Brain, Behavior, & Immunity - Health 10 (2021) 100189

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Full Length Article

Genetic associations of fatigue and other symptoms following breast cancer treatment: A prospective study

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ARTICLE INFO

Keywords:
Cancer-related fatigue
Post-cancer fatigue
Breast cancer
Symptom domain
Cytokine gene polymorphism

ABSTRACT

Background: Cancer-related fatigue, mood disturbances, pain and cognitive disturbance are common after adjuvant cancer therapy, but vary considerably between individuals despite common disease features and treatment exposures. A genetic basis for this variability was explored in a prospective cohort.

Methods: Physical and psychological health of women were assessed prospectively following therapy for early stage breast cancer with self-report questionnaires. Participation in a genetic association sub-study was offered. Indices for the key symptom domains of fatigue, pain, depression, anxiety, and neurocognitive difficulties were empirically derived by principal components analysis from end-treatment questionnaires, and then applied longitudinally. Genetic associations were sought with functional single nucleotide polymorphisms (SNPs) in pro- and anti-inflammatory cytokine genes - tumour necrosis factor (TNF)-α (−308 GG), interferon (IFN)-ɣ (+874 TA), interleukin (IL)-10 (1082 GA and −592 CA), IL-6 (−174 GC), IL-1β (−511 GA).

Results: Questionnaire data was available for 210 participants, of whom 111 participated in the genetic sub-study. As expected, symptom domain scores generally improved over several months following treatment completion. Tumour and adjuvant treatment related factors were unassociated with either severity or duration of the individual symptom domains, but severity of symptoms at end-treatment was strongly associated with duration for each domain (all $p < 0.05$). In multivariable analyses, risk genotypes were independently associated with: fatigue with IL-6 -174 GG/GC and IL-10 -1082 GG; depression and anxiety with IL-10 -1082 AA; neurocognitive disturbance: TNF-α −308 GG; depression IL-1β (all $p < 0.05$). The identified SNPs also had cumulative effects in prolonging the time to recovery from the associated symptom domain.

Conclusions: Genetic factors contribute to the severity and duration of common symptom domains after cancer therapy.

1. Introduction

Although it is well recognised that cancer survivors experience adverse physical and psychological outcomes after cancer treatment (Saligan et al., 2015), early identification and management of those at highest risk remains challenging. Clinical and demographic factors may help in defining those at increased risk, as there remains considerable variability in the severity and duration of symptoms despite comparable exposure to cancer treatments (Abrahams et al., 2016, 2018; Bower et al., 2018; Orre et al., 2008).

The illness experience during and after cancer treatment is complex with both physical and psychological elements. In mental health research, complex behavioural symptoms in disorders with a recognised heritable component, such as major depression or schizophrenia, have

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https://doi.org/10.1016/j.bbih.2020.100189
Received 29 October 2020; Received in revised form 4 December 2020; Accepted 6 December 2020
Available online 8 December 2020

been divided into domains with recognisable and stable characteristics to investigate potential genetic associations (Braff and Light, 2005; Cannon and Keller, 2006). The most extensively investigated symptom domain is suicidal behaviours, which cross primary psychiatric diagnostic boundaries, and has been associated with genetic variations in the serotonin transporter gene (SLC6A4, 5-HTTLPR) (Fanelli and Serretti, 2019).

Symptom domains have also been described in the acute sickness response to infection (Piraino et al., 2012), with unique associations identified between the domains of fatigue, pain, mood disturbance, and neurocognitive difficulties, and functional single nucleotide polymorphisms (SNPs) in genes encoding pro- and anti-inflammatory cytokines. In this previous study, the high-producing T allele of interferon (IFN)-γ +874 T/A was associated with fatigue, the high-producing C allele of interleukin(IL)-10 -592 C/A was protective against neurocognitive difficulties, as well as the low-producing A allele of IL-10 -592 and the high-producing G allele IL-6 -174 G/C being associated with mood disturbance.

As there are clear similarities between the symptom domains in the acute sickness response and those commonly reported during and following adjuvant cancer therapy, it is reasonable to postulate that this concept of symptom domains will also apply following cancer treatment, perhaps with a common genetic basis related to inflammation. In addition, persistent immune activation leading to pro-inflammatory cytokine production has been proposed as a mechanism underpinning post-cancer fatigue (PCF) (Bower, 2014; Saligan and Kim, 2012), a syndrome which is closely analogous in clinical characteristics to the fatigue syndrome which may follow acute infections such as infectious mononucleosis, and also the more heterogeneous chronic fatigue syndrome (Bennett et al., 2007; Hickie et al., 2006; Strawbridge et al., 2019; Blundell et al., 2015). Previous studies seeking associations between circulating cytokine levels and such post-cancer symptoms have yielded conflicting results (Orre et al., 2008, 2011; Cameron et al., 2012; Wratten et al., 2004; Bower et al., 2007; Bower and Lamkin, 2013). Although preliminary work has been done in the cancer setting investigating associations between SNPs in cytokine genes and symptoms during or after adjuvant therapy (Bower et al., 2013; Jim et al., 2012; Kober et al., 2016), the findings have been inconsistent and the concept of symptom domains has not been addressed.

This study investigated the relationships between SNPs in cytokine genes and common symptom domains in a well-characterised, prospective cohort of women completing breast cancer therapy.

2. Materials and methods

2.1. The cohort

The Follow-Up after Cancer (FolCan) study was a prospective cohort of 210 women with early stage breast cancer who were followed for 12 months after completion of adjuvant treatment (Goldstein et al., 2012). Women aged 18 years and over, with stage I or II breast cancer, and no significant medical or psychiatric comorbidities were recruited following cancer surgery, but prior to adjuvant therapy (Goldstein et al., 2012). Demographic data and information on medical and psychiatric history was collected at baseline through structured interviews. Clinical data including type of surgery, tumour histology, menopausal status and adjuvant treatments were extracted from the medical record. Self-report questionnaires were completed at baseline, on completion of adjuvant chemotherapy and/or radiotherapy, and then at 1, 3, 6, 9 and 12 months. All Folcan participants were offered enrolment in a genetics sub-study provided they were of Caucasian descent and willing to provide a blood sample at each study time point for immunological and genetic testing. Ethics approval was obtained from the Human Research Ethics Committees of participating hospitals and all participants gave written informed consent.

2.2. Questionnaires

At each study time point, physical and psychological symptoms were assessed using the Somatic and Psychological Health Report (SPHERE) questionnaire (Hickie et al., 2001), the Physical Symptom Checklist (PSC) (Hickie, Davenport et al., 2006), the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) (Aaronson et al., 1993), and the Blatt-Kupperman Menopausal Index (BKMI) (Blatt et al., 1953). In addition to these measures, participants also completed the Brief Disability Questionnaire (BDQ) (Von Korff et al., 1996) as a measure of overall functional impairment. The SPHERE and PSC code symptoms on a 0–2 scale ("never or some of the time", "a good part of the time" or "most of the time") whereas the BKMI and EORTC-QLQ-C30 utilise a 0–3 scale ("not at all", "a little", "quite a bit", "very much"). For consistency across instruments, scores of 0 and 1 on the BKMI and EORTC-QLQC30 were therefore collapsed.

2.3. Symptom domains

The symptom domains within the post-cancer illness were derived by principal component analysis (PCA) using end-treatment symptom data from the SPHERE, PSC, EORTC-QLQC30 and BKMI. The PCA was conducted with oblique (direct oblimin) rotation. Symptom items were sequentially removed until all remaining items had a loading score >0.4 and the first component accounted for >30% of the variance. If a symptom item was duplicated (e.g. SPHERE- "headache" and PSC- "have you had a headache") the item with the lower loading score on the first component was removed. The final solution included all symptom items with potential relevance to each of the designated symptom domains of fatigue, pain, depression, anxiety and neurocognitive difficulties (7, 8, 8, 6, and 5 symptom items respectively). The reliability of each of the PCA-derived symptom domains was assessed with Cronbach's alpha.

The PCA-derived symptom domain loadings were applied to the relevant items in the self-report data at end-treatment, and 3, 6, 9 and 12 months post-treatment. The symptom domain scores were split for genetic association analysis with scores of zero designated as 'Low' symptom severity as compared to participants scoring 1 or more, who were designated as 'High' symptom severity.

2.4. Genotyping and cytokine measurement

Peripheral blood mononuclear cells (PBMC) and sera were collected, separated and stored at −80 °C under endotoxin-minimised conditions. DNA was extracted from PBMC (Wizard DNA kit; Promega) and quantified using Nano-DropR ND-1000 (Biolab), with quality verified by agarose gel electrophoresis. Genomic DNA was sent to the Australian Genome Research Facility (AGRF) for genotyping (Sequenom MassARRAY) of functional SNPs in pro- and anti-inflammatory cytokine genes implicated in the acute sickness response, including IFN-γ (+874 T/A, rs2430561), TNF-α (−308 G/A, rs1800629), IL-1β (−511 C/T, rs16944), IL-6 (−174 G/C, rs18000795) and IL-10 (−1082 G/A, rs1800896 and -592 C/A, rs1800872).

Sera from the end-treatment time point were subsequently thawed and concentrations of the cytokines, IL-1β, IL-6, IL-10, TNF-α, and IFN-γ analysed in a bead-based multiplex immunoassay system (BioPlex; Bio-Rad, Hercules, CA).

2.5. Statistics

Demographic, clinical and treatment variables for the genetic substudy participants and non-participants were compared using two-tailed unpaired *t* tests for continuous variables, and Chi-square tests (χ^2) or Fisher's exact test for categorical variables where appropriate.

Conformation with Hardy–Weinberg equilibrium was tested for each SNP in the genetic substudy participants. Genetic associations of each SNP with symptom domains was assessed in a logistic regression model

adjusted for age as a covariate. Odds ratios (ORs) and 95% confidence intervals (CI) were determined. Time to recovery Kaplan-Meier curves were developed with log-rank analysis to evaluate the effects of genotype and allelic combinations on symptom duration.

For longitudinal analyses, if self-report data was missing for a subject for a given time point a score was imputed as a simple average of the adjacent time points. If no subsequent self-report data was available, subjects were censored at the time of the last data collected. Logistic regression was used to analyse whether clinical or demographic variables predicted end-treatment symptom domain scores. Linear regression was used to test for associations between clinical or demographic variables as well as the end-treatment symptom scores, and the duration of each symptom domain.

Mean serum cytokine levels grouped by genotype and symptom domain were compared using t-tests.

Analyses were conducted using SPSS (SPSS statistics v25, IBM, Armonk, NY, USA) and PLINK (http://zzz.bwh.harvard.edu/plink//). P values < 0.05 were considered to indicate statistical significance.

3. Results

3.1. The cohort

Self-reported symptom data was available at end-treatment for 210 women, of whom 111 participated in the genetic association substudy (Fig. 1). The clinical and demographic features of the participants are shown in Table 1. As Caucasian ethnicity was an inclusion criterion, the genetic association substudy cohort was significantly more likely to be Australian-born and to have English as a first language ($p = 0.02$ and 0.01), but no other significant differences were seen.

3.2. Symptom domains

Symptom domains for fatigue, pain, depression, anxiety, and neurocognitive disturbance, were derived by PCA. Each of the scales demonstrated internal consistency with Cronbach's alpha >0.87 (Supplementary Table 1). The derived scales were comparable to PCA solutions based on data from 3 month to 6 month timepoints, rather than end treatment (data not shown). The PCA derived scales were further

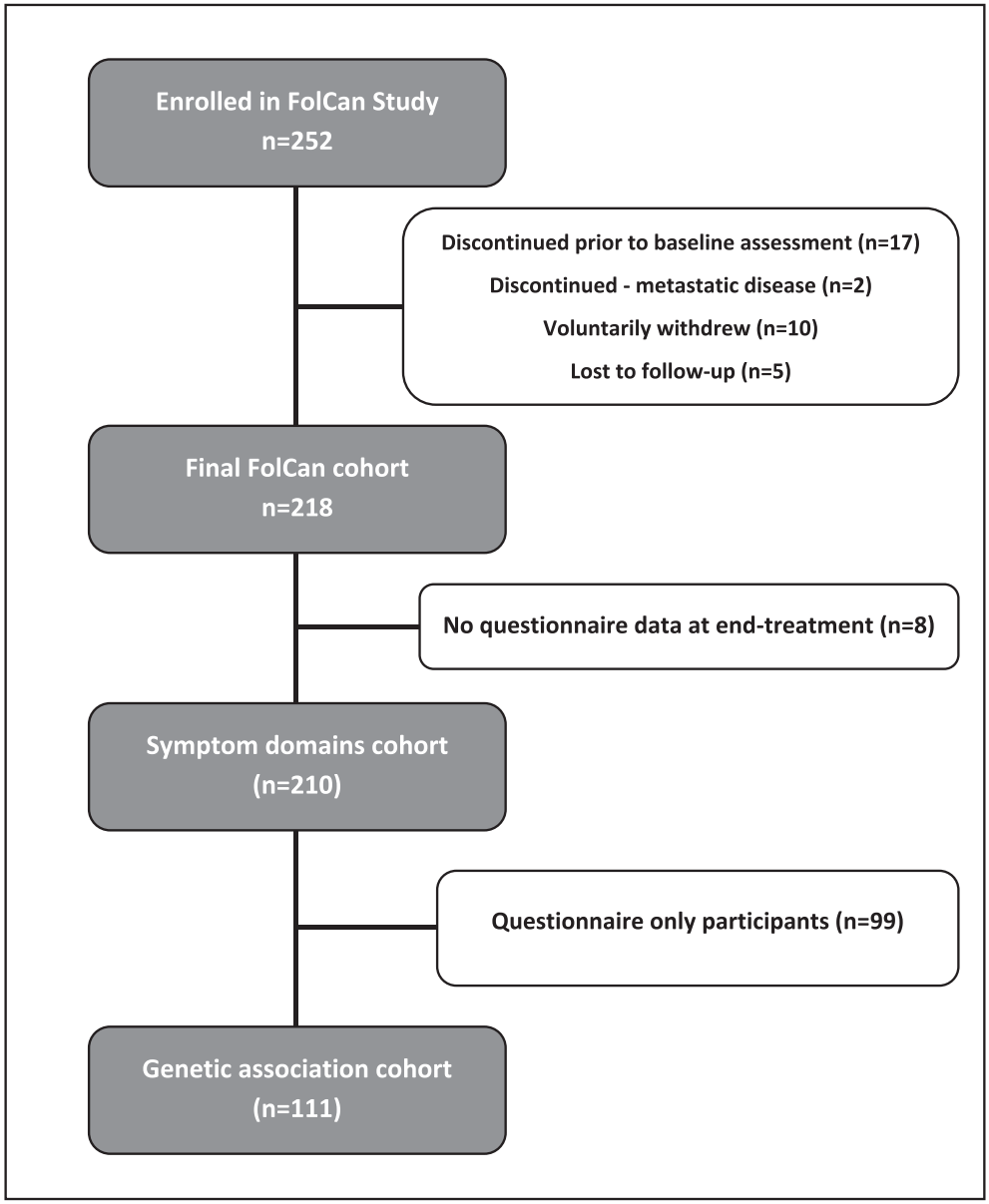

Fig. 1. The FolCan and genetic substudy cohorts.

Table 1
Clinical and demographic features of FolCan cohort and genetics sub-cohort.

	Questionnaire only cohort (n = 99) n (%)	Genetics sub-cohort (n = 111) n (%)	P value
Demographics			
Age, years - mean (SD)	51.8 (10.6)	52.8 (10.1)	0.490
Australian born	64 (65)	86 (77)	**0.020**
First language English	87 (88)	106 (95)	**0.010**
Completed ≥ 12 years education	66 (67)	61 (55)	0.210
Married or defacto	77 (78)	87 (78)	0.720
Pre-menopausal at baseline	47 (47)	53 (48)	0.840
Tumour characteristics			
Size, (mm) - mean (SD)	21.9 (12.9)	22.3 (14.6)	0.820
Positive lymph nodes	34 (34)	36 (32)	0.750
Oestrogen receptor positive	66 (67)	73 (66)	0.400
Surgical treatment			
Mastectomy	31 (31)	42 (38)	0.280
Axillary clearance	78 (79)	81 (73)	0.390
Adjuvant treatment			
Chemotherapy	61 (62)	75 (68)	0.230
Radiotherapy	71 (72)	75 (68)	0.680
Hormonal therapy	49 (49)	48 (43)	0.440

validated by correlation with self-reported disability. High symptom scores for each symptom domain were strongly associated with more "days out of usual work or other role in the past few weeks" (Table 2). These differences in functional status by severity of the individual symptom domains were consistently evident at each timepoint (data not shown).

Regression analysis showed that for the cohort as a whole, there were no clinical or demographic variables associated with symptom severity at final treatment, apart from '>20 h/week of paid work' with High severity of the Pain symptom domain (p = 0.003).

Comparison of the High and Low severity groups of the genetics sub-cohort for each of the symptom domains is seen in Table 3.

Symptom domain scores generally fell over time after cancer treatment, including for fatigue (p < 0.001), depression (p = 0.002), and anxiety (p = 0.004), but not for pain or neurocognitive disturbance (both p > 0.1). For all symptom domains, only the symptom severity at end-treatment, but not any of the clinical or demographic variables, predicted the duration of the symptoms (Pain p = 0.026, all others p < 0.0001).

3.3. Genetic associations with symptom domain severity

The call rates in the SNP genotyping were greater than 97.3%, with an overall pass rate of 99.1% for all subjects. All SNPs were in Hardy–Weinberg equilibrium (p > 0.07).

Table 2
Disability as measured by days in the last month out of usual role.

Symptom domain	Low severity		High severity		
	n[a]	Mean days out of role (SD)	n[a]	Mean days out of role (SD)	P
Fatigue	56	2.5 (6.1)	150	7.1 (9.5)	**<0.001**
Pain	112	4.7 (7.7)	93	7.3(10.1)	**0.04**
Depression	133	4.2 (7.5)	74	8.8 (10.5)	**0.001**
Anxiety	139	4.5 (7.8)	68	8.5 (10.4)	**0.006**
Neurocognitive disturbance	137	4.5 (7.7)	70	8.5 (10.3)	**0.005**

[a] Numbers vary slightly depending on missing datapoints.

Table 3
Comparison of the High and Low severity groups in the genetics sub-cohort for each symptom domain.

Symptom domain	Low severity		High severity		
	n (%)[a]	Mean scale score	n (%)[a]	Mean scale score	P
Fatigue	29 (26.4)	0	81 (73.6)	4.68	**<0.001**
Pain	58 (52.7)	0	52 (47.3)	3.75	**<0.001**
Depression	72 (64.9)	0	39 (35.1)	4.16	**<0.001**
Anxiety	75 (67.6)	0	36 (32.4)	3.00	**<0.001**
Neurocognitive disturbance	81 (73.0)	0	30 (27.0)	2.61	**<0.001**

[a] Numbers vary slightly depending on missing datapoints.

The associations between cytokine genotypes and symptom domain severity scores at end-treatment are shown in Table 4. Logistic regression analyses including age as a co-variate were conducted. For the fatigue symptom domain, both dominant and recessive models revealed that the high-producing G allele of the IL-10 -1082 SNP was associated with reduced fatigue (p = 0.041, OR:0.29; p = 0.046, OR:0.36 respectively). Under the recessive model, the low producing CC allelotype of the IL-6 -174 SNP was associated with reduced fatigue (p = 0.012, OR:0.27). Furthermore, those subjects who were homozygous for the IL-6 -174 C allele and also carried a G allele at the IL-10 -1082 SNP were significantly less likely to experience severe fatigue than those who were homozygous for the IL-10 -1082 A allele and had a G allele at IL-6 -174 (p = 0.004,OR:0.26).

The dominant and recessive models for depression showed that the high-producing G allele of the IL-10 -1082 SNP was associated with protection against severe depression (p = 0.0002, OR:0.18; p = 0.023, OR:0.23, respectively). By contrast, under the dominant inheritance model, carrying a high-producing A allele of the IL-1β −511 G/A SNP was associated with increased susceptibility to severe depression (p = 0.030, OR:2.61). Subjects who were homozygous for both IL-10 -1082 G and IL-1β −511 G alleles had a greatly reduced risk of experiencing depression, compared with subjects who were homozygous for the IL-10 -1082 A allele and had an A allele for IL-1β −511 (p = 0.003, OR:0.031).

For anxiety, the high-producing G allele of the IL-10 -1082 SNP was associated with a decreased risk of anxiety (dominant model, p = 0.0002, OR:0.18; and for the recessive model, p = 0.040, OR:0.26). A weaker association with severe neurocognitive disturbance was also found with the TNF-α −308 G allele (p = 0.030, OR:0.34). None of the polymorphisms was associated with pain severity.

3.4. Genetic associations with symptom duration

The allelic combinations of SNPs in IL-10, IL-1β and IL-6 associated with severity at end-treatment were also associated with the duration of symptoms. Significant, cumulative effects on time to recovery were seen for the SNPs associated with fatigue, depression and anxiety. Subjects who carried the protective genotypes against the severity of fatigue symptom domain (i.e homozygosity for IL-6 -174 C and heterozygosity for IL-10 -1082G) had symptomatic fatigue for a median of 0 months (range 0–12), compared with those with the risk genotypes (heterozygosity for IL-6 -174 G and homozygosity for IL-10 -1082 A) who experienced significant prolonged fatigue for 9 months (range 0–12) ($\chi 2$ = 5.80, p = 0.016; Fig. 2A). Subjects who carried the protective genotypes against severe depression (IL-1β −511 GG and IL-10 -1082 GG) were symptomatic with depression for a median of 0 months (range 0–12), whereas those who had risk genotypes (IL-1 β −511 AA or AG and IL-10 -1082 AA) experienced depression for 6 months (range 0–12) ($\chi 2$ =

Table 4
Associations between symptom domain severity scores and SNPs in cytokine genes.

SNP	SNP position	A1[a]	A2[b]	MAF[c]	Symptom domain	Dominant model		Recessive model	
						Adjusted P value	OR (95% CI)	Adjusted P value	OR (95% CI)
rs1800872	IL-10 -592	A	C	0.2658	Fatigue	0.112	2.11 (0.84–5.28)	0.998	<0.0001 ([d])
					Pain	0.555	0.80 (0.38–1.69)	0.651	1.44 (0.30–6.93)
					Depression	0.733	1.15 (0.52–2.56)	0.414	1.93 (0.40–9.41)
					Anxiety	0.181	1.75 (0.77–3.99)	0.319	2.24 (0.46–10.91)
					Neurocognitive disturbance	0.994	1.00 (0.43–2.36)	0.805	0.80 (0.14–4.52)
rs1800896	IL-10-1082	G	A	0.4595	Fatigue	**0.041**	0.29(0.09–0.95)	**0.046**	0.36 (0.13–0.98)
					Pain	0.439	0.72 (0.32–1.65)	0.394	0.67 (0.26–1.70)
					Depression	**<0.001**	0.18 (0.07–0.45)	**0.023**	0.22 (0.06–0.81)
					Anxiety	**<0.001**	0.18 (0.07–0.45)	**0.040**	0.26 (0.07–0.94)
					Neurocognitive disturbance	0.128	0.50 (0.20–1.22)	0.116	0.35 (0.09–1.30)
rs16944	IL-1B −511	A	G	0.3519	Fatigue	0.475	1.38(0.57–3.35)	0.556	0.69 (0.20–2.38)
					Pain	0.078	0.50 (0.23–1.08)	0.143	0.40 (0.12–1.37)
					Depression	**0.030**	2.61 (1.10–6.23)	0.857	1.12 (0.33–3.73)
					Anxiety	0.673	1.20 (0.52–2.78)	0.403	0.56 (0.14–2.20)
					Neurocognitive disturbance	0.850	1.09 (0.45–2.62)	0.299	0.43 (0.09–2.10)
rs1800629	TNF-α −308	A	G	0.2162	Fatigue	0.713	0.85 (0.35–2.06)	0.999	452000000.0 ([d])
					Pain	0.627	1.21 (0.56–2.60)	0.605	0.53 (0.05–6.01)
					Depression	0.504	0.76 (0.33–1.72)	0.836	0.77 (0.07–8.97)
					Anxiety	0.802	1.11 (0.49–2.54)	0.289	3.77 (0.32–43.69)
					Neurocognitive disturbance	**0.030**	0.34 (0.13–0.90)	0.896	1.18 (0.10–13.70)
rs1800795	IL-6 -174	C	G	0.412	Fatigue	0.120	0.46 (0.17–1.23)	**0.012**	0.27 (0.10–0.75)
					Pain	0.914	1.04 (0.47–2.30)	0.650	0.81 (0.32–2.04)
					Depression	0.333	0.66 (0.29–1.52)	0.940	0.96 (0.36–2.60)
					Anxiety	0.843	0.92 (0.39–2.15)	0.437	0.66 (0.23–1.90)
					Neurocognitive disturbance	0.365	0.66 (0.28–1.61)	0.236	0.49 (0.15–1.61)
rs2430561	IFN-γ +874	A	T	0.4595	Fatigue	0.463	1.44 (0.54–3.81)	0.577	1.39 (0.44–4.39)
					Pain	0.781	0.89 (0.38–2.07)	0.119	0.45 (0.17–1.23)
					Depression	0.776	1.14 (0.46–2.81)	0.900	0.94 (0.34–2.61)
					Anxiety	0.565	0.77 (0.31–1.88)	0.378	0.61 (0.20–1.84)
					Neurocognitive disturbance	0.339	1.64 (0.60–4.49)	0.754	0.84 (0.27–2.56)

[a] A1 Allele 1.
[b] A2 Allele 2.
[c] MAF Minor allele frequency.
[d] No CI where the observation frequency <5.

10.83, p = 0.001; Fig. 2B). Subjects with the protective genotypes against anxiety (heterozygosity or homozygosity for IL-10 -1082 G) were symptomatic in this domain for a median of 0 months (range 0–12), whereas those with the risk genotypes (homozygosity for IL-10 -1082A) experienced anxiety for 6 months (range 0–12) ($\chi 2 = 5.49$, p = 0.019; Fig. 2C). There was no significant association between the TNF-α −308 SNP and time to recovery from neurocognitive disturbance.

3.5. Cytokines

Stored sera from the end-treatment time point was available for cytokine analysis in 94 subjects. Serum cytokine levels did not differ between subjects in the High and Low severity groups for any of the symptom domains for which a genetic association was identified (IL-1β/depression p = 0.23; IL-6/fatigue p = 0.52; IL-10/fatigue p = 0.32, IL-10/depression p = 0.79, IL-10/anxiety p = 0.64; TNF-α/neurocognitive disturbance p = 0.40) (Supplementary Table 2). Additionally, no differences were seen in serum cytokine levels analysed according to the cytokine genotypes (Supplementary Table 2).

4. Discussion

The findings in this study support the notion that the symptom domain concept can be meaningfully applied to the apparently complex constellation of clinical features that are commonly reported by patients during and after adjuvant cancer therapy. Five empirically derived indices were identified which characterise the symptom domains of fatigue, pain, depression, anxiety and neurocognitive difficulties. Consistent with clinical experience individual severity scores for each symptom domain varied widely between individuals at end-treatment, yet the relative dominance of each were stable over time within individuals (data not shown) (Goldstein et al., 2012). Further, symptom severity for each symptom domain correlated strongly with degree of disability, arguing that all make meaningful contributions to the overall illness complex and associated functional impairment. Finally, each of these symptom domains was found to be associated with functional SNPs in pro- and anti-inflammatory cytokine genes, with most of these associations impacting also on the duration of the illness experience.

It is notable that none of the cancer or treatment-related variables were significantly associated with severity of any of the symptom domains, which contrasts with a meta-analysis which reported fatigue to be associated with both late stage cancer and type of treatment (Abrahams et al., 2016) – this may reflect the relatively small sample size and the exclusion of individuals with advanced cancer from the present study. By contrast, the lack of association is analogous to findings in other settings, where severity and relative dominance of fatigue and related symptoms were not related to the individual initiating pathogen, or to treatments such as interferon-α (Piraino et al., 2012; Russell et al., 2019). In combination these data suggested that these symptom domains represent a relatively stereotyped set of host-determined responses to an insult - in this case adjuvant breast cancer therapy. This concept is supported by recent studies describing predisposing individual patterns of fatigue or

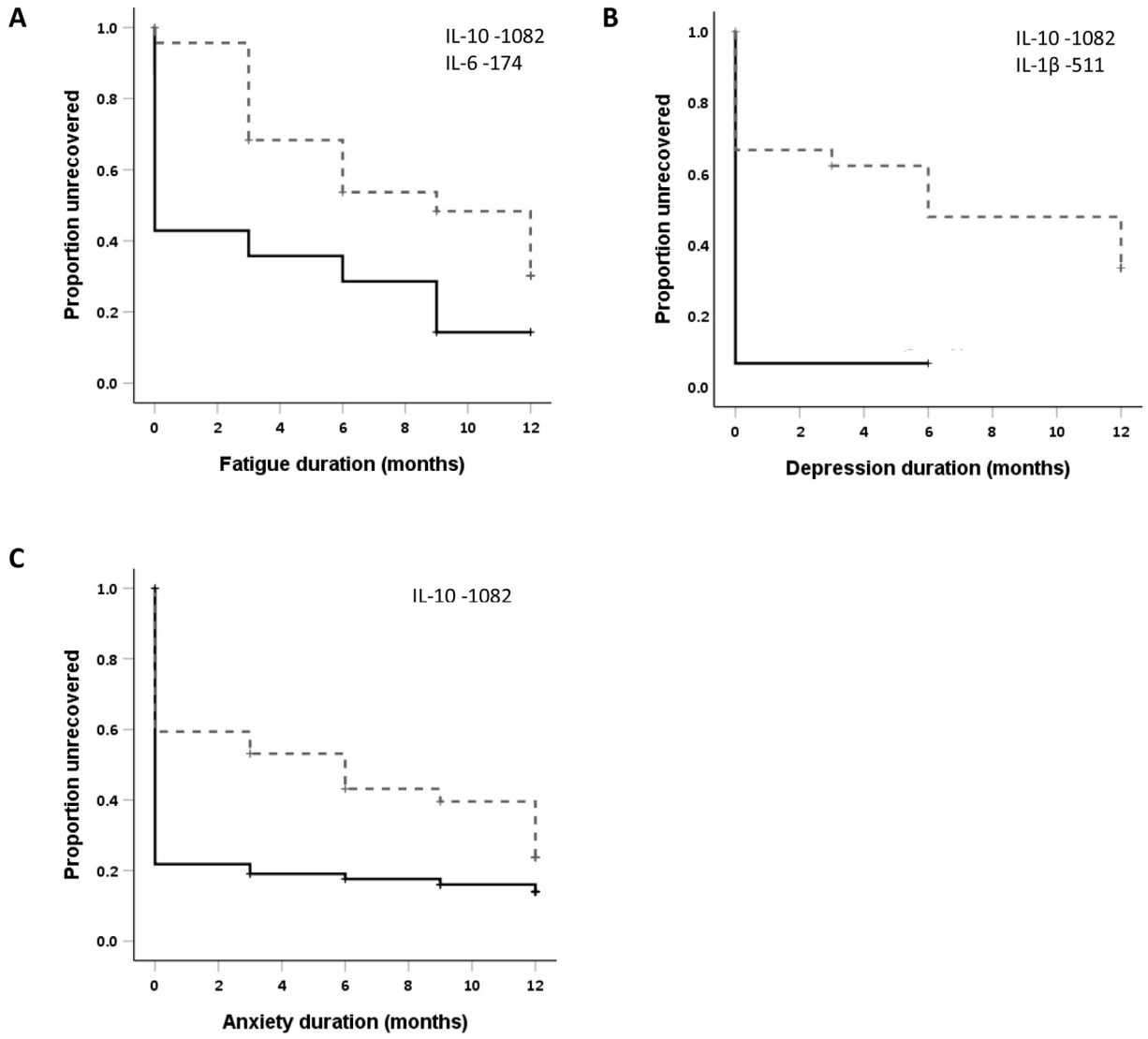

Fig. 2. Survival curves for fatigue, depression, and anxiety symptom domains by risk or protective genotypes.
(**A**) Duration of fatigue by protective genotype (IL-6 -174 CC and IL-10 -1082 GG or GA) compared with the risk genotype (IL-6 -174 CG or GG and IL-10 -1082 AA). (**B**) Duration of depression by protective genotype (IL-1β −511 GG and IL-10 -1082 GG) compared with the risk genotype (IL-1 β −511 AA or AG and IL-10 -1082 AA). (**C**) Genetic effect of protective genotypes IL-10 -1082 GG or GA on duration of the anxiety symptom domain compared with the risk genotype (IL-10 -1082 AA). N = 94 for all graphs. Solid lines are protective genotypes, dashed lines are risk genotypes. The zero timepoint refers to end-treatment. Kaplan-Meir survival analysis.

depression, and their association with trajectories of symptoms during and after cancer (Bower et al., 2018).

Some of the genetic SNPs associated with symptoms in this study have been previously described in the post-cancer setting, as reviewed by Wang et al. (2017). Post cancer fatigue has been associated with the G allele of the IL-6 -174 SNP (Bower et al., 2013), as well as higher fatigue with the A allele of IL-1β −511 and lower fatigue with the C allele of IL-10 rs3024496 SNPs (Kober et al., 2016). Others have not found such an association, however one study did not include IL-10 and was conducted much later (3–4 years) after treatment for breast cancer (Reinertsen et al., 2011), and by contrast the other evaluated patients only to 4 months after treatment in relation to a range of cancer types (Dhruva et al., 2015). In contrast to our findings, the C allele in the IL-6 -174 SNP was associated with fatigue frequency, duration and intrusiveness compared to the GG genotype in prostate cancer, however this small (n = 53) study was conducted during active hormone therapy and all subjects were male (Jim et al., 2012). Interestingly, the high-producing G allele of the IL-10 -1082 SNP has also been associated with a lower risk of depression in patients with end-stage renal disease (Holtzman et al., 2012) and following stroke (Kim et al., 2011).

Others have also reported an association between the G allele of the TNF-α −308 SNP and fatigue (Bower et al., 2013; Dhruva et al., 2015), but no such association was seen here. A non-significant trend towards association between the GG genotype of TNF-α −308 and memory complaints has been reported (Bower et al., 2013), which is consistent with the association with more severe neurocognitive difficulties in the present study. Interestingly, the GG genotype of the TNF-α −308 SNP has also been reported to be associated with poorer attentional processing in healthy individuals (Beste et al., 2010).

Whilst identification of these individual genetic associations is extremely informative, it would be simplistic to attribute causality of any post-cancer treatment symptom to a single functional SNP. The effect of paired risk genotypes on time to symptom recovery seen in our cohort provides evidence that the symptom domains are complex polygenic phenomena. This is supported by work in the post-infection setting, where the high-risk IFN-γ +874 TT and IL-10 -592 AA genotypes (associated with high levels of pro-inflammatory IFN-γ and low levels of anti-inflammatory cytokine IL-10) had a synergistic effect on time to recovery

from acute viral infection (Vollmer-Conna et al., 2008). Bower and colleagues have also described additive effects of IL-6, IL-1β and TNF-α cytokine SNPs on cross-sectional rates of fatigue among breast cancer survivors (Bower et al., 2013). The present study supports and expands on these findings, by demonstrating a sustained relationship over time which impacts on symptom recovery and ongoing disability.

The lack of a relationship between symptom domain severity and serum cytokine levels in the present study is unsurprising, given previous findings from a longitudinal nested case control series in this same cohort which found no differences in serum cytokine levels at any of the study time points between 13 cases with post-cancer fatigue and 15 matched controls who were free of persistent fatigue (Cameron et al., 2012). Previous research exploring relationships between circulating cytokines and symptoms in cancer survivors has also largely yielded negative results (Orre et al., 2008, 2011; Wratten et al., 2004; Bower et al., 2007, 2009). Taken together, these findings support the notion that genetically-determined differences in cytokine production may act largely within the central nervous system. Much cytokine signalling occurs in an autocrine or paracrine fashion in a tissue microenvironment, and thus serum levels may bear little relationship to central activity. Microglial cells have been postulated to be central to this process as both targets and producers of cytokines in the central nervous system, and so it may be in this site that genotype-driven cytokine expression has its biological effect (Wang et al., 2020). Others have suggested that even very low circulating cytokine levels may be sufficient to affect complex brain functions, although this hypothesis is speculative (Pollmächer et al., 2002). The associations between SNPs and cytokines are likely to be as inducers or regulators of other molecules such as neurotransmitters rather than direct effects.

The limitations of this study should be acknowledged, in particular related to the sample size, which should be viewed as a proof of concept analysis. As such, a relatively liberal approach to statistical significance was taken and no corrections were made in relation to multiple testing, with a view to validation in a larger dataset. Nevertheless, it is reassuring that the genotypes identified using these methods demonstrated strong associations with the clinical trajectories found on the time-to-recovery Kaplan-Meier analyses, supporting a biologically plausible role for these immunomodulatory SNPs. Unfortunately, sample size has been a challenge common to most of the published literature in the field, with sample sizes generally less than 200 individuals (Bower et al., 2013; Jim et al., 2012). The genetic associations found in the present study and others should be validated in larger patient cohorts, and arguably could be embedded in large prospective adjuvant trials given the growing focus of minimising protracted symptoms and side effects of treatment in cancer survivors. The choice of instruments used in this study may also have influenced the results (Feng et al., 2019; Lacourt et al., 2018; Schvartsman et al., 2017). The SPHERE and PSC group together individuals with low symptom frequency and no symptoms on each question item with a score of zero. This has the advantage that it discounts trivial symptoms, but may reduce the sensitivity of the instruments. The symptom domain scales derived will therefore tend to underestimate the overall symptom burden in patients, which may limit the capacity to find significant associations.

In conclusion, these findings support the notion that the variable and heterogeneous symptom experience of individuals after cancer therapy may at least partially be a consequence of an inherent, genetic predisposition to these symptoms. In this developing era of personalised medicine, it may be possible to not only tailor cancer therapies to target somatic gene aberrations in the tumour, but also to predict those individuals at risk of significant problems after cancer therapy based on genotype, in order to allow effective targeting of preventative or remedial interventions from limited health care resources to those most in need.

Declaration of conflict of interest

All authors declare no conflict of interest.

Acknowledgements

The larger FolCan study was supported by a grant by the Susan G. Komen for the Cure Foundation, while the work comprising the symptom domain substudy was supported by a Pfizer Australia Cancer Research Award. ARL was supported by an NHMRC Practitioner Fellowship (No. 1137587).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2020.100189.

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