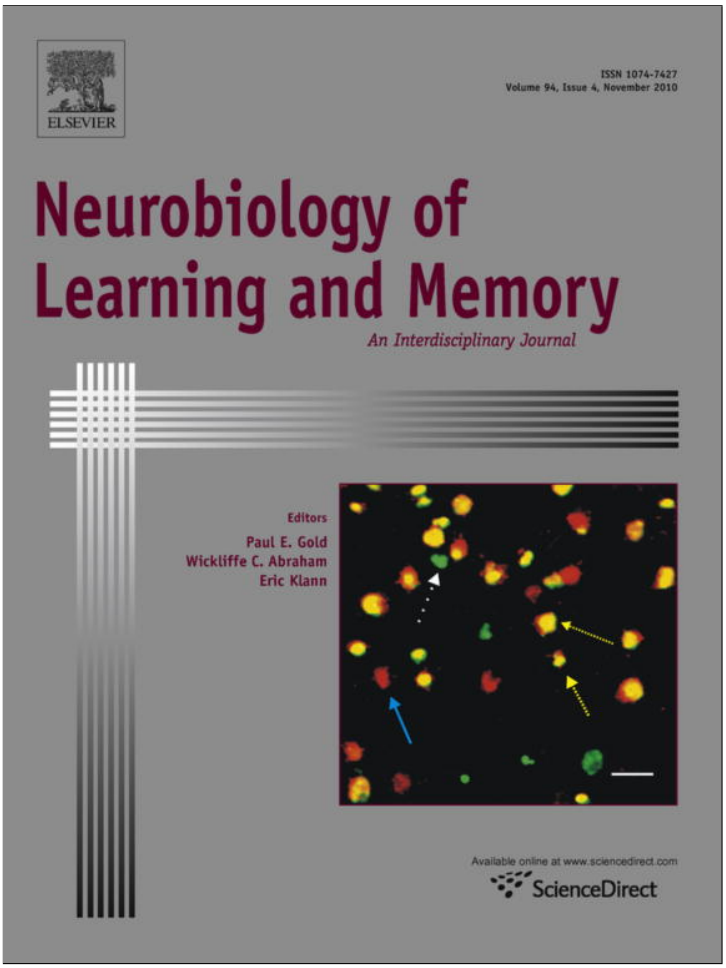

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The Reelin (*RELN*) gene is associated with executive function in healthy individuals

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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

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(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.

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.

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 2
Selection of single nucleotide polymorphisms within *RELN* gene.

Gene pos.	SNPs (MAF ⩾ 0.05)	# of tagging SNP	Mean r^2	Selected SNPs	Position	Function	Alleles	MAF	Alleles captured	Prediction (STAMPA)
				rs4621738	103621551	Intron 1	AG	0.432 (G)	21	
				rs4460306	103621132	Intron 1	AC	0.491 (C)	13	
				rs2299403	103555914	Intron 2	GT	0.208 (T)	11	
				rs39367	103473365	Intron 3	CT	0.412 (T)	34	
Chromosome 7				rs528528	103389085	Intron 6	CT	0.492 (T)	19	
7q22.2–22.3				rs2215535	103298865	**Intron 7**	CT	0.350 (T)	4	
1036,29,963–1031,12,231	1079	813	**0.961**				C = > G			
				rs362691	103251161	**Exon 22**	Leu-Val	0.133 (C)	9	88%
				rs2249372	103238210	Intron 24	AG	0.351 (A)	12	
				rs2711870	103226907	Intron 28	AG	0.308 (A)	8	
				rs2229860	103205827	**Exon 34**	C = > G			
							Pro-Arg	0.008 (G)	1	
				rs2528856	103142795	Intron 52	CT	0.305 (C)	7	
				rs2711844	103132863	Intron 57	AC	0.233 (A)	7	

RELN, Reelin; SNP, single nucleotide polymorphism; MAF, minor allele frequency; r^2, linkage disequilibrium statistic (Carlson, Reider, Nickerson, Eberle, & Kruglyak, 2005). MAF data relates CEPH population from HapMap Phases I and II (International, HapMap, and Consortium, 2005).

tagging SNPs in conjunction with one or more tagged SNPs was 0.961 (Table 2).

Genotyping of the selected 12 *RELN* tagging SNPs was carried out following published protocols applying the multiplex genotyping assay iPLEX™ for use with the MassARRAY platform (Oeth et al., 2007), yielding a genotyping completion rate of 97.2%. Genotypes were determined by investigators blinded for the study.

2.2.4. Statistical analyses

Differences of means of continuous variables between groups were tested using two-sample *t*-test (Table 1). Multivariate analysis of covariance (MANCOVA) was performed to investigate the association between *RELN* SNPs and perseverative errors (MCST in %) considering age, gender, MWTB IQ and BDI as covariates (Table 3). In case individual genotypes had small numbers, they were collapsed to a combined genotype. Bonferroni correction for multiple comparisons was carried out post hoc for 12 SNPs yielding a corrected *p*-value of $p = 0.004$. While no corrections for multiple comparisons were made during parsing of the haplotype analyses, *p*-values derived from haplotype analyses were compared to the corrected *p*-value of $p = 0.004$.

To reduce computational demands and because of a relatively small sample size, haplotypes were analysed with using "sliding window" approach with a three marker window size. Haplotypes were inferred using the expectation maximation (EM) algorithm from unphased genotype data and a global test of haplotype association was initially performed taking the most common haplotype as baseline and comparing all other haplotypes simultaneously. Statistically significant haplotypes were then explored individually and tested for potential confounders such as gender, age, BDI, and log-transformed MWTB IQ. All associations were assessed under additive, dominant, and recessive models of inheritance and Akaike's information criterion was used for selecting among models (Akaike, 1974). Three SNPs were set to create the haplotypes because of computational limitations and decreasing frequency of longer haplotypes. All computations were performed using SimHap v1.0.2 software (Carter, McCaskie, & Palmer, 2008).

Table 3
Relationship between 12 *RELN* single nucleotide polymorphisms (SNPs) and percentage perseverative errors of the modified card sorting test (MCST) among healthy subjects ($N = 98$) using multivariate analysis of covariance (MANCOVA).

12 *RELN* SNPs	MANCOVA*	
	F-value	*p*-Value*
rs2215535	1.39	0.092
rs2229860	0.01	0.950
rs2249372	6.97	*0.002***
rs2299403	3.07	*0.05*
rs2528856	3.87	*0.027*
rs2711844	4.30	*0.019*
rs2711870	7.14	*0.0019***
rs362691	0.42	0.522
rs39367	0.68	0.511
rs4460306	0.87	0.423
rs4621738	1.44	0.248
rs528528	1.59	0.215

* *p*-Value from MANCOVA with covariates: age, gender, MWTB IQ, BDI.
** *p*-Value withstands adjusted *p*-value (Bonferroni correction for 12 tests): $p = 0.004$.

3. Results

3.1. RELN gene and neuropsychological performance

Table 3 presents results of MANCOVAs (covariates age, gender, MWTB IQ, BDI) analysing the association between individual *RELN* SNPs and perseverative errors (in %). Among the 12 *RELN* SNPs, five SNPs (rs2249372, rs2299403, rs2528856, rs2711844, rs2711870) showed significant associations ($p \leqslant 0.05$) with perseverative errors (in %). Two of the 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: $p = 0.004$). For those two SNPs, we repeated MANCOVA with the combined AA/AG genotype vs. GG genotype due to small numbers of the AA genotype in both SNPs ($N = 4$ and $N = 6$, respectively). We found that the associations between both SNPs and perseverative errors (in %) of the MCST were even stronger (rs2711870: $F_{1,38} = 14.48$; $p = 0.0004$ and rs2249372: $F_{1,38} = 14.10$; $p = 0.0005$). A comparison of means of error processing shows for both SNPs better performance (less errors) of the GG vs. AA/AG genotypes (rs2711870: GG = 3.9, *SE* = 0.4 vs. AA/AG = 6.0, *SE* 0.6; rs2249372: GG = 3.8, *SE* = 0.4 vs. AA/AG = 5.8, *SE* = 0.6). Verbal working memory was not associated with any of the *RELN* SNPs (data not shown).

As presented in Table 4, haplotype analyses of the *RELN* gene showed significant associations between three haplotypes and perseverative errors in various models of inheritance (adjusted for age, gender, BDI, MWTB IQ). The CAA haplotype contained SNPs rs2249372 and rs2711870 the two most significant single SNPs from previous single SNP analyses withstanding Bonferroni correction for multiple testing (as shown in Table 3) as well as SNP rs2229860 residing in a protein-coding region representing a vari-

Table 4
Association of *RELN* gene haplotypes with perseverative errors.

SNP	Haplotype	Frequency	Copy	Additive model				Dominant model				Recessive model			
				Coefficient	*SE*	*P*	Mean	AIC	Coefficient	*SE*	*P*	Mean	AIC	Coefficient	*SE*	*P*	Mean	AIC
rs2229860	CAA	0.2666 (N = 53)	0	–	–	–	4.0543	504.4	–	–	–	4.0561	502.4	–	–	–	4.7993	507.6
													0.0283					
rs2711870			1	1.792	0.853	0.0381	5.8464	NS	1.787	0.814	*0.0302*	5.8432						NS
rs2249372			2	1.752	1.717	0.3103	5.8060							1.007	1.719	0.5600	5.8060	
rs362691	GCT	0.2300 (N = 45)	0	–	–	–	4.1431	489.6	–	–	–	4.1489	503.7	–	–	–	4.5645	489.6
													NS					
rs2215535			1	0.911	0.839	0.2770	5.0546	4.748×10^{-5}	1.487	0.955	0.1250	5.6358						1.782×10^{-5}
rs528528			2	9.300	2.016	1.36×10^{-5}	13.4433							8.879	1.989	2.32×10^{-5}	13.4433	
rs528528	TCT	0.0649 (N = 13)	0	–	–	–	4.4743	501.5	–	–	–	4.4745	500.7	–	–	–	4.7853	505.0
rs39367			1	2.661	1.431	0.0525	7.1353	0.0302	2.980	1.366	*0.0288*	7.4541	0.0272					NS
rs2299403			2	6.636	3.620	0.0703	11.1100							6.325	3.705	0.0913	11.1100	

SNP, single nucleotide polymorphism; copy, copy number of haplotypes; *SE*, standard error; *P*, *p*-value; AIC, Akaike's information criterion; NS, non-significant model.

ant of Reelin with leucin in the mentioned position. This haplotype seems to increase preservative error in a dominant manner.

While the CAA haplotype (rs2229860–rs2711870–rs2249373, $p = 0.0302$) and the TCT haplotype (rs2299403–rs39367–rs528528, $p = 0.0288$) following a dominant model of inheritance showed moderately strong associations with perseverative errors, the most striking result was found for the GCT haplotpye (rs362691–rs2215535–rs528528) in an additive ($p = 1.36 \times 10^{-5}$) and recessive ($p = 2.32 \times 10^{-5}$) model of inheritance. The GCT haplotype contains the functional SNP rs362691 coding the prolin variant of the Reelin protein and another two intronic SNPs. Although these three SNPs comprising the GCT haplotype are not associated with the preservative error phenotype when analysed individually (see Table 3), this haplotype shows a striking increase in mean preservative errors from 4.56 (zero or one copy of the haplotype) to 13.44 with two copies of the haplotype in a recessive model of inheritance. A recessive model of inheritance is a common model of inheritance in multifactorial cognitive processes and diseases, such as in neuropsychiatric disorders.

Overall, haplotype analyses suggest that various SNPs of the *RELN* gene appear to be individually and in haplotypes related to perseverative error processing in healthy individuals. While the significant results of the two haplotypes in a dominant model of inheritance would no withstand Bonferroni correction of multiple testing ($p = 0.004$), the GCT haplotype showing a strong association with perseverative error processing survived Bonferroni correction for multiple testing.

4. Discussion

In this study we investigated the association between a wide range of genetic variants of *RELN* (covering 88% of the *RELN* gene) and cognitive set-shifting (perseverative error processing) in healthy individuals. Among 12 SNPs in the *RELN* gene, the SNPs rs2711870 and rs2249372 showed significant associations with perseverative error processing. For both SNPs, the A-alleles were related to higher numbers of perseverative errors as compared to the GG genotype. Haplotype analyses confirmed the association between the *RELN* gene variants and perseverative error processing, and this effect was best seen under a recessive model of inheritance withstanding Bonferroni correction for multiple testing. Given the relative high frequency of two of the CAA and BCT haplotypes (26.7% and 23.0%), it can be concluded that they may have impact on perseverative error processing in a significant number of healthy individuals. Although the haplotype analyses need to be interpreted with caution in this relatively small sample and require replication in larger samples, the GCT haplotype with a robust result for a recessive mode of inheritance points to the most important finding of this study.

This GCT haplotype contains a SNP rs362691 leading to a Leu-Val amino acid change. It is hypothesized that this amino acid change is functionally relevant in conjunction with the other two SNPs of the haplotype for higher cognitive functions such as perseverative error processing rather than as an individual SNP. In contrast, the CAA haplotype that contains a functional SNP (Pro-Arg amino acid change) plus two other SNPs with strong significant associations with perseverative error processing in single analyses, failed to show a degree of statistical significance withstanding Bonferroni correction for multiple testing in the haplotype analyses. The relevance of the SNP rs362691 as a putative marker involved in complex high-cognitive function is underlined by previous reports that found this SNP to be involved in ventricular enlargement in schizophrenia (Gregorio et al., 2009). Since set-shifting, as an index of cognitive control, is presumed to be mediated by the prefrontal cortex (Nakahara, Hayashi, Konishi, & Miyashita, 2002) a

region classically involved in neuropsychiatric illnesses such as schizophrenia, the specific SNP rs362691 appears to be a promising putative marker of some neuropsychiatric diseases such as schizophrenia, but possibly not of other illnesses such as autism (Dutta et al., 2008; He et al., in press). However, several studies suggest other SNPs of the *RELN* gene to be involved in a variety of neuropsychiatric disorders (see Section 1 for details).

The human *RELN* gene is large in size and contains an enormous amount of introns. Alternative splicing and microRNAs are the most probable factors influencing quality and quantity of the Reelin protein expression during embryonal as well as adult live. Distinct patterns of Reelin expression in structures such as olfactory bulb, retina, and spinal cord suggest that the protein might be endowed with different functions (Lambert de Rouvroit, Bernier, Royaux, de Bergeyck, & Goffinet, 1999). It is suggested that two alternative evolutionary conserved splicing events result in different forms of the protein, both of which are regarded as functionally important (Lambert de Rouvroit et al., 1999). In the context of our results, it is suggested that the combination of several genetic factors rather than a single mutation has implications for intricate expression–controlling mechanisms within the *RELN* gene.

Previous studies have shown that Reelin is involved in the molecular mechanisms of cognitive functions. For the current study, previous findings showing that the expression of Reelin in medial prefrontal cortical areas is associated with executive functions in Wistar rats (Cassidy et al., 2010) is most important. Likely, the underlying mechanism observed in the Cassidy et al. (2010) study may underlie the observed effects on cognitive set-shifting in humans, too. In particular, genetic differences may be associated with an altered (maladaptive) wiring within the medial prefrontal cortex that may ultimately lead to the observed differences at the behavioural level. As Reelin also alters mechanisms of synaptic neural transmission (e.g. Groc et al., 2007; Herz & Chen, 2006) these functional effects may also contribute to the observed differences, besides a possible alteration in the morphology of relevant brain areas.

From our haplotype analyses and the hypothesized mechanism by which the *RELN* gene may influence cognitive processes, we conclude that the GCT haplotype with a recessive model of inheritance appears most likely to be relevant for multifactorial complex cognitive tasks such as cognitive set-shifting. Since Reelin has shown to be relevant for two major processes of neurodevelopment and adult multifactorial neuropsychiatric conditions such as schizophrenia and bipolar disorder with psychosis, we hypothesize that the GCT haplotype of the *RELN* gene might prove to be relevant for general cognitive deficits in those neuropsychiatric disorders. Further sufficiently powered clinical genetic studies especially in younger adults being at risk for schizophrenia or mood disorders are required in order possibly identify the *RELN* gene as a risk gene for early cognitive impairment and/or as a susceptibility gene for cognitive components of neuropsychiatric disorders. This suggestion is supported by previous findings that report on the involvement of the *RELN* gene in a broad range of neuropsychiatric disorders rather than in specific psychiatric disorders only (Fatemi et al., 2000, 2005; Kato & Dobyns, 2003; Knuesel et al., 2009; Skaar et al., 2005; Suzuki et al., 2008).

The individual after Bonferroni correction for multiple testing significant SNPs rs2249372 and rs2711870 are located in non-translating regions (intron 23, position 10,323,8210 and intron 28, position 10,32,26,907, respectively), both not resulting in an amino acid change in the Reelin protein. Interestingly, recent studies show that nucleotide changes in intronic regions may result in clinical manifestation (Lin, Miller, & Ying, 2006; Meerson, Cacheaux, Goosens, Sapolsky, Soreq, & Kaufer, 2010) since sequences in these non-protein-coding regions can encode microRNAs (miRNAs), which are responsible for RNA-mediated gene silencing through RNA interference (Lin et al., 2006). A role of miRNAs in the nervous system has been described over the past 10 years clearly showing that miRNA-dependent posttranscriptional gene regulation plays pivotal roles at all stages of neural development, for example, neural differentiation, morphogenesis, and plasticity (Christensen & Schratt, 2009; Schratt, 2009). More specifically, miRNAs have been show to be implicated in neurodevelopmental (Martino, di Girolamo, Orlacchio, & Datti, 2009) and neurodegenerative disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease (Boissonneault, Plante, Rivest, & Provost, 2009; Lukiw, Zhao, & Cui, 2008; Packer, Xing, Harper, Jones, & Davidson, 2008) and cognitive function such as memory (Sanchez-Carbente Mdel & Desgroseillers, 2008). Since the *RELN* gene has also been associated with neurodevelopment and neurodegeneration (see introduction for details) and cognitive function such as cognitive control as shown with our data, it can be hypothesized that the SNPs rs2249372 and rs2711870 could be a part or close to miRNA participating in Reelin protein expression regulation. However, the currently available database 'miRBase' does not indicate that miRNAs are found in the region of chromosome 7 under investigation in our data. At this stage it cannot be answered with confidence if this missing link is a real finding or if it suggests that miRNAs have not been investigated in the region of interest in chromosome 7. Future work is required to clarify this important question. Finally, more genetic and epidemiological research has to be undertaken to clarify the possible role of these two individual SNPs in Reelin expression control.

Although the literature lacks a clear mechanistic rationale to explain the observed association, our data support the role of the *RELN* gene in cognitive set-shifting processes. Specifically, the roles of the functional SNP rs362691 and the intron SNPs rs2249372 and rs2711870 as either causative genetic variants, or as biomarkers associated with another, possibly functionally relevant polymorphism in linkage disequilibrium with this SNP remains to be investigated.

In conclusion, we have presented that in healthy individuals complex cognitive functions such as set-shifting (perseverative error processing) show a strong association with the *RELN* gene involving a single haplotype (recessive mode of inheritance) with one functional and two non-functional SNPs. The possible specific role of the functional SNP reported in 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.

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