

Copy number variations and risk for schizophrenia in 22q11.2 deletion syndrome

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22q11.2 Deletion Syndrome (22q11.2DS) is a common microdeletion syndrome with congenital and late-onset features. Testing for the genomic content of copy number variations (CNVs) may help elucidate the 22q11.2 deletion mechanism and the variable clinical expression of the syndrome including the high (25%) risk for schizophrenia. We used genome-wide microarrays to assess CNV content and the parental origin of 22q11.2 deletions in a cohort of 100 adults with 22q11.2DS (44 with schizophrenia) and controls. 22q11.2DS subjects with schizophrenia failed to exhibit *de novo* CNVs or any excess of novel inherited CNVs outside the 22q11.2 region. There were no significant effects of parental origin of the 22q11.2 deletion, deletion length, parental age or family history on expression of schizophrenia. There was no evidence for a general increase of *de novo* CNVs in 22q11.2DS. A novel finding was the relative paucity of males with *de novo* 22q11.2 deletions of paternal origin ($P = 0.019$). The Y chromosome may play a mediating role in the mechanism of 22q11.2 deletion events during spermatogenesis, resulting in the previously observed excess of maternal *de novo* 22q11.2 deletions. Hemizyosity of the 22q11.2 region appears to be the major CNV-related risk factor for schizophrenia in 22q11.2DS. The results reinforce the need for further efforts to identify specific molecular mechanisms underlying this expression and to identify the 1% of patients with schizophrenia who carry 22q11.2 deletions.

INTRODUCTION

22q11.2 deletion syndrome (22q11.2DS), also known as velocardiofacial syndrome or DiGeorge syndrome (MIM nos 188400, 192430), is the most common microdeletion syndrome, occurring in approximately one in every 4000 live births (1). *De novo* 22q11.2 deletions (approximately 90%) are more common than inherited deletions (approximately 10%) in newly diagnosed patients. The high recurrence rate of these mutations is related to the presence of low-copy repeat sequences, which predispose the 22q11.2 region to structural rearrangement (2,3). A slight excess of *de novo*

22q11.2 deletions arising on the maternally inherited chromosome is unexplained (4,5).

The 22q11.2DS phenotype is highly variable with features including velopharyngeal insufficiency, congenital cardiac defects and learning difficulties. Most 22q11.2DS cases, however, share similar 3 Mb hemizygous microdeletions, suggesting that factors other than deletion length might contribute to variable clinical expression (6,7).

Adults with 22q11.2DS have a rate of schizophrenia approaching 25%, whereas in the general population approximately 1% of schizophrenia is associated with 22q11.2 deletions (8,9). It is likely that there are elevated rates of

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Table 1. 22q11.2 deletions in 94 adult probands with 22q11.2 deletion syndrome (22q11.2 DS)

Deletion type	Current study Subjects with 22q11.2DS (<i>n</i> = 94 probands)		Estimates of 22q11.2 deletion breakpoints using Affymetrix NspI 250K SNP array and 3 algorithms (NCBI Build 35)				Clinical FISH probe ^b	Previous study qPCR (<i>n</i> = 44) ^c (7)
	Total	Confirmed origin of 22q11.2 deletion	Proximal start point	Distal end point	Minimum length (Mb)	Similar results		
<i>n</i>	%	Inherited ^a (<i>n</i> = 5)					<i>De novo</i> (<i>n</i> = 65)	
Short variant	1	1	1	16, 323, 500	18, 285, 270	2.0	-/+	-
Long nested	1	1		17, 269, 780	19, 067, 004	1.8	-/+	Yes (<i>n</i> = 1)
Short nested	3	3	2	17, 269, 780	18, 697, 764	1.4	-/+	-
Short proximal variant 1	1	1	1	17, 412, 288	18, 697, 764	1.3	-/+	-
Proximal variant 1 ^a	2	2	1	17, 412, 288	19, 786, 161	2.4	-/+	Yes (<i>n</i> = 2)
Proximal variant 2	1	1	1	17, 490, 048	19, 786, 161	2.3	-/+	-
Atypical 1 ^b	1	1	1	17, 774, 335	19, 786, 161	2.0	+/+	Yes (<i>n</i> = 1)
Atypical 2 ^b	1	1	1	18, 147, 862	19, 786, 161	1.6	+/+	Yes (<i>n</i> = 1)
Typical ^c	83	88	4	17, 269, 780	19, 786, 161	2.5	-/+	Yes (<i>n</i> = 39) ³

Typical start and end points of 22q11.2 deletions are shaded. Recurrent start and end points are shown in bold.

^aOne subject was assumed to have inherited a proximal variant 1 deletion (DNA unavailable for copy number variation (CNV) analysis from the mother with 22q11.2 DS but clinical fluorescence *in situ* hybridization (FISH) results had confirmed a 22q11.2 deletion). Four inherited typical 22q11.2 deletions were confirmed with CNV results available from parents.

^b-/+ Denotes hemizygous 22q11.2 deletion on standard clinical FISH testing using TUPLE1 or N25 probe. +/+ Denotes 22q11.2 deletion not detected on clinical FISH testing. Two atypical 22q11.2 deletions were confirmed with FISH using probe COMT in a quantitative polymerase chain reaction (qPCR) study (7). Confirmatory FISH data were thus available for all 100 subjects with 22q11.2DS (94 probands, five transmitting parents, one half sibling).

^cqPCR results using 21 markers available for the 39 subjects in the current study with apparently typical 22q11.2 deletions showed that 34 subjects had the same typical 22q11.2 deletion and five had variant distal breakpoints beyond the resolution of the single nucleotide polymorphisms (SNPs) in this study (7). The SNP array study provided more details on proximal breakpoints but less details on distal breakpoints than the qPCR study (7).

spontaneous mutations in schizophrenia in the general population (10). In addition to recurrent 22q11.2 deletions, recent reports (11–14) provide some evidence for elevated rates of rare novel CNVs and/or recurrent CNVs in schizophrenia. These studies suggest that CNVs at loci other than 22q11.2 may contribute to the expression of schizophrenia in 22q11.2DS. To test this hypothesis and to examine characteristics of the 22q11.2 deletion itself, we scanned the genomes of a cohort of 100 Canadian adults diagnosed with 22q11.2DS.

RESULTS

Genome-wide copy number variation

Table 1 summarizes the 22q11.2DS-related deletions observed in this study. Table 2 summarizes CNVs detected elsewhere in the genome. We found no evidence for an excess of CNVs or any specific targeted CNV regions in subjects with 22q11.2DS and schizophrenia, or in 22q11.2DS overall compared with controls. We were also able to compare the CNV content between 65 unaffected parents in whom *de novo* 22q11.2 deletions were confirmed not to have arisen and 53 unaffected parents on whose chromosome 22 the 22q11.2 deletions were confirmed to have arisen as *de novo* events. These analyses showed no significant differences between these two groups of parents in number, size and proportion of losses of genome-wide CNVs (Supplementary Material, Table S1).

There was only one confirmed *de novo* CNV outside the 22q11.2 region (a 636 kb gain at 4q35.1) that occurred in a non-psychotic 22q11.2DS proband. This is within the expected rates, given a *de novo* mutation rate of approximately 1% at

this resolution of analysis in control populations (15). Inspection of approximately 100 inherited CNVs in subjects with 22q11.2DS revealed on average fewer regions of interest in this study than in our recent study of autism spectrum disorders (16). There was a novel recurrent/overlapping CNV at 18p11.31 in four subjects with 22q11.2DS and schizophrenia, three with 8.5–10 kb losses that were not validated with quantitative polymerase chain reaction (qPCR) and one with a confirmed 338 kb gain inherited from an unaffected parent. Although a possible region of interest for psychotic disorders (17,18), the CNV region contained no genes. Supplementary Material, Tables S2 and S3 show all CNVs, besides the major 22q11.2 deletion, in subjects with 22q11.2DS and their unaffected parents, respectively.

22q11.2 deletions and related copy number variations

The majority (83/94; 88.3%) of probands with 22q11.2DS had the common 3 Mb 22q11.2 deletion. The other 11 probands carried one of eight variants of the 22q11.2 deletion (Table 1). Single nucleotide polymorphism (SNP) array results broadly agreed with qPCR results previously published for 44 of the subjects (7) (Table 1). Subjects with schizophrenia carried the typical (39/44; 88.6%) or one of the three variant 22q11.2 deletion breakpoints.

We also examined the 22q11.2 deletion region for CNVs that could predispose to deletion formation. As expected in this region, there were polymorphic CNVs in some parents, including two on whose chromosome the deletion had arisen. A rare 142 508 bp loss (17 269 780–17 412 288), involving the typical 22q11.2 deletion's proximal breakpoint, was found

Table 2. Genome-wide copy number variants (CNV) in 22q11.2 deletion syndrome (22q11.2 DS) with and without schizophrenia and in unaffected parents

	250K Affymetrix array 22q11.2DS study						Unaffected parents ^a (n = 122)	500K Affymetrix array (13) Popgen controls (n = 500)			
	22q11.2DS patients ^a (n = 95)			22q11.2DS Schizophrenia versus non-psychotic ^b				All 22q11.2DS patients versus unaffected parents ^b			
	All (n = 95)	Schizophrenia (n = 42)	Non-psychotic (n = 53)	Statistic	df	P		Statistic	df	P	
Total number of CNV	494	209	285				657			3695	
Stringent ^b	216	91	125				286			1558	
Average CNV per genome	5.2	5	5.4				5.4			7.4	
Stringent ^b	2.3	2.2	2.4	z = -1.20	-	0.2	2.3	z = -0.43	-	0.7	3.1
Median size in kb (range)	177 (3–1987)	177 (3–1987)	181 (3–1987)				194 (2–2731)			151	
Stringent ^b	212 (14.8–1987)	211 (24.7–1987)	215 (14.8–1987)	z = -0.36	-	0.7	206 (3–2731)	z = -0.51	-	0.6	224
Number >1 Mb CNV (% of total)	45 (9.1%)	19 (9%)	26 (9.1%)				72 (10.9%)			343 (9%)	
Stringent ^b	28 (13%)	13 (14.3%)	15 (12%)	$\chi^2 = 0.24$	1	0.6	46 (16.1%)	$\chi^2 = 0.95$	1	0.3	250 (16%)
Losses, % of total	36%	35.4%	36.5%				37.6%			41%	
Stringent ^b	40.3%	36.3%	43.2%	$\chi^2 = 1.05$	1	0.3	40.6%	$\chi^2 = 0.004$	1	1.0	30%
Number of novel CNV (% of total)	100 (20.2%)	42 (20.1%)	58 (20.4%)				117 (18.1%)			690 (19%)	
Stringent ^b [number of different loci involved]	39 (18.1%) [39]	18 (19.8%) [18]	21 (16.8%) [21]	$\chi^2 = 0.32$	1	0.6	44 (15.4%) [43]	$\chi^2 = 0.64$	1	0.4	237 (15.2%) [219]

^aAll 22q11.2 deletions shown in Table 1 were excluded as CNV in the 22q11.2DS patient group. No unaffected parent had a 22q11.2 deletion as shown in Table 1. CNV data for Popgen controls (13) are provided for comparison. Sample derivations are described in the text.

^bAnalysis using stringent data set, i.e. called using more than one algorithm or array (see Materials and Methods for further details).

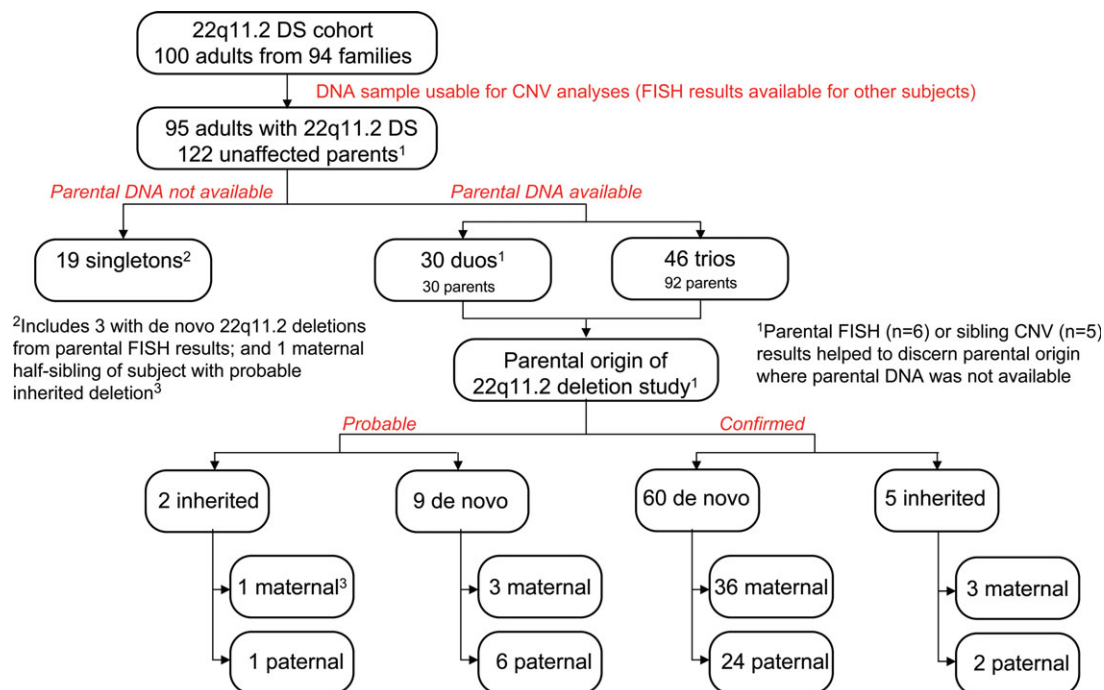


Figure 1. 22q11.2DS, copy number variants (CNVs) and parental origin of deletion. Summary of samples used for genome-wide CNV analyses and of results for analyses of parental origin of 22q11.2 deletions. Confirmed results for parental origin of deletion are based on CNV and fluorescence *in situ* hybridization (FISH) data for 46 trios and 19 duos. In six cases, FISH results allowed inference of a *de novo* ($n = 5$) or inherited ($n = 1$) 22q11.2 deletion when parental DNA was unavailable and, in five cases CNV results for siblings helped confirm parental origin. Probable results are based on CNV and FISH data for 11 duos, supplemented with clinical data for the parent whose DNA was unavailable for study.

with one algorithm in a proband and unaffected father on whose chromosome arose the 22q11.2 deletion with an atypical proximal breakpoint about 360 kb distal at 17 774 335 (Atypical 1, Table 1). The rarity of this CNV in control populations (0.2–0.8%) (16,19) makes it possible that it was a factor in the origin of this atypical deletion which is estimated to comprise <1% of all 22q11.2 deletions identified (6). However, there was no evidence that this or any other CNV in the 22q11.2 region predisposed to other 22q11.2 deletion events.

Parental origin of 22q11.2 deletions and expression of schizophrenia

Expression of schizophrenia was not related to inherited or *de novo* status of 22q11.2 deletions. Of the probands with inherited 22q11.2 deletions (Fig. 1), three of seven (42.9%; one confirmed, two probable) had schizophrenia. Of the 65 probands with confirmed *de novo* deletions (Table 1), in whom parental origin of deletion could be resolved for 60 (Fig. 1), there were no significant differences in parental origin of *de novo* 22q11.2 deletion between the schizophrenia (14/26, 53.9% maternal) and non-psychotic groups (22/34, 64.7% maternal; $\chi^2 = 0.72$, $df = 1$, $P = 0.39$), or in mean intellectual level (IQ) between probands with maternal or paternal origin of *de novo* 22q11.2 deletion (70.8, SD 7.9 versus 71.9, SD 9.5, respectively; $t = -0.48$, $df = 22$, $P = 0.63$). IQ results were similarly non-significant within the schizophrenia subgroup (data not shown).

Other factors examined also showed no evidence that they significantly affected risk for schizophrenia in 22q11.2DS.

In first-degree relatives, with fluorescence *in situ* hybridization (FISH) data confirming no 22q11.2 deletion, there was a family history of psychotic illness in two of 44 (4.5%) subjects with schizophrenia and one of 56 (1.8%) in the non-psychotic group. There were no significant differences between these groups in maternal or paternal age (data not shown).

Sex distribution and parental origin of 22q11.2 deletions

Table 3 shows the sex distribution of probands with confirmed *de novo* 22q11.2 deletions and known parental origin. As previously observed in 22q11.2DS, we found a non-significant excess of *de novo* maternal deletions (60%; $P = 0.12$). Further analyses showed that this was associated with sex of the offspring, with males significantly less likely than females to have a *de novo* 22q11.2 deletion of paternal origin [relative risk (RR) = 0.44, 95% CI = 0.20–0.94; $P = 0.019$]. This appeared to be due to an unexpected paucity of males with deletions arising on paternally inherited chromosomes (Table 3). Results were similar for an expanded sample (Fig. 1) including nine subjects with 'probable' *de novo* origin of 22q11.2 deletion (RR = 0.47, 95% CI = 0.25–0.91; $P = 0.014$). Similar rates of subjects with schizophrenia were present in all four of the possible groups (data not shown) and there was no significant difference in sex distribution between the schizophrenia (20 male, 24 female) and non-psychotic (24 male, 32 female) 22q11.2DS groups ($\chi^2 = 0.07$, $df = 1$, $P = 0.80$).

We expanded our examination of skewed sex distribution to include unaffected full sibling live births. Tests of equal

Table 3. Confirmed parental origin of *de novo* 22q11.2 deletions and sex distribution in 22q11.2 deletion syndrome (22q11.2 DS)

Proband with 22q11.2DS	Parental origin of <i>de novo</i> 22q11.2 deletion				Analyses							
	Maternal		Paternal		Chi-square of equal proportions of parental origin			Relative risk (RR) for maternal origin of deletion in males		Overall chi-square of parental origin by sex		
	<i>n</i>	(%)	<i>n</i>	(%)	χ^2	df	<i>P</i>	RR	95% CI	χ^2	df	<i>P</i>
Males	20	(33.3)	6	(10.0)	7.54	1	0.006	1.63	1.08–2.47	5.48	1	0.019
Females	16	(26.7)	18	(30.0)	0.12	1	0.73					
Total (<i>n</i> = 60)	36	(60.0)	24	(40.0)	2.40	1	0.12					

proportions showed no significant differences in the six families with male probands of paternal *de novo* origin 22q11.2 deletions (three brothers, five sisters), 18 families with female probands of paternal origin (18 brothers, 21 sisters), 16 with female probands of maternal origin (14 brothers, 16 sisters) and 20 families of male probands of maternal origin (18 brothers, 14 sisters). There were also no significant differences in maternal or paternal age between subjects with maternal or paternal *de novo* origin of deletion overall or when male and female probands were examined separately (data not shown).

DISCUSSION

Our results suggest that the expression of schizophrenia in individuals with 22q11.2DS primarily arises from effects related to the 22q11.2 deletion itself. There was no indication that other CNVs elsewhere in the genome or that the parent of origin of *de novo* 22q11.2 deletions influenced the schizophrenia phenotype. An interesting observation was that the previously observed maternal excess of 22q11.2 deletions appears to be related to a relative paucity of 22q11.2 deletions of paternal origin in males, that is, in those deletions arising during spermatogenesis and segregated with a Y chromosome in the resulting sperm.

Expression of schizophrenia in 22q11.2DS

In contrast to the recent reports for schizophrenia in the general population (11–14), we found no evidence for an overall increase in novel genome-wide CNVs in 22q11.2DS. There was also no indication in our sample that specific recurrent CNVs, other than 22q11.2 deletions, were associated with expression of schizophrenia. While some rarer CNVs may be involved in increasing risk for schizophrenia in individual cases in the general population (11,13,14,20), these do not appear to be a necessary additional mechanism in the expression of schizophrenia in individuals with 22q11.2 deletions. We found no significant effects of parental origin of the 22q11.2 deletion, deletion length, paternal age or family history of psychotic illness on developing schizophrenia in 22q11.2DS. Our results are consistent with those showing no relationship of parental origin of *de novo* 22q11.2 deletions and expression of attention deficit hyperactivity disorder or obsessive–compulsive disorder in 22q11.2DS (21). Previous results using small samples that have suggested a parental origin of deletion effect on neuropsychiatric expression (22) are likely to be spurious. Collectively, these results provide

no support for imprinting effects on neuropsychiatric expression in 22q11.2DS (23). Hemizyosity of the 22q11.2 region appears to be the main factor in neuropsychiatric expression in the syndrome (9).

Notwithstanding our negative statistical results, we compared individual CNVs in our 22q11.2DS sample to those reported in recent studies of schizophrenia from the general population that used various ascertainment strategies but similar CNV detection methods and diagnostic criteria (DSM-IV) (11–14) (Supplementary Material, Table S4). We were particularly interested in determining any overlap between individual non-22q11.2 deletion CNVs in our 22q11.2DS subjects with schizophrenia and those reported in these studies. Five subjects with schizophrenia from our sample had CNVs that shared some similarities in position, though not necessarily loss/gain status or length, to three CNVs reported in these studies. One 22q11.2DS subject with schizophrenia had a 1.48 Mb loss at 15q13.2–15q13.3 (chr 15:28,748,100–30,231,500) that was inherited from an unaffected parent. This CNV, in a region flanked by segmental duplications, has previously been reported in controls (chr 15:28,393,128–30,740,356; variation_8795) (24) as has a large inversion (chr 15:28,524,307–30,602,466) in this region (25). The CNV we observed was similar in position but approximately 100 kb smaller than the 1.54–2.47 Mb losses reported in 15 patients with schizophrenia and eight controls in two multi-site studies with slightly overlapping samples (13,14). Interpretation of these findings will be aided by further data on ethnicity of samples and the phenotype of controls and inheritance of this CNV in schizophrenia. Three subjects with 22q11.2DS and schizophrenia had large (>1.6 Mb) losses at 15q11.2, one of which was inherited from an unaffected parent. We found similar CNVs in 20 (16.4%) unaffected parents who did not transmit this CNV to the offspring with 22q11.2DS and in one non-psychotic proband with 22q11.2DS. These 15q11.2 CNVs show partial overlap with the proximal end of a 471 kb loss observed in 26 cases with schizophrenia and 79 controls in one study (13). Both the large >1.6 Mb and more distal 471 kb CNVs have previously been reported in controls (24). Finally, one subject with 22q11.2DS and schizophrenia had inherited from an unaffected parent a novel 141 kb gain in a 2q31.3 region with no genes but contained within the extent of a 2.5 Mb *de novo* loss reported in a patient with childhood-onset schizophrenia (11). These comparisons with the literature do not seem sufficiently compelling to modify our overall conclusions with respect to CNVs, other than the 22q11.2 deletion, and expression of schizophrenia in 22q11.2DS.

What then underlies the variable expression of schizophrenia in 22q11.2DS? Altered expression of several genes within the 22q11.2 region may be necessary to increase susceptibility of schizophrenia in 22q11.2DS (26–28). There is some evidence that SNPs in certain genes within the 22q11.2 deletion region on the intact homologous chromosome may be involved. For example, we have found significant association with schizophrenia and the *PIK4CA* gene in 22q11.2DS (29) using the same SNPs as those found to be associated in a general population sample of schizophrenia (30). This is in contrast to the negative findings of the COMT functional allele and schizophrenia in 22q11.2DS (31). Other small genetic variants in candidate genes for schizophrenia elsewhere in the genome may also modify the large effects of the hemizygous 22q11.2 deletion, as shown for other phenotypic features of 22q11.2DS (32,33). There also remains the possibility that various individual CNVs increase the likelihood of expressing schizophrenia in 22q11.2DS.

Origin of *de novo* 22q11.2 deletions

We found that males with 22q11.2DS who had a maternal origin of a *de novo* 22q11.2 deletion were represented in a similar proportion to females with either maternal or paternal origin of deletion, but that males had a significantly lower chance of having a paternal origin of the deletion. This is consistent with the meta-analyses of previous studies (4,5) and our own results showing an excess of *de novo* deletions of maternal origin in 22q11.2DS. Previous studies have not consistently documented the sex distribution of patients however, hampering further comparisons. Larger samples will be needed to replicate the paucity of paternal origin of *de novo* 22q11.2 deletions in males which, although significant, could be due to chance in a relatively small sample.

Explaining this finding is difficult. The mechanism underlying deletions in 22q11.2DS mainly involves non-allelic homologous recombination (NAHR) between misaligned low copy repeats during gametogenesis (4,34). It is possible that there is a female bias in NAHR causing *de novo* 22q11.2 deletions (5). Our results suggest however that *de novo* 22q11.2 deletions may occur with approximately equal frequency in oogenesis and during spermatogenesis, but that somehow the Y chromosome affects the likelihood of observing a 22q11.2 deletion. There is a sequence homology between the Yq12 region and the low copy repeats, LCR22-2 and LCR22-4, that are most commonly involved in the 22q11.2 interchromosomal exchanges and NAHR that give rise to 22q11.2 deletions. These regions share a 2.4 kb human satellite sequence subclass involving AT-rich tripartite repeats (35). Babcock *et al.* (35) have posited that these repeat elements may play a structural role in mediating NAHR between LCR22-2 and LCR22-4 if the presence of the Yq12 repeat prevents recombination between the LCRs, thereby enhancing meiotic stability of the 22q11.2 region. Such a mechanism could be related to physical proximity of these heterochromatic regions in the nuclear periphery (35,36). The report of a subject with 22q11.2DS resulting from a *de novo* Y;22 translocation (37) may be consistent with interchromosomal interaction between these chromosomes. To explain the possibility that, in sperm with a 22q11.2 deletion

chromosome, there may be an elevated ratio of those with an X to those with a Y chromosome, however, would appear to implicate the segregation process, as a spermatocyte with a *de novo* 22q11.2 mutation would have both sex chromosomes present. One could speculate that proximity of the Y chromosome to the deletion chromosome 22 could interfere with normal segregation and/or error correction, resulting in an unbalanced, non-viable sperm (38), e.g. with two deletion 22q11.2 chromosomes.

Studies of sperm could help investigate possible mechanisms. Further studies of NAHR and allelic homologous recombination (AHR, i.e. normal meiotic recombination) in 3-generation families are also needed to determine whether polymorphic size of LCRs in the 22q11.2 region (39) or clustering of recombination events within certain families and high rates of AHR in the 22q11.2 region in female meiosis (5) may also play a role in the observed sex distribution of *de novo* 22q11.2 deletions.

Advantages and limitations

This is the largest sample of adults with 22q11.2DS, and one of the largest ever investigated with respect to the parent of origin of *de novo* 22q11.2 deletions (5). Nevertheless, there were only six men with confirmed paternal *de novo* origin of the deletion. There were fewer fathers than mothers available in parent–offspring pairs but a systematic ascertainment bias appears unlikely to explain the results. Although including the largest number of patients with 22q11.2DS-schizophrenia yet studied, our sample size was relatively limited. However, if there were a three-fold excess of genome-wide novel or rare CNVs as proposed in a recent study (11), our sample would have had sufficient power to show this. Few subjects (four of 56, 7.1%) in the non-psychotic group were younger than the median age at onset of psychosis (20.5 years) and there were no trends for differences between the schizophrenia and non-psychotic subgroups on any CNV-related parameter (Table 2), suggesting that misclassifying some subjects as non-psychotic would not have materially affected the results.

CONCLUSIONS

In summary, hemizyosity of the 22q11.2 region seems to confer the major CNV risk for the expression of schizophrenia in 22q11.2DS. While further investigations are required to determine molecular risk factors that mediate *de novo* 22q11.2 deletion events during gametogenesis, the results reinforce the need for genetic counseling of individuals with 22q11.2DS about the risk for schizophrenia and further efforts to identify the 1% of patients with schizophrenia who carry 22q11.2 deletions.

MATERIALS AND METHODS

Subjects with 22q11.2DS

We diagnosed 100 Canadian adults (44 male, 56 female) with 22q11.2DS originating from 94 families; six families had two affected subjects (five parent–offspring pairs and one half-sibling pair). All subjects met clinical screening

criteria for 22q11.2DS in adults (40) and had confirmed chromosome 22q11.2 microdeletions using FISH and probes from the 22q11.2 region (41) (Table 1). Observed ethnicity of probands was 85/94 (90.4%) European, three of 94 (3.2%) African or mixed African, three of 94 (3.2%) Asian and three of 94 (3.2%) other origins, consistent with the genotype data. Probands (median age 31.4; range 17.6–56.3) were ascertained as previously described (8), many through psychiatric sources or referrals, ensuring a high rate of schizophrenia. Informed consent was obtained and the study was approved by the Research Ethics Boards of the University of Toronto, Centre for Addiction and Mental Health and University Health Network.

All 22q11.2DS subjects had direct assessments, review of medical records and information from relatives (8,31,42). Lifetime psychiatric diagnoses were obtained by research psychiatrists using standard methods and DSM-IV criteria (31). Subjects with schizophrenia ($n = 37$), schizoaffective disorder ($n = 6$) and psychotic disorder, not otherwise specified ($n = 1$), met the criteria for major psychotic disorders [mean age at onset 20.9 (SD 5.4) years], collectively termed 'schizophrenia' for this study. The 56 other subjects comprised the non-psychotic subgroup. Intellectual level was assessed using standard methods to obtain Full Scale IQ ($n = 86$) or IQ estimates ($n = 8$) (43). As expected (43), subjects with schizophrenia were significantly older (38.3, SD 9.8 versus 30.6, SD 10.6 years; $t = 3.74$, $P < 0.001$) and had lower mean IQ (68.6, SD 9.7 versus 73.0, SD 10.2; $t = -2.16$, $P = 0.03$) than those in the non-psychotic group.

Parents

We included data for as many of the biological parents of this adult sample as possible; no parental data existed for four probands who were adopted. Mean paternal and maternal ages at birth for 96 subjects with 22q11.2DS were 31.3 (SD 5.8) and 27.9 (SD 4.7) years, respectively. FISH data confirmed the absence of a 22q11.2 deletion in 73 mothers and 64 fathers. Extensive family history data from multiple sources provided information on the demographics and health status of first-degree relatives, including common phenotypic features of 22q11.2 DS.

Copy number variation detection

We genotyped each available DNA sample [99 subjects with 22q11.2DS; 122 unaffected parents (69 mothers, 53 fathers); five unaffected siblings] for approximately 250 000 SNPs with the Affymetrix GeneChip® Human Mapping 250K NspI Array according to established protocols (16,39,44). Duos (proband and one parent) and trios (proband and both parents) comprised the sample sets used to investigate parental origin of *de novo* 22q11.2 deletions and inheritance of genome-wide CNVs (Fig. 1).

NspI array scans were analyzed for CNV content with three algorithms: DNA Chip Analyzer (dChip) (45,46), Copy Number Analysis for GeneChip (CNAG) (47) and Genotyping Microarray based CNV Analysis (GEMCA) (16,48). To minimize potential batch effects, analysis with dChip was performed in groups of approximately 50 probands/subjects according to

when the arrays were hybridized. For CNAG version 2.0, we set the reference pool to include all samples and performed an automatic batch pairwise analysis with sex-matched controls. For GEMCA, we used two designated DNA samples (NA10851 and NA15510) as references for pairwise comparison with all proband experiments. We further filtered these results by including only those CNVs that were common to both pairwise experiments. In all instances, CNVs were merged if they were detected in the same individual by more than one algorithm with the outside probe boundaries (16). In general, CNAG Merged (5.6/genome) and GEMCA (5.5/genome) algorithms show more CNVs per genome than dChip Merged (3.0/genome) (16). We report all data but in analyses we used a 'stringent' data set, i.e. CNVs called by more than one algorithm, because these data have a high rate of validation (16).

Controls

Comparison control data comprised CNVs observed in 500 Europeans from the German PopGen project (16,49) and entries in the Database of Genomic Variants (containing 8006 CNVs at 3933 loci) (24). A CNV was considered to be 'novel' in subjects if it was ≥ 10 kb, contained at least three probes, and at least 20% of its total length was unique when compared with controls. We also compared the results to CNVs found in a cohort of 1152 non-disease controls of European origin from the Ontario population that had been analyzed using the same methods (16,19).

Parental origin of 22q11.2 deletions and other copy number variations

We used SNP data to infer the parental origin of 22q11.2 deletions for trios and a combination of SNP and FISH data for duos. Where parent of origin could not be resolved using SNP data, microsatellite marker analyses were performed using 11 markers (sequence available upon request) across the 22q11.2 region (16). For some duos, parent of origin could be determined but that parent was unavailable for genotypic study. In those cases, we estimated whether 22q11.2 deletions were probably inherited or *de novo* in origin using the available clinical data. We assigned a 'probable inherited' 22q11.2 deletion status if the unavailable parent had two or more major features of 22q11.2DS (40) and a 'probable *de novo*' 22q11.2 deletion status if the unavailable parent had none of these features and other offspring did not meet the clinical criteria for 22q11.2DS (40).

Copy number variation validation of genome-wide copy number variations

We validated all *de novo* and other potentially interesting novel CNVs (16). Position coordinates were determined using NCBI Build 35, and results were confirmed using inheritance patterns and qPCR.

Statistical analysis

Chi-square or two-sided Fisher's exact tests and estimates of relative risk with 95% CI were used to compare categorical

variables. Wilcoxon rank sum tests were used to compare ordinal data, and two-tailed Student's *t*-tests for continuous variables. Main comparisons were between schizophrenia and non-psychotic groups on genome-wide CNVs and parent of origin of *de novo* 22q11.2 deletions; we also examined parental ages at birth and family history of psychotic illness in first-degree relatives. All analyses were performed using SAS 9.1.3 (SAS Institute, Cary, NC, USA). *P*-values <0.05 were considered significant.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* Online.

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