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Dysbindin C–A–T haplotype is associated with thicker medial orbitofrontal cortex in healthy population

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ABSTRACT

The dysbindin (dystrobrevin-binding protein 1) gene has been indicated as one of the most important schizophrenia susceptibility genes. Several genetic variations of this gene have been investigated by using an "intermediate phenotype" approach showing a particular detrimental effect on the prefrontal function in schizophrenic patients. However, the nature of dysbindin function within the brains of healthy individuals is poorly understood, in particular as concerns brain anatomy.

We examine relationships between a previously implicated three marker C–A–T dysbindin haplotype and regional cortical thickness in a wide population genotyped for risk carriers (n = 14) and non-risk carriers (n = 93).

Surface-based analysis of the cortical mantle showed that the dysbindin haplotype was associated with structural differences in the medial orbitofrontal cortex, where the risk carriers showed the highest cortical thickness values and the non-risk carriers the lowest.

Our study extends previous evidence found on schizophrenic patients to the healthy population, demonstrating the influence of dysbindin risk variants on the neuronal architecture of a specific brain region relevant to the neuropathology of schizophrenia.

Introduction

The dystrobrevin-binding protein 1 (DTNBP1) gene, known as the dysbindin gene, is located in chromosome 6p22.3, encodes 40–50 kDa protein, which is widely expressed in neurons of the human brain, and plays a significant role in modulating glutamatergic neurotransmission (Numakawa et al., 2004; Talbot et al., 2006).. Several single nucleotide polymorphisms (SNPs) for this gene, have been investigated in the past few years in worldwide populations (Straub et al., 2002; Schwab et al., 2003; van den Oord et al., 2003; Kirov et al., 2004; Williams et al., 2004; Funke et al., 2004) demonstrating a significant association with schizophrenia, although some inconsistent findings have been reported (Mutsuddi et al., 2006). Moreover, it has been demonstrated that variability in the dysbindin gene contributes to individual variability of cognitive functions at both neurophysiological and behavioral levels in patients with schizophrenia as well as in healthy controls (Burdick et al., 2006; Donohoe et al., 2007, 2008; Fallgatter et al., 2006, 2010).

To investigate the relationships between the biology of schizophrenic risk and specific brain functions *in vivo*, several authors have conducted imaging genetics studies (Markov et al., 2009, 2010; Mechelli et al., 2010; Wolf et al., in press; Fallgatter et al., 2006, 2010). Imaging genetics is a novel approach aimed to characterize the influence of genes on individual variability in cognition and behavior. It is based on the assumption that brain structure and function are more closely related to gene function than trait differences in overt behavior (Mattay et al., 2008). Thus, the study of the "intermediate phenotype" might provide a more direct measurement of the impact of a gene. Several imaging genetics studies have been focused on two specific SNPs: 1018381 and 2619538, both of which have resulted in being risk factors for schizophrenia and associated with altered intermediate brain phenotypes (Markov et al., 2009, 2010; Narr et al., 2009; Mechelli et al., 2010). These findings are complimented by studies of other risk variants investigating haplotypes at this locus. Recently, the C–A–T haplotype (derived from SNPs rs2619539,

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rs3213207 and rs2619538) reported by Williams et al. (2004) has shown a strong association with schizophrenia, reduced dysbindin mRNA expression in human post-mortem analysis of homogenized human brain (Bray et al., 2005), poorer cognitive performance in working memory (Donohoe et al., 2007) and reduced gray matter volume in the prefrontal cortex in patients with schizophrenia (Donohoe et al., 2010). However, the nature of dysbindin function within the brain of healthy individuals is poorly understood, in particular as concerns brain anatomy. Dysbindin gene transcription has been observed in the temporal cortex, entorhinal cortex, prefrontal cortex (including both orbitofrontal and dorsolateral prefrontal cortices) and hippocampus in healthy individuals (Weickert et al., 2004). Dysbindin is involved in the regulation of neuroplasticity (Guo et al., 2009; Talbot et al., 2006) and interacts with 31 proteins involved in cell morphology, synaptic structure and cellular development (Talbot et al., 2006; Guo et al., 2009). All this evidence highlights the importance of investigating the neuroanatomical correlates of genetic variations of this gene in healthy populations, in order to understand how genes affect brain physiology and anatomy to mediate vulnerability to physiopathology of schizophrenia (Viding et al., 2006).

Thus, the aim of this study is to provide, for the first time in healthy individuals, evidence of morphological changes associated with the C–A–T schizophrenia-risk haplotype by using an *in vivo* cortical thickness measurement. Given the aforementioned abundant evidence showing how this haplotype affects both brain function and anatomy of the prefrontal cortex in patients with schizophrenia, we hypothesized a similar effect also in healthy individuals.

Methods

Participants

One hundred and seven healthy individuals (Caucasian, age-range: 18–73) were considered eligible for this study. Subjects were recruited from universities, community recreational centers and hospital personnel from 2005 to 2009. Inclusion criteria were: (1) right handedness, according to the Edinburgh Handedness Inventory (Oldfield, 1971); (2) vision and hearing sufficient for compliance with testing procedures; (3) neuropsychological scores above the cutoff scores, corrected for age and educational level, identifying normal cognitive level in the Italian population (see Neuropsychological assessment). Exclusion criteria were: (1) major medical illnesses, known or suspected history of alcoholism or drug dependence and abuse during lifetime and family history of major psychotic disorders such as schizophrenia and bipolar disorders; (2) mental disorders (i.e., schizophrenia, mood disorders, anxiety disorders, personality disorders or any other significant mental disorder), according to DSM-IV criteria assessed by the Structured Clinical Interviews for DSM-IV Axis I (SCID-I) (First et al., 1997a) and Axis II (SCID-II) (First et al., 1997b) and/or neurological disorders diagnosed by an accurate clinical neurological examination; (3) dementia, according to DSM-IV criteria or mild cognitive impairment according to Petersen criteria (Petersen et al., 1999) and confirmed by the administration of the Mental Deterioration Battery (MDB) (Carlesimo et al., 1996); (4) Mini Mental State Examination (MMSE, (Folstein et al., 1975)) score<27; (5) presence of vascular brain lesions, brain tumor and/or marked cortical and subcortical atrophy on MRI scan. All subjects were biologically unrelated, white/Caucasian residents of Italy, and all provided written informed consent to participate in the genetic studies. The study procedures were undertaken in accordance with the guidance of Santa Lucia Foundation Ethics Committee. All participants were genotyped based on the high risk haplotype (C–A–T; n 14) and non-risk haplotype (n 93) of the dysbindin gene (Williams et al., 2004) (see Genetic analysis).

Neuropsychological assessment

Two trained neuropsychologists, who were blind to the aim of the study, conducted the cognitive assessment, which was performed within 15 days of MRI acquisition. We selected the following tests from the MDB (Carlesimo et al., 1996) in order to provide information about the functionality of different cognitive domains such as: verbal memory (Rey's 15-word Immediate Recall (RIR) (cut-off score<28.53) and Delayed Recall (RDR) (cut-off score<4.69)), short-term visual memory (Immediate Visual Memory (IVM) (cut-off score<13.85)) and logical reasoning (Raven's Progressive Matrices' 47 (PM47) (cut-off score<18.96)). As "executive functioning" denotes a set of different cognitive abilities that are involved in complex, goal-directed thought and behavior, the following executive dimensions were assessed: a) attention control, b) set-shifting and c) working memory (Spoletini et al., 2009). In order to assess abilities of attention control and inhibition, we administered the Stroop test (ST) (Stroop, 1935). Time of performance was chosen as a measurement. Set-shifting or cognitive flexibility was assessed using the Modified Wisconsin Card Sorting test (MWCST) (Heaton et al., 1993); the number of perseverative/no-perseverative errors was chosen as a measurement. In order to measure verbal, spatial and visual working memory, we administered the n-back test. In this test, participants were required to continuously monitor a sequence of verbal/spatial/visual stimuli (a total of 22 items for each task, visually presented on a screen) and to select items that appeared as n-back items in any sequence. The number of correct responses was generally considered as an index of working memory performance (Spoletini et al., 2009). In this study we only considered highly cognitive demanding n−2 level performances.

Although none of the participants met the criteria for mood or other psychiatric disorders, we further investigated for the presence of depression and anxiety symptoms using the Hamilton Depression rating scale (HDRS) and the Hamilton Rating Scale Anxiety (HAM-A), respectively (Hamilton, 1959, 1967).

Genetic analysis

The dysbindin "risk" haplotype identified by Williams et al. (2004) is derived from alleles C–A–T at (SNPs) rs2619539, rs3213207 and rs2619538. All SNPs were genotyped using Custom Taqman SNP Genotyping Assays from Applied Biosystems on an ABI7900 real-time PCR instrument (Applied Biosystems). Duplicate genotyping in a subset of the total sample from which this MRI sample was drawn (192 of 1200 cases and controls) indicated a genotyping error level of <1%. The haplotype analysis was performed using the program PHASE ver 2.1.1 (Stephens et al., 2001). The program PHASE implements a Bayesian statistical method for resolving the haplotype combination using genotype data from unrelated subjects. The three SNPs of the dysbindin haplotype were reconstructed and the best pair haplotype was assigned to each subject. The subjects carrying at least one copy of the risk haplotype C–A–T were included in the risk group. Two of the subjects in our risk group were carriers of two copies of the C–A–T-risk haplotype. The SNP rs2619539 was in Hardy–Weinberg equilibrium ($p = 0.928$). The SNPs rs3213207 and rs2619538 did not deviate significantly from the Hardy–Weinberg equilibrium with respective p-values of 0.489 and 0.611.

Magnetic resonance imaging

Each of the 107 participants underwent the same imaging protocol with a whole-brain T1-weighted scan using a 3 T Allegra MR imager (Siemens, Erlangen, Germany) with a standard quadrature head coil. Whole-brain T1-weighted images were obtained in the sagittal plane using a modified driven equilibrium Fourier transform (MDEFT)

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(Deichmann et al., 2004) sequence (TE/TR = 2.4/7.92 ms, flip angle 15°, voxel-size $1 \times 1 \times 1$ mm^3).

Cortical thickness analysis

MRI-based quantification of cortical thickness was performed using *Freesurfer* (v. 4.05) software package (http://surfer.nmr.mgh.harvard.edu). This method has been previously described in detail (Dale et al., 1999; Fischl et al., 1999; Fischl and Dale, 2000). The procedure involves segmentation of white matter, tessellation of the gray/white matter junction, inflation of the folded surface, tessellation patterns and automatic correction of topological defects in the resulting mainfold. Cortical thickness measurements were obtained by reconstructing representations of the gray/white matter boundary and the cortical surface. The distance between these two surfaces was calculated individually at each point across the cortical mantle. This method uses both intensity and continuity information from the entire 3D MRI volume in segmentation and deformation procedures to construct representations of cortical thickness. The maps are created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity. The entire cortex in each individual subject was then visually inspected, and any inaccuracies in Talairach-transformed, skull stripped and segmentation were manually corrected and re-inspected. Thickness measurements can be mapped onto the "inflated" surface of each participant's reconstructed brain, thus allowing visualization without interference from cortical folding. Maps were smoothed using a circularly symmetric Gaussian kernel across the brain/cortical surface with a standard deviation of 12.6 mm and averaged across participants using a non-rigid high-dimensional spherical averaging method to align cortical folding patterns. This procedure provides accurate matching of morphologically homologous cortical locations among participants on the basis of each individual's anatomy while minimizing metric distortions, resulting in a mean measure of cortical thickness for each group at each point on the reconstructed surface. This spherical morphing procedure was used to construct the brain maps differences in cortical thickness.

For each hemisphere, differences in cortical thickness between haplotype groups were tested by computing a general linear model (GLM) of the effects of "group" on cortical thickness at each vertex controlling for the effects of age and gender considered as covariates in this model. A False Discovery Rate (FDR) of $p \leq 0.05$ was applied to correct for multiple comparisons. For a FDR of 5% our data implied an appropriate threshold of significance at $p = 0.00005$, corresponding to a t-value of $= 4.67$. Furthermore, the relationship between the detected regional cortical thickness abnormalities and neuropsychological scores was investigated by correlational analysis using the Spearman rank-order coefficient ($p < 0.05$). In particular, the regions showing significant morphological changes were used to create regions of interest (ROIs) on a standard brain that were mapped back to each individual subject using spherical morphing to find homologous regions across subjects. A mean thickness score over each location was calculated for each subject and used for correlation analysis. The Cohen's *d* (Cohen, 1998) was calculated as a measure of the effect sizes, which indicates the magnitude of mean differences (using the estimated marginal means) in SD units.

Voxel-based morphometry analysis

In order to strengthen our data we performed a further distinct whole-brain MRI measurement assessing characteristic patterns of cortical volume: Voxel-Based Morphometry (VBM) (see Supplementary materials). VBM analysis perfectly confirmed the findings reported by using cortical thickness measurements.

Statistical analysis

Statistical analyses for demographic data (Table 1) were performed with Statistical Package for Social Sciences software—SPSS (version 12.0, Chicago IL, USA). Assumptions for normality were tested for all continuous variables. Normality was tested using the Kolmogorov–Smirnov test. All variables were normally distributed, except for the number of years of formal education. ANOVAs, Mann–Whitney U-test and χ^2 were used to assess potential differences between the haplotype groups for all demographic variables. All statistical analyses had a two-tailed α level of <0.05 for defining significance.

Results

Mean (or median) scores on demographic and cognitive variables are presented for both risk and non-risk haplotype groups in Table 1. All variables were not different between the two groups. Fig. 1 presents the statistical differences in the analysis of cortical thickness across the entire mantle between the haplotype groups. After the FDR correction for multiple comparisons and using age and gender as covariates, a significant effect was detected only on the right medial orbitofrontal cortex where the risk-haplotype group showed the highest value (2.59 ± 0.34 mm) and the non-risk haplotype group the lowest (2.24 ± 0.41 mm) ($F_{1,102} = 10.7$; p-level < 0.001; Cohen's $d = 2.08$). When the threshold for significance was lowered to $p \leq 0.01$ without correction for multiple comparisons, the risk-haplotype group had a more pronounced thickening of the right cuneus, cingulate cortex and the left lateral/medial orbitofrontal cortex. There were no suprathreshold peaks indicating thinning of the cortex in the risk haplotype group.

We further performed regression analyses in order to investigate whether neuropsychological scores are associated with the detected significant changes in the morphology of right orbitofrontal cortex. No significant correlations were detected in either group.

Table 1
Group demographics for cortical thickness analysis.

Demographic data	Risk haplotype	Non-risk haplotype	*p* values
N	14	93	
Gender (f/m)	9/5	55/38	
Age (years)	36.8 ± 14.9	39.73 ± 15.2	0.49[a]
Educational level (years)	16.5 (8–21)	15 (5–24)	0.56[b]
Verbal memory			
RIR	47.8 ± 7.8	44 ± 7.5	0.1[a]
RDR	9.6 ± 1.7	9.7 ± 2.4	0.81[a]
Short-term visual memory			
IVM	19.5 ± 1	19.4 ± 1.5	0.88[a]
Logical reasoning			
PM 47	28.5 ± 2.2	28.4 ± 3.5	0.97[a]
Executive function			
ST read (time, s)	14.2 ± 3.9	13.6 ± 2.7	0.48[a]
ST color (time, s)	16.1 ± 2.5	17.6 ± 3.9	0.17[a]
ST color-word (time, s)	28.6 ± 7.4	31 ± 9.9	0.39[a]
MWCST PE	0.1 ± 0.5	0.4 ± 1	0.51[a]
MWCST No PE	0.6 ± 0.7	0.8 ± 1.1	0.52[a]
Verbal n-back 2-back	16.2 ± 3.5	15.3 ± 5.5	0.62[a]
Spatial n-back 2-back	15.2 ± 3.5	15.3 ± 5	0.41[a]
Visuo-spatial n-back 2-back	17.6 ± 2.6	15.8 ± 5.1	0.77[a]
Psychological variables			
HAM-A	5.4 ± 4.7	5.2 ± 3.4	0.85[a]
HDRS	3.8 ± 3.2	3.4 ± 3.1	0.67[a]

Data are given as mean values (SD) or median values (range) when appropriate. RIR and RDR, Rey's 15-word Immediate and Differite Recall; IVM, Immediate Visual Memory; PM 47, Raven's Progressive Matrices' 47; ST, Stroop task. MWCST PE and No PE, Modified Wisconsin Card Sorting test, perseverative and no perseverative errors. HAM-A, Hamilton Rating Scale Anxiety. HDRS, Hamilton Depression Rating Scale.
[a] One-way ANOVA.
[b] Mann–Whitney test.

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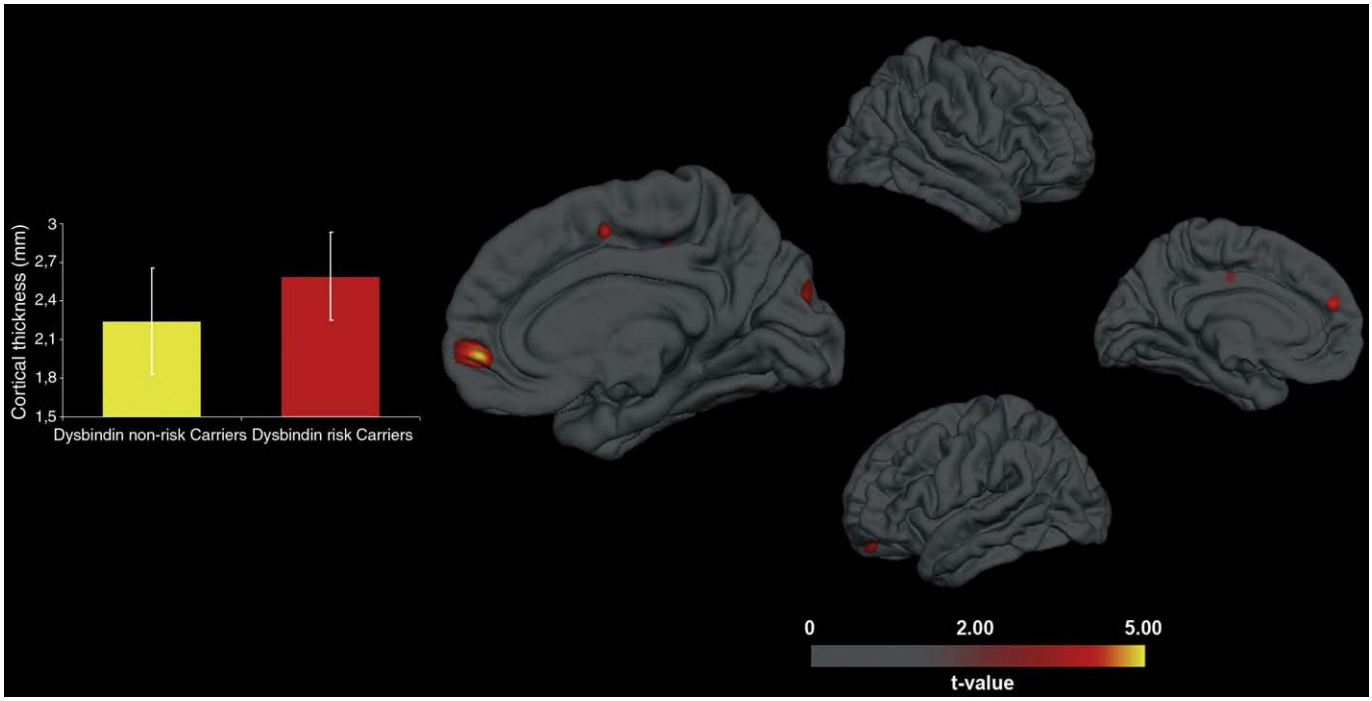

Fig. 1. Whole brain vertex-wise analysis of cortical thickness for haplotype groups. Mean difference maps were generated by aligning and averaging brain MRIs across participants in spherical space to demonstrate the main cortical thickness differences between the two groups at each point on the cortex. Maps are presented on the pial cortical surface that shows the differences between individual carriers and non-carriers of the dysbindin-risk haplotype. The results were thresholded by a conventional criterion for correction for multiple comparisons (FDR<0.05). In particular, results are thresholded using t-values, where a t-value of 2 displays all significant differences with p-level<0.01, and a t-value of 5 shows all significant differences of p-level<0.00001. For a FDR<0.05 our data implied an appropriate threshold of significance at p-level = 0.00005, corresponding to a t-value = 4.67. The regions displayed in yellow on the brain maps all met this criterion for significance and represent areas where individuals carrying the risk haplotype had significantly thicker cortices than their non-carrier counterparts. We also display thickness differences at subthreshold levels (an uncorrected threshold of p<0.01) in red in order to examine the full extent of cortical thickness differences across the cortical surface. The graphs show the mean thickness (±SD) by haplotype of the region showing the maximum effect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Discussion

We present compelling new evidence that the C–A–T schizophrenia-risk haplotype (Williams et al., 2004) in the dysbindin gene is associated with morphological changes in healthy individuals. In particular, we showed a selective effect on the right medial orbitofrontal cortex where the individuals carrying the risk haplotype showed the highest mean cortical thickness value.

Several lines of evidence have highlighted the role of dysbindin in modulating and regulating prefrontal function (Wolf et al., in press; Donohoe et al., 2007, 2010; Markov et al., 2010; Fallgatter et al., 2006, 2010), in particular in the orbitofrontal cortex that presents a high expression of this protein (Weickert et al., 2004). In agreement with this evidence, a recent fMRI study demonstrated reduced brain activity in this prefrontal area during the execution of emotional tasks in carriers of a risk dysbindin variant (Wolf et al., in press). Our structural data are consistent with the reported influence of the genetic variants in the dysbindin gene on function of the orbitofrontal cortex, a key region implicated in the neuropathology of schizophrenia (Moghaddam and Homayoun, 2008). The vulnerability of the orbitofrontal cortex as well as the overall prefrontal cortex in patients with schizophrenia has been widely demonstrated (Davidson and Heinrichs, 2003; Rapoport et al., 2005; Nesvåg et al., 2008), and large parts of these studies support the neurodevelopmental model of schizophrenia (Rapoport et al., 2005). In fact, it has been proposed (Nesvåg et al., 2008) that the aberrant morphological changes detected on the prefrontal cortex (Davidson and Heinrichs, 2003; Nesvåg et al., 2008) may reflect an altered maturation (i.e. neural migration or organization) in the developing brain of patients with schizophrenia. Consequently, research of all candidate genes affecting brain development are of primary interest to elucidate the mechanisms that account for this specific vulnerability. The dysbindin gene has been included as a candidate gene in the neurodevelopmental model of schizophrenia (Rapoport et al., 2005).

A new finding of our imaging genetic study is the presence of the increased cortical thickness in carriers of risk haplotype with respect to the non-risk carriers. Recently, Donohoe et al. (2010) reported significant atrophy of the occipital and prefrontal cortices in schizophrenic patients carrying the same haplotype. This inverse association that would seem to be counterintuitive has been confirmed by recent morphological and electrophysiological studies investigating the effect of other dysbindin risk alleles, such as SNP 1018381 (Narr et al., 2009) and SNP 2619528 (Fallgatter et al., 2006, 2010). Using cortical thickness, Narr et al. (2009) demonstrated that risk status is associated with cortical thinning, particularly in the temporal cortex, in patients but with thickness increasing in controls. Moreover, Fallgatter et al. (2006, 2010) found the same opposite effect of dysbindin genetic variants on prefrontal function in healthy controls and schizophrenic patients. In particular, the schizophrenic patients carrying the risk allele showed a diminished prefrontal activity whereas the same allele was associated with the increased prefrontal function in healthy controls. To explain these findings all these authors propose attractive hypotheses. Narr et al. (2009) hypothesized a protective role of cortical thickening in healthy controls. Otherwise, Fallgatter et al. (2010) proposed an inverted "U-shaped relation" between glutamatergic tone (regulated by dysbindin level) and cognitive/prefrontal functioning to explain the inverse relation found in schizophrenic patients and controls.

An alternate (speculative) explanation might be that our findings and those provided by Narr et al. (2009) and Donohoe et al. (2010) could be related to other biological pathways enhanced by the low/high dysbindin expression: i.e., epistatic effects between dysbindin and neurotrophic factors. As pointed out by several authors (Talbot et al., 2004; Williams et al., 2005; Wolf et al., in press; Fallgatter et al.,

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2010) dysbindin may confer its susceptibility to schizophrenia through its impact on glutamatergic neurotransmission. Through this effect, it has been demonstrated that the dysbindin gene also has direct and indirect effects on cell growth, cell division, cell differentiation, cell migration and cell survival (Kalkman, 2006). Recent imaging genetics studies have highlighted the hypothesis that these effects could be more prominent on the orbitofrontal cortex where brain activity is profoundly influenced by genetic dysbindin variants (Wolf et al., in press). The glutamatergic neurotransmitter system has been frequently suggested as being involved in the pathophysiology of schizophrenia (Kim et al., 1980; Moghaddam and Krystal, 2003). Indeed, in the prefrontal cortex of subjects with schizophrenia, inhibitory neurotransmission appears to be altered as indicated by the decreased expression of mRNA encoding markers, such as the 67-kilodalton isoform of glutamate decarboxylase (GAD67), an enzyme for GABA synthesis (Akbarian et al., 1995; Volk et al., 2000). Several lines of evidence demonstrate that the signaling mediated by brain-derived neurotrophic factor (BDNF) is involved in these altered mechanisms of GABA neurons in schizophrenia (Hashimoto and Lewis, 2006) since that BDNF has a crucial role in modulating inhibitory neural transmission *per se*. In fact, chronic treatment with BDNF enhances depolarization-induced releases of glutamate and GABA in cultured cortical neurons (Takei et al., 1997). Moreover, genetic variants (such as Val66Met) of the BDNF gene have also been tested for association with schizophrenia, both at phenotypic and at intermediate phenotypic levels (Spalletta et al., 2010; Ho et al., 2007), revealing a profound impact on schizophrenia-related brain regions, such as the prefrontal cortex. Again, additional indirect evidence involved the SNAP25 protein. In fact, in primary cortical neuronal culture, dysbindin appears to influence exocytotic glutamate release via upregulation of molecules in presynaptic machinery (Numakawa et al., 2004) such as SNAP25, a protein that is known to be involved in synaptic vesicle fusion and neurotransmitter release (Sudhof, 1995). Several *in vitro* studies demonstrated that BDNF significantly enhanced the expression of the synaptic protein SNAP25 in cultured neurons (Feng et al., 1999; Yee et al., 2003). These combined findings would seem to support our hypothesis, but at this moment there is no evidence linking directly the biological effect of the dysbindin protein with the BDNF molecule. Thus, on the basis of our results, the discussion surrounding the biological relationship between a thicker medial prefrontal cortex and the dysbindin-risk haplotype remains speculative until direct evidence on this issue is available.

There are some limitations in this study that need to be discussed. First, we did not examine other implicated dysbindin single or multi-marker risk variants in this study, but instead focused on three-SNP haplotype that has been previously implicated (Williams et al., 2004; Donohoe et al., 2007, 2008, 2010) and this could explain the lack of significant association between dysbindin and cognitive performance in our study. In fact, at phenotypic level there is evidence of the impact of the C–A–T haplotype only in the patients with schizophrenia (Donohoe et al., 2007, 2008, 2010). No evidence of the effect of this risk variant has been provided for healthy controls, as revealed for other single SNPs (Burdick et al., 2006; Fallgatter et al., 2006, 2010). Future studies that compare the relative impact of the various risk variants within the same study design are warranted to disentangle the independent or interactive effects on brain anatomy and cognitive performance. Second, this work could suffer from inherent limitations of studying genetic effects on brain anatomy in humans (i.e. sample size). Critically small effect sizes of genes involved in complex phenotypes have necessitated collection of data in very large samples, often in the hundreds, to identify such effects. Although the size of our sample was large enough to detect significant effects at intermediate phenotypic level, few subjects carrying the high risk allele have been detected (n 14). Nonetheless, we believe that earlier neurobiological evidence (Weickert et al., 2004; Wolf et al., in press) and the fact that the statistical data survived correction for multiple comparison (FDR<0.05) speak to the robustness of our findings.

In conclusion, our data demonstrated that the three-marker C–A–T dysbindin haplotype identified by Williams et al. (2004) is associated with altered brain morphology of the medial orbitofrontal cortex in healthy individuals. Although the biological mechanisms underlying dysbindin–thicker cortex relationship are not clear, our findings complement previous observations on brain morphology of schizophrenic patients (Donohoe et al., 2010). We retained that thickness measurements of this brain region may represent a new promising morphometric endophenotype in elucidating the biological function of this gene on the neural systems relevant to the pathophysiology of schizophrenia (Nesvåg et al., 2008). However, it is important to bear in mind that the lack of significant association with behavioral measures (as previously reported by Donohoe et al. (2010)) and the fact that we examined only healthy controls might question the generalization of our findings to schizophrenic patients. Moreover, further neurobiological investigations are strongly warranted to determine if the influence on the prefrontal anatomy represents a direct effect of dysbindin or a consequence of dysbindin interaction with other genetic factors (i.e., neurotrophins).

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