

EDITORIAL

Of genes and twins¹

The two paradigms that now dominate the increasingly active field of psychiatric genetics – genetic epidemiology and gene-finding methods – are well illustrated by five papers in this issue of *Psychological Medicine*. Genetic epidemiology, which uses the classical ‘work-horse’ methods of family, twin and adoption studies, infers the action of genetic and environmental risk factors by observing the pattern of resemblance of traits or disorders in various classes of relatives. Methods that are utilized in genetic epidemiology can range from great simplicity, such as the odds ratio of a disorder in first-degree relatives of affected *versus* matched control probands, to substantial complexity, as seen in some advanced multivariate twin-family models (Truett *et al.* 1994). Of the quasi-experimental methods available to psychiatric geneticists that can tease apart the effects of genetic and shared-environmental factors (nature *versus* nurture), the twin method has become increasingly popular. This is probably due to the increasing availability of large population-based or volunteer twin registries (see the Oct 2002 issue of *Twin Research* on ‘Twin Registers as a Global Resource for Genetic Research’) that can be utilized to study psychiatric and drug abuse disorders. By contrast, due to changing social circumstances in most Western countries, adoption, even in the Scandinavian countries where most of the classic studies have been done, is becoming so rare that new adoption studies with younger age cohorts are hardly feasible. By contrast, gene-finding methods, which utilized variants of two different methods of linkage or association, have the goal of determining, on the human genome, the location and potential identity of susceptibility genes. In the last two decades in psychiatric genetics, gene-finding methods have moved from a position on the periphery of the field, where they were viewed with considerable scepticism, to being, without doubt, the dominant scientific paradigm.

A REVIEW OF THE FIVE PSYCHIATRIC GENETICS PAPERS IN THIS ISSUE – GENE FINDING METHODS

In the first study, Pooley and colleagues (2003) studied six genetic polymorphisms in genes broadly involved in serotonergic function in a sample of 129 subjects with deliberate self-harm (DSH) and 329 comparison subjects. Of these six polymorphisms, one – the A779 allele in tryptophan hydroxylase (TPH) – was significantly different in cases and controls.

One important methodological issue in association studies is population stratification. False positive (or false negative) results could emerge if cases and controls are not matched in their ethnic background. Pooley and colleagues were certainly aware of this issue as samples of both cases and controls were all Caucasian subjects whose four grandparents were all born in the UK.

The major interpretative challenge in human association studies is to distinguish true from false positive findings. Several recent reviews have documented what many have suspected for a long-time – that a substantial proportion of positive association results do not survive the test of replication (Ioannidis *et al.* 2003; Lohmueller *et al.* 2003).

What do we know about the results of this paper that might help us evaluate these possibilities? One useful approach is to adopt a ‘quasi-Bayesian’ perspective. What is the prior probability that the TPH-DSH association reflects a true finding? Much evidence supports the role of the serotonergic systems in DSH. Several previous studies have obtained similar findings either with the A779 polymorphism or with a nearby polymorphism A218. However, negative reports have also

¹ Address for correspondence: Professor K. S. Kendler, Department of Psychiatry, PO Box 980126, Richmond, VA 23298-0126, USA.

appeared. Finally, the variant alleles at this polymorphism in TPH might have functional consequences.

Given what might be seen as moderate prior probability, how much do the results of this study add? While the sample is of good size, and is indeed larger than most that have previously looked at this question, its power for the detection of small effect size associations is modest. Assuming a frequency of the A allele in controls of the 34% observed in their sample, the authors would not have 80% power to detect a significant effect at the 5% level until the allele frequency in their DSH group was 44% (equal to an OR of 1.54). This is a rather strong effect and shows that large sample sizes are needed in association studies to provide high power to detect the small effects that are plausible for many psychiatric disorders. The statistical signal obtained by the authors (a *P* value just under 5%) is also modest. The authors tested six different gene variants and did not correct for multiple testing. They argue, with justification, that at least for their TPH results, they were replicating prior findings and thus should avoid the 'tax' of multiple testing. But they did test the other loci and concerns can be made about the 'experiment-wise' type I error rate. While no unambiguous result is obtained by our 'quasi-Bayesian' analysis, the most plausible interpretation is that this study provides a small amount of additional data to support the hypothesis that variation in TPH might be related to risk for DSH.

In a second study, Johansson *et al.* (2003) examine a much-studied functional repeat length polymorphism in the serotonin transporter (5-HTTLPR) in individuals with seasonal affective disorder (SAD) and then perform a pooled analyses across all studies known to them. The interpretation of these results raises the same set of issues reviewed above. One major positive report appeared in 1998 demonstrating, in a case-control design, a significant association between SAD and the 'long' variants of 5-HTTLPR (Rosenthal *et al.* 1998). The OR in this original report (2.4) was quite substantial. Johansson and colleagues had unsuccessfully attempted to replicate this finding in 2001 and in this report try again. The persistence with which these authors have pursued this issue is particularly noteworthy in a field of enquiry that probably overvalues initial positive reports, especially with modest sample sizes, and undervalues well done non-replications.

Their current report included a total of 147 SAD cases from a wide variety of countries and 115 controls matched for age, sex and ethnicity. Not even a hint of differences in allele frequencies were found in the two groups in this study (OR = 0.93). The authors then examined the four relevant case-control studies known to them including the original positive report. They find no evidence of statistical heterogeneity across the studies and then combined them. In aggregate, they found no evidence for association. In trying to replicate an initial positive result, it might be more prudent to exclude that report from subsequent analyses. In that case, the results are even more clearly negative, producing an OR (for the short allele) of 1.01. The null hypothesis of course can never be proved, but the consistency of these results certainly support the hypothesis that the original result of 5-HTTLPR in SAD was a false positive. This pattern – a strongly positive initial report followed by multiple negative or much weaker reports – is rather frequently seen in the literature on association studies (Lohmueller *et al.* 2003).

In addition to examining clinically defined SAD cases, Johansson *et al.* also examined the relationship between the 5-HTTLPR and seasonal mood variation in the general population as measured by self-report. They used 'extreme sampling' comparing individuals in the upper and lower 5% of the population distribution, a method shown to produce substantial increases in power. They report five different analyses of this data examining genotypes, allele frequency, and additive, dominant and recessive models. Only one of these – the recessive model – was statistically significant, although trends were seen in other analyses. No correction was applied for the multiple models tested. They then present a summary of scores on self-report measures of seasonality of mood and 5-HTTLPR genotypes in a series of controls, SAD patients and non-seasonal unipolar patients. With a combined sample of 721 individuals, none of these results were statistically significant. The authors conclude in their abstract that their results '... provide modest evidence for an effect (of 5-HTTLPR) on seasonality'. This is probably an overstatement as their results with seasonality were in aggregate quite negative. But, this illustrates the problem

with such studies. Multiple tests are done and one is statistically positive. How should these results be viewed?

The interpretation of association studies in psychiatric genetics continues to be extremely challenging for readers (and editors!). The greatest concern is probably the high likely proportion of false positive results. The combination of low *a priori* probabilities and low power with even moderately liberal alpha levels (*P* values) will produce a very high proportion of false positive results (Crowe, 1993). Furthermore, as perhaps demonstrated by the 5-HTTLPR-SAD story, there is substantial asymmetry between the relative ease with which an initial positive result can be generated, compared to the substantial effort required to put such a result 'to rest' if it is indeed false. After all, it is formally impossible to demonstrate that there is no effect of the 5-HTTLPR on SAD. This leads to the conclusion, adopted by some leading journals, of only publishing positive association studies given either very strong initial results or independent replication. Those interested in a sober review of the some of the substantial methodological and interpretative difficulties with case-control association studies might profitably consult one of more of the following (Sullivan *et al.* 2001; Little *et al.* 2002; Cardon & Palmer, 2003; Colhoun *et al.* 2003; Ioannidis *et al.* 2003; Lohmueller *et al.* 2003).

GENETIC EPIDEMIOLOGY

This issue contains three studies using a genetic epidemiological paradigm. Each of these studies examines twins utilizing structural equation modeling and illustrates an important, more 'advanced' model examining: (i) longitudinal data (Rijdsdijk *et al.* 2003); (ii) multiple phenotypes (Linney *et al.* 2003); and (iii) genotype \times environmental interaction (Kendler *et al.* 2003).

In the first such study, Rijdsdijk *et al.* (2003) examine, in two waves of female twins from the volunteer St Thomas UK Adult Twin Registry, the oft-studied General Health Questionnaire (GHQ). Their initial sample was large (1950 complete pairs) while the number that completed a second wave was much smaller (360 pairs). As the authors note, given that the GHQ is designed as a measure of current psychiatric state (assessing symptoms over 'the last couple of weeks'), one might assume that genetic influences ought to be quite minimal. However, focusing first on the total GHQ scores, they found that the shared environment makes little of no contribution and estimates of heritability are $\sim 40\%$. The pattern of results for the subscales differs little except for lower heritability for social dysfunction.

Because the authors had longitudinal data (the two waves differed by a mean of 3.5 years), they could ask more interesting questions. In particular, they could calculate the genetic and (individual-specific) environmental correlations between their two times of measurement. These correlations reflect the degree to which the genetic or environmental factors the influence scores at time 1 are the same as those that impact at time 2. These same estimates can be re-parameterized to answer the following intriguing question – what proportion of the observed stability in GHQ scores over time is due to genetic *versus* environmental influences? For both the total scores and the subscales, the results were largely the same. Genetic correlations were high (in the range of +0.70–0.80), while environmental correlations were much more modest (all less than +0.30). Except for the social dysfunction scale, genetic factors accounted for $>65\%$ of the cross-time correlation in GHQ. Finally, the authors examined the 'tails' of the distribution of scores and found, perhaps contrary to expectation, that heritability of those with especially high or low levels of general distress were quite similar to those found in the entire sample.

These findings have several interesting implications, largely in line with the previous literature. First, it suggests that, from a genetic perspective, the difference between 'state' and 'trait' measures in the area of psychopathology is quite a bit less than one might think (Duncan-Jones *et al.* 1990). This is perhaps unsurprising, since one major trait in nearly all personality schemas (John, 1990) is neuroticism or emotional stability – the relatively stable tendency to display high *versus* low levels of dysphoric symptoms. As noted by the authors, a number of other studies have examined state measures of symptoms of depression or anxiety in twin samples (Jardine *et al.* 1984; Kendler *et al.*

1994) and found moderate levels of heritability, usually between 20 and 40 %. This level of heritability is not much lower than that commonly found for the classic trait measures of personality like neuroticism (Loehlin, 1992). Secondly, in adulthood, genetic influences on levels of common psychiatric symptoms are probably rather stable (Kendler *et al.* 1993; Foley *et al.* 2001; McGue & Christensen, 2003). This may differ, for example, from what is seen across puberty (Silberg *et al.* 2001). Thirdly, environmental influences are much less stable than genetic influences. This may be a result of 'real' environmental risk factors, such as stressful life events, that have substantial but short-term effects (Surtees, 1989) or result from 'errors' of measurement which in these analyses are confounded with true individual specific environmental effects. Fourthly, as a consequence of points 2 and 3, much of the phenotypic continuity in human populations in adulthood – the tendency for some individuals to stably demonstrate high *versus* low levels of symptoms – may be due to genetic factors. Finally, physicians are often fond of the idea that genetic factors are much more important for severe 'clinical' levels of symptoms than they are for the milder levels commonly as seen in the general population. However, this preconception may not be well empirically supported especially in the area of anxiety and depressive symptoms (Kendler *et al.* 1992).

In the second twin study in this issue, Linney *et al.* (2003) examined, in 928 complete twin pairs obtained from the Institute of Psychiatry Volunteer Twin Registry, responses to self-report items assessing five dimensions of schizotypal or 'psychosis-proneness' traits: Unusual experiences (Un-Exp), Cognitive Disorganization (CogDis), Introvertive Anhedonia (IntAn), Delusional Thinking (DT) (as assessed by Peters Delusion Inventory) and the Schizotypal Personality Scale (STA) scale. Univariate twin modelling indicated that twin resemblance was due solely to genetic factors and heritabilities were in the range of 35–50 %. The authors then submitted four of these scales (not including STA) to multivariate genetic modelling. The best-fit model included two common factors each of which were substantially heritable (estimates of 0·59 and 0·71, respectively) and were uncorrelated. The first factor had high loadings on DT and UnExp and the second factor had high loadings on IntAn. Both factors loaded moderately on CogDis.

As reviewed by the authors (see their Table 1), the results of their scale by scale twin analysis are consistent with previous studies. Like the vast majority of personality and symptom-based measures, schizotypal symptoms as assessed by self-report in general population samples, are moderately heritable with little impact of shared environmental factors. The multivariate findings are of greater interest. The suggestion that schizotypal symptoms were separable into multiple dimensions goes back several decades (i.e. 'cognitive-perceptual' v. 'social-interpersonal' (Siever & Gunderson, 1983)) and had been validated using standard methods (e.g. factor analysis, Kendler & Hewitt, 1992). However, as suggested by the authors, multivariate genetic methods have not previously been applied to a broad set of schizotypal items in a large twin sample. Their findings suggest two separable and rather highly heritable 'schizotypal' liability dimensions. One of these dimensions influences risk for 'positive' (delusion-like and hallucination-like) symptoms while the other impacts on risk for 'negative' (introversion/social withdrawal) schizotypal symptoms. In these analyses, the CogDis scale was influenced by both factors. The similarity of this 'schizotypal' symptom structure to that proposed for schizophrenia (Liddle, 1987) is self-evident. This parallel has taken on particular interest in the light of findings from a large-scale family study suggesting etiologic continuity between the two domains (Fanous *et al.* 2001). In that study, symptom dimensions in schizophrenic probands predicted scores on the expected schizotypal dimensions in their unaffected relatives.

The final twin paper in this issue examines the central question of genotype \times environment interaction. Common sense (and most of our analytical models) assumes that genetic and environmental risk factors add together to produce an overall liability to illness. However, medicine and psychiatry have many examples in which genes not only influence overall risk of illness, but also modulate the pathogenic impact of environmental stressors. In this report (Kendler *et al.* 2003), Kendler and colleagues utilize 957 personally interviewed adult female–female twin pairs from the population-based Virginia Twin Register to examine whether a general measure of dysfunction in

the family of origin moderates the impact of genetic risk factors on the personality trait of neuroticism. As noted by the authors, adoption studies of a range of psychiatric and drug abuse syndromes have suggested increased genetic effects in pathogenic rearing environments. Would a similar effect be seen for personality?

An innovative structural equation modelling approach was used that permits a quantitative measure of the environment to separately moderate the impact of genes and other environmental risk factors. The results were clear if unexpected. While the mean levels of neuroticism increase substantially and the variance more modestly with increasing levels of reported family dysfunction, the best-fit model involved no moderation at all. That is, the role of genetic and environmental effects on neuroticism were constant across levels of family dysfunction. As noted by the authors, what they called a scalar model – in which there is a total increase in variance of neuroticism with increased levels of dysfunction but the proportion of variance due to genes and environmental is unchanged – also fit the data well. However, both of these models provided a considerably better fit than what they called the ‘moderator’ model, which predicted increasing heritability of neuroticism with increasing levels of family dysfunction. The authors point out that this negative result could be due to low power of this new method, although a recently published power analysis suggests this is unlikely (Purcell, 2002).

As recently reviewed (Kendler, 2001), a number of twin studies on a range of behavioural traits suggest that trait heritability may increase in environments that are less restrictive and/or provide more opportunity for ‘niche-picking’ (i.e. the smart child who augments her own intelligence by spending a lot of time reading at the library). These results suggest that dysfunctional families might provide more opportunity for the development of neuroticism in a high risk individual. While attractive, the results of Kendler *et al.* suggest that this model is incorrect. Perhaps the liability to neuroticism is relatively ‘hard-wired’ and the magnitude of gene expression insensitive to environmental variation.

CONCLUDING THOUGHTS

These five papers well illustrate the current diversity of methods and interpretative questions in the field of psychiatric genetics. It is a sign of health that the field contains two vibrant contrasting set of methods with different strengths and limitations. It should be a source of considerable interest to see how the two subfields of genetic epidemiology and gene-finding methods develop in the coming years. The hope is the gene-finding methods will be successful in identifying genes of modest to moderate effect size for key psychiatric disorders. There is some evidence that this may be now occurring especially in schizophrenia (Harrison & Owen, 2003). For genetic epidemiology, I predict a growth of interest in two areas: (*i*) intermediate phenotypes – that is the tracing the pathway from genes to psychiatric illness; and (*ii*) development – clarifying how genetic and environmental risk factors interact over time and across key biopsychosocial transitions to produce individuals at high risk for disease.

KENNETH S. KENDLER

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