Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Brain, Behavior, and Immunity 26 (2012) 552–558

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Named Series: Fatigue, Brain, Behavior, and Immunity

Genetic associations of fatigue and other symptom domains of the acute sickness response to infection

B. Piraino [a], U. Vollmer-Conna [b], A.R. Lloyd [a,*]

[a] Inflammation and Infection Research Centre, School of Medical Sciences, University of New South Wales, Australia
[b] School of Psychiatry, University of New South Wales, Australia

ARTICLE INFO

Article history:
Received 11 August 2011
Received in revised form 2 December 2011
Accepted 19 December 2011
Available online 29 December 2011

Keywords:
Acute sickness response
Fatigue
Genetic association
Cytokine
Polymorphisms

ABSTRACT

The acute sickness response to infection is a conserved set of changes in physiology and behaviour, featuring fever, fatigue, musculo-skeletal pain, disturbed mood, and cognitive difficulties. The manifestations differ somewhat between individuals, including those infected with pathogens which do not have genetic variability – suggesting host determinants.

Principal components analysis (PCA) was applied to acute phase, self-report symptom data from subjects in the Dubbo Infection Outcomes Study ($n = 296$) to empirically derive indices of *fatigue*, *pain*, *neurocognitive difficulties*, and *mood disturbance*, as well as overall illness *severity*. Associations were sought with functional single nucleotide polymorphisms (SNPs) in the cytokine genes, interleukin (IL)-6, tumour necrosis factor (TNF)-α, interferon (IFN)-γ, and IL-10.

The summed individual symptom indices correlated with overall *severity* and also with functional status. The relative contribution of individual symptom domains to the overall illness was stable over time within subjects, but varied between subjects with the same infection.

The T allele of the IFN-γ +874 T/A SNP was associated with increased *fatigue* ($p = 0.0003$; OR: 3.3). The C allele of the IL-10 −592 C/A SNP exerted a protective effect on *neurocognitive difficulties* ($p = 0.017$; OR: 0.52); while the A allele for the IL-10 −592 SNP was associated with increased *mood disturbance* ($p = 0.044$; OR: 1.83), as was the G allele of the IL-6 −174 G/C SNP ($p = 0.051$; OR: 1.83).

The acute sickness response has discrete symptom domains including *fatigue*, which have unique genetic associations. These data provide novel insights into the pathophysiology of fatigue states.

1. Introduction

The initial immune response to infection by a wide range of pathogens triggers a set of stereotyped clinical manifestations including fever, fatigue, hypersomnia, hyperalgesia, anorexia, disturbed mood, and cognitive difficulties – termed the acute sickness response. The changes in physiology and behaviour which characterise the acute sickness response are also well documented in a wide range of vertebrate species indicating strong evolutionary conservation of the phenomenon (Hart, 1988; Konsman et al., 2002). This response is initiated in the periphery by elements of the activated immune system, and is mediated by the action of pro-inflammatory cytokines and other acute phase proteins. The acute sickness response is ultimately translated into central nervous system events via activation of resident microglial cells, secondary production of mediators, and ultimately altered neural transmissions (Vollmer-Conna, 2001).

We have previously shown that during the acute sickness response, the severity of the illness correlates with the production of pro-inflammatory cytokines in the periphery (Vollmer-Conna et al., 2004). However, in relation to any given acute infection, the illness manifestations cannot simply be equated to the acute sickness response, as the symptoms and signs are also attributable to local pathogen-mediated tissue injury. For example in primary *Varicella-zoster Virus* (VZV) infection, the vesicular skin rash is due to viral replication and cytolysis of epithelia and other skin cells, as well as local immunopathology (Arvin, 2001), whereas the systemic features of chickenpox such as fever, loss of appetite, and malaise are likely to be purely immunologically driven.

Although the acute sickness response consists of a relatively uniform set of symptoms and behaviours, it is very evident in clinical practice that there is significant variability in the frequency and severity of specific illness manifestations experienced by one individual in comparison to another during acute infection with the same pathogen, including infection with microorganisms which do not feature significant genetic variability. This is illustrated by case series of patients with Severe Acute Respiratory Syndrome (SARS) in which the reported prevalence of systemic illness

* Corresponding author. Address: Inflammation and Infection Research Centre, School of Medical Sciences, University of New South Wales, Sydney 2052, Australia. Tel.: +61 02 9385 2534; fax: +61 02 9385 1514.
E-mail address: a.lloyd@unsw.edu.au (A.R. Lloyd).

0889-1591/$ - see front matter Crown Copyright © 2012 Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2011.12.009

manifestations such as myalgia, range from 7% to 50% (Fan et al., 2006; Muller et al., 2006), and approximately 30% of patients report chills and rigors, whereas the remainder do not (Booth et al., 2003; Wang et al., 2004; Fan et al., 2006; Muller et al., 2006). As analysis of variability in the genome of the SARS Corona virus (SARS CoV), has revealed very limited alterations between isolates (Ruan et al., 2003; Salemi et al., 2004), it is unlikely that these variations in individual symptoms relate to changes in the pathogen.

We have previously found that the overall *severity* of the acute sickness response to several different pathogens is associated with functional polymorphisms in pro-inflammatory cytokine genes (Vollmer-Conna et al., 2008), indicating a strong host genetic influence on the response. Given the observed variations within the detailed pattern of the acute sickness response to a given pathogen, we therefore sought to test the hypothesis that the pathophysiological basis of relatively discrete 'symptom domains' within the overall illness complex may also be differentially influenced by genetic polymorphisms. This notion of symptom domains is analogous to the concept of 'endophenotypes', which arose relatively recently out of the substantive challenges of pathophysiological research into the common, complex, and heritable disorders of mental health, including major depression and bipolar disorder (Cannon and Keller, 2006). The intent of this concept was to divide behavioural symptoms into recognisable and stable characteristics with a likely (or proven) genetic association. The assumptions underlying the concept are firstly that genes influencing susceptibility to mental disorders are likely to be mediated via multiple neurotransmitter systems affecting multiple neurobehavioural processes such as attention, memory, stress sensitivity, and emotional regulation (Cannon et al., 2001); and secondly, that identification of genetic or other biological correlates with an endophenotype is likely to be less complex than that of the illness phenotype overall (Gottesman and Gould, 2003). Although this field is still in its infancy, one of the best examples is in the context of studies of suicidal behaviour, in which impulsive–aggressive behaviour is an endophenotype, and with which polymorphisms in the serotonin receptor-1B gene (5-HT_{1B}) have been associated (Zouk et al., 2007).

In this study, the endophenotype concept has been applied to comprehensive clinical datasets describing symptom domains of the acute sickness response associated with both viral and non-viral pathogens from a prospective cohort study of infection outcomes (Hickie et al., 2006)and validation of the endophenotypes was completed by demonstration of associations with functional polymorphisms in cytokine genes.

2. Materials and methods

2.1. Subjects

A total of 296 unambiguously Caucasian, adult subjects [mean age 34.2 years, 49% women], with complete data sets, were selected from participants in the Dubbo Infection Outcomes Study (DIOS, $N = 396$) (Hickie et al., 2006). Subjects were recruited into DIOS, after written informed consent, subsequent to presentation to their family physician and serological testing indicated acute Epstein Barr Virus (EBV), Ross River Virus (RRV), or Q fever infection. All participants were recruited shortly after the acute febrile phase of their illness, and no greater than 6 weeks after the onset of symptoms. The relevant institutional review boards approved this study.

2.2. Self-report measures

After enrolment participants were followed up at 2–3 weeks, 4–6 weeks, and at three monthly intervals thereafter until 12 months post infection. At enrolment, the study nurse conducted a baseline medical and psychiatric interview and recorded the date of onset of symptoms, as well as demographic characteristics including a detailed description of the subject's ethnicity. At each visit a wide range of physical and psychological symptoms of acute illness were assessed using two self-administered questionnaires; the Somatic and Psychological Health Report (SPHERE) (Hickie et al., 2001) and the Physical Symptoms Checklist (PSC) (Hickie et al., 2006) – (see online Supplementary material for copies of the questionnaires). The impact of the illness on functional capacity was recorded at each time point using the Brief Disability Questionnaire (Von Korff et al., 1996).

2.3. Endophenotype indices

Principal components analysis (PCA) of baseline symptom data collected by the SPHERE and PSC (51 symptom items) was used to derive indices of the individual symptom domains or endophenotypes of the acute sickness response. An initial analysis of symptom question items with a report rate >30% revealed that the items fell into four broad symptom domains: *pain*, *fatigue*, *neurocognitive difficulties*, and *mood disturbance*. Items relevant to each endophenotype were further selected by face validity and included in the initial PCA for each domain. For symptom items which were duplicated (e.g., SPHERE Q1 – 'headache' and PSC Q7 – 'had a headache'), the item with the lower loading score on the first component in the PCA was removed. The initial PCAs were then refined by sequential removal of non-salient (loading score <0.4) symptom items and ensuring that the first component accounted for >30% of the variance. The optimal solutions contained items uniquely relevant to the designated symptom domain: *mood disturbance* – 6 items (SPHERE Q's 2, 7, 18, 19, 20, 21, 24, 34), *pain*-8 items (SPHERE Q's 4, 5, 10, 11, 12, 31; PSC Q's 9, 10), *fatigue* – 6 items (SPHERE Q's 6, 13, 14, 15, 26; PSC Q15), and *neurocognitive difficulties* – 3 items (SPHERE Q's 3, 16, 27) (see online Supplementary material for individual item loading scores).

2.4. Illness severity index

The baseline symptom data collected by the SPHERE and PSC were also used to derive an overall index of illness *severity* by PCA in order to relate the contribution of the individual symptom domains to the illness complex as a whole. In preliminary data preparation, symptom items with a low report rate (<30%) were excluded (17 items). PCA was then conducted on the remaining 34 symptom items. In determining the optimal solution symptom items were then sequentially removed until all remaining items had a loading score >0.4 (salient variables) and the first component accounted for >30% of the variance, allowing for an index based on 25 items which encapsulated a spectrum of severity (see online Supplementary material for individual item loading scores).

2.5. Selection of comparison groups

One hundred subjects with scores constituting the top and bottom thirds of the distribution of the empirically-derived symptom domains were selected to optimally represent the extremes of each domain. Subjects with equivalent scores at the cut-off ($N = 100$) were included into the respective extreme. The comparison groups were labelled for subsequent analysis as 'high' – to represent a high symptom endophenotype and as 'low' to represent the low symptom endophenotype.

2.6. Duration of illness

The SOMA subscale of the SPHERE which records somatic symptoms (Hadzi-Pavlovic et al., 2000), was used to measure illness duration (Hickie et al., 2006). A conservative measure of illness duration was designated as the time in days of continuous SOMA positivity (score $\geqslant 3$ out of a possible 12) from the symptom onset date plus half the time between the last SOMA positive date and first SOMA negative (<3) date. Illness duration was not derived for 18 subjects due to incomplete longitudinal data sets.

2.7. DNA extraction and genotyping

DNA was extracted from peripheral blood mononuclear cells (PBMCs) (Wizard DNA kit; Promega) and quantified using NanoDropR ND-1000 (Biolab), before the quality was verified by agarose gel electrophoresis. Genomic DNA was sent to the Australian Genome Research Facility (AGRF) for genotyping by Sequenom MassARRAY® of functional polymorphisms in pro- and anti-inflammatory cytokines implicated in the acute sickness response, including interferon (IFN)-γ (rs2430561), tumour necrosis factor (TNF)-α (rs1800629) and IL-6 (rs18000795) genes, as well as interleukin (IL)-10 (rs1800896 and rs1800872).

2.8. Statistical analysis

Statistical analyses were performed using SPSS for Windows version 15 (SPSS Inc., Chicago, IL, USA). Factor analysis (principal component method) was used to derive the illness severity and symptom domain (endophenotype) indices. Multiple regression analysis was used to demonstrate independence of the overall sickness response from age, sex and infection type. Logistic regression analyses then served to determine the relative impact of specific cytokine genotypes and endophenotype outcomes with relevant covariates (age, sex, and infection type) included in the models.

Correlation analyses were used to assess bi-variate relationships. Analyses of the consistency of the endophenotype-scores over time (referred to as temporal stability) was additionally examined via intraclass correlation coefficients. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Symptom domains of the acute sickness response

Four symptom domains, or endophenotypes, within the illness complex were derived by PCA. Within each symptom domain, comparison groups were derived to optimally represent the extremes (the top and bottom tertiles) of the distribution of the PCA scores. Table 1 summarises the characteristics of the extreme groups.

3.2. Severity of the acute sickness response

The principal component reflecting the overall illness severity featured the fundamental symptoms of the acute sickness response (e.g., fatigue, musculoskeletal pain, cognitive disturbance, sleep disturbance). Multiple regression analysis of the scores verified that the index was not predicted by age, sex or infection type ($R^2_{adj} = 0.012$, $F(3.281) = 2.11$, $p = 0.1$). Two disability measures from the BDQ questionnaire were used to validate the illness severity index. Positive correlations were found between the illness *severity* score and the reported 'number of days spent in bed' ($r(225) = 0.121$, $p = 0.001$), and 'number of days spent away from usual activities' ($r(224) = 0.33$, $p < 0.001$).

3.3. Relative severity of symptom domains

Initial validation of the individual symptom domain indices was sought by correlation of the sum of these indices with the overall illness *severity* scores for each subject. Spearman's correlations between the percentile ranks for each subject in illness *severity* and the summed percentile ranks of the symptom domains revealed a strong correlation ($r_s(294) = 0.971$, $p < 0.0001$). The summed endophenotype scores correlated with the measures of disability including 'number of days spent in bed' ($r(225) = 0.20$, $p = 0.002$), and 'number of days spent away from usual activities' ($r(224) = 0.31$, $p < 0.001$).

Further investigation of the composition of the illness complex revealed several interesting findings. As depicted in Fig. 1, the overall illness *severity* varied between subjects with the same infection, as well as across infection sub-cohorts. Secondly, within a given infection group, although overall illness *severity* may have been comparable in some subjects, the relative contributions from the individual symptom domains making up the illness complex varied between these subjects.

3.4. Temporal stability of symptom patterns

The observed variation in the severity of individual symptom domains, suggested that each individual's acute sickness response may be dominated by one or more symptom domains over another. If this is host determined, in subjects with prolonged illness this may be expected to be temporally stable. To investigate this notion, indices for all subjects ($N = 168$) for whom data were available for at least 3 months were derived at 2 weeks, 6 weeks, and 3 months post enrolment. The relative severity and stability of each symptom domain is illustrated in four subjects with persisting symptoms in Fig. 2. Fig. 2A reveals an 'equally' distributed illness complex which was stable over time; while Fig. 2B–D show illness responses that were dominated by *pain*, *mood disturbance*, and *fatigue* respectively. The stability of symptom dominance across time for each endophenotype was reflected by significant correlations in scores from adjacent assessment points. In addition, the overall consistency of scores within endophenoptypes across all time points was evidenced by significant intraclass correlations (see Table 2 for both).

Table 1
Characteristics of subjects in the high and low extreme groups for each of the symptom domains.

Severity	Fatigue		Pain		Mood disturbance		Neurocognitive difficulties	
	High	Low	High	Low	High	Low	High	Low
No. of subjects	100	102	100	102	100	100	104	130
Mean age in years (±SD)	32.3 (14.6)	37.4 (16.1)	36.1 (13.2)	32.5 (15.8)	31.7 (13.3)	36.5 (15.9)	33.5 (14.4)	35 (15.4)
M:F ratio	45:54	63:32	52:48	49:42	42:58	59:34	44:59	76:47
Infection type (EBV:RRV:QF)	51:23:26	37:41:24	34:40:26	60:13:30	54:21:25	45:31:24	49:23:32	57:43:30

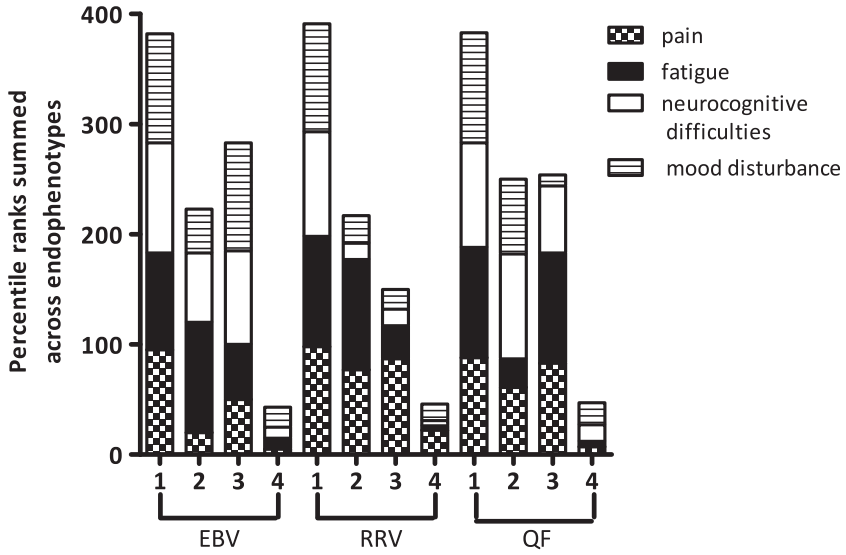

Fig. 1. Relative severity of symptom domains of the acute sickness response. Summed rank scores of the four individual symptom domains demonstrated variations in overall illness *severity*, as variations in the relative severity of individual symptom domains between subjects within the one infection group. For instance, RRV subjects 2 and 3 have comparable overall illness *severity* as well as comparable severities for each of the symptom domains. By contrast, EBV subjects 2 and 3, and QF subjects 2 and 3 have comparable overall illness *severity*, but varied severities the of the individual symptom domains which make up the illness complex.

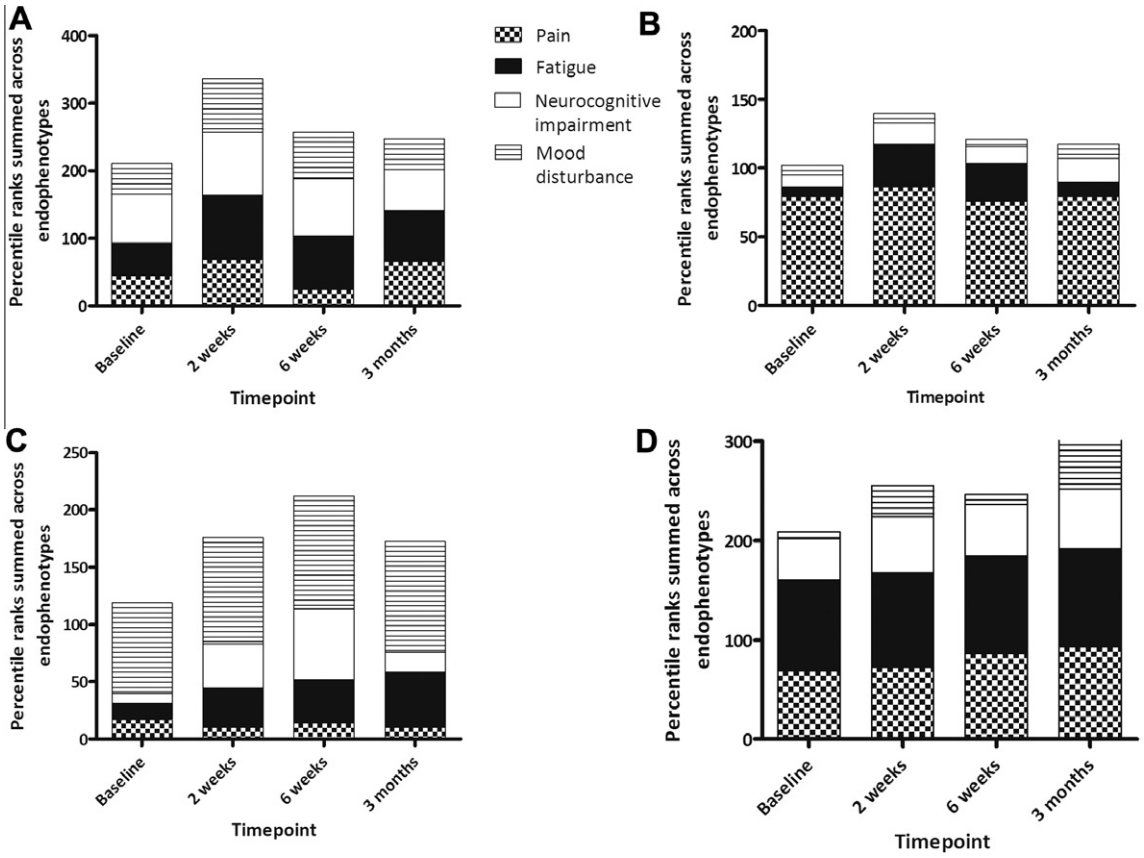

Fig. 2. (A–D) Temporal stability of dominant symptom domains. The four stacked column graphs illustrate stable patterns of symptom domain dominance in selected subjects with prolonged illness after acute infection, including an equally distributed symptom complex (A), and symptom complexes dominated by *pain* (B), *mood disturbance* (C), and *fatigue* (D).

3.5. Association of cytokine genotypes with symptom domain severity

Genotypes for all candidate SNPs were in Hardy–Weinberg equilibrium (all $p > 0.2$). Regression modelling to predict extreme scores in the *fatigue* domain produced a highly significant solution [$\chi^2(5) = 30.1$; $p < 0.001$] which indicated that the putative high cytokine producing T allele of the IFN-γ +874 T/A SNP was the best predictor of severe fatigue [$p < 0.001$; OR: 3.6 (95% confidence interval (CI): 1.8–7.2]. In addition, females were more likely grouped in the high fatigue extreme [$p = 0.01$; OR: 2.3 (95% CI:

Table 2
Consistency measures for scores obtained for each of the symptom domains across four assessment times ($N = 168$).

	Fatigue *r*	Pain *r*	Mood disturbance *r*	Neurocognitive difficulties *r*
Baseline–week 2	0.60	0.66	0.60	0.50
Week 2–week 6	0.70	0.76	0.80	0.63
Week 6–3 months	0.61	0.67	0.62	0.58
ICC (95% CI)	0.56 (0.49–0.64)	0.62 (0.55–0.69)	0.55 (0.47–0.63)	0.47 (0.40–0.56)

r = Pearson correlations, ICC = intraclass correlation coefficient; CI = confidence interval.
P values for all statistics presented in this table are <0.001.

1.2–4.6], while individuals with RRV infection were less likely to suffer from extreme fatigue [$p = .02$; OR: 0.35 (95% CI: 0.15–0.85]. The best solution for extreme scores in the pain symptom domain [$\chi^2(5) = 21.6$; $p = 0.001$] identified RRV infection as the best and only predictor for high pain severity [$p = 0.004$; OR: 3.5 (95% CI: 1.5–8.1]; this model also included the IL-6 SNP, however the association with the high producing G allele failed to reach significance ($p = 0.08$).

The model for *neurocognitive difficulties* [$\chi^2(5) = 21.6$; $p = 0.001$] showed that female sex was the strongest predictor for neurocognitive difficulties during acute illness [$p = 0.001$; OR: 2.9 (95% CI: 1.6–5.4]. In contrast, subjects homozygous for the high producing C allele of the IL-10 −592 C/A SNP were significantly less likely to experience *neurocognitive difficulties* [$p = 0.005$; OR: 0.44 (95% CI: 0.25–0.78], as were those with RRV infections [$p = 0.03$; OR: 0.42 (95% CI: 0.19–0.90]. The best solution for severe symptoms in the mood domain [$\chi^2(6) = 29.1$; $p < 0.001$] also identified female sex as the strongest risk for severe mood disturbance during acute infection [$p < 0.001$; OR: 3.9 (95% CI: 1.9–7.9]. This model included both the IL-6 −174 G/C and the IL-10 −592 C/A SNPs. The putative high producing G allele of the IL-6 −174 G/C SNP was associated with greater mood disturbance [$p = 0.027$; OR: 2.2 (95% CI: 1.1–4.4], while the IL-10 −592 C allele exerted a protective effect [$p = 0.014$; OR: 0.44 (95% CI: 0.23–0.85]. Moreover, those individuals who had both the G allele of the IL-6 −174 and an A allele for IL-10 −592 (i.e., a high IL-6 low IL-10 combination) had a greatly increased risk for mood disturbance [$p = 0.004$; OR: 5.3 (95% CI: 1.7–16.2].

No other allelic combinations were associated with an increase in the strength of the association with the endophenotypes.

4. Discussion

This is the first systematic phenotypic and immunogenetic analysis of the different components of the acute sickness response in the setting of natural infection in humans. The illness *severity* phenotype described here was ubiquitous across infection types, yet also highlighted heterogeneity within the varied pattern and duration of the response. This heterogeneity was further explored via empirically-derived indices describing endophenotypes or symptom domains within the overall illness complex. These indices were validated by demonstration of significant differential associations with polymorphisms in cytokine genes previously implicated in the acute sickness response (Vollmer-Conna et al., 2008).

As expected, there was a robust correlation between illness *severity* and the level of reported disability in the acute illness, supporting the validity of this measure in the clinical setting. Consistent with previous analyses in the DIOS cohort (Hickie et al., 2006; Vollmer-Conna et al., 2008), the illness *severity* phenotype was not dependent on age, sex or infection subtype. This finding is concordant with the recognised spectrum of clinical illness severity for each of the pathogens studied here. Infectious mononucleosis due to primary EBV infection occurs in 25–50% of adolescents and adults who become infected – the remainder have mild or subclinical illness (Vetsika and Callan, 2004); similarly the subclinical: clinical ratio for RRV infection ranges from 1.2–3.0 (Harley et al., 2001); and almost 60% of QF infection cases in adults are asymptomatic (Maurin and Raoult, 1999).

It is also evident in clinical practice that there is significant variability in the pattern of symptoms experienced by one individual in comparison to another during acute infection with the same pathogen. For example, in infectious mononucleosis due to EBV infection the prevalence of sore throat, headache and malaise ranged from 70–88%, 37–55%, and 43–76%, respectively across several studies (Marrie, 2000; Mylonas et al., 2002; Mandell et al., 2005). Consistent with these observations, when the acute sickness response was systematically divided into symptom domains, it became evident that the prevalence and severity of each endophenotype varied between subjects infected with the same pathogen.

Although the illness complex as a whole and its *severity* were independent of demographics, females were over-represented in the high severity groups for *fatigue*, *neurocognitive difficulties*, and *mood disturbance*. This finding is consistent with previous studies showing that women are more likely to report both physical and psychological symptoms than men (Gijsbers van Wijk et al., 1991; Barsky et al., 2001), with one study demonstrating that most physical symptoms are typically reported at least twice as commonly by women in a healthy, disease-free setting (Kroenke and Spitzer, 1998). It has been proposed that as a result of their reproductive role, women are more attentive to bodily changes (Gijsbers van Wijk et al., 1991; Barsky et al., 2001), and/or have heightened interoceptive awareness which allows them to sense internal disruptions more easily; and may thus be more likely to report symptoms (Gijsbers van Wijk et al., 1991; Barsky et al., 2001). Gender socialisation roles may additionally impact on this phenomenon (Gijsbers van Wijk et al., 1991; Barsky et al., 2001). Interestingly, although no sex difference in seroprevalence or clinical illness rates are reported for EBV, men are more commonly symptomatic with acute QF than women (at a ratio of 2.5:1), despite equal seroprevalence (Tissot Dupont et al., 1992). Studies in a mouse model of QF suggest this may be attributable to the effects of oestrogens on the host response (Leone et al., 2004).

Administration of recombinant cytokines, or activation of cytokine production by immunogenic stimulation (e.g., via administration of lipopolysaccharide) produces the symptom complex characteristic of the acute sickness response (Dantzer, 2004). In particular, the pro-inflammatory cytokines, IL-1β, IL-6, and TNF-α have been most studied and each shown to induce fever, fatigue, anhedonia, anorexia, depressed mood and to perturb cognitive functions. On the other hand, the anti-inflammatory cytokine, IL-10 has been shown to inhibit the symptoms of the acute sickness response (Krzyszton et al., 2008). In our previous study in DIOS demonstrated strong correlations between symptom severity during the acute illness and *ex vivo* production of IL-1β and IL-6 (Vollmer-Conna et al., 2004). In particular, symptoms of mood disturbance were associated with IL-6 production, whereas fatigue

and other physical symptoms were better correlated with IL-1 production, raising the suggestion that individual cytokines may influence the generation of different components of the symptom complex.

We have previously shown significant associations between more severe acute infective illness and protracted illness duration, with polymorphisms in the IFN-γ and IL-10 genes (Vollmer-Conna et al., 2008). The data presented here specifically implicate the high-producing T allele of the IFN-γ gene in the production of *fatigue*. This allele is associated with increased binding of transcription factors to a putative NF-κB site (Pravica et al., 1999, 2000), resulting in increased cytokine production. Patients undergoing treatment with recombinant IFN-γ report symptoms typical of the acute sickness response, although only fever, headaches and chills, but not self-reported fatigue or other symptoms have been found to be more frequent in the treated subjects than those receiving placebo in controlled trials (Gallin et al., 1991; Raghu et al., 2004). However, only brief self-report items, rather than systematic delineation of symptom domains, was undertaken in the adverse event reporting in these trials.

We have also previously shown a significant association between the A allele of the IL-10 −592 C/A SNP with increased illness *severity*. In the present study further associations were demonstrated with greater *mood disturbance* and increased *neurocognitive difficulties*. The A allele of this SNP has been associated with decreased production of IL-10 and a bias towards pro-inflammatory responses (Turner et al., 1997; Kung et al., 2010). IL-10 knockout mice feature a worsened acute sickness response upon challenge with lipopolysaccharide, notably increased 'fatigue' evidenced by reduced psychomotor coordination in a running test and shortened time to volitional fatigue (Krzyszton et al., 2008). In addition, increased 'depression-like' changes in behaviour has been observed in these mice, evidenced by decreased latency to immobility and longer immobilization periods in a forced swimming test – changes that were reversible by the administration of IL-10 (Mesquita et al., 2008).

Carriers of the allele correlating with increased production of IL-6 (−174 G) had greater *mood disturbance* in the data reported here. This finding is particularly interesting as a substantive body of literature has documented elevated serum levels of IL-6 in patients with major depression (Maes et al., 1997; Kubera et al., 2000; Bob et al., 2010; Dowlati et al., 2010). In combination these data suggest shared mechanisms underpinning both the typically short-lived mood disturbance in the context of acute infection, and the more substantive and sustained phenomenon of major depression.

We have documented significant and synergistic effects of a genetically-determined imbalance in pro- and anti-inflammatory cytokine production on the severity and duration of the acute sickness response (Vollmer-Conna et al., 2008). In the present study, this synergistic association was only evident in relation to *mood disturbance* and the IL-6/IL-10 SNP combination, potentially suggesting that the other endophenotypes are more specifically associated with individual cytokines.

The systematic analysis of the spectrum of symptoms of the acute sickness response described here supports the concept of a universal, yet somewhat heterogeneous, host phenomenon made up of coherent, temporally stable symptom domains or endophenotypes. Further exploration of the genetic associations with these endophenotypes is likely to provide unique insights into the more sustained and severe disorders which feature similar symptoms, including post-infective fatigue syndrome and major depression.

Acknowledgments

We appreciate the support of the general practitioners, the public health unit, and the diagnostic pathology services in the Dubbo region and the enduring cooperation of the participants in the research. This project was funded in part by National Health and Medical Research Council of Australia (NHMRC) Project Grants (157092 and 157062), Mason Foundation, and a Cooperative Research Agreement with the US Centers for Disease Control and Prevention (U50/CCU019851-01). AL is supported by a NHMRC Practitioner Fellowship (510246).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbi.2011.12.009.

References

Arvin, A. M., 2001. Varicella-zoster virus. Fields Virology. D. M. Knipe and P. M. Howley. Lippincott-Raven, Philadelphia. 2, 2731–2768.
Barsky, A.J., Peekna, H.M., Borus, J.F., 2001. Somatic symptom reporting in women and men. J. Gen. Intern. Med. 16, 266–275.
Bob, P., Raboch, J., Maes, M., Susta, M., Pavlat, J., Jasova, D., Vevera, J., Uhrova, J., Benakova, H., Zima, T., 2010. Depression, traumatic stress and interleukin-6. J. Affect. Disord. 120, 231–234.
Booth, C.M., Matukas, L.M., Tomlinson, G.A., Rachlis, A.R., Rose, D.B., Dwosh, H.A., Walmsley, S.L., Mazzulli, T., Avendano, M., Derkach, P., Ephtimios, I.E., Kitai, I., Mederski, B.D., Shadowitz, S.B., Gold, W.L., Hawryluck, L.A., Rea, E., Chenkin, J.S., Cescon, D.W., Poutanen, S.M., Detsky, A.S., 2003. Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. JAMA 289, 2801–2809.
Cannon, T.D., Keller, M.C., 2006. Endophenotypes in the genetic analyses of mental disorders. Annu. Rev. Clin. Psychol. 2, 267–290.
Cannon, T.D., Gasperoni, T.L., van Erp, T.G., Rosso, I.M., 2001. Quantitative neural indicators of liability to schizophrenia: implications for molecular genetic studies. Am. J. Med. Genet. 105, 16–19.
Dantzer, R., 2004. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. Eur. J. Pharmacol. 500, 399–411.
Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., Lanctot, K.L., 2010. A meta-analysis of cytokines in major depression. Biol. Psychiatry 67, 446–457.
Fan, C.K., Yieh, K.M., Peng, M.Y., Lin, J.C., Wang, N.C., Chang, F.Y., 2006. Clinical and laboratory features in the early stage of severe acute respiratory syndrome. J. Microbiol. Immunol. Infect. 39, 45–53.
Gallin, J.I., Malech, H.L., Weening, R.S., et al., 1991. A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease. The International Chronic Granulomatous Disease Cooperative Study Group. N. Engl. J. Med. 324, 509–516.
Gijsbers van Wijk, C.M., van Vliet, K.P., Kolk, A.M., Everaerd, W.T., 1991. Symptom sensitivity and sex differences in physical morbidity: a review of health surveys in the United States and The Netherlands. Women Health 17, 91–124.
Gottesman, I.I., Gould, T.D., 2003. The endophenotype concept in psychiatry: etymology and strategic intentions. Am. J. Psychiatry 160, 636–645.
Hadzi-Pavlovic, D., Hickie, I.B., Wilson, A.J., Davenport, T.A., Lloyd, A.R., Wakefield, D., 2000. Screening for prolonged fatigue syndromes: validation of the SOFA scale. Soc. Psychiatry Psychiatr. Epidemiol. 35, 471–479.
Harley, D., Sleigh, A., Ritchie, S., 2001. Ross River virus transmission, infection, and disease: a cross-disciplinary review. Clin. Microbiol. Rev. 14, 909–932, table of contents.
Hart, B.L., 1988. Biological basis of the behavior of sick animals. Neurosci. Biobehav. Rev. 12, 123–137.
Hickie, I., Davenport, T.A., Hadzi-Pavlovic, D., Koschera, A., Naismith, S.L., Scott, E.M., Wilhelm, K.A., 2001. Development of a simple screening tool for common mental disorders in general practice. Med. J. Aust. 175, S10–S17.
Hickie, I., Davenport, T., Wakefield, D., Vollmer-Conna, U., Cameron, B., Vernon, S.D., Reeves, W.C., Lloyd, A., 2006. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. BMJ 333, 575–580.
Konsman, J.P., Parnet, P., Dantzer, R., 2002. Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci. 25, 154–159.
Kroenke, K., Spitzer, R.L., 1998. Gender differences in the reporting of physical and somatoform symptoms. Psychosom. Med. 60, 150–155.
Krzyszton, C.P., Sparkman, N.L., Grant, R.W., Buchanan, J.B., Broussard, S.R., Woods, J., Johnson, R.W., 2008. Exacerbated fatigue and motor deficits in interleukin-10-deficient mice after peripheral immune stimulation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 295, R1109–R1114.
Kubera, M., Kenis, G., Bosmans, E., Zieba, A., Dudek, D., Nowak, G., Maes, M., 2000. Plasma levels of interleukin-6, interleukin-10, and interleukin-1 receptor antagonist in depression: comparison between the acute state and after remission. Pol. J. Pharmacol. 52, 237–241.
Kung, W.J., Lin, C.C., Liu, S.H., Chaung, H.C., 2010. Association of interleukin-10 polymorphisms with cytokines in type 2 diabetic nephropathy. Diabetes Technol. Ther. 12, 809–813.

Leone, M., Honstettre, A., Lepidi, H., Capo, C., Bayard, F., Raoult, D., Mege, J.L., 2004. Effect of sex on Coxiella burnetii infection: protective role of 17beta-estradiol. J. Infect. Dis. 189, 339–345.

Maes, M., Bosmans, E., De Jongh, R., Kenis, G., Vandoolaeghe, E., Neels, H., 1997. Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. Cytokine 9, 853–858.

Mandell, G.L., Bennett, J.E., Dolin, R. (Eds.), 2005. Mandell, Douglas, and Bennett;s principles and practice of infectious diseases. Elsevier, Churchill Livingstone, New York.

Marrie, T.J., 2000. Q fever. CRC Press, Inc., Florida.

Maurin, M., Raoult, D., 1999. Q fever. Clin. Microbiol. Rev. 12, 518–553.

Mesquita, A.R., Correia-Neves, M., Roque, S., Castro, A.G., Vieira, P., Pedrosa, J., Palha, J.A., Sousa, N., 2008. IL-10 modulates depressive-like behavior. J. Psychiatr. Res. 43, 89–97.

Muller, M.P., Richardson, S.E., McGeer, A., Dresser, L., Raboud, J., Mazzulli, T., Loeb, M., Louie, M., 2006. Early diagnosis of SARS: lessons from the Toronto SARS outbreak. Eur. J. Clin. Microbiol. Infect. Dis. 25, 230–237.

Mylonas, A., Brown, A., Carthew, T., McGrath, B., Purdie, D., Pandeya, N., Vecchio, P., Collins, L., Gardner, I., de Looze, F., Reymond, E., Suhrbier, A., 2002. Natural history of Ross River virus-induced epidemic polyarthritis. Med. J. Aust. 177, 356–360.

Pravica, V., Asderakis, A., Perrey, C., Hajeer, A., Sinnott, P.J., Hutchinson, I.V., 1999. In vitro production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. Eur. J. Immunogenet. 26, 1–3.

Pravica, V., Perrey, C., Stevens, A., Lee, J.H., Hutchinson, I.V., 2000. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. Hum. Immunol. 61, 863–866.

Raghu, G., Brown, K.K., Bradford, W.Z., Starko, K., Noble, P.W., Schwartz, D.A., King Jr., T.E., 2004. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. N. Engl. J. Med. 350, 125–133.

Ruan, Y.J., Wei, C.L., Ee, A.L., Vega, V.B., Thoreau, H., Su, S.T., Chia, J.M., Ng, P., Chiu, K.P., Lim, L., Zhang, T., Peng, C.K., Lin, E.O., Lee, N.M., Yee, S.L., Ng, L.F., Chee, R.E., Stanton, L.W., Long, P.M., Liu, E.T., 2003. Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. Lancet 361, 1779–1785.

Salemi, M., Fitch, W.M., Ciccozzi, M., Ruiz-Alvarez, M.J., Rezza, G., Lewis, M.J., 2004. Severe acute respiratory syndrome coronavirus sequence characteristics and evolutionary rate estimate from maximum likelihood analysis. J. Virol. 78, 1602–1603.

Tissot Dupont, H., Raoult, D., Brouqui, P., Janbon, F., Peyramond, D., Weiller, P.J., Chicheportiche, C., Nezri, M., Poirier, R., 1992. Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. Am. J. Med. 93, 427–434.

Turner, D.M., Williams, D.M., Sankaran, D., Lazarus, M., Sinnott, P.J., Hutchinson, I.V., 1997. An investigation of polymorphism in the interleukin-10 gene promoter. Eur. J. Immunogenet. 24, 1–8.

Vetsika, E.K., Callan, M., 2004. Infectious mononucleosis and Epstein-Barr virus. Expert Rev. Mol. Med. 6, 1–16.

Vollmer-Conna, U., 2001. Acute sickness behaviour: an immune system-to-brain communication? Psychol. Med. 31, 761–767.

Vollmer-Conna, U., Fazou, C., Cameron, B., Li, H., Brennan, C., Luck, L., Davenport, T., Wakefield, D., Hickie, I., Lloyd, A., 2004. Production of pro-inflammatory cytokines correlates with the symptoms of acute sickness behaviour in humans. Psychol. Med. 34, 1289–1297.

Vollmer-Conna, U., Piraino, B.F., Cameron, B., Davenport, T., Hickie, I., Wakefield, D., Lloyd, A.R., 2008. Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. Clin. Infect. Dis. 47, 1418–1425.

Von Korff, M., Ustun, T.B., Ormel, J., Kaplan, I., Simon, G.E., 1996. Self-report disability in an international primary care study of psychological illness. J. Clin. Epidemiol. 49, 297–303.

Wang, J.T., Sheng, W.H., Fang, C.T., Chen, Y.C., Wang, J.L., Yu, C.J., Chang, S.C., Yang, P.C., 2004. Clinical manifestations, laboratory findings, and treatment outcomes of SARS patients. Emerg. Infect. Dis. 10, 818–824.

Zouk, H., McGirr, A., Lebel, V., Benkelfat, C., Rouleau, G., Turecki, G., 2007. The effect of genetic variation of the serotonin 1B receptor gene on impulsive aggressive behavior and suicide. Am. J. Med. Genet. B Neuropsychiatr. Genet. 144B, 996–1002.