

Complex genetic and environmental relationships between psychological distress, fatigue and immune functioning: a twin study

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ABSTRACT

Background. Although there is considerable support for adverse relationships between states of psychological and somatic distress and immune response, there is little evidence in humans of the relative contribution of genetic and environmental factors.

Methods. This study utilized a twin methodology to examine the interplay between psychological distress, fatigue and immune function. We recorded a number of measures of distress, including conventional depression and anxiety as well as the somatic symptom of prolonged fatigue, and immune responsiveness (by delayed-type hypersensitivity skin response) in 124 normal adult twin pairs (79 monozygotic, 45 dizygotic).

Results. While there were strong genetic influences on the psychological distress and fatigue factors (only some of which are common to both), familial aggregation of immune responsiveness arose mainly from environmental factors shared by both members of a twin pair. Phenotypic correlations between psychological and immune measures were negligible, but multivariate genetic modelling revealed that these masked larger genetic and environmental correlations of opposite sign. Negative environmental effects of psychological distress and fatigue on immune responsiveness were countered by a positive genetic relationship between psychological distress and immune function.

Conclusions. Our study suggests that current psychoneuroimmunological hypotheses in humans need to be modified to place increasing importance on the individual's genotype. In this cohort immune responsiveness varied in response to a complex interplay of genetic and environmental factors. Additionally, although psychological distress and fatigue had some shared genetic determinants, independent genetic and environmental risk factors for fatigue were also identified.

INTRODUCTION

A large body of clinical and basic research has now provided support for the basic psycho-immunological hypothesis. That is, a variety of behavioural stressors in laboratory animals, and states of distress in humans, are associated with significant changes in immune responsiveness

(Keller *et al.* 1983; Kiecolt-Glaser *et al.* 1986, 1987; Irwin *et al.* 1988, 1991, 1992; Schleifer *et al.* 1989; Taylor & Janney, 1992; Schedlowski *et al.* 1993; Maes *et al.* 1994). The most robust effects in humans occur in patients with severe depressive disorders (Hickie *et al.* 1990, 1993*a*) and involve principally reductions in markers of cell-mediated immunity (CMI) rather than the humoral immune response. Limited longitudinal data suggest that such changes are state-dependent and resolve with treatment of the depressive episode (Irwin, 1993, 1994; Hickie &

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Hickie, 1991). The impact of specific acute or chronic psychological stressors and less severe depression in humans appears more variable (Kiecolt-Glaser *et al.* 1987, 1988; Irwin *et al.* 1990). Changes in immune response may well depend on the severity of intermediate psychological responses, notably the development of anxiety and/or depressed mood (Hall, 1987; Ader *et al.* 1995).

When evaluating the psychoimmunological hypothesis in clinical populations many confounding factors are relevant. These include the effects of sampling bias (e.g. hospitalization), concurrent psychotropic medications, co-morbid medical and psychological disorders, and substance use (e.g. cigarettes and alcohol). Further, inferences drawn from certain clinical cohorts may be extremely limited. For example, the severe depressive disorders result in widespread alterations to the neuroendocrine network and sympathetic nervous system, which may induce levels of immune alteration rarely encountered in less severe or non-clinical settings (Hickie & Hickie, 1991; Hickie *et al.* 1993*a, b*; Maes *et al.* 1994). Additionally, experimentation with disease models in animals has suggested that the organism's current health status also modulates its response to stress (Tapp & Natelson, 1988). Therefore, epidemiologically-relevant tests of the psychoimmunological hypothesis should combine non-clinical cohorts with sensitive and relevant measures of CMI.

As both immunological responsiveness and psychological traits are underpinned by both genetic factors and past and current environmental effects, the relative contribution of each of these factors, (and the possibility that some genetic or environmental factors are shared) requires detailed examination. In fact, any association between psychological distress and immune responsiveness may be explained by variation in a common genetic or environmental factor. Standard clinical studies in humans do not provide mechanisms for studying these possible effects. Relevant experimentation with laboratory animals, however, has demonstrated the importance of variable genetic factors in modulating the pathological effects of stressful environments (Pare & Kluczynski, 1997).

Combined behavioural and neurophysiological studies within twin populations offer a unique opportunity to explore the complex relationships

between genetic factors, effects of shared environmental influences – likely to occur in early childhood, and environmental factors unique to individuals – more likely to occur in later life when twins have left the family home. The technique has been used largely in recent years to test various aetiological models of the common mental disorders (Kendler *et al.* 1986, 1987). In the situation, however, where both psychological states and laboratory markers are examined in MZ and DZ twins, one can gain some leverage on the vexed question of whether direct causal relationships exist between such clinical and laboratory markers or whether common genetic or environmental factors account for associations.

In patients with severe depression and patients with chronic fatigue syndrome (CFS) we have explored the utility of the 'CMI Multitest' (Merieux, France) of delayed-type hypersensitivity skin response (DTH) as an *in vivo* marker of CMI (Lloyd *et al.* 1989, 1992; Hickie *et al.* 1993*a, b*). The 'CMI Multitest' assesses DTH skin response to a battery of known antigens and its validity as a clinical measure of immune function has been well demonstrated (Hersh *et al.* 1971; Golub *et al.* 1974; Gmur *et al.* 1976; MacLean, 1988; Poenaru & Christou, 1991). In non-clinical settings the commonest forms of psychological distress include non-specific somatic symptoms such as fatigue, as well as the typical symptoms of anxiety and depression (Pawlikowska *et al.* 1994; Hickie *et al.* 1996, 1997). Consequently, when assessing psychological distress in non-clinical settings we have utilized both conventional psychological measures such as the General Health Questionnaire (GHQ, Goldberg & Williams, 1988), in combination with specific instruments such as the Schedule of Fatigue and Anergia (SOFA) to detect cases of prolonged fatigue and somatic distress (Hickie *et al.* 1996).

Our aims in this study were, first, to test the psychoimmunological hypothesis within a representative non-clinical sample utilizing more extensive notions of psychological distress and a clinically-valid *in vivo* measure of CMI and, secondly, to examine the relative contribution of genetic, early environmental and current environmental contributions to both immunological and psychological phenotypes. To this end, we have measured psychological distress, fa-

tigue, and immune responsiveness in an unselected sample of 124 twin pairs (79 monozygotic, 45 dizygotic) aged 31–84 years.

METHOD

In the course of a larger study of genetic risk factors for alcoholism (Heath *et al.* 1997) and depression, the opportunity arose to perform *in vivo* assessment of CMI by DTH. The CMI kit is expensive and the procedure is labour intensive so resources were available to perform DTH testing on only about 5% (265/5889) of the larger sample. The main criteria for inclusion were that twins both presented for testing together, both were willing to take part and lived in geographic proximity to our testing centres in Melbourne, Sydney and Canberra. After obtaining informed consent, twin pairs were tested. The 'CMI Multitest' was applied to the volar aspect of the left forearm by trained nursing personnel. After 48 h the area was examined (by nursing staff and/or the subject) for responses to the antigens. Disregarding any erythema, indurated areas were measured in two diameters by the subject and the mean diameter recorded for each site. The sum of all the diameters was recorded. If the glycerin control was positive, the subject's results were discarded.

The reliability of self-reading of the DTH response has been established during the course of this study and is reported in detail elsewhere (Bennett *et al.* 1999). In summary, we found that subjects were highly reliable in measuring their own induration diameters and then reporting them by telephone. This overcomes a major methodological limitation for use of the CMI kit in large epidemiological studies. Without self-reading the researcher has to either call the subject back to the research site or travel to visit the subject 48 h after administration.

At the time of the CMI test, the twins completed two self-report questionnaires, the 10 item SOFA-Community version, a screening test for chronic fatigue/neurasthenia (Hickie *et al.* 1996) and the 12-item GHQ (Goldberg & Williams, 1988), a screening test for psychiatric morbidity. These questionnaires give the subjects four options for a response. The responses for each of the 10 items on the SOFA were scored dichotomously. Responses of 'none or a little' or 'some of the time' receiving a score of '0' and

'good part of the time' and 'most of the time' given a score of '1'. The scores were then summed to yield a total SOFA score from 0 to 10 (Hickie *et al.* 1996). Similarly, the 12 items of the GHQ were scored dichotomously, and the scores summed for a total score of 0 to 12.

Zygoty diagnosis

Zygoty of twins was decided on the basis of their responses to standard questions about similarity and the degree to which others confused them. Any inconsistency between co-twins in these responses was followed up with requests for photographs which usually cleared up any confusion. We have shown that diagnosis by these criteria is very accurate compared with objective genetic marker typing (Martin & Martin, 1975).

Statistical methods

Data were analysed using PRELIS 2.12 and LISREL 8.12 (Joreskog & Sorbom, 1993). Correlations between variables are calculated on the assumption that underlying each variable is a continuum of liability that is normally distributed in the population. Categorical variables arise when thresholds, often arbitrary, are imposed on this liability continuum. PRELIS estimates polychoric correlations between categorical variables, Pearson correlations between continuous measures, and polyserial correlations between continuous and categorical variables (Joreskog & Sorbom, 1993).

Because of its skewed distribution, we have applied a logarithmic transformation to the immune responsiveness measure. The psychological variables, which have relatively few categories (13 for psychological distress 'DIS' and 11 for fatigue 'FAT') and skewed distributions (most people with low scores and a long tail to the right), have been treated as categorical.

Significant twin correlations establish the fact that there is familial aggregation for the measures of interest. Our task, however, is to distinguish between the possible mechanisms by which this familial likeness may arise. One useful method is via structural equation modelling as implemented in LISREL, Mx, or similar packages (Neale & Cardon, 1992). One can conceive of three broad causes of variation, two of which (additive genetic influences 'G' and common environment 'C') make family mem-

bers more alike than random pairs of individuals, and one of which (unique environmental experiences 'E') makes MZ twins and siblings different. The task then is to decide which combination of these three parameters provides the most parsimonious explanation for the observed pattern of MZ and DZ twin correlations.

The methods of structural equation modelling are readily extended to the more complex questions of the relationship between variables, in which one is trying to discover not only the *sources* of covariation (G, C, E), but the pattern or structure in which these differentially influence the covarying measures. We have therefore, used the Cholesky decomposition to dissect this (Neale & Cardon, 1992). Each source of covariance between n variables is decomposed into a series of n factors, the first factor loading on all variables, the second factor loading on all but the first variable, the third factor loading on all but the first two variables, and so on until the last factor loads on only the n th variable. Models were fitted in LISREL 8.12 using maximum likelihood, since numbers of pairs were too small to estimate reliably asymptotic covariance matrices to use weighted least squares. We began by specifying a complete decomposition for three sources of variance – additive genes, shared environment, and individual environment. This full model was then simplified by successive dropping of non-significant parameters i.e. by seeing whether dropping a parameter resulted in a significant increase in the goodness-of-fit chi-square. The same principles of parsimony were applied in arriving at the preferred model (Neale & Cardon, 1992).

RESULTS

The sample for this DTH substudy was predominantly female (81%) with a mean age of 46.4 years (s.d. = 11.7). The full sample of the larger study was 65% female and slightly younger (44.8 for females, 42.7 for men), and this suggests that the greater cooperativeness and availability of older females was an important factor in participation. This would be of concern if it produced a major bias with respect to our target variables. We cannot address this directly because we do not have DTH, GHQ or SOFA data for the larger sample. However, we do have DSM-III-R diagnoses of major depression for

the entire sample and these occur in 14.8% (889/5995) of the entire sample compared with 14.0% (37/265) of the DTH tested subsample. This rate of DSM-III-R depression is very similar to prevalences reported from other large community samples (Blazer *et al.* 1994) and suggests no major bias on this, the principal dimension of interest in our study.

Immunological 'anergy' (i.e. no DTH response to any antigen in the test battery), in this group was higher than that found in our earlier control series, particularly for female subjects (Hickie *et al.* 1995), (males 9% v. 3% and females 31% v. 6%). Of the total sample ($N = 248$) 9.2% were categorized by the SOFA as fatigue cases (FAT), while 12% described current psychological distress with GHQ scores of ≥ 3 (DIS).

Estimating twin correlations

Complete data were available for 124 twin pairs, comprising 67 monozygotic (MZ) female pairs, 12 MZ male pairs, 26 dizygotic (DZ) female pairs, 5 DZ male and 14 DZ unlike sex pairs. These latter groups are too small for the calculation of polychoric correlations and must either be discarded or pooled into total MZ *versus* total DZ groups. A concern in pooling over sexes within zygosity groups is that sex differences in means will inflate twin correlations. Since there are considerable sex differences in the immune variables and their putative psychological risk factors, we entered sex as a covariate in order to remove such an inflationary effect. Age is always a potential confounding factor in studies such as this, but in our sample we found only negligible correlations with DIS, FAT and IMM (-0.12 , -0.02 and 0.01 respectively).

Cross-twin-cross-trait correlations were estimated using PRELIS for the complete MZ (79 pairs) and DZ twin group (45 pairs) with sex (0 = female, 1 = male) as a categorical covariate. GHQ and SOFA scores were treated as categorical variables whereas 'total score' (the sum of the average induration diameters for all seven antigens was log transformed (total mm + 1) and then treated as continuous. Correlations are shown in Table 1 for both MZ and DZ twins. The twin1–twin2 correlations for the three key variables are highlighted in bold. It can be seen that for GHQ and SOFA the MZ correlations are roughly twice their DZ counterparts (0.56

Table 1. Polychoric correlations for MZ (above diagonal) and DZ (below diagonal) pairs for measures of psychological distress (DIS), fatigue (FAT), and immune responsiveness (IMM) for twin 1 and twin 2. Correlations with sex (female = 0, male = 1) are also shown. Co-twin correlations for each measure are shown in bold

	Monozygotic (79 pairs)						
	¹ DIS1	² FAT1	³ IMM1	⁴ DIS2	⁵ FAT2	⁶ IMM2	SEX
DIS1	<i>1.00</i>	0.32	-0.15	0.56	0.43	0.40	0.04
FAT1	0.39	<i>1.00</i>	-0.07	0.45	0.50	0.34	0.06
IMM1	0.20	-0.29	<i>1.00</i>	0.19	-0.03	0.32	0.28
DIS2	0.27	-0.17	-0.21	<i>1.00</i>	0.57	0.28	0.06
FAT2	0.21	0.27	-0.02	0.43	<i>1.00</i>	0.23	-0.24
IMM2	-0.13	-0.20	0.33	-0.29	0.11	<i>1.00</i>	0.42
SEX	-0.18	-0.03	0.02	-0.09	0.23	0.17	<i>1.00</i>

Dizygotic (45 pairs)						

¹ Psychological distress (GHQ) score – twin 1.

² Fatigue (SOFA) score – twin 1.

³ Immunological responsiveness score (IMM) – twin 1.

⁴ Psychological distress (GHQ) score – twin 2.

⁵ Fatigue (SOFA) score – twin 2.

⁶ Immunological responsiveness score (IMM) – twin 2.

versus 0.27 and 0.50 versus 0.27 respectively), indicating additive genetic control of familial aggregation for depression and fatigue. On the other hand, we see that the MZ and DZ correlations for immune responsiveness (IMM) are approximately equal (0.32 versus 0.33), indicating that shared family environment is largely responsible for any familial resemblance in this variable.

Fitting multivariate genetic and environmental models

Our task, however, is to explore not only the causes of individual differences for the separate variables but also the causes of covariation between them. The focus of our interest is covariation between immune suppression and the psychological factors of distress and fatigue. In our sample, the phenotypic correlation between distress and fatigue is 0.45, consistent with our notion that they represent somewhat different types of distress (Hickie *et al.* 1997, 1998). However, their correlations with immune responsiveness are only -0.04 and 0.09 respectively. It may seem, therefore, that there is little reason to proceed with further analyses, but the trivial phenotypic correlations do not necessarily tell us about genetic and environmental factors influencing covariation. Indeed, it is possible to find a zero phenotypic correlation which masks strong genetic and environmental

correlations of opposite signs (Heath & Martin, 1990). It is only by multivariate genetic analysis (Neale & Cardon, 1992) that we can estimate the relative contributions of genes and environment to covariation between measures.

We begin by specifying a complete Cholesky decomposition for three sources of variance – additive genes, shared environment, and individual environment. We simplify this by dropping non-significant parameters and the final model, shown in Fig. 1, fits no worse than the complete decomposition (likelihood ratio chi-square (8) = (4.58).

The principal genetic features of this model are as follows. First, there is a common genetic factor accounting for 62% (i.e. 0.79²) of the variance in GHQ which also accounts for 31% of the variance in fatigue. Most interestingly this genetic factor which increases depression and fatigue also has an increasing effect on immunological responsiveness (IMM), accounting for 8% (0.28²) of the total variance in IMM. Secondly, there is also genetic variance specific for fatigue accounting for 22% (0.47²) of its variance. Thirdly, shared environment accounts for 19% (0.44²) of the variance in IMM.

Individual environmental variance also subsumes any errors of measurement and so it is to be expected that the vertical paths from E1, E2 and E3 to the corresponding first, second and third variables (DIS, FAT, IMM) will be large.

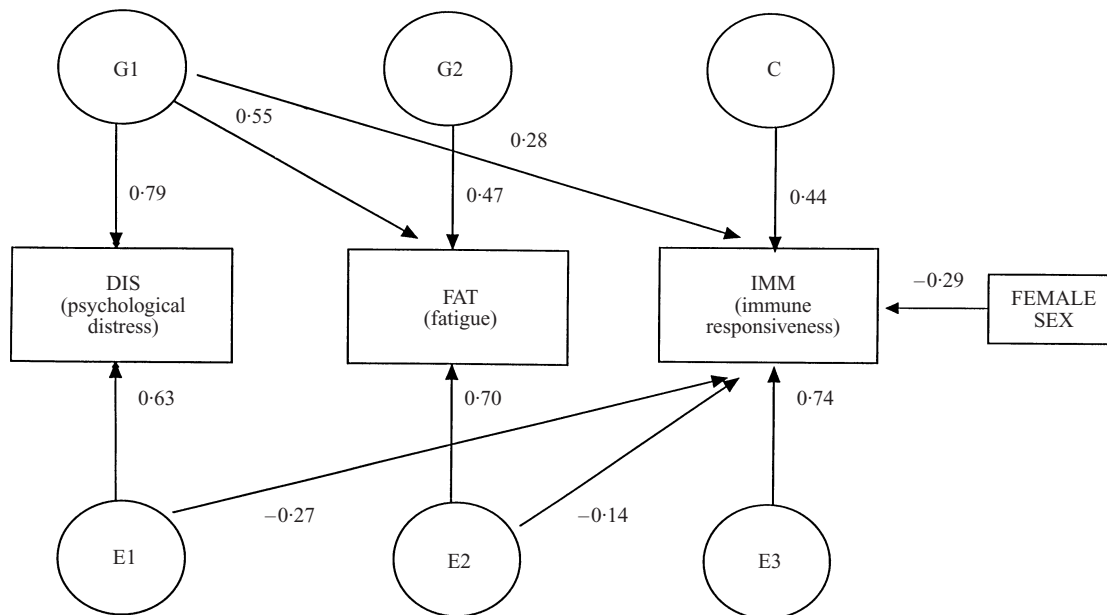


FIG. 1. Path diagram showing latent genetic and environmental influences (shown in circles) on the measured phenotypes 'DIS', 'FAT' and 'IMM' (shown in squares). G1 and G2 represent additive genetic factors, C is a shared environmental factor (influencing only 'IMM') and E1, E2 and E3 are unique environmental factors. Only significant paths ($\alpha = 0.05$) as judged by likelihood ratio tests are shown. The influence of sex on 'IMM' is also accounted for so as not to bias the other parameter estimates.

However, most interesting is the negative path from E1 to IMM, such that environmental influences which increase DIS reduce IMM, accounting for 7% (-0.27^2) of its variance and this is highly significant ($\chi^2 = 12.87$). This apparently counterbalances the positive genetic correlation between DIS and IMM, as seen by the positive path (0.28) from G1 to IMM ($\chi^2 = 11.85$). Finally, we see a parallel negative environmental correlation between fatigue and IMM but only accounting for 2% of the variance of IMM (-0.14^2), although this is still significant ($\chi^2 = 4.19$).

DISCUSSION

There is now considerable support for the hypothesis that states of distress in humans are associated with impaired immune responsiveness. To investigate the genetic and environmental antecedents of psychoneuroimmunological associations, and of the common psychological and somatic forms of distress, we have measured distress, fatigue and immune responsiveness in 124 normal adult twin pairs (79 MZ, 45 DZ).

The application of the twin methodology using both behavioural and immunological measures provides a unique test of the psychoimmunological hypothesis, as well as novel insights into the likely aetiology of somatic and psychological distress. The key features of the model (see Fig. 1) can be summarized as: (i) a common genetic factor (G1) underpins the expression of psychological distress and fatigue, but also has an enhancing effect on immune function; (ii) a second independent genetic factor (G2) contributes to fatigue only; (iii) a shared (possibly early) environmental factor (C) contributes strongly to immune responsiveness but appears to have no relationship with the psychological or fatigue variables; (iv) a first individual environmental factor is associated with increased psychological distress and decreased immune responsiveness (E1); (v) a second independent individual environment factor (E2) contributes to increase fatigue but is also associated with reduced immune responsiveness; and (vi) a third individual environment factor (E3) contributes to immune responsiveness only.

Our major aim in this study was to test the psychoimmunological hypothesis that current

emotional distress is associated with reduced immune responsiveness in a non-clinical cohort. Factors E1 and E2 in our model indicated clearly that the two most common forms of distress in the community, namely mixed anxiety/depression (indicated by GHQ caseness) and fatigue (indicated by SOFA caseness), have a significant inverse relationship with immune responsiveness, as assessed by a functionally-relevant *in vivo* marker. That is, the study provides strong evidence for the notion that environmental factors which increase psychological distress and fatigue are also associated with reduced CMI. The model however, additionally also provides us with data concerning the relative genetic and environmental contributions to the dimensions of psychological and somatic distress, and CMI.

While there are strong genetic influences on the two psychological risk factors (only some of which are common to both), surprisingly, familial aggregation of immune responsiveness arises mainly from environmental factors shared by both members of a twin pair. Such factors could include common exposure to relevant antigens in childhood. Phenotypic correlations between psychological and immune measures were however negligible, but multivariate genetic modelling revealed that these masked larger genetic and environmental correlations of opposite sign. Negative environmental effects of distress and fatigue on immune responsiveness were balanced by a positive genetic relationship between distress and immune function. This suggests that those psychoneuroimmunological hypotheses, which imply a uniform and negative environmental effect of distress, regardless of the host's genotype, are overly simplistic. In humans, it appears that there is a much more complex interplay of genetic and environmental factors at work. Future psychoimmunological studies need to examine the possible interactions of genetic and environmental factors, by making use of relevant twin and family designs.

Limitations of the study

The novel findings presented here must be assessed in the context of the limitations of this study, which include the following points.

(i) A relatively small sample size, so that our parameter estimates are unstable. Additionally, this increases the type II error rate so that salient

effects may have been too readily discounted; in particular, it is possible that there are quite large shared environmental influences on one or more of the variables that we have failed to detect because of insufficient sample size (Martin *et al.* 1978).

(ii) Because of our small sample size, our treatment of sex differences was not ideal; there were too few males (48 v. 200 females) to subdivide our sample further, so we coped with the sex difference in immune response by regressing it out as if it were a continuous variable, which clearly it is not. The instability of the polychoric correlations with sex when the sample is further subdivided is exemplified by the four different correlations of fatigue with sex in Table 1 (-0.03 , 0.22 , 0.06 , -0.24); however, since the overall correlation was negligible, the path from sex to FAT was omitted from the model, as was the path from sex to DIS. Separate from the issue of mean differences between sexes, or differences in prevalences, is the issue of the causes of variation within a sex. There is no necessary reason why the causes of variation in males should be the same, either in magnitude or in kind, as those in females. This is the specific reason for the inclusion of opposite sex pairs in the ideal twin design; if their correlation is substantially lower than those of DZ same sex pairs this suggests genetic, or family environmental influences specific to one sex. Ideally one treats each sex-zygosity twin group separately for the purposes of modelling sex-limited effects (see Neale & Cardon, 1992, chap. 11) but our sample size was not big enough to permit this.

(iii) Given the practical constraints of the study, not all our measures were ideal. Only short forms of the psychological tests rather than detailed case finding interviews were used, so decreasing their reliability.

(iv) Our measure of immune responsiveness relied on self-measurement, though we have demonstrated the reliability of this method (Bennett *et al.* 1999).

Conclusion

Despite these limitations and, hence, the need for replication of the key findings, the study makes an important contribution to two controversial areas of psychiatry. First, while providing support for the basic notion that distress decreases immune responsiveness, it does how-

ever, suggest a more complex interplay of genetic and environmental factors. Secondly, it provides evidence that while some genetic factors result in both psychological distress and fatigue, separate genetic and environmental factors that predispose to fatigue can be identified separately. Given the wider debate as to the nosological status of fatigue states, this study supports the notion that such disorders may constitute relatively independent forms of distress (Hickie *et al.* 1997, 1998).

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