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Schizophrenia Candidate Gene *ERBB4*: Covert Routes of Vulnerability to Psychosis Detected at the Population Level

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Prior genetic and functional evidence established *ERBB4* as a probable schizophrenia susceptibility gene that may confer risk via modulating brain information processing dependent on the integrity of frontotemporal brain circuitry. Utilizing retrospective data drawn from the cross-sectional population-based Athens Study of Psychosis Proneness and Incidence of Schizophrenia (ASPIS) (n = 1127), we attempted to independently replicate and further extend previous findings by examining the effects of *ERBB4* gene variants on 3 broad population–based psychosis-related phenotypes: verbal working memory (VWM), trait schizotypy, and stress-induced subclinical psychotic experiences (PE). Three common *ERBB4* single nucleotide polymorphisms that were previously associated with schizophrenia and impaired frontotemporal-related information processing (rs7598440, rs839523, and rs707284), their haplotypes, and corresponding diplotypes were tested. VWM performance was significantly associated with rs839523 and rs707284 markers even after correction for multiple testing, thus validating reported findings that have implicated *ERBB4* gene variation on working memory. No associations were detected between these *ERBB4* variants and trait schizotypy. However, we were able to detect a significant effect of rs7598440 marker on PE expressed under stressful environmental conditions. Combined haplotype analysis of the above 3 markers, identified a "yin-yang" pattern of association, confirmed at the diplotype level. While GGG haplotype homozygotes were associated with "protective" effects on VWM performance and PE, AAA "risk" haplotype carriers were associated with worse VWM performance and simultaneously exhibited significantly elevated PE. This dual, possibly pleiotropic, impact on frontotemporal circuitry and increased sensitivity to psychosocial stress may represent subtle manifestations of *ERBB4*-related vulnerability to psychosis, expressed at the population level.

Key words: schizotypy/working memory/psychotic symptoms/schizophrenia/stress/polymorphism

Introduction

Since the initial work of Stefansson et al[1] that identified *NRG1* as a potential susceptibility gene for schizophrenia, accumulating evidence continues to support the role of the *NRG1-ERBB4* signaling pathway as a promising mechanism for vulnerability to psychosis.[2,3] The *NRG1* receptor *ERBB4* is coded for by a gene, which spans 1.16 Mb on chromosome 2q34 and is 1 of 4 members of the mammalian *ERBB* family of transmembrane tyrosine kinases.[3] Among the *ERBB* proteins, *ERBB4* is the best characterized in terms of its function in the Central Nervous System. Of the other 3 *ERBB* receptors that are involved in *NRG1* signaling, *ERBB2* lacks an active ligand-binding domain and *ERBB3* lacks an active catalytic domain because both of these receptors must form heterodimers for signal transduction. In contrast, *ERBB4* can form functional homodimers as well as heterodimers. As yet, there is no evidence that *ERBB2* and *ERBB3* are susceptibility genes for schizophrenia and, unlike *ERBB4* mutant mice, mice that carried mutations in *ERBB2* and *ERBB3* did not produce behaviors that were analogous to animal models of schizophrenia.[4] The *ERBB4* gene also is among several that have been identified as being

disrupted by microdeletions or microduplications in patients with schizophrenia.[5] Despite emerging evidence that the gene for *ERBB4* may influences risk for schizophrenia, when considered together with *NRG1*,[3,6–8] few but important studies suggest direct association of *ERBB4* variants with schizophrenia. In a large sample ($n = 296$) of schizophrenia families and control individuals ($n = 370$), we reported association for two 3-markers *ERBB4* haplotypes, one including rs7598440, rs839541, and rs839523 around exon 3.[9] For this latter haplotype, we were able to confirm this finding in a sample of 51 African-American families (National Institute of Mental Health Genetics Initiative). Moreover, in a second study of unrelated Ashkenazi, schizophrenia patients and matched controls association were detected with 3 out of 19 single nucleotide polymorphisms (SNPs) along the *ERBB4* gene from one linkage disequilibrium (LD) block surrounding exon 3: rs707284, rs839523, and rs7598440.[10] In addition, Silberberg et al[10] reported that this schizophrenia risk haplotype around exon 3 is associated with altered expression of certain splice variants of the gene (CYT-1 isoform) in schizophrenia dorsolateral prefrontal brain. This finding was confirmed by Law et al[11] who found main effects for rs839523, rs707284, rs7598440, and a core-risk haplotype surrounding exon 3 on expression of the exon 26 (CYT-1) isoform of *ERBB4* mRNA transcript in dorsolateral prefrontal cortex (DLPFC). These studies strongly suggest that splice-variant-specific expression of *ERBB4* may be the basis for the association of this gene with schizophrenia. A recent study showed that *NRG1* and *ERBB4* protein levels were increased in the prefrontal cortex of patients with schizophrenia.[12] Taken together, these few studies suggest that splice-variant-specific expression of *ERBB4* may be the basis for the association of this gene with schizophrenia, and collectively, that *ERBB4* may independently exert its effects on schizophrenia susceptibility.

Schizophrenia is thought to be a complex "polygenic" disorder in which at least some of the variation in liability is due to multiple common alleles found in the general population, which individually contribute only a very small fraction of the overall risk.[13] Therefore, the effects of specific risk gene variations can be investigated in the general population as well, ie, in healthy subjects, even in the absence of the overt behavioral abnormalities seen in patients, provided the employed phenotypic measures are related to genetic risk for schizophrenia and that they are sensitive enough to detect genetic effects.[14,15] Such phenotypes genetically linked with schizophrenia, often referred as "intermediate phenotypes" with which the disorder presumably shares a degree of overlapping genetic liability, include structural and functional brain alterations, neurocognitive deficits, and schizotypal personality traits or symptoms. These are expressed as quantitative traits with different intensities across a broad phenotypic spectrum, ranging from patients to their unaffected relatives and extending to the (disease-free) general population.

Recent evidence that *ERBB4* may indeed impact on intermediate cognitive phenotypes of schizophrenia is provided by Nicodemus et al[3,9] and Konrad et al.[15] In the first study, *ERBB4* haplotype variability (rs228908, rs3791709, and rs4673628) was associated with verbal working memory (VWM) test scores in healthy controls that have been shown to be related to increased genetic risk for schizophrenia. Similarly, Konrad et al[15] reported that *ERBB4* genetic variations (rs7598440, rs839541, rs839523, and rs707284) may confer risk for schizophrenia illness via its impact on left frontotemporal connectivity in human brain. Finally, Nicodemus et al[3] showed that interactions of *ERBB4* SNP rs1026882 with *NRG1* pathway partners at the DLPFC, increased inefficiency in cortical processing during a standard 2-back working memory task in healthy subjects. While collectively these studies may be viewed as supportive for the potential effect of *ERBB4* variability on cognitive intermediate phenotypes such as working memory, they do not address the possibility that *ERBB4*-related vulnerability may be mediated via alternative routes, for example, impacting on psychological rather than cognitive intermediate phenotypes, such as enduring schizotypal traits or subclinical psychotic experiences (PE).

We first reported on the utility of adopting a population-based cognitive subclinical psychosis phenotype approach, to study the potential effect of candidate susceptibility genes for schizophrenia.[16] Subclinical psychosis phenotypes such as schizotypal traits and/or PE are now recognized to be substantially heritable exhibiting familiar, temporal, developmental, and etiological continuity with the clinical disorder.[17,18] We hypothesized that these subclinical phenotypes may serve as potential targets of schizophrenia susceptibility alleles that are common in the general population. This strategy has been recently gaining theoretical and empirical momentum,[19,20] reinforced by the notion that self-reported PE are strongly and inversely age dependent, thus representing a broadly distributed phenotype that may be the developmental albeit transient expression of genetic liability to psychosis.[21,22] Within this context of gene association studies in apparently healthy populations, a phenotype of interest that has not been explored before is stress-induced subclinical PE. Gene-environment interactions may be an important mechanism in explaining between-subject differences in risk for psychosis after exposure to psychosocial stress.[23,24] Powerful evidence for the role of stress in the etiology of psychosis comes from the study of elevated expression of psychosis after semi-experimental stressful conditions such as army induction.[25] Extending this rational and in accordance with previous gene-environment work on this cohort, in which PE (as measured with SCL90R-CP) were found to be modulated by *COMT* variability,[26] we hypothesized

that psychosocial stress may operate in synergy with candidate schizophrenia genes such as *ERBB4* to increase vulnerability for psychosis at the population level.

As part of an ongoing project in which several candidate genes for schizophrenia have been selectively examined based on prior genetic and functional evidence,[16,26,27] we set out in this study to attempt replication of previous reported associations by evaluating in a large cohort of apparently healthy young males whether 3 common *ERBB4* variants, previously associated with risk for schizophrenia, impact on intermediate phenotypes linked with the disorder, namely aspects of working memory that exhibit a degree of heritability and are broadly dependent on frontotemporal brain function. Furthermore, in the context of the continuum hypothesis of psychosis and upon as yet limited molecular evidence of gene-environment interactions operating in psychosis susceptibility, we further set out in this study to explore the hypothesis that *ERBB4* variants may impact on psychosis-related psychological constructs such as self-rated schizotypy and stress-induced PE. Potential associations detected with specific schizotypy constructs/symptoms might help elucidate and refine *ERBB4*-dependent effects on the broader psychosis phenotype.

Participants and Methods

Participants

All 2243 young healthy male conscripts aged 18–24 years that were admitted in 8 consecutive waves to the National Basic Air Force Training Center in Tripoli, Greece, were eligible to participate in the Athens Study of Psychosis Proneness and Incidence of Schizophrenia (ASPIS). Military service is compulsory in Greece, and all healthy men are recruited and assigned to the different army corps by random assignment. The sample, therefore, is representative for the Greek population of this age stratum. The compulsory aspect of the military training in Greece also offers a unique natural setting to study genetic differences in stress sensitivity because the level of environmental exposure to stress was datable, similar for all recruits and not associated with genetic variation (ie, no confounding by gene-environment correlation). After obtaining written informed consent, DNA was extracted from mouthwash samples. Of the 2243 conscripts, 214 did not participate with genetic testing. Two thousand one hundred and forty-two subjects performed at least some of the cognitive tasks assessing vigilance of attention and aspects of working memory, and 1955 conscripts participated by filling in a psychometric battery of self-administered questionnaires.[28] No conscript was excluded due to medical conditions as they were deemed to be healthy following prior medical screening. An a priori selected subsample of 1127 conscripts with multiple valid cognitive and self-reported psychometric scores across the entire battery was used for further analyses in this study, biasing thus the sample toward participants with an increased acceptance rate of the procedures. This study was approved by the Bioethics and Medical Deontology Committee of the University Mental Health Research Institute, Athens, Greece.

Subclinical Psychosis Assessment

Within the first 2 weeks of military induction, conscripts completed a psychometric battery of self-administered questionnaires. The assessment battery included, among others, assessment of lifetime schizotypal traits with the Schizotypal Personality Questionnaire (SPQ) and the Symptom Checklist 90-Revised (SCL90-R) was also completed.[28–31] The SPQ is a 74-item questionnaire that assesses all 9 aspects of the Schizotypal Personality Disorder (SPD) according the *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised* (American Psychiatric Association). High SPQ scorers in this conscript sample are predictive of an independent diagnosis of SPD upon interview.[30] The SCL90-R is a comprehensive self-report questionnaire of 90 questions, which covers a broad range of state psychiatric symptoms experienced in the first 2 weeks of military induction. According to the SCL90-R manual, the 6-item dimension "paranoid ideation" is characterized by projective thoughts, hostility, suspiciousness, grandiosity, centrality, fear of loss of autonomy, and delusions. The dimension "psychoticism" (10 items) includes items indicative of a withdrawn, isolated schizoid life style as well as items representing symptoms of psychosis and schizophrenia such as hallucinations and thought broadcasting. We combined the scores of "paranoid ideation" and "psychoticism" subscales into a mean total score (SCL90-R "combined psychosis" score or SCL90R-CP) as a measure of PE. This combined SCL90-R psychosis score was also computed in previous publications that used this composite variable as a proxy measure of subclinical psychosis in the general population.[26,32,33] The SCL90R-CP has been recently found to possess a degree of predictive validity since persistence over time of high SCL90R-CP scores in adolescents and young adults in the German population predicted 40% of new onset clinically relevant psychosis in the Munich Early Developmental Stages of Psychopathology Study.[34]

Cognitive Assessment

Conscripts underwent an extensive interview of computerized neurocognitive tests.[16,35] We chose to include in this study our verbal 2-back working memory paradigm,[29] based on prior evidence of association with *ERBB4* variants. VWM performance as assayed by our 2-back paradigm is very similar to the *N*-back task that was demonstrated to be sensitive to *ERBB4* variability, albeit to different *ERBB4* SNPs and haplotypes,[3,11]

and it presumably activates broadly similar frontotemporal circuitry as the oddball attentional-working memory paradigm employed by Konrad et al.[15] Furthermore, we considered recent in vitro and in vivo evidence showing that since ErbB4 expression in mouse cortex is largely restricted to GABAergic interneurons,[36] *ERBB4* function may be linked to working memory performance in normal individuals and patients with schizophrenia. In accordance with our previous work, we a priori excluded data from further analyses, if the central index of performance (*d'*) of verbal 2-back was <0, if there were ≥3 unsuccessful trials (of 5).

DNA Extraction, SNP Selection, and Genotyping

Mouthwash samples for DNA extraction were chosen as described previously to obtain a better procedure acceptance rate.[37] For *ERBB4*, we chose to genotype SNPs reported previously to be associated with schizophrenia and cognitive deficits in Caucasian and Ashkenazi samples. We therefore genotyped rs707284, rs839523, and rs7598440 in accordance with Silberberg et al,[10] Nicodemus et al,[9] Law et al,[11] and Konrad et al.[15] Genotyping was performed using commercially available TaqMan 5′-exonuclease allelic discrimination assays (Applied Biosystems) (details available on request). Genotype reproducibility was routinely assessed by regenotyping a subsample for selected SNPs in an independent laboratory (Erasmus Medical Center) and was generally >99%.

Statistical Analysis

We quantified total SPQ and SCL90R-CP raw scores as continuous variables as in our previous studies.[16,26,35] Mean scores on demographics, psychometric, and cognitive variables were compared between the 1127 conscript subsample under investigation in this study and the nongenotyped sample. We assessed genotypic compliance with the Hardy-Weinberg (HW) law using an exact test and we estimated the D′ and r^2 coefficients for SNP pairs using Haploview 4.0.[38] Standard linear regression models were conducted in order to assess the effect of each SNP on continuous phenotypic variables (allele-load or allele-based additive models), using SPSS 18 statistical software (SPSS Inc.). All statistical tests are 2-tailed, and correction for multiple testing was applied using the Bonferroni procedure. Given the number of tests performed (3 SNPs × 3 phenotypes = 9 tests), we acquired a conservative significance level of $P = .006$. In post hoc analyses, we included age, education level, and IQ as plausible covariates. Three-marker haplotypes were reconstructed with UNPHASED 3.1.3 software, and association testing was examined utilizing the individual haplotype option provided in UNPHASED.[39] Haplotypes with frequency <5% were excluded from further analysis. Haplotypes for each participant were also reconstructed with PHASE 2.1 software, and this was utilized to estimate diplotype status of each participant.[40] One-way ANCOVA was used to compare the mean scores of intermediate phenotypes with respect to status of increasing diplotype risk load. Despite the expected erosion of power in diplotype construction ($n = 829$), the process is only restricted by random missing genotype calls. In this study, given our adjusted significance level (α set to .006) and our sample size, we had 90% statistical power to detect small effect sizes ($f^2 = 0.02$).

Table 1. Single Nucleotide Polymorphisms (SNPs) Analyzed and Genotype Information in the ASPIS Study

Marker (dbSNP)	rs7598440	rs839523	rs707284
Chromosomal region	2q34	2q34	2q34
Location (bp)	212.501.443	212.524.334	212.547.291
Alleles (major/minor)	A/G	G/A	G/A
Minor allele frequency (MAF)	0.44	0.30	0.35
Genotype counts	AA 333 AG 541 GG 226	GG 529 GA 463 AA 104	GG 425 GA 501 AA 145
Genotype call rate (%)	97.6	97.2	95
HWE exact *P* value	.81	.89	.95

Note: dbSNP, marker identification number according to NCBI SNP build 131; HWE, Hardy-Weinberg equilibrium; ASPIS, Athens Study of Psychosis Proneness and Incidence of Schizophrenia.

Results

SNPs characteristics and genotyping statistics are shown in table 1. The frequencies in the ASPIS sample of each 1 of the 3 SNPs were not significantly deviant from HW equilibrium. The 3 *ERBB4* polymorphic markers analyzed herein cover 46 kb worth of genomic DNA, spanning exon 3 (University of California, Santa Cruz Genome browser, http://genome.ucsc.edu). Online supplementary table 1 shows that there is moderate LD between all 3 SNPs in our sample. Participants did not differ in terms of their schizotypal personality scores (total SPQ score) (mean 36.6 vs 38.2, $P = .053$), SCL90R-CP (PE) scores (mean 1.80 vs 1.75, $P = .41$), and central index of VWM performance scores (mean 2.56 vs 2.52, $P = .36$) compared with the nongenotyped conscript sample, thus excluding potential bias in the selection of the sample under investigation.

Table 2 provides the results from the linear regression tests of association between SNPs genotypes and total SPQ score, PE score, and 2-back working memory task. As can been seen, all 3 markers were nominally associated with VWM performance. However, after conservative Bonferroni correction for multiple comparisons, 2 of the

Table 2. Single-Marker Association Results With Continuous Phenotypes

	rs7598440		rs839523		rs707284	
Phenotype	*P* Value	Increasing Allele[a]	*P* Value	Increasing Allele	*P* Value	Increasing Allele
Schizotypal traits (SPQ)	.369	A	.904	A	.364	A
PE (SCL90R-CP)	**.006**	A	.048	A	.072	A
Verbal working memory	.031	G	**.005**	G	**.006**	G

Note: P values <.05 that survived Bonferroni correction for multiple comparisons are in bold. SPQ, Schizotypal Personality Questionnaire; PE, stress-induced psychotic experiences; SCL90R-CP, Symptoms Checklist 90-Revised combined psychosis score.
[a]Allele associated with higher phenotypic values.

3 association signals remained significant ($\beta = -.086$, $P = .005$, $R^2 = .008$ for rs839523 and $\beta = -.087$, $P = .006$, $R^2 = .008$ for rs707284), while convergent evidence for association with PE score was detected only for rs7598440 ($\beta = -.088$, $P = .006$, $R^2 = .008$). We note that the association of rs7598440 with PE was markedly strengthened when a recessive genetic model was assumed in our analysis ($\beta = -.11$, $P = .0004$). Considering VWM, a similar trend for greater recessive effects was observed for all 3 markers but with marginal statistical significance. Furthermore, multiple regression analysis including 2 markers at a time showed that all 3 likely reflect the same effect through LD. Plausible confounding effects were tested by entering age, education, and IQ as covariates, but results were identical (not shown). Next, we tested VWM and PE phenotypic variables as covariates of each other in our standard regression models. Association results remained significant ($P < .02$) with no considerable change of beta regression coefficient after covariation, suggesting thus a pleiotropic genetic effect of these 3 *ERBB4* variants on the psychosis-related phenotypes reported in this study.

Haplotype analysis revealed 5 common (>5%) haplotypes in the Greek population. The individual effects of each of the 5 common haplotypes on VWM performance, schizotypy (total SPQ score), and PE are shown in table 3. The 2 most common haplotypes GGG and AAA are associated with VWM and PE scores. The GGG haplotype was associated ($P = .019$) with enhanced VWM performance, and the complementary AAA haplotype was associated ($P = .008$) with decreased VWM performance (ie, risk). As regards PE, the GGG haplotype was associated ($P = .014$) with decreased SCL90R-CP scores, and the complementary AAA haplotype was associated ($P = .03$) with elevated SCL90R-CP scores (ie, risk).

Following diplotype reconstruction, similar results were observed. We implemented one-way ANOVA in order to compare the mean scores of VWM with respect to the categories of diplotype risk load. As shown in figure 1, we detected an overall significant difference in VWM scores between the 3 diplotype groups ($F_{2,781} = 4.64$, $P = .01$, partial $\eta^2 = .013$). With respect to PE, the effect of carrying at least one risk haplotype copy (AAA) was significant as well ($F_{2,734} = 5.85$, $P = .003$, partial $\eta^2 = .017$). In order to validate the potential pleiotropic effect observed at the single-marker association analysis, we examined at the diplotype level, the effects of *ERBB4* diplotype risk load on these 2 psychosis-related phenotypes by entering VWM score as the dependent variable and PE score as a potential confounder and vise-versa. Therefore, 2 ANCOVA models were employed that confirmed the independent effects observed at the single SNP level. The effect of diplotype risk load on VWM performance remained significant after adjusting for PE ($F_{3,689} = 2.98$, $P = .031$), and similarly, after considering VWM as a covariate, the effect of diplotype risk load on PE score was also found to be significant ($F_{3,689} = 3.69$, $P = .012$).

Table 3. *ERBB4* Haplotype Structure in the ASPIS Sample and Individual Haplotype Association Results

		Schizotypy (SPQ Score)		PE (SCL90R-CP Score)		Verbal Working Memory	
Haplotype	Frequency (%)	add.value	*P* Value	add.value	*P* Value	add.value	*P* Value
AGG[a]	20.3	0	.536	0	.385	0	.791
AGA	5.6	−0.13	.294	−0.62	.583	0.1	.468
AAA	30.3	−0.62	.903	0.03	**.031**	−0.1	**.008**
GGG	43.8	−0.85	.274	−0.09	**.014**	0.09	**.019**

Note: Nominal significant *P* values (<.05, 2-tailed) are in bold. SPQ, Schizotypal Personality Questionnaire; PE, stress-induced psychotic experiences; SCL90R-CP, Symptoms Checklist 90-Revised combined psychosis score; add.value, estimated additive genetic value relative to the reference haplotype; ASPIS, Athens Study of Psychosis Proneness and Incidence of Schizophrenia.
[a]Reference haplotype

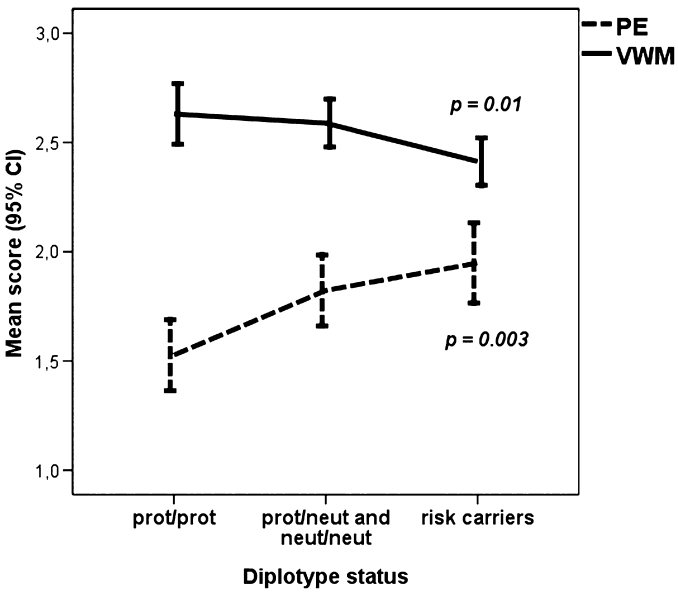

Fig. 1. Graphical representation of *ERBB4* diplotype effects ($n = 829$) on verbal working memory (VWM) and stress-induced psychotic experiences (PE). Haplotypes were defined as protective (GGG), neutral (AGG or AGA), and risk (AAA) according to their impact on the above phenotypic variables. Individuals were divided according to diplotype status into 3 groups, protective homozygotes ($n = 207$), neutral homozygotes and protective/neutral heterozygotes ($n = 346$), and risk haplotype carriers ($n = 276$). Mean scores (±95% CI) are shown.

Discussion

We report here associations of common *ERBB4* gene variants, previously associated genetically and functionally with schizophrenia susceptibility (rs7598440, rs839523, and rs707284), with working memory performance and stress-induced subclinical PE at the population level, in a large cross-sectional sample of healthy Greek Air force recruits. The *ERBB4* effects on these 2 hypothesized routes of vulnerability for psychosis were detected at the single SNP, haplotype and diplotype level.

The association between *ERBB4* variants and VWM adds to prior evidence, supporting the notion that *ERBB4* variability may increase risk for psychosis via modulating frontotemporal circuitry. Law et al[11] provide gene expression data showing that rs7598440/A allele, rs839523/A allele, and rs707284/A allele, lead to overexpression of the CYT-1 *ERBB4* transcript in the prefrontal cortex (DLPFC). This finding might confer a potential functional explanation of the working memory impairment observed in AAA risk haplotype carriers, suggesting that CYT-1 transcript overexpression may negatively modulate prefrontal-dependent cognitive tasks such as VWM performance. Similarly, elevated CYT-1 levels in prefrontal cortex might trigger specific molecular pathways that provoke PE in healthy individuals. This later notion is in line with the finding of higher CYT-1 transcript DLPFC expression levels in schizophrenia patients compared with controls,[10] demonstrating that CYT-1 may regulate key physiological processes implicated in DLPFC proper function, which could control the transition from a subclinical to a clinical state. The association between *ERBB4* variants and PE (as measured with SCL90R-CP), but not schizotypal traits, suggest that *ERBB4* potential psychotogenic effects are stress sensitive rather than associated with modulation of stable schizotypal personality traits. This introduces the possibility of gene (*ERBB4*)-environment (psychosocial stress) interaction operating in psychosis vulnerability related to this gene. It could be argued that SCL90R-CP scores at military induction represent trait rather than state attributes because no measures of SCL90R-CP psychopathology were collected prior to conscription. However, an 18-month retest assessment in a small but representative subsample of 194 army recruits at the end of military service, confirmed a reduction of overall SCL90-R scores (ranging from 14% to 53% for individual subscales including SCL90R-CP).[26] This led us to hypothesize that increased PE, as captured by SCL90R-CP, at the induction to military service timepoint may well be attributed to the presence of increased psychosocial stress associated with such factors as separation from family and friends, adjustment to a novel social environment, loss of autonomy and subordination to strict military regulations, and the engagement in everyday military drills.

Moreover, no significant associations between *ERBB4* variants and PE were observed in the retest sample (data upon request). Taken together, our findings introduce the intriguing possibility that *ERBB4* impact on PE is conditioned on psychosocial stress, thus complementing at the molecular level recent epidemiological evidence in which PE measured in the general population (with SCL90R-CP) were exacerbated under the influence of various hypothesized environmental stressors.[22,32,33]

Haplotypic and diplotypic association to VWM and stress-induced psychosis provides further evidence of a functional locus potentially within a haplotype block at least 46 kb, encompassing exon 3 of *ERBB4* gene locus. These effects appear to be independent of each other, suggesting that an as yet unidentified common genetic denominator within this region is responsible for a pleiotropic effect on psychosis vulnerability via 2 independent mechanisms: impact on frontotemporal function (VWM) and increased sensitivity to adverse environmental stimuli (psychosocial stress), though we cannot rule out convincingly that both associations reflect a common underlying biologic trait. Both GABAergic and glutamatergic transmission in the prefrontal cortex which are thought to contribute to the pathophysiology of schizophrenia are known to influence *NRG1-ERBB4* signaling pathway.[3]

The combined effect of a risk *ERBB4* haplotype on a phenotype related to psychosis (PE) as well as a recognized intermediate phenotype for psychosis (VWM) furthers in our opinion the possibility that *ERBB4* is indeed a psychosis vulnerability gene. We acknowledge however

that only prospective studies will be able to decipher which (if any) of these 2 *ERBB4* driven routes of presumed vulnerability, actually mediate transition to clinically defined psychosis.

In order to minimize false positive results of association, we preselected only 3 *ERBB4* genetic variants that have been associated genetically and functionally with schizophrenia and with brain-related biologic phenotypes. We also restricted multiple phenotypes of interest to VWM based on prior converging evidence of association with *ERBB4*. We also restricted multiple self-rated instruments of interest to the 2 main phenotypes used in our previous work to reflect schizotypy and self-rated psychotic symptoms. Moreover, we applied stringent Bonferroni correction for multiple comparisons at the single SNP level in order to minimize type I error rate in our results. This type of statistical correction is overwhelming conservative because it does not account for putative correlations between genetic markers (LD) or phenotypic variables. *ERBB4* SNPs were in modest to high LD, and therefore, the multiplicity of comparisons is far less than implied at first sight. Furthermore, our approach was to target variants of a gene that already had some indirect or 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. Therefore, and in view of direct comparison intended with Silberberg et al[10] and Law et al,[11] adjustment for multiple comparisons was not additionally performed at the haplotype and diplotype level of analysis. It is thus further acknowledged that several of the identified signals in our study may represent false positives, although this is unlikely if single SNP, haplotype, and diplotype results are to be considered as a whole. Regardless, the interpretation of the modestly significant associations reported herein should be conservative.

It should be noted that the Greek AAA "risk" haplotype is different than the AGG risk haplotype described by Silberberg et al[10] and Law et al.[11] The haplotype frequencies in this Greek cohort differ substantially from those in the Ashkenazi-Jewish cohort. This alone indicates that the population structure in these cohorts is quite different and that different risk haplotypes may be expected, each may be however restricted to the particular ethnic group under examination. Secondly, it may be that the effects observed in our cohort are not exerted by the whole haplotype but rather only by part of it, eg, the first SNP (rs7598440), in which the risk associated nucleotide (A) was previously identified by Silberger et al[10] and Law et al[11] (albeit being the minor and not the major allele as in this cohort) or by rs839523 and rs707284 which are in tighter LD. Furthermore, the genetic variations in *ERBB4* that we studied are noncoding intronic variants, likely monitoring other potential causal variants in tight LD with the markers studied herein. It is acknowledged therefore that *ERBB4* case-control association studies in the Greek population may assist in validating the risk variants and haplotypes for psychosis proposed herein from the population perspective.

It should also be considered that *ERBB4* putative psychogenic effects are more likely to be exerted not in isolation but rather within a biological context that is presumably dependent on other genes (epistasis) and/or polygenic. Current work in progress will attempt to probe potential epistatic interactions between *NRG1*, *NRG3*, and *ERBB4* gene variants using machine learning algorithms, a viable approach for detecting higher order epistasis that avoids some of the obstacles of more traditional statistical approaches.[3]

In conclusion, we provide here convergent evidence that select cognitive functions and PE induced under environmental stress, may represent 2 independent biologically related phenotypes, which are genetically modulated by *ERBB4* variation. This might represent subtle or covert manifestation of *ERBB4*-related vulnerability to psychosis expressed 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 larger sample sizes and more detailed molecular examination of *ERBB4* gene modulation should be encouraged in order to validate the results reported herein.

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Supplementary Material

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

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