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Variation in Psychosis Gene *ZNF804A* Is Associated With a Refined Schizotypy Phenotype but Not Neurocognitive Performance in a Large Young Male Population

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Genetic variability within the *ZNF804A* gene has been recently found to be associated with schizophrenia and bipolar disorder, although the pathways by which this gene may confer risk remain largely unknown. We set out to investigate whether common *ZNF804A* variants affect psychosis-related intermediate phenotypes such as cognitive performance dependent on prefrontal and frontotemporal brain function, schizotypal traits, and attenuated psychotic experiences in a large young male population. Association analyses were performed using all 4 available self-rated schizotypy questionnaires and cognitive data retrospectively drawn from the Athens Study of Psychosis Proneness and Incidence of Schizophrenia (ASPIS). DNA samples from 1507 healthy young men undergoing induction to military training were genotyped for 4 previously studied polymorphic markers in the *ZNF804A* gene locus. Single-marker analysis revealed significant associations between 2 recently identified candidate schizophrenia susceptibility variants (rs1344706 and rs7597593) and a refined positive schizotypy phenotype characterized primarily by self-rated paranoia/ideas of reference. Nominal associations were noted with all positive, but not negative, schizotypy related factors. *ZNF804A* genotype effect on paranoia was confirmed at the haplotype level. No significant associations were noted with central indexes of sustained attention or working memory performance. In this study, *ZNF804A* variation was associated with a population-based self-rated schizotypy phenotype previously suggested to preferentially reflect genetic liability to psychosis and defined by a tendency to misinterpret otherwise neutral social cues and perceptual experiences in one's immediate environment, as personally relevant and significant information. This suggests a novel route by which schizophrenia-implicated *ZNF804A* genetic variation may confer risk to clinical psychosis at the general population level.

Key words: schizophrenia/aberrant salience/schizotypy/paranoia/psychosis/*ZNF804A*

Introduction

One of the most promising candidate genes to emerge from genome-wide association studies (GWASs) of psychosis is *ZNF804A*. O'Donovan et al[1] undertook a GWAS of schizophrenia with follow-up in approximately 17 000 subjects comprising 2 further replication stages. Although the statistical evidence in schizophrenia was short of genome-wide significance, that threshold was surpassed when the phenotype was expanded to include bipolar disorder ($P = 9.96 \times 10^{-9}$). The T-allele of rs1344706 in *ZNF804A,* showing the strongest association signal with schizophrenia, also showed an association with bipolar disorder in the above study and predicted elevated manic symptomatology in an independent study,[2] demonstrating that variation at this locus has an effect on illness susceptibility across the traditional diagnostic boundaries. Association between rs1344706, recently acknowledged as a functional polymorphism,[3] and the broader psychosis phenotype was confirmed in a nonoverlapping large multiethnic European sample,[4] although not every dataset surveyed has shown positive association.[5] A recent meta-analysis of rs1344706 in all available datasets provided strong evidence for association with schizophrenia ($P = 2.54 \times 10^{-11}$) and the

extended psychosis phenotype, and there was no evidence for heterogeneity across studies.[6] Association with *ZNF804A* variants has expanded to include potentially new candidates such as rs7597593 that was found to be preferentially associated with schizophrenia in the Irish Case–Control Study of Schizophrenia.[7] In a recent study, Zhang et al[8] reported that the association with rs7597593 in several large public GWAS datasets may show sex modulation, being especially strong in females. Overall, there is now substantial evidence for the involvement of *ZNF804A* in psychosis vulnerability.

An enduring hypothesis to explain many inconsistencies in association genetic studies of schizophrenia is that risk variants may modulate intermediate phenotypes associated with increased risk for psychosis rather than the disorder itself.[9] Candidate intermediate phenotypes of schizophrenia with which the disorder presumably shares a degree of overlapping genetic liability include structural and functional brain alterations, neurocognitive deficits, and enduring schizotypal personality traits. Recent evidence that *ZNF804A* may indeed affect intermediate cognitive phenotypes is provided by Esslinger et al,[10] who demonstrated that among normal volunteers who were assessed with an established neurocognitive probe, the N-back working memory task, the rs1344706 risk allele was associated with a pattern of relatively abnormal connectivity in the human brain, including reduced connectivity within the dorsolateral prefrontal cortex (DLPFC) and decreased uncoupling between the DLPFC and hippocampus, which parallels findings with the same paradigm reported in patients with schizophrenia. Of note, the above observations were validated by other groups,[11,12] supporting the modulatory role of *ZNF804A* in human cognitive functioning. In addition, Walter et al[13] found differences in medial prefrontal and left temporoparietal cortical activations during a theory of mind task, again in healthy carriers of the common rs1344706 risk allele, and Walters et al[14] have reported results with rs1344706 that further demonstrate that the inherent complexity in the relationships between genotype and phenotype will defeat overly simplistic attempts in proposing mechanisms of action. They found in 2 independent samples that the strength of the effect on clinical risk increased when patients with lower intelligence quotients (IQs) were excluded from the analysis, even though no single-nucleotide polymorphism (SNP) effect on IQ was found. These results suggested that in contrast to the postulated effects of *ZNF804A* on neural connectivity, variations within this gene may not be strongly associated with standard cognitive intermediate phenotypes in controls and patients with schizophrenia as they appeared to delineate an illness subtype in which cognitive deficits were not a strong feature. Recent studies in healthy individuals provide fairly clear evidence that standard structural brain endophenotypes are not targets of *ZNF804A* variability: For example, Donohoe et al[15] found no significant rs1344706 effect on brain volumes in normal individuals, which was successfully replicated by Cousijn et al[16] in a much larger sample of 892 volunteers.

Collectively, these studies highlight the lack of *ZNF804A* effect on general cognitive abilities and structural intermediate phenotypes, at least in healthy subjects. On the other hand, the above studies do not address the possibility that *ZNF804A*-related vulnerability may be mediated via alternative routes, eg, by affecting psychological rather than cognitive intermediate phenotypes, such as enduring schizotypal personality traits or subclinical psychotic experiences that are considered to be in an etiological continuity with the disorder and that are suggested to share genetic variation with the clinical phenotype.[17]

We first reported on the utility of adopting a population-based cognitive-psychological intermediate phenotype approach to study the potential effect of candidate susceptibility genes for schizophrenia.[18] In view of recent conflicting reports, we set out to evaluate in a large cohort of healthy young males whether 4 SNP variants, and their corresponding haplotypes in the *ZNF804A* gene locus, affect intermediate cognitive phenotypes of schizophrenia, namely aspects of cognitive performance that exhibit a degree of heritability and are broadly dependent on prefrontal or frontotemporal brain function. We further set out to evaluate whether *ZNF804A* gene variations affect such schizophrenia-related psychological constructs as self-rated schizotypy and stress-induced psychotic experiences. Potential associations detected with specific schizotypy constructs/symptoms and/or cognitive performance might help elucidate and refine *ZNF804A* effects on the broader psychosis phenotype and add validity to the evidence of association with clinical diagnosis.

Method

Participants

Participants at the time of data collection were undergoing a mandatory military training program at the Greek Air Force during the first 2 weeks of admission to the National Basic Air Force Training Center in Tripolis, Greece. Within the ASPIS (Athens Study of Psychosis Proneness and Incidence of Schizophrenia), 8 such consecutive separate waves of conscripts were assessed. From a total of 2130 eligible healthy young males aged 18–24 years, retrospective data were drawn from a pool of 1507 selected young male responders who had valid scores across the entire neurocognitive battery, thus biasing the sample toward participants with an increased acceptance rate of the procedures. All conscripts had received a standardized screening interview by a team of military doctors of different specialties in order to exclude serious medical conditions, including documented diagnosis of psychotic disorders and substance dependence. Military service is compulsory in Greece, and all healthy men are recruited and assigned to the different army corps by random assignment. All the

individuals included in this study were of Greek family background (both parents of Greek origin).

Self-Rated Subclinical Psychosis

Within the first 2 weeks of military induction, conscripts completed a psychometric battery of self-administered questionnaires The assessment battery included assessment of (a) lifetime schizotypal personality traits, with the Schizotypal Personality Questionnaire (SPQ),[19–21] (b) unusual body perceptual experiences, with the Perceptual Aberration Scale (PAS)[22], (c) attenuated psychotic experiences, with the Community Assessment of Psychic Experiences (CAPE)[23] and (d) an index of stressed-induced psychotic symptoms, with the combined psychosis score from the Symptom Checklist 90-Revised (SCL90R).[24,25] The SPQ is a 74 "yes-no" item questionnaire that assesses 9 aspects of the Schizotypal Personality Disorder (SPD) according to the Diagnostic and Statistical Manual of Mental Disorders (Revised).[27] Analysis of the factorial structure of the SPQ as defined by the responses of this sample has been achieved through confirmatory factor analysis (CFA), which indicated that the best fit to the data was provided by a 4-factor model, comprising Positive, Negative, Disorganization, and Paranoid factors.[20] This 4-factorial model of schizotypy has been recently replicated by other research groups,[26] and importantly, high SPQ scorers in this conscript sample were predictive of an independent diagnosis of Schizotypal Personality Disorder upon SCID II interview.[21] The CAPE is a 40-item, self-rated questionnaire, allowing for a 4-scaled response to the lifetime presence of a broad range of "toned down" psychosis symptoms. It has a 3-factor structure of positive, negative, and depression dimensions extracted from the CFA. The original validation study of this schizotypy instrument was based on this conscript population.[23]

The PAS is a 35-item, yes-no, self-rated questionnaire. The SCL90R is a comprehensive "state" self-report questionnaire of 90 questions, which covers a broad range of psychiatric symptoms experienced within the previous 2 weeks. We combined the scores of "paranoid ideation" and "psychoticism" subscales into a mean total score (hereafter "psychosis" score). This was also done in previous publications that used this composite variable as a proxy measure of psychosis proneness in the general population.[25,28] The study was approved by the Bioethics and Medical Deontology Committee of the University Mental Health Research Institute.

Cognitive Assessment

Conscripts underwent an extensive interview of computerized neurocognitive abilities. We chose to include in this study those available cognitive measures related broadly to prefrontal and frontotemporal brain function, namely, sustained attention, with the Continuous Performance Task-Identical Pairs version (CPT-IP)[29] and verbal and spatial working memory, with the N-back task.[30] In accordance with our previous work, we a priori decided to exclude data from further analyses if the central index of performance (d') on CPT-IP and 2-back was <0 and if there were ≥3 unsuccessful trials (of 5) for verbal and spatial 2-back tasks.

SNPs Selection and Genotyping

After written informed consent was obtained, mouthwash samples for DNA extraction were selected as described previously.[31] Four common (minor allele frequency, MAF > 0.2) SNPs within the *ZNF804A* gene locus were chosen for genotyping from among the 1507 selected young male conscripts. The SNPs were rs7597593, rs1344706, rs4667001, and rs3731834. Marker rs1344706 was originally associated with schizophrenia in a GWAS by O'Donovan et al.[1] We selected rs7597593 from the study of Riley et al[7] because it provided the strongest association in the Irish Case–Control Study of Schizophrenia. The G-allele at rs4667001 (missense SNP) was associated with an increase in *ZNF804A* expression.[6] Genotyping was performed using Taqman 5′-exonuclease allelic discrimination assays (Applied Biosystems). Genotype reproducibility was routinely assessed by re-genotyping 10% of the samples and was generally >99%.

Statistical Analysis

We quantified the preselected psychometric and neurocognitive central indexes of performance as continuous dependent variables. We assessed separately the 4 schizotypal latent factors (or dimensions) of SPQ and the positive and negative CAPE factors[23] that emerged from applying CFA to this dataset.[20,23] We opted to examine post hoc potential association with individual SPQ subscales or CAPE items, only if convincing signals of association emerged across the *ZNF804A* locus with any of the latent constructs derived from CFA. Genotypic compliance with the Hardy-Weinberg law was assessed with an exact test. In our sample, SPQ, SCL90R "psychosis" score, and all cognitive scores were normally distributed and analyzed with linear regressions. We examined whether the number of minor allele copies was associated with each quantitative outcome (allele-load or allele-based additive models), using PLINK software v1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/).[32] PAS as well as CAPE positive and negative symptom subscales were log-transformed before analysis due to deviation from normality. However, individual CAPE items were skewed and therefore nonparametric Kruskal-Wallis tests were utilized as appropriate. In our single-SNP analyses, we corrected for multiple comparisons tested (11 phenotypes ×4 SNPs) by applying the false discovery rate (FDR) procedure according to Benjamini and Hochberg,[33] allowing for 5% false-positive results (significant association if $P_{FDR} < .05$). We note, however, that the FDR correction could be considered overly conservative in this dataset given the

nonindependence between the statistical tests performed, due to high correlations between the phenotypic outcomes and the SNPs analyzed (supplementary tables S1, S2). Furthermore, all significant associations in the allele-load regression analysis were individually confirmed by nonparametric permutation testing in order to minimize plausible spurious effects. Further, 2- and 3-marker haplotypes were reconstructed in PLINK, which uses the expectation-maximization algorithm to estimate haplotype structures and their frequencies. Only haplotypes with frequency >1% were considered for further analysis. The effect of each haplotype was tested using linear models and empirical significance was obtained by performing 10 000 permutations of the data (Max(T) procedure in PLINK). The main analyses did not adjust for nongenetic factors. However, in a post hoc analysis, we adjusted for age, IQ, and their interaction. Given the sample size and an additive genetic model, we have sufficient power (80%) to detect genotype effects that may explain individual differences in phenotypic variance of $R^2 = 0.012$.

Results

The mean age of the individuals was 21.0 years (SD = 1.9), and mean number of years of schooling was 13.0 years (SD = 2.0). In total, 1507 healthy young male conscripts were genotyped for the 4 previously studied SNPs within the *ZNF804A* gene locus. Marker characteristics and genotype information for each marker are summarized in table 1. Genotype frequencies for each SNP studied were not deviant from those expected for genotypes in Hardy-Weinberg equilibrium (all $P > .3$). As depicted in supplementary table S1, based on D' values, moderate to high levels of linkage disequilibrium (LD) were observed between rs1344706 and rs7597593 markers ($r^2 = 0.32$) and between rs1344706 and rs4667001 ($r^2 = 0.427$). However, marker rs3731834 showed negligible LD correlation with the other 3 markers in our sample ($r^2 < 0.13$) and, thus, we did not include it in subsequent haplotype association analysis. For each marker tested, there was no difference between demographic variables and *ZNF80A* genotype status (*t* test $P > .1$).

As shown in table 2, our primary allele-load regression analysis showed that 2 *ZNF804A* SNPs were significantly associated with the SPQ paranoid schizotypy factor: rs7597593 (β = –.03, $R^2 = 0.013$, $P = .00008$, corrected $P_{FDR} = .004$) and rs1344706 (β = .026, $R^2 = 0.009$, $P = .0009$, corrected $P_{FDR} = .02$). Furthermore, we detected an individual nominally significant effect of the originally GWAS-identified SNP rs1344706 on SPQ disorganization factor (β = .022, $P = .009$, corrected $P_{FDR} = .081$). No association was observed with the "core" positive and negative SPQ schizotypy factors. In a post hoc regression analysis on SPQ subscales that loaded on the SPQ paranoid factor[20], we noted that the association between *ZNF804A* variants and paranoia was driven mainly by the SPQ "ideas of reference" subscale and was markedly stronger for rs7597593, compared to the other 3 markers located at the 3′ end (β = –.04; $R^2 = 0.014$, $P_{FDR} = .003$).

In line with the results obtained from SPQ, individuals carrying the major allele C for the most prominent marker rs7597593 exhibited nominally significant elevated scores in both psychosis-related phenotypes PAS and CAPE positive factor (β = –.028; $P = .009$ for PAS; β = –.011; $P = .0097$ for CAPE positive factor), showing an association trend after correcting for multiple comparisons ($P_{FDR} = .081$; table 2). Notably, in the subsequent exploratory analysis and in agreement with the effect of rs7597593 on SPQ paranoia/ideas of reference, rs7597593 effect on positive CAPE factor (18 items) was driven by associations with 2 items related to a conceptually similar phenotype: "Do you ever feel as if things in magazines or on TV were written especially for you?" ($P = .002$); and "Do you ever feel as if you are being persecuted in some way?" ($P = .003$). However, in our single-marker analysis, the 2 previously reported psychosis risk alleles, (A) at rs7597593 and (T) at rs1344706, were associated with decreased scores in SPQ factors as well as the PAS and CAPE positive factor, apparently exhibiting a "protective" rather than "risk" effect. Regarding the effect of *ZNF804A* variants on stress-induced psychotic symptoms, as indexed by the SCL90R combined psychosis score, none of the variants showed convincing evidence of association, although rs1344706 was nominally associated ($P = .035$) with this "positive" psychosis index. Similarly, no associations could be observed with any of the cognitive measures included in this study (table 3).

Table 4 shows the 2- and 3-marker haplotype frequencies found in this Greek population, as well as the individual association results. Haplotype analyses yielded results generally consistent with the single-SNP analyses and did not provide much additional insight on certain "risk" allele combinations. Specifically, when the 4 SPQ schizotypy factors were explored, only the paranoid factor score was significantly associated with *ZNF804A* haplotype variability. Similarly, nominal associations were also observed for PAS and CAPE positive factor (data available upon request). As seen in table 4, individuals carrying the rs7597593-C, rs1344706-C, and rs4667001-A alleles (CCA haplotype) expressed significantly elevated SPQ paranoid factor scores (β = .027, adjusted $P = .005$ after 10 000 permutations) compared with the carriers of the complementary psychosis risk alleles (TAG haplotype), who demonstrated a fairly "protective" effect against paranoid features (adjusted $P = .01$). These effects remained similar when age, IQ, and their interaction were entered as confounding factors in our model. Furthermore, to disentangle whether the 2 significantly associated markers (rs7597593, rs1344706) in our single-SNP analysis represent independent effects or whether they reflect the same signal due to LD, a 2-SNP conditional haplotype analysis was performed.

Table 1. *ZNF804A* Single-Nucleotide Polymorphisms (SNPs) Analyzed and Genotype Frequencies in the ASPIS Study

SNP ID (dbSNP)	rs7597593	rs1344706	rs4667001	rs3731834
Alleles (major/minor)	C/T	A/C	A/G	C/G
Region	2q32.1	2q32.1	2q32.1	2q32.1
Location (bp)	185.241.825	185.486.673	185.509.992	185.511.609
Intermarker distance (bp)	0	244.848	23.319	1.617
Description	Intronic	Intronic	Missense	Missense
Sample size	1507	1507	1507	1507
Count 1/1	519	526	488	886
Count 1/2	690	714	720	504
Count 2/2	216	217	245	74
Missing frequency	0.054	0.033	0.036	0.029
Minor allele frequency (MAF)	0.372	0.381	0.401	0.216
HWE exact test *P* value	.618	.351	.483	.821

Note: HWE, Hardy-Weinberg Equilibrium; dbSNP, marker reference number according to NCBI/SNP build 131 database.

Table 2. Single-Marker Linear Regression Association Results With Psychosis-Related Traits (n = 1507)

	rs7597593		rs1344706		rs4667001		rs3731834	
Phenotype	Beta	*P* value	Beta	*P* value	Beta	*P* value	Beta	*P* value
Schizotypal traits								
SPQ positive	−.011	.026	.008	.092	−.006	.245	.007	.227
SPQ negative	−.007	.17	.006	.312	−.003	.591	.004	.554
SPQ disorganized	−.02	.067	.022	.009**(.081)**	−.015	.075	.009	.329
SPQ paranoid	−.03	.00008**(.004)**	.026	.0009**(.02)**	−.019	.011	.02	.082
Ideas of reference	−.04	.00005**(.003)**	.032	.001**(.02)**	−.022	.072	.027	.053
Suspiciousness	−.029	.007	.025	.021	−.025	.022	.017	.183
Social anxiety	−.022	.07	.011	.355	−.016	.176	.016	.246
Perceptual aberrations								
PAS	−.028	.0086**(.081)**	.01	.016	−.017	.123	.014	.272
Psychotic experiences								
CAPE positive	−.011	.0097**(.081)**	.003	.488	−.003	.389	.008	.092
CAPE negative	−.008	.118	.003	.55	.002	.746	.004	.487
SCL90R 'psychosis'	−.001	.59	.009	.035	−.003	.096	.012	.236

Note: SPQ, Schizotypal Personality Questionnaire; PAS, Perceptual Aberration Scale; CAPE, Community Assessment of Psychic Experiences; SCL90R, Symptoms Checklist 90-Revised. FDR-corrected *P* values for multiple comparisons are shown in bold.

The haplotype association with SPQ paranoid factor disappeared when conditioned on rs7597593 (P = .68) but remained significant when conditioned on rs1344706 (P = .022), indicating that the observed genetic effect was actually driven by rs7597593.

Discussion

We report a significant association between *ZNF804A*, a strong candidate gene for psychosis with a refined "positive" schizotypy phenotype primarily encompassing paranoia/ideas of reference, in a large cross-sectional study of 1507 young male conscripts undergoing initiation to military service. First, at the single-marker level of analysis, highly significant association was detected between 2 out of 4 tested *ZNF804A* variants and the paranoid schizotypy factor derived from confirmatory factor analysis of the SPQ. The strongest detected signal was between rs7597593 and the SPQ paranoid factor, surviving a stringent correction for multiple hypothesis testing. The association with paranoia for both the schizophrenia susceptibility SNPs, rs7597593 and rs1344706, was mainly attributable to association with the SPQ "ideas of reference" subscale (best $P = 5\times10^{-5}$, uncorrected). The *ZNF804A* association with paranoia was confirmed at the haplotype level. Interestingly, both schizophrenia candidate susceptibility SNPs were simultaneously nominally associated with perceptual aberrations, indicating that *ZNF804A* variability may also be tagging a schizotypy phenotype associated with spatial and temporal unity of self, considered a core feature of schizophrena liability.[34,22] Additionally, the genome-wide psychosis implicated marker rs1344706 was associated with the paranoid state and also showed a trend association (FDR-corrected P = .08) with the SPQ disorganization factor. The above finding partially replicates a recent report in which, however, paranoid traits were not

Table 3. Association Results With Cognitive Performance Indexes

	Sustained Attention (CPT-IP task)		Verbal Working Memory (N-Back Task)		Spatial Working Memory (N-Back Task)	
SNP ID	Beta	*P* Value	Beta	*P* Value	Beta	*P* Value
rs7597593	−.072	.041	.012	.718	.02	.611
rs1344706	.019	.602	−.008	.806	−.026	.519
rs4667001	−.026	.452	.035	.402	.006	.982
rs3731834	.035	.389	−.028	.478	−.007	.969

Table 4. Individual Haplotype Association Results Using SPQ Paranoid Factor as the Phenotypic Outcome

SNPs	Haplotype	Freq (%)	Beta	Nominal *P* Value	Adjusted *P* Value[a]
rs7597593	CC	37.8	.026	.001	**.012**
rs1344706	TA	37.4	−.032	.00001	**.001**
—	CA	22.8	.009	.333	ns
—	TC	2.0	.003	.918	ns
rs7597593	CCA	37.9	027	.001	**.005**
rs1344706	TAG	25.1	−.028	.001	**.01**
rs4667001	CAG	15.7	.002	.876	ns
—	TAA	12.0	−.024	.049	ns
—	CAA	7.0	.017	.304	ns
—	TCA	1.6	−.01	.785	ns

Note: Only significant *P* values (<.05) are shown in bold; ns, nonsignificant.
[a]Adjusted *P* values after running 10 000 permutations.

separately investigated.[35] In line with the above findings, it is also worth noting that a recent report implicated the same rs1344706 risk allele to elevated personalizing bias (tendency to blame others) among normal volunteers, which represents a behavioral trait closely related to paranoid ideation.[36] Our results might be viewed as supplementary to the GWAS results from O'Donovan et al,[1] which revealed genome-wide association of psychosis to the *ZNF804A* locus, as it perhaps further refines the phenotypic effects of the gene. We hypothesize that positive schizotypal traits such as paranoia/ideas of reference, psychological features traditionally linked with genetic vulnerability to psychosis,[37] may be an important phenotypic target of *ZNF804A* variability. Furthermore we propose that *ZNF804A* variability complements at the molecular level previous psychometric studies utilizing the SPQ in relatives of patients, which demonstrated consistently that ideas of reference best reflect the genetic liability to schizophrenia.[38–40] We acknowledge, however, that only prospective studies will be able to decipher whether this *ZNF804A*-driven route of presumed vulnerability actually mediates transition to clinically defined psychosis.

Second, we demonstrate here that the genetic associations with the examined schizotypy-related outcomes were mainly attributable to rs7597593, because the effect of rs1344706 was shown to arise from its high correlation with rs7597593. Notably, similar observations were made by Riley et al[7] with the clinical phenotype in the Irish Case–Control Study of Schizophrenia and by Zhang et al[8] in a large case–control analyses from public GWAS datasets. Also, as shown by Williams et al,[6] rs1344706 remained the most strongly associated marker tested in the gene after sequencing exonic regions in and around it, but rs7597593 was not typed in that study. The above findings perhaps suggest that multiple biologically active genetic variants are located toward the 5′ end of the gene and that more detailed sequencing efforts of affected individuals is warranted. Furthermore, rs7597593, which is not covered by the Affymetrix genome-wide 6.0 array, and thus not reported upon in previous GWASs, was suggestively associated in this study with positive rather than negative aspects of schizotypy across most of self-rated schizotypy questionnaires and with the same allele directionality. It would be difficult to dismiss these phenotypes—consistent signals of association—on the basis of potential type I error or due to the psychometric properties of any individual self-rated schizotypy scale. On the contrary, we propose that they may be viewed as providing convergence and discriminant validity for rs7597593 affecting a common underlying aspect of positive schizotypy, which is related to ideas of reference and perceptual distortions. Further item-to-item exploratory and confirmatory factorial analysis could be attempted in the future in order to further refine the phenotypes associated with rs7597593 and rs1344706.

ZNF804A SNPs and haplotypes were not associated with our measures of sustained attention and working memory ability, thus adding to the accumulating evidence that standard neurocognitive-intermediate phenotypes dependent on the integrity of prefrontal and frontotemporal function are probably not a major target of *ZNF804A* variability, at least amongst healthy individuals. Nevertheless, the lack of functional–imaging-data acquisition in this cohort prohibits us from reporting on the possibility that functional imaging intermediate phenotypes are suitable targets of *ZNF804A* variability, as suggested by other groups.[10]

Despite the fact that the association with paranoid traits withstands a multiple-testing correction, the allelic direction of association was opposite to what we expected and is, at least on the surface, somewhat counterintuitive. Previously identified risk-associated alleles of rs7597593 (T) and rs1344706 (A) were, in this study, associated with a significant reduction rather than increase in all instruments of positive schizotypy used, both in the single-SNP and haplotype analyses. In order to exclude possible technical issues in genotyping procedure, which could explain the above observation, we a posteriori obtained external validation of our results

by typing marker rs1344706 in an independent laboratory (Erasmus Center, Rotterdam, data available upon request), obtaining identical directionality and significance level. The observation of opposing associated alleles is, however, a relatively frequent phenomenon, especially when studying populations with different genetic backgrounds.[41,42] Explanations for this phenomenon include differences in LD with reverse correlation coefficients, with a causal variant at another locus or interactive effects with other genomic variants that carry different common alleles in different populations (in this case, Greek vs northern European). As demonstrated recently, the impact of a specific genetic variant on a phenotype may depend on the genetic background of the population, the constellation of many other variants with small effects across the genome. Studies on intellectual disability and autism have shown that normal synaptic function happens within a range of glutamate receptor protein synthesis and that genetic variation tipping the balance in either direction can result in the same deleterious effect, in this case, autism.[43] A consequence of this result is that a mild effect that increases protein synthesis would be beneficial if the population average is slightly closer to the low side of the balance and deleterious in the opposite case. This is one concrete example on how allelic alterations that increase risk in one population could be protective in another. It is possible that this or other mechanisms could be the explanation for our results. This allele reversal likely represents an indirect indication that other tagged causal variants that cannot be detected in conventional association studies are truly responsible for the observed association in our study and also in previous reports that examined the same common markers. Furthermore, as Clarke and Cardon[42] have shown, the probability of observing a significant allele flip is negligible when an allele flip is not genuine. Alternatively, this significant allele flip may suggest that at the subclinical level, ZNF804A genotype exerts a rather protective effect that plausibly becomes a true predisposition factor in clinical states where additional genetic alterations/defects are present. However, a replication attempt in an independent sample from the same population would possibly resolve this issue and help disentangle whether this is a spurious finding.

Along these lines, we acknowledge that, several, if not all, of the identified signals in our study might still be false positives and thus the interpretation of the modestly significant associations should be conservative. However, our primary aim was to target variants of a gene that already had some direct support for involvement in the pathogenesis of schizophrenia and therefore the prestudy probability of significant associations was not negligible as in a hypothesis-free, discovery-oriented approach. Consequently, the multiplicity of comparisons is far less than implied at first sight. While 3 of the 4 SNPs are now considered functional or lead to conservative amino acid substitutions, it is likely that the bulk of the signal is due to multiple unobserved causal variants in LD with the 2 (rs7597593, rs1344706) intronic SNPs that showed the strongest association in this study.

These results can be viewed as providing further support for at least some overlapping genetic underpinnings for the subclinical and clinical manifestations of psychosis. In conclusion, we report here that particular aspects of "positive" schizotypy rather than aspects of cognitive function may serve as targets of *ZNF804A* variation. The refined phenotype most sensitive to *ZNF804A* variability is characterized by ideas of reference and suggestively of distortion of perceptual experiences, which bears a striking resemblance to "aberrant salience"[44], the process hypothesized to underlie the formation of delusions and hallucinations. Based on this apparent similarity we can only speculate that *ZNF804A*, a gene strongly associated with clinical psychosis and derived "atheoretically" by GWAS, may mediate risk by indirectly impacting on a tendency to misconstrue external/internal cues which is arguably central to the psychosis experience. This is reflected by the clinically unobservable or covert effects of *ZNF804A* variation at the population level. This work also may offer further support for the strategy of adopting subclinical, population-based phenotypes and cognitive intermediate phenotypes in order to explore the genetic underpinnings of clinical psychosis. Further prospective and case–control studies that may incorporate more detailed molecular examination of *ZNF804A* gene modulation are encouraged in order to validate the results reported herein.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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