Neurobiology of Learning and Memory 94 (2010) 446–451

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

The Reelin (*RELN*) gene is associated with executive function in healthy individuals

Bernhard T. Baune [a,1,*], Carsten Konrad [b,d,e,1], Thomas Suslow [b,f], Katharina Domschke [b], Eva Birosova [a], Christina Sehlmeyer [b,d], Christian Beste [c]

[a] Department of Psychiatry and Psychiatric Neuroscience, School of Medicine and Dentistry, James Cook University, QLD, Australia
[b] Department of Psychiatry, University of Münster, Münster, Germany
[c] Institute for Cognitive Neuroscience, Department of Biopsychology, Ruhr-Universität Bochum, Germany
[d] Interdisciplinary Center for Clinical Research (IZKF), University of Münster, Germany
[e] Department of Psychiatry and Psychotherapy, University of Marburg, Germany
[f] Department of Psychosomatic Medicine and Psychotherapy, University of Leipzig, Leipzig, Germany

ARTICLE INFO

Article history:
Received 8 May 2010
Revised 26 July 2010
Accepted 16 August 2010
Available online 19 August 2010

Keywords:
RELN
Neurodevelopment
Perseverative errors
Schizophrenia
Healthy individuals

ABSTRACT

Executive functions such as set-shifting and maintenance are cognitive processes that rely on complex neurodevelopmental processes. Although neurodevelopmental processes are mainly studied in animal models and in neuropsychiatric disorders, the underlying genetic basis for these processes under physiological conditions is poorly understood. We aimed to investigate the association between genetic variants of the Reelin *(RELN)* gene and cognitive set-shifting in healthy young individuals. The relationship between 12 selected single nucleotide polymorphisms (SNPs) of the *RELN* gene and cognitive set-shifting as measured by perseverative errors using the modified card sorting test (MCST) was analysed in a sample of $N = 98$ young healthy individuals (mean age in years: 22.7 ± 0.19). Results show that in individual MANCOVA analyses two of five significant SNPs (rs2711870: $F_{2,39} = 7.14$; $p = 0.0019$; rs2249372: $F_{2,39} = 6.97$; $p = 0.002$) withstood Bonferroni correction for multiple testing (corrected *p-value:* $p = 0.004$). While haplotype analyses of the *RELN* gene showed significant associations between three haplotypes and perseverative error processing in various models of inheritance (adjusted for age, gender, BDI, MWTB IQ), the GCT haplotye showed the most robust finding with a recessive model of inheritance ($p = 2.32 \times 10^{-5}$) involving the functional SNP rs362691 (Leu-Val amino acid change). Although our study strongly suggests the involvement of the *RELN* gene in cognitive set-shifting and maintenance, our study requires further exploration as well as replication of the findings in larger samples of healthy individuals and in clinical samples with neuropsychiatric disorders.

1. Introduction

The underlying molecular mechanisms of neurodevelopment may affect complex cognitive function such as learning, memory and executive function (Brigman, Padukiewicz, Sutherland, & Rothblat, 2006; Herz & Chen, 2006; Qiu et al., 2006). Disruptions in some of the responsible proteins for neurodevelopment such as the Reelin pathways may increase the risk for neuropsychiatric disorders such as schizophrenia, bipolar disorder, major depression, Alzheimer's disease, autism and lissencephaly presenting with various domains of impaired cognitive function (Fatemi, Earle, & McMenomy, 2000; Fatemi et al., 2005; Kato & Dobyns, 2003; Knuesel et al., 2009; Skaar et al., 2005; Suzuki et al., 2008). Findings on the relevance of the Reelin (*RELN*) gene for executive function and working memory in schizophrenia have been supported by human and animals studies (Brigman et al., 2006; Cassidy, Mulvany, Pangalos, Murphy, & Regan, 2010; Wedenoja et al., 2008). Moreover, *RELN* promoter-region hypermethylation (Abdolmaleky et al., 2005; Grayson et al., 2005) and selective down-regulation of *RELN* have been detected in post-mortem schizophrenic brains (Guidotti et al., 2000).

Reelin is a protein that is involved in the regulation processes of neuronal migration and positioning in the developing brain (D'Arcangelo, 2006; Zhang, Assadi, Roceri, Clark, & D'Arcangelo, 2009). Although Reelin appears to play a major role in neurodevelopment, research on the role of Reelin in cognitive function in healthy young humans seems to be spare. Investigations, however, of the role of the *RELN* gene in cognitive performance and in executive function in particular in the young developing brain may provide insight into vulnerability factors for the development of neuropsychiatric disorders (Crews & Boettiger, 2009; O'Hearn, Asato, Ordaz, & Luna, 2008; Schubert & McNeil, 2007; Stevens, Skudlarski, Pearlson, & Calhoun, 2009). Set-shifting functions are one instance of executive functions and are mediated via medial prefrontal areas

* Corresponding author. Fax: +61 7 4781 6841.
E-mail address: Bernhard.Baune@jcu.edu.au (B.T. Baune).
[1] These authors contributed equally to this manuscript and should be regarded as joint first author.

1074-7427/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2010.08.002

(e.g. Floresco, Zhang, & Enomoto, 2009; Kehagia, Murray, & Robbins, 2010). Especially the medial prefrontal cortex (mPFC) has been shown to be sensitive to changes in Reelin expression, which correlates with deficits in executive functions (Cassidy et al., 2010). Based upon this we hypothesized that the *RELN* gene impacts cognitive set-shifting functions in healthy individuals. We investigated the role of 12 tagging SNPs of the *RELN* gene in cognitive set-shifting as a measure of executive function in healthy subjects.

2. Material and methods

2.1. Sample

The cross-sectional study was performed as part of a larger study effort to investigate genetic influences on cognitive and electrophysiological processes (Beste et al., 2010). A sample of 98 (31 males, 67 females) genetically unrelated, healthy subjects of Caucasian descent (mean age of 22.7 ± 0.19) was recruited by newspaper announcement. All subjects underwent a detailed screening interview to exclude any current or previous medical and psychiatric disorders. No gender differences were observed for perseverative errors (MCST in %), IQ (MWTB IQ) and depressive symptoms (BDI) (see Table 1 for details). MCST (range: 0–20.3%; normally distribute data according to Kolmogorov–Smirnov test: $p = 0.52$) was not related to age ($F_{1,9} = 0.91$; $p = 0.52$). Hardy–Weinberg equilibrium was examined using the program Finetti provided as an online source (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl; Wienker TF and Strom TM). Gender was equally distributed across genotype groups of all 12 SNPs (Kruskal–Wallis-Test (H-Test); data not shown). The distribution of the genotypes of the 12 SNPs in the *RELN* gene did not significantly differ from the expected numbers calculated on the basis of observed allele frequencies according to Hardy–Weinberg equilibrium.

The study was approved by the local ethics committee of the University of Muenster, Germany. Participants gave written informed consent after full explanation of all study procedures.

2.2. Neuropsychological measures

2.2.1. Modified card sorting test (MCST) and premorbid intelligence: MWTB IQ

A modified version of the Wisconsin card sorting test (MCST) (Heaton, 1981) was used which resembles the modification developed by Nelson (Nelson, 1976). This modified version seems to have motivational and interpretative advantages (Nelson, 1976). When the standard Wisconsin card sorting test is used, it is not always possible to identify what strategy the subject is employing, since 80 of the 128 cards share two or three attributes with a stimulus card. Before the test, subjects were provided with part of the sorting rules and were trained with an automated test version, in which the stimulus card appeared on the screen [part of the neuropsychological tests version 2.2. developed by Ille, Kapitza, and Vogelgesang (1992)]. They were told that one sorting category was colour and that the sorting rule would change during the test. Use of these modified instructions was intended to minimize motivational reasons for performance deficits. The MCST was presented on an IBM-compatible microcomputer. The subject sorted the cards by pressing one of four response-card buttons on a keyboard. Feedback ('right' or 'wrong') was provided acoustically and visually on the screen after the sort. The subject had 20 s to choose a card. The criterion for shifting category was six correct responses. The test was stopped after six categories had been completed. In addition, there were no test cards sharing two or more attributes with a stimulus card. These modifications were made to obtain greater clarity in categorizing errors. The main measure from the MCST used in this study is called perseverative errors. All participants were tested individually in a quite room free from auditory and visual distractions. Perseverative errors result from ignoring changing rules. For example, if the rule instructs to sort cards by colour, and the participant does so, but when the instructed rule changes, e.g. to sort by shape and the participant still sorts cards by colour, this is defined as a perseverative error. An example from day-to-day life might be that a password is requested to login onto the computer, an error message occurs and despite the obvious error, the user enters the same password repeatedly.

Premorbid intelligence was assessed with a multiple choice verbal intelligence test (Mehrfachwahl–Wortschatz–Intelligenztest MWTB IQ) (Lehr, 2005). The MCST and MWTB were given as part of a test battery which included seven information-processing measures including the "Digits backward" for assessment of verbal working memory. We selected the MCST to analyse the association with genetic variants of the *RELN* gene since perseverative errors and the *RELN* gene are discussed to be involved in impaired executive function, such as in schizophrenia (Brigman et al., 2006; Wedenoja et al., 2008). The tests were administered in a fixed sequence of presentation.

2.2.2. Depressive symptoms

In order to exclude depressive symptoms, Beck's Depression Inventory (Beck & Beck, 1972; Hautzinger, Bailer, Worall, & Keller, 1995) was applied at the time of the screening interview (BDI: mean 3.5 ± 0.69; *t*-test for differences between male/female subjects: $df = 94$, $p = 0.77$). All diagnostic and psychometric evaluations were performed by experienced clinical raters.

2.2.3. SNPs selection and genotyping

The entire sequence of the *RELN* gene contains more than 1079 single nucleotide polymorphisms (SNPs) of which 813 SNPs have minor allele frequency (MAF) > 5% (International, HapMap, and Consortium, 2007). We used various techniques to limit the number of SNPs assessed to the most relevant. We initially constructed the linkage disequilibrium (LD) pattern of the CEPH population of the HapMap Phase II genotype data to identify tagging SNPs by an aggressive tagging approach (minor allele frequency, MAF > 5% and $r^2 > 0.8$) using Gevalt v2 software package (Davidovich, Kimmel, & Shamir, 2007). The region analysed included about 517.7-kb of the *RELN* gene between the positions 103112230 and 103629962 at chromosome 7 (human genome coordinates hg18). Ultimately, we reduced SNP numbers by assessing the ability of limited numbers of the tagging SNPs to predict the total SNP population using Stampa algorithm (Halperin, Kimmel, & Shamir, 2005). With this approach 88.0% of the variation in the gene was captured using 12 tagging SNPs (Table 2). The mean r^2 of individual

Table 1
Sample ($N = 98$) characteristics across gender.

	Gender (mean ± *SE*)		*t*-Test *p*-Value
	Female ($N = 67$)	Male ($N = 31$)	
Age	22.4 ± 0.24	23.2 ± 0.35	0.032
MWTB IQ	107.3 ± 1.4	109.8 ± 1.8	0.153
Perseverative errors (MCST in %)	4.5 ± 0.41	5.6 ± 0.83	0.10
Verbal working memory (digits backward)	7.6 ± 0.26	8.2 ± 0.35	0.13
BDI	3.7 ± 0.39	3.2 ± 0.54	0.77

MCST = modified card sorting test.
MWTB IQ = premorbid intelligence (Mehrfachwahl–Wortschatz–Intelligenztest).
BDI = Becks Depression Inventory.