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Short Communication

Germline DNA copy number variation in individuals with Argyrophilic grain disease reveals *CTNS* as a plausible candidate gene

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Abstract

Argyrophilic grain disease (AGD) is a progressive neurodegenerative disease of the human brain that has never been associated to a particular gene locus. In the present study, we report the results of a CNV investigation in 29 individuals whose anatomopathologic investigation of the brain showed AGD. Rare CNVs were identified in six patients (21%), in particular a 40 kb deletion at 17p13.2 encompassing the *CTNS* gene. Homozygote mutations in *CTNS* are known to cause cystinosis, a disorder characterized by the intralysosomal accumulation of cystine in all tissues. We present the first CNV results in individuals presenting AGD and a possible candidate gene implicated in the disorder.

Keywords: Argyrophilic grain disease, copy number variations, CNVs, array-CGH, *CTNS*.

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Argyrophilic grain disease (AGD) is a neurodegenerative disorder of the aged human brain morphologically characterized by the presence of argyrophilic grains (AG) mainly in limbic areas. Several studies have shown frequent association of AGD with other tauopathies such as Alzheimer's and Pick's diseases, and its prevalence increases significantly with age, present in up to 40% of all 85 years or older individuals (Tolnay and Clavaguera, 2004). However, the cause of AGD remains unknown; the disease seems to be sporadic but genetic studies have failed to discover a link between AGD and a particular gene locus.

The contribution of DNA copy number variations (CNVs) in the phenotypes of various complex diseases has been widely demonstrated over the past years. In fact, CNVs are increasingly recognized to be a prevalent form of common genetic variation in the human population. Even though a great number of studies have demonstrated the role of CNVs in the etiology of several neuropsychiatric disorders (Lee and Lupski, 2006; Cook and Scherer, 2008) there are no reports of specific CNVs related do AGD. Here, we identified rare constitutive CNVs in individuals with AGD, in particular one at 17p13.2 that points to deletions in the cystinosin, lysosomal cystine transporter gene, CTNS, as strong candidate to cause AGD.

The Brain Bank of the Brazilian Aging Brain Study Group (BBBABSG) (Grinberg *et al.*, 2007) provided DNA from blood samples of 29 individuals with AGD. The individuals of our group ranged in age from 50 to 89 and included females and males. Table 1 presents the characterization of all cases investigated in this study. The neuropathological diagnosis of AGD was based, at least, on the presence of AG in hippocampal CA1 area (CA - *Cornu Ammonis*) and entorhinal region, as well as in pretangles in hippocampal CA2 area in sections immunostained with the phosphor-tau antibody (PHF1, 1:1000, gift from Peter Davies, NY) according to accepted criteria (Braak and Braak, 1987). The subject's clinical and functional statuses were assessed through a knowledgeable informant based on a validated clinical protocol. The protocol includes a series of semi-structured scales and questionnaires that cover ma-

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Table 1 - Case profiles of the all subjects with Argyrophilic grain disease investigated in this study.

Case	Age at death (years)	Gender	Stages of senile changes			Additional pathology	Cause of death
			NFT	SP	LB		
1	85	F	3	A	-	-	Chronic obstructive pulmonary disease
2	89	F	2	0	-	VD	Pulmonary thromboembolism
3	85	F	3	B	-	-	Esophageal cancer
4	84	F	0	0	-	-	Acute pulmonary edema
5	78	M	2	A	-	VD	Peritonitis
6	85	F	2	0	-	-	Aortic aneurysm
7	79	M	0	0	-	-	Gastric ulcer
8	82	F	2	0	-	-	Aortic aneurysm
9	72	F	1	0	-	VD	Ischemic heart disease
10	75	F	2		B	PD	Pulmonary thromboembolism
11	67	F	1	0	-	-	Pneumonia
12	76	F	3	0	-	-	Heart failure
13	80	M	3	A	-	-	Pneumonia
14	73	F	3	0	-	-	Myocardial sclerosis
15	81	F	4	C	-	AD+VD	Aortic aneurysm
16	59	M	1	0	-	-	Pneumonia
17	80	M	2	0	-	-	Hypertensive heart disease
18	86	M	4	0	-	-	Heart failure
19	58	M	1	0	-	-	Coronary artery disease
20	50	M	1	0	-	-	Pneumonia
21	76	F	0	0	-	-	Perforated gastric ulcer
22	76	M	0	0	-	-	Myocardial infarction
23	80	M	0	0	-	-	Myocardial infarction
24	88	F	3	0	-	-	Ischemic heart disease
25	69	F	1	0	-	-	Pulmonary thromboembolism
26	83	F	3	A	-	-	Myocardial infarction
27	85	F	3	0	-	-	Pneumonia
28	89	M	2	0	-	-	Duodenal ulcer bleeding
29	80	M	3	A	-	-	Chronic obstructive pulmonary disease

NFT=neurofibrillary tangle, Braak stage; SP=senile plaque, CERAD (Consortium to Establish a Registry for Alzheimer's Disease); LB=Lewy body; VD=vascular dementia; PD=Parkinson disease; AD=Alzheimer's disease.

jor functional abilities and were validated for assessment with an informant (Grinberg *et al.*, 2007). BBBABSG's procedures were approved by the Ethical Board of University of São Paulo Medical School and the next-of-kin agreed to participate and signed an informed written consent.

To identify CNVs we performed oligonucleotide comparative genomic hybridization based on microarrays (array-CGH) using a whole-genome platform containing ~180.000 oligonucleotides (180k platform) (Oxford Gene Technologies, UK). Briefly, samples were labeled with Cy3- and Cy5-deoxycytidine triphosphates by random priming. Purification, hybridization and washing were carried out as previously reported (Krepischi *et al.*, 2010). Scanned images of the arrays were processed using Feature Extraction software and data were analyzed with the Genomic Workbench software, both from Agilent Technologies. To distinguish CNVs, we used the Aberration Detection Method 2 statistical algorithm (ADM2) with a sensitivity threshold of 6.7. A genomic segment was considered duplicated or deleted when the $\log_2$ ratio of the Test/Reference fluorescent intensities of a given region encompassing at least three probes was above 0.3 or below -0.3, respectively. Detected CNVs were compared to CNV data from oligoarray studies documented in the Database of Genomic Variants (DGV). The relevant CNVs were validated by dye-swap hybridizations, and only a mirror result would be confirmatory of the CNVs presence.

The array-CGH analysis revealed rare CNVs (rare defined as frequency < 0.1% of population, based on DGV) in six individuals among the 29 with AGD, none of them re-

current. To exclude that these rare CNVs represent common variants in the Brazilian population, we compiled CNV data obtained with the same 180K array-CGH platform from more than 400 independent samples studied in our laboratory for reasons other than dementia. None of the rare CNVs documented in this study were detected in these subjects. Table 2 summarizes the rare CNVs identified in our group and shows the genes encompassed by these genomic imbalances. Any of the rare CNVs detected are potential candidates for the investigated phenotype; however, we highlighted the genomic 40 kb microdeletion of at 17p13.2 that includes the *CTNS* gene as especially interesting due to its relevant gene content and the lack of reports on copy number changes affecting this gene. Figure 1 shows the validation of this CNV made by reverse labeling hybridization, where it is possible to see that the alteration is mirrored.

Table 2 - Descriptions of all rare copy number variations identified in the individuals with Argyrophilic grain disease.

Case	Chr	Cytoband	Start site	End site	CNV type	Size (kb)	Gene (s)
2	10	p13	14996608	15043743	del	44	MEIG1
3	9	p24.1	5763235	5881920	del	70	KIAA1432, *ERMP1*
4	**17**	**p13.2**	**3527628**	**3560118**	**del**	**40**	**SHPK**, *CTNS*
14	1	p31.1	78594557	79456374	dup	789	GIPC2, MGC27382, PTGFR, IFI44L, ELTD1
17	12	q12.1	25723651	25755485	del	30	*IFLTD1*
21	5	q14.2-q14.3	82630761	82851414	dup	103	*XRCC4*, *VCAN*

Genomic positions based on GRCh37 Build reference sequence. Highlighted in bold is a CNV with relevant gene content for the investigated phenotype. CNV= copy number variation; del = deletion; dup = duplication.

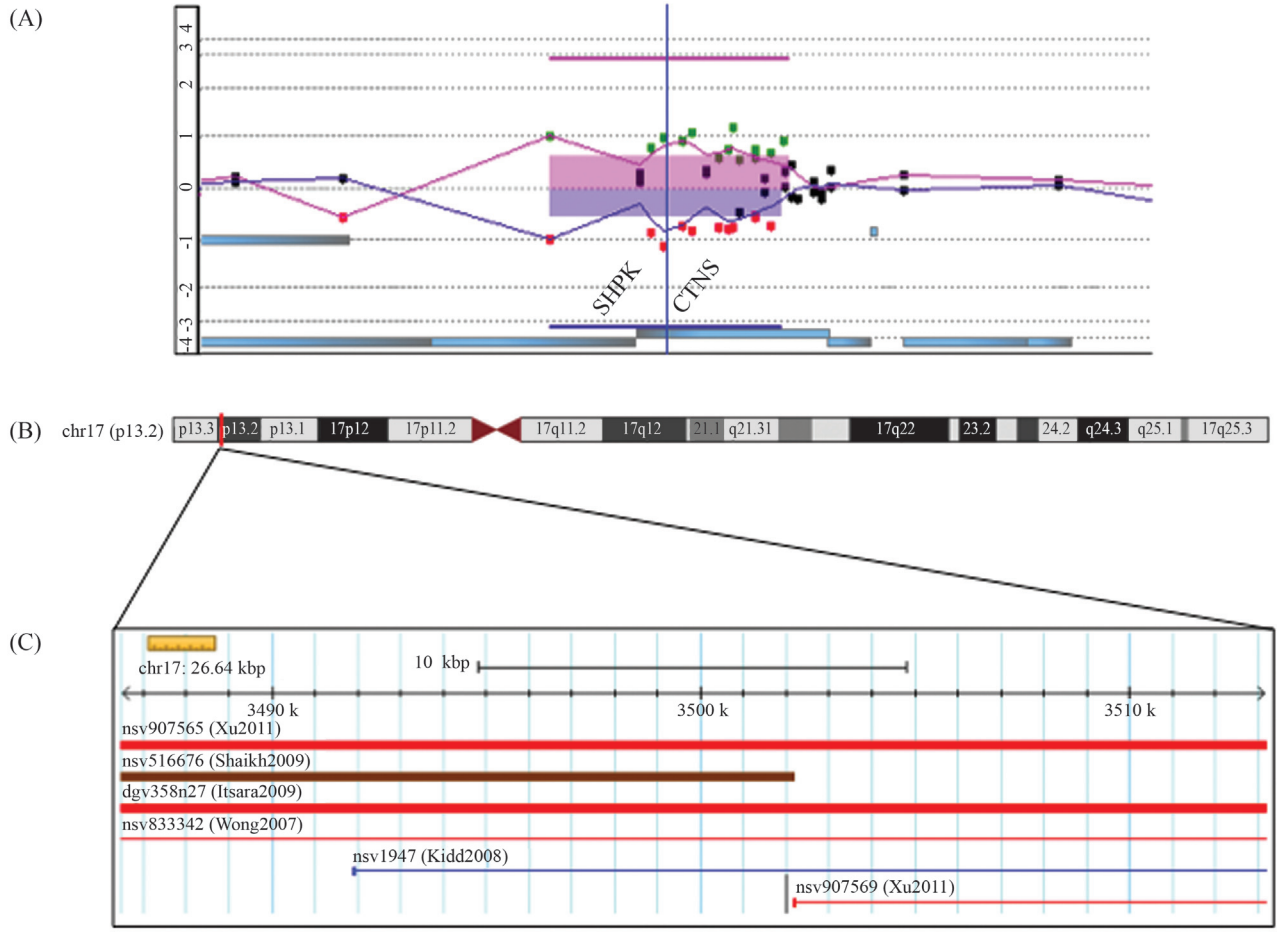

Figure 1 - The 17p13.2 rare CNV detected in a subject with Argyrophilic grain disease. (A) Array-CGH profile of the genomic segment containing the CNV, and its reverse labeling hybridization (image based on the Genome Workbench software); (B) Ideogram of chromosome 17 showing the position of the CNV (small vertical red bar); (C) Region covered by the CNV. CNV loci reported in the general population are indicated by blue (gain), red (loss) and brown (complex rearrangement) bars in the Database of Genomic Variants (DGV) (image derived from the DGV, freeze September 2013).

The *CTNS* gene encodes a seven-transmembrane domain protein that functions to transport cystine out of lysosomes. Its activity is driven by the H^+ electrochemical gradient across the lysosomal membrane. Mutations in this gene cause cystinosis, a rare autosomal recessive disorder characterized by the intralysosomal accumulation of cystine in all tissues (Kalatzis and Antignac, 2002). The most common mutation associated with this rare disease is a deletion of 65 kb presented in homozygosity that also includes the *SHPK* gene. The protein encoded by this latter gene has weak homology to several carbohydrate kinases, a class of proteins involved in the phosphorylation of sugars as they enter the cell, inhibiting return across the cell membrane (Wamelink *et al.*, 2008). Our results show a heterozygous deletion in the *CTNS* gene encompassing the region of this common mutation associated with cystinosis. Literature data demonstrate that the brain is one of the last organs to be affected by the progressive cystine accumulation (Cochat *et al.*, 1986). Cognitive impairments have been documented in some cystinosis patients, which presented deficit in visual-spatial memory (Trauner *et al.*, 1988; Scarvie *et al.*, 1996). Additionally, an investigation showed that *Ctns* -/- knockout mice present a severe age-related memory deficit (Maurice *et al.*, 2009). Therefore, this evidence makes *CTNS* a good candidate gene for susceptibility to AGD.

In conclusion, this is the first study to identify a rare CNV at 17p13.2 with AGD and links this disease with a particular gene locus, the *CTNS*.

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