(The International HapMap Consortium, 2005). We re-genotyped 46 of these samples along with the case control sample to provide a measure of genotyping accuracy. All genotypes were called blind to sample identity and affected status.

2.4. Statistical analysis

Tests of genotypic and allelic association were performed using contingency tables. Haplotype analyses were performed using the EM algorithm and a permutation test as implemented in program EH plus (Zhao et al., 2000) for global significance. Association of specific haplotypes was estimated with Cocaphase (Dudbridge, 2003). LD values were calculated using the ldmax program within the GOLD software (Abecasis and Cookson, 2000).

3. Results

Genotype data for SNP2, rs2494738, rs3803304 and SNPA from our assays in the same 90 CEPH DNA samples used in the International HapMap Project were 100% concordant with HapMap data. 100% concordance was also achieved between genotype data of 46 CEPH DNA samples typed in our initial assay optimisation stage and the same samples contained within our case control sample set for all 10 SNPs.

Genotype data were in Hardy Weinberg equilibrium for both cases and controls for all SNPs. No significant differences between cases and controls were observed for any single markers by allele (Table 1) or genotype (data not shown).

We specifically tested those marker combinations reported to yield the most significantly associated haplotypes by Emamian et al. (2004) including the core haplotype, (SNP2/SNP3, TC), those of Schwab et al. (2005), (SNP1/SNP2a/SNP3, GTC) and Ikeda et al. (2004), (SNP3/SNP4/SNP5, CGG and CGA as well as seven other overlapping haplotypes also significantly associated in the Japanese sample). Table 2 summarizes the most significant haplotypes with the ancestral alleles marked as *. Marker combination SNP2/SNP3/SNP4 which gave the most significant results in the Emamian study, gave a global p-value of 0.08 in our sample. However, the TCG haplotype for this marker combination which gave nominal significance in the initial Emamian study ($p=0.0006$) and also in that of Schwab et al. (2005), ($p=0.02$) was not significantly associated in our sample, $p=0.37$, although a non significant trend was observed in the previously reported direction (Table 2). Our case and control frequencies for this haplotype were 0.19 and 0.17 respectively, compared to 0.17 and 0.10 in the German sample (Schwab et al., 2005) and 0.15 in the US sample of European origin of Emamian et al. (2004).

Global haplotype analysis of markers (SNP2/SNP3) forming the core two-marker haplotype of Emamian et al. (2004) revealed no significant evidence for association (global $p=0.09$) nor did specific analysis of the TC phased core haplotype ($p=0.41$). However, a specific phased 4 marker haplotype (SNP2/SNP3/SNP4/SNP5, TCGG, Table 2) which was also significant in the Emamian study, $p=0.004$, was associated in our sample, $p=0.04$ (case freq=0.13, control freq=0.10). The same haplotype was not significantly associated in either the German (Schwab et al., 2005) or in the Japanese (Ikeda et al., 2004) samples, ($p=0.11$ and 0.18 respectively), (personal communications).

The most significant haplotype of (SNP1/SNP2a/SNP3 GTC, Table 2) reported by Schwab et al. (2005) was not associated in our sample, ($p=0.51$), although the trend with frequencies of 0.18 and 0.16 in our cases and controls respectively, compared to 0.17 and 0.09 in the German sample was in the same direction.

Global haplotype analysis of SNP3/SNP4/SNP5 which was the most significant haplotype in the Japanese study of Ikeda et al. (2004) was significantly associated (uncorrected $p=0.04$) in our sample. The largest difference in haplotype frequency for this combination was 5% for the haplotype CGG (Table 2, $p=0.016$). The same haplotype was also significantly associated in the same direction in the Japanese sample (Ikeda et al., 2004), $p=0.014$, although frequencies in the samples are different (CGG=0.27 in Japanese controls vs. 0.17 in Cardiff controls). The most significant haplotype associated in the Japanese sample was SNP3/SNP4/SNP5 CGA, ($p=0.0001$), (Ikeda et al., 2004). This was not significantly associated in our sample. The haplotype frequencies in our sample and in that of Ikeda et al. (2004) are substantially different (Table 2). Ikeda et al. (2004) also reported 7 overlapping haplotypes with individual p-values less than 0.05. None of these was significantly associated in our sample.

Although we selected the markers predicated on single locus (i.e. pairwise tagging), in order to try to capture the effect of unknown variants that are not represented in HapMap, we undertook 2 and 3 marker haplotype analysis across all the markers including those additional SNPs recommended by our tagging procedure. We obtained evidence for association for haplotypes of SNP1/SNP3/SNP4, global $p=0.04$, which overlaps physically with the most significant haplotypes reported by Schwab et al. (2005) and Emamian et al. (2004). On closer inspection, the effect came from two haplotypes with frequencies of

less than 5%, (GTG case=0.009, control=0.018, $p=0.06$ and ACG, case=0.043, control=0.023, $p=0.006$). Allele C of SNP3 is common to our significant haplotype (SNP1/SNP3/SNP4, ACG) and the most significant haplotypes of Emamian et al. (2004) and Schwab et al. (2005), whilst allele G of SNP4 is common to our SNP1/SNP3/SNP4, ACG haplotype and the SNP2/SNP3/SNP4, TCG haplotype of Emamian et al. (2004) and Schwab et al. (2005).

4. Discussion

Following the initial report (Emamian et al., 2004) and mixed replication evidence (Ohtsuki et al., 2004; Ikeda et al., 2004; Ide et al., 2006; Schwab et al., 2005; Liu et al., 2006; Bajestan et al., 2006) we sought to provide further evidence for association between schizophrenia and polymorphisms in *AKT1*. The question of when the evidence for association between disease and gene is convincing is a vexing one for several reasons. Ideally, such evidence would come from repeated demonstration of a directional association (even if not significant) between a disorder and a specified allele such that pooled or meta-analyses demonstrates a clear highly significant directional effect. However, when based upon indirect association, replication of specified alleles may not be easily obtained due to a mixture of population differences in allelic heterogeneity at the locus, patterns of LD, allele frequencies, phenotypic variation relevant to the associated allele, or exposure to environmental variables with which a risk allele interacts (O'Donovan and Owen, 1999). Moreover, mathematical analyses show that where the true effect size of a susceptibility allele is weak, opposite alleles may be genuinely associated with disease even in populations with similar LD measures, allele frequencies, and identical effect sizes at the functional locus (Moskvina and O'Donovan, in press). Given the above, while association to the same allele across studies should at least be sought, it cannot be a prerequisite for considering a study as supportive of association between disease and a gene. Instead, we believe it is legitimate to view association to any allele or haplotype that both survives honest appropriate correction for multiple testing and is based on a well-designed quality-controlled study as significant evidence for replication at the gene-level. When multiple studies meet this criterion, as we consider to be the case for example for dysbindin (Williams et al., 2005b), then in our view, the evidence can be considered convincing.

Our single marker data for AKT1 provide no evidence for association with schizophrenia, but haplotype analysis showed some trends similar to the existing data, albeit, none that remain significantly associated in the context of multiple testing. When associated haplotypes from all of these studies are aligned (Table 2), it is apparent that the most significant risk haplotypes across studies overlap, making the correction for multiple testing over conservative. (Of the p-values reported in Table 2, the most significant haplotype of Schwab et al. (2005) is already corrected for multiple testing by simulation (Becker and Knapp, 2004). The most significant haplotype in the Emamian study ($p=0.006$) is uncorrected, but remains significant after adjustment for the comparisons made in the study, ($p<0.04$).

In Table 2 where we show the most significant haplotype reported from each study and compare these specific haplotypes across the published data and with our own data, the SNP3/4, alleles CG combination occurs in 6/7 of the significant haplotypes. This trend also extends to the Japanese sample (Ikeda et al., 2004), but not in the Iranian sample (Bajestan et al., 2006) where the only significant haplotype contains the A allele at SNP 4. Also, when all ethnicities are considered together, a trend for a longer overlap is observed with SNPs 3/4/5, CGG in the significant risk haplotypes of both this, the initial positive and the Japanese study, (4/10 significant haplotypes). However, given that alleles C and G are respectively the major alleles at SNP3 and SNP4, and are present on numerically more haplotypes than SNP3 allele T, this may simply be chance rather than a reflection of a genuine pattern in the data. Nevertheless, similar directions of effect were observed in our sample for the haplotypes most significantly over-represented in samples of European origin. Further confidence could be achieved if the four marker haplotype (SNP2/SNP3/SNP4/SNP5, TCGG) which was associated in the original positive study and in our own data was also significant in the German study and Japanese samples, particularly since (SNP3/SNP4/SNP5, CGG) was also significant in the Japanese sample. However, the TCGG haplotype was not significantly associated in the German sample, $p=0.11$ (personal communication) although the trend was in the same direction, and the same haplotype was of low frequency in the Japanese sample of Ikeda et al. (2004), (personal communication) and was not significant in the Japanese sample of Ohtsuki et al. (2004), (personal communication).

Our haplotype frequencies were broadly similar to those other samples of European, origin (Emamian et al., 2004; Schwab et al., 2005), although when compared to the German sample (Schwab et al., 2005), both our case and control frequencies were more similar to the German cases than to the German controls (Table 2). Comparison of LD patterns across studies showed D'

values for our sample to be very similar to those of Emamian et al. (2004) and also to those of Schwab et al. (2005) and the CEPH sample (Table 3). Schwab et al. (2005) also suggested increased recombination in the region between SNP2a and SNP3 based on a decrease in D' in this region compared to neighbouring regions. By genotyping all markers in the same CEPH individuals used in the international HapMap project, our data agree with this finding (data not shown).

4.1. Conclusions

In a bid to replicate association of *AKT1* with schizophrenia, we genotyped those polymorphisms that provided evidence for association in samples of both European and Japanese origin and also undertook a pairwise analysis across the locus in a large UK based case control sample. None of our findings survive correction for even modest degrees of multiple testing, and therefore we must conclude that our study does not provide robust support for the hypothesis. However, in our sample, we find (uncorrected) support for a phased 4 marker haplotype that was significantly associated in the original positive association study (Emamian et al., 2004) and which also shows non-significant trends in the same direction in the only other sample of white European origin (Schwab et al., 2005), (p=0.11, 2-tailed) and, notwithstanding a very low frequency, in the positive Japanese study (Ikeda et al., 2004), (p= 0.18). This phased haplotype also shares 3 alleles with a significant phased 3 marker haplotype in a Japanese sample (Ikeda et al., 2004). Thus, while our study does not provide strong support, the current evidence for association between *AKT1* across different studies remains intriguing, and worthy of further investigation.

5. Contributors

NN, HW, SD, LC, TP performed laboratory assays. NN performed the data-analysis and drafted the manuscript. VM and RS advised on data-analysis. NW participated in the design of the study. IN was responsible for data-management. MI and NI provided haplotypic data and analysis from an independent sample. MOD and MJO participated in the design of the study, interpretation of the data, and drafting of the manuscript. All authors read and approved the final manuscript.

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7. Conflicts of interest

The author(s) declare that they have no conflicts of interest.

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References

Abecasis, G.R., Cookson, W.O., 2000. GOLD-graphical overview of linkage disequilibrium. Bioinformatics 16, 182–183.

Bajestan, S.N., Sabouri, A.H., Nakamura, M., Takashima, H., Keikhaee, M.R., Behdani, F., et al., 2006. Association of AKT1 haplotype with the risk of schizophrenia in Iranian population. Am. J. Med. Genet. B Neuropsychiatr. Genet. 141, 383–386.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265.

Becker, T., Knapp, M., 2004. A powerful strategy to account for multiple testing in the context of haplotype analysis. Am. J. Hum. Genet. 75, 561–570.

Dudbridge, F., 2003. Pedigree disequilibrium tests for multilocus haplotypes. Genet. Epidemiol. 25, 115–121.

Emamian, E.S., Hall, D., Birnbaum, M.J., Karayiorgou, M., Gogos, J.A., 2004. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. Nat. Genet. 36, 131–137.

Georgieva, L., Moskvina, V., Peirce, T., Norton, N., Bray, N.J., Jones, L., et al., 2006. Convergent evidence that oligodendrocyte lineage transcription factor 2 (OLIG2) and interacting genes influence susceptibility to schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 103, 12469–12474.

Hawskins, J.R., Khripin, Y., Valdes, A.M., Weaver, T.A., 2002. Miniaturized sealed-tubes allele-specific PCR. Human Mutat. 19, 543–553.

Ide, M., Ohnishi, T., Murayama, M., Matsumoto, I., Yamada, K., Iwayama, Y., 2006. Failure to support a genetic contribution of AKT1 polymorphisms and altered AKT signaling in schizophrenia. J. Neurochem. 99, 277–287.

Ikeda, M., Iwata, N., Suzuki, T., Kitajima, T., Yamanouchi, Y., Kinoshita, Y., et al., 2004. Association of AKT1 with schizophrenia confirmed in a Japanese population. Biol. Psychiatry 56, 698–700.

Liu, Y.L., Fann, C.S., Liu, C.M., Wu, J.Y., Hung, S.I., Chan, H.Y., et al., 2006. Absence of significant associations between four AKT1 SNP markers and schizophrenia in the Taiwanese population. Psychiatr. Genet. 16, 39–41.

Moskvina, V., O'Donovan, M.C., in press. Detailed analysis of the relative power of direct and indirect association studies and the implications for their interpretation. Hum. Hered.

Myakishev, M.V., Khripin, Y., Hu, S., Hamer, D.H., 2001. High-throughput SNP genotyping by allele-specific PCR with universal energy-transfer-labeled primers. Genome Res. 11, 163–169.

O'Donovan, M.C., Owen, M.J., 1999. Candidate-gene association studies of schizophrenia. Am. J. Hum. Genet. 65, 587–592.

Ohtsuki, T., Inada, T., Arinami, T., 2004. Failure to confirm association between AKT1 haplotype and schizophrenia in a Japanese case-control population. Mol. Psychiatry 9, 981–983.

Owen, M.J., Holmans, P., McGuffin, P., 1997. Association studies in psychiatric genetics. Mol. Psychiatry 2, 270–273.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.

Schwab, S.G., Hoefgen, B., Hanses, C., Hassenbach, M.B., Albus, M., Lerer, B., et al., 2005. Further evidence for association of variants in the AKT1 gene with schizophrenia in a sample of European sib-pair families. Biol. Psychiatry 58, 446–450.

The International HapMap Consortium, 2005. A haplotype map of the human genome. Nature 437, 1299–1320.

Williams, H.J., Glaser, B., Williams, N.M., Norton, N., Zammit, S., MacGregor, S., et al., 2005a. No association between schizophrenia and polymorphisms in COMT in two large samples. Am. J. Psychiatry 162, 1736–1738.

Williams, N.M., O'Donovan, M.C., Owen, M.J., 2005b. Is the dysbindin gene (DTNBP1) a susceptibility gene for schizophrenia? Schizophr. Bull. 31, 800–805.

Wing, J.K., Cooper, J.E., Satorius, N., 1974. The Measurement and Classification of Psychiatric Illness. Cambridge University Press, Cambridge, UK.

Wing, J.K., Babor, T., Brugha, T., 1990. SCAN: schedules for the clinical assessment in neuropsychiatry. Arch. Gen. Psychiatry 47, 589–593.

Zhao, J.H., Curtis, D., Sham, P.C., 2000. Model-free analysis and permutation tests for allelic associations. Hum. Hered. 50, 133–139.