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C9orf72 G_4C_2 repeat expansions in Alzheimer's disease and mild cognitive impairment

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ABSTRACT

C9orf72 G_4C_2 repeat expansion is a major cause of amyotrophic lateral sclerosis and frontotemporal lobar degeneration. Its role in Alzheimer's disease (AD) is less clear. We assessed the prevalence of G_4C_2 pathogenic repeat expansions in Flanders-Belgian patients with clinical AD or mild cognitive impairment (MCI). In addition, we studied the effect of non-pathogenic G_4C_2 repeat length variability on susceptibility to AD, and on AD cerebrospinal fluid (CSF) biomarker levels. A pathogenic repeat expansion was identified in 5 of 1217 AD patients (frequency <1%). No pathogenic expansions were observed in patients with MCI (n = 200) or control individuals (n = 1119). Nonpathogenic repeat length variability was not associated with AD, risk of conversion to AD in MCI individuals, or CSF biomarker levels. We conclude that pathogenic *C9orf72* G_4C_2 repeat expansions can be detected in clinical AD patients and could act as a contributor to AD pathogenesis. Non-pathogenic repeat length variability did not affect risk of AD or MCI, nor AD biomarker levels in CSF, indicating that *C9orf72* is not a direct AD risk factor.

1. Introduction

A pathogenic expansion of a hexanucleotide (G_4C_2) repeat in the regulatory region of *C9orf72* (Fig. 1) was recently identified as a major cause of disease in the amyotrophic lateral sclerosis (ALS)–frontotemporal lobar degeneration (FTLD) spectrum of neurodegeneration (DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011). *C9orf72* repeat expansions exceeding 30 G_4C_2 units have been suggested to be pathological (Renton et al., 2011), although a much higher number of G_4C_2 repeats have been detected, depending on the sensitivity of the molecular genetics technology used to size the G_4C_2 repeat itself (i.e. fluorescence in situ hybridization [FISH] and Southern blot assay) (DeJesus-Hernandez et al., 2011; Renton et al., 2011). A heterozygous pathogenic G_4C_2 repeat expansion in *C9orf72* explains 4% to 8% of patients with isolated ALS or FTLD, and 25% to 40% of familial ALS or FTLD worldwide (Majounie et al., 2012b), but is particularly common in patients or families with a mixed phenotype of both FTLD and ALS, explaining up to 85% of the occurrence of disease (Gijselinck et al., 2012). In line with this, detailed clinical investigations on cohorts of pathogenic repeat expansion carriers demonstrate a relatively high prevalence of cognitive dysfunction and frontal symptoms in mutation carriers with ALS, and of motor neuron disease in mutation carriers with FTLD (Chio et al., 2012; Mahoney et al., 2012). Nevertheless, substantial clinical heterogeneity is observed between mutation carriers and within families, providing evidence for a wider phenotypic spectrum associated with this genetic defect (Mahoney et al., 2012; Stewart et al., 2012). Psychosis or other psychiatric symptoms appear to be prominent features (Arighi et al., 2012; Mahoney et al., 2012; Snowden et al., 2012). Pathogenic repeat expansions have been identified in patients with atypical clinical presentation, i.e. clinically diagnosed with olivopontocerebellar degeneration, atypical Parkinsonian

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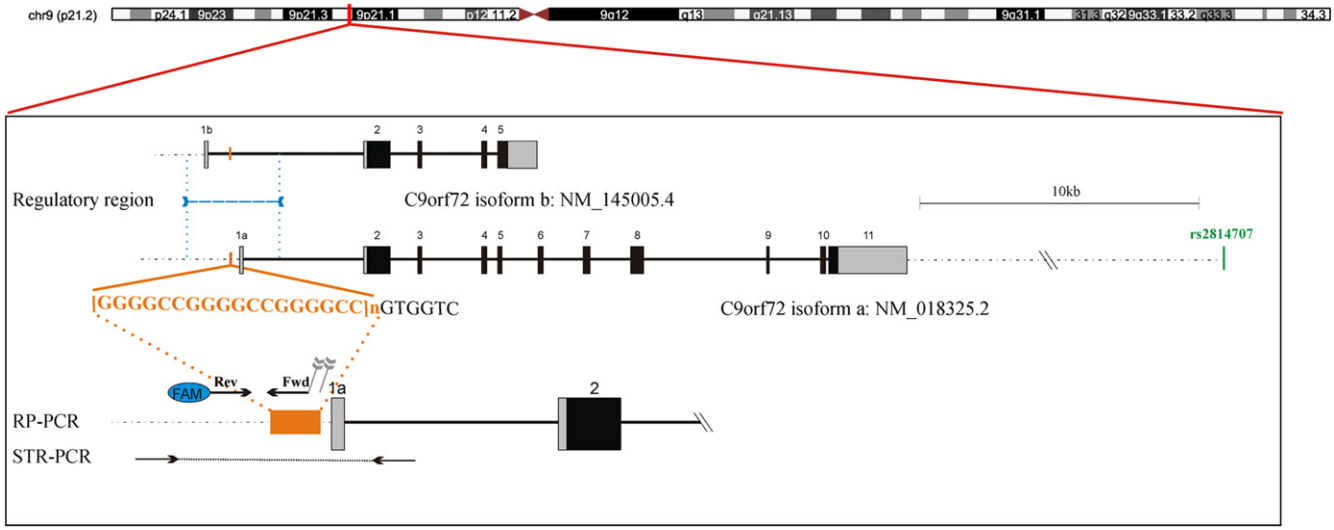

Fig. 1. Schematic representation of *C9orf72* gene and genotyping techniques. Schematic representation of the location of the 2 major isoforms of *C9orf72* gene (coding regions are represented in black and noncoding regions in gray) and of rs2814707 on chromosome 9p21 (green). In orange, the position of the G_4C_2 repeat within the regulatory region (blue) is depicted, and the orange sequence presents 3 complete G_4C_2 repeat units. The letter "n" indicates a variable G_4C_2 repeat length, with number of units ranging from 2 to ~30 in control individuals, and more than 30 in patients. The first 2 exons of the isoform a (NM_018325.2) are enhanced to show the location of the primer pairs used for RP-PCR (the primer anchor sequence is represented in gray) and STR-PCR to genotype the G_4C_2 repeats in the AD and MCI cohorts. The two *C9orf72* gene isoforms, rs2814707, G_4C_2 repeat and primer pairs for the genotyping have been represented on the reverse complement strand relative to their original position on the reference genome (NCBIbuild37 - hg19).

syndrome, corticobasal syndrome (Lindquist et al., 2012) or slowly progressive behavioral variant FTD (Khan et al., 2012). Also, a family-based study detected a pathogenic repeat expansion in 6 patients clinically diagnosed with probable AD in 3 of 342 families (Majounie et al., 2012a). In a second series of 114 AD patients with early age at onset and cerebrospinal fluid (CSF) biomarker profile typical of AD, 3 carried a G_4C_2 expansion (Wallon et al., 2012). In 2 additional series of 568 and 424 AD patients, no pathogenic repeat expansions were detected (Rollinson et al., 2012; Xi et al., 2012). An additional cohort of 1184 AD patients was recently investigated by Kohli et al. and resulted in the identification of 9 patients carrying the G_4C_2 expansion of which 3 were autopsy confirmed (Kohli et al., 2012). These studies suggest that, albeit rare, *C9orf72* repeat expansions may underlie a spectrum of neurodegenerative brain disease phenotypes, such as Alzheimer's disease (AD), with repercussions for clinical decision making. A general role of *C9orf72* in different neurodegenerative diseases was recently proposed (Satoh et al., 2012), because of co-expression of C9orf72 and UBQLN1 in dystrophic neurites distributed in the CA1 region and the molecular layer in the hippocampus of AD and non-AD brains, suggesting that C9orf72 in dystrophic neurites is involved in the homeostasis of protein degradation by acting together with UBQLN1 (Satoh et al., 2012).

In this study, we assess the prevalence of pathogenic *C9orf72* repeat expansions in a large prospective cohort of Flanders-Belgian patients with clinical diagnosis of AD (n = 1217) or mild cognitive impairment (MCI; n = 200), an intermediate stage of cognitive decline that may convert to AD. We extend these analyses to explore whether repeat expansions of intermediate length might convey risk to develop AD and/or could influence the CSF biomarker levels of amyloid-β peptide ($A\beta_{1\text{-}42}$), total tau (T-tau), and tau phosphorylated at threonine 181 ($P\text{-}tau_{181P}$).

2. Material and methods

2.1. Patient/control cohort

The Flanders-Belgian study cohort consisted of 1417 patients, 1217 AD patients (mean age at onset, 74.5 ± 8.9 years; 65.2% women) and 200 MCI individuals (mean age at onset, 72.4 ± 8.8 years; 50.7% women), and another group of 1119 healthy control individuals (mean age at inclusion, 65.1 ± 13.6 years; 56.5% women).

The majority of the AD and MCI cohort was ascertained at the Memory Clinic of the ZNA Middelheim and Hoge Beuken, Antwerp, Belgium (P.P.D.D. and S.E.) as part of a prospective study of neurodegenerative and vascular dementia in Flanders, the Dutch-speaking region of Belgium (Engelborghs et al., 2003; Engelborghs et al., 2006). Consensus diagnosis of possible and probable AD was given by at least 2 neurologists based on the National Institute of Neurological and Communication Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984). Another subset of patients was collected at the Memory Clinic of the University Hospitals of Leuven, Gasthuisberg, Leuven, Belgium (R.V. and M.V.) as part of a prospective study on the molecular genetics of cognitive impairment (Bettens et al., 2010) using the same clinical assessments and biosampling schemes. Each AD patient underwent a neuropsychological examination, including Mini-Mental State Examination (MMSE) (Folstein et al., 1975), and structural and/or functional neuroimaging (Bettens et al., 2010). For a subset of patients (n = 292; mean age at onset, 76.7 ± 8.2 years; 62.3% women), CSF levels of $A\beta_{1\text{-}42}$, T-tau, and $P\text{-}tau_{181P}$ were available as part of the diagnostic work-up, determined with commercially available single parameter enzyme-linked immunosorbent assay (ELISA) kits (Innogenetics, Ghent, Belgium). Previous mutation screening revealed 1 patient with a known pathogenic *APP* mutation, 6 patients with a *PSEN1*, and 3 patients with a *GRN* known pathogenic mutation, respectively. Diagnosis of MCI was based on Petersen's diagnostic criteria (Petersen 2004). During follow-up, 62 of the MCI patients (31%) converted to AD.

The control cohort consisted of unrelated individuals, without neurological or psychiatric antecedents or neurological complaints or without organic disease involving the central nervous system, examined at the Memory Clinic of ZNA Middelheim and Hoge Beuken, Antwerp, Belgium (P.P.D.D. and S.E.)