Chromosome 22 Deletions and Suicidal Behavior in Schizophrenia

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Keywords

Copy number variant · Suicide · Schizophrenia · Chromosome 22

Abstract

Background: Studies have shown that the overall copy number variant (CNV) load is associated with schizophrenia. Schizophrenia is a mental disorder that is frequently associated with suicidal behavior. **Methods:** We recruited 263 patients with schizophrenia from the Centre for Addiction and Mental Health. The Columbia Suicide Severity Rating Scale was used to assess the presence of lifetime suicide attempt. Genotyping was completed using the Illumina Omni 2.5 chip. We tested the association between deletion events on chromosome 22 with suicide attempt in our schizophrenia sample. **Results:** There was no significant difference between suicide attempters and non-attempters considering the presence/absence of deletion events on chromosome 22. **Conclusion:** Although our results did not show a significant association between deletions on chromosome 22 and suicide attempt in schizophrenia, CNV studies may reveal important, novel insights and open further investigation for the treatment of neuropsychiatric diseases.

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Introduction

The suicide rate in patients with schizophrenia (SCZ) is approximately 5% [1]. In comparison, suicide attempts occur at a much higher rate, ranging from 30 and up to 49% in some studies [2–4]. Specifically, for SCZ, a shorter life-span is partly attributable to elevated suicide rates [5]. Like the general population, the best predictors to identify individuals most at risk to commit suicide are those with a history of previous suicidal attempts [6–8]. Other known risk factors for suicide in SCZ include recent stressful life events [9] and specific personality traits such as alexithymia [10]. Although the anti-suicide effect of specific antipsychotics has been reported [11], analyzing DNA variations (e.g., SNPs, copy number variation [CNV], and epigenome modifications) is a promising method of identifying SCZ patients at heightened risk for suicide.

SCZ is a severe mental health disorder with high heritability. One important heritable factor believed to contribute to the development of SCZ is CNV [12]. CNVs are deletions or duplications of a segment of the genome usually >1 kb. Evidence suggests that copy number variants have a robust and reproducible impact on SCZ [13]. 22q11.2 deletion represents one of the greatest known genetic risk factors for the development of psychosis. Genes within the 22q11.2 locus have been implicated in the etiology of idiopathic psychosis [14].

Fig. 1. Analysis of subjects with and without deletion events ($N = 263$). There were more subjects with deletions than without in both algorithms (0 = absence of deletion events; 1 = presence of at least 1 deletion event).

To date, nobody has tested the hypothesis that a larger number of CNVs could confer susceptibility for suicidal behavior in SCZ. Our hypothesis was that SCZ subjects with CNV deletions on chromosome 22 would demonstrate an increased risk for suicide attempt. Our specific aim was to analyze the impact of any chromosome 22 CNV deletion events in conferring risk for suicide attempt in our SCZ sample.

Methods

From the Centre for Addiction and Mental Health (CAMH) in Toronto (ON, Canada), 263 participants were recruited between the ages of 18 and 75 in a 5-year period. Each subject selected was diagnosed with SCZ or schizoaffective disorder. From the recruitment, 101 were suicide attempters and 162 participants were non-attempters. The diagnosis of SCZ was confirmed using the Structured Clinical Interview for DSM-IV (SCID-I/P) [15]. The CAMH Research Ethics Board reviewed and approved the study. Written informed consent was obtained from all participating subjects. None of the participants was diagnosed with 22q11.2 microdeletion (velo craniofacial/DiGeorge syndrome). Determination of lifetime suicide attempts was conducted using gold-standard measures for the assessment of suicide behavior by the means of the Columbia Suicide Severity Rating Scale (C-SSRS) and the Beck Scale for Suicidal Ideation (BSS) [16, 17].

Genomic DNA was extracted from saliva or white blood cells using standard high-salt methods [18]. The Illumina Omni 2.5 chip was used to genotype 2.5 M SNPs in our pilot sample. Log ratio values were calculated using the normalized intensity for each marker R using the software GenomeStudio.

The chromosome 22 CNV analysis was performed using SNP and Variation Suite (SVS) version 8 (Golden Helix) implementing a segmentation-based algorithm [19]. A confirmation analysis was

performed with PennCNV. PennCNV, which is a free software tool for CNV detection from SNP genotyping arrays, performs a hidden Markov model that integrates multiple sources of information to conclude CNV calls for individual genotyped samples [20]. However, Golden Helix SNP and Variation Suite (SVS) applies a segmentation algorithm using only the signal intensity data to detect CNVs on either a univariate or multivariate basis [21]. The univariate method considers only 1 sample at a time and was designed for detecting rare and/or large CNVs. However, the multivariate method considers all samples simultaneously and was designed for detecting small and common CNVs [19]. Both algorithms can handle the Illumina array.

In this study, suicide attempt lifetime was the main grouping variable. We used the number of deletion events on chromosome 22 as genetic predictors. Approximately 33,000 probes were used to scan chromosome 22 for CNV. SVS CNV analysis was performed using the univariate method considering only one sample at a time. We performed the SVS Copy Number Analysis Module (CNAM) segmenting algorithm to determine deletion events across chromosome 22. The array was run in duplicate for 4 subjects.

The nonparametric Mann-Whitney U test was used to compare the total number of chromosome 22 deletions detected in suicide attempters and non-attempters. Presence and absence of deletion events were compared between suicide attempters and non-attempters by Pearson's χ^2 test. The statistical analyses were performed using IBM SPSS Statistics version 24.

Results

We have analyzed 263 subjects with respect to the chromosome 22 deletions. We analyzed 4 subjects in duplicate to confirm the reliability of the deletion analysis. SVS CNV analysis showed 164 subjects with at least one

Fig. 2. The total number of deletion events using the CNAM analysis (SVS) and HMM model (PennCNV) in our sample ($N = 263$). There were more subjects with deletions than without. CNAM, Copy Number Analysis Module; HMM, hidden Markov model.

Fig. 3. Length distribution of deletions. Several large deletions were identified in SVS CNV analysis (left). PennCNV confirmed a few of the large deletion events (right).

deletion on chromosome 22, while the analysis with PennCNV results in 166 subjects with at least one deletion on chromosome 22 (Fig. 1). There were 99 subjects in SVS CNV analysis versus 97 subjects in the PennCNV analysis with no deletions on chromosome 22 (Fig. 1).

When we compared subjects with deletion and without deletion in suicide attempters and nonattempters, we found no significant difference ($\chi^2 = 0.03$; 1 df, $p = 0.42$).

There were no differences between attempters and non-attempters in terms of number of deletion events using the Mann-Whitney U test ($z = -0.327$; $p = 0.744$).

We also looked at the number of deletion for each subject. The maximum number of deletions per subjects detected by SVS was 18; however, the maximum number of deletions detected by PennCNV was 69 (Fig. 2). Although there were 80 subjects with a single deletion in SVS CNV

analysis, the number of subjects with single deletion we found by PennCNV was 62. The distribution of the length of the deletions in the SVS CNV analysis indicated several large duplications; however, only a few of them were confirmed by PennCNV (Fig. 3). When we looked at the size of the deletion between attempters and nonattempt-

ers, we found no significant difference ($z = -0.570$; $p = 0.569$). Figures 4–8 show our CNV analysis in suicide attempters and nonattempters separately.

Tables 1 and 2 show the demographic characteristics of our sample. Age ($p = 0.017$), duration of illness ($p = 0.002$), number of hospitalizations ($p = 0.00005$), and number of psychotic episodes ($p = 0.0003$) were significantly different between suicide attempters and non-attempters (Table 2). Among the clinical characteristics, only family history of suicidal behavior was significantly associated with suicide attempt (Table 3). We also analyzed the effect of several covariates on the number of deletion events. Our results suggest that the white European ethnicity has some effect on the number of deletions detected by both algorithms, SVS ($p = 0.02$) and PennCNV ($p = 0.052$) (Table 4).

Fig. 4. ROC curve for deletion events detected by PennCNV and SVS. ROC, receiver operating characteristic.

Table 1. Difference between attempters and nonattempters

Characteristics	Group			
	nonattempters		attempters	
	<i>n</i>	%	<i>n</i>	%
Total sample	162	61.6	101	38.4
White European		84		87.1
Female		30.9		33.7
Employed		35.7		30.4
Unemployed		60.7		63.0
Retired		3.6		6.5
Single		86.7		73.6
Married		6.7		13.2
Divorced		6.7		13.2

Fig. 5. Analysis of subjects with and without deletion events (suicide attempters [$n = 101$]).

Fig. 6. Analysis of subjects with and without deletion events (nonattempters [$n = 162$]).

Fig. 7. Chromosome 22 deletions distribution in nonattempters ($n = 162$).

Fig. 8. Chromosome 22 deletion distribution in suicide attempters ($n = 101$).

Table 2. Demographic characteristics

	Suicide attempters		Nonattempters		<i>p</i> value
	mean	SD	mean	SD	
Age	46.39	11.91	42.43	13.55	0.017
Age at onset	21.80	7.32	23.39	7.82	0.102
Duration of illness, years	23.71	12.65	18.78	12.73	0.002
Hospitalizations, <i>n</i>	7.29	6.67	4.24	4.98	0.00005
Psychotic episodes, <i>n</i>	8.54	11.50	4.53	4.89	0.0003
Duration of untreated psychosis, months	24.27	57.81	30.46	73.08	0.486

SD, standard deviation.

Table 3. Clinical characteristics

	Suicide attempters		Nonattempters		<i>p</i> value
	<i>n</i>	%	<i>n</i>	%	
Alcohol abuse		30		34	0.139
Substance abuse		37.6		28	0.118
Marijuana		22.5		29.5	0.458
Smoker		44.6		41.6	0.641
Cigarettes per day, <i>n</i>	9.5		7.5		0.193
Family history of suicide		34.4		19.9	0.012
Family history of schizophrenia		41.9		33.3	0.201

Table 4. Effect of covariates on the number of deletion events

Variable	Spearman's correlation <i>p</i> value (SVS)	Spearman's correlation <i>p</i> value (PennCNV)
Sex	0.038	0.414
Ethnicity	0.024	0.053
Age at onset	0.841	0.565

When we compared the analysis between SVS and PennCNV, we found inconsistent results. However, most of the deletions were detected by both methods; therefore, we reanalyzed our data with only those deletions that were detected by both algorithms. Using McNemar's test, 124 subjects with deletion events and 57 subjects without deletions were detected by both algorithms. To check the sensitivity and the specificity of our algorithms, we used the receiver operating characteristic (ROC) curve in SPSS. The area under the curve (AUC) was 0.666, and the sensitivity and specificity were 76 and 58%, respectively, (Fig. 4).

PennCNV showed consistent results for 3 out of 4 subjects who were run in duplicate; however, the SVS results were consistent only for one of these subjects. With respect to the DNA extracted from saliva and blood samples, there were no differences in deletions between subjects.

Discussion

Copy number variants are deletions or duplications of a segment of DNA usually >1 kb. Evidence suggests that CNVs have a robust and reproducible impact on SCZ [13]. This study is the first CNV analysis that investigates chromosome 22 deletion events in suicide attempters with SCZ. With respect to suicidal behaviors, only 2 studies performed CNV analysis [22, 23]. However, these studies included mood disorder subjects in which the effect of CNVs is less evident than in SCZ. This specific CNV analysis of chromosome 22 does not show differences between suicide attempters and non-suicide attempters.

In this study, SVS and PennCNV were used to investigate chromosome 22 deletion events to compare suicide attempters with SCZ and non-attempters with SCZ. Like other studies that have already described that CNV results differ depending on CNV detection methods, our CNV analysis from two different algorithms resulted in different findings [24, 25]. From these results, it was identified that in some cases, they were consistent. We used 2 different algorithms as it has been recommended using multiple CNV calling algorithms rather than only one [24]. Using multiple CNV calling algorithms can decrease the false-negative results; however, it may also increase the false-positive findings. This heterogeneity in the results from different CNV calling algorithms highlights the necessity of standardizing array data collection, quality assessment, and experimental validation [21].

For this study, two limitations were identified. One limitation was due to the number of subjects recruited. With a small number of individuals studied, it is difficult to analyze rare CNVs. The second limitation identified was that the data used for this study were from different Illumina platforms (Omni 2.5 quad and eight). This may have resulted in different findings as the CNAM algorithm seems to overestimate the size of deletion events.

Future studies should investigate copy number variants in a larger sample of schizophrenic patients assessed for suicidal behavior. This is important as no studies have tested this specific hypothesis. 22q11.2 deletion represents one of the greatest known genetic risk factors for the development of psychosis. Genes within the 22q11.2 locus have been implicated in the etiology of psychosis [14]. Given the fact that genetic studies of suicide focused mainly on SNP analysis, we aimed to look at the effect of chromosome 22 deletions in conferring risk for suicide. The location of SNPs previously associated with suicidal behavior in psychiatric patients [26] did not coincide with CNVs we studied. Although in these preliminary data we have observed no direct CNV effect, there appears to be

several confounding and experimental variables that influence deletion events and their detection. A study by Tombácz et al. [27] examined CNVs in the whole-exome sequencing of suicide victims with major depressive disorder finding no significant association.

Nevertheless, future studies may investigate CNVs in suicide attempt and ideation to determine whether the suicide phenotype is impacted by deletions and duplications across the entire genome. Moreover, since large CNVs in suicidal behavior are rare, future studies may use other techniques to increase the chance of detecting CNVs related to this phenotype [22].

Statement of Ethics

The study protocol was approved by the CAMH Institutional Research Ethics Board.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

Ali Bani-Fatemi analyzed the data and led the preparation of the manuscript. Vincenzo De Luca developed the study design, provided expert advice, and aided in manuscript revisions. The other authors helped with the data collection and the analysis.

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