Genes, Brain and Behavior
Official publication of the International Behavioural and Neural Genetics Society

Genes, Brain and Behavior (2011) **10:** 223–227 doi: 10.1111/j.1601-183X.2010.00657.x

ZNF804A may be associated with executive control of attention

Z. Balog[‡], I. Kiss[‡] and S. Kéri[*,†,‡]

[†]*Department of Physiology, University of Szeged, Faculty of Medicine, Szeged, and* [‡]*National Psychiatry Center, Budapest, Hungary*
**Corresponding author: Dr Szabolcs Kéri, National Psychiatry Center, Balassa u. 6, Budapest H1083, Hungary. E-mail: szkeri@phys.szote.u-szeged.hu*

***ZNF804A*, encoding the transcription factor zinc-finger protein 804A, is a genome-wide supported psychosis gene associated with schizophrenia and bipolar disorder. However, only little information is available on the role of *ZNF804A* regarding the cognitive phenotype of psychosis. In this study, we investigated the relationship between the single-nucleotide polymorphism rs1344706 (A/C, A = risk allele) in *ZNF804A* and attention in 200 healthy volunteers. We used the attention network test, which was designed to separate the three main components of attention (alerting, orienting and executive control). Results showed a significant association with the executive control network: the A/A genotype and the A-allele were associated with increased reaction time when conflicting information was present. In contrast, rs1344706 was not related to alerting and orienting. These results suggest that the genome-wide supported psychosis risk variant of *ZNF804A* is associated with altered executive control (larger conflict effect), which is a potential endophenotype of psychotic disorders.**

Keywords: Attention, executive control, psychosis, schizophrenia, *ZNF804A*

Received 17 July 2010, revised 31 August 2010 and 20 September 2010, accepted for publication 22 September 2010

Recent evidence from large genome-wide association studies and their replications indicates that the single-nucleotide polymorphism rs1344706 (A/C, A = risk allele) in the *ZNF804A* gene (2q32.1) is associated with psychotic disorders, including schizophrenia and bipolar disorder (International Schizophrenia Consortium 2009; O'Donovan *et al*. 2008; Riley *et al*. 2010; Steinberg *et al*. 2010; Williams *et al*. in press; Zhang *et al*. in press). *ZNF804A* encodes the transcription factor zinc-finger protein 804A, which displays a slightly increased expression in post-mortem brain tissue of patients with schizophrenia (Riley *et al*. 2010). Consistent with this neuropathological observation, the risk A-allele is associated with increased gene expression (Riley *et al*. 2010). It has been shown that *ZNF804A* is a target of *Hoxc8*, which suggests that it is involved in neurodevelopmental processes (Chung *et al*. in press). The deletion of the chromosome region containing *ZNF804A* may be associated with developmental delay, mental retardation and behavioral disturbances (Cocchella *et al*. 2010).

To date, however, there are only few studies investigating the relationship between *ZNF804A* and cognitive functions (Esslinger *et al*. 2009; Lencz *et al*. 2010; Walters *et al*. 2010). Impaired cognition is a characteristic feature of psychotic disorders and may be related to the genetic background of these maladies (Bora *et al*. 2009; Cannon & Keller 2006; Reichenberg & Harvey 2007). Attention is one of the most important cognitive phenotypes for psychosis (Luck & Gold 2008). Posner and Petersen (1990) postulated that attention is mediated by three brain networks: alerting, orienting and executive control. The alerting network is responsible for achieving and maintaining an alert state (e.g. responding faster when a cue precedes the target and thus alerts the observer), whereas the orienting network regulates the selection of information from sensory input (e.g. responding faster when a cue preceding the target signifies where the target will appear in the visual field). Finally, the executive control network enables us to select from conflicting and competing responses via appropriate rules, goals and context. Using the attention network test (ANT), which was designed to assess the three components of attention, Fan *et al*. (2005) showed thalamic and neocortical activations during alerting, whereas orienting activated parietal areas and frontal eye fields. Executive control activated the anterior cingulate cortex, together with an extensive frontal and posterior neocortical network. Evidence suggests that the executive control network displays the most prominent impairment in schizophrenia (Gooding *et al*. 2006; Neuhaus *et al*. 2007; Opgen-Rhein *et al*. 2008; Urbanek *et al*. 2009; Wang *et al*. 2005), although the results are heterogeneous (Allen *et al*. 2003). However, converging evidence suggests that executive dysfunction is present in the majority of patients with schizophrenia (Reichenberg & Harvey 2007).

Here, we tested the hypothesis that rs1344706 in the *ZNF804A* gene is associated with attention in healthy volunteers. Given that *ZNF804A* is a candidate gene for psychotic disorders, and in schizophrenia the executive control network may be the most impaired component of attention, we hypothesized that *ZNF804A* is mainly related to the executive control network.

Materials and methods

Participants

Volunteers ($n = 200$) were members of the community recruited via acquaintance networks and advertisements. All participants

Table 1: Characteristics of the participants

ZNF804A rs1344706 genotypes	A/A	A/C	C/C
Number of participants	70	92	38
Male/female	50/20	64/28	27/11
Age (years)	39.6 (8.3)	42.5 (8.4)	40.3 (10.6)
Years of education	14.6 (4.2)	13.9 (5.0)	14.0 (4.8)
IQ	109.6 (10.5)	108.5 (10.3)	110.0 (11.7)
Socioeconomic status	39.5 (11.5)	34.3 (15.9)	37.4 (12.1)

Data are mean (standard deviation) with the exception of the number of participants and the gender ratio. Participants with different genotypes did not differ ($P > 0.1$, *t*-tests).

were of Caucasian origin and received the following assessments: (1) Structured Clinical Interview for DSM-IV axis I disorders (SCID-CV) (First *et al*. 1996) to exclude severe psychopathology; (2) the revised version of the Wechsler Adult Intelligence Scale (WAIS-R) (Wechsler 1981) and (3) the Hollingshead Four-Factor Index (Cirino *et al*. 2002) to evaluate socioeconomic status. Table 1 shows the demographic data and the *ZNF804A* genotype distribution. All participants gave informed consent and the study was approved by the official ethics board.

Attention network test

The ANT is suitable for the assessment of alerting, orienting and executive control of attention (Fan *et al*. 2002). In a trial of the test, an arrow pointing to the left or the right direction is presented. The arrow appears either below or above a fixation point. The task is to press a left or a right button according to the direction of the arrow. In some trials, cues indicate when (time cue) or where (spatial cue) the arrow is presented. The reduction of reaction time due to the time cue reflects the performance of the alerting network. The reduction of reaction time due to the spatial cue gives information about the orienting network. To assess executive control, the arrow is flanked by two other arrows on each side pointing either in the same or in the opposite direction (congruent and incongruent information). The increase in reaction time in the incongruent condition as compared with the congruent condition characterizes executive control to process and resolve conflicting information.

One trial of the ANT consists of five consecutive events. First, a fixation cross is presented (exposure time: 400–1600 milliseconds). Second, an asterisk appears for 100 milliseconds, indicating either where the target arrow will be presented (spatial cue, above or below the fixation point) or when the target would be presented (center cue, replacing the fixation point, or double cue, above and below the fixation cross). In the no-cue condition, only the fixation cross is presented. Third, there is a post-cue fixation period of 400 milliseconds. Fourth, the target arrow is presented either alone or flanked by two arrows. Then the participant responds by pressing the appropriate button to indicate the direction of the target arrow.

Each participant receives a practice block of 24 trials. There are three blocks of 96 trials during which all 48 trial types are presented two times [4 (cue types: no, spatial, center, double) × 3 (flanker: no, congruent, incongruent) × 2 (positions: above, below) × 2 (directions: left, right)].

Genotyping

Genomic DNA was extracted from venous blood samples. *ZNF804A* rs1344706 was genotyped using TaqMan bioassay (Applied Biosystems, Foster City, CA, USA), as described by Riley *et al*. (2010). There was a duplicate run to check genotyping accuracy (error rate <2%). The distribution of the genotypes did not deviate from the Hardy–Weinberg equilibrium ($P > 0.5$; Table 1).

Data analysis

The data from the ANT were entered into repeated measures analyses of variance (ANOVAs) in which genotype or allele was the independent variable and mean errors or reaction time characterizing attention network type (alerting, orienting and executive) was the dependent variable. Fisher's least significant difference (LSD) tests were used for *post hoc* comparisons. Linear regression analyses were used to evaluate how genotype and alleles predicted ANT performances. Student's *t*-tests (two-tailed) were used for the comparison of demographical characteristics. The level of statistical significance was set at $P < 0.05$.

Results

There were no significant differences among genotypes in mean reaction time [A/A: 525.3 milliseconds (SD = 101.1), A/C: 516.4 milliseconds (SD = 111.1), C/C: 515.2 milliseconds (SD = 71.4); $P = 0.82$]. The ANOVA showed no significant main effect of genotype or an interaction between genotype and attention network type in the case of mean errors [A/A: neutral: 0.6 (SD = 0.4), incongruent: 3.6 (SD = 2.5), congruent: 0.2 (SD = 0.08); A/C: neutral: 0.5 (SD = 0.4), incongruent: 3.7 (SD = 2.7), congruent: 0.1 (SD = 0.07); C/C: neutral: 0.6 (SD = 0.5), incongruent: 3.7 (SD = 3.2), congruent: 0.2 (SD = 0.1); $P > 0.5$].

Figure 1 depicts the mean reaction time values for the orienting, alerting and executive attention network for each genotype. The main effect of attention network type was significant ($F_{2,394} = 697.0$, $P < 0.0001$). The main effect of genotype was not significant ($P = 0.83$), but

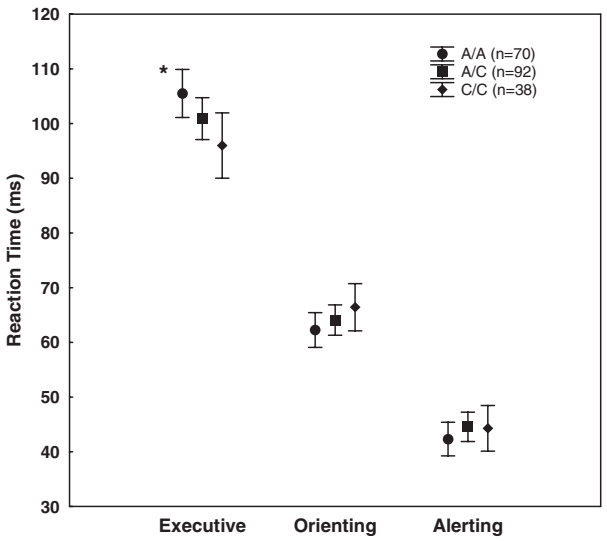

Figure 1: Association between *ZNF804A* rs1344706 polymorphism (A/C, A = risk allele) and attention at the level of genotypes. Differences in reaction times were used to characterize attention networks: alerting = targets (no cue) − targets (center cue); orienting = targets (center cue) − targets (spatial cue); executive (conflict) = incongruent targets − congruent targets. Error bars indicate 95% confidence intervals. *A/A > C/C, $P < 0.05$ (Fisher's LSD test).

there was a two-way interaction between genotype and attention network type ($F_{4,394} = 3.41$, $P < 0.05$). Fisher's LSD tests conducted on this two-way interaction showed increased reaction time in the A/A group relative to the C/C group for the executive network ($P < 0.05$). No other pairwise comparisons among genotypes reached the level of statistical significance ($P > 0.5$) (Fig. 1).

In the case of the executive network, the regression analysis showed that the genotype accounted for 3% of variance on the reaction time ($F_{1,198} = 6.67$, $P < 0.05$, $\beta = -0.18$, $r^2 = 0.03$). In the case of the orienting and alerting network, it was less than 1% and did not reach the level of statistical significance ($P > 0.1$).

In the allele-level analysis (A/A + A/C vs. C/C, A = risk allele), the main effect of attention network type was significant ($F_{2,396} = 457.15$, $P < 0.0001$). The main effect of allele was not significant ($P = 0.55$), but there was a two-way interaction between allele and attention network ($F_{2,396} = 3.99$, $P < 0.05$). Fisher's LSD tests showed a significant difference between A-carriers and non-carriers for the executive network ($P < 0.05$), but not for the orienting and alerting network ($P > 0.1$) (Fig. 2).

In the case of the executive network, the regression analysis showed a significant predictive effect of alleles on reaction time ($F_{1,198} = 4.20$, $P < 0.05$, $\beta = -0.14$, $r^2 = 0.02$). This effect was not present in the case of the orienting and alerting network ($P > 0.2$).

When gender and age were included in the regression analysis, we observed no significant predictive effect in their case or a genotype/allele by gender or age interaction ($P > 0.1$). We also conducted an ANOVA in which gender was the independent variable. This ANOVA showed no significant main effect of gender, but the gender by attention network type interaction was significant ($F_{2,396} = 3.30$, $P < 0.05$). This interaction was because of the prolonged response time in the executive condition in females relative to males ($P < 0.05$) (Fig. 3).

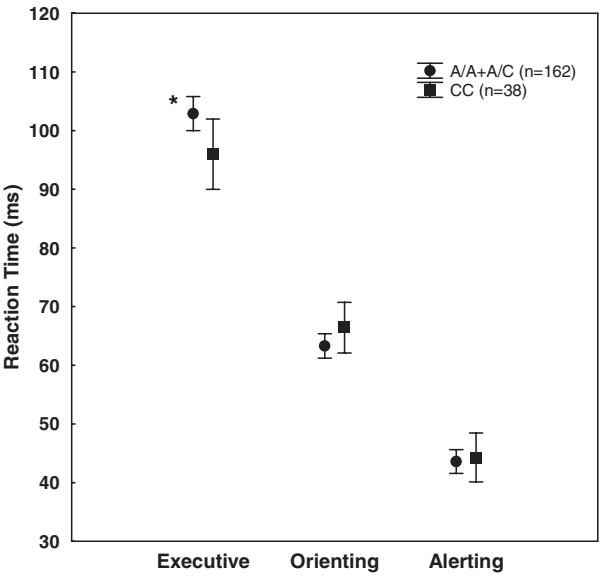

Figure 2: Association between *ZNF804A* rs1344706 polymorphism (A/C, A = risk allele) and attention at the level of alleles. Error bars indicate 95% confidence intervals. *A-carriers > non-carriers, $P < 0.05$ (Fisher's LSD test).

Figure 3: Gender differences in reaction time. *$P < 0.05$ (Fisher's LSD test).

Discussion

In this study, we showed that the risk genotype and allele of *ZNF804A* are associated with a probably less efficient functioning of the executive control network in healthy volunteers. Orienting and alerting were not associated with this genetic variant. These results are consistent with the finding that *ZNF804A* is a susceptibility gene for psychotic disorders, given that executive dysfunction is a prominent alteration in these mental illnesses. However, this single polymorphism had a weak effect on executive control, accounting for 3% of variance on task performance, which was manifested as a slower reaction time in risk variant carriers. The gender effect, which suggests that in healthy controls females show slightly slower responses in the executive condition relative to males, is related to the results of previous studies (Opgen-Rhein *et al*. 2008; Urbanek *et al*. 2009). Urbanek *et al*. (2009) showed that the difference between patients with schizophrenia and healthy controls can be attributed to the decreased conflict effect in men with schizophrenia, whereas the conflict effect was slightly elevated in women with schizophrenia. However, we found no interaction between gender and *ZNF804A* variants.

The results of the present study seem to be in contrast with the findings of Walters *et al*. (2010), who surprisingly found better cognitive functions (episodic and working memory) in patients with schizophrenia who carried the *ZNF804A* risk variant. In healthy controls, *ZNF804A* was not associated

with cognitive functions (Walters *et al*. 2010). The apparent discrepancy between the results of Walters *et al*. (2010) and the present study may stem from task differences. In the ANT, executive network functioning characterizes how efficiently participants are able to resolve conflicting information (arrows pointing in different directions). Participants with the *ZNF804A* risk variant exhibited an increase in reaction time in the incongruent condition relative to the congruent condition (larger conflict effect) (Posner & Petersen, 1990), which reflects slower responding but not increased number of errors. Interestingly, Opgen-Rhein *et al*. (2008) found a smaller conflict effect in patients with schizophrenia (Wang *et al*. 2005), which may lead to premature responses and more errors. If this hypothesis is true, our results resonate with the findings of Walters *et al*. (2010); the increased conflict effect in risk variant carriers may be protective against premature wrong responses when incongruent information is present. However, it is not known how executive control is modulated by the genotype when a mental illness is present. Therefore, our results should be replicated in patients with schizophrenia and bipolar disorder to explore how *ZNF804A* is related to attention in clinical samples.

Regarding the neuroanatomical correlates of genetic variations, Esslinger *et al*. (2009) found that healthy carriers of the *ZNF804A* rs1344706 risk genotypes exhibited gene dosage-dependent alterations in functional connectivity of left and right dorsolateral prefrontal cortex and hippocampus during a working memory task, which is similar to that found in patients with schizophrenia. Lencz *et al*. (2010) reported that healthy individuals who were *ZNF804A* risk allele homozygotes showed larger total white matter volumes than carriers of the non-risk allele, but these individuals showed reduced gray matter volumes in the angular gyrus, parahippocampal gyrus, posterior cingulate cortex and medial orbitofrontal cortex. The risk allele was also associated with an impaired visuomotor performance on the trail making A test (Lencz *et al*. 2010).

In previous genetic imaging studies, genes related to the functioning of the executive control network were associated with activation in anterior cingulate cortex (Fan *et al*. 2003; for review, see Posner *et al*. 2007). Opgen-Rhein *et al*. (2008) showed a significant effect of *catechol-O-methyltransferase* (*COMT*) Val108/158 Met polymorphism on executive control in schizophrenia and in healthy controls (Reuter *et al*. 2007). Converging evidence from human and animal studies suggests that cooperation between dorsolateral prefrontal cortex and anterior cingulate cortex is necessary for successful conflict-induced behavioral adjustment, which requires executive control processes (Mansouri *et al*. 2009). Further studies are warranted to learn how *ZNF804A* interacts with other genes associated with attention.

In conclusion, the data from the present study suggest that the common variant of the *ZNF804A* gene, which is a genome-wide supported risk variant for psychotic disorders, is associated with increased response latency on a task assessing the executive control network of attention. However, the replication of these results is necessary in an independent sample. Future studies should explore how *ZNF804A* affects brain functions and how it interacts with other genes related to cognition and mental illness.

References

Allen, D.N., Goldstein, G. & Warnick, E. (2003) A consideration of neuropsychologically normal schizophrenia. *J Int Neuropsychol Soc* **9**, 56–63.

Bora, E., Yucel, M. & Pantelis, C. (2009) Cognitive endophenotypes of bipolar disorder: a meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives. *J Affect Disord* **113**, 1–20.

Cannon, T.D. & Keller, M.C. (2006) Endophenotypes in the genetic analyses of mental disorders. *Annu Rev Clin Psychol* **2**, 267–290.

Chung, H.J., Lee, J.Y., Deocaris, C.C., Min, H., Kim, S.H. & Kim, M.H. (in press). Mouse homologue of the schizophrenia susceptibility gene *ZNF804A* as a target of Hoxc8. *J Biomed Biotechnol* (in press).

Cirino, P.T., Chin, C.E., Sevcik, R.A., Wolf, M., Lovett, M. & Morris, R.D. (2002) Measuring socioeconomic status: reliability and preliminary validity for different approaches. *Assessment* **9**, 145–155.

Cocchella, A., Malacarne, M., Forzano, F., Marciano, C., Pierluigi, M., Perroni, L., Faravelli, F. & Di Maria, E. (2010) The refinement of the critical region for the 2q31.2q32.3 deletion syndrome indicates candidate genes for mental retardation and speech impairment. *Am J Med Genet B Neuropsychiatr Genet* **153B**, 1342–1346.

Esslinger, C., Walter, H., Kirsch, P., Erk, S., Schnell, K., Arnold, C., Haddad, L., Mier, D., Opitz von Boberfeld, C., Raab, K., Witt, S.H., Rietschel, M., Cichon, S. & Meyer-Lindenberg, A. (2009) Neural mechanisms of a genome-wide supported psychosis variant. *Science* **324**, 605.

Fan, J., McCandliss, B.D., Sommer, T., Raz, A. & Posner, M.I. (2002) Testing the efficiency and independence of attentional networks. *J Cogn Neurosci* **14**, 340–347.

Fan, J., Fossella, J., Sommer, T., Wu, Y. & Posner, M.I. (2003) Mapping the genetic variation of executive attention onto brain activity. *Proc Natl Acad Sci U S A* **100**, 7406–7411.

Fan, J., McCandliss, B.D., Fossella, J., Flombaum, J.I. & Posner, M.I. (2005) The activation of attentional networks. *Neuroimage* **26**, 471–479.

First, M.B., Spitzer, R.L., Gibbon, M. & Williams, J.B.W. (1996) *Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV)*. American Psychiatric Press, Washington, DC.

Gooding, D.C., Braun, J.G. & Studer, J.A. (2006) Attentional network task performance in patients with schizophrenia-spectrum disorders: evidence of a specific deficit. *Schizophr Res* **88**, 169–178.

International Schizophrenia Consortium, Purcell, S.M., Wray, N.R., Stone, J.L., Visscher, P.M., O'Donovan, M.C., Sullivan, P.F. & Sklar, P. (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **460**, 748–752.

Lencz, T., Szeszko, P.R., Derosse, P., Burdick, K.E., Bromet, E.J., Bilder, R.M. & Malhotra, A.K. (2010) A schizophrenia risk gene, *ZNF804A*, influences neuroanatomical and neurocognitive phenotypes. *Neuropsychopharmacology* **35**, 2284–2291.

Luck, S.J. & Gold, J.M. (2008) The construct of attention in schizophrenia. *Biol Psychiatry* **64**, 34–39.

Mansouri, F.A., Tanaka, K. & Buckley, M.J. (2009) Conflict-induced behavioural adjustment: a clue to the executive functions of the prefrontal cortex. *Nat Rev Neurosci* **10**, 141–152.

Neuhaus, A.H., Koehler, S., Opgen-Rhein, C., Urbanek, C., Hahn, E. & Dettling, M. (2007) Selective anterior cingulate cortex deficit during conflict solution in schizophrenia: an event-related potential study. *J Psychiatr Res* **41**, 635–644.

O'Donovan, M.C., Craddock, N. *et al*. (2008) Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet* **40**, 1053–1055.

Opgen-Rhein, C., Neuhaus, A.H., Urbanek, C., Hahn, E., Sander, T. & Dettling, M. (2008) Executive attention in schizophrenic males and the impact of COMT Val108/158Met genotype on performance on the attention network test. *Schizophr Bull* **34**, 1231–1239.

Posner, M.I. & Petersen, S.E. (1990) The attention system of the human brain. *Annu Rev Neurosci* **13**, 25–42.
Posner, M.I., Rothbart, M.K. & Sheese, B.E. (2007) Attention genes. *Dev Sci* **10**, 24–29.
Reichenberg, A. & Harvey, P.D. (2007) Neuropsychological impairments in schizophrenia: integration of performance-based and brain imaging findings. *Psychol Bull* **133**, 833–858.
Reuter, M., Ott, U., Vaitl, D. & Hennig, J. (2007) Impaired executive control is associated with a variation in the promoter region of the tryptophan hydroxylase 2 gene. *J Cogn Neurosci* **19**, 401–408.
Riley, B., Thiselton, D., Maher, B.S., Bigdeli, T., Wormley, B., McMichael, G.O., Fanous, A.H., Vladimirov, V., O'Neill, F.A., Walsh, D. & Kendler, K.S. (2010) Replication of association between schizophrenia and ZNF804A in the Irish case-control study of schizophrenia sample. *Mol Psychiatry* **15**, 29–37.
Steinberg, S., Mors, O., Børglum, A.D. *et al*. (2010) Expanding the range of ZNF804A variants conferring risk of psychosis. *Mol Psychiatry* [Epub ahead of print].
Urbanek, C., Neuhaus, A.H., Opgen-Rhein, C., Strathmann, S., Wieseke, N., Schaub, R., Hahn, E. & Dettling, M. (2009) Attention network test (ANT) reveals gender-specific alterations of executive function in schizophrenia. *Psychiatry Res* **168**, 102–109.
Walters, J.T., Corvin, A., Owen, M.J. *et al*. (2010) Psychosis susceptibility gene *ZNF804A* and cognitive performance in schizophrenia. *Arch Gen Psychiatry* **67**, 692–700.
Wang, K., Fan, J., Dong, Y., Wang, C.Q., Lee, T.M. & Posner, M.I. (2005) Selective impairment of attentional networks of orienting and executive control in schizophrenia. *Schizophr Res* **78**, 235–241.
Wechsler, D. (1981) *Wechsler Adult Intelligence Scale – Revised Manual*. Psychological Corporation, New York, NY.
Williams, H.J., Norton, N., Dwyer, S. *et al*. (in press) Fine mapping of ZNF804A and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder. *Mol Psychiatry* (in press).
Zhang, R., Lu, S.M., Qiu, C., Liu, X.G., Gao, C.G., Guo, T.W., Valenzuela, R.K., Deng, H.W. & Ma, J. (in press) Population-based and family-based association studies of ZNF804A locus and schizophrenia. *Mol Psychiatry* (in press).

Acknowledgments

The study was supported by the Hungarian Research Fund (OTKA NF72488, K68594). The author is indebted to Andrea Szakács and Zsolt Halas for assistance in data collection.