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PIN1 promoter polymorphisms are associated with Alzheimer's disease

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Abstract

In our study, we analyzed the coding and promoter regions of the PIN1 gene in a group of 111 Alzheimer's disease (AD) patients looking for a possible genotype–phenotype correlation. The presence of SNPs – which could affect and modify the clinical phenotype of AD patients was also investigated.

We identified two single nucleotide polymorphisms (SNPs) at positions −842 (G → C) and −667 (C → T) in the promoter region of the PIN1 gene. Our results evidenced a significantly higher percentage of −842C allele carriers in AD subjects with respect to healthy controls. We found that this allele significantly raised the risk of developing AD (OR 3.044, CI 1.42–6.52). The −842 and −667 SNPs were in linkage disequilibrium and combined to form haplotypes. The CC haplotype conferred a higher risk of developing AD (OR 2.95, confidence interval 1.31–6.82).

Finally, protein expression analyses revealed that subjects carrying the −842 CC genotype or the CC haplotype showed reduced levels of the PIN1 protein in peripheral mononuclear cells.

Keywords: PIN1; Genetic polymorphisms; Alzheimer's disease; Genotype–phenotype correlation; Neurofibrillary degeneration

1. Introduction

Neurofibrillary tangles (NFTs) are prominent lesions in a large subset of neurodegenerative diseases, including Alzheimer's disease (AD), which are characterized by paired helical filaments (PHFs) composed of the microtubule-associated protein Tau.

In normal situations, Tau plays a role in the modulation of the functional organization and structure of neurons by regulating microtubules assembly [20,7]; in NFTs, instead, Tau is hyperphosphorylated on serine or threonine residues preceding proline and this abnormal phosphorylation is responsible for Tau aggregation and abolishes its ability to bind microtubules and promote microtubule assembly. Interestingly, the increased proline-directed phosphorylation of Tau and other proteins appears to precede tangle formation and neurodegeneration in AD [14,4].

Phosphorylated serine/threonine–proline motifs (like those found in NFTs) can exist in two distinct conformations, whose conversion in some proteins is catalysed by PIN1: PIN1 is in fact a peptidil-prolil-*cis*-trans isomerase that specifically isomerizes phosphorylation of a serine or threonine that precedes proline. The PIN1 protein – characterized by a carboxy-terminal catalytic domain as well as by a WW amino-terminal protein–protein interaction domain –

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is mainly expressed in neurons at higher levels than in most other postmitotic cells, where it regulates the dephosphorylation and functioning of several mitotic phosphoproteins, many of which are increased in AD [14,6].

Lu et al. [11] hypothesized that PIN1 can restore the function of phosphorylated Tau and may prevent or reverse the paired helical filaments (PHFs) formation in AD. In their study, they demonstrated that PIN1 WW domain binds hyperphosphorylated Tau from AD brains, but not Tau from age-matched healthy brains; they also proved that PIN1 is capable of restoring the biological function of phosphorylated Tau in vitro.

Overexpression of hyperphosphorylated Tau in AD brains can cause an increased association of these molecules in the tangles that might lead to depletion of the soluble form of PIN1 in neurons; indeed, the level of soluble PIN1 in the brains of AD patients is greatly reduced if compared to that in age-matched control brains.

There are also increasing evidences that AD might be related to an aberrant reactivation of the cell cycle and apoptosis in neurons and that PIN1 can play a pivotal role in this [19,14,15].

Moreover, the gene encoding the PIN1 protein – consisting of four exons and spanning over more than 14 kb – maps on 19p13.2, a locus recently associated with late-onset AD [21].

In our study, we analyzed both coding and promoter regions of the PIN1 gene in a cohort of 111 AD patients looking for a possible genotype–phenotype correlation between PIN1 gene nucleotide sequence variations and AD. We also investigated the presence of SNPs, which could affect and modify the clinical phenotype of AD patients.

2. Methods

2.1. Patients and controls

One hundred and eleven AD patients (79 F/32 M, mean age 79.47 ± S.D. 6.30) and 73 non-demented sex- and age-matched healthy controls (HC 50 F/23 M, mean age 79.98 ± S.D. 6.36) were enrolled for this study. All patients were Caucasian, living in Northern Italy and selected from a larger ambulatory population cared for at the Geriatric Department of the Ospedale Maggiore IRCCS, University of Milan, Italy. There were no significant differences between the groups in age or education level.

Diagnosis of probable AD was performed according to standard clinical procedures and following the DMS IV and NINCDS-ADRDA criteria [17]. The cognitive and functional performances were assessed using mini-mental state evaluation (MMSE), activities of daily living (ADL), instrumental activities of daily living (IADL) as well as an extensive neuropshycological evaluation. Every subject had undergone a recent brain magnetic resonance imaging (MRI)/computed tomography (CT) scan. Criteria for the diagnosis of normal cognition were as follows: (1) no active neurological or psychiatric disorders; (2) any ongoing medical problems or related treatments not interfering with cognitive function; (3) a normal neurological exam; (4) no psychoactive medications; (5) independently functioning community dwellers.

In order to minimize the risk of possible inflammatory processes, all subjects selected showed no clinical signs of inflammation (e.g. normal body temperature, no concomitant inflammatory condition) and normal blood chemistry levels (red blood cell sedimentation rate, albumin, transferrin and C reactive protein plasma levels).

Informed consent was obtained from all subjects or their relatives. The study protocol was approved by the Ethics Committee of the University Hospital.

2.2. Genotyping

Whole blood was collected by venipuncture in Vacutainer tubes containing EDTA (Becton Dickinson Co., Rutherford, NJ).

Genomic DNA was extracted by salting-out method as described in scientific literature [16]. DNA concentration and purity were determined by spectrophotometric analysis.

Amplifications of PIN1 coding (four exons) and promoter regions (1150 bp upstream the ATG codon) were performed by using primers (Table 1) designed using the software Primer Express 2.0 (Applied Biosystems, Foster City, CA) according to the human sequences available in GenBank (NM_006221 range = chr19:9807013–9821356 for the coding region, AF501321 for the promoter).

PCR reactions were carried out in a GeneAmp 9700 Thermal cycler (Applied Biosystems, Foster City, CA) using PCR buffer 1×, 1 unit of Taq Gold, 0.2 mM dNTPs and variable concentrations of $MgCl_2$ (from 1 to 2.5 mM). The cycling was performed with an initial denaturation for 10 min at 95 °C, followed by 36 cycles at 95 °C for 30 s, at the annealing temperature (T_a) for 30 s (see Table 1 for the different T_a used), at 72 °C for 30 s with a final extension to 72 °C for 7 min. PCR products were observed – under UV light – in a 2% agarose gel stained with ethidium bromide.

DNA sequencing of PCR products was performed using the BigDye Terminator Cycle Sequencing Ready Reaction Kit 2.0 (Applied Biosystems, Foster City, CA). DNA sequences were run on an automated ABI Prism 3100 Genetic Analyser (Applied Biosystems, Foster City, CA). Sequences were handled using SeqScape 1.0 Software.

ApoE genotypes were determined by PCR amplification of a 234 base-pair fragment of exon 4 of the ApoE gene, followed by digestion using Cfo1, according to protocols already described in scientific literature [18]. Restriction patterns were revealed by 2% agarose gel electrophoresis.

2.3. Protein expression analysis

Peripheral mononuclear cells (PBMCs) of 25 subjects (AD patients and healthy controls chosen to be representative