Phenotypic categorization of putative pathogenic CNVs in a population of Autism Spectrum Disorder patients

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Copy Number Variants in Autism Spectrum Disorder

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 and others suggest that rare Copy Number Variants (CNVs), characterized by submicroscopic chromosomal deletions and duplications, are more frequent in ASD cases compared to controls, and may play an important role in susceptibility to this disorder[2]. However, to adequately assess pathogenicity, a detailed characterization of each patients' CNVs and clinical phenotype is required. The goal of this study was to establish the clinical and etiological relevance for ASD of potentially pathogenic CNVs identified in a Portuguese population sample by whole genome CNV analysis, through the detailed characterization of CNVs and correlation with clinical phenotypes. A genome-wide CNV screening of 342 ASD patients, carried out by the AGP using 1M SNP microarrays[2], identified a total of 14218 CNVs in this sample. We selected for further characterization 149 genic CNVs (CNVs containing genes or parts of genes) present in 127 individuals, using the following criteria: 1) CNVs that contained implicated/candidate genes for ASD; 2) CNVs in genomic regions known to be implicated/candidate for ASD; 3) CNVs in regions associated with syndromes presenting ASD symptoms; and 4) high confidence CNVs that did not overlap more than 20% with controls in available databases. We explored recurrence rates, genic content, regulatory elements, inheritance patterns and phenotypic correlations.

CNV characterization and distribution

The 149 genic CNVs selected were identified in 127 individuals (12 females and 115 males), ranged from **5 Kb to 3 Mb**, **58%** were **deletions**, and included from one gene (67% of all genic CNVs) to 25 genes in a single CNV. Large CNVs (>500 Kb) were more frequently duplications. Although most CNVs (82%) were present in a single individual, 26 CNVs (17.2%), distributed across 14 genomic regions, were present in more than one individual, and some encompassed regions/genes implicated in autism[1,2], such as **16p13.11 (N=3), *PARK2* (N=5), and *VPS13B* (N=3)**, as well as putative novel genes for ASD (*eg UBC, SLC25A4, TERT*). Network analysis of the 309 genes mapping to genic CNVs, using the String software[3], yielded a network including 67 genes (Fig.1). This network is enriched in the following Biological Processes: **regulation of apoptotic processes** (14 genes; p-value=0.05), and **PPAR and ErbB signaling pathways** (5 genes; p-value=0.02 in both cases). This data adds **novel genes for autism** to the list of potential candidates, and **identifies biological pathways** not previously associated with ASD.

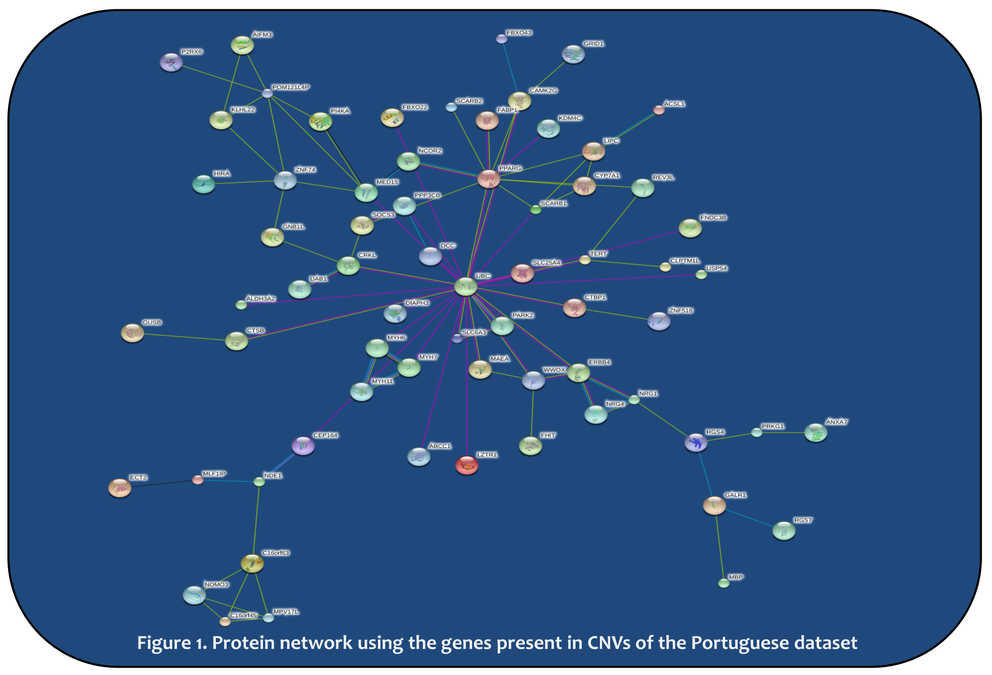

Figure 1. Protein network using the genes present in CNVs of the Portuguese dataset

Phenotypic correlations

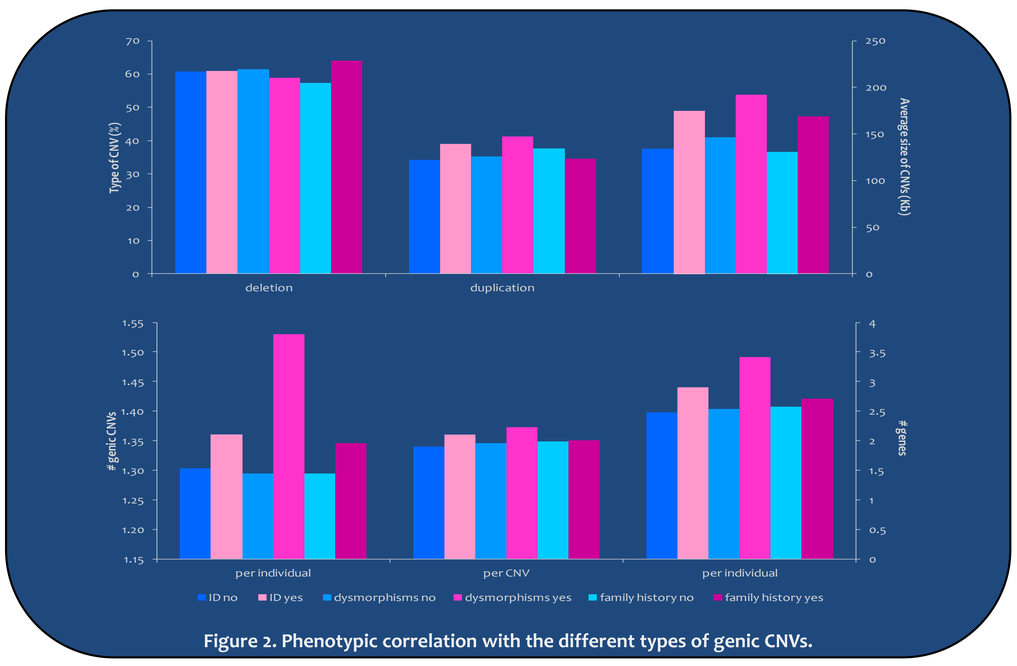

Figure 2. Phenotypic correlation with the different types of genic CNVs.

This patient sample consisted of individuals with no severe intellectual disability (IQ>35) and no gross chromosomal aberrations or obvious dysmorphisms, but included some subjects with minor dysmorphisms or macrocephaly. We explored differences in the type, size and number of the genic CNVs and in the number of genes implicated, in relation to different phenotypic categories (Fig. 2): 1) intellectual disability (ID): no (N=79) and yes (IQ=35-69; N=59); 2) minor dysmorphisms: no (N=122) and yes (N=17); and 3) family history of neuropsychiatric disorders: no (N=61) and yes (N=78). As observed in previous studies, there were more deletions than duplications, regardless of the phenotype. In general, **individuals with ID, dysmorphisms and/or family history, had a higher burden of genic CNVs, higher number of genes affected** (both *per* CNV and *per* individual) **and a larger average CNV size.** At least 7 individuals presented CNVs disrupting genes frequently identified in ASD patients, which constitute an etiological diagnosis. **Most CNVs contained only one gene, and were present in a single individual, regardless of phenotypic category, thus reinforcing the role of rare variants in this disease and the large heterogeneity in ASD etiology.**

CNV inheritance and parental personality traits

We further evaluated correlations between data for autistic traits in the parents and CNV inheritance, using the Broad Autism Phenotype Questionnaire (BAPQ) and the Social Responsiveness Scale (SRS) (Fig. 3). **A significant excess of autistic traits was observed in the fathers that transmitted a CNV, mainly in the "aloof" personality, which is defined as lacking interest in social interaction.** Paternal inheritance does not explain all changes, indicating a putative maternal contribution. Using SRS questionnaire results from parents and probands, we also calculated familial correlations for *de novo* or inherited CNVs and all parent-offspring and parental pair types (data not shown). While there were **no SRS correlations between any parent-offspring types** for *de novo* or inherited CNVs, a **significant correlation between the SRS results from both parents supports the idea of assortative mating in ASD.**

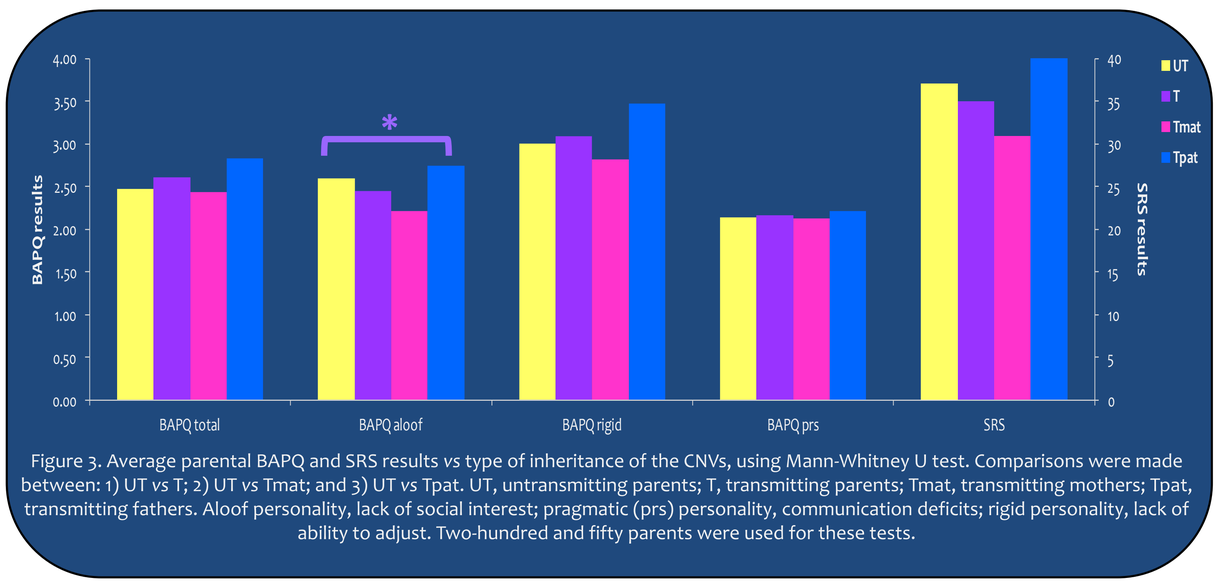

Figure 3. Average parental BAPQ and SRS results *vs* type of inheritance of the CNVs, using Mann-Whitney U test. Comparisons were made between: 1) UT *vs* T; 2) UT *vs* Tmat; and 3) UT *vs* Tpat. UT, untransmitting parents; T, transmitting parents; Tmat, transmitting mothers; Tpat, transmitting fathers. Aloof personality, lack of social interest; pragmatic (prs) personality, communication deficits; rigid personality, lack of ability to adjust. Two-hundred and fifty parents were used for these tests.

REFERENCES: 1. Betancur C. (2010) *Brain Res* **1380**:42-77; 2. Pinto D. *et al.* (2010) *Nature* **466**:368-72; 3. Franceschini A. et al. (2013) *Nucleic Acids Res*. 41:D808-15.

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