

Published in final edited form as:

Biol Psychiatry. 2008 March 1; 63(5): 449–457.

AKT1 is associated with schizophrenia across multiple symptom dimensions in the Irish Study of High Density Schizophrenia Families (ISHDSF)

Dawn L Thiselton¹, Vladimir I Vladimirov¹, Po-Hsiu Kuo¹, Joseph McClay², Brandon Wormley¹, Ayman Fanous^{1,3}, Francis A O'Neill⁴, Dermot Walsh⁵, Edwin JCG Van den Oord², Kenneth S Kendler¹, and Brien P Riley¹

¹Dept of Psychiatry, Virginia Commonwealth University, USA ²Center for Biomarker Research and Personalized Medicine, Dept of Pharmacy, Virginia Commonwealth University, USA ³Dept of Psychiatry, Georgetown University, and the Washington VA Medical Center ⁴Dept of Psychiatry, The Queens University, Belfast, Ireland ⁵The Health Research Board, Dublin, Ireland.

Abstract

Background—The phosphatidylinositol 3-kinase (PI3K)-AKT signal transduction pathway is critical to cell growth and survival. *In vitro* functional studies indicate that the candidate schizophrenia susceptibility gene *DTNBP1* influences AKT signaling to promote neuronal viability. The *AKT1* gene has also been implicated in schizophrenia by association studies and decreased protein expression in the brains of schizophrenic patients.

Methods—The association of *DTNBP1* in the Irish Study of High Density Schizophrenia Families (ISHDSF) prompted our investigation of *AKT1* for association with disease in this sample. Eight SNPs spanning *AKT1* were analyzed for association with schizophrenia across 4 definitions of affection, and according to Operational Criteria Checklist of Psychotic Illness (OPCRIT) symptom scales. We examined expression of *AKT1* mRNA from post-mortem brain tissue of schizophrenic, bipolar and control individuals.

Results—No single marker showed significant association, but the risk haplotype previously found over-transmitted to Caucasian schizophrenic patients was significantly under-transmitted in the ISHDSF ($0.01 < p < 0.05$), across all OPCRIT symptom dimensions. Exploratory haplotype analysis confirmed association with schizophrenia toward the 5' end of *AKT1* ($0.008 < p < 0.049$, uncorrected). Consistent with reduced AKT1 protein levels reported in schizophrenic brain, we found significantly decreased RNA levels in prefrontal cortex of schizophrenic individuals.

Conclusions—The replication of association of *AKT1* gene variants in a further Caucasian family sample adds support for involvement of AKT signaling in schizophrenia, perhaps encompassing a broader clinical phenotype that includes mood dysregulation. We show that AKT1 signaling may be

Address for correspondence: Dawn L Thiselton PhD, Dept of Psychiatry, Virginia Commonwealth University, Biotech 1, Suite 110, 800 E. Leigh St, USA, 23298-0424, Tel: (804) 828 3048, Fax: (804) 828 1471, email: dlthiselton@vcu.edu.

The authors declare no financial conflict of interest

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compromised in schizophrenic and bipolar patients via reduced RNA expression of specific AKT isoforms.

Keywords

AKT1; schizophrenia; association; gene expression; clinical features

Introduction

Schizophrenia (MIM 181500) is a severe psychiatric illness with complex etiology and lifetime prevalence of ~1% worldwide (1). Family, twin and adoption studies have consistently demonstrated a genetic component to schizophrenia, together with developmental and environmental influences (1,2). Current pathophysiological models posit the disease to be the cognitive, perceptual and behavioural outcome of subtle alterations in brain development occurring long before clinical symptoms, and emphasize glutamatergic, dopaminergic and GABAergic systems (3).

In recent years, positional candidate genes have been widely studied for association with schizophrenia. A parallel approach toward identifying susceptibility variants is via 'functional candidate' genes or molecular pathways based on disease etiology. Alterations in intracellular signaling pathways are known to be important in treating neuropsychiatric disorders, e.g. two medications for bipolar disorder and schizophrenia, lithium and clozapine (respectively), enhance AKT signaling (4). Indeed, as a key signaling intermediate downstream of dopamine receptor D2 (DRD2), the best-established target of antipsychotic drugs, AKT is instrumental in normal dopaminergic transmission and expression of dopamine-associated behaviours (4). DNA sequence variants in *AKT1* were associated with bipolar disorder following linkage to 14q32 (5), and more recently with schizophrenia (6).

AKT1 belongs to a serine/threonine kinase family, including AKT2 and AKT3, integral to a signal transduction pathway (with phosphatidylinositol-3 kinase (PI3K) and glycogen synthase kinase 3 β (GSK3 β)) that regulates cellular functions such as survival, growth, transcriptional regulation and nutrient metabolism (7). AKT1 activation can suppress apoptosis in a transcription-independent manner. AKT1, AKT2 and AKT3 are encoded by separate genes, each playing unique as well as common roles in cells: *Akt1* knockout mice are growth retarded, *Akt2* knockout mice develop diabetes-like symptoms through impaired insulin response, and *Akt3* knockout mice show reduced brain size (reviewed in 8). AKT1, although ubiquitous, is highly expressed in brain, hence critically involved in neuronal survival, but also important for neuronal excitability and synaptic plasticity by directly regulating GABA-A receptor mediated synaptic inhibition (9). It appears to be involved in neurodevelopment and working memory formation, domains potentially impaired in schizophrenia (10,11).

The study of Emamian *et al* (6) provided converging lines of evidence to link dysregulation of AKT signaling with schizophrenia. Reduced AKT1 protein levels and phosphorylation of its substrate GSK3 β were found in brain tissue of schizophrenic patients, while C57Bl/6 mice treated with haloperidol displayed increased phosphorylation of total AKT and GSK3 β . Furthermore, DNA variants within the *AKT1* gene were associated with schizophrenia in 268 families of European origin. Most significantly associated was SNP rs3730358 in intron 3 ($p=0.05$), with one haplotype (rs1130214-rs3730358-rs2498799, alleles T-C-G) spanning intron 2-intron 7, over-transmitted to affected offspring (1.8:1, $p=0.0006$) (Fig.1). The *AKT1* core risk haplotype (rs1130214-rs3730358, T-C, $p=0.001$) was associated with lower AKT1 protein levels in lymphocyte-derived cell lines of schizophrenia patients.

Association between *AKT1* and schizophrenia has been replicated in 3 independent studies: 79 Caucasian sib-pair families (12), a Japanese 507 case –437 control sample (13) and an Iranian 321 case –383 control sample (14). Schwab et al (12) found association with rs3730358 ($p=0.027$), but more significantly with 5'UTR SNPs, rs1130214 ($p=0.011$) and rs10149779 ($p=0.002$). Most significant association was detected with haplotype rs3803300-rs10149779-rs3730358 ($p=0.001$), although others were also over-transmitted to affected offspring, including the T-C-G risk haplotype of Emamian *et al* (6;Fig.1). Ikeda *et al* (13) found most significant association with rs2494732, in intron 7 ($p=0.004$), and with haplotypes overlapping the Emamian *et al* 'risk' haplotype at the 3' end (Fig.1). The most significant haplotype rs3730358-rs2498799-rs2494732 (C-G-A) was more frequent in controls than cases, indicative of a 'protective' effect. Bajestan *et al* (14) showed modest evidence of association with schizophrenia in the Iranian population with a different haplotype of the 5 original SNPs of Emamian *et al* (6). Norton *et al* (15) in a UK 673 case –716 control sample recently reported weak evidence for association ($p=0.04$, uncorrected) with a 4-marker haplotype encompassing the Emamian *et al* core haplotype. However, a second Japanese case-control sample (16), and Japanese and Taiwanese family-based studies (17,18) detected no association between these SNPs and schizophrenia. A family-based study in the Finnish population also failed to demonstrate association between *AKT1* or *DTNBP1* and schizophrenia (19).

Functional studies suggest that defective PI3K-AKT signaling in schizophrenic brains may be due to decreased expression of dysbindin (*DTNBP1*), which may normally support neuronal viability through a neurotrophic effect via AKT signaling (20). Defective dysbindin-AKT signaling may therefore produce long-term vulnerability leading to psychiatric illness. The presence of a high-risk *DTNBP1* haplotype in the ISHDSF previously implicating dysbindin in the etiology of schizophrenia (21), prompted our investigation of association between *AKT1* and schizophrenia in this sample.

Materials and Methods

Subjects and phenotypes

The ISHDSF sample comprises 265 high-density schizophrenia families with 1408 individuals available for genotyping (22). All participating individuals gave appropriate informed consent to the study. The sample was divided into 4 concentric diagnostic categories for analysis purposes, ranging from narrow to very broad spectrum disease. Briefly, D2 (core schizophrenia) includes schizophrenia, poor-outcome schizoaffective disorder and simple schizophrenia, D5 (narrow psychosis spectrum) additionally includes schizotypal personality disorder and all other non-affective psychotic disorders, D8 (broad spectrum) additionally includes mood-congruent and incongruent psychotic affective illness, paranoid, avoidant and schizoid personality disorders, while D9 (very broad spectrum) includes family members with any psychiatric disorder. All patients with history of any psychotic episode were assessed by K.S.K. for lifetime psychotic symptoms using the Operational Criteria Checklist of Psychotic Illness (OPCRIT; 23), to reflect both symptom severity and chronicity.

The Stanley Foundation postmortem brain series (SBS) donated by the Stanley Medical Research Institute comprises DNA and total RNA extracted from dorsolateral prefrontal cortex (Brodmann's area 46) of 35 individuals each with schizophrenia, bipolar disorder and controls (24).

SNPs and genotyping

We assessed 8 *AKT1* SNPs, 4 common to prior positive studies (rs1130214, rs2494732, rs2498799, rs3730358) and 4 additional SNPs (<http://www.hapmap.org/>) to increase marker density and include the 3'UTR (rs2494746, rs3803304, rs2498802, rs2498804). SNP

rs3803300 (6), failed to perform well by template-directed dye-terminator incorporation with fluorescence polarization detection (FP-TDI).

SNP genotyping was performed by FP-TDI using AcycloPrime SNP detection kits (PerkinElmer Life Sciences, Boston, MA), manufacturer's instructions, and an automated allele scoring platform (25). PCR and single-base extension primers were designed manually and are available on request. Average genotyping completion rate was 93%. Genotypes were examined for incompatibilities within families by PEDCHECK v1.1 (26), unlikely recombinations by MERLIN (27), and were corrected as necessary. Where errors could not be unambiguously resolved, respective marker genotypes were deleted for the whole family.

Statistical methods

Genotype deviation from Hardy-Weinberg equilibrium (HWE) and pairwise intermarker linkage disequilibrium (LD) were assessed using HAPLOVIEW v3.2 from genotypes of unrelated founders (28). We performed association analysis by transmission disequilibrium test (TDT) using TRANSMIT v2.5.4 (29) and PDTPHASE v4.0, an implementation of the pedigree disequilibrium test (PDT) with extensions to deal with uncertain haplotypes and missing data (30,31). *P* values were empirically derived from 10,000 bootstrap replicates in each TRANSMIT analysis to allow inclusion of multiple affected offspring per family, and multiple families per pedigree. PDTPHASE creates a measure of marker-disease associations for each pedigree, averaged from that defined for each constituent triad and discordant sibpair, thus has greater power over methods using only a subset of the data. PDTPHASE was also the analytic tool in the original *AKT1* association with schizophrenia (6). Throughout, we analyzed only haplotypes of frequency >2% to avoid the application of statistics to small numbers of chromosomes and multiple rare alleles/haplotypes.

Association analysis was performed in stages across diagnostic categories: 1) Single SNPs 2) A replication study, specifically examining reported *AKT1* schizophrenia risk haplotypes 3) Investigation into etiological heterogeneity, by testing the original risk haplotype (6) for association with OPCRIT symptom dimensions 4) False Discovery Rate control (32) following this directed testing phase, to minimize risk of Type I errors 5) 'Sliding windows' analysis of 2-4 marker haplotypes and 6) In the absence of 'tag SNPs', exploratory analysis of all 2-4 SNP haplotypes for association with disease. The large number of possible haplotypes precludes presenting all these exploratory data systematically. Therefore, the latter two stages remain uncorrected for multiple testing and are presented with that caveat. The statistical significance of these exploratory findings, though based on empirical *p* values obtained by simulation, should be viewed accordingly.

Quantitative real-time PCR

Expression of *AKT1*, *AKT2*, and *AKT3* was analyzed by quantitative real-time PCR using SYBR Green I on a Bio-Rad (Hercules, CA) iCycler platform, normalized against two reference genes *GAPDH* and *TBP* by the $2^{-\Delta\Delta C_T}$ algorithm (33;34), implemented in the Relative Gene Expression Macro Software Package (Bio-Rad Inc). For details of the real-time PCR experiments, statistical analysis of gene expression levels, and *AKT1* and *DTNBPI* haplotype ascertainment see Supplementary Methods.

Bioinformatics

50bp of DNA sequence encompassing rs1130214 was analyzed for transcription-factor binding sites using MatInspector (<http://www.genomatrix.de>; 35) and putative splice sites using the Berkeley *Drosophila* Genome Project predictor (http://www.fruitfly.org/seq_tools/splice.html). VISTA was used to define conserved regions

in *AKT1* genomic sequence surrounding rs1130214 (36). Other features were ascertained from the University of California Santa Cruz genome browser (<http://genome.ucsc.edu/>).

Results

Table 1 lists the *AKT1* SNP locations and allele frequencies in the ISHDSF, which are similar to those of Emamian *et al* (6). *AKT1* has 14–15 known exons (depending on splice variant) spanning ~24.2kb (Fig.1). Genotype frequencies of all SNPs were in HWE (data not shown).

Linkage disequilibrium (LD) map

Pairwise intermarker LD in the ISHDSF revealed 2 haplotype ‘blocks’ across *AKT1*, with strong LD between 5’ SNPs rs1130214-rs2494746, then further 3’ between rs3730358-rs2498804 (Fig.2). We see a similarly complex LD structure to that reported in cases by Ikeda *et al* (13), with low LD between rs3803304-rs2498799 (SNPs 4-6 in Fig.1) in the midst of otherwise high intermarker LD. This may contribute to the haplotypic diversity of *AKT1* and underlie the lack of tag SNPs for either block (Fig.2).

Single marker association analysis

AKT1 single markers showed no association with schizophrenia, although rs1130214 and rs2494732 show a modest trend ($0.10 < p < 0.20$, two-tailed) towards significance with TRANSMIT and PDTPHASE across several diagnostic categories (Supplemental Table 1). Interestingly, rs2494732 was the most significant SNP of Ikeda *et al* (13; $p=0.0037$), whereas Schwab *et al* (12) found rs1130214 to be associated with disease ($p=0.011$).

Haplotype replication

Several *AKT1* haplotypes have been associated with schizophrenia: in Caucasians (6,12), haplotype [rs1130214-rs3730358]-rs2498799 (T-C-G, SNPs [1-3]-4 in our study; Fig.1) and in a Japanese sample (13), association is more pronounced toward 3’ *AKT1* (Fig.1). Given the ethnicity of our sample, we focused on the Caucasian over-transmitted risk haplotype T-C-G (rs1130214-rs3730358-rs2498799; 6). We see a contrasting trend towards *under*-transmission in the ISHDSF, with less striking over-transmission of the all-common-allele haplotype G-C-G (Table 2 and Table 3). This effect is most evident with TRANSMIT for haplotype rs1130214-rs2498799 (SNPs 1-4; global $p=0.002$ at D5; Table 2) and with PDTPHASE, for haplotype rs1130214-rs3730358 (SNPs 1-3, the ‘core’ haplotype of Emamian *et al* (6); global $p=0.031$ at D9; Table 3). Our results also contrast those of Schwab *et al* (12) who consistently saw *over*-transmission of the T allele of rs1130214 (SNP 1), in 2 versions of the ‘risk’ haplotype (T-C-G and T-T-A; Fig.1). We see the T-T-A haplotype at similar frequency in the ISHDSF (~9%), trending towards over-transmission to affected individuals (data not shown). The overall picture in the ISHDSF (over-transmission of SNPs 1-3-4 haplotype G-C-G, under-transmission of T-C-G) becomes stronger with broader diagnosis, D5-D9 (especially D5; Table 2 and Table 3).

AKT1 ‘risk haplotype’ analysis with OPCRIT symptom scales

To clarify the putative association between *AKT1* and schizophrenia in the ISHDSF, we examined transmission of the Caucasian risk haplotype (6) within OPCRIT symptom dimensions. From factor analysis of OPCRIT item lifetime ratings for ISHDSF subjects, five factors were extracted and used to derive scales; negative symptoms, delusions, hallucinations, mania and depression (37). For each scale, patients were divided into high and low-scoring categories to perform a family-based association test appropriate for our sample and design (38). Multiple thresholds (upper 20%, 30%, 40%) were used, as threshold delineation is somewhat arbitrary, above which patients were coded affected, and below, unaffected.

We tested, for each OPCRIT factor at each threshold, for TD to affected offspring of the *AKT1* haplotype rs1130214-rs2498799 (SNPs 1-4; T-G) using TRANSMIT (29). This haplotype tagged the Emamian *et al* risk haplotype T-C-G (SNPs 1-3-4) in the ISHDSF and showed significant evidence for association (albeit *under*-transmission) in the whole sample (Table 2). As for the schizophrenia diagnostic categories, the T-G haplotype was significantly *under*-transmitted for all symptom dimensions at the 40% threshold (Table 4). Associations are less significant at more stringent thresholds, perhaps as power decreases with less “affected” individuals. However, at the 30% threshold, significant associations remain for hallucinations ($p<0.003$) and depression ($p<0.023$) (Table 4).

Control of false discoveries

The analysis of single markers, haplotype replication and OPCRIT analysis, generated in total 150 significance tests. To control for risk of false discoveries we used the adaptive (39) and more conservative step-up (32) FDR control methods. The FDR is robust to effects of correlated tests, hence especially applicable to this study (32,40,41). An FDR=0.1 provides a reasonable balance between control of false positives and power to detect true association (40). For FDR<0.1 the necessary sample size required to detect true effects increases dramatically. Using the adaptive method, we estimated the proportion of true null hypotheses to be as low as $p_0=0.6$. Allowing an FDR=0.1 gave a p value threshold =0.017, whereby 17 of 150 tests were significant. The step-up method, with p_0 assumed =1, gave a p value threshold =0.004, whereby 7 tests were significant. All are with the rs1130214-rs2498799 (T-G) haplotype, D5 and D9 diagnostic categories as before (Table 3), and with negative, hallucinations, mania and depression OPCRIT symptom factors (Table 4).

Exploratory analysis – ‘sliding window’ haplotypes

This analysis revealed a trend towards association at the 5’ of *AKT1*, with increasing significance moving from 2 to 4 SNP windows (Table 5 and Table 6). With PDTPHASE, strongest associations were with broadest diagnostic category D9 (haplotypic $p=0.008$, global $p=0.017$), then D5, with no association at D2, core schizophrenia (Table 5). Similar to the replication study, a pattern emerged of over-transmission of all-common-allele haplotypes, and under-transmission of these haplotypes with the rs1130214 rare allele (T) (SNP 1; Table 5). For TRANSMIT, this pattern held but with strongest associations at D5 (haplotypic $p=0.025$, global $p=0.030$; Table 6). No consistent evidence for association was observed for 3’ marker haplotypes (data not shown).

Exploratory analysis – all 2-4 SNP haplotypes

The pattern of over-transmission of all-common-allele SNP haplotypes including SNP 1 (rs1130214;G) and under-transmission with the rare T allele was observed, D5 and D9 maintaining strongest associations (Supplementary Table S2). Interestingly, haplotype 1-5 (rs1130214-rs2494732) whose 2 SNPs singly showed trends toward significance (Supplementary Table S1), is most significantly associated with TRANSMIT/PDTPHASE at broadest diagnostic category D9 ($0.0002<global\ p<0.045$; Supplementary Table S2). We saw no association with ‘risk’ haplotypes of Ikeda *et al* (13), or the tested portion of the Bajestan *et al* (14) risk haplotype.

AKT gene expression in the Stanley brain series

We investigated whether the reduced AKT1 protein in schizophrenic brain (6) was reflected in lower levels of *AKT1* mRNA, and analyzed transcriptional levels of *AKT2* and *AKT3*, no different between cases and controls at the protein level, yet which have functional redundancy with AKT1 as well as specific roles (6,8). Decreased *AKT1* expression was evident in dorsolateral prefrontal cortex of both schizophrenic and bipolar patients compared to controls,

significantly for schizophrenics, and of moderate effect size (Fig.3A; $p=0.029$, Cohen's $d=0.44$). A non-significant reduction in *AKT2* expression was observed in schizophrenic and bipolar brain compared to controls (Fig.3B), whilst no difference between diagnostic groups in *AKT3* expression level was detected (Fig.3C). Analysis of potential confounder variables, *AKT1* and *DTNBP1* haplotypic effects are described in the Supplementary Results.

In silico functional analysis of rs1130214

Putative function for this SNP was investigated owing to its complex role in haplotype association both here and in prior studies (6,12). VISTA analysis revealed that it lies upstream of the *AKT1* 5'UTR in a region conserved in mammals and rodents and of high regulatory potential (Supplementary Fig S1;42). Although within an alternative 5' exon (Supplementary Fig S1), it is not predicted to affect splicing (data not shown). However, rs1130214 lies within a cluster of functional STAT3 binding sites and the T allele abolishes a putative E2F transcription factor binding site (Supplementary Fig. S1), so it potentially influences regulation of *AKT1* expression (8).

Discussion

As is increasingly observed in complex disease association studies, reports on *AKT1* and schizophrenia reveal inconsistent replication of a particular allele or allelic combination, yet replication at a broader level, i.e. the SNP or haplotype and ultimately the gene itself (43,44). Potential reasons for such diverse findings are sample size, heterogenous phenotypes, marker sets, allele frequencies or LD structure, genetic admixture, multiple testing issues, and indeed true heterogeneity in variants that influence disease.

In this study we sought to replicate association of *AKT1* with schizophrenia in the ISHDSF, prompted by the susceptibility haplotype in this sample within the dysbindin (*DTNBP1*) gene (21). Although no single *AKT1* marker showed association with disease, the data were consistent with global association of common alleles for selected SNP haplotypes including rs1130214, and a striking *under*-transmission of these haplotypes with the rare allele (T) at rs1130214. This contrasts with Emamian *et al* (6) and Schwab *et al* (12) who found this allele in over-transmitted haplotypes, but is similar to Ikeda *et al* (13) in the most significant association being a 'protective' (albeit different) haplotype. Our study is not unique – an increasing number of publications are replicating a previously reported disease-marker association with the risk allele(s) reversed. One explanation may be the 'Flip-Flop Phenomenon' of Lin *et al* (45), whereby such associations may indeed be confirmations, with the 'flip-flop' due to interactive effects, haplotypic heterogeneity or linkage disequilibrium with a causal variant at another locus.

There is still debate over the 'level of replication' required to demonstrate true replication of association. Sullivan (46) has explored the capacity of association studies to produce false positive findings and impact of various definitions of replication. He defines 4 levels of replication, A-D, being highly precise (same SNP, statistical test/phenotype, direction of association) and D very loose (any SNP, test, direction). Our study corresponds to definition B (same SNPs, test, different direction), predicted to give 0–10% false replications. We therefore consider it unlikely that our results represent false positive findings.

We note in our study the slight difference in results between TRANSMIT and PDTPHASE (e.g. Table 2 and Table 3). This is due to differences in how several key factors are treated in their algorithms (family structure, frequency of missing parental genotypes, number of informative families and assumed genetic model) creating differences in power. Nicodemus *et al* (47) have evaluated various algorithms commonly used to implement TD-based tests, employing the joint null hypothesis of no linkage and no association, hence their results are

applicable to our study. Although overall, PDTPHASE may be considered most rigorous for extended pedigrees, we included TRANSMIT as it was required for the FDR approach to correct for multiple testing, recovers missing parental data efficiently from unaffected sibling data, and with no linkage to 14q32, whereby using multiple families within pedigrees was permitted, TRANSMIT can use the maximal amount of data present in the sample. Both algorithms have strengths for use with the ISHDSF, both broadly concur, thus results of both are presented accordingly.

Many *AKT1* haplotypes including rs1130214 appeared to be associated with schizophrenia in the ISHDSF to varying degrees. However, results from our exploratory haplotype analysis may have emerged through multiple testing and reflect false positives. It is unlikely that rs1130214 is an appreciable risk variant itself as single marker association does not reach significance. That it has functional relevance is suggested by bioinformatic analysis, which indicates a transcriptional regulatory role. Our data is consistent overall with prior Caucasian studies in highlighting *AKT1* 5' in schizophrenia susceptibility.

The C allele of rs3730358 (in the T-C-G haplotype) was associated with schizophrenia (6, 12) and in the core risk haplotype, rs1130214-rs3730358 (T-C), with decreased AKT1 protein levels in lymphocyte cell lines (6). However, lymphocytes from normal Caucasians homozygous for the C-G portion of the haplotype T-C-G have higher levels of AKT1 protein, and lowered response to apoptotic stimuli, than those harbouring the T-A haplotype (48). As we found no clear relationship between haplotype and *AKT1* transcription in the SBS, further studies are required to elucidate any differential effect in schizophrenic patients (associated with *AKT1* or not) versus controls. We did observe reduced expression of total *AKT1* RNA from schizophrenic and bipolar brain compared to controls, statistically significant for the schizophrenic cases ($p=0.029$), and consistent with the reduced AKT1 protein in schizophrenic brain (6). We found no significant differences between cases and controls in total RNA expression of *AKT2* and *AKT3*, reflecting again the protein level (6), and pinpointing *AKT1* especially for a role in schizophrenia.

Familial factors are thought not only to influence liability to psychiatric illness but also specific clinical features (49). Along with shared environment, a major phenotype modifier is genetic, specific susceptibility alleles/haplotypes conferring liability to particular clinical subtypes (37,50). The significant under-transmission in our study of the Emamian *et al* risk haplotype across all OPCRIT symptom dimensions may indicate an effect on risk for psychosis unspecific to symptom factors. This may be consistent with the strongest associations being at broader diagnostic categories, which include individuals with mood disorders. Using this approach at other schizophrenia risk loci, associations were identified with 1–2 symptom factors (37,51). Prior *AKT1*/schizophrenia associations in Caucasian samples (6,12) are with core schizophrenia or schizoaffective disorder (our D2 category). However, our results suggest the *AKT1* effect to be general in nature, and underscore the possibility that *AKT1* plays a role in multiple psychiatric syndromes. Although clear differences exist between schizophrenia and bipolar disorder (52), neuropharmacological studies implicate dopamine dysregulation in both, and molecular studies suggest the conditions may share predisposing genes (53).

In summary, our data is consistent with global association for *AKT1* haplotypes including SNP rs1130214 with schizophrenia in the ISHDSF. We note an equally striking *under-transmission* of haplotypes comprising the same markers but with the rare allele at rs1130214, in contrast to prior reports. We highlight the 5'UTR of *AKT1* in disease association, and emphasise the role of genes, not specific alleles or haplotypes, as 'units or replication' in association studies (43). We conclude that whilst *AKT1* may play a minor role in susceptibility to schizophrenia in the ISHDSF, it appears to impact risk for a class of psychiatric syndrome with symptoms of schizophrenia and mood dysregulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Special thanks go to all the patients and family members who participated in this study. We are grateful also to Dr Frank Dudbridge for helpful suggestions implementing PDTPHASE for our sample, and to some very useful comments from the reviewers. This work was supported by NIH Grant No. MH041953.

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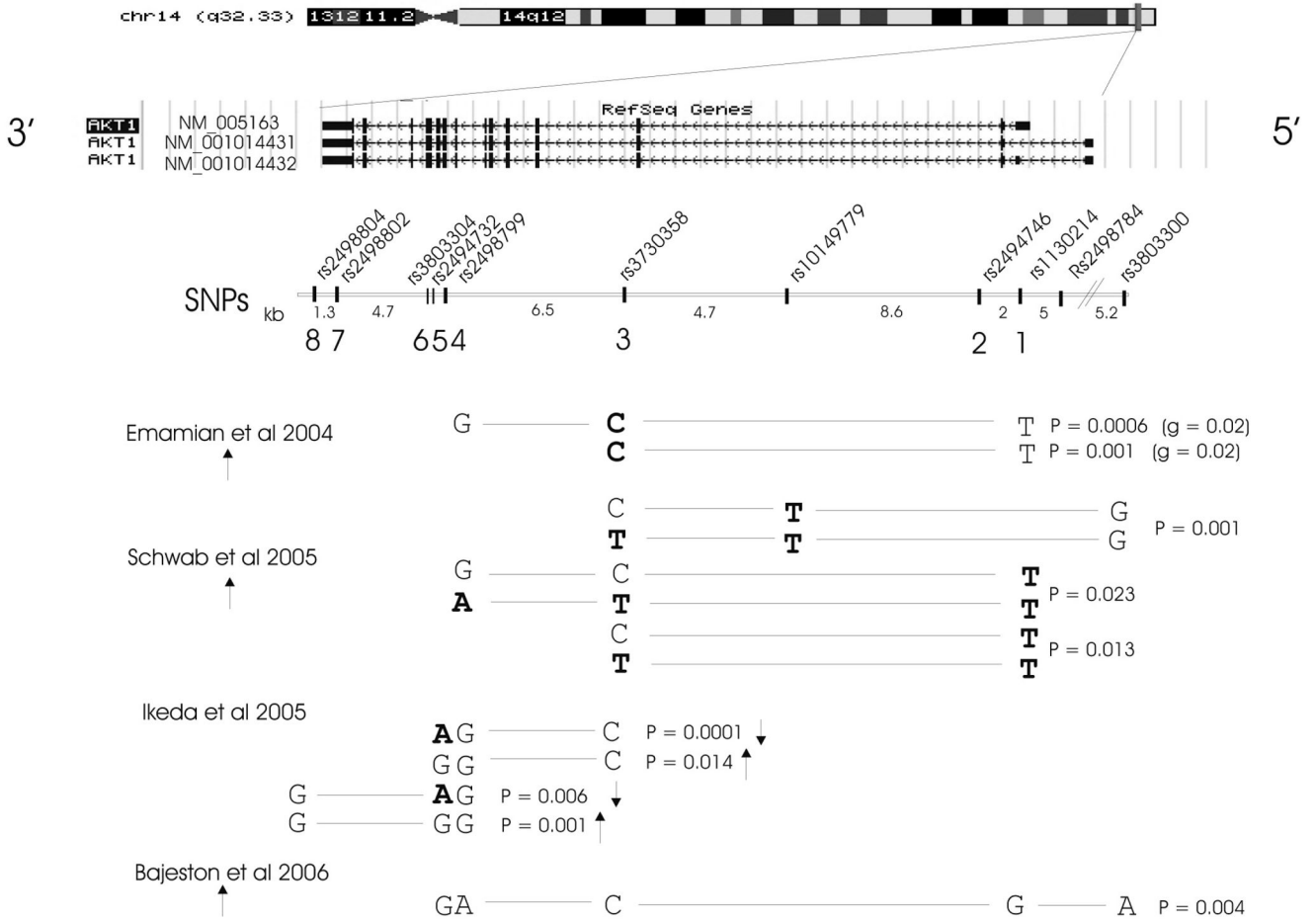


Fig. 1. Most significant *AKT1* SNP haplotypes associated with schizophrenia to date. Exon locations of the 3 main alternative *AKT1* transcripts are shown at the top (<http://genome.ucsc.edu/>, human genome build 35). SNPs are positioned underneath with intervening distances in kilobases. Numbers 1–8 refer to SNPs typed in our study. The *p* values of the most significant haplotypes are given for each report of association between *AKT1* and schizophrenia. The arrows depict over-representation (up arrow) or under-representation (down arrow) in cases. SNP alleles highlighted in bold are significantly associated with schizophrenia as single SNPs. Marker order and haplotypes are given in the reverse order here to that in the text, in accordance with the orientation of the gene on the UCSC genome browser.

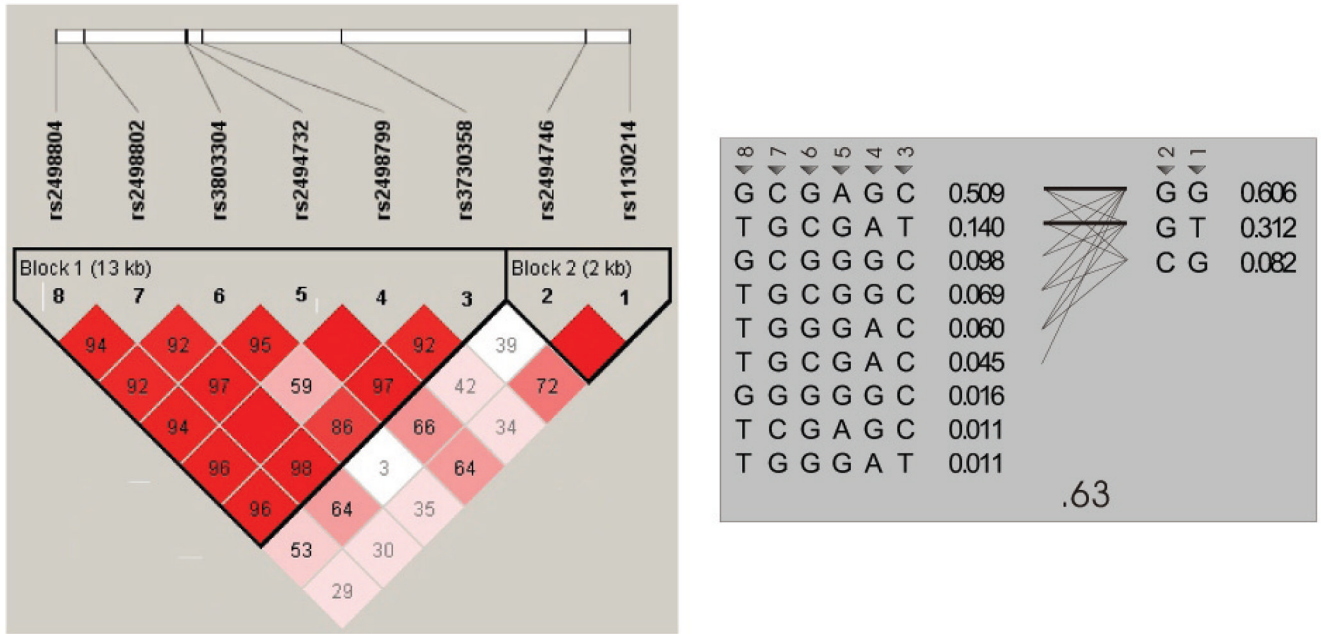
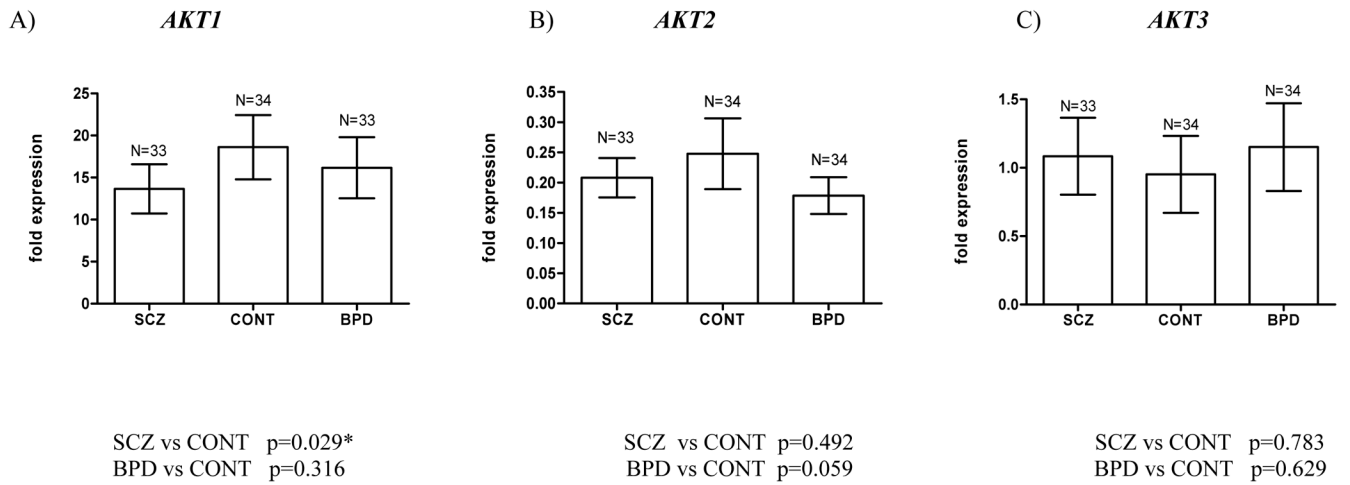


Fig. 2. LD map of the *AKT1* region in the ISHDSF. The map is viewed through Haploview v 3.2 (the solid spine rule for block structure is shown), with gene direction 3'–5'. Pairwise LD is indicated by the numbers (related to D') and depth of red colour. SNPs and chromosomal positions are indicated along the top. The region spans ~26kb. Despite apparent 'blocks', the haplotypes on the right show no tag SNPs.

**Fig. 3.**

AKT gene expression relative to reference genes *GAPDH* and *TBP* analyzed by real-time qPCR of the Stanley Consortium brain series. Expression differences between diagnostic groups were evaluated by one-tailed Mann-Whitney U test for *AKT1* (SCZ vs CONT) and two-tailed tests for all other comparisons.

Table 1
Position and allele frequencies of *AKT1* SNPs used in this study

SNP number	SNP name	Position (bp)*	<i>AKT1</i> gene location#	Distance (bp)	Major allele	Minor allele	Minor allele frequency in ISHDSF
1	rs1130214	104,330,779	5' UTR	2015	G	T	0.31
2	rs2494746	104,328,764	Intron 2	11312	G	C	0.08
3	rs3730358	104,317,452	Intron 3	6513	C	T	0.17
4 ⁺	rs2498799	104,310,939	Exon 9	702	G	A	0.25
5	rs2494732	104,310,237	Intron 11	46	A	G	0.45
6	rs3803304	104,310,191	Intron 11	4704	G	C	0.28
7	rs2498802	104,305,487	3'UTR	1347	C	G	0.34
8	rs2498804	104,304,140	3'UTR		G	T	0.34

* University of California at Santa Cruz (UCSC) Human Genome Browser Gateway assembly May 2004

Based on *AKT1* gene transcript variant 1 (NM_005163) which has 14 exons

+ rs2498799 is a synonymous coding variant

Table 2 Caucasian *AKT1* haplotype replication by TRANSMIT. Significant *p* values and haplotypes are highlighted in bold.

DIAG	HAPLO	* Alleles	No.fams#	<i>p</i> val	Obs	Exp	Freq	Global P
D2	1-3	↑ G-C	326	0.079	693	678	0.64	0.039
		↓ T-C		0.056	183	196	0.20	
		↑ G-C	363	0.036	842	822		
		↓ T-C		0.078	223	236		
D5	1-3	↑ G-C	376	0.125	899	884		0.208
		↓ T-C		0.178	247	258		
		↑ G-C	405	0.057	1111	1089		
		↓ T-C		0.056	322	339		
D2	1-4	↑ G-G	321	0.106	604	589	0.56	0.009
		↓ T-G		0.010	171	190	0.19	
		↑ G-G	359	0.045	731	712		
		↓ T-G		0.003	205	228		
D5	1-4	↑ G-G	372	0.068	783	765		0.009
		↓ T-G		0.009	228	248		
		↑ G-G	403	0.071	964	945		
		↓ T-G		0.003	299	324		
D2	1-3-4	↑ G-C-G	326	0.130	607	593	0.55	0.095
		↓ T-C-G		0.024	172	187	0.09	
		↑ G-C-G	363	0.087	731	714		
		↓ T-C-G		0.023	207	225		
D5	1-3-4	↑ G-C-G	376	0.133	781	765		0.121
		↓ T-C-G		0.054	230	245		
		↑ G-C-G	405	0.085	957	937		
		↓ T-C-G		0.017	301	320		

* up and down arrows indicate over- or under-transmission to affected individuals

number of families with transmissions to affected offspring

Table 3 Caucasian *AKT1* haplotype replication by PDPHASE. Significant *p* values and haplotypes are highlighted in bold.

DIAG	HAPLO	* Alleles	<i>p</i> val	Trio T	Trio NT	Aff Sib	Unaff Sib	Freq	Global <i>P</i>
D2	1-3	↑ G-C	0.257	700	675	925	909	0.65	0.199
		↓ T-C	0.114	181	204	302	301	0.19	
D5	1-3	↑ G-C	0.045	850	817	1001	959		0.051
		↓ T-C	0.070	219	246	305	315		
D8	1-3	↑ G-C	0.062	907	879	973	949		0.087
		↓ T-C	0.102	244	265	310	311		
D9	1-3	↑ G-C	0.047	1123	1091	801	770		0.031
		↓ T-C	0.014	319	343	245	265		
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D2	1-4	↑ G-G	0.380	614	590	836	814	0.57	0.429
		↓ T-G	0.139	162	180	273	275	0.16	
D5	1-4	↑ G-G	0.121	744	709	915	874		0.190
		↓ T-G	0.049	194	219	280	299		
D8	1-4	↑ G-G	0.133	797	762	894	863		0.263
		↓ T-G	0.086	217	237	287	295		
D9	1-4	↑ G-G	0.158	985	947	736	695		0.086
		↓ T-G	0.010	281	308	227	247		
<hr/>									
D2	1-3-4	↑ G-C-G	0.274	620	593	850	829	0.56	0.337
		↓ T-C-G	0.097	164	185	279	283	0.17	
D5	1-3-4	↑ G-C-G	0.080	747	712	917	882		0.149
		↓ T-C-G	0.050	198	224	285	303		
D8	1-3-4	↑ G-C-G	0.090	798	764	896	872		0.250
		↓ T-C-G	0.083	220	242	292	299		
D9	1-3-4	↑ G-C-G	0.092	982	947	737	700		0.087
		↓ T-C-G	0.010	289	314	230	255		

* up and down arrows indicate over- or under-transmission to affected individuals

Within-family vertical transmission of *AKT1* haplotypes rs1130214-rs2498799 (SNPs 1-4, alleles T-G) for the 5 dimensions of the OPCRIT scale. All individuals affected with psychosis and with available OPCRIT data (n=749) were analyzed. Number of families with transmissions to affected offspring is shown in parentheses. *p* values (χ^2 , 1df) are highlighted using * <0.05 , ** <0.01 .

Table 4

Threshold	Negative Symptoms	Delusions	Hallucinations	Mania	Depression
20%	0.641 (140)	0.15 (115)	0.003** (184) ^d	0.089 (108)	0.12 (101)
30%	0.226 (179)	0.081 (169)	0.003** (184) ^d	0.345 (168)	0.023* (140)
40%	0.003** (225)	0.024* (201)	0.008** (255)	0.002** (221)	0.004** (186)

N.B. "a" denotes that the two thresholds resulted in the same cutoff score on the Hallucinations scale, which is comprised of only seven items. The data are identical at both levels, and the two separate entries represent a single test.

Table 5
Significant *sliding windows' AKT1 SNP haplotypes (in bold) by PDTPHASE.

DIAG	HAPLO	Alleles	p val	Trio T	Trio NT	Aff Sib	Unaff Sib	Freq	Global P
D2	1-2	G-G	0.217	650	626	863	849	0.60	0.188
		T-G	0.240	76	85	90	111	0.31	
D5	1-2	G-G	0.098	788	759	930	900		0.264
		T-G	0.125	371	393	473	490		
D8	1-2	G-G	0.116	841	816	905	897		0.304
		T-G	0.400	405	423	483	484		
D9	1-2	G-G	0.028	1043	1014	752	716		0.078
		T-G	0.072	517	540	388	406		
D2	1-2-3	G-G-C	0.133	638	607	857	833	0.58	0.107
		T-G-C	0.140	179	201	302	301	0.18	
D5	1-2-3	G-G-C	0.037	769	735	913	870		0.078
		T-G-C	0.049	215	243	301	314		
D8	1-2-3	G-G-C	0.054	818	789	888	866		0.133
		T-G-C	0.075	239	262	306	310		
D9	1-2-3	G-G-C	0.022	1010	977	734	693		0.017
		T-G-C	0.009	314	339	241	265		
D2	1-2-3-4	G-G-C-G	0.154	580	550	806	771	0.52	0.176
		T-G-C-G	0.136	163	183	282	283	0.16	
D5	1-2-3-4	G-G-C-G	0.045	697	664	857	812		0.078
		T-G-C-G	0.043	197	223	283	301		
D8	1-2-3-4	G-G-C-G	0.057	742	712	841	808		0.216
		T-G-C-G	0.075	219	240	290	298		
D9	1-2-3-4	G-G-C-G	0.035	912	881	691	641		0.046
		T-G-C-G	0.008	287	312	228	253		

* up and down arrows indicate over- or under-transmission to affected individuals.

Table 6
Significant 'sliding windows' AKT1 SNP haplotypes (in bold) by TRANSMIT.

DIAG	HAPLO	Alleles	*	p val	Obs	Exp	Freq	Global P
D2	1-2	G-G	↑	0.083	648	632	0.59	0.078
			↓	0.494	313	324	0.32	
D5	1-2	G-G	↑	0.055	783	765	0.32	0.079
			↓	0.116	374	388		
D8	1-2	G-G	↑	0.165	837	823	0.32	0.191
			↓	0.239	407	418		
D9	1-2	G-G	↑	0.047	1039	1018	0.32	0.128
			↓	0.178	518	532		
D2	1-2-3	G-G-C	↑	0.037	628	610	0.56	0.037
			↓	0.059	181	193		
D5	1-2-3	G-G-C	↑	0.025	756	734	0.19	0.030
			↓	0.059	219	234		
D8	1-2-3	G-G-C	↑	0.114	806	789	0.162	0.162
			↓	0.145	243	255		
D9	1-2-3	G-G-C	↑	0.028	993	969	0.142	0.142
			↓	0.079	317	332		
D2	1-2-3-4	G-G-C-G	↑	0.097	566	551	0.51	0.111
			↓	0.046	170	183		
D5	1-2-3-4	G-G-C-G	↑	0.105	675	659	0.18	0.067
			↓	0.031	206	222		
D8	1-2-3-4	G-G-C-G	↑	0.194	719	706	0.278	0.278
			↓	0.066	228	242		
D9	1-2-3-4	G-G-C-G	↑	0.138	878	862	0.296	0.296
			↓	0.045	297	314		

* up and down arrows indicate over- or under-transmission to affected individuals.