Characterization and expression analysis of a CNV at chromosome 10q22 encompassing 14 genes in an autistic patient

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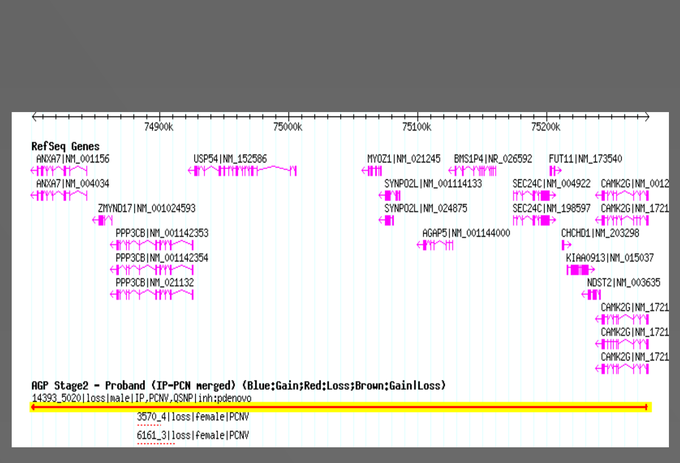
Genetics of Autism Spectrum Disorders

Autism Spectrum Disorders (ASD) have a strong genetic component, with an estimated heritability of over 90%[1]. Recent studies carried out by the Autism Genome Project (AGP) consortium suggest that rare Copy Number Variants (CNV), characterized by submicroscopic chromosomal deletions and duplications, are more frequent in ASD compared to controls, and may play an important role in susceptibility to this disorder[2-7]. However, to adequately assess pathogenicity, a detailed characterization of patients CNVs is required.

A *de novo* deletion encompassing 14 genes

We have been characterizing potentially pathogenic rare CNVs identified by the AGP whole genome CNV analysis of 1,275 ASD individuals. CNV validation in patients and parents and characterization were performed by qPCR and Long-range PCR. One autistic patient showed a rare deletion absent in 4964 controls of European ancestry with no psychiatric disease history. This deletion was located at 10q22, and encompassed 14 genes, including *ANXA7*, *ZMYND17*, *PPP3CB* and *CAMK2G* (Figure 1). We validated this CNV as *de novo*, and accurate breakpoint determination showed that it is smaller than predicted by CNV identification algorithms, including only part of *CAMK2G*. We found that a 39-nucleotide addition occurred with the deletion, a mutational mechanism previously observed in other CNVs (Figure 2). Expression analysis of *ANXA7*, *ZMYND17* and *PPP3CB* in this patient, in comparison with controls, is ongoing.



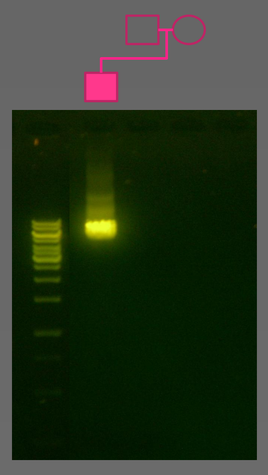


Figure 1. A *de novo* deletion located at 10q22, encompassing 14 genes, in an autistic patient was predicted using three algorithms for CNV detection. Experimental validation with qPCR and LR-PCR confirmed a ~ 477Kb deletion. The gel shows the result of a LR-PCR using primers outside the CNV region. The deletion is only observed in the child, making it possible to obtain amplification.

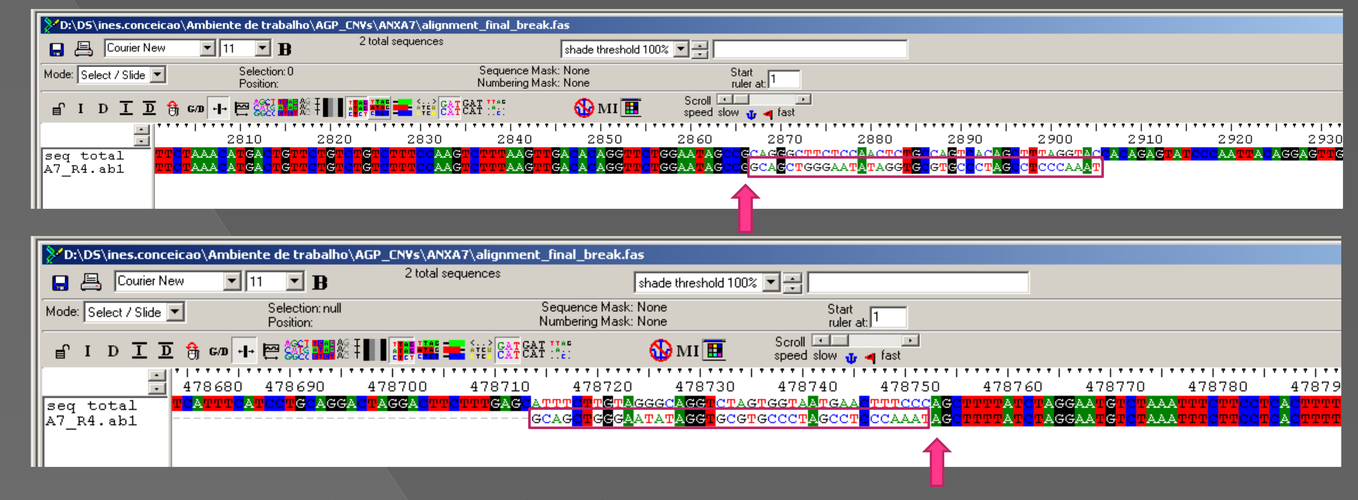


Figure 2. Breakpoint identification in both 5´and 3´regions of the *de novo* deletion in chromosome 10, using primer walking sequencing and alignment with the reference sequence of the human genome ("seq total", without deletion). The arrows indicate the position of cut. A 39-nucleotide addition was observed (pink box), with no conservation with the remaining sequence. This is one of the two major breakpoint signatures known to exist[8].

Common pathways between autism and schizophrenia?

A recent study[9] identified a genetic association of the *ANXA7*, *PPP3CB* and *ZMYND17* region with schizophrenia, and significant expression alterations in schizophrenic patients. *ANXA7* encodes Annexin7, involved in membrane fusion;. *PPP3CB* plays an important role in synaptic plasticity, learning and memory. *ZMYND17* has no known function. Our results suggest that alterations in these genes may be risk factors co-observed in autism and schizophrenia. Additional genetic and functional studies may lead to a better understanding of the common pathways between these neuropsychiatric disorders.

The role of Annexins in ASD

Interestingly, we have identified CNVs in other *Annexin* genes, namely an inherited duplication in the Annexin 1 gene (*ANXA1*) present in 12 patients and 10 parents and no control. *ANXA1* plays a central role in anti-inflammatory response and neuroprotection, contributing to brain homeostasis[10]. The same breakpoint in all individuals was observed (Figure 3) and three new polymorphisms were identified in the 3'UTR in three patients, one of them in a putative miRNA binding site (Figure 4).

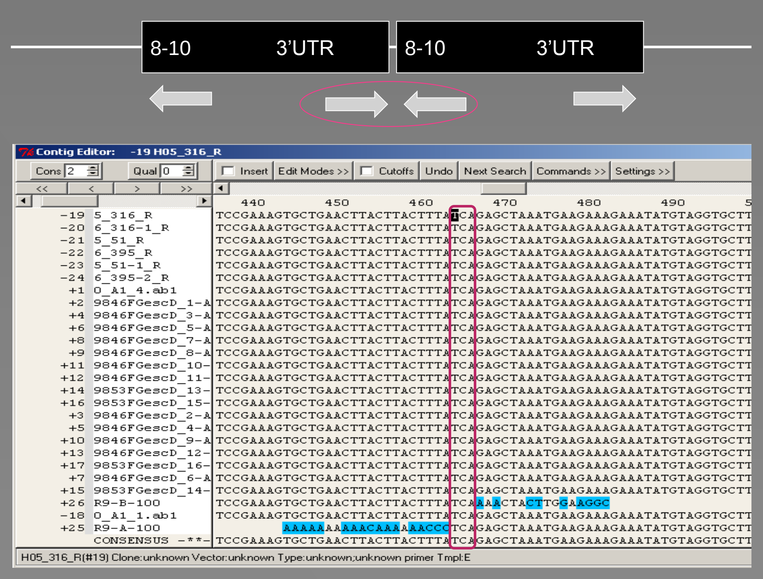


Figure 3. Deletion breakpoint identification in all the 12 patients and 9 parents. We designed primers located in the 3'UTR and pointing outwards. Only in those cases where the duplication occurred, there was amplification. Interestingly, the breakpoints are all located in the same position, and seems to be mediated by a sequence of microhomology of three nucleotides (TCA).

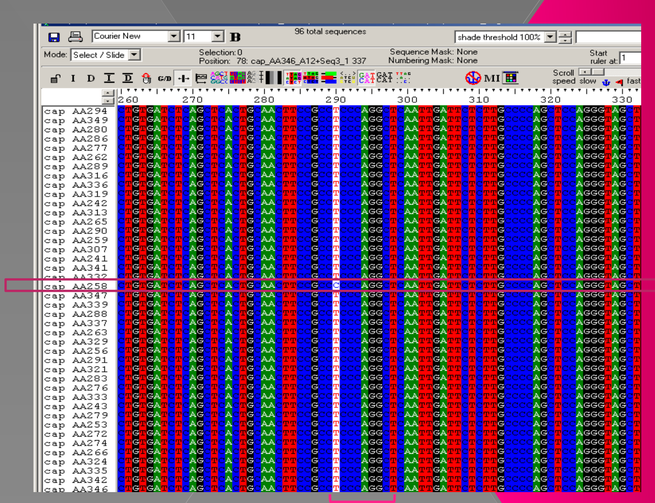


Figure 4. The sequencing of the *ANXA1* 3'UTR is ongoing and, so far, we identified a 2101-nucleotide variant in one patient. Using PITA software[11] we identified this region as a putative binding site for miR-1254-4 and miR-661.

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