European Journal of Medical Genetics 59 (2016) 26–31

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

Clinical research

ADDing a piece to the puzzle of cognition in schizophrenia

Marta Bosia [a, b, *], Alessandro Pigoni [b], Laura Zagato [c], Lino Merlino [c], Nunzia Casamassima [c], Cristina Lorenzi [b], Adele Pirovano [a], Enrico Smeraldi [a, b], Paolo Manunta [a, c], Roberto Cavallaro [b]

[a] Università Vita-Salute San Raffaele, Milan, Italy
[b] Department of Clinical Neurosciences, IRCCS San Raffaele Scientific Institute, Milan, Italy
[c] OU Nephrology and Dialysis, IRCCS San Raffaele Scientific Institute, Milan, Italy

ARTICLE INFO

Article history:
Received 5 March 2015
Received in revised form
2 December 2015
Accepted 21 December 2015
Available online 24 December 2015

Keywords:
Adducin
Schizophrenia
Psychotic disorders
Cytoskeleton proteins
Cognition
Synaptic plasticity
Neurodevelopment
Genetics

ABSTRACT

The biological bases of cognitive impairment in schizophrenia are poorly understood and may lie in insults in neurodevelopment, leading to alterations in critical structures. Synapses proteins are claimed to have etiopathogenic roles and more direct effects on core cognitive functions. Adducins family proteins seem of great interest, as they are fundamental constituents of synapses, involved in actin cytoskeleton assembly-disassembly, responsible of synaptic plasticity. *ADD2* is more prominently expressed in brain tissues and influences memory and learning, commonly impaired in schizophrenia.

In the present study we tested 342 patients with schizophrenia for three common adducins genetic variants, *ADD1* rs4961, *ADD2* rs4984 and *ADD3* rs3731566, reported to have significant effects on circulatory system in humans. Neuropsychological measures were evaluated with the Brief Assessment of Cognition in Schizophrenia (BACS), a broad battery evaluating core cognitive domains.

The analysis showed significant effects of *ADD2* genotype on almost every cognitive domain. Moreover, significant interactions between *ADD1* and *ADD3* were also observed on some BACS subtests, namely Symbol Coding and Verbal Memory.

Our findings suggest that adducins are involved in cognitive impairment in schizophrenia. This effect may result both from a direct mechanism affecting synaptic building and plasticity and indirectly as a consequence of vascular insults.

1. Introduction

Schizophrenia is a devastating chronic mental illness, affecting almost 1% of the general population, with onset in late adolescence and early adulthood, characterized by cognitive and functional deficits, leading to social disruption and poor functional outcome (Keefe and Harvey, 2012). In the past decades there has been a significant increase in knowledge about molecular mechanisms underlying the disease and its genetic basis, however a clear picture is still lacking (Bosia et al., 2015).

A neurodevelopmental component in the etiopathogenesis of schizophrenia has been claimed for a long time, based on clinical evidences of subtle abnormalities prior to disease's onset, such as neurological soft signs (Varambally et al., 2012; Dong-Min et al., 2013; Hirjak et al., 2013). A renewed interest in the neurodevelopmental hypothesis has been raised from recent works. The involvement of risks and insults during a critical period in the etiopathogenesis of the disease has been claimed, thus underlying the role of pathways implicated in synaptic formation and plasticity (Hayashi-Takagi and Sawa, 2010). The focus has been on genes codifying for structural proteins involved in morphology, stability, growing and plasticity of synapses, suggested to play a role in neurophysiologic alterations observed in psychiatric and neurodegenerative disorders (Shirendeb et al., 2012; Guilmatre et al., 2014). Among these, adducin family proteins appear of great interest, as they are constituents of synaptic structures, such as dendritic spines and growth cones of neurons, in which they are expressed at high levels. Adducins are involved in assembly-disassembly of actin cytoskeleton, responsible of synaptic plasticity and able to modulate synaptic strength through a variety of mechanisms (Stevens and Littleton, 2011). Recent studies showed

* Corresponding author. Department of Clinical Neurosciences, IRCCS San Raffaele Scientific Institute, Via Stamira d'Ancona 20, 20127 Milan, Italy.
E-mail address: bosia.marta@hsr.it (M. Bosia).

http://dx.doi.org/10.1016/j.ejmg.2015.12.012
1769-7212/

that stable assembly of new synapses depends on the presence of β-adducin, while disassembly involves β–adducin phosphorylation through PKC, and both processes are required for superior cognitive functions such as learning and memory (Bednarek and Caroni, 2011).

Adducins are heterodimeric membrane proteins ubiquitously expressed in three different forms (α, β, γ), encoded by three genes (*ADD1*, *ADD2* and *ADD3*). While α and γ-adducins are abundantly expressed in most tissues, the β form is present mostly in erythrocytes and in brain tissues, indicative of a different function of the three genes (Matsuoka et al., 2000). In animal studies, genetic manipulations are reported to affect neuronal connections and underlying behavioral performance. β-adducin KO mice show an hippocampal impairment in both Long Term Potentiation (LTP) and Long Term Depression (LTD), associated with deficits in learning and motor coordination (Rabenstein et al., 2005). Phenotypic analysis of α-adducin KO worms demonstrated that the gene is required for consolidation of synaptic plasticity and influences short and long-term memory (Vukojevic et al., 2012).

The role of inter-individual genetic variability is less known. *ADD* genes polymorphisms have been mostly studied in relation to nephrogenic hypertension, showing an effect on hypertension, its clinical consequences and antihypertensive response to drugs (Manunta et al., 2007; Citterio et al., 2010; Kalita et al., 2011). In details, the antihypertensive drug rostafuroxin proved to act specifically on primary hypertension caused by genetic variants of adducin, probably underlying a direct interaction with adducin polymorphisms (Lanzani et al., 2010; Citterio et al., 2011). There is only one study evaluating *ADD1* polymorphisms on cognition in humans, showing a significant association between genotype and episodic memory measures in healthy controls (Vukojevic et al., 2012).

These data support the hypothesis that adducins may affect neuropsychological performance, through their direct involvement in synaptic plasticity, underlying memory and learning, and a possible indirect effect leading to vascular insults in the brain. This evidence could be of great relevance in schizophrenia, as adducins may have an etiopathogenic role and a significant effect on core cognitive functions. In the present study we thus analyzed, in a sample of patients affected by schizophrenia, possible effects of three previously studied adducins polymorphisms, *ADD1* G1532T (rs4961), *ADD2* C1797T (rs4984) and *ADD3* IVS11 + 386A > G (rs3731566), on performance in cognitive domains, that are typically impaired in the disease.

2. Patient data

A sample of 342 Caucasian biologically unrelated outpatients with schizophrenia was recruited at the San Raffaele Scientific Institute of Milan (Italy). Inclusion criteria were: diagnosis of schizophrenia meeting DSM-IV-TR criteria, I.Q.≥ 70, treatment with a stable dose of the same antipsychotic in monotherapy since at least 3 months and good response to treatment (defined as a reduction of 30% or more in PANSS Total Score after 3 months of treatment). Exclusion criteria were: psychiatric comorbidities, concomitant psychiatric treatments except benzodiazepines, substance abuse, neurological disorders and brain injury. After a complete description of the study, informed consent to participation was obtained. The protocol followed the principles of the Declaration of Helsinki and was approved by the local Ethics Committee.

3. Methods

3.1. Genotyping

Genomic DNA was extracted from whole blood by manual extraction, using the "Illustra blood genomic Prep Midi Flow kit" (GE Healthcare, Milan, Italy).

ADD1 Gly460Trp missense mutation deriving from G1532T polymorphism (rs4961) was analyzed by using the forward primer 5′-AAGGTGGGAATTGAAGAGACTCTCA-3′, the reverse primer 5′-CAACTATGCAGATGACCTTTGCTTT-3′, and the Taqman MGB probes VIC-TCTGGAAATGTCAAATAGTAA and FAM-CTGGAAATGTCAAGTAGTAA.

ADD2 C1797T (rs4984) polymorphism, a silent exonic mutation, was detected using the forward primer 5′-TCCTTCATCAAAACACACCTACCAAT-3′, the reverse primer 5′-CTGGTCCGGCCGTGT-3′, and the Taqman MGB probes VIC-TTCTTCAGTGTTGCCC and FAM-TTCAGCGTTGCCC.

The intronic IVS11 + 386A > G polymorphism of *ADD3* (rs3731566) was analyzed using the forward primer 5′-AAGGTGGGAATTGAAGAGACTCTCA-3′, the reverse primer 5′-CAACTATGCAGATGACCTTTGCTTT-3′ and the Taqman MGB probes VIC-TCTGGAAATGTCAAATAGTAA and FAM-CTGGAAATGTCAAGTAGTAA.

All of the primers and the Taqman probes were purchased by Life Technologies Inc. (Foster City, CA, USA), and the polymorphisms were investigated by using the Taqman ABI Prism 7900 Sequencer Detector (Life Technologies, Inc.). Genotypes were determined by real-time fluorescent measurements (Livak, 1999).

All of the subjects were genotyped for *ADD1*, *ADD2*, and *ADD3* polymorphisms.

3.2. Clinical and neuropsychological assessment

Basic clinical and demographic data were collected from clinical reports.

Psychopathology was assessed by means of Positive and Negative Syndrome Scale for Schizophrenia-PANSS (Kay et al., 1987), administered by trained psychiatrists. Neuropsychological measures were evaluated with the Brief Assessment of Cognition in Schizophrenia-BACS (Keefe et al., 2004), a broad battery of neuropsychological tests evaluating core cognitive domains that are typically impaired in schizophrenia. It consists of the following tests: verbal memory (words recall); working memory (digit sequencing); token motor task (psychomotor speed and coordination); speed of processing (symbol coding); verbal fluency (semantic and letter production) and planning (Tower of London). Others neuropsychological domains were assessed with computerized Wisconsin Card Sorting Test (WCST), for the evaluation of cognitive flexibility, and Continuous Performance Test (CPT), for the evaluation of attention (Stratta et al., 2004). Every test was administered by a trained psychologist.

Analyses on neuropsychological performance were performed on T scores for BACS subtests, number of completed categories for WCST, as a measure of executive functions more specifically related to abstract thinking (Bosia et al., 2010), and number of "misses" (i.e. number of targets not responded to) for CPT, a measure of sustained attention. BACS T scores were calculated from Italian normative data (Anselmetti et al., 2008).

3.3. Statistical analysis

The effects of the three polymorphisms were investigated using a dominant model. We analyzed *ADD1* G1532T (rs4961) testing TT + GT versus GG and *ADD2* C1797T (rs4984) testing TT + CT