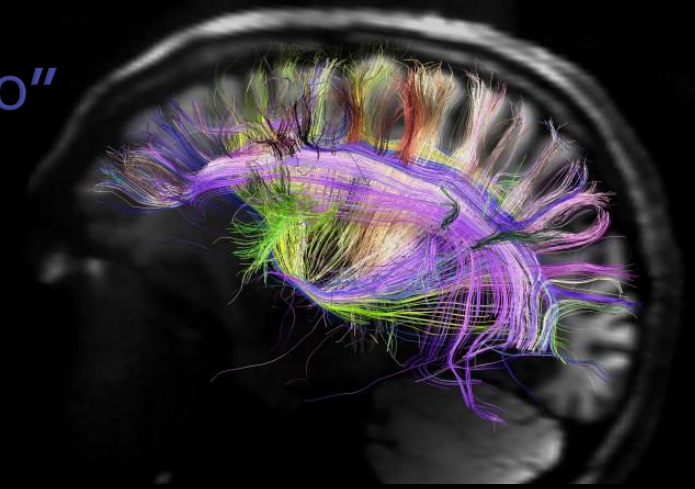


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Unidade de I&D – Grupo de Neurogenética e Saúde Mental

“SINAPSE: À PROCURA DE PISTAS PARA A ETIOLOGIA DAS PERTURBAÇÕES DO ESPETRO DO AUTISMO”



Prevalência das PEA



Elsabbagh et al., 2012



- * Portugal continental e Açores (2000)
- * Crianças nascidas entre 1990-1992
- * Prevalência em PC: **9.2/10 000**
- * Prevalência nos Açores: **15.6/10 000**
- * **Rácio : - 2.9:1**

Oliveira et al., 2007

Table II: Number of children in Portugal surveyed, referred by teachers, selected for assessment, observed, and diagnosed with ASD, and prevalence estimates per 10 000 children in target population for school-year 1999 to 2000 by geographic region

Region	AD estimates			Geographic region	Surveyed children, n	Referred children, n	Selected children, n (% referred)	Children observed, n (% selected)	Children diagnosed with ASD, n (% observed)	Prevalence of ASD per 10000 children (95% confidence interval)
	Median	Range	Number of estimates							
Europe	19	7-39	16	Norte	24 386	73	60 (82.2)	55 (91.7)	28 (50.9)	6.0 (5.0-7.5)
America	22	11-40	7	Centro	10 585	46	46 (100.0)	45 (97.8)	23 (51.1)	12.5 (9.6-15.0)
Western Pacific	12	2.8-94	12	Lisboa e Vale do Tejo	19 359	90	78 (86.7)	70 (89.7)	50 (71.4)	12.3 (10.0-14.0)
South East Asia	—	—	1	Alentejo	2895	9	6 (66.7)	5 (83.3)	3 (60.0)	7.0 (3.0-11.0)
Eastern Mediterranean	—	—	0	Algarve	2090	8	8 (100.0)	7 (87.5)	3 (42.9)	2.4 (0.3-5.0)
Africa	—	—	0	Unknown ^a	163	—	—	—	—	—
All	17	2.8-94	36	Mainland total	59 478 *	226	198 (87.6)	182 (91.9)	107 (58.8)	9.2 (8.1-10.0)
				Azores	83 17 *	25	24 (96)	23 (95.8)	13 (56.5)	15.6 (8.0-23.0)

Arquitetura genética das PEA

FATORES DE RISCO GENÉTICOS:

SÍNDROMES COM PEA

~10%

- X Frágil, Esclerose Tuberosa, Neurofibromatose, Rett, Timothy, Potocki-Lupski.

ANOMALIAS CROMOSSÓMICAS RARAS

~5%

- Duplicação materna 15q11-q13, Trissomia 21, 45X (S. de Turner), 47XYY (S. de Jacobs), 47XXY (S. de Klinefelter)

CNVs EM REGIÕES/GENES ETIOLÓGICOS

~5%

- 16p11.2, dup7q11.23, 22q11.2, 1q21.1, 15q13.3, dup17p12, 3q37-q38

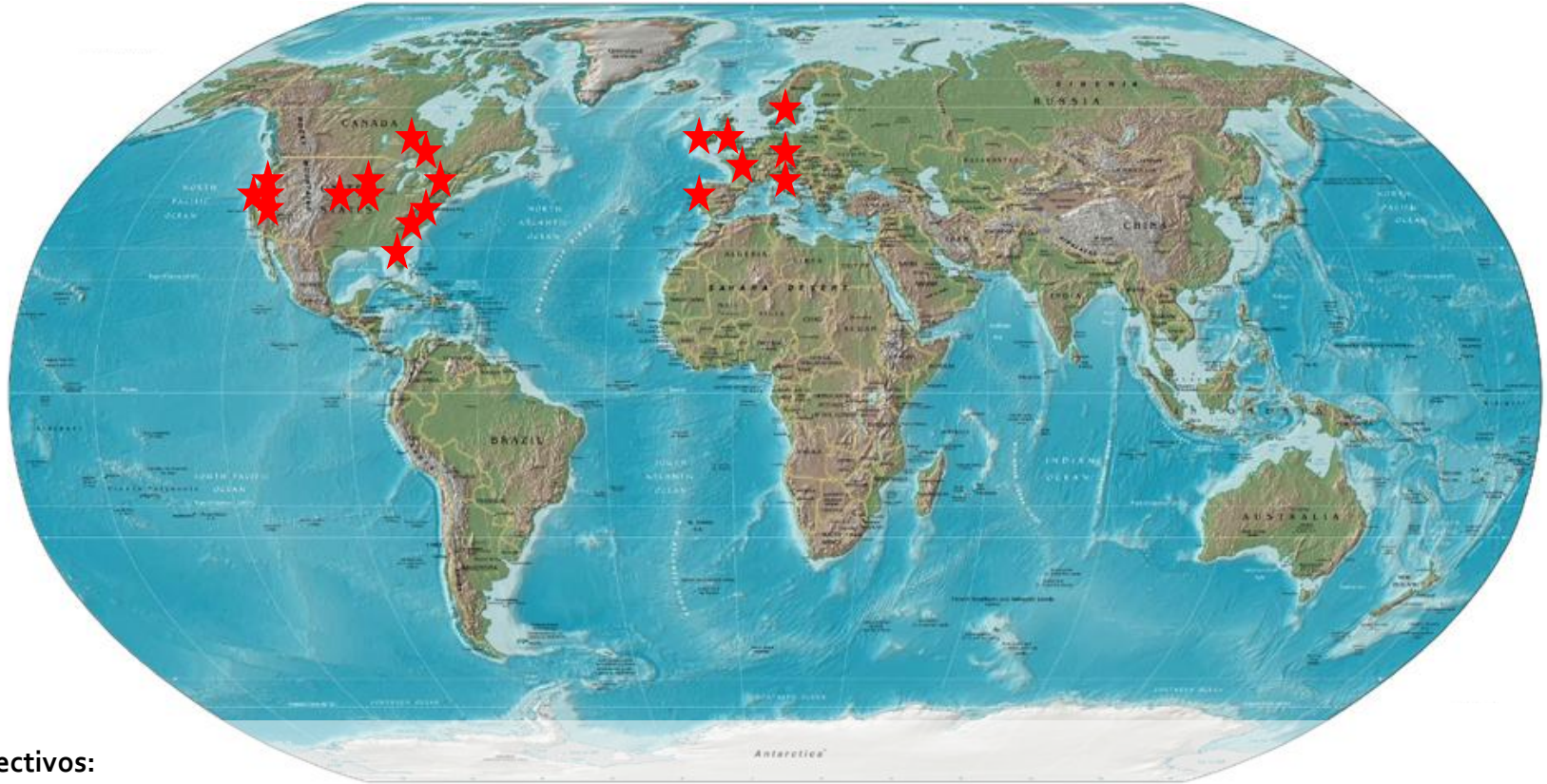
GENES COM ELEVADA PENETRÂNCIA

~5%

- *PTCHD1/PTCHD1AS*, *NRXN1*, *SHANK1*, *SHANK2*, *SHANK3*, *NLGN3*, *NLGN4x*, *NRXN3*, *CNTNAP2*, *DPP6*

25%

The Autism Genome Project



Objectivos:

- Identificação dos factores genéticos de susceptibilidade para o autismo e de vias fisiológicas afetadas
- Tradução de resultados de investigação para a prática clínica: diagnóstico
- Desenvolvimento de *guidelines* para a prática clínica
- Tradução de resultados de investigação para a prática clínica: alvos terapêuticos

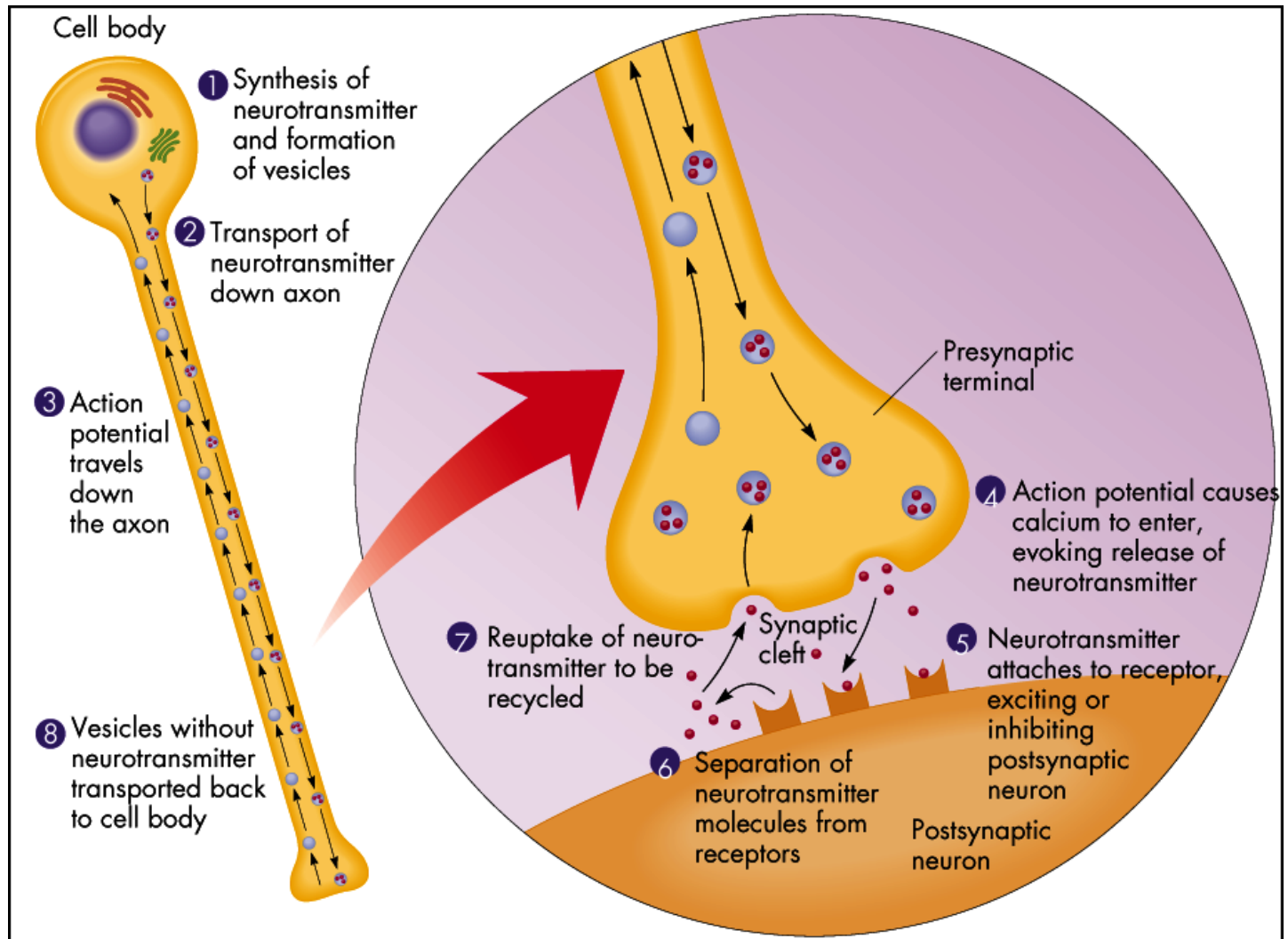
Objetivo

Os rastreios genómicos tem contribuído para a identificação de vários genes candidatos para a etiologia do autismo que convergem em vias biológicas/fisiológicas comuns.

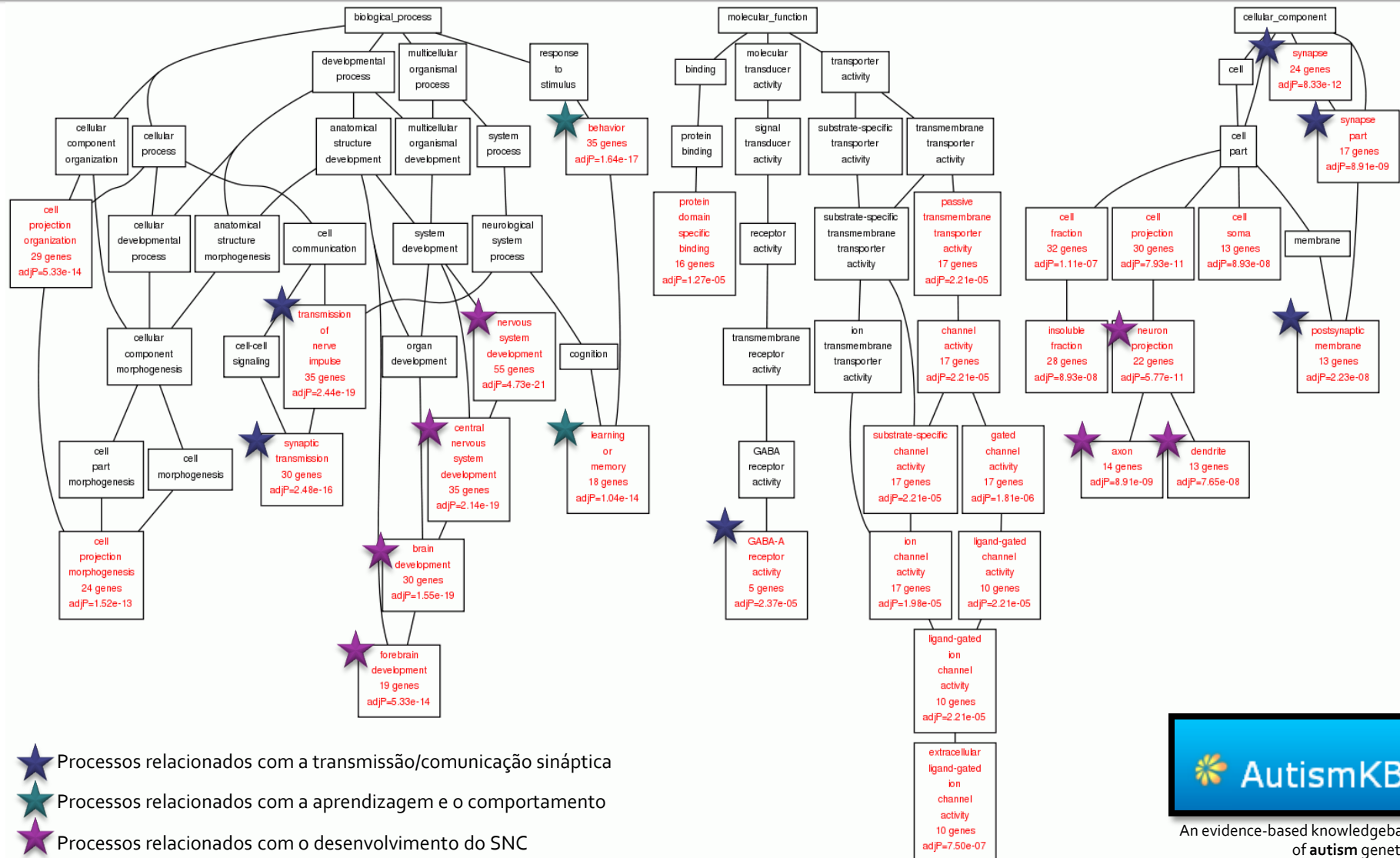


O objetivo deste trabalho consiste na **identificação de genes sinápticos** com variantes estruturais, identificadas por análise completa do genoma, relevantes para a etiologia das PEA para **estudos genéticos e caracterização funcional**.

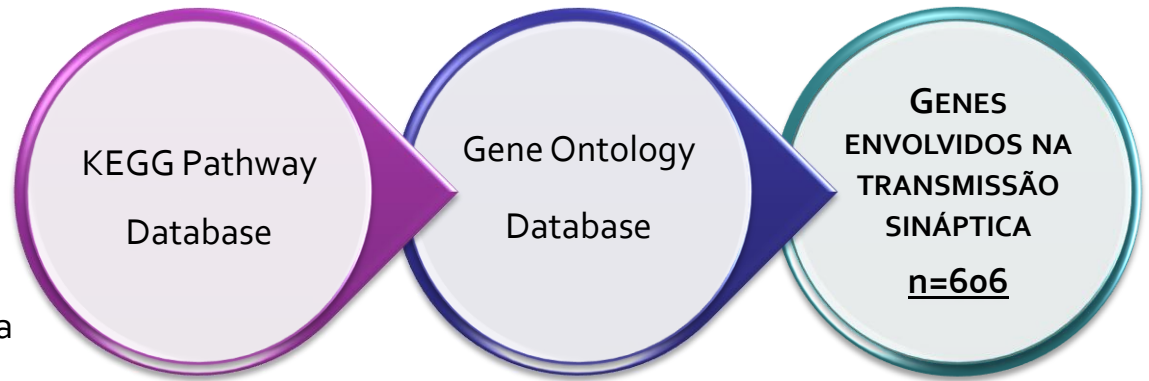
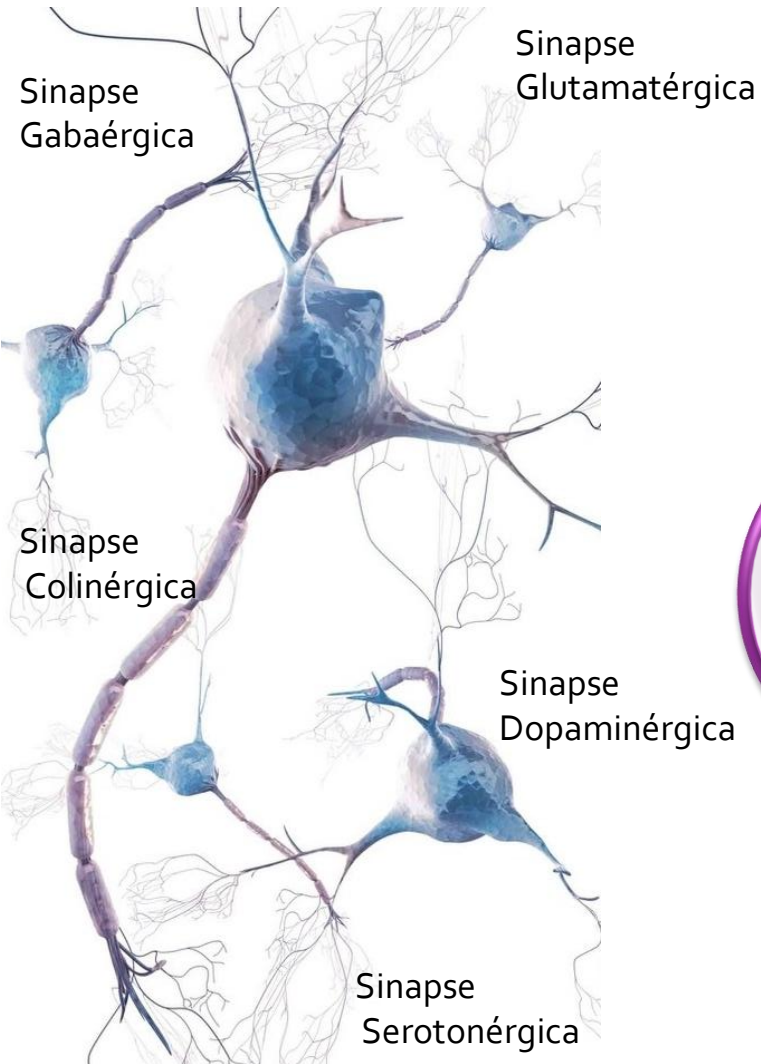
Transmissão sináptica



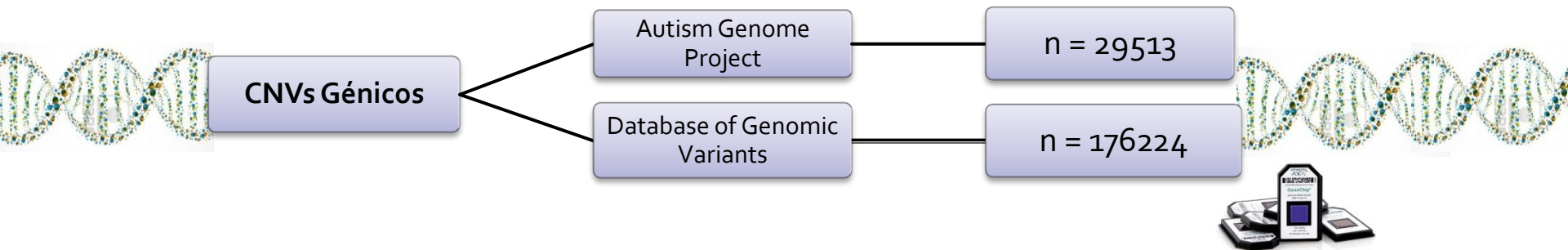
Processos biológicos implicados nas PEA



Genes envolvidos na transmissão sináptica



CNVs em genes sinápticos I



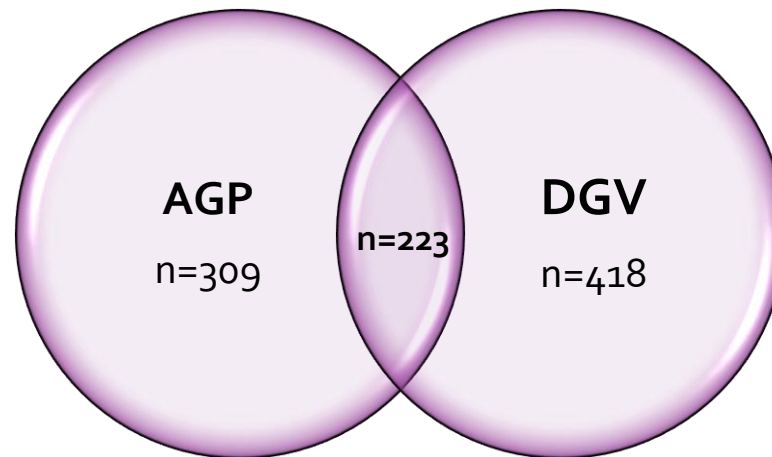
	AGP (A=29513)	DGV (B=176224)
CNVs com genes sinápticos	Ao n=1534 (5.20%)	Bo n=6334 (3.59%)
CNVs sem genes sinápticos	A1 n= 27979 (94.80%)	B1 n=169890 (96,41%)

Teste de Fisher (Ao/A vs Bo/B) : $p < 2.2 \times 10^{-16}$, OR=1.47

A PROBABILIDADE DE OCORRÊNCIA DE CNVs EM GENES SINÁPTICOS É MAIOR NA POPULAÇÃO DO AGP DO QUE NA DGV.

CNVs em genes sinápticos II

Genes sinápticos (n= 606)



Overlap com controlos (SAGE (n=1287), OHI(n=431), PopGen (n=1123), *Shaik et al.*(n=1320))

5 Genes apresentam CNVs exclusivamente em indivíduos com PEA

PLA2G1B

PPP2R3C

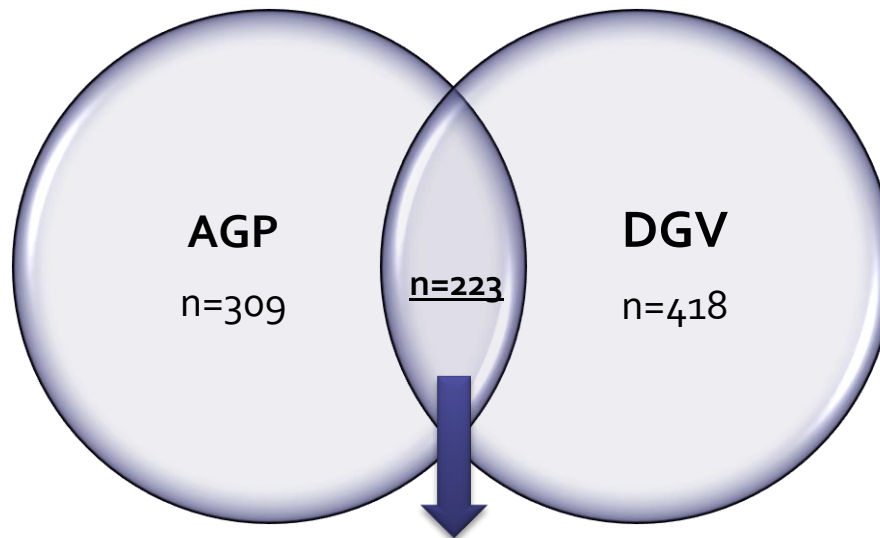
KCNK7

ADCY7

RAPSN

CNVs em genes sinápticos III

Genes sinápticos (n= 606)



Cálculo da carga genética de CNVs em cada gene: AGP vs. DGV (Teste de Fisher)

15 Genes estatisticamente significativos

<50% de *overlap* com controles (SAGE (n=1287), OHI(n=431), PopGen (n=1123), *Shaik et al.*(n=1320))

CNVs em genes sinápticos IV

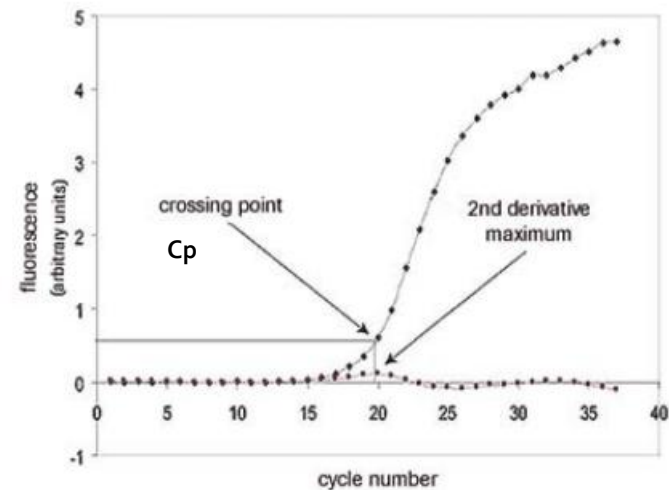
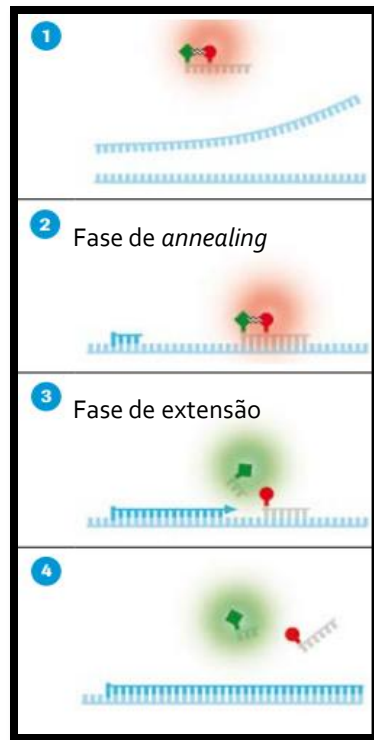
Tabela 1. Lista de genes com CNVs na população do AGP e da DGV, com significado estatístico e com overlap inferior a 50% com bases de dados.

Gene name	p-value (Fisher's exact test)	Adjusted p-values (FDR)	DGV CNVs (n)	AGP overall CNVs (n)	AGP CNVs (<50% overlap) (n)
★ <i>NF1</i>	< 2.2e-16	1.0285 e-14	21	44	14
★ <i>ERBB4</i>	1.354e-13	4.2075 e-09	252	116	5
<i>PRKACB</i>	3.58 e-12	1.3389 e-10	15	25	25
★ <i>GABRG1</i>	1.685 e-09	3.5114 e-05	5	15	4
<i>HRAS</i>	1.722 e-07	0.0029	12	16	5
<i>HCN1</i>	2.294 e-06	0.0203	24	19	17
★ <i>CHRNA7</i>	0.0012	0.0122	78	28	3
<i>TH</i>	0.0002	0.0032	6	8	8
<i>GNG2</i>	0.0002	0.0032	4	7	7
<i>AKAP9</i>	0.0008	0.0091	8	8	8
<i>PPP3CB</i>	0.0008	0.0091	8	8	8
<i>PTGS2</i>	0.0011	0.0119	4	6	6
<i>JAK2</i>	0.0017	0.0157	7	7	3
<i>CALML6</i>	0.0050	0.0327	2	4	2
<i>NISCH</i>	0.0050	0.0327	2	4	4

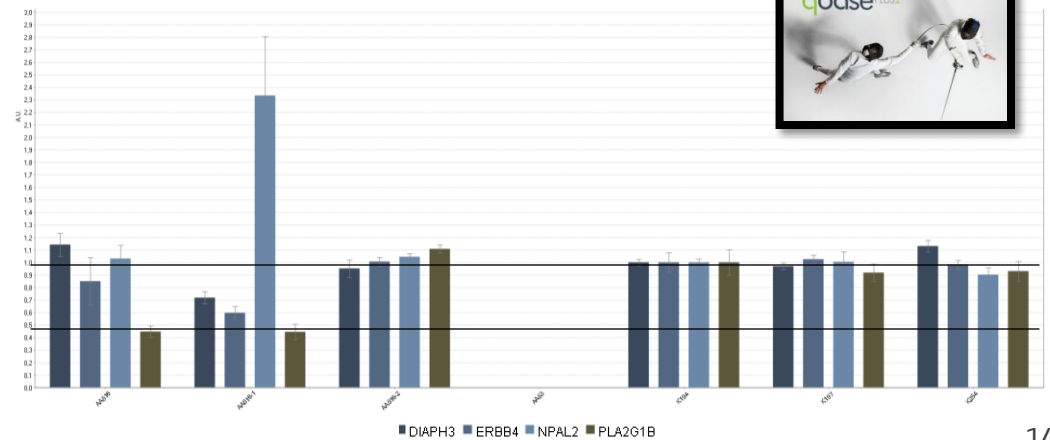
Validação de CNVs: qPCR (Universal Probe Library system)

Porquê validar???

Quantificação absoluta segundo o método do máximo da 2ª derivativa



O aumento de fluorescência emitida pelo reporter correlaciona-se com a acumulação de moléculas libertadas e com a quantidade de produto de PCR



Resultados de validação



Tabela 2. Resultados de validação dos CNVs relevantes que contêm genes sinápticos.

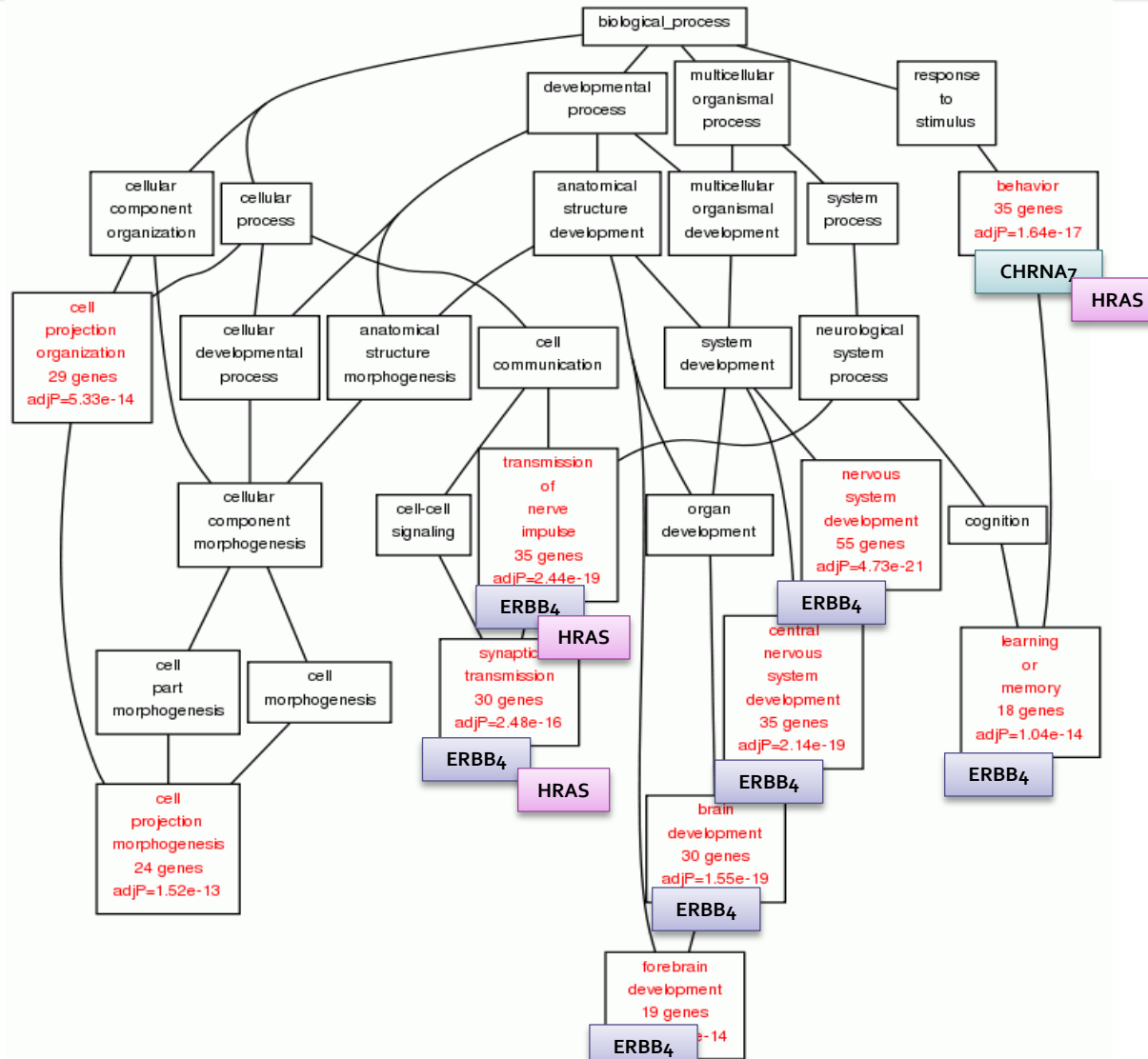
Gene	Number of samples (CNVs<50% overlap)	Number of samples tested	Validation results	CNV type	Inheritance
<i>PLA2G1B</i>	1	1	Validated	Loss	Maternal
<i>RAPSN</i>	4	2	False positive	--	--
<i>ERBB4</i>	5	1	Validated	Loss	Maternal
<i>PRKACB</i>	25	1	False positive	--	--
<i>HRAS</i>	5	1	Validated	Gain	Not tested
<i>HCN1</i>	17	2	False positive	--	--
<i>CHRNA7</i>	3	2	Validated	Loss	Maternal/Paternal
<i>TH</i>	8	1	Validated	Gain	Not tested
<i>GNG2</i>	7	1	False positive	--	--
<i>PPP3CB</i>	8	3	Validated (2)	Loss/Gain	De novo/Maternal

CNVs em genes sinápticos validados

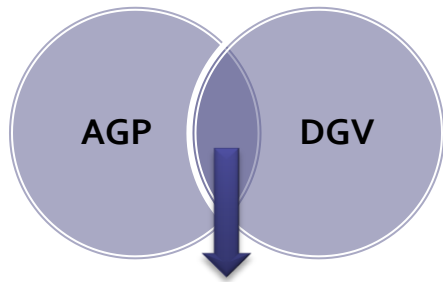


Gene ID	Gene name 	Protein function 	KO Mouse neurological phenotypes 
<i>PLA2G1B</i>	Phospholipase A2, group IB (pancreas)	Phospholipases are a group of enzymes that hydrolyze phospholipids into fatty acids and other lipophilic molecules. PLA is subdivided into PLA1 which cleave phospholipids at the sn-1 ester bond and PLA2, which cleave at the sn-2 bond. Their most common substrate is phosphatidylcholine, which generates lysophosphatidylcholine and arachidonic acid. Phospholipases are ubiquitously expressed and have diverse biological functions including roles in inflammation, cell growth, signaling and death and maintenance of membrane phospholipids.	--
<i>ERBB4</i>	Tyrosine kinase-type cell surface receptor HER4	Plays an essential role as cell surface receptor for neuregulins and EGF family members. Required for normal development of the embryonic central nervous system, especially for normal neural crest cell migration and normal axon guidance. Acts as cell-surface receptor for the neuregulins NRG1, NRG2, NRG3 and NRG4 and the EGF family members BTC, EREG and HBEGF.	--
<i>HRAS</i>	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	The products encoded by these genes function in signal transduction pathways . Ras proteins bind GDP/GTP and possess intrinsic GTPase activity.	--
<i>CHRNA7</i>	Cholinergic receptor, nicotinic, alpha 7 (neuronal)	The nicotinic acetylcholine receptors (nAChRs) are members of a superfamily of ligand-gated ion channels that mediate fast signal transmission at synapses . Alpha7 is a subunit of the neuronal nicotinic receptor that can combine to form homomeric receptors. Presynaptic alpha7 nAChRs are present on glutamate terminals and facilitate transmitter release in various brain regions , including the hippocampus, cortex and ventral tegmental area. Nicotine acting at alpha7 nAChRs can enhance hippocampal LTP, and alpha7 nAChRs are associated with attentional processes and working memory . This gene is located in a region identified as a major susceptibility locus for juvenile myoclonic epilepsy and a chromosomal location involved in the genetic transmission of schizophrenia.	(1) Abnormal nicotine-mediated receptor currents; (2) Abnormal brain morphology; (3) Failure of neuromuscular synapse presynaptic differentiation; (4) Abnormal seizure response to pharmacological agent.
<i>TH</i>	Tyrosine hydroxylase	The protein encoded by this gene is involved in the conversion of tyrosine to dopamine . Plays an important role in the physiology of adrenergic neurons as it is the rate-limiting enzyme in the synthesis of catecholamines . Mainly expressed in brain and adrenal glands.	--
<i>PPP3CB</i>	Protein phosphatase 3, catalytic subunit, beta isozyme	Calcium-dependent, calmodulin-stimulated protein phosphatase. This subunit may have a role in the calmodulin activation of calcineurin.	(1) Hyperactive behaviour

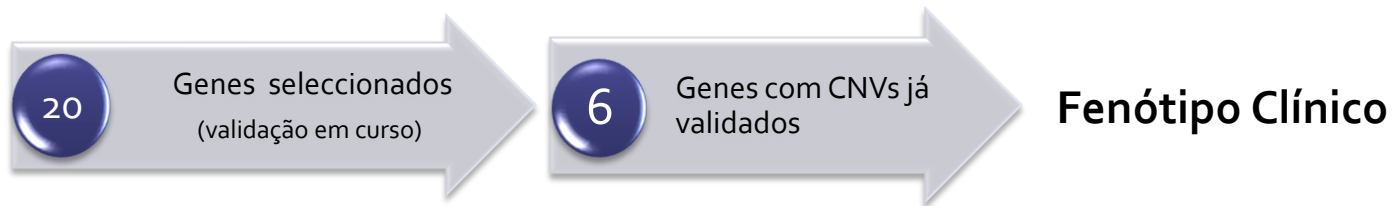
CNVs implicados na transmissão sináptica



Conclusões



- **Genes sinápticos** com CNVs nas duas populações apresentam um “burden” maior na população do AGP o que poderá, em conjunto com outros fatores genéticos constituir um **fator de risco** para o desenvolvimento das PEA.



Conclusões

- As variantes encontradas exclusivamente no AGP, dada a sua ausência num grande número de controlos, representam excelentes **candidatos para a etiologia das PEA.**

- **Já descritas:**

Outros estudos de CNVs (*KCNK7*, Nord et al., 2011; *PPP2R3C*, Levy et al., 2011);

Estudos de *linkage* (*RAPSN*, Youan et al., 2003 e Spence et al., 2006);



- **Nova variante identificada - deleção no gene *PLA2G1B*.**

Até ao momento apenas foram descritos ganhos neste gene candidato (*Szatmari et al., 2007; Marshall et al., 2008; Sanders et al., 2011*);



- **Estudos funcionais:** alterações **transcricionais** e de **expressão** dos genes afetados.

Obrigada!!

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