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RESEARCH ARTICLE

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No evidence of association between either Modic change or disc degeneration and five circulating inflammatory proteins

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Abstract

Introduction: Intervertebral disc degeneration and Modic change are the main spinal structural changes associated with chronic low back pain (LBP). Both conditions are thought to manifest local inflammation and if inflammatory proteins translocate to the blood circulation could be detected systemically. The work here assesses whether the presence of disc degeneration is associated with detectable blood level changes of five inflammatory markers and whether chronic LBP is associated with these changes.

Materials and Methods: Two hundred and forty TwinsUK cohort participants with both MRI disc degeneration grade and Modic change extent, and IL-6, IL-8, IL-8 TNF, and CX3CL1 protein blood concentration measurements were included in this work. Linear mixed effects models were used to test the association of blood cytokine concentration with disc degeneration score and Modic change volumetric score. Association of chronic LBP status from questionnaires with disc degeneration, Modic change, and cytokine blood concentration was also tested.

Results: No statistically significant association between disc degeneration or Modic change with cytokine blood concentration was found. Instead, regression analysis pointed strong association between cytokine blood concentration with body mass index for IL-6 and with age for IL-6 and TNF. Mild association was found between IL-8 blood concentration and body mass index. Additionally, LBP status was associated with Modic change volumetric score but not associated with any cytokine concentration.

Conclusions: We found no evidence that Modic change and disc degeneration are able to produce changes in tested blood cytokine concentration. However, age and body mass index have strong influence on cytokine concentration and both are associated with the conditions studied which may confound associations found in the literature. It is then unlikely that cytokines produced in the disc or vertebral bone marrow induce chronic LBP.

KEYWORDS
aging, degeneration, inflammation, pain

1 | INTRODUCTION

Chronic low back pain (LBP) is the leading cause of years lived with disability.[1] While chronic LBP has several etiological factors, intervertebral disc (IVD) degeneration (IVDD) is leading risk factor.[2,3] IVDD is characterized by the deterioration of IVD structure and loss of function and is an age-related condition; however, in some individuals it occurs at an accelerated rate and can be extremely painful. Magnetic resonance imaging (MRI) is used to identify IVDD for its high anatomic detail of spinal tissues,[4] and several IVDD-linked features are examined. Most commonly, measurements include IVD narrowing, disc protrusion or bulge development, osteophyte formation on nearby vertebrae, and changes in disc MRI intensity signals due to disc dehydration. Sambrook et al. used a summary score of disc height, bulge, osteophytosis, and signal intensity changes extracted from MRI and reported a heritability of 74% at the lumbar spine.[5] Modic change (MC) are bone marrow (BM) lesions in the vertebrae visible on MRI and have been associated with IVDD and LBP.[6–8] MC are divided into three types: MC type I (MCI) is characterized by low signal intensity in T1 and high intensity disc on T2 MRI where fibrous tissue replaces BM indicating inflammation, MC type II (MCII) indicates fatty conversion of BM and vascularization in the disc-bone junction, and MC type III (MCIII) indicates formation of sclerotic tissue in the endplate.[9,10] The inflammation with fibrosis has been corroborated in MCI and MCII tissue extracted during lumbar surgery.[9] MCI is associated with an increased rate of bone remodeling, while reduced bone formation characterizes MCII and in MCIII BM became sclerotic tissue.[11] Nevertheless, sclerotic tissue has also been found in mixed types (MCI/MCII and MCII/MCIII).[12] MC has numerous risk factors. IVDD normally coincides with MC and many risk factors are shared between both. Aging, spinal deformities, and female sex increase MC physiological risk factors, environmental risk factors include smoking, obesity, and overloading,[13–16] and MC heritability has been estimated at 30%.[15]

IVDD and MC co-occur with shared features (such endplate defects and herniations), yet the direction of causality is unknown. It is probably complex and likely to vary between individuals.[9,16–18] The size of BM changes and IVDD are proportionally correlated.[19] And endplate damage is thought to link BM changes with disc changes. It has been suggested that in contrast to age-related BM changes, MC starts at the interphase between disc and vertebra (endplates) and progresses to the inner BM (diaphysis).[20,21] Endplate damage affects crosstalk between BM and the IVD by increasing the transit of metabolites, extracellular matrix (ECM) degrading enzymes, inflammatory, and regulatory factors.[22–24] MC, especially MCI, has been associated with inflammatory processes.[21,25] MC explants contain both granulation and fibrotic tissue, suggested an indicator of inflammation cycles and healing attempts.[26] The presence of MC has been associated with different inflammation processes such as macrophage infiltration to the disc[27] (which produce inflammatory proteins such as IL-6 and TNF[27]) and increases in levels of C-reactive protein (CRP) in the BM.[28] MC occurs in conjunction with increased endplate degeneration, and it has been posited inflammatory, angiogenic, and neurogenic factors account for the association between MC and BP.[28] There are several lines of evidence to suggest endplate damage is a IVDD initiator[29,30]; however these reports have been only partially replicated.[31] The co-expression of inflammatory and fibrogenic genes in the adjacent discs and MC presenting BM serves as evidence of a synergistic and coordinated participation of both tissues initiating and perpetuating inflammation and fibrogenesis.[26,27] Moreover, MC (both type I and II) adjacent discs demonstrate increased production of inflammatory mediators such as IL-6, IL-8,[31] and higher incidence in LBP symptomatic subjects,[32] further suggesting a combined inflammatory response of both tissues.[31,33] Indeed, disc cells have natural production of inflammatory factors such as interleukins with pro-inflammatory effects (e.g., IL-1, IL-6, IL-18), interleukins with anti-inflammatory effects (e.g., IL-10 and IL-6), chemokines (e.g., CXCL1, CXCL8, CX3CL1), and TNF among others.[34]

MC, as a potential cause of LBP, is diagnosed using MRI, yet clinical protocols in the UK and many other countries do not recommend MRI scanning for nonspecific back pain. Thus, a straightforward analysis of blood biomarkers could offer a relatively accessible solution for improved and earlier clinical decision-making and treatment.[35] Therefore, whether MC derived inflammatory markers are detectable in blood is being actively studied as part of attempts to identify clinical biomarkers. A recent study found significant correlations between MCI size and IL-6 blood levels and between blood and BM CRP levels in patients who underwent spinal fusion.[36] Similarly, serum concentrations of different chemokines (such as CCL2 and CCL5), cytokines (IL-8 and IL-15), and TNF were increased in chronic LBP patients with MC (mostly MCI but also MCII) compared with healthy controls (neither LBP, nor MC).[37] In contrast, in a study including chronic LBP patients, there were no significant differences in IL-1β, IL-6, IL-8, and TNF levels between those with or without MC.[38] While blood inflammation marker levels remain a tantalizing prospect of a diagnostic biomarker for IVDD, consistent results are yet to be reported.[39,40] Thus, while there is evidence of inflammatory serum biomarkers for MC and IVDD, literature to date is not consistent and often studies are hampered by small study sizes and lack appropriate consideration of confounding factors.

Advances in proteomic technologies enable the analysis of large sample sets with high sensitivity, allowing the detection of small changes of proteins.[41,42] Thus, this study aimed to investigate whether serum levels of IL-6, IL-8, IL-10, and TNF correlated with MC size, IVDD score, and chronic LBP in a large population sample while considering various confounding factors including age, BMI, and smoking history (Figure 1). Additionally, because fractalkine (CX3CL1) has recently been identified as a neuroactive chemokine that causes crucial neuronal sensitization in chronic LBP[43] and it is produced by disc cells,[34] we also investigated whether MC and IVDD were associated with a systemic increase of its concentration.

2 | METHODS

2.1 | Ethics

This retrospective study was conducted utilizing data collected under TwinsUK BioBank ethics, approved by North West—Liverpool Central

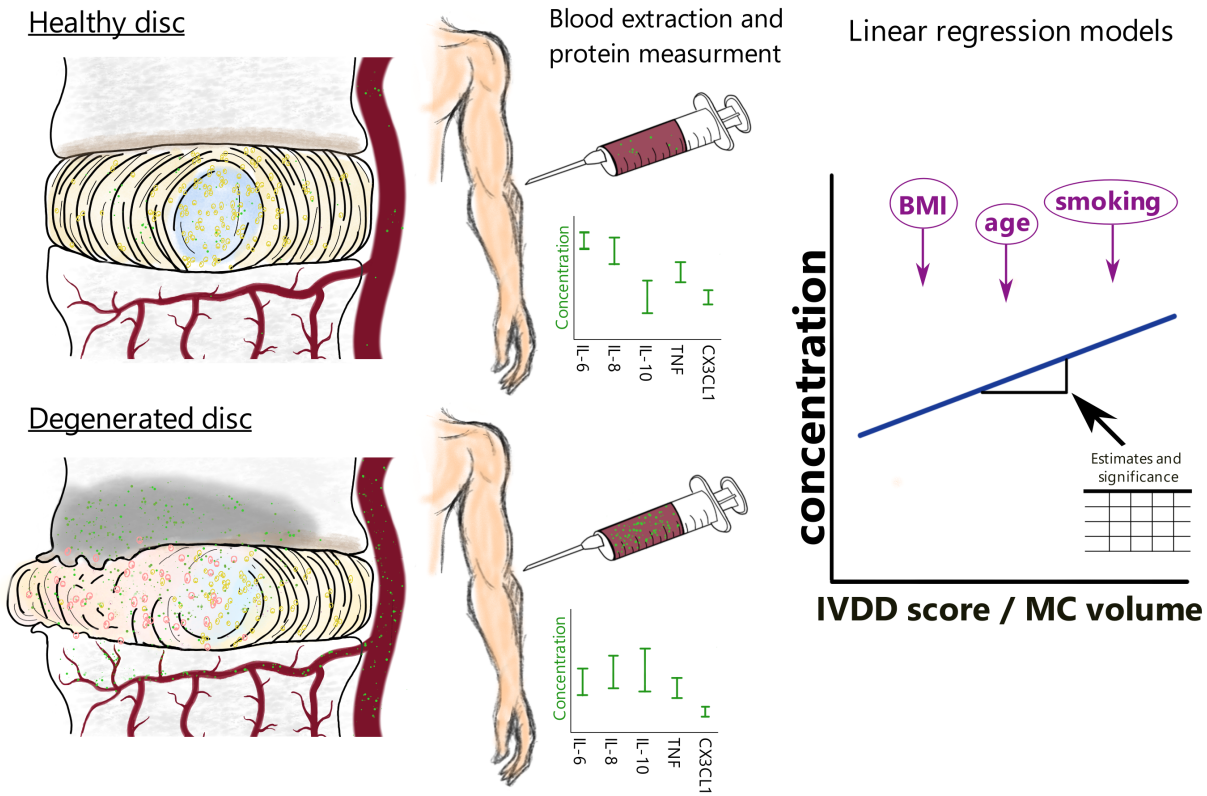

FIGURE 1 Graphical representation of the aim and plan. A healthy disc is shown on the top left. A degenerated disc, presenting the main features of disc degeneration and Modic change, is shown on the bottom left. Systemic blood from participants is extracted and inflammatory protein concentrations are then measured. Inflammatory protein concentrations are then regressed on the disc degeneration score and Modic change volume adjusting for age, BMI, and smoking. Regression estimates, their deviation, and significance are finally tabulated. BMI, body mass index; IVDD, intervertebral disc degeneration score; MC, Modic change.

Research Ethics Committee (REC reference 19/NW/0187), IRAS ID 258513. This approval supersedes earlier approvals granted to TwinsUK by the St Thomas' Hospital Research Ethics Committee, later London—Westminster Research Ethics Committee (REC reference EC04/015), which have now been subsumed within the TwinsUK BioBank.

2.2 | Study participants

All participants were drawn from the TwinsUK cohort, the largest and most extensively characterized adult twin cohort in the world. It was originally created in 1992 to study osteoporosis and osteoarthritis, both highly prevalent in women. Thus, initial recruit included a few hundred predominantly female volunteers. Over the years the cohort has been rapidly expanded to over 15 000 male and female monozygotic (MZ) and dizygotic twins, however, still with a higher predominance of females. TwinsUK participants are followed annually with questionnaires and quadrennially with clinical visits in order to record their health status and retrieve biological samples (e.g., blood samples).[44]

Between 1995 and 2000, a random subgroup of twins was invited for spine MRI scanning. From those, 832 had successful MRI scanning and IVDD and MC coding from which a subset underwent follow-up MRI 10 years later and 350 were successfully coded for IVDD and MC. In parallel, between 1997 and 2018 fasting blood samples from two subgroups of the TwinsUK cohort were selected to undergo the Olink® proteomics assay in two different batches, respectively.

In total, 436 participants were included in at least one time point protein analysis (1999–2018) and one spinal MRI coding (1995–2009) group. In cases where multiple blood samples were available the one closest in date to the MRI scan was selected. Participants having more than 5 years' difference between blood sample and MRI were excluded, leaving 240 participants in the study (Figure 2 shows a summary flow diagram).

2.3 | Proteomic data extraction and preprocessing

Blood from TwinsUK volunteers was collected at clinical visit and then centrifuged to obtain serum which was stored frozen at −80°C. Serum inflammatory markers were quantified using Olink® Target 96 Inflammation Panel, which measures 92 inflammatory proteins (https://Olink.com/products-services/target/inflammation). Olink® utilizes Proximity Extension Assay (PEA) which allows high precision analysis of multiple proteins. PEA uses antibodies linked to DNA complementary tags. Upon binding with the protein, proximity of complementary tags allows for amplification through PCR.[45] This amplification process increases precision of detection and quantification of small quantities of protein.

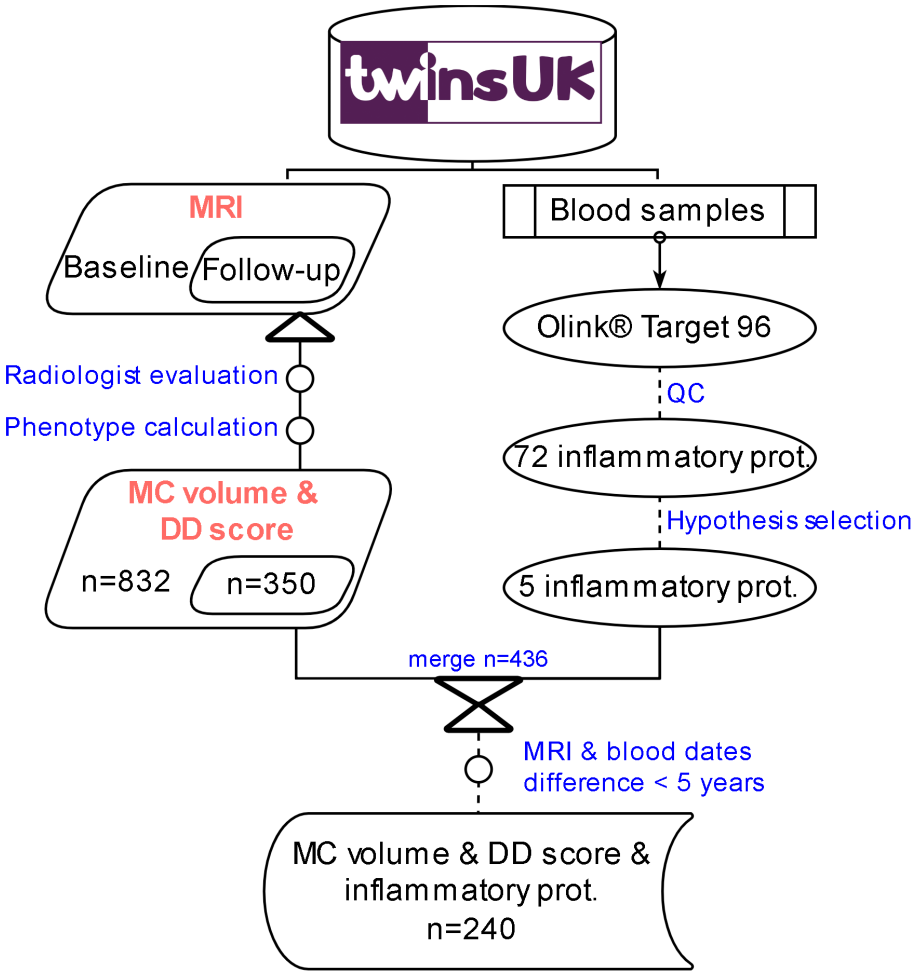

FIGURE 2 Flow diagram of the selection and filtering of the data for inclusion in this study. Dash lines indicate filtering the data.

Protein concentration is expressed as normalized protein expression using Olink® arbitrary unit in log2 scale (NPX). Normalization between the two batches had been executed according to company guidelines (https://Olink.com/faq/data-normalization/). Briefly, 16 samples were included in both proteomic batches to compare and adjust for batch effects, allowing a robust and effective sample merge. For each protein, pairwise differences of the 16 bridge samples were calculated and the median was subtracted from one of the dataset measurements. From the 92 inflammatory proteins included in the Olink platform, 20 did not reach the limit of detection (based on negative control on each analysis plate) of the assay on at least 30% of the samples. Thus, 72 were suitable for further inclusion in analysis. In order to maintain power of the study, we pre-selected five proteins for analysis based on previous reports of associations with IVDD and MC (IL-6, IL-8, IL-10, TNF, CX3CL1).

2.4 | Spine MRI specifications and phenotypes extraction

T2-weighted sagittal imaging had been performed using a Siemens (Munich, Germany) 1.0 T superconducting magnet scan in the baseline MRI scanning and a 1.5 T magnet scan for the follow-up series. Sagittal series of images had been obtained for cervical, thoraco-lumbar, and lumbar spine. Lumbar spine (L1–L5) disc images had been coded for disc height loss, disc bulge, anterior osteophyte formation, and changes in signal intensity of nucleus pulposus each using a four-point scale (0–3), where 0 represents healthy/normal disc and 3 represents maximal degenerative state. Scores were then summed for each disc level and for all five disc levels, giving an IVDD score range from 0 to 60 as previously described.[30,46,47] Similarly, MC axial area and sagittal depth had been formerly calculated on T2-weighted MRI in both rostral and caudal vertebrae regardless of the MC type.[6,15] Briefly, MC axial area was defined as number of vertebral divisions, four quadrants, and a central circular area, presenting MC. MC sagittal depth was defined as the extension of MC in the vertebral body height. Axial area and sagittal depth were multiplied to calculate a MC volumetric score. Rostral and caudal volumes were summed at each disc to give a quantitative MC volumetric score of each level. Next, five lumbar disc level volumetric scores were summed to get an individual measure.[6,15] Size and prevalence of MC in higher spine levels are both very reduced in higher levels thus they were not considered in this study.

2.5 | Low back pain definition

Twins from TwinsUK undergo a series of clinical and laboratory tests, which include a nurse-led interview, and regularly respond to

questionnaires relating to their health and lifestyle. On those questionnaires, they are asked about their lifetime history of LBP. Each twin completes the questionnaires independently. The questionnaires contain written questions as well as a mannequin pain diagram that allowed for an assessment of location, severity, and duration of pain. In the diagram, LBP is defined as pain located between the 12th rib and the gluteal sulcus. Two questionnaires were used for this study. The first questionnaire was answered at the time of MRI scanning, using the UK Medical Research Council Nurses Study format, and the procedure is described elsewhere.[48] The second questionnaire used the London Fibromyalgia Epidemiology Symptom Screening Questionnaire format.[6,49] Utilizing answers from both questionnaires, chronic LBP was defined as a binary variable where cases presented a total duration of LBP longer than a month according to the first questionnaire or 3 months according to the second questionnaire, as described previously.[50]

2.6 | Study design—models and variables

Different model designs were considered. A simplified model construction examined the relationship between protein concentration and MC and IVDD, along with the necessary twin relationship adjustment (family membership was introduced as a random effect). Logarithmic in base 10 transformation (log10) was applied to all protein concentrations to achieve normally distributed concentrations, assessed with Kolmogorov–Smirnov test. Next, protein concentrations below and above the 1.5 interquartile range from the first and third quartiles, respectively, were treated as outliers and removed from the main analysis. Normality was confirmed by Kolmogorov test. Sensitivity analysis without outlier removal can be found in the supporting information in Data S2. Individual protein concentrations were used as response, and MC and IVDD as predictor variables, giving a total of five models (one for each protein). These models were extended by including additional adjustments (fixed effects) for age, BMI, and smoking status (extended models). To exploit the capability of twin relationship, a cotwin control regression model was included. Cytokine concentrations were regressed on between and within twin-pair differences of MC, IVDD, BMI, and smoking status using all twin pairs included in the study and only MZ twin pairs as indicated elsewhere.[51] Between twin-pair age differences were also included. An extra model was defined to test associations of chronic LBP with MC, IVDD, and protein concentration which also included the confounders of the extended model. Chronic LBP was set as response and the five cytokines, MC, DD, and the common risk factors as predictors. A detailed summary of the different models used can be found in the supporting information in Data S2.

2.7 | Statistical analysis

Data processing and cleaning was performed in Python (v3.10.8). General linear regression models were determined using LMER and GLMER (logistic regression for the LBP model) functions from lme4 R package (v1.3-31). To assess correct fitting and non-violation of assumptions of the linear models, normality of the residuals was confirmed through QQ plots and Shapiro–Wilk test. Quantitative predictors (age, BMI, IVDD score, and MC volumetric measure) were scaled and centered (mean subtracted). Absence of collinearity was confirmed through variance inflating factor (VIF), which estimates the inflation of the regression coefficient of a predictor due to multicollinearity in the model. Typically, values below 5 indicate acceptable collinearity.[52] To assess for multiple test correction, p-value significance threshold was modified using Bonferroni correction using a base alpha of 0.05.

Supplementary methods are available in the supporting information in Data S1 and include details of the power calculation, preprocessing data transformation, and fold change calculations from linear regression estimates.

3 | RESULTS

3.1 | Study subjects and inflammatory proteins

The 240 subjects included (see Figure 2) were females with a mean age of 63.5 years old (43–79 years) and mean BMI of 26 kg/m^2 (18–44 kg/m^2). Sixty of the subjects were MZ twins, 98 dizygotic twins, and 82 did not have their twin relatives included. Table 1 provides demographic information. Forty-eight percent of the participants presented MC at least at one lumbar disc. Thirty-three percent of the participants presented MC volume different from zero at one disc, 11% at two discs, and 3%, 1%, and <1% at three, four and all lumbar discs investigated, respectively. MC presence was increased in lower lumbar levels (from 5% on L1/2 disc to 24% on L5/S1 disc). Similarly, the mean MC volumetric score increased linearly from higher to lower levels ($R^2 = 0.97$) ranging from 6.08% at L1/2 to 11.81% at L5/S1 discs.

Age and BMI and MC size were significantly correlated (Spearman's correlation p-value 0.04 and 0.03, respectively). There was a strong correlation of IVDD with age and BMI ($p = 1.05E{-}8$ and 5.44E−3 respectively). Smoking status (never, former, and current; 129, 89, and 24 subjects, respectively) did not show significant

TABLE 1 Demographics of the subjects from TwinsUK.

$N = 240$	Mean	SD	Range
Age [years]	63.48	7.30	43–79
BMI [kg/m^2]	26.11	4.32	17.84–43.75
IVDD	22.35	7.01	1–51
MC score[a]	0	12	0–50

Note: Participants are females ($n = 240$).
Abbreviations: BMI, body mass index; IVDD, intervertebral disc degeneration score; MC, Modic change.
[a]Variable with non-normal distribution; median instead of mean and interquartile range instead of standard deviation are provided.

TABLE 2 Distribution descriptors of proteins concentration (NPX).

	Mean (log10 scale)	SD (log10 scale)	*N*
IL-6 [NPX]	2.52 (0.39)	0.55 (0.09)	232
IL-8 [NPX]	5.06 (0.70)	0.44 (0.04)	229
IL-10 [NPX]	3.62 (0.56)	0.42 (0.05)	226
TNF [NPX]	2.90 (0.46)	0.39 (0.06)	235
CX3CL1 [NPX]	4.69 (0.67)	0.45 (0.04)	235

Note: Concentration means and standard deviations of the five proteins concentration. Logarithmic (base 10) was used to achieve normality of the distribution, and their values are showed within parentheses.
Abbreviation: NPX, normalized protein expression units.

correlations either with MC volumetric measures or IVDD scores. Age and BMI did not show a significant correlation ($p = 0.43$).

Preprocessing (outlier removal) of the five protein measurements lead to slightly different sample size for the study of each protein with a minimal sample size of 226 measurements for IL-6. Further descriptors of the protein concentrations after processing can be found in Table 2. A scatter plot of the data is shown in Figure 3. There were correlations found within concentrations of the inflammatory markers IL-6, IL-8, IL-10, and TNF. CX3CL1 concentration was only correlated with IL-8 and IL-10 concentrations.

3.2 | Association of inflammatory proteins concentration and MC and IVDD

Using the simplified model (without risk factor adjustment), no correlations between MC score and any inflammatory protein were found (Table 3). There were correlations before BF correction between IVDD and IL-8 and CX3CL1 (p-values 0.046 and 0.049 respectively) but they were lost after BF correction (adjusted p-value threshold 0.01).

In the extended model, which includes adjustment for known risk factors, IL-6 and IL-8 concentrations were significantly impacted by variations in BMI (Table 4). An increase in BMI of 10 units (kg/m^2) corresponded to increases in IL-6 and IL-8 concentrations of 1.47-fold (CI: 1.30–1.68) and 1.13-fold (CI: 1.03–1.25) around their mean values, respectively. Similarly, an increase of 10 years of age was linked to increases in IL-6 concentration of 1.12-fold (CI: 1.04–1.21) and TNF of 1.09-fold (CI: 1.03–1.15) in mean values. Smoking showed an increasing trend with IL-6 and IL-8 before BF correction. Neither MC nor IVDD were significantly correlated with protein concentrations.

Sensitivity analyses were performed by applying the extended model to investigate the extent of the effect of outlier removal (supporting information in Data S2, Table S1). Results showed no important change. Additionally, dispersion of outliers along the different risk factors, MC and IVDD shows no visual trend of outliers toward any specific value(s) of risk factors and phenotypes (Figure 3).

In agreement to last results, cotwin control regression model using MZ and DZ pairs ($n \sim 79$ pairs) resulted in significant association of between twin-pair BMI difference with IL-8 concentration, while its association with IL-6 failed to reach the adjusted significance of 0.01 (p-value = 0.011). Also in concordance, between twin-pair age differences were associated with IL-6 and TNF concentrations. Either between or within MC and IVDD twin-pair differences were associated with any cytokine concentration, both using DZ and MZ twin pairs and MZ alone (see supporting information in Data S2, Tables S2 and S3).

3.3 | Association of LBP with MC, IVDD, and inflammatory proteins concentration

To assess the association of chronic LBP with blood cytokines concentration and MC and IVDD scores, chronic LBP status was regressed against MC, IVDD, inflammatory proteins, and risk factors. Only MC was associated with increased risk of experiencing chronic LBP (Table 5). The odds ratio for chronic LBP increased by 1.06 for every unit increase in MC volumetric score unit (CI: 1.02–1.15). There were no correlations between chronic LBP and any of the systemic inflammatory proteins investigated.

4 | DISCUSSION

To test if MC and IVDD promote systemic inflammation through cytokine translocation to the blood flow, this study investigated the MC and IVDD relationship with blood concentration of five selected inflammation proteins, which have been previously associated to MC and chronic LBP conditions in literature. Other studies with different designs have investigated such relationships but have reported different results. In the current study, a large population sample and quantitative measures of MC and IVDD were utilized. Two models were applied and compared, improving on previous designs with the inclusion of confounders (common risk factors). When no confounders were considered (simplified model), there was a trend of increasing IL-8 and CX3CL1 concentrations with increasing IVDD measures but there was no statistically significant correlation between serum concentrations of any of the five proteins and MC or IVDD, in either model. When common risk factors were accounted for in the analysis, age and BMI showed a strong influence on IL-6, one of the most meaningful inflammatory proteins for MC and IVDD and thus one of the most studied. TNF and IL-8 were also influenced by age and BMI, respectively. Not surprisingly, the link between IL-6 and obesity has been demonstrated in many studies.[53–56] Rodrigues et al. found changes in IL-6 levels in high BMI patients with diabetes mellitus type 2, but no differences in IL-10 nor TNF, echoed by the results of our study.[55] Similarly, Charles et al. found an association between BMI and IL-6 levels, but no BMI association with IL-10 levels.[54] Further in line with our results, IL-8 has been reported positively correlated with BMI in different studies.[57–59] Again, concordantly with the current report, there is strong evidence of the involvement of IL-6 and TNF concentrations in aging and related disorders.[60–62]

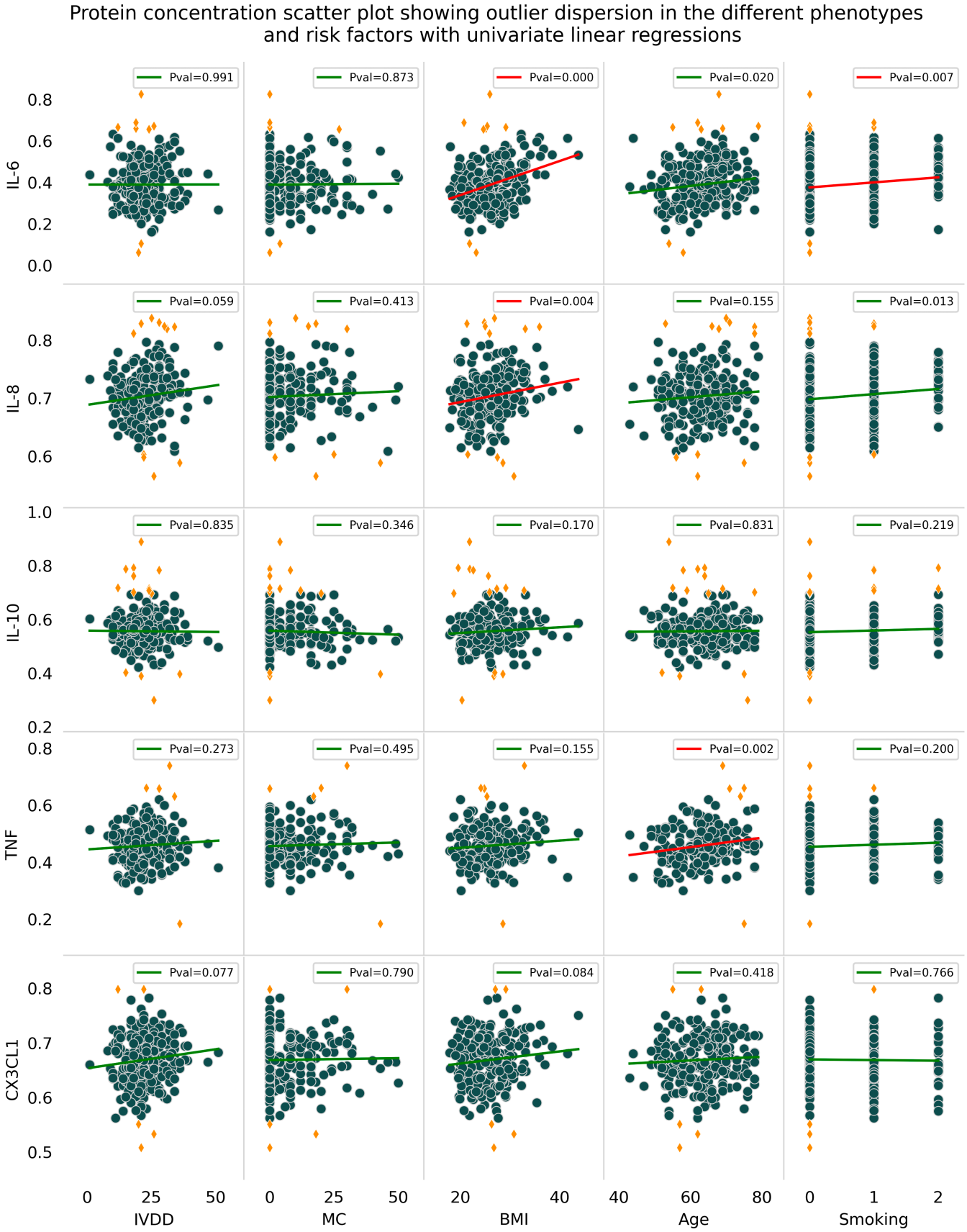

FIGURE 3 Scatter plot of protein concentration. Statistical outliers are colored in orange. Lines corresponding to univariate linear regression, in read regressions with significant estimates (p-value <0.01). Protein concentration in logarithmic scale. Age is expressed in years and smoking status corresponds to never, former, and current smoker. BMI, body mass index [kg/m^2]; IVDD, intervertebral disc degeneration score; MC, Modic change.

TABLE 3 Association of systemic cytokine levels with MC and IVDD—simplified model.

	Predictors	Estimate	Std. error	*p*-value
IL-6	MC	2.69E−04	6.70E−04	0.689
	IVDD	−5.64E−05	9.81E−04	0.954
IL-8	MC	−2.17E−05	2.74E−04	0.937
	IVDD	7.86E−04	3.91E−04	0.046
IL-10	MC	−2.58E−04	3.57E−04	0.471
	IVDD	8.72E−05	5.25E−04	0.868
TNF	MC	9.61E−05	4.18E−04	0.818
	IVDD	4.61E−04	6.07E−04	0.448
CX3CL1	MC	−1.89E−04	2.89E−04	0.514
	IVDD	8.55E−04	4.32E−04	0.049

Note: Linear regression assessing the relationship of MC and IVDD with each protein concentration. Only adjustment for twin relationship is included (simplified model). *p*-value significance threshold is 0.01 after multiple test correction.
Abbreviations: IVDD, intervertebral disc degeneration score; MC, Modic change.

TABLE 4 Linear regression results of cytokines association with MC and IVDD with risk factors adjustment—extended model.

	Predictors	Estimate	Std. error	*p*-value
IL-6	MC	−9.60E−05	6.07E−04	0.875
	IVDD	−1.64E−03	9.49E−04	0.086
	Age	**2.70E−03**	**8.61E−04**	**0.002**
	BMI	**8.46E−03**	**1.31E−03**	**<0.001**
	Smoking	1.78E−02	8.32E−03	0.034
IL-8	MC	−1.09E−04	2.70E−04	0.688
	IVDD	4.69E−04	4.15E−04	0.260
	Age	4.79E−04	3.82E−04	0.211
	BMI	**1.52E−03**	**5.85E−04**	**0.010**
	Smoking	8.13E−03	3.59E−03	0.024
IL-10	MC	−4.18E−04	3.58E−04	0.245
	IVDD	0.00E+00	5.66E−04	1.000
	Age	2.33E−04	5.06E−04	0.646
	BMI	1.06E−03	7.84E−04	0.179
	Smoking	6.33E−03	5.04E−03	0.211
TNF	MC	6.60E−05	4.14E−04	0.873
	IVDD	−1.71E−04	6.46E−04	0.791
	Age	**1.75E−03**	**5.76E−04**	**0.003**
	BMI	1.24E−03	8.84E−04	0.163
	Smoking	7.12E−03	5.60E−03	0.205
CX3CL1	MC	−1.93E−04	2.93E−04	0.510
	IVDD	7.19E−04	4.70E−04	0.127
	Age	1.31E−04	4.42E−04	0.768
	BMI	1.06E−03	6.49E−04	0.103
	Smoking	−1.66E−03	3.98E−03	0.676

Note: Results summary of the linear regressions assessing the relationship of MC and IVDD with each protein concentration. Adjustment for twin relationship, age, BMI, and smoking are included (extended model). *p*-value significance threshold is 0.01 after multiple test correction from a significance of 0.05. Corresponding significant predictors in bold.
Abbreviations: BMI, body mass index; IVDD, intervertebral disc degeneration score; MC, Modic change.

Therefore, when studying cytokines systemically, risk factors known to influence cytokine levels must be considered. It is essential when cytokine concentrations are measured in conditions that co-occur with those same risk factors; that is, IVDD and MC with age and BMI. Two studies to date have investigated links between blood inflammatory biomarkers and the presence of MC.[37,38] A study of 34 LBP participants did not find differences in IL-1β, IL-6, IL-8, and TNF blood concentrations comparing presence and absence of MC.[38] In contrast, Karppinen et al. showed that LBP patients had significantly lower serum concentrations of IL-8 and TNF (among other proteins) compared to pain-free controls without MC.[37] Unfortunately, neither of the previous studies included an analysis of the potential confounding effects of BMI, which may account for the nil or surprising results reported. One may expect MC participants to have increased pro-inflammatory cytokines, in opposition to these findings. When only participants with chronic LBP are included in a study the effect of commonly used anti-inflammatory medications may explain the reduction in pro-inflammatory markers.[63]

Boisson et al. (2021) included participants with LBP (with and without MC) while the current study utilized standard population samples (TwinsUK—which includes those with and without LBP). Population cohorts have intrinsically less selection bias, allow a clearer examination of confounding factors, and generate more generalizable to general population results.[64] Additionally, they permit large sample sizes and indeed the current study is the largest reported to date and its design is powerful enough (see Data S1) to detect concentration changes lower than those previously reported. Notwithstanding this, it is important to note that the participants used in this study are relatively old (mean age 63.48 ± 7.30 years) and this could potentially hinder associations with MC and IVDD, as inflammatory phases of these conditions are less likely to be active and inflammatory marker levels may be remnants of previous disease processes. This occurrence has previously been reported in similar conditions such as rheumatoid arthritis[65] and osteoarthritis.[66] Nevertheless, Boisson et al.'s results agree with the current study, they did not show a relationship between MC and systemic cytokines levels, even when their participants were younger.[38]

Similarly, sex has been reported to influence levels of inflammatory markers.[67,68] The sample used in this analysis comprised only females and results may not be generalizable to males. Together, these described design differences could explain divergent results. Additionally, other confounders or conditions may play important roles in cytokine systemic concentration levels, such as other comorbidities or medications (i.e., anti-inflammatory medications). To evaluate the potential confounding effect of anti-inflammatory drug usage, we examined recurrent use of nonsteroidal anti-inflammatory drugs (NSAIDs) in the analyzed dataset, utilizing TwinsUK longitudinal

TABLE 5 Association of LBP with cytokines, MC, and IVDD.

Predictors	Estimate	Std. error	*p*-value
MC	**0.057**	**0.220**	**0.007**
IVDD	0.023	0.210	0.440
IL-6	0.899	2.267	0.692
IL-8	5.010	5.494	0.362
IL-10	0.107	4.065	0.979
TNF	6.418	3.907	0.100
CX3CL1	−0.970	4.624	0.834
Age	−0.165	0.208	0.429
BMI	−0.119	0.196	0.543
Smoking	0.104	0.269	0.699

Note: Results summary of the linear regression assessing the relationship between LBP with the different protein concentrations, MC and IVDD. Adjustments for twin relationship, BMI, age, and smoking are included. *p*-value significance threshold is 0.05. Corresponding significant predictors in bold.

Abbreviations: BMI, body mass index; IVDD, intervertebral disc degeneration score; LBP, low back pain; MC, Modic change.

records of medication usage. No significant differences in cytokine concentration were observed in the individuals with recurrent NSAID use (8.75%, 21 out of 240 participants), with *p*-values ranging from 0.17 to 0.91. Genetic background may also be an important player since cytokine levels and response have high heritability estimates.[69] Indeed, genetic studies (including GWAS) of cytokine production associated with different infectious agents has led to the discovery of genetic variants that modulate cytokine production.[70,71] Such variants hinder the effects of MC and IVDD in blood cytokine concentration levels. The use of twin models, specifically including only MZ twins, should bypass this analytic hindrance, however, the cotwin control analysis showed no association between the cytokines and MC and/or IVDD. If MC and IVDD do have small effects on blood protein concentrations, the biological impacts may be negligible, since, as discussed, BMI and age, both so strongly associated with IVDD and MC, have a large impact on the systemic inflammation profile.

While systemic levels of the five inflammatory proteins tested were not associated with IVDD and MC in this study, there is compelling evidence that local inflammatory factors produce changes in both MC (BM)[21,72] and IVDD (disc tissue).[73,74] BM hosts different immune cell types, which naturally produce these cytokines,[75] and BM vascularization could lead to the rapid translocation of BM-produced cytokines to the blood flow leading to potential biomarkers of BM pathology, as shown for different BM conditions.[76,77] Differently, IVD is an immune-privileged tissue,[74,78] within an intact IVD, the native cells of the disc themselves synthesize and respond to pro-inflammatory cytokines such as IL-1 and IL-6,[79] which can lead to decreased ECM synthesis (aggrecan, collagen) and to increased production of ECM degrading enzymes.[34,73,80] For further detail see review by Bermudez-Lekerika et al.[74,80,81] The regulation of cytokine synthesis by IVD cells is complex and includes mechanical stimuli, nutrient deprivation, and disc cell senescence.[74,81] Additionally, endplate or annulus fibrosus damage can lead to blood vessel ingrowth and immune cell infiltration (which produces cytokines), thus enabling crosstalk of IVD with systemic regulators and modifying disc homeostasis.[74] The implication of increased production of cytokines in disc is speculated to have several downstream effects including the initiation or propagation of IVDD phenotypes.[78,82] This study did not test whether an increase in blood cytokines is predictive or causative of IVDD. The focus was solely on determining the association between inflammation in MC and IVDD and detectable changes in blood cytokine concentration.

Unfortunately, in this study IL-1β was not analyzed due to the proteomics platform utilized, however, the cytokines produced as downstream effects of IL-1β (such as IL-6 and IL-8) were included giving an indication of this pathway activation. It is possible that other cytokines may be used as blood biomarkers. The cytokines examined in this study correspond to those reported as produced in the disc, known to initiate a catabolic response in the disc, or those most commonly associated with IVDD and which were available in our data. In this study, only T2-weighted MRI scans were available, preventing differentiation between MC types. A more detailed stratification of subjects could enhance result comprehensiveness. Similarly, IVDD was assessed using a summary score. It is possible that each IVDD trait may have a different etiology and individual analysis could lead to revealing results. However, increasing the number of variables again would result in penalty for performing multiple tests and reduce analytical power. Nevertheless, previous studies showed that correlation of the independent traits and LBP was the same as the summary score.[83] Additionally, data for MC and IVDD were only available for lumbar levels. Including cervical and thoracic levels could further expand and strengthen the analysis.

This study also examined to what extent MC, IVDD, and the five proteins, could explain the presence of LBP. As previously demonstrated in TwinsUK data, LBP has been associated with MC.[6] However, none of the inflammatory marker levels were increased in the presence of chronic LBP in our study. These findings show no evidence that MC and chronic LBP relationship may be due to traditional inflammation markers within the systemic circulation. Furthermore, the evidence that LBP produces differences in blood concentration of inflammatory proteins is uncertain as the literature shows conflicting results. Koch et al. reported IL-6 plasma concentration was increased in LBP but no differences were found in IL-1β and TNF concentrations.[84] Similarly, Licciardone et al. found that increased IL-6 concentrations were associated with more severe pain reporting from LBP patients.[85] In contrast, neither IL-6 nor IL-10 were associated with serum concentrations in chronic LBP when compared to healthy controls according to Luchting et al.[86] and TNF concentration demonstrated higher in a study including 29 LBP patients and 29 pain-free controls.[87] None of these studies used association methods that included covariates control (such as age and BMI). Conflicting results may also arise from large interpersonal variance between included participants. Thus, a longitudinal study investigating differences in non-LBP and LBP states of the same participants would be appropriate and encouraged. Moreover, CX3CL1 was included in the analysis here to assess if there was a direct link between MC, IVDD, and neural sensitization. No association was found between CX3CL1 and

chronic LBP, IVDD, or MC. It is still unclear how MC and IVDD cause chronic LBP and how the communication occurs between the tissues of the spine and the systemic flow. Pain is inextricably linked to psychological and neurological factors,[88,89] which must not be overlooked but whether there is a link between psychological factors and spinal conditions was beyond the scope of this study. It is possible that LBP requires stratification before biological associations can be understood.

In the datasets used here, there were time differences between protein quantification and MRI scanning therefore for some participants, temporal bias may have been introduced in the analysis, producing deviated effects. A maximum threshold of a 5-year time difference was set to diminish the effect of this gap, although reassuringly 45% of the participants included in the analysis had their MRI and blood extraction on the same day. Association analyses using the extended model and the LBP regression were repeated for this subset of participants (supporting information in Data S2, Tables S4 and S5). This subgroup analysis did not show substantial differences.

In this study we showed no evidence that either MC size or IVDD produce a change in the blood concentration of five key proteins involved in the fundamental inflammatory pathway. In contrast, we demonstrated the strong link between blood inflammatory protein concentrations and common IVDD and MC risk factors such as BMI and age. In addition, our results show that MC has a strong correlation with chronic LBP but no evidence that this link is due to a systemic inflammation crosstalk through the five studied cytokines. We did not find any evidence that MC and IVDD increased CX3CL1 blood concentration levels and therefore no suggestion neural sensitization is the link between the spine conditions and chronic LBP.

In summary, our findings in a generous sample of women suggest previous reports of increased systematic inflammatory markers in individuals with MC and IVDD may reflect other causes of inflammation such as increased BMI. We do not find evidence that the five studied cytokines cause chronic LBP through changes in systemic blood concentration.

AUTHOR CONTRIBUTIONS

The authors confirm their contributions to the paper as follows: RC and FMKW contributed to the study conception and design; DV, AN, and GL were responsible for data collection and distribution; RC led the analysis and interpretation of results supervised by MBF, CLLM, and MBF; RC led manuscript and figures preparation. IGS, FMKW, and CLLM reviewed and edited the manuscript. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data are available upon request in line with our ethics guidelines via the cohort site (https://twinsuk.ac.uk/resources-for-researchers/access-our-data/).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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