Synaptic transmission: looking for clues to Autism Spectrum Disorders (ASD) etiology in Copy Number Variants containing synaptic genes

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Background

Copy Number Variants (CNVs) play an important role in susceptibility to ASD, often mediated by the deletion or duplication of genes involved in synaptic structure and function. Increasing evidence suggests a central role for defects in synaptic structure and function in the pathogenesis of non-syndromic ASD. In this study we tested the hypothesis of an enrichment in CNVs encompassing synaptic transmission genes in ASD.

Methodology

To test our hypothesis we first queried the information available in public databases on synaptic pathways (KEGG and GO databases) to obtain a list of 606 genes involved in synaptic transmission. We intersected this gene list with the results of a large genomic screening for CNVs, carried out by the Autism Genome Project (AGP) in 1590 Caucasian ASD patients, and CNV information on the Database of Genomic Variants (DGV) to test if the frequency of CNVs encompassing synaptic genes is higher in the AGP population than in control subjects. Both cases and controls were matched for ancestry and CNV detection methodology (SNP arrays). Further, we use this gene list to identify genes in CNVs present exclusively on the AGP dataset, when comparing with the DGV and additional control datasets (Table 1).

CNVs in ASD subjects are enriched in Synaptic genes

❖ The intersection of the synaptic pathways gene list with AGP and DGV CNV data revealed a significantly increased burden of CNVs encompassing synaptic genes in ASD patients compared to controls (Fisher's exact test $P = 2.2x10^{-16}$,OR=2.06).

❖ 45,2% of ASD cases present CNVs encompassing synaptic genes compared to 30,7% of controls. Most ASD patients and controls had only one synaptic CNV *per* individual, however 12,8% of ASD cases presented more than one CNV encompassing synaptic genes, against 5,6% in controls. (Figure 1).

❖ Closer inspection of the clinical history from a subset of 137 patients showed a co-occurrence of synaptic CNVs with brain morphology alterations in 5,1% of patients and neuropsychiatric conditions in relatives in 6,6%. For example, in one male patient diagnosed with ASD and moderate intellectual disability (ID), deletions in *PLA2G4A, AKT3, PRKACB, HTR2B, CALY* and a duplication of *CACNA1F* genes co-occurred with bilateral atrophy of the parietal brain region; another ASD patient with moderate ID presented a deletion of the *AKT3* and *HCN1* genes associated with atrophy of the right region of the hippocampus and had a brother and father with schizophrenia; a female ASD patient with moderate ID with duplications encompassing the *TH, HRAS, GNG13* and *CAMK2B* genes presented epilepsy and had a brother with ASD.

Table 2. Contingency table for burden calculation.

	AGP	DGV
CNVs encompassing synaptic genes	1024 (2.95%)	193 (1.43%)
CNVs not encompassing synaptic genes	34753 (97.05%)	13461 (98.57%)

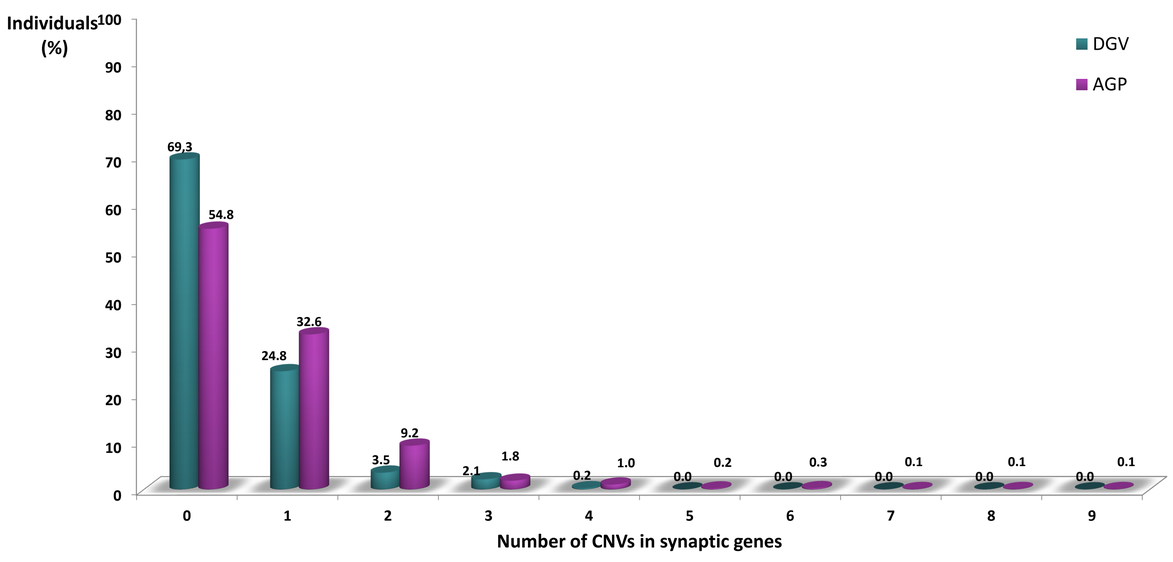

Figure1. CNVs encompassing synaptic genes *per* individual.

Conclusion

The present results confirm an excess of structural alterations encompassing synaptic genes in ASD and reaffirms the genetic complexity underlying ASD etiology. It highlights novel candidates for genomic sequencing and functional studies, as well as synapse-related pathways possibly compromised in this disorder.

REFERENCES: Pinto D. *et al.* Functional impact of global rare copy number variation in autism spectrum disorders. (2010) Nature 466: 368-372.; Krzywinski, M. *et al.* Circos: an Information Aesthetic for Comparative Genomics. (2009) Genome Res 19:1639-1645; Iafrate A.J., *et al.* Detection of large-scale variation in the human genome. Nat Genet. (2004) 36(9):949-51. KEGG pathway database: Kyoto Encyclopaedia of Gene and Genomes. Available at: http://www.genome.jp/kegg/pathway.html; The Gene Ontology Consortium. Gene ontology: tool for the unification of biology. Nat. Genet. (2000) 25(1):25-9.

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Table1. Control datasets.

Control Dataset	Number of samples	Genotyping platforms
Database of Genomic Variants[1,2,3,4]	427	Illumina HumanHap 300 BeadChip, Illumina HumanHap 550 BeadChip, Illumina Human 1M array, Affymetrix Genome Wide Human SNP array 6.0.
The Children's Hospital of Philadelphia CNV project[2]	1320	Illumina HumanHap 550 BeadChip
Study of Addiction: Genetics and Environment (SAGE)[3]	1261	Illumina Human 1M array
PopGene[4]	1123	Affymetrix Genome Wide Human SNP array 6.0
Ottawa Heart Genomics Study[4]	1234	Affymetrix Genome Wide Human SNP array 6.0
Total number of control samples	5365	--

Beyond the usual suspects

❖ A total of 91 genes that were deleted and/or duplicated exclusively in the AGP dataset were identified.

❖ Some of the synaptic genes identified in the ASD dataset CNVs have previously been described in ASD patients, like *SHANK1, GABRA5, GABRG1 GRM5, GRIN3B, GRIK2, MBP, ERBB4* and *PLCB1* (Figure 2).

❖ However, novel genes not listed as ASD-candidate or implicated genes were also identified in this dataset:

Novel genes: *PLA2G4A, PPP3CB, GNG2, ADCY7, ADCY8, ADRBK1, ADRBK2, PPP2R3C, PPP3CB.*

Genes belonging to the same gene family of ASD candidate genes: *SYN2* (Synapsin gene family)*; CHRNA4* (Superfamily of ligand-gated ion channels)*; PLCB3 (*Phosphoinositide phospholipase C beta enzyme family).

Genes implicated in other neuropsychiatric disorders: *CHRNA4, SLC6A3, SYN2, DLG2.*

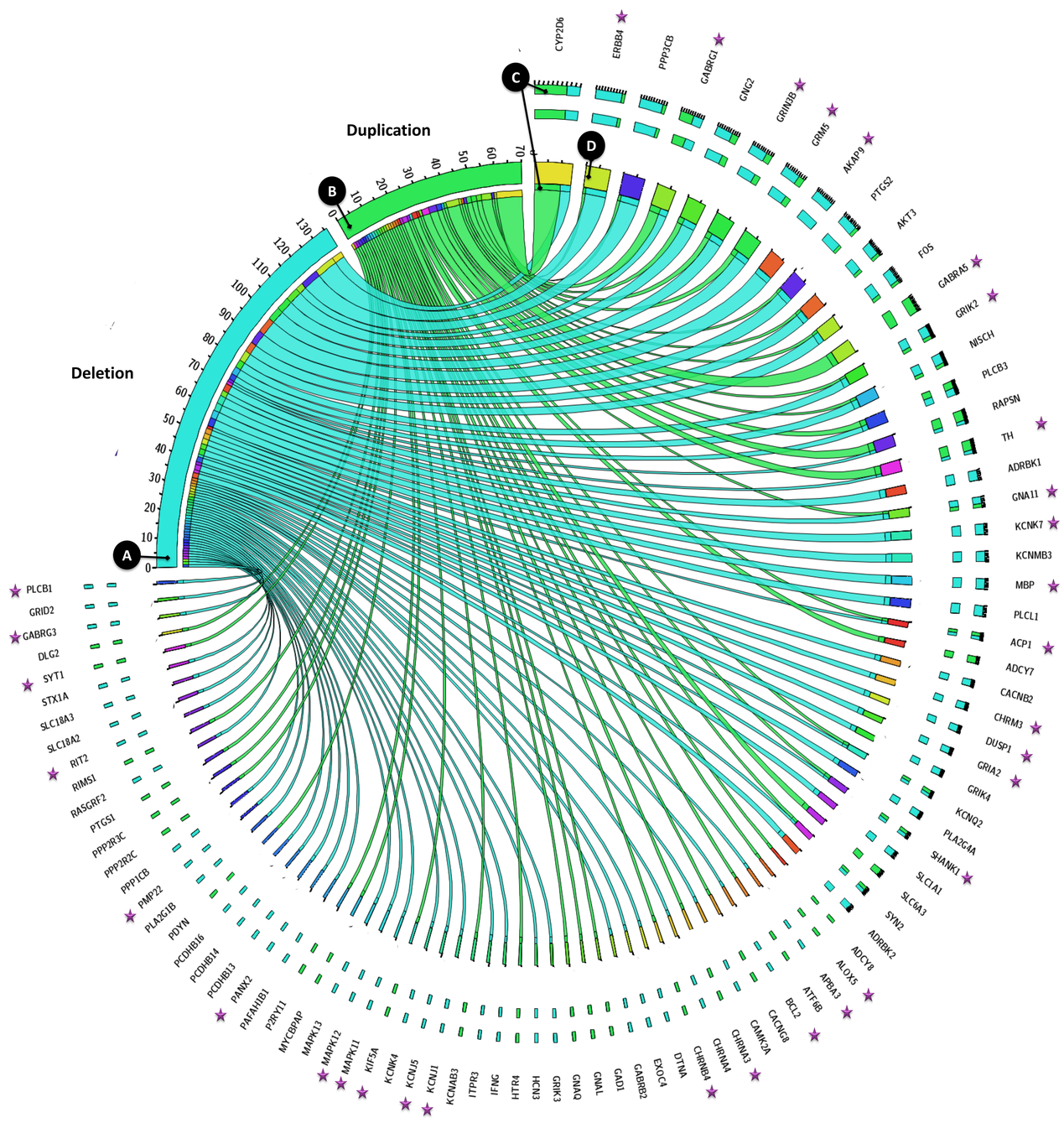

Figure2. Circus plot representing the CNVs deleting or duplicating genes involved in synaptic functions and exclusively identified in ASD probands. In a total of 206 CNVs, deletions (A) were shown to be more frequent (2:1 ratio, N=136 CNVs encompassing synaptic genes) than duplications (B) (N=70 CNVs encompassing synaptic genes). The type of structural variation *per gene* is represented by the color of the ribbon or ribbons (C) connected with gene specific segments (D) (blue for deletions and green for duplications). Genes are shown in a clockwise distribution, starting with those present in a higher number of CNVs. The purple star represents genes previously implicated in ASD etiology (according to information available in the AutismKB database on CNVs detected using SNPs microarrays, not present in control subjects and with Caucasian ancestry) that are also present in our dataset.