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Reciprocal duplication of the Williams-Beuren syndrome deletion on chromosome 7q11.23 is associated with schizophrenia

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Abstract

Background—Several copy number variants (CNVs) have been implicated as susceptibility factors for schizophrenia (SZ). Some of these same CNV also increase risk for autism spectrum disorders (ASD), suggesting an etiologic overlap between these conditions. Recently, *de novo* duplications of a region on chromosome 7q11.23 were associated with ASD. The reciprocal deletion of this region causes Williams-Beuren syndrome (WBS).

Methods—We assayed an Ashkenazi Jewish cohort of 554 SZ cases and 1014 controls for copy number variation (CNV), using a high-density genome-wide array. An excess of large rare and *de novo* CNV were observed, including a 1.4 Mb duplication on chromosome 7q11.23 identified in two unrelated patients. To test whether this 7q11.23 duplication is also associated with SZ, we obtained data for 14,387 SZ cases and 28,139 controls from seven additional studies with high-resolution genome-wide CNV detection. We performed a meta-analysis, correcting for study population of origin, to assess whether the 7q11.23 duplication is associated with SZ.

Results—We find duplications at 7q11.23 in 11 of 14,387 SZ cases with only 1 in 28,139 controls (unadjusted odds ratio, 21.52, 95% CI: 3.13-922.6, p-value 5.5×10^{-5} ; adjusted odds ratio 10.8, 95% CI: 1.46-79.62, p-value 0.007). Of three SZ duplication carriers with available detailed retrospective data, all show social anxiety and language delay premorbid to SZ onset, consistent with both human studies and animal models of the 7q11.23 duplication.

Conclusion—We have identified a new CNV associated with SZ. Reciprocal duplication of the Williams syndrome deletion at chromosome 7q11.23 confers an approximately 10-fold increase in risk for SZ.

Keywords

Schizophrenia; 7q11.23 duplication syndrome; Psychiatric genetics; Schizophrenia genetics; Autism; Williams-Beuren syndrome

Introduction

Schizophrenia (SZ) is a severe psychiatric disorder that represents a significant public health burden, affecting 1% of the population worldwide(1). It has long been recognized that genetic factors must play a role in susceptibility, but until recently identification of such risk factors has proven elusive(1). Landmark studies of genomic structural variation in SZ, first published in 2008, established that there is an excess of large, rare copy number variants (CNVs) in SZ populations(2-5). Since then, this has become one of the most exciting and consistently replicated findings in psychiatric genetics. Currently at least ten specific CNV loci have been replicated in multiple reports and can thus be regarded as confirmed risk factors(3, 5-14). The first of these reports include two large studies with thousands of patients, where recurrent deletions were found at three new loci: 1q21, 15q11 and 15q13, in addition to the previously identified 22q11 deletion(5, 15). Several studies soon followed, implicating deletions at 17p12(16) and 3q29(7, 8), and duplications at 16p11(12). These variants have the following properties in common: they are all large, involving many genes; they are rare, at a frequency far less than 1% even in SZ populations; and they all carry substantial risk for SZ, with estimated odds ratios between 3-30(7, 14). Smaller variants have been identified in specific genes, including deletions in neurexin1 (NRXN1)(9, 10, 17) and contactin-associated protein-like 2 (CNTNAP2), another member of the neurexin family. (11). Duplication at the vasoactive intestinal peptide receptor 2 (VIPR2) locus is also associated with high risk for schizophrenia(7, 13). One surprising finding is that many of the CNVs associated with SZ are also significantly more common in individuals with intellectual disability (ID), autism, and epilepsy(16, 18-21), advancing the concept of a neurodevelopmental link between these disorders(22).

In the current study we initially assessed a sample of 554 independent SZ cases and 1014 controls, all of Ashkenazi Jewish (AJ) descent. We limited our analysis to large rare CNVs in keeping with previous reports. We replicate previous findings of rare and *de novo* CNVs, finding a significant excess of these events in SZ cases compared to controls. Among these rare events, we discovered two duplications on chromosome 7q11.23, including one confirmed *de novo* event. A subsequent meta-analysis of 14,387 SZ cases and 28,139 controls confirmed the overrepresentation of this duplication in SZ cases, with an estimated odds ratio of 10.8 (95% CI: 1.46-79.62, p-value 0.007). *De novo* duplications at this identical locus were previously reported in an autism population(23), further supporting a shared etiology between autism and schizophrenia.

Methods and Materials

Study subjects

SZ Cases—Ashkenazi Jewish individuals affected with SZ (n=615) were recruited nationally over a six-year period. Cases were eligible for inclusion in these analyses if the proband met DSM-IV criteria for a SZ diagnosis and all four grandparents were of Ashkenazi Jewish descent. When available, parental DNAs were also collected. Probands were assessed for psychiatric illness according to an established consensus-based procedure,

as described in(24) and in the Supplement. No subject in our study had a previous clinical genetic diagnosis.

Controls—Control subjects were selected from three cohorts: a study of Crohn’s disease in the Ashkenazim (n=258), a study of neuromuscular disease (Parkinson’s and dystonia) in the Ashkenazim (n=266), and the Ashkenazi Jewish Control Registry (AJCR) hosted at Johns Hopkins University (n=538). Control subjects from the Crohn’s and neuromuscular cohorts were not screened for psychiatric disease; AJCR controls were administered a questionnaire about psychiatric conditions.

Meta-Analysis—Seven additional samples were incorporated into our meta-analysis, ultimately totaling 14,387 SZ cases and 28,139 unaffected controls. Details of each sample, including ascertainment criteria and genotyping platform, are included in the Supplement.

For the Ashkenazi Jewish sample ascertained at Johns Hopkins, all recruitment methods and protocols for collection of clinical data and blood samples were approved by the Johns Hopkins institutional review board, and informed consent was obtained from all individuals. All other data, including data for the meta-analysis, were fully anonymized prior to receipt at Emory University.

Genotyping and identification of CNVs—DNA from the Ashkenazi sample was extracted using the Gentra Puregene kit at Johns Hopkins University. All DNA used for this study was extracted from blood (no cell line DNA was used). Genotyping was performed using the Affymetrix Human Genome-Wide SNP Array 6.0 at Emory University. Genotypes were called using the Birdseed algorithm, as implemented in Affymetrix power tool software (version 1.12.0). SNPs with completion rates < 90% were excluded, rendering 816,284 autosomal SNPs for analysis. For CNV analysis, normalization and log ratio data calculation was obtained using the Affymetrix power tools software (version 1.12.0). Log(2) ratio data for autosomes were extracted and analyzed using three algorithms: GLAD(25), GADA(26), and BEAST(27). CNVs called by only a single algorithm were removed from analysis. Putative CNV intervals were filtered by size (> 100 kb), number of SNPs in the CNV interval (> 20 SNPs), and CNV-interval SNP homozygosity rates. CNVs > 500 kb were validated by a second array (Illumina Human OmniExpress v1 genotyping array), qPCR, or PCR across deletion breakpoint. See Methods in the Supplement for specific details on validation methodologies.

Definition of “rare CNV”—We filtered variants with the goal of excluding typical polymorphic events. To facilitate comparison with our earlier study(8), we excluded variants that had > 50% overlap with a CNV in the database of genomic variants (DGV, <http://projects.tcag.ca/variation/>, Nov 02, 2010 update) for deletions and duplications separately. We also excluded any variant with a frequency > 0.5% at any single locus.

Statistical analysis—Permutation-corrected p-values for overall CNV burden were calculated using PLINK. All odds ratios, associated confidence intervals, and p-values were calculated using the R statistical software package. Meta-Analysis statistics were calculated with the Cochran-Mantel-Haenszel exact test, stratified by study, in the R statistical package(28, 29).

Results

Rare and *de novo* CNV in the AJ population

There was a 1.29-fold excess of rare CNVs > 100 kb (see Supplement), which became more pronounced for CNV > 500 kb. In the 554 AJ SZ cases compared to 1014 controls, 5.6% of cases vs. 2.4% of controls had at least one rare CNV > 500 kb (OR: 2.44, P-value 0.001), consistent with previous reports(2-5). The OR is strongest for deletions > 1 Mb (OR 5.1, p-value 0.004) (Table 1). These data harbor known susceptibility variants, including three 22q11 deletions and four 16p11 duplications, which are now considered established risk factors for SZ (see data and Table S4 in the Supplement). For 292 of these SZ cases, both parental DNAs are available, enabling us to estimate the *de novo* rate for large (> 500 kb) rare CNV in our sample. We find 6 *de novo* events, or a rate of 2.1%. We compared this to the *de novo* rate in non-psychiatric trios, reviewed by Kirov et al (2012)(30), where a rate of 0.8% was reported (27 *de novo* CNV in 3495 trios). This difference is statistically significant (OR: 2.69, p-value 0.038). Our *de novo* rate of 2.1% is identical to that reported in SZ (2.1% in the study by Kirov et al (2012)(30)) and lower, but not significantly so, than the reported *de novo* rate in simplex ASD trios(23), (2.1% vs 3.9%, p-value 0.13).

Meta-Analysis of the 7q11.23 region

The six *de novo* CNV > 500 kb in the AJ sample included one 1.3 Mb *de novo* duplication on chromosome 7q11.23. A duplication at this locus was also identified in an additional unrelated proband (parental DNAs were unavailable for inheritance testing) (Figure 1). Given the recurrent nature of this duplication, and its involvement in ASD, we were motivated to investigate its frequency in additional SZ cohorts. This was the only variant tested in additional samples. We analyzed datasets where we could obtain the raw intensity files, to enable consistent data processing (Table S3 in the Supplement). In total, we found 11 duplications in 14,387 cases (0.076%) and one in 28,139 controls (0.0035%) (Table 2 and Figure 2), for an overall odds ratio of 21.5 (95% CI: 3.13-922.6, p-value 5.5×10^{-5}). Although such a large CNV would be found with any of the array platforms used and there is no reason to suspect population differences for a CNV with such a high pathogenic effect and *de novo* rate, we nevertheless also calculated the Mantel-Haenszel corrected OR for this duplication, leading to a corrected OR of 10.8 (p-value 0.007). These data therefore indicate the 7q11.23 duplication is a significant new risk factor for SZ.

Discussion

We have identified a new risk factor for schizophrenia, a 1.4 Mb duplication on chromosome 7q11.23. Two prior studies reported single 7q11.23 duplication carriers in their SZ case populations patients(30, 31). In this current study we find the duplication in 0.076% of SZ patients and establish a statistically significant enrichment compared to controls. The 7q11.23 duplication syndrome has previously been described (OMIM #609757) in the context of intellectual disability and autism(23, 32, 33). It is found in 0.1 - 0.12% (39/32,587(34); 16/15,749(35)) of patients referred for cytogenetic testing because of developmental delay/congenital malformations/ASD(34, 35). We note that prior studies of the 7q11.23 duplication phenotype involve mainly pediatric patients. As the typical age of onset for psychosis in schizophrenia is in early adulthood, risk for psychosis in 7q11.23 duplication carriers is therefore not captured. Studies including adult patients are limited and omit psychiatric evaluation. For example, a 2009 study of twelve 7q11.23 duplication carrier probands with intellectual disability revealed seven were inherited; phenotypic descriptions of the parents neglected an evaluation for psychosis(32). Similarly, a 2011 study did not conduct psychiatric assessments on the eight adults identified with 7q11.23 duplication syndrome(33). We therefore may have uncovered a previously unrecognized aspect of the

natural history of 7q11.23 duplication syndrome: adult risk for schizophrenia. This is reminiscent of 22q11 deletion syndrome, which is typically identified as a pediatric condition but carries substantial risk for schizophrenia in early adulthood. (36, 37). A longitudinal study of 7q11.23 duplication carriers would ideally assess the true risk for schizophrenia in this population.

It is possible we have ascertained adult individuals with autism rather than schizophrenia. However, the frequency of the 7q11.23 duplication in ASD patients is estimated at 4 in 3816, or 0.1048% (23). Our study observes the 7q11.23 duplication 11 times in 14,573 individuals with schizophrenia. If we assume for the moment that all 11 duplication carriers have ASD, not SZ, and that this duplication is at the reported frequency of 0.1048 in the ASD population, that would imply 72% of all our SZ are actually misclassified ASD. Ascertainment procedures of SZ patients for the studies included herein are methodical, detailed, and comprehensive, and a 72% misclassification rate is highly unlikely. Assuming a more reasonable misclassification rate of 10% would imply 1 or 2 out of the 11 deletions are due to mischaracterized ASD. Since we observe the duplication 11 times, we conclude that any adult autism patients included in our sample cannot account for the enrichment we observe.

The 1.4 Mb region on chromosome 7q11.23 is flanked by segmental duplications (Figure 2), which are likely mediating non-allelic homologous recombination, giving rise to the duplication and reciprocal deletion. These rearrangements are typically *de novo* events: Sanders et al found that 4 out of 4 duplications were *de novo* (23); Girirajan et al found of 9 informative duplications, 5 were *de novo* (an additional 30 duplications in this study did not have parental DNAs available for inheritance testing) (34). The reciprocal deletion gives rise to the well-characterized Williams-Beuren syndrome (OMIM #194050), first described in the early 1960s (38, 39). The duplication syndrome has only been described more recently. In 2005, a single patient with the duplication and severe language delay was described (40); later, description of the microduplication syndrome (OMIM #609757) was expanded to include speech delay and other variable characteristics, such as ID, hypotonia, congenital heart defects, social interaction difficulties and, less often, epilepsy (32). More recently, *de novo* duplication of this region was linked to ASD, where hallmarks of the phenotype are diminished development of language and poor social communication (23, 41). In one of the largest and most comprehensive studies to date, Velleman and Mervis describe the cognitive and behavioral profile of 7q11.23 duplication syndrome derived from both literature reports and their own direct evaluation of 30 children with the duplication (33). They confirm both language delay and social anxiety in 7q11.23 duplication carriers, and find separation anxiety to feature prominently among these individuals (33, 42). In fact, these authors suggest that this separation anxiety, which can manifest with selective mutism, could resemble an autism phenotype (33). In a research setting the authors document social phenomena including eye contact, pleasurable engagement with a parent, imaginative play, and other reciprocal social interactions inconsistent with autism (33). While further study will be required to confirm or refute the autism-7q11.23 duplication relationship, our current data support a link between social anxiety, language delay, and the 7q11.23 duplication. Both SZ duplication carriers in the Ashkenazi sample report severe social anxiety and language delay (data in the Supplement). Similarly, a Bulgarian duplication carrier with SZ also had speech delay and is described as socially withdrawn (30). The mean age at onset of the 11 SZ duplication carriers we report here is 20.7 (SD = 8.3 years), however two cases had an onset in childhood (aged 7 and 8 years), suggesting that the age at onset could be quite early in a proportion of carriers. In light of the WBS phenotype, it is possible there may be a quantitative relationship between dosage of the 7q11.23 region and verbal and social skills, though more detailed phenotypic data will be necessary to investigate this hypothesis.

Heterogeneity is a hallmark of schizophrenia. Among any population of SZ patients, there is a wide spectrum of differences in clinical presentation, course, and outcome. It has long been suspected that this spectrum reflects heterogeneity in underlying etiology, and current CNV data support this notion. In our sample, we note that the two unrelated patients in the AJ cohort had similar early childhood morbidity, including obsessions and compulsions, eating disorders, and language delay. Though more research is required, including detailed phenotypic studies and collection of retrospective data on duplication carriers, our findings nevertheless suggest the 7q11.23 region may define a specific subtype of SZ. Categorizing patients by etiologic variants like this may ultimately serve to reduce the vast heterogeneity seen in this disorder.

We find duplications at 7q11.23 in SZ cases at a frequency of 0.08%, similar to the frequency of this duplication in ASD (0.1%, four out of 3816 cases)(23). In two large samples of individuals referred for array CGH testing with a heterogeneous mixture of phenotypes, including congenital abnormalities, developmental delay, and intellectual disability, this duplication is seen in 39 out of 32,587 individuals (0.12%) (34), and 16 of 15,749 individuals (0.1%) (35), respectively. Thus, this duplication is seen with similar frequencies in patients with either SZ, ASD, or ID, echoing results from other CNVs associated with both SZ and ASD. Notably, thus far only a single duplication has been described in controls; most carriers are found in ID, autism, or SZ cohorts, underscoring the high pathogenicity of this duplication, which is similar to the 3q29 or 22q11 deletions(7, 14).

In summary, we find that the 7q11.23 duplication is a new risk factor for the development of SZ. This duplication shares the hallmarks of other SZ-associated CNVs: it is rare, has a large effect size, and is associated not only with schizophrenia, but also with autism and ID, supporting an etiologic link between these phenotypes. Future study incorporating comprehensive data collection will be required to understand the apparently different behavioral manifestations of the 7q11.23 deletion and duplication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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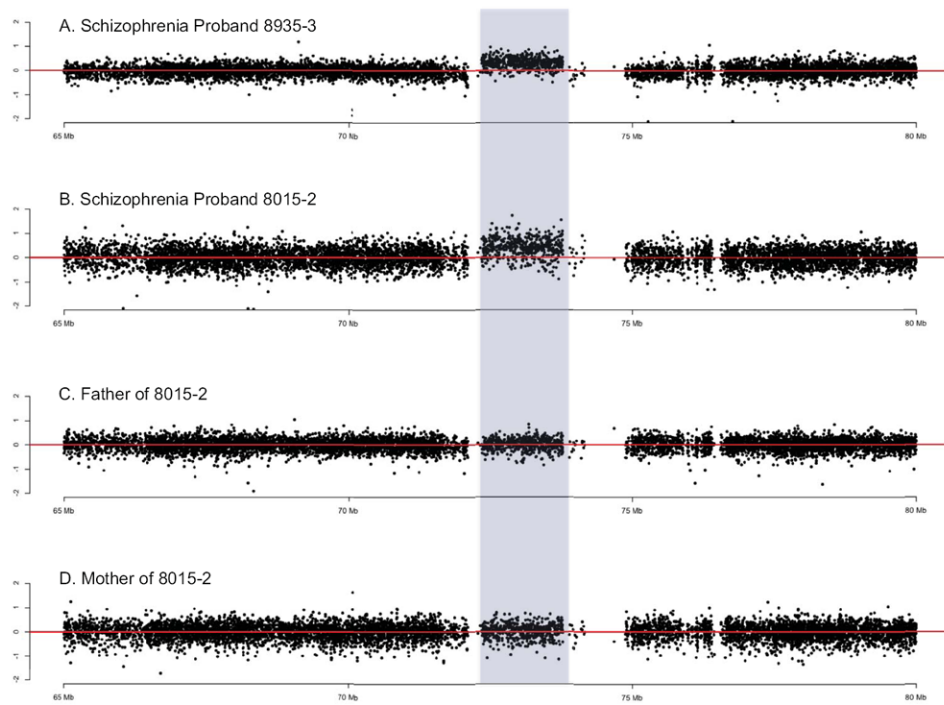


Figure 1. Raw \log_2 ratio data are shown for 15 Mb on chromosome 7q (65 Mb – 80 Mb) flanking the duplication region, for two unrelated AJ schizophrenia probands carrying the 7q11.23 duplication (panels A and B). Shading indicates the duplicated region. The parents of SZ proband 8015-2, who do not carry the duplication, are also shown (panels C and D).

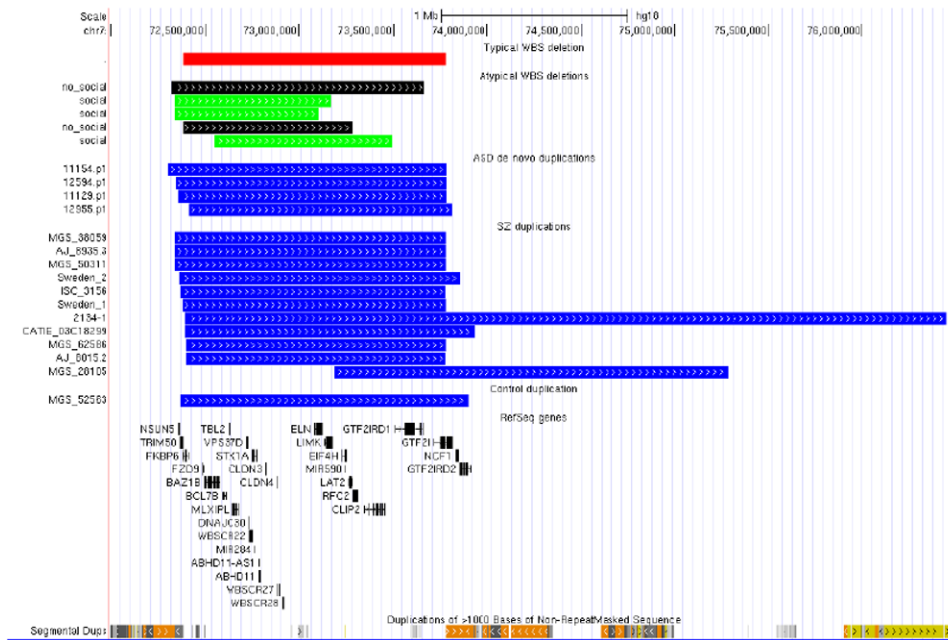


Figure 2. Eleven duplications found in SZ patients are shown against the context of previously reported events at the 7q11.23 locus, including typical and atypical WBD deletions (with highly social or not highly social phenotypes noted), de novo duplications in ASD patients, and genes in the WBS region.

Table 1

Excess rare variants in 554 SZ cases vs 1014 controls

Type of variant	Number of cases with at least 1 variant	Number of controls with at least 1 variant	p-value	Odds Ratio (95% CI)
Rare CNV > 500 kb	31 (5.6%)	24 (2.4%)	0.0013	2.44 (1.37-4.4)
Deletions > 500 kb	13 (2.3%)	8 (0.8%)	0.018	3.02 (1.15-8.46)
Duplications > 500 kb	18 (3.2%)	16 (1.6%)	0.044	2.09 (1.00 – 4.43)
Rare CNV > 1 Mb	19 (3.4%)	10 (1%)	0.0012	3.56 (1.56 – 8.64)
Deletions > 1 Mb	11 (2%)	4 (0.4%)	0.0043	5.11 (1.51 – 22.1)
Duplications > 1 Mb	8 (1.4%)	6 (0.8%)	0.097	2.46 (0.74 – 8.65)

All odds ratios and p-values calculated using a two-sided Fisher's exact test in R.

Table 2

Meta-Analysis of the 7q11.23 duplication in SZ cohorts

Sample	Cases	Case 7q11.23 Duplications	Controls	Control 7q11.23 Duplications
Ashkenazi	554	2*	1014	0
MGS(44)	3945	4	3611	1
ISC(3)	3063	1	3181	0
Bulgarian Trios(30)	662	1**	638	0
Japanese(45)	575	0	564	0
UK(4, 14)	471	0	13,036	0
CATIE(46)	738	1	289	0
Sweden(47)	4379	2	5806	0
Totals	14,387	11	28,139	1
Raw OR	21.52 (95% CI: 3.13-922.6, p-value 5.5×10^{-5})			
MH-corrected OR	10.78 (95% CI: 1.46-79.62, p-value 0.007)			

* one *de novo* event;** *de novo* event