RELEVANCE OF COMMON AND RARE CNVs FOR AUTISM ETIOLOGY

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Background and Methods

Recent reports by the Autism Genome Project (AGP) consortium and other groups show that Copy Number Variants (CNVs), while individually rare, collectively may explain a large fraction of the etiology of Autism Spectrum Disorders (ASD). 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. Analysis of the Autism Genome Project genome-wide CNV results using 1M SNP microarray[1] identified a total of 14218 CNVs in 342 Portuguese probands. We selected 292 CNVs, present in 191 individuals (19 females and 172 males), 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 containing genes associated with syndromes with 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 clinical correlations.

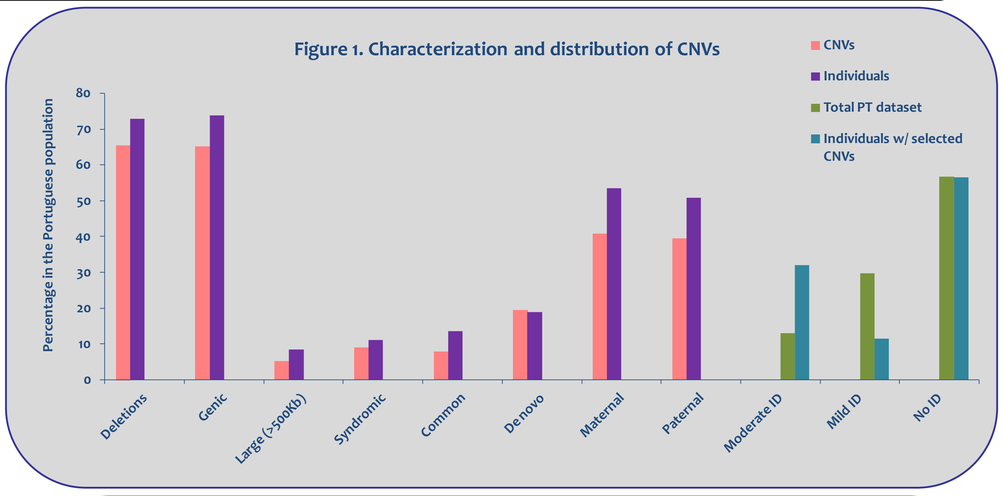
Common and Rare Genic CNVs in autism etiology

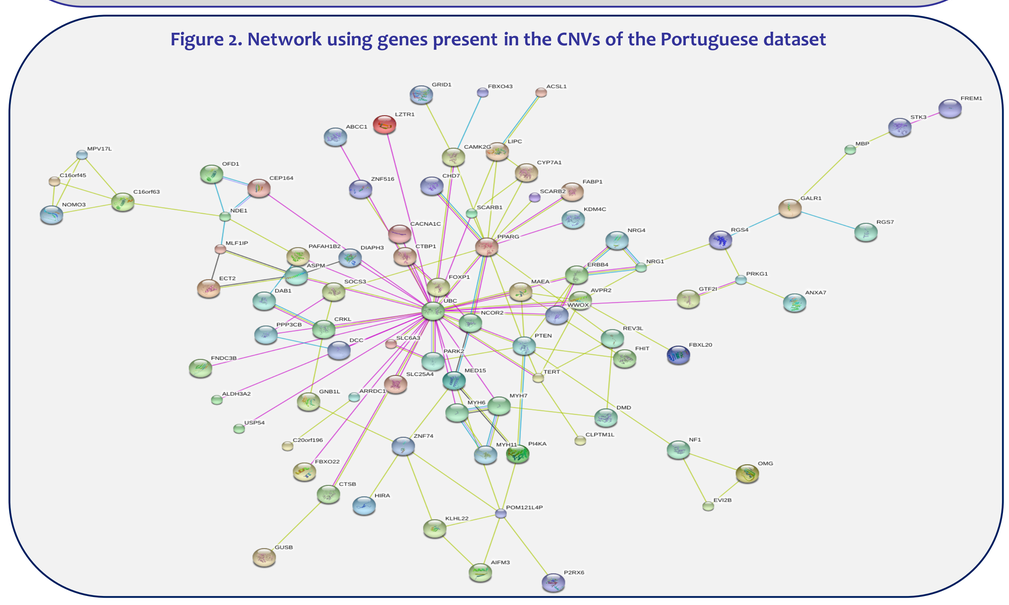
The identified 292 CNVs ranged from about 5 Kb to 3.7 Mb, with 65% being deletions (Fig.1). Large CNVs (>500 Kb) were more frequently duplications than deletions. There were 190 (65%) genic CNVs, ranging from one gene (48% of all genic CNVs) to 25 genes in a single CNV. Although most CNVs (61%) were present in a single individual, 24 "common" CNVs (8.8%), defined as CNVs with a frequency of 1% or higher in the sample population, were identified in 26 individuals. Each of these "common" CNVs were present in 3 to 6 individuals, and encompassed candidate genes for autism[1,2], such as *DPYD* (N=5), *PARK2* (N=6), and *VPS13B* (N=3).

Comparing the IQ levels of the individuals with the selected CNVs with the total Portuguese dataset, the percentage of individuals with 35>IQ>49 (moderate intellectual disability, ID) is higher (13% and 32%, respectively). This suggests that the selected CNVs have an impact on ID. The proportion of moderate ID/mild ID/ and normal cognition is maintained when considering only genic or large CNVs.

Using the 314 genes present in the genic CNVs, a network was built using String software[3], which included 85 of these genes (Fig.2). This network is enriched in the following Biological Processes and Kegg Pathways:

	Term	p-value (FDR)	# genes	Genes
GO Biological Processes	central nervous system development	0,030	14	FOXP1, AVPR2, ASPM, NCOR2, **PTEN**, CHD7, DCC, SLC6A3, NDE1, **PARK2**, ALDH3A2, **PRKG1**, MBP, STK3
	regulation of cellular catabolic process	0,035	11	**FABP1**, SCARB1, PAFAH1B2, **PTEN**, ECT2, **PARK2**, MYH6, FBXO22, RGS4, **PRKG1**, RGS7
Kegg pathways	PPAR signalling pathway	0,048	5	**FABP1**, PPARG, CYP7A1, ACSL1, UBC
	ErbB signalling pathway	0,048	5	CAMK2G, ERBB4, NRG1, NRG4, CRKL





CNV inheritance and parental personality traits

We further correlated data for autistic traits in the parents, using the BAPQ and SRS questionnaires, with the type of inheritance (inherited *vs de novo*; Figure 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. However, the paternal inheritance does not seem to explain all changes, indicating a putative maternal contribution. Also, we calculated familial correlation for all pair types, using the SRS questionnaire results from parents and probands (Table 1). A significant correlation between the SRS results from both parents supports the idea of assortative mating in ASD.

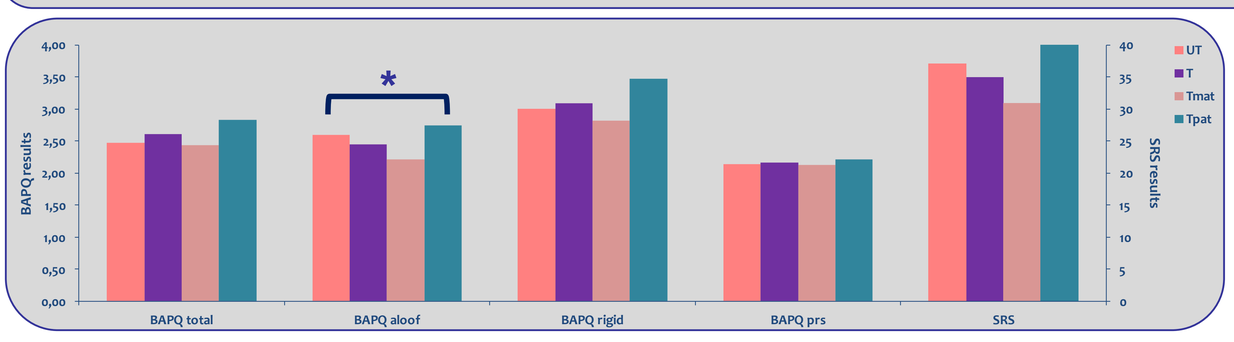


Figure 3. Average parental BAPQ and SRS results *vs* type of inheritance of the CNVs, using Mann-Whitney U test. Comparisons were made between: 1) UT *vs* T; 2) UT *vs* Tmat; and 3) UT *vs* Tpat. UT, untransmitting parents; T, transmitting parents; Tmat, transmitting mothers; Tpat, transmitting fathers. 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.

Table 1. Familial correlation results using FCOR program from S.A.G.E. package[5].

	de novo CNVs			inherited CNVs		
	Count	Correlation	p-value	Count	Correlation	P-value
parent:offspring	12	0.393	0.35	109	-0.075	0.53
father:son	6	0.758	0.015*	49	-0.039	0.79
mother:son	6	-0.152	0.18	51	-0.025	0.86
father:daughter	-	-	-	4	-0.75	0.13
mother:daughter	-	-	-	5	-0.069	0.095
father:mother	9	0.75	0.003**	67	0.57	0.00***

Lately, a lot of interest has been directed to the contribution of rare variants to the etiology of ASD. Here, we show that both rare and common variants are likely to play an important role, since the genes they encompass are functionally inter-connected. We observe an enrichment in genes implicated in central nervous system development and catabolic processes. This subset of CNVs seems to be enriched with pathogenic CNVs, since it affects a higher percentage of individuals with moderate ID. Other clinical correlations, namely with ASD severity, are under study. We further find a trend for an excess of sub-threshold autistic traits in the CNV transmitting parents of children with ASD, particularly for paternal transmission of aloof personality.

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