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Exploration of Large, Rare CNVs Associated with Psychiatric and Neurodevelopmental Disorders in Individuals with Anorexia Nervosa

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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. Other authors have no interests to disclose.

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Abstract

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 size. We conducted a case-only genome-wide CNV survey in 1,983 female AN cases included in the Genetic Consortium for Anorexia Nervosa (GCAN). 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. Additionally, one case had a deletion in the 13q12 region, overlapping with a deletion previously reported in two AN cases. As a secondary aim, we also examined our sample for CNVs over 1 Mb in size. Out of the 40 instances of such large CNVs which were not previously implicated for AN or neuropsychiatric phenotypes, two of them contained genes with previous neuropsychiatric associations, and only five of them had no associated reports associated 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.

Keywords

anorexia nervosa; eating disorders; copy number variation; neuropsychiatric disorders; rare variation

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 (Boraska *et al.*, 2014, Wang *et al.*, 2011, Duncan *et al.*, 2016). Considering the important role copy number variants (CNVs) play in psychiatric phenotypes (Bergen *et al.*, 2012, Cooper *et al.*, 2011, International Schizophrenia, 2008, Levinson *et al.*, 2011, Malhotra and Sebat, 2012, Sanders *et al.*, 2011, Szatkiewicz *et al.*, 2014), studies are needed to help elucidate their role 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 Mb 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 purpose of the present study was to assess the prevalence of large, rare CNVs previously associated 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 2,907 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). Subject 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 felt that a case-only analysis was necessary to ensure the correctness and validity of our results. Genotype data for 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).

Since GCAN included DNA samples derived from various tissues (i.e., blood, buccal epithelium, saliva, or cell lines), we removed all non-blood samples to ensure high-quality and high-confidence CNV calls that are not confounded by DNA source, which led to a sample size of 1,983 AN cases. Our analysis pipeline, which followed the steps outlined by Szatkiewicz *et al* (Szatkiewicz *et al.*, 2014), is summarized in Figure 1, and additional information about the process is available in Supplementary Methods and Supplementary Table 1.

CNV calling was performed using PennCNV (June 2011 version)(Diskin *et al.*, 2008, Wang *et al.*, 2008, Wang *et al.*, 2007) with parameters appropriate for the Illumina 660W-Quad chip (Illumina Inc., San Diego, CA). All other analyses were conducted using PLINK (Purcell *et al.*, 2007) and R (R Development Core Team, 2011). We implemented strict

cutoffs for SNP-, sample-, and CNV-level QC procedures (Supplementary Methods). We examined AN cases for large CNVs previously reported to be associated with schizophrenia, autism, intellectual disability, or developmental delay (Levinson *et al.*, 2011, Malhotra and Sebat, 2012, Sullivan *et al.*, 2012, Sanders *et al.*, 2011, Cooper *et al.*, 2011)(Table 1). We considered all CNV events >100 kb that had > 50% reciprocal overlap within ± 20 kb of a known psychiatric or neurodevelopmental CNV (i.e., at least 50 kb overlap with a known CNV was required). We also assessed the presence of the 1.5 Mb 13q12 deletion previously reported in two AN cases (Wang *et al.*, 2011)(Table 1). As a secondary aim, we searched for any CNVs which are > 1 Mb 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 < 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 2,724 high-confidence, rare (<1% MAF), large (>100 kb) CNVs in 1,983 cases. Factors affecting the number of CNVs detected include array type used and analytic tools employed (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 subject, a rate comparable to what has previously been reported 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. Additionally, 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 Figure 1); however, whether this CNV is AN-specific is uncertain due to the case-only design of our study.

Following candidate CNV analysis, we next examined our dataset for the presence of rare (minor allele frequency [MAF] < 1%) CNVs over 1 Mb in size. We identified 42 regions with such 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 phneotypes (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 previously associated 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 which 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 and Psychosis Endophenotypes International Consortium, 2016).

We were unable to find previous reports of or frequency information for five of the 42 rare and large CNV events. While two of them (2p25.1 and 2p25.2) do not contain 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). Also of note, 10 cases had CNV events (eight duplications and two deletions) in the Xp22.31 region (chrX: 6458166-8141017; Table 2). The follow-up qPCR experiment examining one deletion and one duplication from this region successfully validated these CNVs (details in Supplementary Information). There were no reports of CNVs with > 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 and 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 previously described in two cases with AN (Wang *et al.*, 2011). Of note, 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 > 50% reciprocal overlap with the CNV in our study (Cooper *et al.*, 2011, Uddin *et al.*, 2015, Pinto *et al.*, 2007). While 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, there were 40 instances of rare and large CNVs observed in AN cases. While many of them contained OMIM disease genes, only two of these events (3p26.3 and Xp11.23) had previously associations with psychiatric/neurodeveopmental phenotypes. However, 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 dabatases were observed in schizophrenia cases but not controls in the PGC CNV database, thus suggesting a potential association with schizophrenia that requires replication. While there were 10 instances of CNV events in the Xp22.31 region in AN cases in our study (validity of which was confirmed via qPCR), 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.

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Although this region includes *STS*, a gene associated with X-linked ichithyosis (a family of skin disorders), there are no known associated medical phenotypes seen in females. Of note, 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.

While the present study is the largest AN CNV analysis conducted 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 Mb+ CNVs observed in our sample. The GCAN/WTCCC3 AN genomic dataset is challenging due to ancestral heterogeneity of the component samples, which required us to apply strict QC cutoffs. Furthermore, the WTCCC3 controls used in the primary GWAS 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, lack of detailed clinical phenotype information prevented us from conducting a more in-depth examination of whether there are clinical manifestations associated with these CNVs in patients with AN. While 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 true prevalence in individuals with AN. Future directions include examining a well-matched large case-control sample (ideally around 5,000 cases, which would confidently allow for the assessment of known CNVs with 0.1% frequency implicated in other psychiatric disorders in AN) in order 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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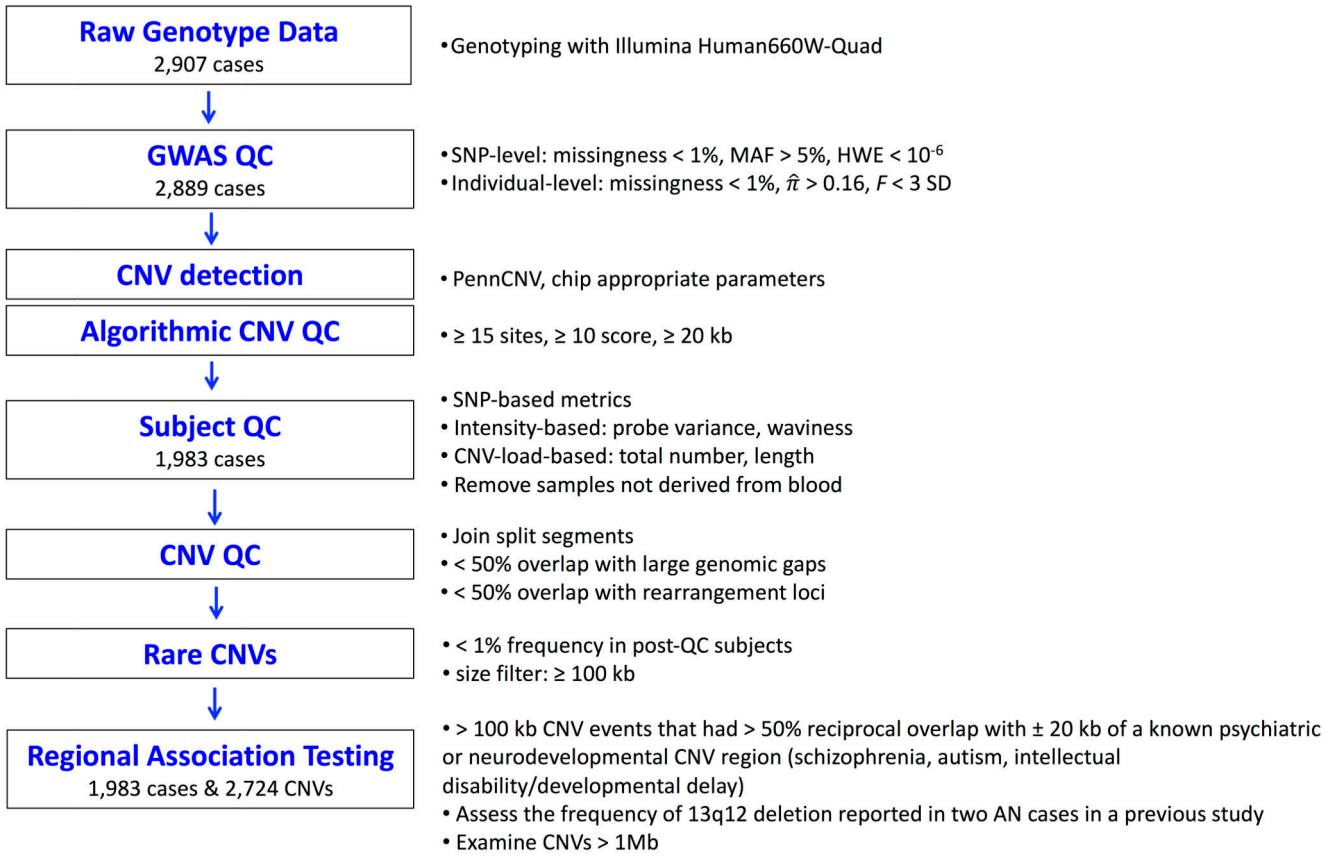

Figure 1.
Experimental workflow and CNV datasets

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Table 1

List of psychiatric and neurodevelopmental CNV regions examined in 1,983 AN cases

Region	Coordinates (hg19)	Findings from the Literature	Deletion count in AN	Duplication count in AN
1q21.1	chr1:145000000-148000000	Deletion and duplication in SCZ; duplication in AUT; deletion and duplication in ID/DD	0	0
2p16.3	chr2:50100000-51200000	Deletion in SCZ; deletion in ID/DD; deletion in AUT	0	0
3q29	chr3:195700000-197300000	Deletion in SCZ; deletion in ID/DD	0	0
7q11.23	chr7:72700000-74100000	Deletion and duplication in ID/DD; duplication in AUT	0	0
7q36.3	chr7:158800000-158900000	Duplication in schizophrenia	0	0
13q12	chr13:23528685-24897901	Deletion in AN (reported in two cases in a previous study)	1	0
15q11.2	chr15:23600000-28400000	Deletion and duplication in SCZ; deletion and duplication in ID/DD; duplication in AUT	0	0
15q13.3	chr15:30900000-32500000	Deletion in SCZ; deletion and duplication in ID/DD; deletion and duplication in AUT	1	0
16p11.2(1)	chr16:28822499-29042499	Deletion in SCZ; deletion in ID/DD	0	0
16p11.2(2)	chr16:29500000-30200000	Duplication in AUT; duplication in SCZ	1	0
17q12	chr17:34800000-36200000	Deletion in SCZ; deletion in ID/DD; deletion in AUT	0	0
22q11.21	chr22:18700000-21800000	Deletion in SCZ; deletion and duplication in ID/DD; deletion and duplication in AUT	0	0

Abbreviations: AN = anorexia nervosa; AUT = autism; ID/DD = intellectual disability/developmental delay; SCZ = schizophrenia

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Table 2

Genomic coordinates for the 40 regions containing large (> 1Mb) CNVs in AN 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	chr1:106195966-107267115	Deletion	No	Yes	
1q31.3	chr1:194453253-195527078	Deletion	No	Yes	
2q13	chr2:111392259-113098812	Deletion	Yes	Yes	• *BUB1* (colorectal cancer with chromosomal instability) • *MERTK* (retinitis pigmentosa 38)
2q14.3	chr2:123030939-126370246	Deletion	No	Yes (1 schizophrenia case)	
2p25.2	chr2:5713005-6973874	Duplication	No	No	
2p25.1	chr2:7284752-8332717	Duplication	No	No	
2p12	chr2:83011373-84155060	Deletion	Yes	No	
3p26.3	chr3:2062298-3363379	Deletion	Yes	Yes	• *CRBN* (intellectual disability, autosomal recessive)
4q35.1	chr4:186563188-188247352	Duplication	Yes	Yes	• *CYP4V2* (Bietti crystalline corneoretinal dystrophy) • *F11* (Factor XI deficiency, autosomal dominant or recessive)
4q35.2	chr4:188317381-189536211	Duplication	Yes	Yes	
5p14.1	chr5:26942758-28114207	Duplication	Yes	No	
5q11.2	chr5:53865607-55220819	Deletion	No	No	• *IL31RA* (amyloidosis)
5q13.2	chr5:68865034-70307359	Duplication	Yes	No	• *SMN1* (spinal muscular atrophy)
8q23.2	chr8:110934142-114949589	Duplication	No	No	
8p22	chr8:13369061-14658615	Duplication	Yes	Yes	• *DLC1* (deletion associated with colorectal cancer)
8p21.3	chr8:19723503-21556732	Duplication	No	No	• *LPL* (familial combined hyperlipidemia; lipoprotein lipase deficiency) • *LZTS1* (esophageal squamous cell carcinoma)
8p21.1	chr8:28557627-30585738	Duplication	No	No	• *GSR* (hemolytic anemia due to glutathione reductase deficiency)

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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
10q21.1	chr10:55198707-56731004	Duplication	No	Yes (schizophrenia cases)	• *PCDH15* (deafness, autosomal recessive 23; Usher syndrome, type 1D/F or 1F)
12q12	chr12:43628705-44755194	Duplication	Yes	No	• *IRAK4* (*IRAK4* deficiency; invasive pneumococcal disease)
13q33.1	chr13:103682440-104973086	Duplication	Yes	No	• *SLC10A2* (primary bile acid malabsorption)
16p12.3	chr16:16859801-18165043	Deletion	Yes	Yes	• *XYLT1* (Desbuquois dysplasia 2)
16q23.3	chr16:82185320-83665269	Duplication	Yes	Yes	
17q12	chr17:31825116-33030020	Duplication	Yes	Yes	
18q22.3	chr18:68814612-69928013	Duplication	Yes	No	
20p13	chr20:3392871-4622756	Deletion	No	Yes (1 schizophrenia case)	• *PANK2* (HARP syndrome; neurodegeneration with brain iron accumulation)
20p12.3	chr20:7102986-8575671	Duplication	No	Yes	• *PLCB1* (Epileptic encephalopathy, early infantile)
22q11.21	chr22:18941457-20279159	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	chr22:23690325-24996630	Duplication	Yes	Yes	• *IGLL1* (agammaglobulinemia 2) • *SMARCB1* (Coffin-Siris syndrome 3; somatic rhabdoid tumors) • *SPECC1L* (facial clefting; Opitz GBBB syndrome, type II) • *UPB1* (beta-ureidopropionase deficiency)
Xp11.23	chrX:48291665-52255360	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)

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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
					• *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:6458166-7517325	Duplication	No	Yes	
Xp22.31	chrX:6458166-7980930	Duplication	No	Yes	
Xp22.31	chrX:6458166-8068292	Duplication	No	Yes	
Xp22.31	chrX:6458166-8135053	Deletion[d]	No	Yes	
Xp22.31	chrX:6458166-8141017	Duplication[d]	No	Yes	• *STS* (X-linked ichthyosis)
Xp22.31	chrX:6516735-8068292	Duplication	No	Yes	
Xp22.31	chrX:6564943-7745286	Duplication	No	Yes	
Xp22.31	chrX:6664300-8115453	Duplication	No	Yes	

[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