POE) in patients with major psychosis and suicide attempts (De Luca et al., 2007). However, molecular studies investigating the transmission of maternal and paternal alleles lack power due to the fact that the number of informative meiosis is halved (from the mother or from the father). The aim of this complementary study is to investigate 102C methylation status in brain tissue from suicide victims and controls included in our previous allelic expression analysis, to investigate the methylation level in peripheral leukocytes from suicide attempters with schizophrenia and bipolar disorder, and to investigate the POE of the C102T in a larger sample of suicidal patients.

2. Methods

2.1. DNA methylation analysis

We matched ten suicide cases for genotype, diagnosis, age of death and sex to ten controls who died from other causes donated from The Stanley Medical Research Institute (SMRI) array collection. The suicide group consisted of nine bipolars and one subject with schizophrenia (Table 1). All subjects were C102T heterozygous. The control group consisted of 7 bipolar and 3 schizophrenics (4 male and 6 female) with a mean age of 43.6 ± 11.04. Genomic DNA from BA46 dorsolateral preforntal cortex (DLPFC) was analyzed from the same samples analyzed for the differential allele expression (De Luca et al., 2007). Even though the sample and the locus are the same this analysis adds new information since it considers DNA methylation rather than RNA differential allelic analysis that was performed in our previous publication (De Luca et al., 2007).

The C102T SNP is a well-known polymorphic CpG site within exon1 of HTR2A that is recognized by the enzyme HpaII. Brain DNA was treated with HpaII restriction enzyme using a modified version of the protocol outlined by Polesskaya et al. (2006). The genomic DNA was pooled for suicide victims and non-suicide brains from SMRI. The pooled DNA was divided into two aliquots for both groups. One aliquot was treated with HpaII and the other was untreated. 40 ng samples of genomic DNA (in a volume of 2 μl) from each subject were incubated at 37 °C for 8 h with HpaII, followed by 5 min at 65 °C for HpaII inactivation. Methylation levels of 102CpG were measured with quantitative PCR (Q-PCR; ABI PRISM® 7000 Sequence Detection System) using TaqMan® Assays-by-Design (ABI). The forward primer sequence was 5′-GACACCAGGCTCTACAGTAATGAC-3′ and the reverse primer was 5′-CGACTGTCCAGTTAAATGCATCAGA-3′. Quantitative measurement of the methylation status of the 102CpG site was carried out on the ABI PRISM® 7000 with the following allele-specific, fluorescent-labelled probes: VIC- 5′-CTTCTCC**G**GAGTTAAA-3′ and 6FAM-5′- CTTCTCC**A**GAGTTAAA-3′. The relative amplification of the T allele that is not affected by HpaII was used to normalize the quantification of the methylated C allele.

The quantified cytosine levels in HpaII-treated DNA were divided by the cytosine levels in untreated DNA, allowing the calculation of the percentage of methylated cytosine within the sample. After the PCR, a 100% unmethylated HpaII region would generate no amplicons (complete template digestion). All reactions were performed in quadruplicate and a standard curve was generated for the probe using untreated DNA, composed of known serial dilutions, in order to establish the relationship between the threshold cycle (T_c) and the relative amount of cytosine. Furthermore, we analyzed 48 schizophrenics (24 attempters vs. 24 non-attempters) and 57 bipolar subjects (29 attempters vs. 28 non-attempters) from our existing DNA samples used in previous genetic association studies (De Luca et al., 2007; De Luca et al. 2005). The attempters and non-attempters were group-matched by gender and age and all subjects were C102T heterozygous and white –Caucasian ethnicity. The DNA from white blood cells was extracted using the high salt extraction method (Lahiri and Nurnberger, 1991).

Table 1
Demographic characteristics of the subjects from SMRI sample

	Suicide subjects	Control subjects
Sex M/F	4:6	4:6
Principal axis I diagnosis, No. (%)		
Schizophrenia	1 (10%)	3 (30%)
Bipolar disorder	9 (90%)	7 (70%)
Mean ± SD		
Age of death	40.4 ± 10.68	43.6 ± 11.04
Liftime alcohol abuse[a]	2.40 ± 2.01	2.10 ± 2.31
Liftime drug abuse[a]	1.70 ± 1.83	2.10 ± 2.08
Time in the hospital (years)	0.200 ± .163	0.267 ± .269
Age of onset (years)	26.6 ± 11.2	24.2 ± 6.3
PMI (hours)	41.0 ± 17.6	26.9 ± 14.8
Brain pH	6.45 ± .31	6.30 ± 0.22

[a] Lifetime alcohol and drug abuse were assessed on a semi-quantitative scale, with the following order: 0 = little or none, 1 = social, 2 = moderate in the past, 3 = moderate at time of death, 4 = heavy in the past, 5 = heavy at time of death.