Copy Number Variation in a Follow-up of Adults with ASD: a Behavioral Phenotype-Genotype Study

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Introduction

Autistic Spectrum Disorders (ASD) are a set of complex, early-onset neurodevelopmental disorders. Recent studies have revealed a complex genetic landscape for ASD, with many potential genes involved. Little is known about the long-term outcome of individuals with ASD who were diagnosed in childhood, and no studies to date have examined genetic correlates of their level of functioning as adults. Copy number variation appears to play an important role in ASD. An increasing number of studies have identified both inherited and de novo copy number variants in ASDs (see Cook & Scherer, 2008 for review). Microarray technology is providing the opportunity for discovery of deletions and insertions across the genome. Here we present an initial exploratory evaluation of copy number variation (CNV) in an ongoing follow-up study of adults with ASD.

Materials and Methods

Subjects were recruited from a state-wide sample of 222 children and adolescents diagnosed with DSM-III autism in the 1980's. A subset of subjects with autism and average intellectual ability in childhood were re-contacted for assessment of adult outcome (Farley 2009). Evaluations included diagnostic testing; assessments of cognitive ability and adaptive functioning; and characteristics of current living status. A composite score of overall functioning was given: Very Poor, Poor, Fair, Good, or Very Good (Howlin 2004, Farley 2009).

Genotyping was done on 10 of these individuals, first using Affymetrix 250k arrays, and then Affymetrix 6.0 arrays (with the exception of individual #10 who has not had Affy 6.0 genotyping done yet). CNV analysis was performed using Affymetrix Genotyping Console (GTC) for Affymetrix 6.0 arrays. All .CEL files analyzed were "in bounds" according to default software QC/MAPD parameters. Copy number segment reporting was conducted using a minimum of 5 markers per segment, and a minimum genomic size of 100kb per CNV segment. For 250k arrays, both the Affymetrix GTC and Golden Helix CNAM were used for CNV calls and segmenting. CEU (Utah) HapMap samples were used as controls.

Results

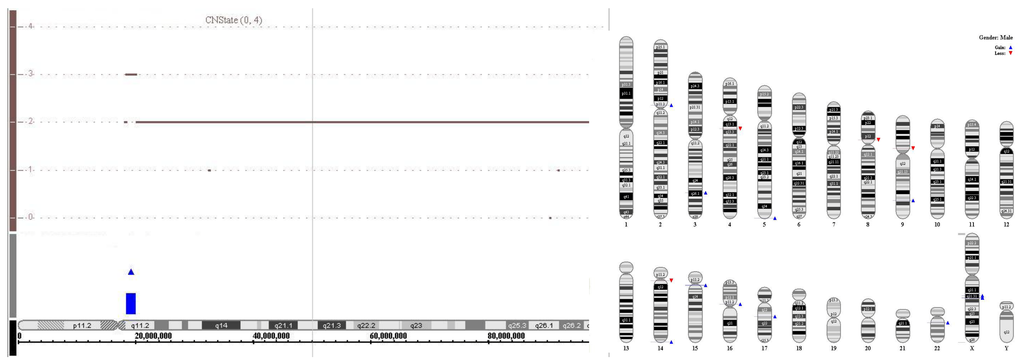
Table 1. Description of CNVs and Phenotype of 10 adults with ASD

ID	Gender	Age	Notes	Outcome*	#CNVs (6.0)	Cytoband	Size (kb)	250k GTC**	250k GH***	6.0 GTC	Ref
1	Male	26	MDD, ADHD	Fair	22	dup 15q11	603	yes	yes	yes	1
						dup 17q21	324	yes	yes	yes	2
2	Male	24	Divorced, some college	Very Good	22	dup 15q11	1378	yes	yes	yes	1
						del 17q21	133	yes	yes	yes	2
3	Male	24	College grad	Very Good	24	del 15q11	331	no	yes	yes	1
4	Male	26	MDD, ADHD, Tourette	Fair	17	dup 15q11	1023	yes	yes	yes	1
5	Male	17	Seizures		9	del 3p14	2628	yes	yes	yes	3
						dup 22q13.33	243	yes	yes	yes	4
6	Male	17	Large head circumference, IQ 39		13	dup 15q11	104	yes	yes	no	1
7	male	46	Married,High IQ, MDD		18	dup 15q11	696	yes	yes	no	1
8	Male	30	OCD, GAD, MDD	Fair	21	dup 15q13	496	yes	yes	yes	1
						del 15q11	435	yes	yes	yes	1
9	Male	44	Seizures, Bipolar, Psychosis	Good	16	dup 15q11	696	yes	yes	yes	1
						dup 17q21	249	yes	yes	yes	2
10	Female	32	Seizures, MR		N/A	17p13.1	4774	yes	yes	n/a	5

* Outcome summary based on a composite score of overall functioning: Very Poor, Poor, Fair, Good, or Very Good (Howlin 2004, Farley 2009).
** Affymetrix Genotyping Console
*** Golden Helix Copy Number Analysis Module

A number of CNVs detected in our preliminary exploration have been previously implicated in ASD or related conditions. Maternal duplications of chr15q11-13 are present in an estimated 1% of ASD (Hogart 2008). The 17q21 region has been implicated by linkage and CNV analyses of ASD (for review see Kumar 2009). The 3p14.1 region and the 17p13.1 region have both previously been implicated in ASD CNV studies (Sebat 2007, Christian et al., 2008). The duplication at 22q13.33 is a region upstream of SHANK3, a gene encoding synaptic scaffolding proteins that has been associated with ASD in a number of studies (Kumar et al., 2009).

Table 3. Copy Number Variation on Chromosome 15 of individual #4 showing a large 15q11 duplication.



Discussion

Our initial analysis reveals a high number of CNVs in these adult subjects with ASD, and many of the CNVs noted have been previously implicated in autism or related conditions. A higher percentage of CNVs than expected was found in the region of chromosome 15q11. All CNVs reported were detected using at least two software algorithms (i.e. Affymetrix GTC and Golden Helix), or on two different platforms (Affymetrix 250K and Affymetrix 6.0). Many more CNVs were detected that were not consistently seen across algorithms. As expected, the Affymetrix 6.0 platform detected more CNVs than the lower density 250K array.

Genotyping has been completed for many more individuals in our extended pedigrees with ASD; however, follow-up phenotyping as adults is pending. After re-contacting these subjects, we will use a trait-based approach to examine possible ties between specific phenotypic characteristics of autism and CNVs. We plan to confirm these initial results by further comparison of ASD CNVs with controls from other healthy populations. We also plan to validate these findings using quantitative PCR, due to the possibility of false positive results. Further analysis is also underway to determine which of these CNVs are transmitted in families, and which are de novo mutations.

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