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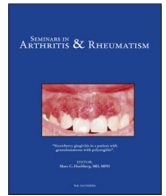
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A systematic review and meta-analysis of the prevalence of small fiber pathology in fibromyalgia: Implications for a new paradigm in fibromyalgia etiopathogenesis

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ABSTRACT

Objectives: Fibromyalgia is a condition which exhibits chronic widespread pain with neuropathic pain features and has a major impact on health-related quality of life. The pathophysiology remains unclear, however, there is increasing evidence for involvement of the peripheral nervous system with a high prevalence of small fiber pathology (SFP). The aim of this systematic literature review is to establish the prevalence of SFP in fibromyalgia.

Methods: An electronic literature search was performed using MEDLINE, EMBASE, PubMed, Web of Science, CINAHL and the Cochrane Library databases. Published full-text, English language articles that provide SFP prevalence data in studies of fibromyalgia of patients over 18 years old were included. All articles were screened by two independent reviewers using a priori criteria. Methodological quality and risk of bias were evaluated using the critical appraisal tool by Munn et al. Overall and subgroup pooled prevalence were calculated by random-effects meta-analysis with 95% CI.

Results: Database searches found 935 studies; 45 articles were screened of which 8 full text articles satisfied the inclusion criteria, providing data from 222 participants. The meta-analysis demonstrated the pooled prevalence of SFP in fibromyalgia is 49% (95% CI: 38–60%) with a moderate degree of heterogeneity, ($I^2 = 68\%$). The prevalence estimate attained by a skin biopsy was 45% (95% CI: 32–59%, $I^2 = 70\%$) and for corneal confocal microscopy it was 59% (95% CI: 40–78%, $I^2 = 51\%$).

Conclusion: There is a high prevalence of SFP in fibromyalgia. This study provides compelling evidence of a distinct phenotype involving SFP in fibromyalgia. Identifying SFP will aid in determining its relationship to pain and potentially facilitate the development of future interventions and pharmacotherapy.

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Introduction

Fibromyalgia syndrome is characterised by chronic widespread pain, sleep disturbance, fatigue and cognitive impairment, which have a major impact on quality of life [1, 2]. Symptoms include tender and stiff muscles, joints and tendons with multiple tender points,

which are often extremely painful to touch [3] without grossly demonstrable tissue inflammation, deformity, or damage [4].

The underlying etiology of fibromyalgia is yet to be fully elucidated. Dysfunction of the central, autonomic and peripheral nervous systems, alteration of neurotransmitters, endocrine and immune systems, external stressors and psychological aspects have been implicated in the common symptom of widespread pain in fibromyalgia. There continues to be debate around the relative contributions of the central nervous system (CNS) and peripheral nervous system (PNS) in the pathogenesis of fibromyalgia [5]. Disturbed pain processing with central sensitisation,

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identified by increased responsiveness to stimuli has been demonstrated in fibromyalgia. Increased central hyperexcitability including wind up of ascending pathways has been implicated in the generation of widespread pain in fibromyalgia [6, 7]. A meta-analysis of magnetic resonance imaging (MRI) based studies (voxel-based morphometry, functional MRI, or resting state-fMRI) in fibromyalgia showed region-specific changes in grey matter volume, a decreased functional connectivity in the descending pain-modulating system, and increased activity in the pain matrix related to central sensitisation [8]. In addition, single photon emission computed tomography (SPECT) studies have also shown decreased regional cerebral blood flow in the caudate and thalamus of patients with fibromyalgia compared with matched healthy controls [9–11]. However, the temporal relationship of CNS pathology and whether it is a primary defect in fibromyalgia, remains to be established. Other evidence suggests neurotransmitter abnormalities in fibromyalgia are central to the development of pain including elevated insular glutamate, abnormal dopamine response to pain and transmission and metabolism of serotonin leading to dysregulation of pain processing [7, 12–16]. Despite these findings, there is no single unifying CNS pathology present in fibromyalgia that defines this widespread pain state.

While there is no diagnostic gold standard, small fibre neuropathy (SFN) is currently defined as pain and/or par-/or dysesthesias accompanied by findings of small fibre impairment in at least two of the following three tests: neurological examination, quantitative sensory testing, and skin punch biopsy and exclusion of relevant large fibre neuropathy [17]. SFN results in selective impairment of unmyelinated C and thinly myelinated A δ fibers that mediate pain, heat, and cold sensation. Therefore, pathology of small nerve fibers remains a biologically plausible explanation that may at least contribute to the fibromyalgia symptom complex. Indeed, there is a significant sensory symptom overlap in approximately 20–35% of patients with painful neuropathy, suggesting a peripheral neuropathic origin in a subset of people with fibromyalgia [18]. Fibromyalgia has neuropathic pain features, which is often stimulus dependent with hyperalgesia and also exhibits allodynia often in the form of burning or pricking sensations; pain attacks are frequently described [18–21]. Decreased detection thresholds for noxious stimuli such as heat and cold have been demonstrated on quantitative sensory testing [22, 23]. Phenotypic similarities with other peripheral neuropathic pain disorders suggest the underlying mechanism for these symptoms may be of small nerve fiber origin [24], particularly in a subset of individuals. Recently, reduced intra-epidermal nerve fiber (IENF) density (IENFD) has been demonstrated after a sustained increase in insular glutamate in an experimental model of fibromyalgia, suggestive of an underlying pathognomonic neuroplastic process [25]. Several studies in recent years have demonstrated significant small fiber pathology (SFP) in individuals with fibromyalgia [26–33], with a reduction in IENFD [26, 28–30, 33]. Other studies have also demonstrated SFP using corneal confocal microscopy (CCM) [31, 32]. However, SFN needs to be distinguished from SFP in fibromyalgia as the underlying mechanisms causing pathology in small fibers remains to be elucidated and the clinical phenotype of fibromyalgia is distinct from SFN [34]. Furthermore, in a study from the Netherlands the actual prevalence of SFN in the general population was 52.95 cases (60.9 male/45.4 female) per 100,000 inhabitants [35]. The actual background prevalence of pure SFN is significantly lower [35] than SFP in fibromyalgia [26, 28–33].

The aim of this study is to determine the prevalence of SFP in fibromyalgia through a systematic literature review and meta-analysis of published data which have used an objective assessment of small nerve fibers.

Methods

Search strategy

In accordance with PRISMA guidelines, protocol for this systematic review and meta-analysis was developed and subsequently

registered with PROSPERO, (CRD: 42018087277). Electronic literature searches of MEDLINE (access via OVID), EMBASE (access via OVID), PubMed, Web of Science, CINAHL and the Cochrane Library were performed for articles reporting fibromyalgia and SFP. The searches were restricted to English language from inception to April 2018.

A qualified medical librarian and R.G. independently searched the stated databases using varying combinations of the following search terms: 'fibromyalgia', 'fibromyositis', 'fibrositis', 'muscular rheumatism', 'musculoskeletal pain syndrome', 'nonarticular rheumatism', 'periarticular fibrositis', 'rheumatoid myositis', 'tension myalgia', 'myalgia', 'small fibre/fiber neuropathy', 'peripheral neuropathy', 'polyneuropathy', 'painful neuropathy', 'small fibre/fiber sensory neuropathy', 'small fibre/fiber pathology' and 'neuropathy'.

All the search results were combined using Endnote and duplicates were removed by R.G. Reference lists of the primary and secondary literature were manually browsed to identify any additional studies.

Inclusion and exclusion criteria

Studies were included if they (1) were original studies displaying prevalence data for SFP within a fibromyalgia population, (2) concerned adult patients with fibromyalgia diagnosed in accordance with international (American College of Rheumatology) ACR diagnostic criteria [36–38]; (3) included assessment of small nerve fibers in all patients, using skin biopsy, corneal confocal microscopy, laser Doppler imaging (LDI flare), microneurography or quantitative sudomotor axon reflex testing (QSART) 4) were reported in full-text publication. Studies were excluded if they, (1) were not a human study, (2) did not report SFP prevalence within fibromyalgia patients or (3) were not in English language. Only studies using quantitative structural or functional measures of small nerve fiber integrity were included within the systematic review as they objectively quantify small nerve fiber deficits. This is in contrast to alternate methods, which do not localise pathology directly to small nerve fibers i.e. thermal threshold testing. Fibromyalgia was defined in relation to either the 1990 or 2010 ACR criteria as 'widespread' pain, lasting for a period of 3 months or longer, in association with tender points or somatic symptoms as described in their respective protocols.

Two reviewers, (R.G. and K.E.) independently screened all articles and selected those that satisfied the inclusion criteria for full text analysis. The titles and abstracts of articles were screened to remove irrelevant studies and the remaining shortlisted articles were screened in depth for eligibility. The full texts of relevant articles were retrieved, screened and selected using the inclusion and exclusion criteria to compile a set of final articles to be reviewed. Both reviewers made a decision to include or exclude and any discrepancies were put forward to the senior author (U.A.) for final judgement on the inclusion of a study. The process of screening and selection for inclusion were recorded using a PRISMA flowchart (Fig. 1). Before data extraction and quality assessment U.A. screened all articles in order to confirm their eligibility within this study.

Data extraction and quality assessment

Study characteristics, methodology data and results from studies were extracted independently by R.G. and K.E. and any discrepancies between the R.G. and K.E. were reviewed by U.A. Extraction of the first author, study name and country were completed. Subsequently followed by the sample size and participant number within each study group, age, sex, SFP diagnostic technique used, international guideline of fibromyalgia diagnosis and the prevalence number or estimation. The combined extracted data was reviewed by U.A. to ensure accuracy of the data extraction.

The articles were all appraised using a risk of bias tool specifically addressing external and internal validity of the selected studies. In

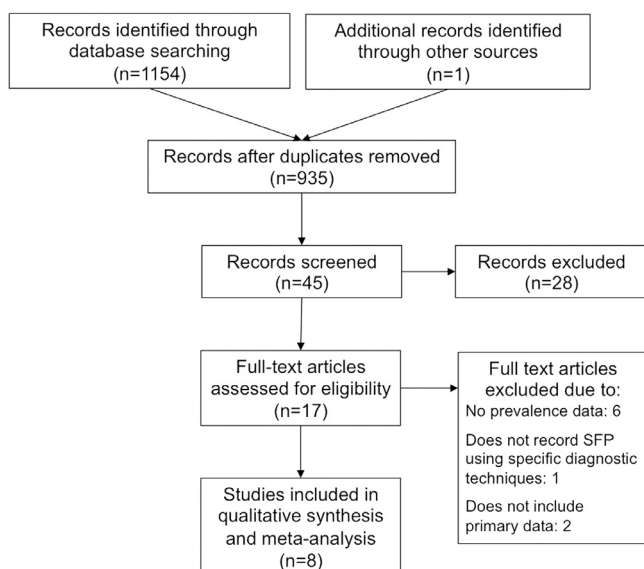


Fig. 1. PRISMA flowchart demonstrating the article screening process.

order to evaluate the quality of selected articles, both R.G. and K.E. independently critically appraised each article using the tool developed by Munn et al. [39]. This critical appraisal tool assesses the risk of bias and the methodological practices that may impact the study's validity [39, 40]. Each article was posed 10 questions; a score of 0 or 1 was recorded representing a 'yes' or 'no' response respectively determining confounding, selection bias, bias related to measurement and data-analysis. A total score was calculated for each article, presenting their overall risk of bias. A total score between 0 and 3 was considered low risk, 4–6 was moderate risk and ≥ 7 was high risk of bias. Any discrepancies in the risk of bias were put forward to the senior author (U.A.) for a final decision.

Description of skin biopsy and CCM

Skin biopsy technique was initially developed at the Karolinska Institute, Sweden and later standardised at the University of Minnesota and at Johns Hopkins University, USA [5]. Skin biopsy analysis of IENF are used for the diagnosis of patients with SFN through the identification of antibodies against protein gene product 9.5 (PGP 9.5). The immunoreaction is used to visualize the number and morphology of the dermal and IENF according to the European Federation of Neurological Societies (EFNS) [41]. A circular punch biopsy, 3–5 mm in diameter is rotated into the skin to obtain a cylindrical specimen which is then fixed, frozen and 50 μm sections are prepared and immunoreacted with antibodies against PGP 9.5 as a pan-axonal marker. IENF crossing the dermal-epidermal junction are counted to provide densities which are referenced against normative ranges to provide diagnostic cut offs [41].

CCM is a rapid non-invasive ophthalmic imaging modality which has been recently pioneered as a surrogate endpoint of peripheral neuropathy. The cornea is the most densely innervated tissue of the human body and receives sensory innervation from the trigeminal ganglion in the form of nerve bundles containing axons. These bundles terminate in the anterior cornea where they form a dense network of unmyelinated axons (19,000–44,000 axons within 90 mm^2 which are small nerve fibers) termed the sub-basal nerve plexus [42]. CCM can image these bundles of axons at 600x magnification. CCM quantifies axonal damage in early neuropathy [43], reliably [44], with high sensitivity and specificity [45, 46] and closely correlates to the severity of IENF loss [47, 48]. CCM methodology and assessment may be viewed in more detail at: <http://www.jove.com/index/Details.stp?ID=2194>.

We have included comparative images (Fig. 2A–C) of IENF in a healthy-volunteer control, a person with fibromyalgia without SFP and a person with fibromyalgia and SFP. We have also included CCM images (Fig. 2D–F) of a healthy-volunteer control and people with fibromyalgia and SFP. This shows SFP as imaged by these two diagnostic modalities.

Data analysis

Each article selected for final analysis was included within a meta-analysis to determine the overall prevalence of SFP in fibromyalgia. Studies were weighted according to the prevalence effect size and the inverse of the study variance in order to generate an I^2 value, serving as a measure of heterogeneity among the studies. I^2 is a measure of inconsistency within the studies' results [49] and reports the percentage of variation amongst studies that is due to heterogeneity and not chance [50]. The meta-analysis was conducted using a generic inverse variance outcome. The prevalence estimate of each study was used as the effect estimate, and the corresponding standard error (SE) for each study was calculated. The SEs for prevalence (p) estimates were derived from the equation $\sqrt{p(1-p)/n}$, where n is the number of participants with completed data in study. Random-effects model were used to generate summary prevalence data displayed (on forest plots) with 95% CIs in view of the higher I^2 value ($> 50\%$ for overall pooled data and subgroup analyses). In addition, individual subgroup forest plots were formed for diagnostic quantitative assessment used in the final selected studies. This also enabled the differences in prevalence estimates between each assessment method to be observed. A funnel plot was created to show possible bias within the meta-analysis results (supplementary material).

All statistical analyses and figure production were undertaken using Review Manager 5.3 (The Cochrane Collaboration, London, UK).

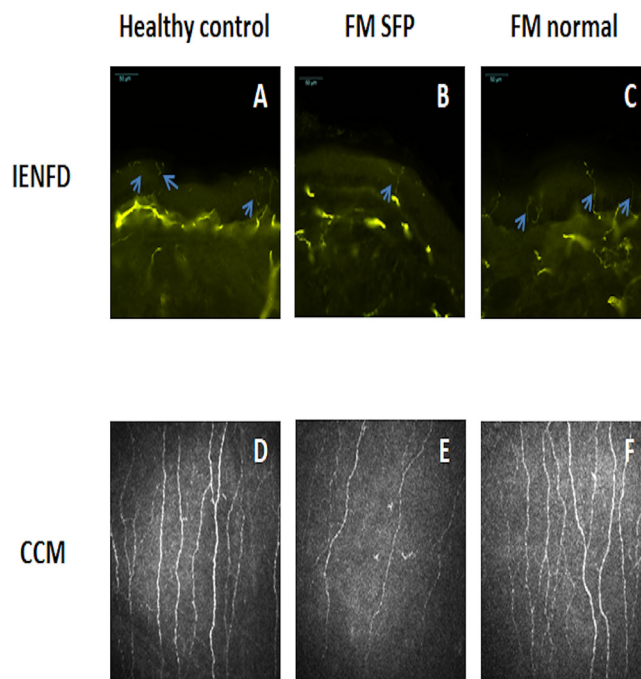


Fig. 2. IENF and CCM images in a healthy volunteer (A, D), a person with fibromyalgia (B, E) showing SFP and a person with fibromyalgia (C, F) showing no SFP. CCM – corneal confocal microscopy, IENF – Intra-epidermal nerve fiber density, FM – fibromyalgia, SFP – small fiber pathology. Permission sought and granted for Fig. 1 from: Üçeyler N, Sommer C. Small nerve fiber pathology. In: Perrot S, Hauser W, eds. Fibromyalgia Syndrome and Widespread Pain: From Construction to Relevant Recognition. Philadelphia, PA. Wolters Kluwer Health; 2019.

Table 1
Data extraction information from all final selected studies.

Author	Country	Sample size	Study group	Group size	Mean age (years ± SD or range)	Sex (Female/Male)	SFP diagnostic technique and criteria for diagnosis	International fibromyalgia guideline	Prevalence number	Prevalence estimate (%)
de Tommaso et al. [51]	Italy	81	Fibromyalgia	21	51 ± 9	18/3	Skin biopsy: IENFD below the 5 ^o percentile cut-off in the thigh, distal leg or finger-tip ^a	2010 ACR criteria	16	76
			Control	60	53 ± 6	50/10				
Giannoccaro et al. [28]	Italy	52	Fibromyalgia	20	40 ± 6	19/1	Skin biopsy: IENFD below 13.5 ENFs/mm in the thigh or 9.5 ENFs/mm in the distal leg	1990 ACR criteria	6	30
			Control	32	^b	^b				
Kosmidis et al. [30]	Greece	80	Fibromyalgia	46	53 (29–76)	41/5	Skin biopsy: IENFD below 3.65 fibres/mm in the distal leg	2010 ACR criteria	16	34
			Control	34	32 (19–84)	18/16				
Leinders et al. [52]	Germany	116	Fibromyalgia	28	51 (39–74)	26/2	Skin biopsy: IENFD below 6 fibres/mm in the thigh or distal leg	1990 ACR criteria	14	50
			Control	88	44 (16–79)	80/8				
Oaklander et al. [27]	USA	57	Fibromyalgia	27	47 (26–68)	20/7	Skin biopsy: IENFD below the 5 ^o percentile cut-off in the distal leg ^a	2010 ACR criteria	11	41
			Control	30	45 (25–65)	24/6				
Oudejans et al. [31]	Netherlands	^b	Fibromyalgia	39	39 (19–58)	36/3	Corneal confocal microscopy: CNFD (< 21.3 no/mm ²), CNFL (< 12.7 mm/mm ²), or CNBD (< 26.7 no/mm ²). Values are below the 5 ^o percentile cut-off.	1990 or 2010 ACR criteria	20	51
Control	^b	^b	^b							
Ramírez et al. [32]	Mexico	34	Fibromyalgia	17	44 ± 5	All female	Corneal confocal microscopy: CNFD (diagnostic cut-off unavailable)	1990 or 2010 ACR criteria	12	71
			Control	17	43 ± 6	All female				
Üçeyler et al. [26]	Germany	155	Fibromyalgia	24	59 (50–70)	22/2	Skin biopsy: IENFD below 8 fibres/mm in the thigh or 6 fibres/mm in the distal leg	1990 ACR criteria	10	42
			Monopolar depression without pain	10	Mean age unknown (39–75)	9/1				
			Control	121	^b	^b				

CNBD – corneal nerve branch density, CNFD – corneal nerve fibre density, CNFL – corneal nerve fibre length, ENF – epidermal nerve fibers, IENFD – intra-epidermal nerve fiber density.

^a Precise cut-off values for IENFD not provided.

^b Information are unavailable.

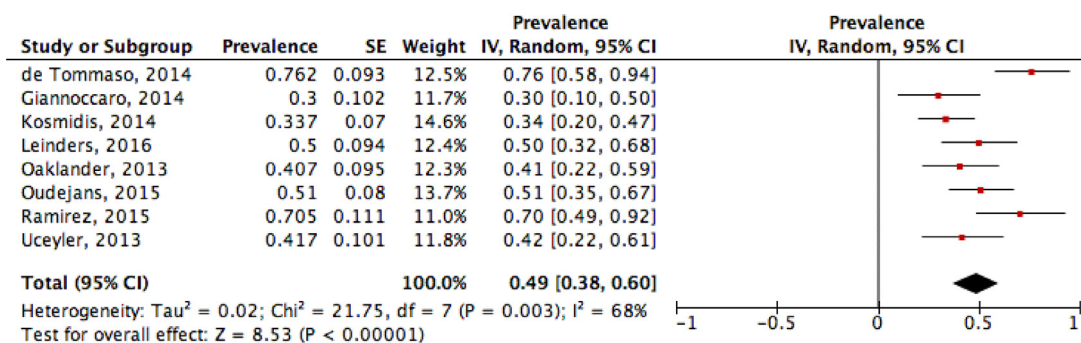


Fig. 3. Forest plot showing overall pooled prevalence estimates of SFP in fibromyalgia. de Tommaso et al. [51]; Giannoccaro et al. [28]; Kosmidis et al. [30]; Leinders et al. [52]; Oaklander et al. [27]; Oudejans et al. [31]; Ramirez et al. [32] and Uceyler et al. [26].

Results

Study characteristics

After the removal of duplicates a total of 935 articles were generated from the electronic database and manual reference searches. A PRISMA flowchart was completed displaying the article exclusions at each stage of screening, (Fig. 1). The titles and abstracts of 45 articles were screened using the inclusion and exclusion criteria. Analysis of 17 full text articles was performed in order to review eligibility and inclusion. Overall, eight articles satisfied the inclusion criteria and underwent data extraction (Table 1) and quality assessment (Supplementary material, Table 2).

Six studies were performed in Europe, [26, 28, 30, 31, 51, 52], one in the USA, [27] and one in Mexico, [32]. Studies had been described by the authors as case-control, cross-sectional or prospective. All studies used the 1990 or 2010 [36, 37] ACR Criteria for the classification of fibromyalgia as a standard definition.

Overall prevalence

The meta-analysis evaluated data from all eight articles, providing eight prevalence estimates; the estimated prevalence ranged between 30% and 76% (Fig. 3). Forest plot analysis showed the random-effects overall prevalence of SFP in fibromyalgia was 49% (95% CI: 38%, 60%) with a moderately high level of heterogeneity, ($I^2=68\%$). Analysis of the overall funnel plot, (Supplementary material, Fig. 5) showed an equal but asymmetrical distribution of studies either side of the overall prevalence estimate.

Subgroup analysis

All selected studies diagnosed small fiber pathology in patients using either a skin biopsy ($n=6$) or corneal confocal microscopy ($n=2$). Skin biopsies were undertaken by; de Tommaso et al. [51], Giannoccaro et al. [28], Kosmidis et al. [30], Leinders et al. [52], Oaklander et al. [27] and Uceyler et al. [26]. The six prevalence estimates

ranged between 30% and 76%, (Fig. 4). Analysis of the skin biopsy meta-analysis displayed a random-effects pooled prevalence of 45% (95% CI: 32%, 59%), with a moderately high level of heterogeneity, ($I^2=70\%$). In comparison, Oudejans et al. [31] and Ramirez et al. [32] assessed SFP using corneal confocal microscopy. This meta-analysis, (Fig. 5) showed the random-effects pooled prevalence was 59% (95% CI: 40%, 78%), with a moderate heterogeneity, ($I^2=51\%$).

Risk of bias

Evaluation of bias and article quality (Supplementary material, Table 2) showed the majority of studies had a low risk of bias. The Giannoccaro et al. [28] study displayed a moderate risk of bias. No sample size calculation was provided; only 20 fibromyalgia patients were studied and there were no details of ethnicity or the reasons for the chosen male/female ratio. Moreover, this study did not explicitly state a specialist or independent clinician reliably measured or reviewed the SFP diagnostic recordings, nor did this study display statistical analysis methods.

Discussion

This meta-analysis estimates a high prevalence of SFP, with 49% of people with fibromyalgia having a structural abnormality of the small nerve fibers and to our knowledge this systematic literature review is the first study to collate data on the overall prevalence of SFP in fibromyalgia. Based on the modified 2010 criteria, approximately 5% of the population are affected by fibromyalgia [53] and this equates to around 1.6 million people with SFP and fibromyalgia in the United Kingdom alone. Fibromyalgia has major implications on morbidity and patients experience a decline in their health-related quality of life with a significant economic burden on health services [54]. In our review, small fiber pathology was defined by objective tests which were either by skin biopsy or corneal confocal microscopy, both of which showed a high prevalence of SFP, challenging the current concept that fibromyalgia is largely a disorder of the CNS.

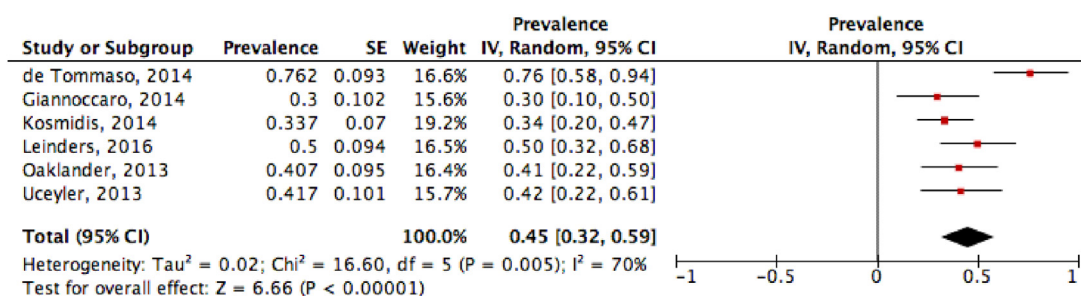


Fig. 4. Forest plot showing prevalence estimates of SFP in fibromyalgia in studies using skin biopsies. de Tommaso et al. [51]; Giannoccaro et al. [28]; Kosmidis et al. [30]; Leinders et al. [52]; Oaklander et al. [27] and Uceyler et al. [26].

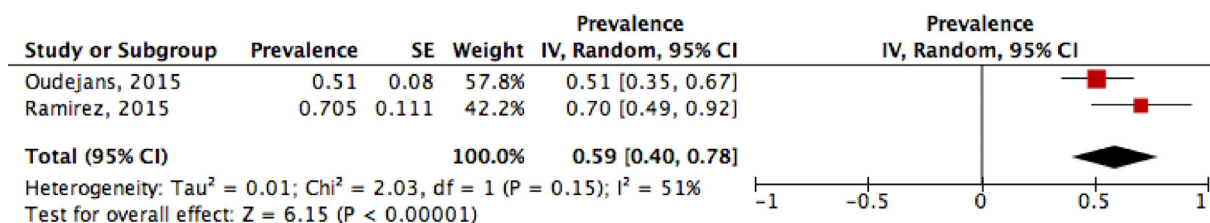


Fig. 5. Forest plot showing prevalence estimates of SFP in fibromyalgia in studies using CCM. Oudejans et al. [31] and Ramirez et al. [32].

We have strengthened our study by imposing strict criteria on the diagnosis of SFP in a priori inclusion criteria with our study conducted to PRISMA guidelines [55]. The tests for statistical heterogeneity amongst articles suggested significant variability for overall pooled data ($I^2 = 68\%$) and skin biopsy data ($I^2 = 70\%$) with moderate variability in data from corneal confocal microscopy ($I^2 = 51\%$). Inconsistencies in the classification of a positive SFP diagnosis between studies may have contributed to the high heterogeneity. In the study by de Tommaso et al. [51], skin biopsies were undertaken in three locations; the thigh, distal leg and finger-tip. In contrast, Giannoccaro et al. [28], Leinders et al. [52] and Üçeyler et al. [26] obtained samples from the thigh and distal leg, whilst Kosmidis et al. [30] and Oaklander et al. [27] obtained biopsies from the distal leg only. de Tommaso et al. [51], showed that 16/21 (76%) patients with fibromyalgia had an epidermal nerve density below the 5th percentile in at least one site from the leg, thigh or fingertip. Uçeyler et al. [26] showed a reduction in IENFD and regenerating nerves stained using GAP43 in both the thigh and distal leg of 24 patients with fibromyalgia. These inconsistencies may limit the reliability of the prevalence estimate and future standardised cut-off values for abnormal IENFD and in particular corneal nerve parameters in fibromyalgia are warranted and will allow for direct comparison in any future prevalence studies.

Abnormal CNS processing is evidenced in a number of studies in fibromyalgia which have shown altered resting and stimulus-evoked regional cerebral blood flow in pain and altered emotional processing regions such as the thalamus, somatosensory cortex, insula, and anterior cingulate cortex [8, 56]. However, the reduction in grey matter volume noted in fibromyalgia patients may be indicative of secondary alterations in central neuroplasticity accompanying affective disorders [57]. Indeed in 'classical' SFN, brain networks tend to alter and deconstruct into functionally independent components (reduced functional connectivity between the anterior cingulate cortex, amygdala and praecuneus), with severity being linked to the degree of cutaneous nerve degeneration [58]. In other pain syndromes such as dysmenorrhoea, patients exhibit adaptive/reactive hyperconnectivity within the sensorimotor cortex [59]. In neither of these conditions is the primary pathological process in the CNS. The preponderance of data regarding SFP supports the notion that besides central sensitisation, structural abnormalities of C-fibers and altered C-fiber efferent function might play a role in fibromyalgia. In addition to reduced fiber densities, in a study of 32 patients with fibromyalgia there was evidence of structural (reduced axon diameter of small nerve fibers) and functional (quantitative sensory testing) abnormalities in the small fibers [33].

Doppler et al. [33] also showed differences in the extent of small fiber axonopathy compared to idiopathic SFP including a greater proportion of ballooned Schwann cells, indicating different pathomechanisms [33]. Schwann cell ballooning and peripheral axonal abnormalities have been demonstrated using electron microscopy in the skin of people with fibromyalgia [60]. Neurogenic inflammation with involvement of C-fibers is evidenced by inflammatory changes in skin biopsies with IgG deposition in the dermis and vessel walls, and the presence of tumour necrosis factor (TNF)- α and interleukin (IL)-6 [61, 62]. In an experimental model, a high fat diet sensitised fibromyalgia-like pain behaviours in mice via increased TNF- α [63]. There is also a link between obesity and fibromyalgia and chronic

pain [64, 65]. In a study of people with obesity and impaired glucose tolerance with SFN, weight loss and exercise improved neuropathic pain and IENFD [66]. Specific symptoms of dysautonomia and paresthesia, both of which are intimately related to small nerve fiber dysfunction may help identify underlying SFP in fibromyalgia, as both symptoms have some diagnostic utility (Area Under the Curve (AUC) 0.729) [67].

There remains considerable scepticism for the role of SFP [68], despite the demonstration of significant small nerve fiber dysfunction and pathology in fibromyalgia. Cutaneous small nerves generate pain and connect to the CNS forming an integral part of pain processing pathways, and small skin nerve fiber dysfunction and pathology have been repeatedly demonstrated in fibromyalgia. Nevertheless there remains considerable scepticism about the relevance of these findings for explaining the patients' pain [51]; Indeed, it has been suggested that some patients in whom fibromyalgia is diagnosed are instead affected by SFN with clinical features mimicking fibromyalgia [69]. And in at least some patients, proximal fiber loss may be greater than, or at least equal to, distal loss, configuring a non-length-dependent pattern more compatible with the widespread pain symptoms of fibromyalgia [69]. An experimental study of Sprague Dawley rats, suggested that increased insular glutamate was associated with a reduction in IENFD [25], thus implying that a central neurotransmitter defect may contribute to SFP. However, we suggest caution in the translational value of an experiment with such small numbers of animals to the human pathogenesis of SFP in fibromyalgia [25].

The peripheral origins of pain are not in doubt in painful neuropathies, however, the question remains if central sensitization is the primary driver in fibromyalgia. SFP also alters small blood vessel function through altered neuropeptide response and upregulation of α -adrenergic receptors [70]. This neurogenic microvasculopathy may explain at least partially, the skeletal muscle perfusion deficits, deep pain, and exercise intolerance characteristic of fibromyalgia and a number of other small fiber neuropathies [71, 72]. This microvasculopathy mediated by small nerve fibers may also contribute to the common symptom of 'brain fog' and provide a putative mechanistic link [73].

There was a limited opportunity to investigate the sources of heterogeneity due to the small number of studies included and lack of recorded patient's characteristics, we therefore only completed a stratified analysis based on the diagnostic modality of SFP. Our study was limited to published data and English language publications which may introduce respective bias. Furthermore, only a single point assessment of small nerve fibers was undertaken in the included studies; however, small nerve fibers degenerate and regenerate [74, 75] and therefore may alter over time. Another limitation of our review was that none of the studies included for prevalence estimates were primarily designed to produce prevalence data and is reflected in the relatively small sample sizes in the included studies (largest study, $n = 47$). Only one study [27] performed a sample size calculation to ensure that adequate numbers were recruited. Two studies recruited from a single centre, namely de Tommaso et al. [51] and Kosmidis et al. [30], thus presence of population bias with an unrepresentative sample is a distinct possibility.

The pooled prevalence estimates were higher with CCM suggesting it may be a more sensitive measure of SFP. CCM quantifies early axonal damage [43], reliably [44], with high sensitivity and specificity [45, 48] and closely correlates to the loss of IENF [47, 48]. As CCM is a rapid, reiterative, non-invasive imaging modality of small nerve fibers it may provide an ideal method to accurately define the prevalence of SFP in large population studies. Larger dedicated prevalence studies are required to augment this meta-analysis and accurately define the contribution of SFP in fibromyalgia. In particular, SFP prevalence in fibromyalgia needs to be determined stratified on the basis of duration of disease which will provide a significant insight in the natural history of this chronic pain condition.

Conclusion

Our meta-analysis shows that the prevalence of SFP in fibromyalgia is 49%. Fibromyalgia and chronic pain has a huge negative impact on quality of life and reduces the ability of our patients to work and function. There needs to be a significant improvement in the understanding of pain in fibromyalgia and the relative contribution of SFP in relation to pain needs to be established.

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Conflict of interest

No potential conflicts of interest relevant to this article were reported.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.semarthrit.2018.08.003.

References

- [1] Wolfe F, Clauw DJ, Fitzcharles M-A, Goldenberg DL, Katz RS, Mease P, et al. The American College of Rheumatology Preliminary Diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res* 2010;62(5):600–10.
- [2] Belt NK, Kronholm E, Kauppi MJ. Sleep problems in fibromyalgia and rheumatoid arthritis compared with the general population. *Clin Exp Rheumatol* 2009;27(1):35–41.
- [3] Bellato E, Marini E, Castoldi F, Barbasetti N, Mattei L, Bonasia DE, et al. Fibromyalgia syndrome: etiology, pathogenesis, diagnosis, and treatment. *Pain Res Treat* 2012;2012:426130.
- [4] Schmidt-Wilcke T, Clauw DJ. Fibromyalgia: from pathophysiology to therapy. *Nat Rev Rheumatol* 2011;7(9):518–27.
- [5] Levine TD, Saperstein DS. Routine use of punch biopsy to diagnose small fiber neuropathy in fibromyalgia patients. *Clin Rheumatol* 2015;34(3):413–7.
- [6] Kindler LL, Bennett RM, Jones KD. Central sensitivity syndromes: mounting pathophysiologic evidence to link fibromyalgia with other common chronic pain disorders. *Pain Manag Nurs: Off J Am Soc Pain Manag Nurses* 2011;12(1):15–24.
- [7] Clauw DJ. Fibromyalgia: an overview. *Am J Med* 2009;122(12, Supplement):S3–S13.
- [8] Cagnie B, Coppieters I, Denecker S, Six J, Danneels L, Meeus M. Central sensitization in fibromyalgia? A systematic review on structural and functional brain MRI. *Semin Arthritis Rheum* 2014;44(1):68–75.
- [9] Foerster BR, Petrou M, Harris RE, Barker PB, Hoeffner EG, Clauw DJ, et al. Cerebral blood flow alterations in pain-processing regions of patients with fibromyalgia using perfusion MR imaging. *AJNR Am J Neuroradiol* 2011;32(10):1873–8.
- [10] Kwiatek R, Barnden L, Tedman R, Jarrett R, Chew J, Rowe C, et al. Regional cerebral blood flow in fibromyalgia: single-photon-emission computed tomography evidence of reduction in the pontine tegmentum and thalami. *Arthritis Rheum* 2000;43(12):2823–33.
- [11] Guedj E, Taieb D, Cammilleri S, Lussato D, de Laforte C, Niboyet J, et al. 99mTc-ECD brain perfusion SPECT in hyperalgesic fibromyalgia. *Eur J Nucl Med Mol Imaging* 2007;34(1):130–4.
- [12] Okifuji A, Hare BD. Management of fibromyalgia syndrome: review of evidence. *Pain Ther* 2013;2(2):87–104.
- [13] Harris RE, Sundgren PC, Craig AD, Kirshenbaum E, Sen A, Napadow V, et al. Elevated insular glutamate in fibromyalgia is associated with experimental pain. *Arthritis Rheum* 2009;60(10):3146–52.
- [14] Pyke TL, Osmotherly PG, Baines S. Measuring glutamate levels in the brains of fibromyalgia patients and a potential role for glutamate in the pathophysiology of fibromyalgia symptoms: a systematic review. *Clin J Pain* 2017;33(10):944–54.
- [15] Wood PB, Schweinhardt P, Jaeger E, Dagher A, Hakyemez H, Rabiner EA, et al. Fibromyalgia patients show an abnormal dopamine response to pain. *Eur J Neurosci* 2007;25(12):3576–82.
- [16] Alnigenis MN, Barland P. Fibromyalgia syndrome and serotonin. *Clin Exp Rheumatol* 2001;19(2):205–10.
- [17] Devigili G, Tugnoli V, Penza P, Camozzi F, Lombardi R, Melli G, et al. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. *Brain* 2008;131(7):1912–25.
- [18] Koroschetz J, Rehm SE, Gockel U, Brosz M, Freynhagen R, Tölle TR, et al. Fibromyalgia and neuropathic pain – differences and similarities. A comparison of 3057 patients with diabetic painful neuropathy and fibromyalgia. *BMC Neurol* 2011;11(1):55.
- [19] Rehm SE, Koroschetz J, Gockel U, Brosz M, Freynhagen R, Tölle TR, et al. A cross-sectional survey of 3035 patients with fibromyalgia: subgroups of patients with typical comorbidities and sensory symptom profiles. *Rheumatology* 2010;49(6):1146–52.
- [20] Kosek E, Ekholm J, Hansson P. Sensory dysfunction in fibromyalgia patients with implications for pathogenic mechanisms. *Pain* 1996;68(2–3):375–83.
- [21] Staud R, Vierck CJ, Cannon RL, Mauderli AP, Price DD. Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. *Pain* 2001;91(1–2):165–75.
- [22] Berglund B, Harju E-L, Kosek E, Lindblom U. Quantitative and qualitative perceptual analysis of cold dysesthesia and hyperalgesia in fibromyalgia. *Pain* 2002;96(1):177–87.
- [23] Geisser ME, Casey KL, Brucksch CB, Ribbens CM, Appleton BB, Crofford LJ. Perception of noxious and innocuous heat stimulation among healthy women and women with fibromyalgia: association with mood, somatic focus, and catastrophizing. *Pain* 2003;102(3):243–50.
- [24] Oaklander AL, Herzog ZD, Downs HM, Klein MM. Objective evidence that small-fiber polyneuropathy underlies some illnesses currently labeled as fibromyalgia. *Pain* 2013;154(11):2310–6.
- [25] Harte SE, Clauw DJ, Hayes JM, Feldman EL, St Charles IC, Watson CJ. Reduced intraepidermal nerve fiber density after a sustained increase in insular glutamate: a proof-of-concept study examining the pathogenesis of small fiber pathology in fibromyalgia. *Pain Rep* 2017;2(3):e590.
- [26] Uceyler N, Zeller D, Kahn AK, Kewenig S, Kittel-Schneider S, Schmid A, et al. Small fibre pathology in patients with fibromyalgia syndrome. *Brain* 2013;136(Pt 6):1857–67.
- [27] Oaklander AL, Herzog ZD, Downs HM, Klein MM. Objective evidence that small-fiber polyneuropathy underlies some illnesses currently labeled as fibromyalgia. *Pain* 2013;154(11):2310–6.
- [28] Giannocaro MP, Donadio V, Incensi A, Avoni P, Liguori R. Small nerve fiber involvement in patients referred for fibromyalgia. *Muscle Nerve* 2014;49(5):757–9.
- [29] Caro XJ, Winter EF. Evidence of abnormal epidermal nerve fiber density in fibromyalgia: clinical and immunologic implications. *Arthritis Rheumatol* 2014;66(7):1945–54.
- [30] Kosmidis ML, Koutsogeorgopoulou L, Alexopoulos H, Mamali I, Vlachoyiannopoulos PG, Voulgarelis M, et al. Reduction of Intraepidermal Nerve Fiber Density (IENFD) in the skin biopsies of patients with fibromyalgia: a controlled study. *J Neurol Sci* 2014;347(1–2):143–7.
- [31] Oudejans L, He X, Niesters M, Dahan A, Brines M, van Velzen M. Cornea nerve fiber quantification and construction of phenotypes in patients with fibromyalgia. *Sci Rep* 2016;6:23573.
- [32] Ramirez M, Martinez-Martinez LA, Hernandez-Quintela E, Velazco-Casapia J, Vargas A, Martinez-Lavin M. Small fiber neuropathy in women with fibromyalgia. An in vivo assessment using corneal confocal bio-microscopy. *Semin Arthritis Rheum* 2015;45(2):214–9.
- [33] Doppler K, Rittner HL, Deckart M, Sommer C. Reduced dermal nerve fiber diameter in skin biopsies of patients with fibromyalgia. *Pain* 2015;156(11):2319–25.
- [34] Uceyler N. Reply *Pain* 2017;158(5):989–90.
- [35] Peters MJ, Bakkers M, Merckies IS, Hoeijmakers JG, van Raak EP, Faber CG. Incidence and prevalence of small-fiber neuropathy: a survey in the Netherlands. *Neurology* 2013;81(15):1356–60.
- [36] Wolfe F. New American College of Rheumatology criteria for fibromyalgia: a twenty-year journey. *Arthritis Care Res* 2010;62(5):583–4.

- [37] Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, et al. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. *Arthritis Rheum* 1990;33(2):160–72.
- [38] Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Hauser W, Katz RL, et al. Revisions to the 2010/2011 fibromyalgia diagnostic criteria. *Semin Arthritis Rheum* 2016;46(3):319–29.
- [39] Munn Z, Moola S, Riitano D, Lisy K. The development of a critical appraisal tool for use in systematic reviews addressing questions of prevalence. *Int J Health Policy Manag* 2014;3(3):123–8.
- [40] Katrak P, Bialocerkowski AE, Massy-Westropp N, Kumar S, Grimmer KA. A systematic review of the content of critical appraisal tools. *BMC Med Res Methodol* 2004;4:22.
- [41] Lauria G, Cornblath DR, Johansson O, McArthur JC, Mellgren SI, Nolano M, et al. EFNS guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy. *Eur J Neurol* 2005;12(10):747–58.
- [42] Müller LJ, Marfurt CF, Kruse F, Tervo TM. Corneal nerves: structure, contents and function. *Exp Eye Res* 2003;76(5):521–42.
- [43] Petropoulos IN, Alam U, Fadavi H, Asghar O, Green P, Ponirakis G, et al. Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. *Diabetes Care* 2013;36(11):3646–51.
- [44] Petropoulos IN, Manzoor T, Morgan P, Fadavi H, Asghar O, Alam U, et al. Repeatability of in vivo corneal confocal microscopy to quantify corneal nerve morphology. *Cornea* 2013;32(5):e83–9.
- [45] Petropoulos IN, Alam U, Fadavi H, Marshall A, Asghar O, Dabbah MA, et al. Rapid automated diagnosis of diabetic peripheral neuropathy with in vivo corneal confocal microscopy. *Investig Ophthalmol Vis Sci* 2014;55(4):2071–8.
- [46] Alam U, Riley DR, Jugdey RS, Azmi S, Rajbhandari S, D'Aout K, et al. Diabetic neuropathy and gait: a review. *Diabetes Therapy: Res Treat Educ Diabetes Relat Disord* 2017;8(6):1253–64.
- [47] Chen X, Graham J, Dabbah MA, Petropoulos IN, Ponirakis G, Asghar O, et al. Small nerve fiber quantification in the diagnosis of diabetic sensorimotor polyneuropathy: comparing corneal confocal microscopy with intraepidermal nerve fiber density. *Diabetes Care* 2015;38(6):1138–44.
- [48] Alam U, Jeziorska M, Petropoulos IN, Asghar O, Fadavi H, Ponirakis G, et al. Diagnostic utility of corneal confocal microscopy and intra-epidermal nerve fibre density in diabetic neuropathy. *PLoS One* 2017;12(7):e0180175.
- [49] Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;21(11):1539–58.
- [50] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;327(7414):557–60.
- [51] de Tommaso M, Nolano M, Iannone F, Vecchio E, Ricci K, Lorenzo M, et al. Update on laser-evoked potential findings in fibromyalgia patients in light of clinical and skin biopsy features. *J Neurol* 2014;261(3):461–72.
- [52] Leinders M, Doppler K, Klein T, Deckart M, Rittner H, Sommer C, et al. Increased cutaneous miR-let-7d expression correlates with small nerve fiber pathology in patients with fibromyalgia syndrome. *Pain* 2016;157(11):2493–503.
- [53] Jones GT, Atzeni F, Beasley M, Fluss E, Sarzi-Puttini P, Macfarlane GJ. The prevalence of fibromyalgia in the general population: a comparison of the American College of Rheumatology 1990, 2010, and modified 2010 classification criteria. *Arthritis Rheumatol* 2015;67(2):568–75.
- [54] Kim S-K, Kim S-H, Lee C-K, Lee H-S, Lee S-H, Park Y-B, et al. Effect of fibromyalgia syndrome on the health-related quality of life and economic burden in Korea. *Rheumatology* 2013;52(2):311–20.
- [55] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009;339:b2700.
- [56] Williams DA, Gracely RH. Biology and therapy of fibromyalgia. *Functional magnetic resonance imaging findings in fibromyalgia. Arthritis Res Therapy* 2006;8(6):224.
- [57] Hsu MC, Harris RE, Sundgren PC, Welsh RC, Fernandes CR, Clauw DJ, et al. No consistent difference in gray matter volume between individuals with fibromyalgia and age-matched healthy subjects when controlling for affective disorder. *Pain* 2009;143(3):262–7.
- [58] Hsieh PC, Tseng MT, Chao CC, Lin YH, Tseng WY, Liu KH, et al. Imaging signatures of altered brain responses in small-fiber neuropathy: reduced functional connectivity of the limbic system after peripheral nerve degeneration. *Pain* 2015;156(5):904–16.
- [59] Wei SY, Chao HT, Tu CH, Li WC, Low I, Chuang CY, et al. Changes in functional connectivity of pain modulatory systems in women with primary dysmenorrhea. *Pain* 2016;157(1):92–102.
- [60] Kim SH, Kim DH, Oh DH, Clauw DJ. Characteristic electron microscopic findings in the skin of patients with fibromyalgia—preliminary study. *Clin Rheumatol* 2008;27(3):407–11.
- [61] Enestrom S, Bengtsson A, Frödin T. Dermal IgG deposits and increase of mast cells in patients with fibromyalgia—relevant findings or epiphenomena. *Scand J Rheumatol* 1997;26(4):308–13.
- [62] Salemi S, Rethage J, Wollina U, Michel BA, Gay RE, Gay S, et al. Detection of interleukin 1beta (IL-1beta), IL-6, and tumor necrosis factor-alpha in skin of patients with fibromyalgia. *J Rheumatol* 2003;30(1):146–50.
- [63] Tian D, Tian M, Zhang L, Zhao P, Cui Y, Li J. High fat diet sensitizes fibromyalgia-like pain behaviors in mice via tumor necrosis factor alpha. *PLoS One* 2018;13(2):e0190861.
- [64] Okifuji A, Hare BD. The association between chronic pain and obesity. *J Pain Res* 2015;8:399–408.
- [65] Oza MJ, Garud MS, Gaikwad AB, Kulkarni YA. Chapter 7 – fibromyalgia syndrome: role of obesity and nutrients A2. In: Watson R R, ed. *Nutrition and functional foods for healthy aging*. Academic Press; 2017:53–63.
- [66] Smith AG, Singleton JR. Impaired glucose tolerance and neuropathy. *Neurologist* 2008;14(1):23–9.
- [67] Lodahl M, Treister R, Oaklander AL. Specific symptoms may discriminate between fibromyalgia patients with vs without objective test evidence of small-fiber polyneuropathy. *Pain Rep* 2018;3(1):e633.
- [68] Oaklander AL. What is the meaning of “small-fiber polyneuropathy” in fibromyalgia? An alternate answer. *Pain* 2016;157(6):1366–7.
- [69] Gemignani F. Fibromyalgia syndrome and small-fiber neuropathy: comment on the article by Caro and Winter. *Arthritis Rheumatol* 2014;66(12):3526–7.
- [70] Littlejohn G. Neurogenic neuroinflammation in fibromyalgia and complex regional pain syndrome. *Nat Rev Rheumatol* 2015;11(11):639–48.
- [71] Albrecht PJ, Hou Q, Argoff CE, Storey JR, Wymer JP, Rice FL. Excessive peptidergic sensory innervation of cutaneous arteriole-venule shunts (AVS) in the palmar glabrous skin of fibromyalgia patients: implications for widespread deep tissue pain and fatigue. *Pain Med* 2013;14(6):895–915.
- [72] Dori A, Lopate G, Keeling R, Pestronk A. Myovascular innervation: axon loss in small-fiber neuropathies. *Muscle Nerve* 2015;51(4):514–21.
- [73] Stewart JM, Medow MS, Messer ZR, Baugham IL, Terilli C, Ocon AJ. Postural neurocognitive and neuronal activated cerebral blood flow deficits in young chronic fatigue syndrome patients with postural tachycardia syndrome. *Am J Physiol – Heart Circ Physiol* 2012;302(5):H1185–94.
- [74] Polydefkis M, Hauer P, Sheth S, Sirdofsky M, Griffin JW, McArthur JC. The time course of epidermal nerve fibre regeneration: studies in normal controls and in people with diabetes, with and without neuropathy. *Brain* 2004;127(7):1606–15.
- [75] Tavakoli M, Mitu-Pretorian M, Petropoulos IN, Fadavi H, Asghar O, Alam U, et al. Corneal confocal microscopy detects early nerve regeneration in diabetic neuropathy after simultaneous pancreas and kidney transplantation. *Diabetes* 2013;62(1):254–60.