Exploration of large, rare copy number variants associated with psychiatric and neurodevelopmental disorders in individuals with anorexia nervosa

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Anorexia nervosa (AN) is a serious and heritable psychiatric disorder. To date, studies of copy number variants (CNVs) have been limited and inconclusive because of small sample sizes. We conducted a case-only genome-wide CNV survey in 1983 female AN cases included in the Genetic Consortium for Anorexia Nervosa. Following stringent quality control procedures, we investigated whether pathogenic CNVs in regions previously implicated in psychiatric and neurodevelopmental disorders were present in AN cases. We observed two instances of the well-established pathogenic CNVs in AN cases. In addition, one case had a deletion in the 13q12 region, overlapping with a deletion reported previously in two AN cases. As a secondary aim, we also examined our sample for CNVs over 1 Mbp in size. Out of the 40 instances of such large CNVs that were not implicated previously for AN or neuropsychiatric phenotypes, two of them contained genes with previous neuropsychiatric associations, and only five of them had no associated reports in public CNV databases. Although ours is the largest study of its kind in AN, larger datasets are needed to comprehensively assess the role of CNVs in the etiology of AN. *Psychiatr Genet* 27:152–158

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Introduction

Anorexia nervosa (AN) is a psychiatric disorder that carries significant morbidity and mortality (Papadopoulos *et al.*, 2009; Arcelus *et al.*, 2011). Multiple lines of evidence suggest a notable genetic component in the etiology of AN. Twin studies have estimated the heritability (h^2) of AN to be 0.5–0.6 (Bulik *et al.*, 2006; Yilmaz *et al.*, 2015), and genomic efforts are underway to elucidate the role of genome-wide common variation in the etiology of AN (Wang *et al.*, 2011; Boraska *et al.*, 2014; Duncan *et al.*, 2016). Considering the important role that copy number variants (CNVs) play in psychiatric phenotypes (International Schizophrenia Consortium, 2008; Cooper *et al.*, 2011; Levinson *et al.*, 2011; Sanders *et al.*, 2011; Bergen *et al.*, 2012; Malhotra and Sebat, 2012; Szatkiewicz *et al.*, 2014), studies are needed to help elucidate their role

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in AN. To date, only one CNV study in AN has been published, and although none of the well-established pathogenic CNVs associated with psychiatric and neurodevelopmental disorders were observed in individuals with AN, the authors reported a novel 1.5 Mbp deletion in 13q12 in two cases (Wang *et al.*, 2011). However, the study was underpowered to detect rare pathogenic CNVs and the preliminary findings pertaining to AN require replication (Wang *et al.*, 2011).

The aim of the present study was to assess the prevalence of large, rare CNVs associated previously with schizophrenia, autism, intellectual disability, or developmental delay in individuals with AN.

Materials and methods

We conducted a case-only genome-wide survey for CNVs in 2907 female AN cases included in the Genetic Consortium for Anorexia Nervosa (GCAN), genotyped as a part of the Wellcome Trust Case Control Consortium 3 (WTCCC3) (Boraska *et al.*, 2014). Patient ascertainment, quality control (QC), and genotyping procedures have been described in detail elsewhere (Boraska *et al.*, 2014). The WTCCC3 controls included in the primary genome-wide association analysis were unavailable for subsequent analyses; therefore, we identified three dbGaP datasets genotyped using the same array as a source of potential controls for our female AN cases of European ancestry. However, despite multiple rounds of rigorous QC, biases pertaining to CNV calling were present. After ruling out ancestry, plate effect, and original affection status reported in dbGaP datasets, we identified differential processing of the intensity files (correction of which was not possible in our case) as the most likely source of this potential bias. Therefore, we decided that a case-only analysis was necessary to ensure the correctness and validity of our results. Genotype data for the majority of the AN cases included in this study are available on the European Genome-phenome Archive (study accession: EGAS00001000913; dataset accession: EGAD00010001034; *https://www.ebi.ac.uk/ega/studies/EGAS00001000913*).

As GCAN included DNA samples derived from various tissues (i.e. blood, buccal epithelium, saliva, or cell lines), we removed all nonblood samples to ensure high-quality and high-confidence CNV calls that are not confounded by DNA source, which led to a sample size of 1983 AN cases. Our analysis pipeline, which followed the steps outlined by Szatkiewicz *et al.* (2014), is summarized in Fig. 1, and additional information on the process is available in Supplementary Methods (Supplemental digital content 1, *http://links.lww.com/PG/A188* and Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/PG/A188*).

CNV calling was performed using PennCNV (June 2011 version) (Wang *et al.*, 2007, 2008; Diskin *et al.*, 2008) with parameters appropriate for the Illumina 660W-Quad chip (Illumina Inc., San Diego, California, USA). All other analyses were carried out using PLINK (Purcell *et al.*, 2007) and R (R Development Core Team, 2011). We implemented strict cutoffs for single-nucleotide polymorphism (SNP) level, sample-level, and CNV-level QC procedures (Supplementary Methods, Supplemental digital content 1, *http://links.lww.com/PG/A188*). We examined AN cases for large CNVs previously reported to be associated with schizophrenia, autism, intellectual disability, or developmental delay (Cooper *et al.*, 2011; Levinson *et al.*, 2011; Sanders *et al.*, 2011; Malhotra and Sebat, 2012; Sullivan *et al.*, 2012) (Table 1). We considered all CNV events more than 100 kbp that had more than 50% reciprocal overlap within ± 20 kbp of a known psychiatric or neurodevelopmental CNV (i.e. at least 50 kbp overlap with a known CNV was required). We also assessed the presence of the 1.5 Mbp 13q12 deletion reported previously in two AN cases (Wang *et al.*, 2011) (Table 1). As a secondary aim, we searched for any CNVs that are more than 1 Mbp in size and checked the Database of Genomic Variants public CNV database (DGV; *http://dgv.tcag.ca*) to determine whether these CNVs were reported in previous studies. A CNV is considered novel if it has less than 50% reciprocal overlap with previously reported CNVs in DGV. We also examined Online Mendelian Inheritance in Men (OMIM; *https://www.omim.org*) for overlap with known disease genes.

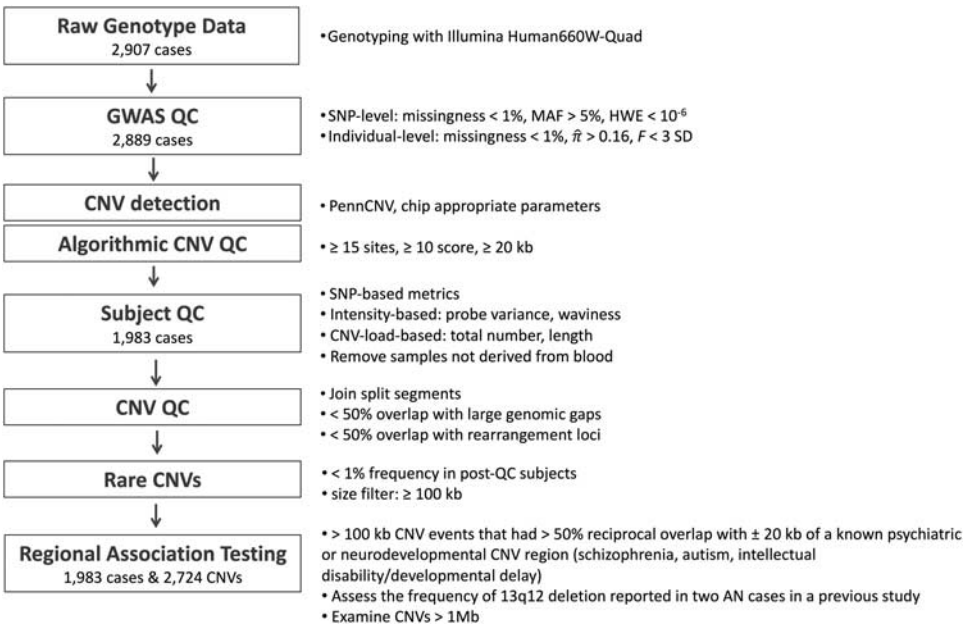
Results

Following rigorous QC, our analysis dataset comprised 2724 high-confidence, rare (< 1% minor allele frequency), large (>100 kbp) CNVs in 1983 cases. Factors affecting the number of CNVs detected include array type used and analytic tools used (detection algorithms and QC procedures), with array type as the primary factor (Pinto *et al.*, 2011; Szatkiewicz *et al.*, 2013; Szatkiewicz *et al.*, 2014). We observed a mean of 1.37 rare, large CNVs per patient, a rate comparable with what has been reported previously in the literature using similar SNP arrays (Szatkiewicz *et al.*, 2014).

We observed two instances of CNVs with at least 50% reciprocal overlap with the regions associated with psychiatric and neurodevelopmental disorders (Table 1). More specifically, there were single instances of deletions in 15q13.3 (associated with autism, schizophrenia, and intellectual disability/developmental delay) and 16p11.12 (distal; deletions not associated with a psychiatric or neurodevelopmental phenotype), respectively. In addition, one AN case had a large deletion in the 13q12 region previously implicated in AN in a preliminary study (hg19 coordinates in Table 1; intensity plot in Supplementary Fig. 1, Supplemental digital content 1, *http://links.lww.com/PG/A188*); however, whether this CNV is AN specific is uncertain because of the case-only design of our study.

Following candidate CNV analysis, we next examined our dataset for the presence of rare (minor allele frequency < 1%) CNVs over 1 Mbp in size. We identified 42 regions with such

Fig. 1



Experimental workflow and CNV datasets. AN, anorexia nervosa; CNV, copy number variant; GWAS, genome-wide association study; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; QC, quality control; SNP, single-nucleotide polymorphism.

Table 1 List of psychiatric and neurodevelopmental copy number variant regions examined in 1983 anorexia nervosa cases

Region	Coordinates (hg19)	Findings from the literature	Deletion count in AN	Duplication count in AN
1q21.1	chr1: 145 000 000–148 000 000	Deletion and duplication in SCZ; duplication in AUT; deletion and duplication in ID/DD	0	0
2p16.3	chr2: 50 100 000–51 200 000	Deletion in SCZ; deletion in ID/DD; deletion in AUT	0	0
3q29	chr3: 195 700 000–197 300 000	Deletion in SCZ; deletion in ID/DD	0	0
7q11.23	chr7: 72 700 000–74 100 000	Deletion and duplication in ID/DD; duplication in AUT	0	0
7q36.3	chr7: 158 800 000–158 900 000	Duplication in SCZ	0	0
13q12	chr13: 23 528 685–24 897 901	Deletion in AN (reported in two cases in a previous study)	1	0
15q11.2	chr15: 23 600 000–28 400 000	Deletion and duplication in SCZ; deletion and duplication in ID/DD; duplication in AUT	0	0
15q13.3	chr15: 30 900 000–32 500 000	Deletion in SCZ; deletion and duplication in ID/DD; deletion and duplication in AUT	1	0
16p11.2(1)	chr16: 28 822 499–29 042 499	Deletion in SCZ; deletion in ID/DD	0	0
16p11.2(2)	chr16: 29 500 000–30 200 000	Duplication in AUT; duplication in SCZ	1	0
17q12	chr17: 34 800 000–36 200 000	Deletion in SCZ; deletion in ID/DD; deletion in AUT	0	0
22q11.21	chr22: 18 700 000–21 800 000	Deletion in SCZ; deletion and duplication in ID/DD; deletion and duplication in AUT	0	0

AN, anorexia nervosa; AUT, autism; ID/DD, intellectual disability/developmental delay; SCZ, schizophrenia.

large deletions and duplications (Table 2), including one instance of 15q13.3 and 13q12 deletions each (Table 1). Out of the remaining 40 CNVs, 18 of them housed a total of 35 genes associated with various Mendelian conditions in OMIM, most of which are not associated with psychiatric or neurodevelopmental phenotypes (Table 2). However, the 3p26.3 deletion observed in one case spans over the *CRBN* gene, which is associated with autosomal recessive intellectual disability. Similarly, two genes (*SHROOM4* and *SYP*) located within the Xp11.23 region (in which one case had a large deletion) have been associated previously with X-linked recessive developmental delay and intellectual disability. A duplication in the 22q11.21 region (which does not have 50% overlap with the 22q11 candidate CNV region) includes *TBX1*, previously linked to velocardiofacial syndrome. Also of interest, three CNV events in our AN database that were not reported in DGV were observed in schizophrenia cases in the PGC CNV database (CNV and Schizophrenia Working Groups of the Psychiatric Genomics Consortium & Psychosis Endophenotypes International Consortium, 2016).

We could not find previous reports of or frequency information for five of the 42 rare and large CNV events. Although two of them (2p25.1 and 2p25.2) do not contain

Table 2 Genomic coordinates for the 40 regions containing large (>1 Mbp) copy number variants in anorexia nervosa cases[a]

CNV region	Coordinates (hg19)	CNV type	Previous report(s) in DGV?[b]	Previous report(s) in PGC?[c]	OMIM disease genes located within CNV and associated phenotypes
1p21.1	chr1: 106 195 966–107 267 115	Deletion	No	Yes	
1q31.3	chr1: 194 453 253–195 527 078	Deletion	No	Yes	
2q13	chr2: 111 392 259–113 098 812	Deletion	Yes	Yes	*BUB1* (colorectal cancer with chromosomal instability); *MERTK* (retinitis pigmentosa 38)
2q14.3	chr2: 123 030 939–126 370 246	Deletion	No	Yes (one schizophrenia case)	
2p25.2	chr2: 5 713 005–6 973 874	Duplication	No	No	
2p25.1	chr2: 7 284 752–8 332 717	Duplication	No	No	
2p12	chr2: 83 011 373–84 155 060	Deletion	Yes	No	
3p26.3	chr3: 2 062 298–3 363 379	Deletion	Yes	Yes	*CRBN* (intellectual disability, autosomal recessive)
4q35.1	chr4: 186 563 188–188 247 352	Duplication	Yes	Yes	*CYP4V2* (Bietti crystalline corneoretinal dystrophy); *F11* (factor XI deficiency, autosomal dominant or recessive)
4q35.2	chr4: 188 317 381–189 536 211	Duplication	Yes	Yes	
5p14.1	chr5: 26 942 758–28 114 207	Duplication	Yes	No	
5q11.2	chr5: 53 865 607–55 220 819	Deletion	No	No	*IL31RA* (amyloidosis)
5q13.2	chr5: 68 865 034–70 307 359	Duplication	Yes	No	*SMN1* (spinal muscular atrophy)
8q23.2	chr8: 110 934 142–114 949 589	Duplication	No	No	
8p22	chr8: 13 369 061–14 658 615	Duplication	Yes	Yes	*DLC1* (deletion associated with colorectal cancer)
8p21.3	chr8: 19 723 503–21 556 732	Duplication	No	No	*LPL* (familial combined hyperlipidemia; lipoprotein lipase deficiency); *LZTS1* (esophageal squamous cell carcinoma)
8p21.1	chr8: 28 557 627–30 585 738	Duplication	No	No	*GSR* (hemolytic anemia due to glutathione reductase deficiency)
10q21.1	chr10: 55 198 707–56 731 004	Duplication	No	Yes (schizophrenia cases)	*PCDH15* (deafness, autosomal recessive 23; Usher syndrome, type 1D/F or 1F)
12q12	chr12: 43 628 705–44 755 194	Duplication	Yes	No	*IRAK4* (*IRAK4* deficiency; invasive pneumococcal disease)
13q33.1	chr13: 103 682 440–104 973 086	Duplication	Yes	No	*SLC10A2* (primary bile acid malabsorption)
16p12.3	chr16: 16 859 801–18 165 043	Deletion	Yes	Yes	*XYLT1* (desbuquois dysplasia 2)
16q23.3	chr16: 82 185 320–83 665 269	Duplication	Yes	Yes	
17q12	chr17: 31 825 116–33 030 020	Duplication	Yes	Yes	
18q22.3	chr18: 68 814 612–69 928 013	Duplication	Yes	No	
20p13	chr20: 3 392 871–4 622 756	Deletion	No	Yes (one schizophrenia case)	*PANK2* (HARP syndrome; neurodegeneration with brain iron accumulation)
20p12.3	chr20: 7 102 986–8 575 671	Duplication	No	Yes	*PLCB1* (epileptic encephalopathy, early infantile)
22q11.21	chr22: 18 941 457–20 279 159	Duplication	Yes	Yes	*TBX1* (conotruncal anomaly face syndrome; DiGeorge syndrome; tetralogy of Fallot; velocardiofacial syndrome) *GP1BB* (Bernard–Soulier syndrome, type B; giant platelet disorder, isolated)
22q11.23	chr22: 23 690 325–24 996 630	Duplication	Yes	Yes	*IGLL1* (agammaglobulinemia 2); *SMARCB1* (Coffin–Siris syndrome 3; somatic rhabdoid tumors); *SPECC1L* (facial clefting; Opitz GBBB syndrome, type II); *UPB1* (β-ureidopropionase deficiency)
Xp11.23	chrX: 48 291 665–52 255 360	Deletion	No	Yes	*WAS* (X-linked neutropenia, severe congenital; Wiskott–Aldrich syndrome; X-linked thrombocytopenia); *GATA1* (X-linked anemia; megakaryoblastic leukemia with or without Down syndrome; X-linked thrombocytopenia); *PQBP1* (renpenning syndrome); *CLCN5* (Dent disease; hypophosphatemic rickets; nephrolithiasis, type I; proteinuria, low molecular weight, with hypercalciuric nephrocalcinosis); *BMP15* (ovarian dysgenesis 2; premature ovarian failure); *EBP* (X-linked dominant hondrodysplasia punctate; MEND syndrome); *SHROOM4* (tocco dos Santos X-linked mental retardation syndrome); *TFE3* (papillary renal cell carcinoma); *SYP* (X-linked intellectual disability); *CACNA1F* [Aland Island eye disease; X-linked cone-rod dystrophy; X-linked night blindness, congenital stationary (incomplete)]; *FOXP3* (X-linked immunodysregulation, polyendocrinopathy, and enteropathy)
Xp22.31	chrX: 6 458 166–7 517 325	Duplication	No	Yes	
Xp22.31	chrX: 6 458 166–7 980 930	Duplication	No	Yes	
Xp22.31	chrX: 6 458 166–8 068 292	Duplication	No	Yes	
Xp22.31	chrX: 6 458 166–8 135 053	Deletion[d]	No	Yes	
Xp22.31	chrX: 6 458 166–8 141 017	Duplication[d]	No	Yes	*STS* (X-linked ichthyosis)
Xp22.31	chrX: 6 516 735–8 068 292	Duplication	No	Yes	
Xp22.31	chrX: 6 564 943–7 745 286	Duplication	No	Yes	
Xp22.31	chrX: 6 664 300–8 115 453	Duplication	No	Yes	

AN, anorexia nervosa; AUT, autism; CNV, copy number variant; DGV, Database of Genomic Variants; ID/DD, intellectual disability/developmental delay; OMIM, Online Mendelian Inheritance in Men; PGC, Psychiatric Genomics Consortium; SCZ, schizophrenia.

[a]This table does not include the 13q12 deletion or the 15q13.3 duplication, and all events are singletons (unless indicated otherwise).

[b]*http://dgv.tcag.ca*; at least 50% reciprocal overlap.

[c]*http://pgc.tcag.ca/gb2/gbrowse/pgc_hg18/*; at least 50% reciprocal overlap.

[d]Observed in two AN cases.

any OMIM disease genes, the remaining three CNV regions (5q11.2, 8p21.1, and 8p21.3) span over OMIM disease genes associated with amyloidosis, hyperlipidemia, hemolytic anemia, and esophageal carcinoma (Table 2). It is also noteworthy that 10 cases had CNV events (eight duplications and two deletions) in the Xp22.31 region (chrX: 6 458 166–8 141 017; Table 2). The follow-up quantitative PCR experiment examining one deletion and one duplication from this region validated these CNVs successfully (details in Supplementary Information, Supplemental digital content 1, *http://links.lww.com/PG/A188*). There were no reports of CNVs with more than 50% reciprocal overlap with Xp22.31 in DGV; however, deletions and duplications in this region were found in both schizophrenia cases and controls as a part of the Psychiatric Genomics Consortium CNV Working Group (CNV and Schizophrenia Working Groups of the Psychiatric Genomics Consortium & Psychosis Endophenotypes International Consortium, 2016).

Discussion

In this case-only analysis, two individuals with AN had large, rare CNVs with over 50% reciprocal overlap with regions associated with psychiatric and neurodevelopmental disorders. The frequency with which these CNVs were observed in our sample is consistent with the literature (Levinson *et al.*, 2011; Szatkiewicz *et al.*, 2014). Furthermore, one AN case in our study had a large deletion in the 13q12 region described previously in two cases with AN (Wang *et al.*, 2011). It is noteworthy that there is no frequency information available for the 13q12 deletion in 1000 Genomes, which suggests that the precise population frequency of this CNV is not well known. However, we found three studies in DGV, which reported CNVs in controls with more than 50% reciprocal overlap with the CNV in our study (Pinto *et al.*, 2007; Cooper *et al.*, 2011; Uddin *et al.*, 2015). Although we cannot rule out a possible AN diagnosis in these individuals, 13q12 deletion does not appear to be AN specific, and its presence in one AN case should be interpreted with caution as larger case–control samples are required to rigorously evaluate the validity of this deletion in AN.

Outside of the well-established neuropsychiatric CNVs, 40 instances of rare and large CNVs were observed in AN cases. Although many of them contained OMIM disease genes, only two of these events (3p26.3 and Xp11.23) had previous associations with psychiatric/neurodevelopmental phenotypes. However, the lack of detailed phenotypic information prevented us from further examining the characteristics of the AN patients with these CNVs. Also of interest, three large CNVs not available in public databases were observed in schizophrenia cases, but not controls in the PGC CNV database, thus suggesting a potential association with schizophrenia that requires replication. Although there were 10 instances of CNV events in the Xp22.31 region in AN cases in our study (whose validity was confirmed by quantitative PCR), this CNV does not appear to be rare and has been reported in healthy controls alongside schizophrenia cases in PGC, and therefore unlikely to be an AN-specific risk CNV. Although this region includes *STS*, a gene associated with X-linked ichithyosis (a family of skin disorders), there are no known associated medical phenotypes observed in females. It is noteworthy that we failed to detect any clinical patterns involving AN age at onset, lowest illness-related BMI, highest-lifetime BMI, or AN subtype among the patients with CNVs in this Xp22.31 region.

Although the present study is the largest AN CNV analysis carried out to date, limitations must be considered. As a case-only design, our analyses focused on the characterization of the large, rare, and well-replicated psychiatric and neurodevelopmental CNVs in individuals with AN, as well as the description of 1 Mbp+ CNVs observed in our sample. The GCAN/WTCCC3 AN genomic dataset is challenging because of the ancestral heterogeneity of the component samples, which required us to apply strict QC cutoffs. Furthermore, the WTCCC3 controls used in the primary genome-wide association studies were unavailable for our analysis. We accessed and tested several dbGaP datasets as controls with the goal of investigating CNV burden and searching for novel CNVs associated with AN; however, several technical issues arose caused by the arrays having been processed separately, ultimately leading to the decision to extract as much information as possible from cases by determining whether CNVs in regions implicated in psychiatric and neurodevelopmental disorders were present in AN cases. Furthermore, the lack of detailed clinical phenotype information prevented us from performing a more in-depth examination of whether there are clinical manifestations associated with these CNVs in patients with AN. Although it is possible that some of the CNVs included in our analysis do not confer risk for AN, our study may have been underpowered to detect a few of these rare CNVs, thus potentially failing to capture their actual prevalence in individuals with AN. Future directions include examining a well-matched large case–control sample (ideally around 5000 cases, which would confidently allow for the assessment of known CNVs with 0.1% frequency implicated in other psychiatric disorders in AN) to assess case–control differences in CNV (e.g. genic and genome-wide) as well as searching for novel CNVs that confer risk to AN.

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Conflicts of interest

C.M. Bulik is a grant recipient from and consultant for Shire Pharmaceuticals on topics unrelated to this submission. P.F. Sullivan advises Pfizer Inc. For the remaining authors there are no conflicts of interest.

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