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Full-length Article

Validation of inflammatory genetic variants associated with long-term cancer related fatigue in a large breast cancer cohort

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ABSTRACT

Background: Studies to date have reported several associations between single nucleotide polymorphisms (SNPs) and cancer related fatigue (CRF), but have been limited by small sample sizes, missing adjustment for relevant covariates or multiple testing, as well as varying CRF definitions, i.e. time and method of assessment. This study aimed to validate previously reported associations using the largest independent breast cancer sample to date and to evaluate further functional cytokine variants in relation to total CRF and all relevant CRF subdomains (physical, cognitive, and affective CRF).

Method: 45 candidate SNPs in inflammatory pathway genes were selected based on previous reports (16 SNPs) or regulatory function (29 SNPs). Breast cancer patients recruited between 2002 and 2005 provided information on CRF at first follow-up (FU1) (N = 1389) and second follow-up (FU2) (N = 950), a median of 6.2 years and 11.7 years respectively after diagnosis. SNP associations were assessed using linear regression models on CRF scores separately for FU1 and FU2. Additionally, patients with persistent fatigue (fatigued at both time-points) were compared to those never fatigued using logistic regression models (N = 684). All analyses were adjusted for relevant covariates. Secondary analyses were conducted for CRF subdomains.

Results: For total CRF none of the previously reported associations were confirmed after correction for multiple testing. The p-value distribution of all SNPs was not different than the one expected by chance. Analyses of CRF subdomains yielded a significant association between TNF-α rs3093662 and persistent physical CRF (Odds Ratio (OR) = 3.23, 95% Confidence Interval (CI) = 1.71–6.10, p = 0.0003).

Conclusion: We were unable to confirm previously reported findings, suggesting that individual SNPs are unlikely to be of clinical utility. Further investigations in well powered studies are warranted, which consider genetic heterogeneity according to subdomains of CRF.

1. Introduction

Cancer related fatigue (CRF) is one of the most common and burdensome side effects of cancer and its treatment, with negative effects on patient's ability to function as well as their quality of life. Prevalence of CRF during treatment ranges from 25% to 99% depending on the population studied and the methods of assessment (Abrahams et al., 2016; Lawrence et al., 2004; Servaes et al., 2002). Approximately two thirds of patients recover from CRF after completion of treatment while one third of cancer survivors suffer from it even years thereafter (Bower et al., 2000; Reinertsen et al., 2009; Schmidt et al., 2012; Servaes et al., 2007; Servaes et al., 2002).

Variability in CRF among breast cancer patients cannot be entirely explained by characteristics of the disease and/or its treatment (Henselmans et al., 2010). While lifestyle factors associated with CRF are well studied (Bower, 2008; Bower et al., 2000; Schmidt et al., 2015; Schmidt et al., 2012), the exact biological mechanisms underlying CRF remain unknown (Saligan et al., 2015; Tariman and Dhorajiwala, 2016). Understanding the aetiology of this multidimensional syndrome is crucial for identifying patients at risk of experiencing persistent CRF and for the development of targeted therapies.

Current research suggests that CRF is caused by a cascade of events, such as inflammatory cytokine production, a dysfunction of the hypothalamic–pituitary–adrenal axis as well as dysregulation of metabolic

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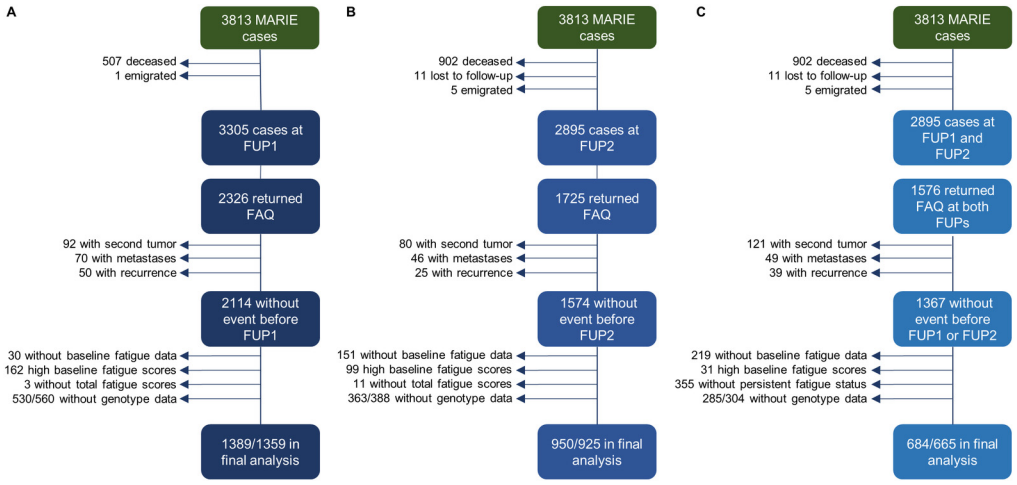

Fig. 1. Patient flow at (A) follow-up 1 (B) follow-up 2 (C) follow-up 1 and 2 combined.

and endocrine functions. To date, most evidence has been accumulated on the role of inflammatory markers in the development of CRF (Saligan et al., 2015; Tariman and Dhorajiwala, 2016). Circulating levels of IL-6 and its receptors have been most commonly investigated with seven studies reporting significant associations with CRF (Clevenger et al., 2012; Courtier et al., 2013; Liu et al., 2012; Meyers et al., 2005; Orre et al., 2009; Pertl et al., 2013; Wang et al., 2010) and only two studies that did not (Kwak et al., 2012; Pusztai et al., 2004). Results found for elevated concentrations of IL-1ra, TNF-α as well as C-reactive protein (CRP) have been less consistent (Saligan et al., 2015). Genetic variants, predominantly single nucleotide polymorphisms (SNPs), have been shown to influence gene expression levels of cytokines (Smith and Humphries, 2009) and have also been assessed with respect to CRF (Saligan et al., 2015; Tariman and Dhorajiwala, 2016). A few variants have been reported by several studies to show associations with CRF, including the TNF-α genomic variant *rs1800629* (Aouizerat et al., 2009; Bower et al., 2013; Dhruva et al., 2015; Jim et al., 2012), IL6 SNP *rs1800795* (Bower et al., 2013; Collado-Hidalgo et al., 2008; Jim et al., 2012), IL1-β variant *rs16944* (Collado-Hidalgo et al., 2008; Jim et al., 2012; Reyes-Gibby et al., 2013a), and IL-4 SNP *rs2243248* (Dhruva et al., 2015; Illi et al., 2012), whereas other SNPs have only been reported to be associated in single studies (Dhruva et al., 2015; Doong et al., 2015; Illi et al., 2012; Miaskowski et al., 2010; Rausch et al., 2010; Reyes-Gibby et al., 2013a; Reyes-Gibby et al., 2013b).

Candidate SNPs examined were mainly located in the promoter, untranslated region (UTR), or coding region of the gene. SNPs that could influence gene expression through further mechanisms, including amino acid changes, exon skipping, proximal promoter variants, distal promoter variants and intronic enhancer variants (Smith and Humphries, 2009), were not considered. Furthermore, studies to date, as reviewed recently, have been underpowered, including 44–599 samples (Saligan et al., 2015; Tariman and Dhorajiwala, 2016), and correction for multiple testing was often ignored even when many SNPs were tested. Reported findings have been inconsistent in direction and magnitude of effect.

Therefore, we aimed to confirm reported associations of inflammatory SNPs with CRF in breast cancer patients as well as to assess additional candidate SNPs with putative functional relevance using a large, independent, well powered prospective study.

2. Material and methods

2.1. Participants

We used data from incident breast cancer patients aged 50–74 years who were recruited into the case–control MARIE (Mamma Carcinoma Risk Factor Investigation) study (Flesch-Janys et al., 2008) conducted in Germany from 2002 to 2005 and re-contacted in 2009 (follow-up 1: FU1) and in 2014 (follow-up 2: FU2) (the MARIE*plus* and MARIE*plus*2 studies). Patients (cases) were eligible if they had a histologically confirmed primary invasive or in situ breast cancer and were a resident of one of the study regions. All patients had undergone breast surgery.

At baseline/recruitment 3813 patients completed a face-to-face interview that collected comprehensive information on personal and lifestyle factors and provided a blood sample. Clinical data, tumor characteristics and treatment data were abstracted from hospital and pathology records. Information on current fatigue was collected at FU1 (median 6.2 years) and FU2 (median 11.7 years) as well as for pre-diagnosis fatigue retrospectively at FU1 using the self-administered Fatigue Assessment Questionnaire (FAQ). Updated information on lifestyle factors at FU1 and FU2 was obtained through computer-assisted telephone interviews. The studies were approved by the ethics committees of the University of Heidelberg and the review board of the Hamburg Medical Council and were conducted in accordance with the Declaration of Helsinki. All study participants provided informed written consent.

Of the 3305 eligible patients at FU1, 2326 (70%) returned the fatigue questionnaire. Patients with recurrences, metastasis or second tumors before FU1 (n = 212), missing pre-diagnosis fatigue information (n = 30), high pre-diagnosis fatigue levels (values ≥ 7 on a 0–10 scale) (n = 162), missing total fatigue scores (n = 3), and missing genotype data (n = 530/560, depending on number of SNPs genotyped) were excluded, so that 1389/1359 patients, respectively, remained for analysis of CRF at FU1 (Fig. 1A). After similar exclusions, a sample of 950/925 remained for CRF at FU2 (Fig. 1B). For the analysis of CRF with respect to both time-points, a total of 684/665 patients were included (see Statistical Analysis) (Fig. 1C).

2.2. Fatigue measurement

The FAQ is a 20-item, multidimensional self-assessment questionnaire that has been validated for a German-speaking population (Beutel et al., 2006; Glaus and Muller, 2001). It covers the physical, affective, and cognitive dimension of CRF and includes one item on sleep disorder. Possible values for these items were 0 = not at all, 1 = a little, 2 = quite a bit, 3 = very much. The total CRF score was calculated by adding these 20 item scores and standardizing the sum to values from 0 to 100. Subscores for all CRF dimensions were derived by using the same procedure for the appropriate items. Mean imputation of missing data was performed if less than half of the questions used to calculate the respective score were missing. The total CRF level in the year before breast cancer diagnosis, retrospectively assessed at FU1,