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RESEARCH NOTE

A microdeletion in Alzheimer's disease disrupts *NAMPT* gene

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DNA copy number variations (CNVs) are recognized to be a prevalent form of common genetic variation in the human genome and their role in normal development and disease has been demonstrated through their impact on gene expression and protein structure (Stankiewicz and Lupski 2010). Here we report on a subject with late-onset Alzheimer's disease (AD) a rare CNV that disrupts the nicotinamide phosphoribosyltransferase (*NAMPT*) gene, which encodes an important enzyme that mediates nicotinamide adenine dinucleotide (NAD) biosynthesis. Recently an interesting study showed that overexpression of the gene for NAD-dependent deacetylase sirtuin-1 (SIRT1) reduces production of amyloid beta (Aβ) and plaques in the brain of AD mice (Donmez *et al.* 2010); disruption of *NAMPT*, therefore, is a likely mechanism of increased production of Aβ and plaques.

The subject was an 83-year-old woman, Caucasian, one year of schooling, investigated by the Brain Bank of the Brazilian Aging Brain Study Group (BBBABSG) (Grinberg *et al.* 2007) with a neuropathological diagnosis of AD (Braak stage 3; CERAD (Consortium to Establish a Registry in AD) moderate; and moderate likelihood that the dementia was caused by AD according to the National Institute on Aging (NIA)–Reagan criteria). After a day of hospitalization due to chest pain and dyspnea, she died because of an acute myocardial infarction. After death, the brain was examined macroscopically, and 15 neurodegenerative-disease-related structures were sampled for microscopic examination according to standard protocol. Neuropathological examinations were carried out using immunohistochemistry following internationally accepted guidelines. CERAD criteria were used to classify the β-amyloid neuritic plaque burden and the distribution of neurofibrillary tangles was classified according to the Braak and Braak staging system (Braak and Braak 1991). Neuropathological diagnosis of AD is made where cases show at least Braak stage III and are CERAD moderate. The usual neuropathological guidelines were used for other dementias and for Parkinson's disease (Grinberg *et al.* 2007). The subject's clinical and functional statuses were assessed through a knowledgeable informant based on a validated clinical protocol. The protocol included a series of semi-structured scales and questionnaires that cover major functional abilities and were validated for assessment with an informant. BBBABSG's procedures were approved by the local ethics committee and an informed written consent was obtained from the next of kin (Grinberg *et al.* 2007).

To identify new candidate genes, we investigated constitutive CNVs by oligonucleotide comparative genomic hybridization based on microarray (array-CGH) using a 180K whole-genome platform (Oxford Gene Technologies, Kidlington, UK), as previously described (Krepischi *et al.* 2012). Briefly, samples were labelled with Cy3-deoxycytidine and Cy5-deoxycytidine triphosphates by random priming. Purification, hybridization and washing were carried out as recommended by the manufacturer. Scanned images of the arrays were processed using Feature Extraction software and data were analysed using Genomic Workbench software, both from Agilent Technologies (Santa Clara,

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USA). To identify 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 fluorescence intensities of a given region showing hybridization with at least three probes was above 0.3 or below −0.3, respectively. All hybridizations were gender-matched and processed in reverse-labelling duplicates; CNVs that were not detected in both dye-swap experiments of the same sample were excluded from the analysis.

The array-CGH analysis of peripheral blood DNA from this woman with late-onset AD revealed six CNVs (table 1). While some of them contained no gene or were commonly variable in copy number in the population, an identified heterozygous 146-kb deletion at 7q22.2, which disrupts the *NAMPT* gene, has not been reported on the Database of Genomic Variants (DGV) (figure 1), a database that compiles published studies on CNVs observed among over 11,000 normal individuals (http://projects.tcag.ca/variation). Further, we have never observed this variant in any of the >1300 individuals studied by array-CGH in our laboratory for reasons others than dementia such as cancer predisposition and intellectual deficiency; therefore, excluding the possibility that this CNV represents a polymorphism in the Brazilian population. Table 1 shows the description of all CNVs identified in the subject of this report.

Owing to their effect on dosage-sensitive genes or genes that are crucial for normal development or maintenance,

Table 1. Copy number variations identified in the subject with late-onset Alzheimer's disease.

Chromosome	Cytoband	Start (bp)	End (bp)	CNV type	Size (kb)	Gene
Chr1	p21.1	104163795	104284223	Del	120	*AMY2A, AMY1A, AMY1C, AMY1B*
Chr3	q26.1	162540988	162618974	Dup	78	
Chr7	**q22.2**	**105923515**	**106069125**	**Del**	**146**	***NAMPT***
Chr9	p21.3	25259001	25347001	Del	88	
Chr15	q26.1	93362475	93474032	Del	112	*CHD2*
ChrX	q21.1	77121672	77123784	Del	2	*MAGT1*

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

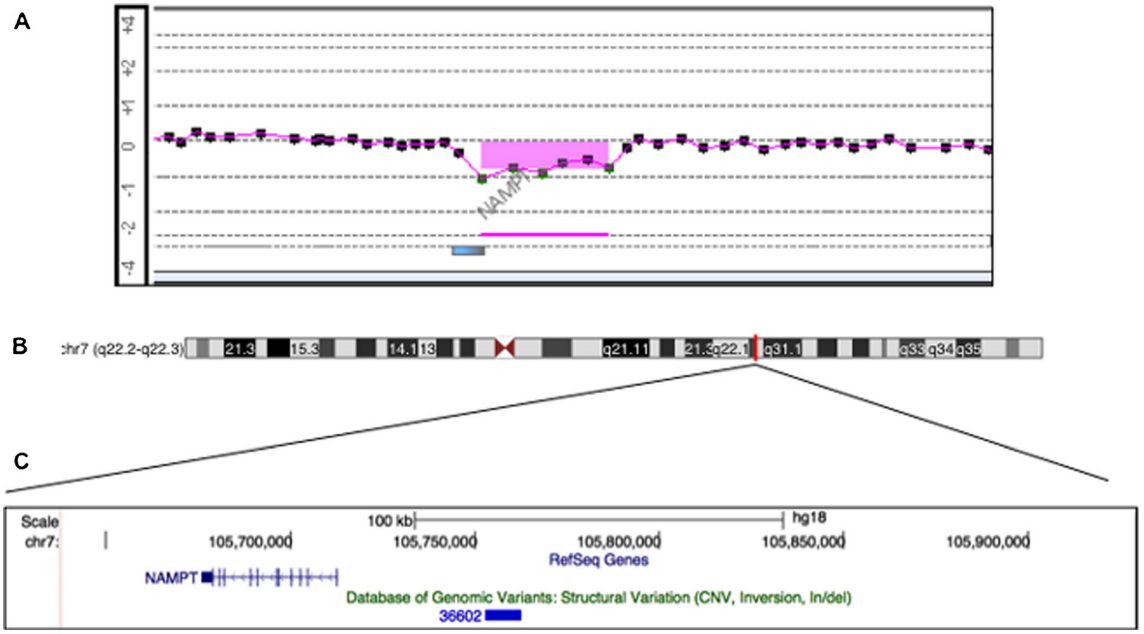

Figure 1. The 7q22.2 rare CNV detected in a subject with late-onset Alzheimer's disease. (A) Array-CGH profile of the genomic segment containing the 7q22.2 deletion (image based on Genome Workbench software). (B) Ideogram of chromosome 7 showing the position of the CNV (small vertical red bar). (C) The *NAMPT* gene, indicated by the blue line in the Reference Sequence (RefSeq) genes track, partially overlaps the 7q22.2 deletion; a CNV reported in the general population (blue bar) maps within the deletion but outside the *NAMPT* gene (image derived from the UCSC Genome Browser, freeze September 2012).

rare CNVs have been implicated in neurodevelopment and neurodegenerative disorders such as autism spectrum disorder, mental deficiency, schizophrenia, depression and Parkinson's disease (Lee and Lupski 2006). However, there are no whole-genome investigations of rare CNVs in patients with late-onset AD. Copy number investigations have been reported for *APOE* and *CR1*, genes whose roles in AD are well established. So far, only common CNVs were shown to be associated with the disease, always with low penetrance (Heinzen *et al.* 2010; Shaw *et al.* 2011). We have now shown in a subject with late-onset AD a never previously reported genomic change that disrupts *NAMPT*, which encodes a key enzyme of NAD biosynthesis. The functional connection between NAMPT-mediated NAD biosynthesis and the enzyme SIRT1 has been demonstrated in different cell types, showing that this pathway regulates important biological events including ageing (Imai 2011). Donmez and colleagues showed that overexpression of NAD-dependent deacetylase SIRT1 prevents Aβ deposition, one of the hallmarks of AD (Donmez *et al.* 2010). SIRT1 decreases the production of Aβ amyloid by deacetylating the retinoic acid receptor β and therefore upregulating the gene for ADAM10, a major component of α-secretase. Additionally, other studies have demonstrated that SIRT1 promotes degradation of phosphorylated Tau by deacetylating it and prevents Tau-mediated neurodegeneration besides regulating memory and synaptic plasticity (Michan *et al.* 2010; Min *et al.* 2010). Altogether, these evidences reveal *NAMPT* as a strong and predictable candidate gene to confer differential risk to AD. The crucial question of whether alterations in the *NAMPT* gene constitute a predisposition factor for AD can only be answered by similar investigations in other cohorts, which we hope will be stimulated by this report.

In summary, our result shows the existence of a novel candidate susceptibility gene for late-onset AD and highlights the importance of rare CNVs as potential disease-susceptibility variants for the disease.

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