DNA copy-number analysis in bipolar disorder and schizophrenia reveals aberrations in genes involved in glutamate signaling

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Using bacterial artificial chromosome (BAC) array comparative genome hybridization (aCGH) at ~1.4 Mbp resolution, we screened post-mortem brain DNA from bipolar disorder cases, schizophrenia cases and control individuals (n = 35 each) for DNA copy-number aberrations. DNA copy number is a largely unexplored source of human genetic variation that may contribute risk for complex disease. We report aberrations at four loci which were seen in affected but not control individuals, and which were verified by quantitative real-time PCR. These aberrant loci contained the genes encoding EFNA5, GLUR7, CACNG2 and AKAP5; all brain-expressed proteins with known or postulated roles in neuronal function, and three of which (GLUR7, CACNG2 and AKAP5) are involved in glutamate signaling. A second cohort of psychiatric samples was also tested by quantitative PCR using the primer/probe sets for EFNA5, GLUR7, CACNG2 and AKAP5, and samples with aberrant copy number were found at three of the four loci (GLUR7, CACNG2 and AKAP5). Further scrutiny of these regions may reveal insights into the etiology and genetic risk factors for these complex psychiatric disorders.

INTRODUCTION

Bipolar disorder and schizophrenia are severe mental illnesses that affect thinking, mood and behavior and cause lifelong disability. While twin, family and adoption studies reveal that both schizophrenia and bipolar disorder have a strong genetic component (1–3), the identification of genetic aberrations associated with these diseases has been elusive. Several chromosomal regions have been implicated as having genetic linkage in multiple studies (4) and finer scale mapping studies have narrowed these intervals and highlighted a number of candidate genes of small effect (5–9). Recent studies have highlighted DNA copy-number differences as a largely under-explored source of human genetic variation (10,11) that may be a common underlying factor in genetic disease (12). Consistent with this notion, evaluation of DNA copy number in schizophrenia and bipolar disorder may yield insights into genetic risk factors for these diseases. Convergent evidence from linkage analysis and the detection of genomic insertion/deletion events has been shown previously to be important in identifying candidate genes for complex CNS disorders. For example, a combination of linkage and cytogenetics data have helped reveal the importance of the parkin (13,14) and alpha-synuclein genes (15,16) in Parkinson’s disease. Further, for schizophrenia and bipolar disorder there is a clear precedent for DNA copy-number variation conferring genetic risk. Velo-Cardio-Facial Syndrome (VCFS) is caused by a 3 Mbp hemizygous microdeletion at 22q11. VCFS patients show an unusually high rate of psychiatric disorders, particularly schizophrenia and bipolar disorder; in fact, VCFS and the 22q11 deletion represent the highest known risk factor for schizophrenia aside from having either parents or a monozygotic twin with the disease (17–19). It is expected that the 22q11 VCFS deletion may predispose to psychotic disorders through haplo-insufficiency of critical developmental genes in this region, or perhaps through unmasking of deleterious polymorphisms in the intact copy. Apart from the VCFS deletion, there are numerous reports of chromosomal

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abnormalities in patients with schizophrenia or bipolar disorder (20). These reports are anecdotal in nature because of the fact that psychiatric cases are not routinely referred for cytogenetic analysis, unless there is some evidence for mental retardation, dysmorphic features or severe behavioral disturbance. Indeed, the association of VCFS with schizophrenia and bipolar disorder was discovered by following a cohort of children with VCFS into adulthood. To date, the tools available for cytogenetic analysis have been limited to: (i) fluorescence in situ hybridization (FISH), a technique that interrogates one or a small number of defined loci; or (ii) low-resolution G-banding techniques. These tools have not allowed a rigorous genome-wide screen at sufficient resolution to detect micro-deletion or micro-insertion events or other aberrations on the order of 3 Mbp aberration that is present in VCFS. We undertook the present study because the scope of chromosomal aberrations causing DNA copy-number changes in schizophrenia and bipolar disorder needs to be further explored.

RESULTS

We used BAC aCGH with 1.4 Mbp resolution to assay DNA copy number in 105 post-mortem brain samples \( n = 35 \) each for schizophrenia, bipolar disorder and controls). Positive findings for copy-number aberrations were verified and extended to a replication sample using quantitative real-time PCR. In the initial screening phase, each of the 105 post-mortem brain DNA samples (schizophrenia, bipolar disorder, control; \( n = 35 \) each) was hybridized against a commercially available reference sample composed of pooled male genomic DNA from six individuals (Novagen). Preliminary interrogation of these data using an arbitrary threshold for calling aberrations (above or below three standard deviations of all nodes on array) and requiring double incidence (i.e. requiring detection of an aberration in at least two individuals from the same diagnostic group) suggested there were as many as 34 aberrant psychosis-specific regions. This phase of analysis also included a comparison with two previous studies (10,11) that allowed previously identified polymorphic copy-number differences to be recognized and subsequently disregarded. Seven polymorphic loci were removed from our preliminary screen leaving 27 psychosis-specific loci with a total of 80 aberrations (64 deletions, 16 insertions). Of interest, we also found 24 copy-number differences (12 decreases, 12 increases) at 12 distinct loci that were restricted to unaffected control individuals but previously unreported. A randomly chosen subset of these preliminary findings was evaluated by quantitative real-time PCR (Taqman, ABI), but only two of the 10 loci showed agreement between the two techniques and only one of these two loci contained a known brain-expressed protein (EFNA5 in BAC clone RP11-252113).

Given the poor validation rate from the preliminary automated analysis we modified our strategy. It was clear from manual inspection of the raw data that the aCGH results showed wide variation in signal-to-noise ratios among individuals and this was making automated detection of single copy-number differences difficult. Variation in data quality was likely related to the starting material (DNA extracted from post-mortem brain tissue). Agarose gel electrophoresis showed DNA to be more intact in some samples than in others (data not shown). In order to improve the probability of finding relevant genes with potential copy-number aberrations, we decided to undertake a much more stringent manual analysis which involved selection of data from the 18 affected samples (12 schizophrenia and six bipolar disorder) for which scatter of data points across the genome was minimal. We screened these 18 array results for BAC clones with large positive or negative log2 ratios (log2 ratios greater than two standard deviations from the mean), small standard deviations among triplicate spots (SD \( \leq 0.2 \)), and relatively high spot fluorescent intensities (log2 fluorescent intensity \( \geq 10 \) on a 16-bit scale). This list of BAC clones was then surveyed for the presence of brain-expressed genes using the UCSC Golden Path web browser (http://genome.ucsc.edu/). Any BAC clone containing a brain-expressed gene was then used to query a MySQL database of all CGH results from the 105-sample set to find those clones in which possible copy-number aberrations were present either in affected samples only, or in which affected samples predominated. A final list of 14 BAC clones was produced by this method. Twelve of these clones contained putative copy-number aberrations in affected samples only. The remaining two clones (CTD-2145H2 and RP11-120P20) contained putative copy-number aberrations in five affected samples and one control sample, and four affected samples and two control samples, respectively. These 14 loci were tested using qPCR and three of the 14 loci were verified (Fig. 1). These loci corresponded to BAC clones RP11-207P5 (1p34.3), RP11-35I10 (22q12.3) and CTD-2055A23 (14q23.3). These three validated aberrations were from the set of 12 putative copy-number differences seen in affected individuals but not controls. The qPCR primer/probe sets used were targeted to the particular brain-expressed gene of interest in each of these regions. BAC clone RP11-207P5 contains the gene GLUR7 (GRK3) that encodes an important kainate receptor subunit; BAC clone RP11-35I10 contains the gene CACNG2, a voltage-gated calcium-channel subunit. Finally, BAC clone CTD-2145H2 contains the gene AKAP5 (AKAP75, AKAP79), which is a member of the A-kinase anchor-protein gene family.

Validation by qPCR involved only those DNAs from the set of 105 samples for which positive results were obtained by aCGH. The GLUR7 and AKAP5 copy-number increases were confirmed in two individuals (one bipolar disorder and one schizophrenia) and one individual (bipolar disorder), respectively. The CACNG2 deletion and the EFNA5 deletion were confirmed in one individual each (bipolar disorder and schizophrenia).

Based on locating aberrations in three genes that are implicated in the glutamate signaling pathway, we tested post-mortem brain DNA from a replication sample of 60 individuals (schizophrenia, bipolar disorder, major depression and unaffected control individuals, \( n = 15 \) each, total \( n = 60 \)) using the qPCR primer/probe sets for genes GLUR7, CACNG2, AKAP5 and EFNA5. [Note: The 15 subjects with major depression were included because testing all samples in this collection from the Stanley Medical Research Institute was a condition for use.] We found qPCR evidence for GluR7
copy number increases in seven individuals, including four bipolar disorder, two major depression and one schizophrenia. No aberrations were detected in control individuals (Table 1). Two of the bipolar disorder cases (SN_13 and SN_35) show haploid copy numbers less than 1.5 (the theoretical value for a duplication of one allele, i.e. a single extra copy) but substantially greater than one (the normal diploid state). Intermediate values for copy number may result from samples containing DNA derived from a mixture of cells, some with normal copy number and some with aberrant copy number at this locus. Results from the other five cases are consistent with a single extra copy of the GLUR7 gene.

We also screened this replication sample set with the EFNA5, CACNG2 and AKAP5 qPCR primer/probe sets. We
found no aberrations with the EFNA5 primer/probe set, one individual (schizophrenia) with a CACNG2 deletion (haploid copy number of 0.72 ± 0.08 and 0.69 ± 0.09 in two independent experiments), and three individuals with amplifications in AKAP5 (one schizophrenia, one bipolar disorder and one major depression, Table 2). There were no aberrations detected in control subjects. Interestingly, one individual (SN_3, a schizophrenia subject) had copy-number aberrations at GLUR7, AKAP5 and CACNG2, and one other individual (SN_19, a major depression subject) had amplifications at both the GLUR7 and AKAP5 loci.

DISCUSSION

We have evaluated DNA copy number in post-mortem brain DNA from schizophrenia and bipolar disorder cases and normal control individuals. Our approach included a screening phase (aCGH at 1.4 Mbp resolution), a validation phase (qPCR) and a replication phase (also using qPCR). We used post-mortem brain samples for this study because data obtained from peripheral tissues would only be revealing in the case of constitutive changes, whereas brain DNA affords the opportunity to detect both constitutive changes and any sporadic somatic copy-number changes within the CNS. The availability of relatively small amounts of genomic DNA from brain tissue in the sample sets used in this study limited our ability to test these samples by aCGH and probably contributed to the relatively low validation rate in the preliminary automated array CGH analysis (two of 10 loci tested including EFNA5 in BAC clone RP11-252I13). In the subsequent manual approach, we identified an additional three loci with copy-number aberrations in affected, but not in control subjects. The BAC clones containing the aberrant regions are: RP11-207P5 (1p34.3), CTD-2055A23 (14q23.3) and RP11-35I10 (22q12.3). These three clones were found to contain the brain-expressed genes GLUR7, AKAP5 and CACNG2, respectively. The qPCR primer/probe sets designed to detect these aberrations were positioned within each of these genes and the results obtained were in agreement with the aCGH findings.

The EFNA5 gene (AF1, AL-1, LERK7) encodes a tyrosine kinase receptor thought to stimulate axon fasciculation (21). The other three genes are implicated in glutamate signaling in the brain. CACNG2 is a voltage-gated calcium-channel gamma subunit (22). The protein product of the mouse version of this gene, stargazin has been shown to modulate AMPA receptor gating and trafficking (23,24). Stargazin has been shown to not only chaperone AMPA receptors to the neuronal cell membrane, but also to remain in a complex with the receptor at the cell surface, reducing the rate of receptor desensitization (24). The function of the human CACNG2 gene product is less clear. It is highly expressed in cerebellum, cerebral cortex, hippocampus and thalamus but has not been shown to modulate the properties of a voltage-dependent calcium channel when heterologously expressed in Xenopus oocytes (25). Of interest, several linkage studies highlight 22q12.3 as a region of interest for psychosis, including a genome-wide scan of 382 sibling pairs with schizophrenia or

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Table 1. GLUR7 copy number increases detected by qPCR in subjects from replication (n = 60) set. The G6PD (X chromosome) locus is also interrogated using a second primer/probe set to provide an independent assay for gender

Table 2. AKAP5 copy number increases detected by qPCR in subjects from replication (n = 60) set. The G6PD (X chromosome) locus provides an independent assay for gender
schizoaffective disorder (26), a study of 65 bipolar disorder families (27), and a genome-wide scan of homogeneous sub-types of schizophrenia families (28). This locus is distinct from the 22q11 VCFS locus. CACNG2 may deserve further investigation as a candidate gene for this region.

The AKAP5 (AKAP75, AKAP79) gene product is a member of the A-kinase anchor-protein gene family, which is predominantly expressed in the cerebral cortex and may anchor the PKA protein to post-synaptic densities, and may be involved in the regulation of post-synaptic events (29,30), including long-term synaptic depression (31). Furthermore, there is evidence of modulation of phosphorylation state and trafficking of the AMPA receptor GluR1 by a complex that includes AKAP79 (32).

Finally, we have found evidence of copy-number increases in GLUR7, a kainate receptor-subunit gene, which has already been proposed to play a role in schizophrenia (33–36). Sokolov showed reduced NMDAR1, GLUR1, GLUR7 and KA1 receptor mRNAs in the frontal cortex of 'neuroleptic-free' schizophrenics (33), whereas Meador-Woodruff et al. (34) detected increased GLUR7 and decreased KA2 mRNA levels in multiple regions of prefrontal-cortex samples from elderly schizophrenics. The GluR7 ser310ala polymorphism has been investigated in two schizophrenia association studies; one positive, involving Caucasian subjects from northern Italy (35) and the other negative, involving Chinese subjects (36). There is also evidence that the GluR7 gene is differentially expressed early in human development and that normal CNS development requires complex regulation of this and other ionotropic glutamate receptor genes (37). Therefore, it is tempting to speculate that psychiatric disease in adulthood may be influenced by the effects of aberrant GluR7 gene dosage during embryonic development. The genomic loci corresponding to BAC clones RP11-207P5, RP11-35I10 and CTD-2055A23 were also compared with the Database of Genomic Variants (10), which contains the results of several large-scale studies of human genomic copy-number variation. No variants were found in these regions, consistent with the possibility (at least in the brain) that copy-number aberrations in these regions are over-represented in individuals with psychotic disorders.

The present study describes a new and complementary approach to finding genetic factors for psychotic disorders. Further scrutiny of the aberrations we have identified, their functional consequence, and their prevalence will hopefully lead to a better understanding of the etiology of these complex psychiatric diseases.

**MATERIALS AND METHODS**

**Patient and control samples**

Genomic DNA from Broca’s Area from SCZ, BPAD and unaffected control subjects (n = 35 each) was obtained from the Stanley Medical Research Institute (SMRI) Array Collection (www.stanleyresearch.org/programs/brain_collection.asp). According to SMRI, exclusion criteria for all specimens included: (i) significant structural brain pathology on post-mortem examination by a neuropathologist, or by pre-mortem imaging; (ii) history of significant focal neurological signs pre-mortem; (iii) history of central nervous system disease that could be expected to alter gene expression in a persistent way; and (iv) documented intelligence quotient less than 70. Additional exclusion criteria for unaffected controls included: (i) age less than 30, thus still in the period of maximum risk; and (ii) substance abuse within 1 year of death or evidence of significant alcohol-related changes in the liver. Based on the medical records, diagnoses were made by two senior psychiatrists using DSM-IV criteria, and when necessary, telephone interviews with family members. Diagnoses of unaffected controls were based on structured interviews by a senior psychiatrist with family member(s) to rule out Axis I diagnoses. The DNA concentration for each sample was determined by fluorometry using pico green dye (Molecular Probes), and calf thymus DNA (BioRad) for preparation of standard curves. DNA was limiting for all samples, and for most, there was only enough material for a single hybridization experiment.

The replication sample comprised genomic DNA extracted from occipital cortex (BA 19) from post-mortem brain tissue from 60 subjects in the SMRI Neuropathology Collection (38).

**Bacterial artificial chromosome array comparative genome hybridization**

Hybridizations were done using HumArray 2.0 (human BAC microarray 2.0) from University of California San Francisco (39). The array consists of 2460 BAC clone representations spotted in triplicate, giving an average resolution of ~1.4 Mbp across the genome. Approximately, 12% of the genome was directly covered. The reference DNA used in each hybridization experiment was commercial Novagen male reference DNA (Cat. No. 70572, EMD Biosciences) which is a pooled batch of genomic DNA from several males, which varies by lot number (36978-2 contained a pool of six male genomic DNAs). Test and reference DNAs were labeled by random priming with the fluorescent nucleotide analogues cy5-dCTP and cy3-dCTP (Amersham), respectively, using a BioPrime labeling kit (Invitrogen). Unincorporated nucleotides were removed using Sephadex G-50 spin columns (Amersham). Mixtures of labeled test and reference DNA (100–200 ng) and Cot-1 DNA (25 μg; Invitrogen) were precipitated by mixing with ethanol and sodium acetate, followed by centrifugation. The precipitated DNA was dissolved in a hybridization mixture composed of 80% DIGEasy hybridization solution (Roche Scientific), 10% sheared salmon sperm DNA (Invitrogen) and 10% purified BSA (New England Biolabs). The hybridization solution was heated to 85°C for 10 min to denature the DNA, then incubated at 45°C for approximately 1–2 h to allow for blocking of genomic repetitive sequences by the Cot-1 DNA. The slides were exposed to 2600 × 100 μJ of UV with a Spectro-linker XL1000 UV Crosslinker (Spectronics Corporation). Each array was covered with an 18 × 22 mm mSeries Lifter slip (Erie Scientific), after which hybridization solution was applied to an open side. Slides were transferred to a prewarmed (45°C) hybridization cassette (Telechem International) containing 2 × SSC in two small reservoirs to prevent evaporation and submerged in water at 45°C for 48–72 h. After hybridization the slides were transferred to Coplin jars and washed once in 80% DIGEasy, 2 × SSC pH 8
7.0 for 15 min at 45°C, three times with 0.1 × SSC, 0.1% SDS for 5 min per wash at room temperature, rinsed four times with 0.1 × SSC for 30 s at room temperature and rinsed briefly in de-ionized distilled water. Slides were then dried in 50 ml conical tubes by centrifugation for 7 min at 350 × g at room temperature. Imaging was done using a Packard Biosciences Scan Array Express instrument at 10 µm resolution. Array images were analysed using an open-source software package, called MIA, as previously described (40).

Quantitative real-time PCR (qPCR)

Primer/probe sets for two separate reference genes, prosta-glandin transporter (PGT) and glucose-6-phosphate dehydrogenase (G6PD) were designed using the repeat-masked reference human genome sequence (NCBI_34; April 2003 release; http://genome.ucsc.edu/; Table 1). The PGT reference gene is a single copy autosomal gene located on chromosome 3, whereas the G6PD gene is located on the X chromosome. All samples, including male and female reference DNA (Novagen) were assayed with both reference primer/probe sets in addition to the primer/probe set for the test locus. To determine relative copy number, 5 ng of genomic DNA was assayed in triplicate in 20 µl reactions containing 1 x final concentration TaqMan Universal Master Mix (ABI part number 4304437), 700 nM of each primer and 200 nM probe. Each experiment was performed using a 384-well optical PCR plate and the ABI 7900HT machine with default cycling conditions. The copy number of the test locus in each case was defined as $2^\triangle \Delta C_T$ where $\triangle C_T$ is the difference in threshold cycle number for the test and reference loci. The gender of each test and reference sample was also determined by comparison of the G6PD X-chromosome copy-number to the PGT autosomal single copy locus copy number producing values of approximately two (female) or one (male). Specific primers and FAM-labeled fluorescent probes used for PCR amplifications were as follows: GLUR7-FAM probe (FAM-CCGGCTTGCTGAAATA-MGBNFQ), CACNG2-for (5'-CTTCGCAAACAGCTACTCTATTTC-3'), GLUR7-rev (5'-CCTGCATCCTCCTGTAGCC-MGBNFQ), AKAP5-for (5'-CACTAGGCTCTTCCAAGGT-3'), GLUR7-FAM probe (FAM-CTTTGGCTGCTTTCTTTC-3'), AKAP5-rev (5'-CTTCGCAAACAGCTACTCTATTTC-3'), CACNG2-for (5'-CTTCGCAAACAGCTACTCTATTTC-3'), CACNG2-rev (5'-CCTACTCTGACAGATCTTTAT-3'), EFNAS5-FAM probe (FAM-CTTGCGAAGAGAATGGCTCCCA-3'), EFNAS5-rev (5'-CCATGCTGATTGCTTCTTCG-3'), EFNAS5-rev (5'-GGACGAACTTTCACTTGGAATGAA-3'), AKAP5-FAM probe (FAM-CTTCGGTCGTCTCATTTCC-3'), AKAP5-rev (5'-CATCCATGCTTTGCTTCAA-3'), AKAP5-rev (5'-GCCAGCTTTGGCTTCA-3').

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Conflict of Interest Statement. None declared.

References

33. Sokolov, B.P. (1998) Expression of NMDAR1, GluR1, GluR7 and KA1 glutamate receptor mRNAs is decreased in frontal cortex of ‘neuroleptic-free’ schizophrenics: evidence on reversible up-regulation by typical neuroleptics. *J. Neurochem.*, 71, 2454–2464.