European Journal of Human Genetics (2011) 19, 588–592

www.nature.com/ejhg

ARTICLE

Analysis of genetic deletions and duplications in the University College London bipolar disorder case control sample

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Genetic deletions and duplications known as copy number variants have been strongly implicated in genetic susceptibility to schizophrenia, autism, attention deficit hyperactivity disorder and epilepsy. The overall rate of copy number variants in the University College London (UCL) bipolar disorder sample was found to be slightly lower than the rate in controls. This finding confirms the results from other studies that have also shown no increased rate of copy number variants in bipolar disorder. However, some rare duplications and deletions were observed only in bipolar disorder cases and not in controls, these included some that had previously been detected only in rare cases of bipolar disorder. We conclude that copy-number variant analysis shows no obvious sharing of the same genetic susceptibility between schizophrenia and bipolar disorder. Copy number variants do not seem to have an important role in susceptibility to bipolar disorder, they may, however, still represent a rare cause of the disease, although the evidence for this is far from clear.
European Journal of Human Genetics (2011) **19,** 588–592; doi:10.1038/ejhg.2010.221; published online 5 January 2011

Keywords: bipolar disorder; genetic susceptibility; deletions; duplications; structural variation; schizophrenia

INTRODUCTION

Bipolar affective disorder (BPAD) is a common mental health disorder that is characterised by periods of mania and depression. BPAD is associated with increased rates of unipolar affective disorder in relatives and with comorbid alcohol abuse,[1,2] as well as with very high rates of suicide.[3] Family, twin and adoption studies have shown that genetic heritability to BPAD is in excess of 80%, which makes bipolar disorder one of the most heritable of the psychiatric disorders.[4–6] Several BPAD genome-wide association studies (GWASs) using up to 1 000 000 single nucleotide polymorphism (SNP) markers have been performed and these data can also be used to detect copy number variants (CNVs).[7–12] The GWASs data have implicated two genes *ANK3* and *CACNA1C* with genome-wide levels of statistical significance.[7] Studies of copy-number variation causing diseases are complicated by the presence of a high background rate of duplications and deletions found across the human genome that are not associated with any disease.[13–16] CNVs seem to have developed through a variety of processes that seem to be dependent and the size of the structural variant.[16] CNVs in specific chromosomal regions have been reported that are appreciably increased in samples of schizophrenia, particularly in early age of onset.[8,17–33] Copy number variants that are shared with schizophrenia but not with bipolar disorder have been found in epilepsy.[29,34–37] CNVs have also been found to be strongly associated with autism, attention deficit hyperactivity disorder and learning disability.[38–50]

There is limited evidence for the involvement of CNVs in susceptibility to bipolar disorder. Zhang *et al*[51] have reported that singleton deletions over 100 Kb in length are more frequent in BPAD cases, a finding which was not replicated by Grozeva *et al.*[52] Lachman *et al*[53] reported an increase in *GSK3β* CNVs in BPAD, but this finding was also not confirmed by Grozeva *et al.*[52] However, there have been reports of rare cases of BPAD with CNVs[32,51–54] and it remains the case that specific rare CNVs may contribute to susceptibility to bipolar disorder. The aim of this study was to assess the frequency of CNVs in the UCL bipolar case control sample and to investigate the location of rare CNVs with reference to bipolar disorder-specific CNVs reported by Zhang *et al*[51] and Grozeva *et al.*[52]

SUBJECTS AND METHODS

Bipolar research subjects

BPAD cases and controls included in the analyses comprised DNA from individuals from the University College London (UCL) bipolar disorder sample collection (see Sklar *et al*[10] and Ferreira *et al*[7] for a description of the case and control samples). Additional UCL Bipolar II samples collected using the same ascertainment criteria as described above were included in the analysis presented in this study.

The UCL Bipolar sample consisted of 97% bipolar 1 cases with psychotic symptoms according to Research Diagnostic Criteria (RDC) categories.[55] The comparison subjects were 510 screened normal volunteers with no personal or first degree family history of any mental disorder. The cases and comparison subjects were selected if both their parents and all four grandparents were of Irish, Welsh, Scottish or English ancestry as defined by an ancestry checklist. Subjects were also included if one of the four grandparents was of European ancestry before the 2004 EU enlargement. U.K. National Health Service (NHS)

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Received 25 August 2010; accepted 12 November 2010; published online 5 January 2011

Table 1 Global copy number variant (CNV) burden in cases and controls

CNV type	*CNV size (Kb)*	*Control*	*Case*	*CNV per control*	*CNV per bipolar*	*BP to control ratio*
Deletion	50–100	223	207	0.473	0.385	0.813
	100–200	150	148	0.318	0.275	0.864
	200–500	86	62	0.183	0.115	**0.630 (0.039)**
	500–1000	9	11	0.019	0.020	1.070
	>1000	4	1	0.008	0.002	0.219
	Total	472	429	1.002	0.797	0.796
	>100	249	222	0.529	0.471	0.892
Duplication	50–100	85	113	0.180	0.210	1.164
	100–200	110	94	0.234	0.175	0.749
	200–500	95	87	0.202	0.162	0.802
	500–1000	13	27	0.028	0.050	1.818
	>1000	10	10	0.021	0.019	0.875
	Total	313	331	0.665	0.615	0.926
	>100	228	218	0.484	0.463	0.956
Deletions and duplications	Total	785	760	1.667	1.413	0.848
	>100	477	440	1.013	0.818	**0.808 (0.036)**
Samples with array data passing QC	471	538				

Bipolar compared with control CNV burden ratios in bold highlight comparisons where the two-sided empirical significance values were less than 0.05 (significance values are shown in parenthesis). Note that these significance values have not been corrected for the multiple comparisons carried out, and that both of the significant findings indicate that fewer rare CNVs are found in cases compared with controls.

Table 2 Global burden of singleton copy number variants (CNVs)

	UCL		*UCL*		*Grozeva et al (WTCCC)*[52]		*Zhang et al*[51]	
	>50 Kb		*>100 Kb*		*>100 Kb*		*>100 Kb*	
Single CNV type	*Cases with CNV*	*Controls with CNV*	*Cases with CNV*	*Controls with CNV*	*Cases with CNV*	*Controls with CNV*	*Cases with CNV*	*Controls with CNV*
Deletion	252 (46.84)	216 (45.86)	143 (26.58)	139 (29.51)	101 (6.0)	173 (6.2)	**162 (16.2)**	127 (12.3)
Duplication	167 (31.04)	166 (35.24)	106 (19.70)	**129 (27.39)**[a]	121 (7.1)	234 (8.3)	197 (19.7)	197 (19.1)
Deletion and duplication	377 (70.07)	353 (74.95)	227 (42.19)	253 (53.72)	187 (11.0)	329 (11.7)	320 (32.0)	299 (29.0)

Bipolar compared with control CNV counts for duplication CNVs, deletions CNVs and both classes of CNVs combined are shown. The frequency (%) of singleton CNV per case or control is shown in parenthesis. Entries marked in bold highlight comparisons where the two-sided empirical significance values were less than 0.05. Note that the significance value has not been corrected for the multiple comparisons carried out, and the significant findings indicate that fewer rare CNV duplications are found in cases compared with controls.
[a]Nominal P=0.033.

multicenter and local research ethics committee approval was obtained. All subjects signed an approved consent form after reading an information sheet and after a description of the study had been given to each subject. All subjects were interviewed by a psychiatrist who used the lifetime version of the Schizophrenia and Affective Disorders Schedule.[55] All the bipolar patients were also rated with the 90-item OPCRIT checklist.[56] Family pedigree diagrams were drawn and drug-treatment response was recorded. DNA was extracted from whole blood using standard nuclear lysis phenol chloroform methods.

Genotyping

Genotyping was performed on the Affymetrix GeneChip Human Mapping 500 K Array Set (Santa Clara, CA, USA) by the Genetic Analysis Platform at the Broad Institute of Harvard and MIT as described previously.[10] The method uses two arrays according to whether the genomic DNA from research subjects was digested with the restriction enzymes NspI or StyI before adaptor nucleotides are ligated with T4 DNA ligase. Because each array can replicate evidence for copy number variants, only cases where data for both arrays was available were analysed for CNVs (546 cases and 517 controls).

CNV Calling

CNV calling was performed using PennCNV software (version 2008 June 26; http://www.openbioinformatics.org/penncnv/).[57] PennCNV uses the total fluorescent intensity signals for both alleles (log R ratio (LRR)) and the B allele frequency (BAF) for each SNP on the genotyping arrays. Standard PennCNV quality control checks are designed for Illumina arrays and are therefore not appropriate for the Affymetrix GeneChip Human Mapping 500 K Array used here (http://www.openbioinformatics.org/penncnv/penncnv_tutorial_affy_gw6.html). Samples, in which more than 100 CNVs were detected by PennCNV were excluded from further analysis (8 BPAD; 46 Controls). CNVs that were smaller than 50 Kb in length or comprised fewer than 10 SNPs were then removed from the analysis, as these were likely to be unreliable CNV calls. CNVs that spanned a centromere or that overlapped at least 50% of their length with regions previously described as being prone to false positives due to somatic mutations were also removed.[22] A further 15 CNVs were also removed that were comprised of SNPs from only one of the two arrays. Finally, for the purpose of rare CNV burden analysis CNVs found in more than 1% of cases and controls were not considered further. A custom track for visualisation of CNVs detected in this paper on the UCSC Genome Browser (University of California, Santa Cruz, CA, USA; http://genome.ucsc.edu/), is available at http://www.ucl.ac.uk/~rejuamc/UCLBPCO.cnv.bed.

Analysis of Rare and singleton CNVs

CNV association analyses were performed with PLINK[58] (version 1.07, http://pngu.mgh.harvard.edu/~purcell/plink/). Empirical significance values (two tailed) were derived with the use of 10 000 permutations.

Table 3 Case only CNVs and supporting evidence from previous studies

Cases (n)	*March 2006 location*	*Genes affected*	*CNV studies*	*Association studies*
5	chr19:58644961–58689358	*ZNF761, ZNF813, ZNF765, ZNF331*	ISC[8]	
4	chr7:75975221–76052734	*UPK3B*		
3	chr1:144439082–144791590	*PDZK1, GPR89A, GPR89C, NBPF11, LOC728912, FAM108A3*	Zhang *et al*[51]	
3	chr19:49581647–49644505	*ZNF285A, ZNF229*		
2	chr1:28399376–28842172	*DNAJC8, ATPIF1, SESN2, MED18, PHACTR4, SNHG3-RCC1, RCC1, TRSPAP1, RAB42, TAF12*		
2	chr2:196772221–197165580	*HECW2*		
2	chr3:8896559–8980146	*RAD18*		
2	chr5:180098728–180099664	*OR2Y1*		
2	chr6:56430743–56816422	*DST*		
2	chr6:57290380–57621335	*PRIM2*		
2	chr6:101953625–102624651	*GRIK2*		Autism[59]
2	chr6:157140777–157572094	*ARID1B*		
2	chr7:34935017–35044178	*DPY19L1*		
2	chr7:88226688–89777622	*ZNF804B, MGC26647, STEAP1, STEAP2, FLJ21062*		
2	chr7:132588362–133401053	*EXOC4*		
2	chr9:111037–169075	*CBWD1*		
2	chr10:50334496–50490772	*ERCC6, PGBD3, CHAT, SLC18A3*	Grozeva *et al*[52]; ISC[8]	Alzheimer's[60–62]
2	chr10:51497689–52053743	*FAM21A, FAM21B, ASAH2, SGMS1*	Grozeva *et al*[52]	
2	chr13:90848887–92317488	*GPC5*		
2	chr14:24044551–24047311	*CMA1*		
2	chr16:15435825–15889948	*C16orf45, KIAA0430, NDE1, MYH11, C16orf63*	Ingason[27]	SCZ[63]
2	chr16:15950934–16296168	*ABCC1, ABCC6, NOMO3,*		
2	chr16:16333234–16351940	*LOC339047*		
2	chr16:68705029–69071678	*PDPR, MGC34761, EXOSC6, AARS, DDX19B, DDX19A, ST3GAL2, FUK*		
2	chr18:27210737–27312663	*DSG4, DSG3*		
2	chr21:36429132–36440730	*CBR3*		

Above table shows the frequency of CNVs that occurred in two or more cases and not in controls, the extent of the genomic region affected and the genes that are duplicated or deleted. Rare CNVs that overlapped with those found in other studies are cited. CNVs containing genes showing allelic association with psychiatric phenotypes are also shown.

Genomic locations presented here are from the March 2006 human genome sequence assembly (UCSC Hg18, National Center for Biotechnology Information build 36). Analyses of CNVs >50 and >100 kb were performed to allow comparison of results from previous studies that only investigated the larger class of CNV.

RESULTS

Global burden of rare CNVs

The numbers of rare CNVs in each size group, their frequencies in cases and controls and the corresponding empirical significance values (where <0.05) are shown in Table 1. Two of the comparisons yielded nominally significant results. In the CNV size range between 200 and 500 Kb, there were significantly fewer deletions (0.183 CNV per control sample *vs* 0.115 per BPAD case; $P=0.039$; see Table 1) and when both deletion and duplication CNVs >100 Kb in size were considered there were also significantly fewer CNVs in the bipolar cases (0.818 per sample) compared with the controls (1.013 per sample; $P=0.036$; see Table 1). Analyses of CNV burden excluding CNVs <100 Kb were performed to allow comparison of the data with that presented in previous studies.

Singleton CNV analysis

The results of singleton CNV analysis are shown in Table 2. This is an attempt to replicate the findings of Zhang *et al*.[51] In contrast to the study of Zhang *et al*, no significant over representation of bipolar singleton deletions were observed. We did however, observe a nominally significant increase in the frequency of control duplication CNVs over 100 Kb in size ($P=0.03$). This finding does not survive correction for the multiple tests carried out. It is noteworthy that there was also a nonsignificant increase in the rate of singleton duplications in the controls of Grozeva *et al*.[52] We also compared the rate of singleton CNVs in cases of BPAD where a first episode of mania occurred at age 18 or below with BPAD samples with a later first episode of mania. No significant differences were observed in the comparison groups (data not shown). However, the rate of singleton deletions among the later age of onset case was higher (0.339) compared with the early onset cases (0.277). This finding is in the opposite direction to that of Zhang *et al*,[51] but in agreement with the findings of Grozeva *et al*.[52]

Bipolar disorder case only CNVs

Although the overall burden of CNVs in cases was not found to be significantly different from that found in controls, we were interested to investigate CNVs found only in two or more bipolar disorder cases that disrupted genes. A total of 26 such CNVs regions were identified (see Table 3). Two of these overlapped with genes that were either duplicated or deleted by CNVs in BPAD cases only in the Grozeva *et al*[52] study, one overlapped with genes also disrupted by the Zhang *et al*[51] BPAD case CNVs, one CNV overlapped with genes also disrupted by ISC[8] schizophrenia case only CNVs, and one CNV included *NDE1* a gene previously found deleted in schizophrenia[27] (see Table 3). Three of the genes in the CNV regions had also previously been found to be associated with neuropsychiatric phenotypes (see Table 3).

DISCUSSION

The results of rare copy-number variation analysis presented here are similar to those of Grozeva *et al*[52] and indicate that the larger type of CNVs that are currently detectable do not have a major role in susceptibility to BPAD. In agreement with Grozeva *et al*,[52] we paradoxically report a small decrease in rare CNV burden in our BPAD cases compared with controls. Also in agreement with Grozeva *et al*,[52] we do not find evidence for a previously reported increase[51] in singleton deletion CNVs in our bipolar disorder cases nor did we find significant age of onset effects with singleton deletion CNVs in our sample. The numbers of samples used in this study are smaller than those of Grozeva *et al*[52] and Zhang *et al*.[51] The microarray technology used in this study was the same as in the study by Grozeva *et al*,[52] however, the calling algorithms and quality control procedures differ slightly. Zhang *et al*[51] used a higher density microarray platform, which might explain the difference between our own and the Grozeva *et al*[52] compared with the study by Zhang *et al*.[51]

There was an overall increase in the rate of CNVs called in our study compared with the rate reported by Grozeva *et al*[52] and Zhang *et al*.[51] Call rates may be very sample dependent with differences in thresholds for CNV calling and the relatively small sample sizes influencing these rates. Grozeva *et al*[52] detected CNVs separately on the two arrays and then combined the CNV calls. Their calling threshold required that 10 SNPs from one array showed consistent evidence for the presence of a CNV. We used a different approach where the intensity (LRR) and allele frequency data (BAF) from the two arrays were combined before CNV calling. Thus, despite the fact that both studies required 10 consecutive SNPs to show consistent evidence for a CNV to be called, the approach used here is likely to be more sensitive. We further required that all CNVs were made up of SNP data from both of the arrays such that localised microarray hybridisation artefacts would be unlikely to produce aberrant CNV calls. Zhang *et al*[51] used a higher density microarray compared with the arrays used in this study therefore, one would expect that their study would have been capable of detecting more CNVs than we report in this study. Again the calling algorithms are different and this may account for the differences in call rates and indeed for the lack of replication in the frequency of singleton deletions in BPAD cases.

The evidence from our own study and from the literature for rare case only CNVs having a role in BPAD is not clear. However, we present a list of CNVs that occurred in two or more cases and indicate those for whom previous bipolar disorder or schizophrenia studies have also found evidence for genes being deleted or duplicated by CNVs or where there is evidence for genetic association with bipolar disorder or related neuropsychiatric phenotypes. Although some of these overlapping findings might be due to chance, future studies will be able to replicate these rare occurrences with greater power.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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