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Full-length Article

The translocator protein gene is associated with symptom severity and cerebral pain processing in fibromyalgia

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ABSTRACT

The translocator protein (TSPO) is upregulated during glia activation in chronic pain patients. TSPO constitutes the rate-limiting step in neurosteroid synthesis, thus modulating synaptic transmission. Related serotonergic mechanisms influence if pro- or anti-nociceptive neurosteroids are produced. This study investigated the effects of a functional genetic polymorphism regulating the binding affinity to the TSPO, thus affecting symptom severity and cerebral pain processing in fibromyalgia patients. Gene-to-gene interactions with a functional polymorphism of the serotonin transporter gene were assessed. Fibromyalgia patients (n = 126) were genotyped regarding the polymorphisms of the TSPO (*rs6971*) and the serotonin transporter (*5-HTTLPR/rs25531*). Functional magnetic resonance imaging (n = 24) was used to study brain activation during individually calibrated pressure pain. Compared to mixed/low TSPO affinity binders, the high TSPO affinity binders rated more severe pain ($p = 0.016$) and fibromyalgia symptoms ($p = 0.02$). A significant interaction was found between the TSPO and the serotonin transporter polymorphisms regarding pain severity ($p < 0.0001$). Functional connectivity analyses revealed that the TSPO high affinity binding group had more pronounced pain-evoked functional connectivity in the right frontoparietal network, between the dorsolateral prefrontal area and the parietal cortex. In conclusion, fibromyalgia patients with the TSPO high affinity binding genotype reported a higher pain intensity and more severe fibromyalgia symptoms compared to mixed/low affinity binders, and this was modulated by interaction with the serotonin transporter gene. To our knowledge this is the first evidence of functional genetic polymorphisms affecting pain severity in FM and our findings are in line with proposed glia-related mechanisms. Furthermore, the functional magnetic resonance findings indicated an effect of translocator protein on the affective-motivational components of pain perception.

1. Introduction

Activated glial cells have been reported in animal models of chronic pain (Milligan and Watkins, 2009; Watkins and Maier, 2005). In humans, glia activation can be studied *in vivo*, using positron emission tomography with ligands for the peripheral benzodiazepine receptor, more frequently referred to as the translocator protein (TSPO). Small amounts of TSPO are expressed by glia in the healthy human brain (Rupprecht et al., 2010), but the expression is up-regulated during glia activation (Nothdurfter et al.,

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2012; Pinna et al., 2006). In clinical conditions, altered expression of TSPO has been reported in patients with various psychiatric (Bloomfield et al., 2016; Pozzo et al., 2012; Setiawan et al., 2015) and neurological (Girard et al., 2011; Zürcher et al., 2015) disorders that are linked to glia activation. Recently, increased thalamic TSPO binding was reported in chronic low back pain patients compared to healthy controls, thus linking glia cell activation to chronic pain in humans (Loggia et al., 2015).

Despite the fact that TSPO is an evolutionary well conserved protein, it's exact biological roles are yet to be determined (Gatliff and Campanella, 2016). TSPO is a mitochondrial membrane protein, important for the regulation of steroid hormone production and was believed to be necesasary for survival. This view was recently challanged by studies showing that TSPO is not necessary for steroid production (Banati et al., 2014; Morohaku et al., 2014) and by demonstrating an overtly normal phenotype of TSPO knockout mice, with the exeption of reduced mitochondrial ATP production in microglia (Banati et al., 2014). The authors speculated that TSPO-mediated changes in ATP production might exert indirect regulatory effects on the energy-dependent steroid biogenesis, particularly under stress challenges, thus influencing the course of inflammatory brain pathology (Banati et al., 2014). However, whereas knockout studies have yielded inconsistent results most likely due to differences in methodology, strains and compensatory mechanisms (Gatliff and Campanella, 2016), the evidence supporting an important role of TSPO in cholesterol metabolism and steroidogenesis is abundant (Gatliff and Campanella, 2016).

Previous studies have shown that by controlling the rate-limiting step in neurosteroid synthesis, TSPO has a large impact on neurosteroids (Costa et al., 2012; Pozzo et al., 2012). Neurosteroids act as potent modulators of synaptic transmission by exerting facilitatory or inhibitory effects on GABA-A receptors, thus affecting mood, cognition and pain (Aouad et al., 2009; Nothdurfter et al., 2012; Pozzo et al., 2012). Depending on their action on the GABA-A receptor subunits, neurosteroids can have analgesic (positive modulators) or hyperalgesic (negative modulators) effects (Scarf and Kassiou, 2011; Svensson et al., 2013). Serotonergic tone may influence which types of neurosteroids are synthesised, with low tone favouring negative modulators (Pinna et al., 2006; Schüle et al., 2011). Thus, whereas TSPO binding affinity regulates the rate of neurosteroid production, serotonergic tone influences if positive or negative neurosteroid modulators are synthesised.

The binding affinity to the human TSPO receptor is genetically determined by a functional polymorphism in the TSPO gene (*rs6971*) (Guo et al., 2013; Mizrahi et al., 2013; Owen et al., 2012; Venneti et al., 2013). This single-nucleotide polymorphism (SNP) substitutes the amino acid alanine 147 into threonine (Ala147Thr) in the C-terminal transmembrane domain containing the cholesterol recognition amino acid consensus sequence (Costa et al., 2009a). The SNP has been shown to affect neurosteroid production (Costa et al., 2009a) and has been associated with psychiatric diagnosis such as panic disorder (Nakamura et al., 2006), adult separation anxiety (Costa et al., 2009b), and bipolar disease (Colasanti et al., 2013).

The amount of serotonin available in the synaptic cleft is genetically regulated by a common, functional polymorphism, the Long Promoter Repeat (*5-HTTLPR*) of the serotonin transporter (5-HTT) gene (*SCL6A4*) (Lesch et al., 1994). The human promoter region of the gene *SLC6A4* coding for the 5-HTT harbors a 43 base-pair (bp) insertion/deletion referred to as the 5-HTT linked polymorphic region (*5-HTTLPR*). This polymorphism consists of a long (L) allele and a short (S) allele, the latter coupled to reduced gene-expression (Lesch et al., 1994). In addition, the promoter region of the *SLC6A4* gene also harbors the single-nucleotide polymorphism (SNP) *rs25531* which includes an A to G substitution (Wendland et al., 2006). The *rs25531* has been shown to further alter the degree of 5-HTT gene expression. The minor G-allele is nearly always in phase with the L-allele of the 5-HTTLPR and has been shown to reduce transcriptional efficacy to the level of the S-allele (Caspi et al., 2010). When studied jointly, as in the present study, the mini-haplotypes constructed from *5-HTTLPR* and *rs25531* are usually referred to as 'tri-allelic' *5-HTTLPR* whereas analysis of only the L/S alleles are termed the 'biallelic' assay. Thus, the tri-allelic *5-HTTLPR* permits the functional division of individuals into high- (LA/LA), intermediate- (LA/LG, SA/LA) or low- (SA/SA, SA/LG) expressors of the 5-HTT (Caspi et al., 2010). This polymorphism affects endogenous pain modulation (Lindstedt et al., 2011) and has been associated with fibromyalgia (FM) (Ablin and Buskila, 2015; Arnold et al., 2013).

FM is characterized by chronic widespread pain and a generalized hypersensitivity to sensory stimuli, often in combination with fatigue, disturbed sleep and psychological distress. FM patients are characterized by pain hypersensitivity (Kosek et al., 1996) and an inability to activate endogenous pain inhibitory mechanisms (Kosek and Hansson, 1997; Lannersten and Kosek, 2010), which has been supported by neuroimaging studies showing augmented and abberrant cerebral pain processing (Gracely et al., 2002; Jensen et al., 2009, 2010, 2012, 2013). Furthermore, glia activation has been suggested in FM patients based on findings of elevated cerebrospinal fluid (CSF) concentrations of interleukin-8 (IL-8), compared to controls and patients with rheumatoid arthritis (Kadetoff et al., 2012; Kosek et al., 2015). The rodent equivalent of IL-8 (CXCL1) is co-localized with TSPO in glia cells (Liu et al., 2016). Furthermore, TSPO agonists regulate the expression of CXCL1 and it's receptor, thus affecting glia to neuron signalling and central sensitisation (Liu et al., 2016). Therefore, the elevated CSF concentrations of IL-8 in FM patients suggest that TSPO associated mechanisms may be involved in the pathophysiology of FM.

In the present study the influence of the functional polymorphism of the TSPO gene on FM symptoms and cerebral pain processing was investigated. We hypothesized that if pain in FM is associated with glia cell activation and TSPO/IL-8 related mechanisms, then genetically inferred differences in TSPO binding affinity would affect FM symptoms. Furthermore, an interaction between the TSPO and the 5-HTT functional polymorphisms would be expected.

2. Materials and methods

2.1. Subjects

Subjects were recruited to a multi-center experimental study (ClinicalTrials.gov identification number: NCT01226784) by newspaper advertisement, where FM patients were randomized to physical exercise or relaxation therapy (Larsson et al., 2015). Only baseline data were used in the current study. Out of 402 patients screened by telephone, 177 were assesed for eligibility at medical examination and 126 completed baseline examination and genotyping and were used for this analysis (Gothenburg n = 38, Linköping n = 41, Stockholm n = 47). The average age was 51 years, range 22–64 years. Inclusion criteria for FM patients were: female, age 20–65 years, and meeting the ACR-1990 classification criteria for FM (Wolfe et al., 1990). The patient characteristics are presented in Table 1. All patients were caucasian. A subgroup (the exercising part of Stockholm cohort) also performed functional magnetic resonance imaging (fMRI), to assess pain-evoked cerebral activations (n = 24, age 25–64 years).

Exclusion criteria were: high blood pressure (>160/90 mmHg), osteoarthritis in hip or knee, other severe somatic or psychiatric