A Twin Study of the Etiology of Prolonged Fatigue and Immune Activation

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Risk factors to prolonged fatigue syndromes (PFS) are controversial. Pre-morbid and/or current psychiatric disturbance, and/or disturbed cell-mediated immunity (CMI), have been proposed as etiologic factors. Self-report measures of fatigue and psychologic distress and three *in vitro* measures of CMI were collected from 124 twin pairs. Crosstwin-crosstrait correlations were estimated for the complete monozygotic (MZ; 79 pairs) and dizygotic (DZ; 45 pairs) twin groups. Multivariate genetic and environmental models were fitted to explore the patterns of covariation between etiologic factors. For fatigue, the MZ correlation was more than double the DZ correlation (0.49 versus 0.16) indicating strong genetic control of familial aggregation. By contrast, for *in vitro* immune activation measures MZ and DZ correlations were similar (0.49–0.69 versus 0.42–0.53) indicating the etiologic role of shared environments. As small univariate associations were noted between prolonged fatigue and the *in vitro* immune measures (r = –0.07 to –0.12), multivariate models were fitted. Relevant etiologic factors included: a common genetic factor accounting for 48% of the variance in fatigue which also accounted for 4%, 6% and 8% *reductions* in immune activation; specific genetic factors for each of the *in vitro* immune measures; a shared environment factor influencing the three immune activation measures; and, most interestingly, unique environmental influences which increased fatigue but also *increased* markers of immune activation. PFS that are associated with *in vitro* measures of immune activation are most likely to be the consequence of current *environmental* rather than genetic factors. Such environmental factors could include physical agents such as infection and/or psychologic stress.

Neuropsychiatric syndromes characterized by prolonged fatigue (PFS) have re-emerged as a focus of epidemiological study and clinical research in medicine and psychiatry (Fukuda et al., 1994; Hickie et al., 1997, 1998; Wessely et al., 1998). Treatment (Deale et al., 1997; Sharpe et al., 1996; Vercoulen et al., 1996), longitudinal (Hickie, Koschera et al., 1999; Merikangas and Angst, 1994; Van der Linden et al., 1999), statistical modelling (Hickie, Lloyd, Hadzi-Pavlovic et al., 1995; Kirk et al., 1999; Koschera et al., 1999) and twin (Farmer et al., 1999; Hickie, Kirk et al., 1999; Hickie, Bennett et al., 1999) studies now support the notion that PFS can potentially be viewed as independent entities from more common forms of psychological distress, such as anxiety and depression. It has been proposed that such general fatigue states, however, also contain a subset of more severe and chronic disorders, termed chronic fatigue syndrome (CFS) (Fukuda et al., 1994). While a variety of immunological, infectious and neuropsychiatric hypotheses have been proposed for the etiology of CFS (Fukuda et al., 1994; Hickie & Lloyd, 1995; Hickie, Lloyd & Wakefield, 1995; Hickie et al., 1997, 1998; Lloyd et al., 1999; Wessely et al., 1998), no consensus has been reached.

It is clear that PFS and CFS may emerge subsequent to certain infectious agents, including viral agents such as Epstein Barr Virus (EBV) (White, Thomas et al., 1995), Ross River Virus (RRV) (Selden & Cameron, 1996) and non-viral infections such as Q fever (Marmion et al., 1996) and Lyme disease (Sigal, 1994). Such infective disorders typically result in acute fatigue and concurrent disturbance of cell-mediated immunity (CMI) (Bennett et al., 1998). Post-infectious fatigue syndromes following EBV can be differentiated clinically from other forms of post-infectious psychiatric disorder (White, Grover et al., 1995). Etiologic theories, however, typically place emphasis on perpetuating psychological factors and/or immunological factors rather than persistent or chronic EBV infection (Straus, 1993; Sumaya, 1991). For example, impaired CMI (due to genetic, infective, toxic or psychological factors) could result in re-presentation of viral antigens and, therefore, ongoing serological or immunological markers of infection (Buchwald et al., 1992; Natelson, 1994; Schmaling, 1996). Importantly, recovery from acute post-infective fatigue is correlated with improvement in CMI (Bennett et al., 1998).

CFS itself has been associated with a wide range of immunological abnormalities, particularly of both *in vitro* and *in vivo* measures of CMI (Lloyd et al., 1989, 1992, 1997). Although an infectious agent may have precipitated these abnormalities, it is also highly likely that such changes occur secondary to a range of genetic and (past and current) environmental factors (Hickie, Bennett et al.,

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1999). Current neuropsychiatric states, such as severe depression (Hickie et al., 1993; Irwin et al., 1990, 1991), are themselves powerful mediators of immune disturbance.

We have argued that a range of neuropsychiatric states characterized by PFS and/or CFS may be due, in part, to excessive cytokine production (Hickie and Lloyd, 1995; Vollmer-Conna et al., 1998). The administration of certain immune products to humans (i.e. the pro-inflammatory cytokines such as interferons and interleukins) results in a range of neuropsychiatric consequences including *profound* fatigue (Mannering & Deloria, 1986; McDonald et al., 1987; Weiss, 1998). Raised levels of these cytokines in animals (in response to infection or immunization) also result in a wide range of behavioral phenomena including reduced psychomotor activity, social withdrawal and increased slow-wave sleep (Hart, 1988). In patients with CFS there has been little direct evidence of raised serum levels of cytokines, though there is preliminary evidence of possible increases within the cerebrospinal fluid (Lloyd et al., 1991). Patients with CFS may have exaggerated release of pro-inflammatory cytokines when peripheral blood mononuclear cells are stimulated with lipopolysaccharide (LPS) (Chao et al., 1991). Similarly, patients with post Q fever fatigue syndromes demonstrate important relationships between fluctuations in pro-inflammatory cytokines and the course of their fatigue (Marmion et al., 1996). Such dynamic tests of immune function place additional importance on the potential role of host-determined regulation of immune response in the pathogenesis of fatigue (Hickie, Bennett et al., 1999).

An overly simplistic view of psychoimmunological relationships (e.g. that adverse behavioral states directly cause negative immune events), may underestimate a more complex interplay between host (e.g. genetic factors, age, gender, health-status) and/or environmental (e.g. social network, exposure to other physical and psychological stressors) factors. Animal studies indicate the contribution of genetic factors to stress-induced disease changes and the ways in which the current health status of the organism may determine its response to further stressful events (Pare & Kluczynski, 1997; Tapp & Natelson, 1988). Previous multivariate modelling of psychological and *in vivo* immunologic data from healthy twins suggested that the current immune status of any individual was a consequence of a complex interplay of host (particularly gender) and other genetic and environmental factors (Hickie, Bennett et al., 1999). In this study we sought to model the etiologic relationships between genetic and environmental determinants of PFS, concurrent psychological distress and key *in vitro* measures of immune activation. Specifically, we focused on those immunological factors that may also be relevant to CFS and other post-infective neuropsychiatric disorders.

Method

Subjects

In the course of a larger study of genetic risk factors for alcoholism and depression (Heath et al., 1997), we enrolled a sub-set of subjects specifically for immunologic testing. These 265 individuals constituted about 5% (265/5,889) of the total sample. Twins were included if both twins presented for testing together, both were willing to take part, lived in close geographic proximity to a testing center and provided informed consent. Recruitment was also limited by the availability of trained staff for only a defined period during the overall study.

Immunologic Tests

Direct assessment of immune function can be achieved by *in vivo* tests (e.g. cutaneous delayed type hypersensitivity [DTH]) and/or *in vitro* measures such as serum estimations of those cytokines, which regulate cellular (interferon-gamma [IFN-γ]) and humoral immunity (interleukin-4 [IL4]). In this study, trained nursing personnel applied the CMI Multitest (Merieux, France), a standard kit for evaluation of DTH, to the volar aspect of the left forearm. After 48 hours the area was examined (by nursing staff and/or the subject) for responses to the seven test and one control 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. The reliability of self-reading of the DTH response was established during the course of this study (Bennett et al., in review).

An indirect assessment of cellular and humoral immunity was determined by serum estimations of IL4 and IFN-γ, the prototypic Th2 and Th1 cytokines important in promoting humoral and cellular immunity, respectively. This was complemented by serum measurements of soluble CD23 (sCD23), which is of predominantly B cell origin (Bansal et al., 1994) and is regulated by the stimulating actions of IL4 and the inhibitory effects of IFN-γ (Delespesse et al., 1991; Gordon, 1992). Serum sCD23 has previously been found to be informative of the balance of cellular and humoral immunity (Bansal et al, 1993). Thus, serum sCD23 was elevated in patients with systemic lupus erythematosus (SLE) and primary Sjögrens syndrome, both of which are associated with a combination of hypergammaglobulinaemia and autoantibody production (Bansal et al., 1992). However, in conditions which are associated with exaggerated cellular immunity, such as celiac and Crohn's disease, serum levels of sCD23 were reduced (Bansal et al., 1993). Thus, a combination of IL4, IFN-γ and sCD23 allowed an assessment of the general pattern of immune response.

Serum sCD23 was measured as previously described using a double monoclonal sandwich ELISA. This utilized the EBVCS2 capture antibody and horse radish peroxidase conjugated BU38 detection antibody (Bansal et al., 1993). IFN-γ was measured, as previously described (Bansal et al., 1997) by double monoclonal sandwich ELISA using the mouse capture and biotinylated detection antibodies from Endogen Inc (Cambridge, MA 02139, USA). IL4 was measured using an identical procedure to the IFN-γ assay in a double monoclonal sandwich ELISA. This employed the paired rat anti-human monoclonal antibodies 8D4-8 for IL4 capture and HRP conjugated MP4-25D2 for the detection step (Pharmingen, San Diego, CA 92191, USA). All sera were analyzed blind and as duplicate neat samples randomly scattered on each plate. The detection limits of both the cytokine assays was 5 pg/ml (indicated by an

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optical density 25% above that of the background and corresponding to a cytokine level of at least 5 pg/ml).

During the acute phase of an infective illness, or shortly after re-presentation of a viral antigen, one would expect to detect raised levels of IFN-γ, but not IL4. During the recovery phase, one would then expect to detect increased levels of IL4 concurrent with the development of neutralizing antiviral antibodies. DTH skin responses may be reduced during both the acute illness and recovery phase but would typically return to pre-illness levels in association with resolution of fatigue and other systemic symptoms (Bennett et al., 1998). Consequently, the detection of raised levels of IFN-γ and/or IL4 in members of the normal population could indicate the presence of recent viral illness, reactivation of viral agents due to impaired immunity or the presence of other medical disorders (e.g. autoimmune disorders). Similarly, reduced DTH skin response could be indicative of these same factors and/or lack of previous exposure to the antigens included in the test kit.

Neuropsychologic Testing

At the time of the CMI test, the twins completed two self-report questionnaires, the 10-item Schedule of Fatigue and Anergia (SOFA) — Community version (Hadzi-Pavlovic et al., 2000; Hickie et al., 1996), a screening test for chronic fatigue/neurasthenia, and the 12-item General Health Questionnaire (GHQ) (Goldberg & Williams, 1988), a screening test for common forms of anxiety and depression. 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' received a score of '0', while 'good part of the time' and 'most of the time' was given a score of '1'. The scores were then summed to yield a total SOFA score between 0 and 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. In a series of clinical and twin studies (Hadzi-Pavlovic et al., 2000; Hickie, Bennett et al., 1999; Hickie, Kirk et al., 1999; Hickie et al., 1996, 2000; Kirk et al., 1999; Koschera et al., 1999), we have established that the GHQ and the SOFA measure only modestly correlated constructs (0.38–0.51), and that multivariate techniques with both twin and longitudinal data support the relative independence of these constructs (Hickie, Bennett et al., 1999; Hickie, Kirk et al., 1999; Hickie, Koschera et al., 1999; Kirk et al., 1999; Koschera et al., 1999; Van der Linden et al., 1999). Patients identified by the SOFA as cases of PFS have typically been ill for more than 12 months (Hickie, Koschera et al., 1999), and are more disabled by their illness than patients with major depression residing in the community (Hickie et al., 2000; Hickie et al., in press). Patients with PFS are at only a weak increased risk of developing pure forms of psychological distress (odds ratio [OR]: 1.4; 95% CI: 0.6–3.4), while patients with typical anxiety and depression are not at an increased risk of developing PFS alone longitudinally (OR: 0.95; 95% CI: 0.2–3.6) (Hickie, Koschera et al., 1999). Patients with PFS constitute approximately 10% of the normal community and about 25% of primary care attendees (Hickie, Bennett et al., 1999; Hickie, Kirk et al., 1999; Hickie et al., 1996, 2000) while patients with CFS constitute approximately 0.05–1% of the community and 1–2% of primary care attendees (Bates et al., 1993; Hickie et al., 1996; Jason et al., 1999; Pawlikowska et al., 1994; Steele et al., 1998; Wessely et al., 1998).

Zygosity Diagnosis

Zygosity 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 on 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 Analysis

Data were analyzed using PRELIS 2.12 and LISREL 8.12 (Jöreskog & Sörbom, 1993). Correlations between variables were calculated on the assumption that underlying each variable there 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 (Jöreskog and Sörbom, 1993).

Because of their skewed distributions, we have applied logarithmic transformations to the *in vivo* CMI measure (i.e. DTH skin response), as well as the *in vitro* measures of immune activation (IL4, IFN-γ and sCD23). 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), have been treated as categorical.

Significant twin correlations establish the fact that there is familial aggregation for the measures of interest. Our task, however, was to distinguish between the possible mechanisms by which this familial likeness may arise. One useful method was structural equation modelling as implemented in LISREL, Mx, or similar packages (Neale & Cardon, 1992). From this, one can conceive of three broad causes of variation, two of which (additive genetic influences 'G' and common environment 'C') make family members more alike than random pairs of individuals, and one of which (unique environmental experiences 'E') makes MZ twins and siblings different. The task then was to decide which combination of these three parameters provided the most parsimonious explanation for the observed pattern of MZ and DZ twin correlations.

The methods of structural equation modelling can be 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, with the first factor loading on all variables, the second factor loading on all but

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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 twin pairs were too small to estimate reliable asymptotic covariance matrices for use with 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 nonsignificant parameters, that is, by determining 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 sub-study was predominantly female (80%) with a mean age of 46.9 years (*SD* 12.1). By comparison, the full sample was 65% female, and slightly younger (44.8 for females, 42.7 for men), suggesting that the greater co-operativeness 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. In the larger study DSM III-R (American Psychiatric Assosication, 1987) diagnoses of major depression were determined and occurred in 14.8% (889/5,995) of the entire sample compared with 13.7% (34/248) of this sub-sample. As this rate of DSM-III-R depression is very similar to prevalences reported from other large community samples (Kessler et al., 1994) it suggests no major bias with regard to recruitment to this study which could be attributed to psychological variables.

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 (Hickie, Hickie et al., 1995) particularly for female subjects (males: 12% versus 3%; and, females: 21% versus 6%). Ranges for the *in vitro* immune measures were IL-4: 0–4707 [mean = 327.7, *SD* = 694] pg/ml; IFN-γ: 0–3335 [mean = 110.3, *SD* = 409.6] pg/ml; and, sCD23: 4.9-770 [mean = 770, *SD* = 66.7] pg/ml, with the percentages of nil scores for IL-4 and IFN-γ being 25.2% and 68.8% respectively.

Of the total sample (n = 248) 8.1% were categorized by the SOFA as fatigue cases (FAT), while 11.3% described current psychological distress with GHQ scores of 3 or more (DIS). There were no direct relationships, however, between these clinical phenotypes and the immune variables (for SOFA cases versus non-cases: IL4, 314 versus 365 pg/ml, *ns*; IFN- γ, 106 versus 112 pg/ml, *ns*; sCD23, 51.4 versus 63.5 pg/ml, *ns*; DTH sites, 1.8 versus 1.3, *ns*; and, for GHQ cases: IL4, 424 versus 353 pg/ml, *ns*; IFN- γ, 114 versus 111 pg/ml, *ns*; sCD23, 87.9 versus 59.3 pg/ml, *ns*; DTH sites, 1.5 versus 1.3, *ns*).

Complete data were available for 124 twin pairs, comprising 67 MZ female pairs, 12 MZ male pairs, 24 DZ female pairs, 5 DZ male and 16 DZ unlike sex pairs. These latter groups were too small for the calculation of polychoric correlations and had to be either discarded or pooled into total MZ versus total DZ groups. One concern in pooling over sexes within zygosity groups was that sex differences in means would inflate twin correlations. Since there were significant sex differences for two of the immune activation variables ($p < 0.05$ for IFN-γ and sCD23), sex was entered as a covariate in order to remove any such inflationary effect. Age is always a potential confounding factor in studies such as this, but in the present sample only negligible correlations with FAT, IL-4, IFN-γ and sCD23 (–0.04, 0.06, zero and 0.06 respectively) were found. Table 1 presents the phenotypic correlations between age, sex, fatigue, psychological distress and the immune variables. Interestingly, the *in vivo* DTH skin response was not correlated with the other three *in vitro* immune activation measures, which were themselves positively inter-correlated. With regard to the psychological variables, DIS showed no relationship to the *in vitro* measures, while FAT demonstrated only small correlations. As we had previously modelled the genetic and environmental determinants of DTH, FAT and DIS from this data set (Hickie, Bennett et al., 1999), and only the *in vitro* measures and FAT were correlated, we only included these variables in the models reported here.

Estimating Twin Correlations

Crosstwin-crosstrait correlations were estimated using PRELIS for the complete MZ (79 pairs) and DZ (45 pairs) twin groups, with sex (0 = female, 1 = male) as a categorical covariate. These correlations are shown in Table 2, with the

Table 1

Phenotypic Correlations of Age, Sex, Fatigue, Psychological Distress, Immune Responsiveness and Immune Activation Variables

	Age	Sex	Fatigue	GHQ	DTH	IL-4	IFN-γ	sCD23
Age	1.00							
Sex	–0.08	1.00						
Fatigue	–0.04	–0.06	1.00					
GHQ	–0.12	–0.11	0.43	1.00				
DTH	–0.05	0.34	0.07	–0.10	1.00			
IL-4	0.06	0.19	–0.12	0.03	0.01	1.00		
IFN-γ	0.00	0.22	–0.11	0.00	0.06	0.60	1.00	
sCD23	0.06	0.23	–0.07	0.03	0.07	0.48	0.78	1.00

Notes: Sex is scored 0–1 for females–males

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twin1-twin2 correlations for the four variables highlighted in bold. It can be seen that for FAT the MZ correlation is greater than twice its DZ counterpart (0.49 versus 0.16), indicating strong genetic control of familial aggregation for fatigue. On the other hand, it can be seen that the MZ correlations for the *in vitro* immune activation variables are only slightly greater than their DZ counterparts (0.69 versus 0.53, 0.51 versus 0.46, and 0.49 versus 0.42, for IL-4, IFN-γ and sCD23 respectively), indicating that shared family environment is an important source of familial resemblance in these variables.

Fitting Multivariate Genetic and Environmental Models

Our task, however, was 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 was on the covariation between fatigue and the *in vitro* measures of immune activation. In our sample, the phenotypic correlations between fatigue and the three immune activation variables were only –0.12, –0.11 and –0.07, respectively. It may seem, therefore, that there was 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 that 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 began by specifying a complete Cholesky decomposition for three sources of variance — additive genes, shared environment, and individual environment. We simplified this by dropping nonsignificant parameters, with the final model, shown in Figure 1, fitting no worse than the complete decomposition (likelihood ratio $\chi^2_9 = 2.4$).

The principal genetic features of this model are as follows. First, there is a common genetic factor (G1) accounting for 48% (i.e. 0.69^2) of the variance in fatigue, which also has a *decreasing* effect on the *in vitro* immune activation measures, accounting for 4%, 6% and 8% (-0.20^2, -0.25^2, -0.29^2) of the total variance in IL4, IFN-γ and sCD23, respectively. Second, there is also genetic variance specific for the *in vitro* immune measures (G2-G4) accounting for 26%, 1% and 10% (0.51^2, 0.11^2, 0.31^2) of the variance in IL4, IFN-γ and sCD23, respectively. Third, a shared environment common factor (C) influences the three immune activation measures, accounting for 32%, 37% and 26% of variance in IL4, IFN-γ and sCD23, respectively.

Individual environmental variance subsumes any errors of measurement, and so it is to be expected that the vertical paths from E1, E2, E3 and E4 to the corresponding first, second, third and fourth variables (FAT, IL4, IFN-γ and sCD23) would be large. However, most interesting are the positive paths from E1 to the immune activation variables, such that environmental influences which increase fatigue also increase IFN-γ and sCD23, although this influence is modest (2% and 1% of variance respectively). This appears to partly counterbalance the *negative* genetic correlations between increasing FAT and the immune activation variables.

Although the three immune activation variables are highly intercorrelated (Table 1), it appears that genetic factors (mediated through fatigue) make only a modest contribution to this association. That is, environmental factors (which may include exposure to infectious agents) are the main determinants of intercorrelation of the immune activation variables. These appear to be mainly environmental exposures shared by co-twins, as evidenced by the large 'C' factor in Figure 1. This may reflect childhood infection history when the twins were exposed to the same antigens. However, we also see that exposures unique to individuals account for some of the co-variation between immune activation measures (the E2 and E3 factors of Figure 1), possibly reflecting infection history in adult life (i.e. when most twins no longer live together) or other immunological disorders specific to the individual.

Conclusions

Considerable debate surrounds the relative etiological contribution of host versus environmental factors to the onset

Table 2

Polychoric Correlations for MZ (above diagonal) and DZ (below diagonal) Pairs for a Measure of Fatigue (FAT) and Immune Activation Variables for Twin1 and Twin2 (Co-twin correlations for each measure are shown in bold)

		Monozygotic (MZ) twins (79 pairs)							
		Twin 1				Twin 2			
		FAT	IL4	IFN-γ	sCD23	FAT	IL4	IFN-γ	sCD23
	FAT	1.00	–0.24	–0.04	–0.09	**0.49**	–0.18	–0.10	–0.07
Twin 1	IL4	–0.27	1.00	0.56	0.51	–0.13	**0.69**	0.50	0.45
	IFN-γ	–0.18	0.81	1.00	0.75	–0.19	0.40	**0.51**	0.38
	sCD23	–0.11	0.62	0.79	1.00	–0.16	0.36	0.50	**0.49**
	FAT	**0.16**	–0.11	–0.06	–0.22	1.00	–0.15	–0.07	0.05
Twin 2	IL4	–0.30	**0.53**	0.46	0.34	0.03	1.00	0.56	0.45
	IFN-γ	–0.33	0.40	**0.46**	0.42	–0.13	0.50	1.00	0.81
	sCD23	–0.39	0.41	0.41	**0.42**	–0.55	0.43	0.80	1.00
		Dizygotic (DZ) twins (45 pairs)							

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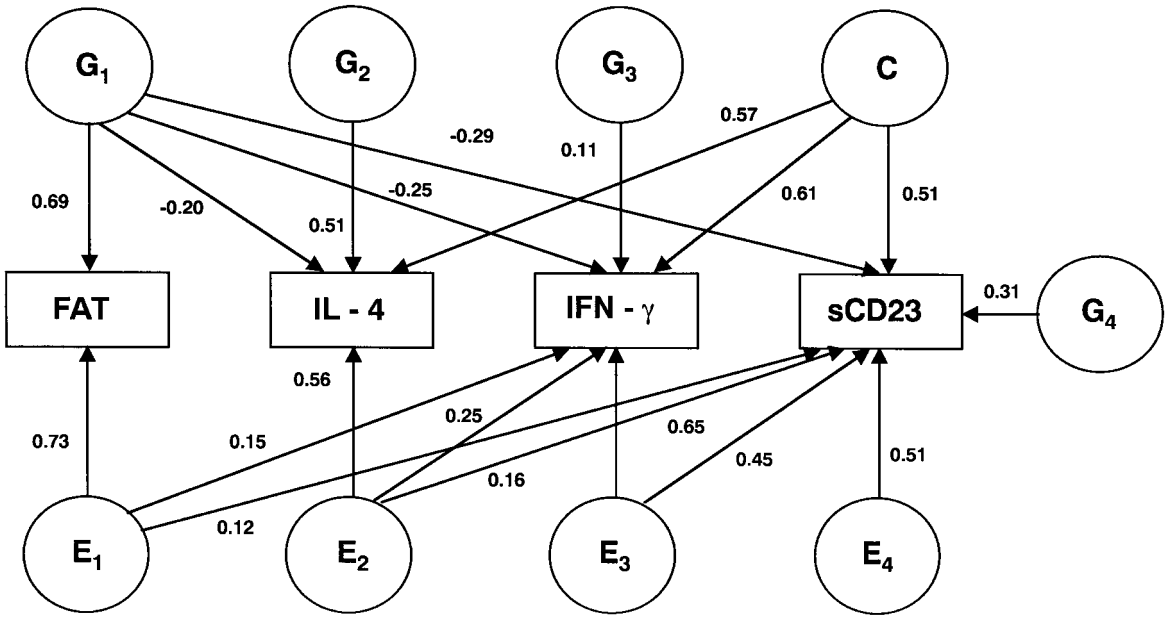

Figure 1

Path diagram showing latent genetic, shared environmental and individual environmental determinants (shown in circles) of the measured phenotypes (shown in squares) of fatigue ('FAT') and immune activation ('IL-4', 'IFN-γ' and 'sCD23'). G1, G2, G3 and G4 represent additive genetic factors, C is a shared environmental factor (influencing 'IL-4', 'IFN-γ' and 'sCD23') and E1, E2, E3 and E4 are unique environmental factors.

of PFS, and specifically CFS. The combination of the twin methodology with measures of immune regulation provides a unique opportunity to model the extent to which key genetic and/or environmental factors contribute to the observed phenotypes of psychological distress, fatigue, and immunological activation in a population-based sample. Such samples contain predominantly healthy individuals but also include individuals with past and/or current medical and psychological disorders. In this sample, almost 14% have a lifetime history of depression, 11% are current cases of psychological disorder and 8% have PFS. None of these particular phenotypes, however, were directly associated with evidence of altered cell-mediated immunity (either in the form of immune activation and/or reduced DTH skin response).

The specific findings from this study are that: (i) those additive genetic factors which *increase* the expression of fatigue are also associated with *less* evidence of immune system activation; (ii) shared environmental factors (probably reflecting shared childhood experiences) make a significant contribution to the familial aggregation of measures of immune activation; and, (iii) unique environmental factors which *increase* fatigue are associated with more evidence of immune activation. Together with our earlier modelling of the relationships between psychological distress, fatigue and other *in vivo* measures or cell-mediated immunity from largely the same twin sample (Hickie, Bennett et al., 1999), we can conclude that for members of the community:

- any genetic determinant (other than gender) of the immune factors measured is substantially due to the genetics of psychological distress. Unexpectedly, this liability appears to result in an increased expression of distress and enhanced immunity;
- shared environmental factors make little contribution to the expression of psychological distress or fatigue, but are key determinants of current immunity. This suggests that while childhood environments contribute little directly to current psychological state, other factors, such as exposure to common antigenic stimuli, including viruses, explain much of the observed similarity in immune response between family members; and,
- unique environmental experiences (such as infection or stress affecting only one twin) result in increased distress and prolonged fatigue and are associated with altered immune function. These findings are generally consistent with current behavioral notions of psychoimmunologic relationships (Ader et al., 1991).

These results also have direct implications for possible risk factors for PFS in the community. First, those persons with a high genetic liability to psychological distress (which may well include fatigue symptoms) will also be characterized by normal immune function. This may appear contrary to current notions of the ways in which psychological disorder is associated with immune dysfunction (Ader et al., 1991) and increased risks to other medical disorders (Bruce et al., 1994; Harris and Barraclough, 1998). Importantly, this finding refers only to the genetic liability for expression of these phenotypes, whose actual presence or absence will be determined by interplay with a wide range of other environmental risk and/or protective factors. These findings are not consistent, however, with the view that a common genetic vulnerability (e.g. 'neuroticism' or other similar

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concepts) underpins any association between psychological distress, fatigue and immune dysfunction.

Second, since shared environmental risk factors emerge as key predictors of current immune function (but not current psychological state) it is likely that some key aspects of the childhood environment, notably childhood exposure to common pathogens such as EBV, have lifelong implications. These findings are certainly consistent with the hypothesis that evidence of immune activation in adults may be due to re-activation of past viral elements rather than exposure to new infective agents.

Third, as environmental factors that increase psychological distress and fatigue are themselves associated with immune dysfunction, a number of possible pathogenic pathways are left open. For example, intercurrent viral infection may increase fatigue and reduce immune responsiveness. Similarly, a major stressor in the adult life of one twin may result in psychological distress, or prolonged fatigue, that is also associated with immune dysfunction.

On the basis of multivariate modelling of clinical phenotypes we had previously suggested that prolonged fatigue syndromes, including CFS, are likely to be heterogeneous in origin (Hickie, Lloyd, Hadzi-Pavlovic et al., 1995). Our twin studies now indicate that while some genetic factors predispose to psychological distress and fatigue, other genetic factors predispose to fatigue alone (Hickie, Bennett et al., 1999; Hickie, Kirk et al., 1999). Notably, those fatigue states which are associated with immune activation (results from this study) and impaired DTH skin response (Hickie, Bennett et al., 1999) are most likely to be the consequence of current *environmental* rather than genetic factors. Such environmental factors could include a wide variety of physical agents and/or psychological stressors. Importantly, it suggests that the specific characteristics of those agents (or stressors) are of etiologic significance. For example, specific viral or non-viral infective agents, or certain interpersonal events (e.g. death of a spouse, marital disharmony) could result in prolonged fatigue and immune activation despite the presence of genetic factors which appear to have opposite effects. The actual clinical phenotype will ultimately depend on the interplay between these various specific environmental and genetic factors. Prospective studies of specific infective agents provide an important methodology for further clarification of the etiological contribution of each agent (Bennett et al., 1998; White, Grover et al., 1995), while alternative genetic methods may lead to clarification of the specific genetic factors involved in the genesis of some other types of prolonged fatigue states, notably CFS (Buchwald et al., 1999).

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