Letter to the Editor

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Two novel genomic rearrangements identified in suicide subjects using a-CGH array

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To the Editor,

Many genes expressed in the central nervous system are candidates for the susceptibility to suicidal behavior (SB) including serotonergic, noradrenergic, and dopaminergic systems [1–3]. We studied the brain-derived neurotrophic factor (BDNF)/tropomyosin receptor kinase B (TrkB) system that plays a critical role in neuronal survival and morphogenesis and in brain plasticity. We ruled out the association between polymorphisms G196A and -281 C/A of the BDNF gene and suicide [4]. Thus, we demonstrated a decreased BDNF expression in Wernicke area of brain of suicide subjects, due to BDNF promoter hypermethylation [5] revealing, for the first time, an association between epigenetics and suicide. Furthermore, we investigated the other component of the system, i.e., the neurotrophic tyrosine receptor kinase (NTRK) 2 gene, that encodes the TrkB receptor, excluding correlations between NTRK2 gene methylation in Wernicke area and suicide [6]. In addition, we evaluated NTRK2 expression of the main TrkB isoforms on Wernicke’s area, i.e., Brodmann’s area 22, that have relevant function in understanding and language [5]. Finally, we demonstrated that some NTRK2 polymorphisms predispose to SB modulating the expression of the different isoforms of the receptor in Wernicke area [7]. All studies performed so far focused on point mutations in candidate genes. Recently, array-comparative genomic hybridization (a-CGH) has allowed the identification of gains and losses of DNA as small as few kilobases enabling a large number of genomic alterations associated with neurological disorders to be identified [8]. However, at present, no studies have been performed to identify genomic alterations in subjects with SB; in order to identify alterations that could be correlated to SB we analyzed by a-CGH DNA samples of brain tissue extracted postmortem from the Wernicke area obtained from 34 suicides (23 females and 11 males) and 34 non-suicide control subjects (21 females and 13 males), aged 17–30 years. The Wernicke’s brain tissue samples were collected in the course of autopsy (Institute of Forensic Medicine, University of Ljubljana) and frozen at –80°C within 26 h from the subject’s death (Biological Bank of the Institut za Varovanje Zdravja, Ljubljana, Slovenia) between 1999 and 2005. The study was performed according to ethical issues of such institution and Helsinki Declaration as revised in 2008 and permission to use the samples was obtained by the institution that

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Unauthenticated
originally collected them. We obtained informed consent to anonymously use all samples for research purposes from next of kin. Data on subjects’ sex, age, drug history, and cause of death were available as well as data on psychiatric diagnoses that were obtained from the physician’s note for autopsy and, in cases when a coroner’s inquest was done in the presence of relatives, their testimony was also taken into account. All subjects were Caucasians and had no documented prior history of psychiatric disorders. pH measurement was performed as previously described [5]. DNA was extracted, from each sample, from a portion of liquid nitrogen-pulverized tissue using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer’s instructions. DNA quality and quantity assessment was assessed using a ND-1000 NanoDrop spectrophotometer (NanoDrop Products, Wilmington, DE, USA). All samples showed a 260/280 ratio between 1.8 and 2.0 and a 260/230 ratio above 1.5, therefore were qualitatively optimal for a-CGH analyses.

DNA samples were analyzed with a CGH Microarray kit 4X180K (Agilent Technologies, Santa Clara, CA, USA). The reference DNA used (Promega, WI, USA) consists in a normalized pool of genomic DNA from seven normal controls. The array used in this study contains 170,334 60-mer oligonucleotides probes covering the whole genome with an average spatial resolution of 13 Kb. DNA digestion, DNA samples were analyzed with a CGH Microarray kit 4X180K (Agilent Technologies, Santa Clara, CA, USA). The reference DNA used (Promega, WI, USA) consists in a normalized pool of genomic DNA from seven normal controls. The array used in this study contains 170,334 60-mer oligonucleotides probes covering the whole genome with an average spatial resolution of 13 Kb. DNA digestion, DNA samples were analyzed with a CGH Microarray kit 4X180K (Agilent Technologies, Santa Clara, CA, USA). The reference DNA used (Promega, WI, USA) consists in a normalized pool of genomic DNA from seven normal controls. The array used in this study contains 170,334 60-mer oligonucleotides probes covering the whole genome with an average spatial resolution of 13 Kb. DNA digestion,
labeling and hybridization were performed according to the manufacturer’s protocols [9, 10]. Microarrays were scanned on an Agilent G2565CA scanner (Agilent Technologies) and image files were quantified using Agilent’s Feature Extraction software (V10.10.1.1); data were visualized with Agilent’s Genomic Work Bench Standard Edition (V6.5.0.58).

Among the suicides evaluated we identified in two subjects genomic rearrangements present in genes previously correlated with human behavior and considered therapeutic targets for patients affected with neurological disorders. One subjects showed an amplification of an area of at least 59 bp at Xp11.3 (chrX:43,516,819–43,516,878, hg19) involving the intron 1 of monoamine oxidase A (MAOA) gene (Figure 1A); a second subject harbored a duplication of about 2.5 Mb located at 22q11.21 (chr22:18,894,835–21,464,119, hg19) involving several genes among which was catechol-O-methyl transferase (COMT) gene (Figure 1B). Quantitative PCR analysis was performed to confirm both rearrangements. Primer pairs were designed using Primer Express 2.0 software (Applied Biosystems) with 20%–80% GC content and 59–60 °C melting temperature; primers selected from the zinc finger protein (ZNF) 80 gene were used to generate a normalizing amplicon (Table 1). Primer specificity was tested using NCBI’s BLAST software. The SYBR Green I based real-time PCR assay was performed on a 7900 Fast Real Time PCR apparatus (Applied Biosystems) using the Fast Start Universal SYBR Green Master Kit (Roche Applied Science, Mannheim, Germany). The assay was carried out in a total volume of 25 μL in triplicates in 96-well optical reaction plates (Applied Biosystems). A relative quantification, using 20 genomic DNA samples, was carried out. The haploid gene copy number was calculated using the comparative Δ-ΔC, method.

Table 1 Quantitative PCR primers used in this study.

<table>
<thead>
<tr>
<th>Primer*</th>
<th>Sequence (5’-3’)</th>
<th>Gene</th>
<th>Product size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>exon 3-F</td>
<td>TGTCATAGGTCGACACCAAG</td>
<td>COMT</td>
<td>102</td>
</tr>
<tr>
<td>exon 3-R</td>
<td>AGTAGGTGTAATGGCCCTCAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>exon 6-F</td>
<td>AGTCCCCGCTCCCTTGGTATG</td>
<td></td>
<td>81</td>
</tr>
<tr>
<td>exon 6-R</td>
<td>AGCTCCAGTGGTCGATGGGCGAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intron 1-2-F</td>
<td>ATGGTGCCGATCTATGGTAAATGT</td>
<td>MAOA</td>
<td>77</td>
</tr>
<tr>
<td>intron 1-2-R</td>
<td>TCAGTTGTGATCTCCAGGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intron 1-2-F</td>
<td>CAGTTGTGAGGACTTGGGAACAATA</td>
<td></td>
<td>81</td>
</tr>
<tr>
<td>intron 1-2-R</td>
<td>GAGAAAGTTTAAAGAATCCTCGACG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>zn80-F</td>
<td>CTTGACGCCGCAGCTTCCTTCT</td>
<td>ZNF80</td>
<td>120</td>
</tr>
<tr>
<td>zn80-R</td>
<td>TAAAGTCTTCTCAGGTGACTGATGTG</td>
<td></td>
<td></td>
</tr>
</tbody>
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*F, sense primer; R, antisense primer.

We speculated that both rearrangements observed by a-CGH might cause an increase in protein expression and activity. In fact, the first variant consists in a duplication of a region in the intron 1 of MAOA, putatively involved in the regulation of gene expression [11], while the other is a large duplication including the whole gene encoding for the COMT enzyme; the subject harboring this duplication has three copies of the COMT gene and should therefore have a significant increase in the enzyme activity. Both alterations are in loci that may have an effect on SB. In fact, MAOA is involved in the catabolism of serotonin; increase in its activity causes a more rapid catabolism and a significant reduction of serotonergic activity that is a well known cause of depression and suicide [3]. For example, a significant increase of MAOA has been found in the hypothalamus of suicide victims [3]. However, polymorphisms that increase MAOA expression are defined risk factors for suicide [1–3]. Moreover, in recent years MAOA gene is a site of high biological plausibility in antisocial spectrum disorders and psychopathy [12]. Similarly, COMT is the key enzyme in the degradation of adrenergic neurotransmitters; a higher activity of the enzyme corresponds to reduced levels of adrenergic neurotransmission. Many studies correlated polymorphisms that enhance COMT activity to depression and suicide [2]. This preliminary study confirms the heterogeneity of genetic determinants of SB; in fact, only two suicide subjects out of 34 analyzed showed genomic alterations including genes potentially related to SB; however, such large rearrangements with possible gain-of-function of specific genes have not been described to date and represent a novel predisposing mechanism in SB pathogenesis. We believe that genomic screening using a-CGH, possibly in combination with other techniques, such as next generation sequencing (NGS), represent powerful diagnostic tools for the identification of altered pathways in subjects considered at risk of SB. An improvement in genotype-phenotype correlation and the identification of alterations in specific genes can be extremely relevant information for the development of prevention programs and pharmacological interventions.

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