

R E V I E W

Molecular foundations of chiropractic therapy

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Summary. *Background and aim:* Alternative medicine is a broad term used to encompass different therapies, including chiropractic. Chiropractic was called “a science of healing without drugs” by its founder, David Daniel Palmer. It is based on the idea that the body has a powerful self-healing ability and that there is a relationship between body structure and function that affects health. In particular, chiropractic assumes that the nervous system controls the human body through nerves branching from the vertebral column and spinal cord. Researchers do not fully understand how chiropractic therapies affect pain, but chiropractic is widely used today to treat chronic pain, such as back pain. Different studies with animal models have demonstrated that chiropractic therapies mediate neuroplasticity, specifically through modulation of neurotrophins. No studies have yet been published on interaction between neurotrophin gene polymorphisms and chiropractic treatment. *Methods:* We searched PubMed with the following keywords: chiropractic, neuroplasticity, neurotrophin gene polymorphism for a panorama of on the molecular mechanisms of chiropractic therapy. *Results:* From the material collected, we identified a set of genes and some functional polymorphisms that could be correlated with better response to chiropractic therapy. *Conclusions:* Further association studies will be necessary to confirm hypotheses of a correlation between single nucleotide polymorphisms in specific genes and better response to chiropractic therapy. (www.actabiomedica.it)

Key words: chiropractic, chiropractic therapy, neuroplasticity, neurotrophins, polymorphism

Chiropractic – a technique based on manipulation of the spine and peripheral nerve endings

Chiropractic is a form of complementary and alternative medicine that focuses on the relationship between major body structures, such as skeleton, muscles and nerves, and patient health. Basic concepts of chiropractic are: i) the body has powerful self-healing ability and ii) body function and structure, especially the spinal cord, are closely related and this relationship may affect health. These two concepts lead to the idea that chiropractic therapy can normalize the relationship between body structure and function and can help the body to heal. The founder of chiropractic, David Daniel Palmer, called it “a science of healing without drugs” (1). Chiropractic is based on the assumption

that the central nervous system controls the human body through peripheral nerves branching from the vertebral column and the spinal cord (2). In the chiropractic view, spinal cord dysfunctions can interfere with the body's innate capacity to heal. Chiropractic emphasizes spinal manipulation. The branches of chiropractic that seem most interesting from a biological point of view are those concerning spinal manipulation and acupuncture/acupressure of peripheral sensory nerve endings (3).

Recent insights have identified inflammation as a local protective response to microbial invasion or injury. It must be well regulated, because deficiencies or excesses of inflammatory response can cause morbidity and mortality. The discovery that cholinergic neurons can inhibit inflammation has changed

the way we understand the link between the nervous system and immune responses. We now know that a basic neural pathway monitors and adjusts the inflammatory response and that an inflammatory input activates a fast subconscious anti-inflammatory response. In his 2002 review, Tracey discussed evidence indicating that stimulation of the “vagus nerve” can prevent inflammation (4). Recent studies of the mechanisms that regulate inflammation have identified a neural mechanism, called the “cholinergic anti-inflammatory pathway”, that inhibits macrophage activation through parasympathetic outflow. The pathway’s name refers to the role of acetylcholine as the principle parasympathetic neurotransmitter, because macrophages exposed to acetylcholine are effectively deactivated (3).

Biological foundations of chiropractic therapy

Massage is a general term for many different techniques involving the application of bodily contact and physical pressure with therapeutic intent. The effects of massage therapy depend on the amount of pressure and the speed of the stroke. For example, slow strokes can evoke systemic relaxation whereas deep strokes increased blood flow to the area. Attempts to define and classify the extensive range of types of massage have sometimes created confusion, but as far as chiropractic is concerned, spinal manipulative therapy (SMT) is the best practice.

One of the effects of spinal manipulation during chiropractic therapy is to stretch spinal muscles. Muscle stretch is a powerful stimulus for up-regulation of a splice product (mechano-growth factor MGF) of the insulin-like growth factor (IGF-1) gene by the stretched muscle. MGF promotes muscle growth and repair (myotrophism) and the growth and repair of neurons (neurotrophism) (5).

Non-noxious mechanical skin stimulation releases nerve growth factor (NGF) in rats. Produced and secreted by brain cortex neurons, NGF is a neurotrophic factor that promotes neuron survival and function (6).

Mechanical vibration massage in rats promotes secretion of NGF by the sub maxillary gland. This secretion accelerates repair of brachial plexus injuries and slows down atrophy of skeletal muscle. Spinal ma-

nipulation is a specific hands-on approach commonly used in chiropractic. Thoracic spinal manipulation can lead to different responses involving the sympathetic nervous system, the endocrine system and the hypothalamic-pituitary axis (7).

Spinal manipulative therapy has been demonstrated to be an effective treatment for acute and chronic back pain (8, 9) although the neuronal mechanisms responsible for the pain-reducing effects are not yet understood (10). The idea of a link between SMT and spinal cord neuroplasticity is gaining interest among researchers, and different studies have demonstrated a connection (11, 12). Interestingly, Guzzetta and colleagues demonstrated that increased body massage and multisensory stimulation affects brain development in humans and rat pups. In particular, visual system maturation seems to be increased by “massage therapy” through modulation of levels of endogenous factors such as IGF-1 in infants. In rat pups, massage also proved effective in increasing IGF-1 levels in the cortex. This suggests that IGF-1 could also be a mediator of the effects of massage therapy on visual development in infants (13). The effects of massage in accelerating visual development in rat pups are not due to the simple act of removing pups from the nest, because they are absent in pups separated from the mother for the same amount of time but not massaged. This supports the idea that massage could be a promoter of brain development and the hypothesis that the level of multisensory stimulation provided by licking/grooming is an important regulator of brain development (14-18).

Chiropractic uses acupressure/acupuncture as well as spinal manipulative therapy to decrease pain and for a wide range of other complaints. Acupuncture is a technique of traditional Chinese medicine (19). Acupuncturists insert hair-thin needles in specific points of the body to balance body energy, stimulate healing and promote relaxation. Researchers do not understand how acupuncture can decrease pain, although the technique is widely used in treatment of neurological disorders such as ischemic stroke, cognitive impairment (20), Parkinson’s disease (PD) (21) and prophylaxis of migraine (22).

Interestingly, insertion of a needle in the human body produced a slow increase in the skin pain thresh-

old, peaking in 30 min, followed by exponential decay after removal of the needle (23). This suggests that chemical mediators are involved, a hypothesis validated by observation of transfer of the analgesic effect on cross-infusion of cerebroventricular fluid from a donor rabbit undergoing acupuncture stimulation to a naive recipient rabbit.

There are currently two theories about how massage and acupuncture could work: i) by stimulating release of endorphins, natural pain-relieving chemicals of the body (23) and/or ii) by influencing the nervous system and the release of chemicals that regulate blood flow and pressure, reduce inflammation and calm the brain (24). It was recently shown that peripheral sensory stimulation by electro-acupuncture could improve the availability and utilization of brain nerve growth factor (NGF). The clinical efficacy of acupuncture on pain and inflammation are based on the stimulation of several classes of sensory afferent fibers. The resulting activation of physiological processes seems to be similar to those resulting from physical exercise or deep massage. Acupuncture stimulation may induce variations in neural activity throughout the nervous system, affecting the synthesis and release of different neuromodulators. A relationship between acupuncture and NGF was recently investigated with a view to synergic clinical use. Administration of NGF with acupuncture and/or SMT in neurological, endocrine and immune diseases could be an interesting therapeutic approach (25).

Neuroplasticity and neuro modulator reflex as effector of chiropractic therapy

Various authors have demonstrated that acupuncture and massage mediate neuroplasticity in animal models (26). Neuroplasticity is defined as the ability of the nervous system to reorganize its structure and function in response to intrinsic and/or environmental demands (27). Neuroplasticity may be involved in physiological or pathological conditions: in physiological conditions, it is mainly related to brain development, learning and memory. The most famous form of physiological neuroplasticity is the process known as “adult hippocampal neurogenesis”, whereby the adult

hippocampus generates functional neurons throughout life (28). In pathological conditions, neuroplasticity seems to be involved in brain injury healing processes.

Neuroplasticity has been identified in the spinal cord and central nervous system, together with its prolonged forms, known as long-term potentiation (LTP) and long-term depression (LTD). In particular, observations of LTP in neurons of the spinal cord and similarities between LTP cell mechanisms and those associated with central sensitization suggest that LTP may play a significant role in establishing major pain conditions. It has also been identified as an amplifying mechanism within the pain system, suggesting its involvement both in acute and chronic pain states (10). If LTP is an important mechanism in pain system regulation, any mechanism that interferes with LTP is of clinical interest. Long-term depression (LTD), found to depend on glutamate release, activation of its NMDARs, and intracellular Ca^{2+} , is suggested to have a relationship with LTP. LTD was initially discovered in the hippocampus and was later identified in other body regions, such as spinal neurons, cerebellum, cortex, basal ganglia and amygdala. The identification of spinal LTD and the consequent demonstration of LTP reversal suggests that LTD is a cell mechanism able to mediate the effects of certain therapies (10).

So how can massage and acupuncture operate in the treatment of diseases and pain? Different studies attribute the underlying mechanisms of mediation of neuroplasticity by acupuncture and SMT to modulation of neurotrophins (NTs) (26). It is well known that NTs are responsible for the growth of neurons during development and for the maintenance of adult neurons. More recently, however, there has been increasing evidence that NTs are also involved in neuron survival and differentiation and even in axonal regeneration in neurological disorders (29, 30).

Genetic determinants of chiropractic response

The expression of NTs, for example by the brain-derived neurotrophic factor (*BDNF*) gene, is induced after cerebral ischemia. Expression peaks within minutes or hours of the event, then quickly returning to normal or even below normal levels. *BDNF* belongs to

a family of neurotrophins that include neurotrophin-3 (NT3), neurotrophin-4 (NT4) and nerve growth factor (NGF) (31). Here we list a series of known polymorphisms, most having a demonstrated functional effect, which could be considered in genetic analysis of predisposition to a positive response to chiropractic care (Table 1). The selected polymorphisms belong to genes involved in neurotrophism, myotrophism and pain sensitivity. Polymorphisms with a weak functional effect, as evaluated by *in vitro* or association studies, were disregarded.

NGF was the first target-derived neurotrophic factor identified. It is fundamental for the develop-

ment and maintenance of peripheral nervous system neurons and for the functional integrity of central nervous system cholinergic neurons (32). Moreover, NGF can affect balanced interplay between the nervous, endocrine and immune systems. It is well known that NGF concentrations increase during stress and play an important role in the hypothalamic-pituitary-adrenal axis (33).

Growing evidence suggests that different polymorphisms in the *NGF* gene can be considered risk factors for conditions such as vascular hypertension (34) and processes such as atherogenesis (35) and inflammation (36).

Table 1. Polymorphisms identified from the literature as potential modifiers of neurotrophin-mediated neuroplastic activity

Gene	Protein	Class	RefSeq	Nucleotide change	Amino acid change	MAF (GnomAD)	Ref
<i>NGF</i>	Beta-nerve growth factor	NTs	rs6330	c.104C>T	Ala35Val	A=0.37015 (91127/246188)	39
<i>BDNF</i>	Brain-derived neurotrophic factor	NTs	rs6265	c.196G>A	Val66Met	T=0.19437 (47821/246030)	40
<i>NGFR</i>	Beta-nerve growth factor receptor	NTs	rs2072446	c.614C>T	Ser205Leu	T=0.05165 (12700/245884)	39
<i>ADRB2</i>	Beta-2 adrenergic receptor	NTs	rs1042713	c.46A>G	Gly16Arg	A=0.42081 (103492/245936)	45
<i>ADRB2</i>	Beta-2 adrenergic receptor	NTs	rs1042714	c.79C>G	Gln27Glu	G=0.31729 (78116/246198)	45
<i>CNTF</i>	Ciliary neurotrophic factor	NTs	rs1800169	c.115-6G>A		A=0.14950 (35961/240538)	46
<i>MSTN</i>	Growth/differentiation factor 8	recovery	rs1805086	c.458A>G	Lys153Arg	C=0.02732 (6704/245366)	53
<i>ACTN3</i>	Alpha-actinin-3	recovery	rs1815739	c.1729C>T	Arg577*	T=0.46060 (113241/245854)	55
<i>NTRK1</i>	High affinity nerve growth factor receptor	pain/NTs	rs6334	c.1656G>A	Gln552His	A=0.22025 (54107/245664)	57
<i>SCN9A</i>	Sodium channel protein type 9 subunit alpha	pain	rs6746030	c.3448C>T	Arg1150Trp	A=0.12050 (18374/152480)	60
<i>COMT</i>	Catechol O-methyltransferase	pain	rs4680	c.322G>A	Val158Met	A=0.46252 (112239/242666)	66

Legend: NTs, neurotrophins; RefSeq, polymorphism accession number as recorded in the database of single nucleotide polymorphisms; MAF, minor allele frequency; Ref, reference.

A nucleotide change in *NGF*, c.104C>T, causes an amino acid change in position 35, p.Ala35Val. This amino acid change is referred to as rs6330 and the more common of the two alleles (C) encodes alanine (Ala). Several studies have indicated that this single nucleotide polymorphism (SNP) can lead to dysfunction in intracellular processing and secretion of NGF. The minor T allele seems to be associated with increased susceptibility to anxiety by association with low vagal activity, while the major C allele seems to be associated with attention deficit hyperactivity disorder (ADHD) (37, 38). More interestingly, rs6330 CC homozygotes show significantly higher NGF median plasma levels of NGF than carriers of the minor T allele (39). These findings suggest an association between certain *NGF* polymorphisms, rs6330 in particular, and response to chiropractic SMT and acupuncture.

The BDNF gene encodes a protein found in the brain and spinal cord. This protein promotes neuron survival by playing a role in the growth, differentiation, maturation and maintenance of these cells. BDNF protein is active in synapses. Synapses change and adapt in response to external factors. The BDNF protein helps regulate synaptic plasticity, which is important for learning and memory.

Several polymorphisms have been identified in *BDNF*. The non-synonymous polymorphism, known as SNP rs6265 or p.Val66Met or c.G>A196, is common and causes a valine (Val) to methionine (Met) substitution at position 66 of the pro-BDNF protein. The replacement of Val by Met impairs neuronal activity-dependent secretion of BDNF (40). Approximately 30-50% of the population is heterozygous or homozygous for this rs6265 polymorphism. The SNP occurs in the 5' pro-domain of *BDNF* and does not affect mature BDNF protein function or BDNF constitutive release, but rather activity-dependent BDNF release, thus influencing intracellular trafficking of pro-BDNF (41).

Recent studies suggest a role of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and their receptor, nerve growth factor receptor (NGFR), in neuropsychiatric disorders, especially Alzheimer's disease (42). The rs2072446 polymorphism in *NGFR* has been associated with risk of Alzheimer's disease, but also as a functional SNP that

could be involved in gene expression and protein secretion (39, 43).

The ADRB2 gene encodes beta2-adrenergic receptor and maps to the 5q32 chromosomal region. The association between polymorphisms in ADRB2 and risk of other diseases has also been studied (44). Moreover, rs1042713 (p.Arg16Gly) and rs1042714 (p.Gln27Glu), non-synonymous polymorphisms in *ADRB2*, have been associated with cognitive function and brain white matter integrity (45).

Ciliary neurotrophic factor (CNTF) is a pleiotropic cytokine of the interleukin-6 family whose myotrophic and neurotrophic effects have been extensively studied (46). Rs1800169 is the most widely studied *CNTF* polymorphism: this SNP leads to a G to A transition close to the boundary between the first intron and second exon. As a result of this change, four nucleotides are inserted in CNTF mRNA, leading to a frameshift mutation and a premature stop codon. By virtue of its functional effect, the rs1800169 polymorphism in *CNTF* could also be considered in relation to response to chiropractic treatment.

Polymorphisms in muscle-related genes can modify muscle healing after injury, so they could also be related to response to chiropractic treatment. Myostatin, encoded by *MSTN*, is a muscle inhibitor peptide that regulates myoblast differentiation with direct consequences on muscle mass and strength. *MSTN* knock-out mice show muscle hypertrophy and greater strength than wild-type mice (47). Null *MSTN* variants are known to express the same phenotype in humans (48) and other mammals (49, 50). Lack of the protein has also been associated with better muscle regeneration after injury (51, 52).

Among common polymorphisms, rs1805086 is receiving growing attention from researchers, and a number of association studies agree that it has functional significance (53, 54).

The ACTN3 gene, encoding the structural skeletal muscle protein α -actinin-3, locates on chromosome 11 and is one of the most interesting genes associated with athletic performance. Previous studies report that the ACTN3 Arg577* variant (rs1815739) seems to be associated with athletic performance in different races but the function of this SNP is unknown (55, 56). Since it is a null mutation and considering its evocative

role in muscle quality, the rs1815739 polymorphism is worth studying in relation to chiropractic response.

The *NTRK1* gene, encoding the high-affinity NGF receptor TrkA, seems to be involved in gene expression and protein secretion. Szczepankiewicz and colleagues demonstrated that NGF serum levels may be influenced by the rs6334 polymorphism in *NTRK1* and that the interaction between this variant and NGF indicates that this pathway may influence NGF protein levels (57). The polymorphism has also been associated with pain perception during acupuncture (58). This involvement led us to consider the rs6334 polymorphism in *NTRK1* as a variation that could be involved in chiropractic response.

Pain is a disturbing non-motor symptom in Parkinson disease (PD) and susceptibility to pain varies widely among these patients. *SCN9A* encodes the NaV1.7 sodium channel and is preferentially expressed in pain-signaling dorsal root ganglion (DRG) neurons (nociceptors) that play a critical role in amplifying small depolarizations, thus increasing the pain signaling gain (59).

Greenbaum and colleagues demonstrated that the non-synonymous rs6746030 polymorphism in *SCN9A* was associated with PD-related pain susceptibility and with PD central and musculoskeletal pain. Since the non-synonymous rs6746030 polymorphism has also proved experimentally to influence the excitability of nociceptive DRG neurons, thus influencing pain sensitivity and susceptibility to chronic pain (60). We focus on this SNP in relation to chiropractic response (61).

The *COMT* gene encodes catechol-O-methyltransferase, the enzyme mainly responsible for catecholamine metabolism and known as a major modulator of synaptic dopamine concentrations in the brain. *COMT* is involved in dopamine degradation at biochemical level and in complex cognitive functions such as cognitive control and working memory (61), and interestingly also pain modulation (62). Indeed, *COMT* is a key regulator in the pain perception pathway and polymorphisms in the gene have been studied in relation to variability in pain perception between individuals (63, 64).

Among the different polymorphisms identified in *COMT*, the rs4680 SNP seems to be involved in the increased synaptic dopamine concentrations in

Met-allele carriers (65). The experimentally detected functional effect of rs4680 revealed a 40% decrease in *COMT* activity associated with the Met108 variant as a consequence of reduced *COMT* protein levels (66). The polymorphism has also been shown to influence the human experience of pain (67). All these findings make it a good candidate for an association study regarding chiropractic response in cases of chronic pain.

Conclusions

As documented by the growing number of scientific papers concerning chiropractic therapy in PubMed over the last 20 years, complementary and alternative medicine and chiropractic in particular are ranking close to traditional medicine, especially but not only in the treatment of chronic pain. Different studies show utilization rates for chiropractic services of 6-12% (68-70). Interestingly, several surveys have found higher use of chiropractic services among middle-aged women (71, 72). As expected, higher chiropractic utilization is reported for patients with chronic pain and back pain (16.1% and 31.0%, respectively) (73, 74). Chiropractors provide a substantial portion of care for patients with various pathologies, including lumbar pain and neck pain (75). Today chiropractic care is available in over 100 countries, most of which have established national chiropractic associations. To the best of our knowledge, no studies regarding interaction between neurotrophin gene polymorphisms and chiropractic have been published; such studies could be interesting for understanding whether certain polymorphisms predispose for response to chiropractic, especially to acupuncture and SMT. Four genes in the family of neurotrophic factors, *BDNF*, *NGF*, *NTF3* and *NTF4*, together with other genes, could be considered for a study into the existence of a genetic association between polymorphisms in these genes and the amount of neurotrophic factors released by chiropractic techniques. Concerning the last two genes, we did not find convincing studies on functional polymorphisms in the literature and they were therefore not considered by this review.

Today it is well known that neuroplasticity plays an important role in neurological rehabilitation. SNPs with

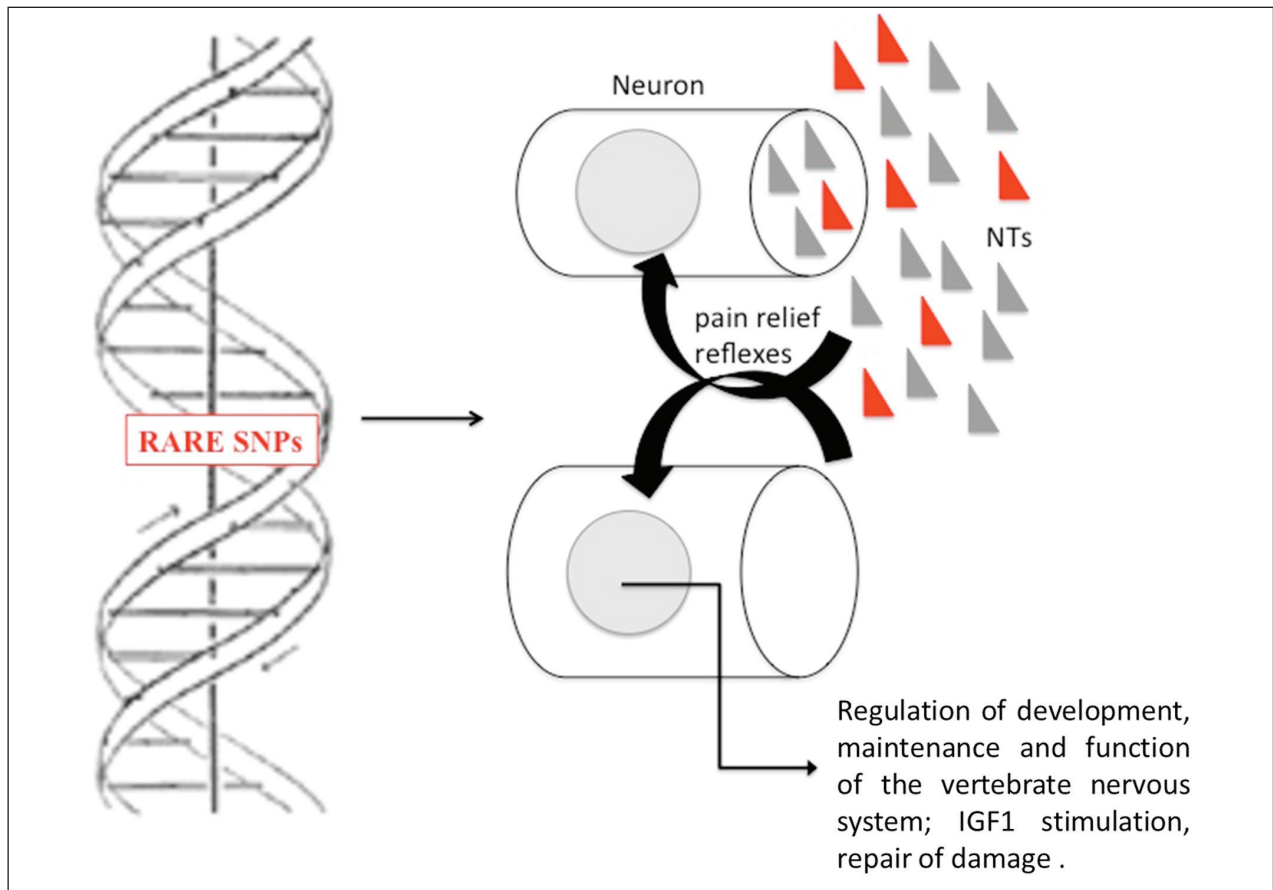


Figure 1. Presence of specific SNPs in genes related to brain function could increase production of neurotrophins (NTs) by neurons and consequently their neuroplasticity, leading to a better response to chiropractic therapy through regulation of development, maintenance and function of the vertebrate nervous system. These specific SNPs could also increase IGF-1 blood levels after stimulation by massage

a demonstrated effect on neuroplasticity may therefore be relevant for neurological rehabilitation (Figure 1). Different authors suggest that individuals with genetic variants associated with neuroplasticity may respond better to therapies involving neuroplastic processes than individuals without such variants (70-72).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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