# COPY NUMBER VARIANTS INVOLVING COMPONENTS OF THE GLUTAMATERGIC SYNAPTIC PATHWAY IN ASD PATIENTS

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## BACKGROUND

Copy Number Variants (CNVs) play an important role in susceptibility to Autism Spectrum Disorders (ASD), in particular when deleting or duplicating genes involved in synaptic structure and function such as glutamatergic synapse genes. Identifying CNVs of etiologic relevance for ASD that include glutamatergic genes may contribute to the understanding of glutamate-related pathogenic mechanisms in this disorder.

## METHODOLOGY

To identify CNVs of potential etiologic relevance that include glutamatergic genes, we crossed the information available in public databases on synaptic pathways (Kyoto Encyclopaedia of Gene and Genomes database) and the results of a large genomic screening for CNVs, carried out by the Autism Genome Project (AGP) in 2184 ASD patients and 8000 healthy controls. In a subset of 342 Portuguese ASD probands, the most interesting CNVs were validated by quantitative or Long Range PCR and clinical correlations further explored.

## AGP POPULATION

We identified a total of 294 CNVs, absent or overlapping less than 20% with CNVs in 8000 control subjects from available databases, that duplicate or delete genes involved in the glutamatergic, dopaminergic, serotonergic, cholinergic or gabaergic synaptic pathways (Figure 1). These CNVs included 181 deletions and 113 duplications. Some genes are shared by several synaptic pathways, while others are specifically involved in the function or structure of a type of synapse. Our results show that genes shared between pathways are more prone to suffer deletions (Figure 2A). This is also true for glutamatergic, serotonergic and cholinergic pathway-specific genes, while gabaergic and dopaminergic pathway genes presented an higher percentage of duplicating events (Figure 2B).

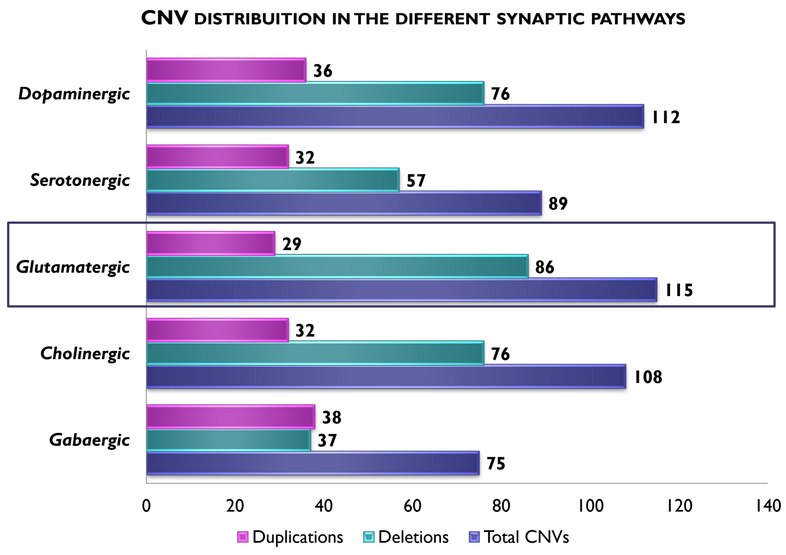


**Figure1.** CNVs deleting or duplicating genes in synaptic pathways identified in ASD probands. Glutamatergic pathway genes are the most frequently deleted, while duplications are more frequent in gabanergic genes.

## GLUTAMATERGIC SYNAPSE

Looking specifically at the glutamatergic synapse, we identified a total of 115 CNVs, including 86 deletions and 29 duplications, encompassing coding genes for proteins involved in glutamatergic neuronal excitability and synaptic plasticity processes (Figure 3 and Table 1). Of these, 20 (14 deletions and 6 duplications) were present in Portuguese probands.

We validated two *de novo* microdeletions of *GRIN3A* and *SHANK3* genes, and maternally or paternally inherited microdeletions of *GRID2* and *RGS7* genes; two duplications of the *ACDY7* gene, one *de novo* and another present in both parents, and a duplication of the *RGS4* gene with maternal inheritance. Validation of the remaining CNVs is ongoing. The probands with CNVs in *ACDY7*, *SHANK3* and *RGS4* genes had a positive family history of ASD and learning difficulties. Three patients presented intellectual disability, but none had epilepsy, although probands with the *RGS4* and *ACDY7* duplications revealed an altered neurological exam and MRI, respectively. Overall, there was no distinct clinical phenotype associated with these alterations.

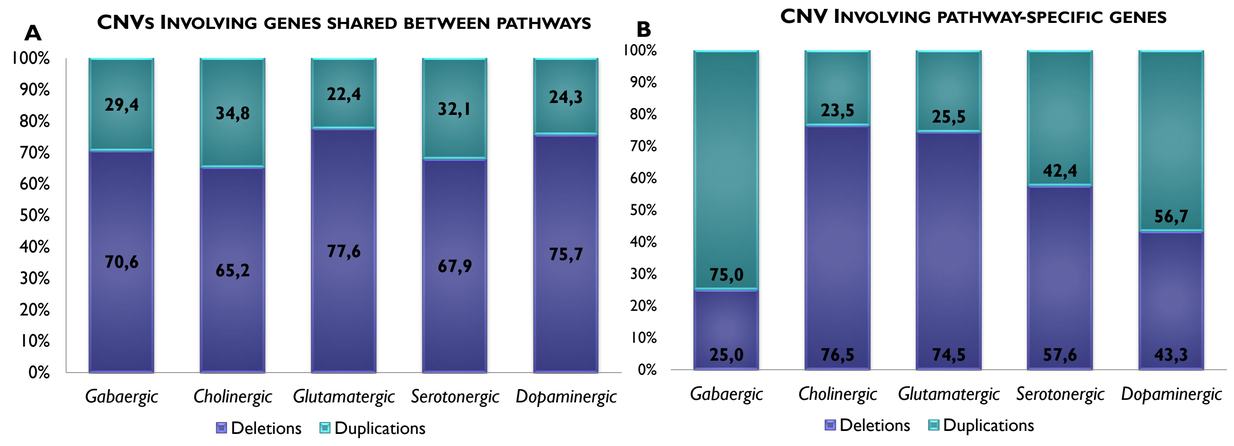


**Figure2.** CNV events involving shared (A) and pathway-specific (B) genes. A single CNV encompassing a gene that is shared between several pathways is likely to be of etiological relevance.

**Table1.** CNVs affecting genes component of the glutamatergic synapse

| PROTEIN FAMILY | DELETIONS (N) | DUPLICATIONS (N) |
|---|---|---|
| **AC** (Adenylyl cyclase class 4/guanylyl cyclase) | *ADCY2* (2) | *ADCY5* (1); *ADCY7* (2) |
| **GRK** (Protein kinase – protein-coupled receptor kinase) | *ADRBK1*(4); *ADRBK2* (2) | - |
| **GLS** (Glutaminase) | - | *GLS* (1) |
| **Gq** (G-protein – Guanine nucleotide-binding protein alpha) | - | *GNAQ* (1) |
| **Gi/o** (G-protein – Guanine nucleotide-binding protein gamma) | *GNG2* (5) | *GNG2* (2) |
| **AMPA** (Glutamate-gated ion chanel – ianotropic glutamate transmembrane receptor) | *GRIA2* (3); *GRID2* (1) | *GRID2* (1) |
| **KA** (Glutamate-gated ion chanel – lanotropic glutamate receptor) | *GRIK2* (3); *GRIK3* (1); *GRIK4* (2) | *GRIK2* (1) |
| **NMDAR** (Glutamate-gated ion chanel – NMDA receptor subtype) | *GRIN3A* (2); *GRIN3B* (5) | *GRIN3B* (2) |
| **mGLUR** (G-protein coupled receptor – Glutamate receptor) | *GMR5* (5); *GMR7* (1) | *GMR5* (2); *GMR8* (1) |
| **IP3R** (InsP3 receptor) | *ITPR3* (1) | - |
| **PLA2** (Phospholipase – Phospholipase A2) | *PLA2G4F* (1); *PLA2G4A* (3) | *JMJD7-PLA2G4B* (1) |
| **PLC** (Phospholipase – Phospholipase C) | *PLCB1* (3); *PLCB3* (2); *PLCB4* (1) | *PLCB3* (2) |
| **PP2B** (Protein phophatase - Calcium-dependent, calmodulin-stimulated protein phosphatase) | *PPP3CA* (1); *PPP3CB* (7) | *PPP3CB* (1) |
| **PKA** (Protein kinase - cAMP-dependent protein kinase) | *PRKACB* (21) | *PRKACB* (4); *PRKCA* (1) |
| **SHANK** (SHANK – scaffold protein) | *SKANK1*(1); *SHANK2* (4); *SHANK3* (2) | *SKANK1* (3); *SHANK3* (3) |
| **EAAT** (Sodium:dicarboxylate (SDF) transporter - Sodium-dependent glutamate/aspartate transporter ) | *SLC1A1* (2); *SLC1A7* (1) | - |
| **RGS** (Regulator of G-protein signaling) | *RGS7* (1) | *RGS4* (1) |

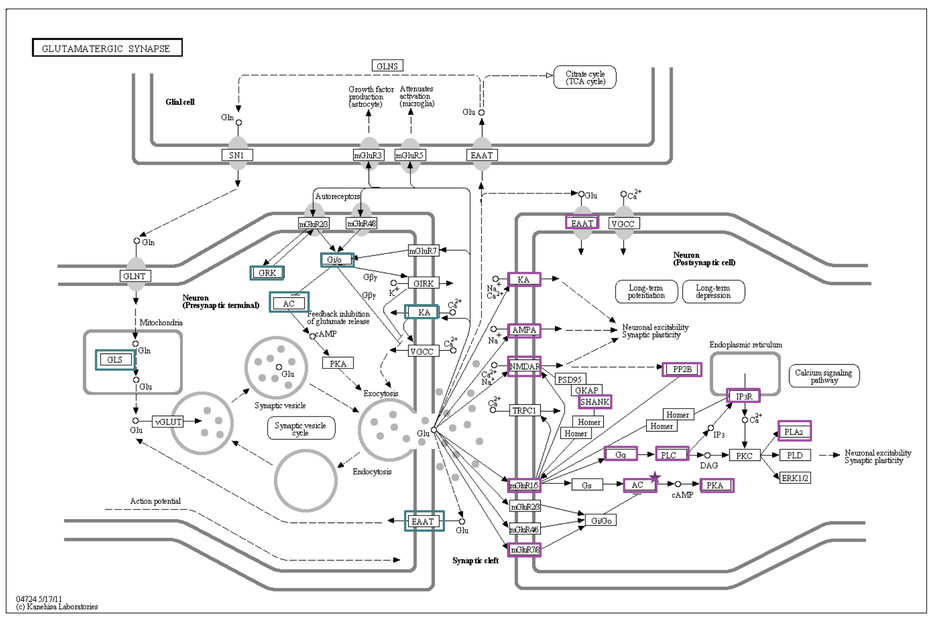


**Figure 3.** Glutamatergic synapse. The CNV identifyed involved genes encoding proteins engaged on synaptic comunication mediated by glutamate.
(Adapted from: http://www.genome.jp/kegg/pathway.html)

## CONCLUSION

Generally, deletions have an higher impact in gene function than duplications. Here we show that genes shared between pathways are more prone to deletions which may suggest that one single CNV may, in fact, interfere with more than one synaptic pathway and have more serious functional consequences.

The present results show more structural alterations encompassing glutamatergic genes relatively to other synaptic pathways , specially those encoding proteins present at the postsynaptic level, reinforcing the putative role of the glutamate pathway in ASD.

**REFERENCES :** Pinto D. *et al.* Functional impact of global rare copy number variation in autism spectrum disorders. (2010) Nature 466: 368-372.; KEGG pathway database: Kyoto Encyclopaedia of Gene and Genomes. Available at: http://www.genome.jp/kegg/pathway.html

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