Pain Medicine 2018; 19: 1010–1014
doi: 10.1093/pm/pnx261

OXFORD

METHODOLOGY, MECHANISMS & TRANSLATIONAL RESEARCH SECTION

Brief Research Report

Common Missense Variant of *SCN9A* Gene Is Associated with Pain Intensity in Patients with Chronic Pain from Disc Herniation

Mateusz Kurzawski, PhD,* Marcin Rut, MD, PhD,† Violetta Dziedziejko, PhD,‡ Krzysztof Safranow, MD, PhD,‡ Anna Machoy-Mokrzynska, MD, PhD,* Marek Drozdzik, MD,* and Monika Bialecka, MD, PhD§

*Department of Experimental and Clinical Pharmacology, Pomeranian Medical University, Szczecin, Poland; †Department of Neurosurgery, SPZZOZ Gryfice, Gryfice, Poland; Departments of ‡Biochemistry and Medical Chemistry and §Department of Pharmacokinetics and Therapeutic Drug Monitoring, Pomeranian Medical University, Szczecin, Poland

Correspondence to: Violetta Dziedziejko, PhD, Department of Biochemistry and Medical Chemistry, Pomeranian Medical University, Szczecin, Powstańców Wlkp 72, Szczecin 70-111, Poland. Tel: +48 91 4661530; Fax: +48 91 4661516; E-mail: viola@pum.edu.pl.

Funding: The study was supported by a grant from the Polish State Committee for Scientific Research of the Ministry of Scientific Research and Information Technology, No. N402386433 and by Pomeranian Medical University.

Conflicts of interest: The authors have no conflicts of interest to declare.

Abstract

Objective. Lumbar intervertebral disk herniation (LDH) is considered one of the major risk factors for lower back pain, mainly caused by irritation of a spinal nerve or its root. One of the genes related to pain perception is *SCN9A*, which encodes the voltage gated sodium channel NaV1.7, a key molecule involved in peripheral pain processing. It had been presented before that a common polymorphism within *SCN9A* (rs6746030: G > A, R1150W) might influence nociception in the general population. Hence, the present study was aimed at investigating the association between *SCN9A* polymorphism and pain sensitivity.

Methods. Pain intensity was measured by means of the visual analog pain scale (VAS) in 176 Caucasian patients with a history of leg and back pain who had been diagnosed with LDH and underwent lumbar discectomy. *SCN9A* polymorphism was determined by means of TaqMan assay.

Results. A significantly higher preoperative back pain intensity was observed among rs6746030 A minor allele carriers, compared with GG homozygotes (VAS = 7.5 ± 2.4 vs 6.5 ± 2.7, $P = 0.012$). Similarly, A allele carriers were characterized by higher values of leg pain prior to surgery (VAS = 7.8 ± 2.3 vs 6.8 ± 2.6, $P = 0.013$). However, postoperative improvement in pain reduction was similar in both groups.

Conclusions. Our results suggest that the *SCN9A* rs6746030 polymorphism may be associated with pain intensity in patients suffering from symptomatic disc herniation.

Key Words. Symptomatic Disc Herniation; SCN9A; Pain; Polymorphism

Introduction

Lower back pain (LBP) is one of the most common musculoskeletal ailments and a major cause for disability, especially in high-income countries. Approximately 5–10% of patients develop chronic lower back pain that lasts longer than three months [1]. There are many different causes for lower back pain, and lumbar intervertebral disk degeneration and herniation (LDH) is considered one of the major risk factors. However, some subjects with LDH do not suffer from chronic pain, and some LPB patients do not show disc degeneration, evaluated by means of magnetic resonance imaging [2,3]. LDH has

possible anatomic and molecular mechanisms, related to a cascade of cellular, biochemical, structural and functional changes developing within the disc and surrounding tissues. Patients who suffer from LBP experience both nociceptive and neuropathic pain; thus discrimination of mechanisms triggering particular types of pain is challenging [4].

The most common type of neuropathic pain in the course of degenerative disk disease is the radicular type, caused by irritation of a spinal nerve or its root due to inflammation, decreased blood supply of root ganglion, or its compression, which initiates and then propogates several distinct cellular and molecular mechanisms involving ion channels, cytokines, and neuropeptides [5]. The magnitude of the degeneration is strongly correlated with pain intensity. However, there is some scientific evidence that indicates an individual genetic predisposition to processing of sensory information, such as pain perception following noxious stimulation, development of pain, and efficacy of analgesics [6].

One of the genes related to pain perception is *SCN9A*, which encodes an α-subunit of the voltage gated sodium channel NaV1.7, highly expressed in nociceptive neurons and recognized as a key molecule involved in peripheral pain processing [7]. Rare mutations in *SCN9A*, severely altering NaV1.7 function, were identified as a causative factor for different human genetic pain disorders. Unlike gain-of-function mutations, causing primary erythromelalgia and paroxysmal extreme pain disorder, nonsense mutations produce complete loss of NaV1.7 function, which results in channelopathy-associated insensitivity to pain [8]. Although subjects with major pain phenotypes are very rare, it has been presented that a common missense polymorphism within *SCN9A* gene (rs6746030: G > A; R1150W) might influence nociception in the general population, and the minor A allele was associated with increased pain scores [9]. Moreover, the latter authors presented that a single amino acid substitution caused by the studied SNP might alter the voltage-dependent slow inactivation of the NaV1.7 channel. The authors demonstrated that the rs6746030 A allele was associated with the increased activity of the NaV1.7 channel, leading to a lower pain threshold in minor allele carriers [9].

So far, no clear relationship between pain intensity in patients with LDH and genetic variants in sodium channel functions has been found. Hence, the present study was aimed at investigating the association between the *SCN9A* gene (rs6746030: G > A) polymorphism and pain sensitivity in patients with a history of leg and back pain, diagnosed with LDH, who underwent lumbar discectomy.

Methods

A total of 270 patients were initially asked to participate in the study, but some refused mainly due to the long distance to clinical center for follow-up. Two hundred thirty-three patients were recruited for the study (clinical evaluation and preoperative visual analog scale [VAS] score measurement) at the Neurosurgery Department of the Medical University of Gdansk, Poland. Fifty-seven did not complete 12-month follow-up, mainly due to no-show, but also diabetes, death of a patient, and dementia. Finally, 176 patients of Caucasian origin (mean age = 46.7 ± 13.2 years, age range = 20–80 years, 41.5% female) diagnosed with lumbar disc herniation who had been subjected to lumbar discectomy were enrolled. The main inclusion criterion was persistent back and leg pain resistant to conservative therapy for at least six months that was associated with lumbar disc herniation on computed tomography or magnetic resonance imaging scans. The surgery, that is, discectomy, was performed at one of the levels from L3 to S1, from the midline standard posterior approach with assistance of an intraoperative microscope, by one of four neurosurgeons.

In order to standardize the clinical indications for surgery, the following were considered the exclusion criteria: spine fracture history, spinal tumors, patients with serious osteoporosis disqualifying for surgery, fixed motor deficit, spinal stenosis, cauda equina syndrome, spine deformities, patients who had progression of symptoms and required immediate surgery, reoperation. The patients with long-term diabetes mellitus with polyneuropathy and those diagnosed with sensorimotor polyneuropathy on a different background were also excluded from the study due to the change in the threshold of pain sensitivity associated with the above diseases. Another exclusion criterion was the treatment with drugs prescribed for neuropathic pain, including antidepressants such as SNRI—duloxetine, venlafaxine, TLPD—amitriptyline, as well as gabapentinoids, for example, pregabalin and gabapentin, due to their potential influence on the assessment of pain severity. The patients with moderate to severe dementia (Mini-Mental State Exmaination < 19) were excluded from the study due to the possible difficulties with objective pain assessment.

The pain intensity was measured by means of the VAS (range 0–10), separately for lower back pain (VASBACK) and leg pain (VASLEG), self-completed by the study participants. The patients who enrolled in the study received a detailed questionnaire concerning the analgesic treatment before and after surgery. The questionnaire asked about the type, daily dose, and frequency of use of analgesics and/or physiotherapeutic treatment. The medical examination was performed at least 72 hours after discontinuation of both nonopioid and opioid analgesics to maximize the objectification of pain during clinical evaluation. All patients were evaluated twice: before the surgery and 12 months postoperation. The study protocol was approved by the local ethics committee, and each patient signed an informed consent form.

Frozen genomic DNA samples (20 ng/μL) were available from the previous study [10]. The SNP within *SCN9A* gene was evaluated using prevalidated allelic

discrimination TaqMan real-time polymerase chain reaction (PCR) assay (assay ID: C__29330435_10, Life Technologies, Carlsbad, CA, USA). PCR thermal profile consisted of initial denaturation at 95 °C for two minutes and 40 cycles at 95 °C for five seconds/60 °C for 15 seconds; all reactions were run using GTXpress master mix (Life Technologies, USA). Fluorescence data were captured using 7500 FAST Real-Time PCR System (Applied Biosystems, Foster City, CA, USA.) after reaction termination.

The consistency of the genotype distribution with the Hardy-Weinberg equilibrium (HWE) was assessed using Fisher's exact test. VAS scores were checked for their concordance with normal distribution using the Shapiro-Wilk test, which showed strong deviation from normality ($P < 0.0001$). Therefore, the scores were subsequently compared between the genotype groups using the non-parametric Mann-Whitney test. P values of $P < 0.05$ were considered statistically significant.

Results

The mean value of VASBACK score among all study participants (±SD) was 6.7 ± 2.7, and it significantly decreased at 12 months after the surgery (4.4 ± 2.9, VASBACK change = 2.3 ± 2.9). Similarly, mean VASLEG values were significantly reduced by the surgical treatment (7.0 ± 2.6 preoperative to 4.0 ± 3.2 postoperative, mean change = 3.0 ± 3.6). The distribution of *SCN9A* rs6746030 genotypes was in concordance with Hardy-Weinberg equilibrium ($P = 0.20$). The GG genotype was determined in 134 patients (76.1%), 37 subjects were heterozygotes (21.0%), and minor homozygous AA genotype was present in five patients (2.8%). Minor allele frequency (MAF = 0.134) was similar to that obtained for the European population in the 1000 Genomes Project (MAF = 0.126, http://www.1000genomes.org). Because the AA homozygous genotype was rare among the study participants, A allele carriers (GA and AA genotypes) were pooled together for further analyses. A significantly higher preoperative back pain intensity was observed among rs6746030 A allele carriers when compared with GG homozygotes (7.5 ± 2.4 vs 6.5 ± 2.7, $P = 0.012$). Similarly, A allele carriers were characterized by higher values of leg pain evaluated by visual scale prior to surgery (7.8 ± 2.3 vs 6.8 ± 2.6, $P = 0.013$). Twelve months after surgery, VASBACK and VASLEG scores were reevaluated, and values were still higher in the A allele carrier group, but the difference was not significant. Improvement in pain reduction (change in VAS score) was similar in both groups. Results are presented in Table 1.

Table 1. VAS scores for chronic back and leg pain in patients stratified by *SCN9A* rs6746030 genotype.

	Genotypes *SCN9A* rs6746030		
	Mean ± SD Median (IQR)		
Parameters	GG (n = 134)	GA+AA (n = 42)	p*
VASBACK			
Preoperative	6.5 ± 2.7 7 (3)	7.5 ± 2.4 8 (2)	0.012
Postoperative	4.3 ± 2.9 4 (5)	4.9 ± 2.7 5 (4)	0.19
Change	-2.2 ± 2.8 -2 (4)	-2.6 ± 3.1 -2 (4)	0.33
VASLEG			
Preoperative	6.8 ± 2.6 7 (4)	7.8 ± 2.3 8 (3)	0.013
Postoperative	3.8 ± 3.1 3 (5)	4.7 ± 3.2 5 (5)	0.11
Change	-3.0 ± 3.6 -2 (6)	-3.1 ± 3.7 -3 (5)	0.87

IQR = interquartile range; VASBACK = scores of visual analogue pain scale (VAS) for lower back pain; VASLEG = VAS scores for leg pain;
*Mann-Whitney U test

Discussion

The pathophysiology of radicular pain is unclear. However, the role of neural compression with axonal dysfunction, ischemia, inflammation, and biochemical influences is postulated. Neuropathic pain, one of the LBP components, produces a large detrimental effect on quality of life, partly due to low treatment efficacy [11]. The understanding of physiological processes or genetic predispositions contributing to persistent LBP may help to identify high-risk patients and target interventions accordingly. An important role of voltage-gated sodium channels in many different pain states has been established in animal models and in humans [12]. Genetic and functional studies have revealed that voltage-gated sodium channel NaV1.7, preferentially expressed in peripheral somatic and visceral sensory neurons and sympathetic ganglion neurons, is a major contributor to pain signaling in humans [13]. The original concept that a nonsynonymous single nucleotide polymorphism (rs6746030: G > A) within the *SCN9A* sequence could influence NaV1.7 sodium channel function was formulated by Estacion et al. The authors identified arginine to tryptophan substitution in a conservative residue of the encoded protein during their studies on erythromelalgia, and subsequently demonstrated that minor A allele produced hyperexcitability [14]. Shortly afterward, Reimann et al. [9] revealed that the *SCN9A* polymorphism determines a difference in the voltage-dependent slow inactivation, with the minor A allele predicting an increase in NaV1.7 activity. The authors observed a significant association between SNP rs6746030 and pain score among osteoarthritis patients, sciatica pain, and phantom pain. In the same

study, in a cohort of 179 Caucasian patients evaluated for pain using a seven-point score one year after lumbar discectomy, an association was marginally significant [9]. In the current study, we have observed an association of *SCN9A* rs6746030: G > A minor allele carrier status and higher pain intensity in patients with lumbar disc herniation. This association was significant prior to surgery, but not when evaluated 12 months postoperation. As a consequence of surgery, most patients experienced overall relief from pain symptoms, and mean VAS scores significantly decreased, regardless the patient's genotype. Hence, it is probable that the effect of the rs6746030 SNP is more pronounced in the case of higher intensity of pain stimuli, and markedly lower after surgical treatment. However, one cannot directly compare the results of different investigations, mainly due to differences in study design, but other factors, including ethnic differences, may also play a role.

The results of association studies on *SCN9A* polymorphism and pain that followed the original investigation were often contradictory. In the study, which recruited patients from three different cohorts, no genotype-dependent differences in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain scores were observed in any of the cohorts. However, when all the study subjects were pooled together, significantly increased odds for multiple regional pain were observed in minor allele carriers [15]. Another population-based study negatively verified the association between *SCN9A* rs6746030 genotypes and chronic widespread pain [16]. It is worth mentioning that the latter study analyzed available data from four different populations and that methods of pain assessment and widespread pain classification criteria differed from one another. Pain analysis is problematic due to its subjective nature and difficulties in developing reliable scales characterizing pain. The method of pain assessment used in the current study (self-evaluation of VAS scores at one time point before and one time point after the operation) is one of its limitations, as choosing an appropriate and reliable method of pain assessment may have impact on study outcome. Moreover, quality of life and other functional parameters were not measured. However, it was presented by Landmark et al. [17] that a single evaluation of chronic pain intensity can replace multiple assessments with high sensitivity and specificity pain, as long as an appropriate method is applied.

In turn, a significant association between *SCN9A* rs6746030 minor allele carrier status and interstitial cystitis/bladder pain syndrome was reported, but the study was performed in a relatively small number of subjects, and the results must be viewed as preliminary [18]. As for non-Caucasian studies, it was reported that *SCN9A* rs6746030 may be associated with postoperative pain intensity (evaluated by numerical rating scale) in Chinese women who underwent gynecological laparoscopic surgery and refused postoperative patient-controlled analgesia [19]. The same authors investigated a set of tag SNPs within the *SCN9A* gene for their potential association with pain sensitivity. Both mechanical and heat pain sensitivity were measured in a large cohort of young women, and despite a low frequency in the studied population, rs6746030 was associated with some measures of mechanical pain, that is, sensitivity to pricking pain [20]. Different results from alternatively designed studies may suggest that the SNP is associated with the severity of pain rather than the presence or absence of pain [9,16].

As our previous paper [10] analyzed the association of *COMT* polymorphisms with pain intensity in the same group of LDH patients, we performed additional analyses to find whether *SCN9A* and *COMT* polymorphism are associated with each other and whether the currently reported associations between *SCN9A* polymorphism and pain intensity are independent of previously reported associations between *COMT* polymorphisms and pain intensity. The analyses showed that there was no association ($P > 0.3$, chi-square test) between rs6746030 *SCN9A* polymorphism and any of the four previously studied *COMT* polymorphisms (rs4680, rs6269, rs4633, rs4818) in our patients. This result could be expected as the *SCN9A* gene is located on chromosome 2 and the *COMT* gene on chromosome 22. Multivariate linear regression analysis showed that the associations of *SCN9A* rs6746030 allele A with higher preoperative lower back and leg pain intensity were independent of the associations with *COMT* polymorphisms as they remained significant ($P < 0.03$) when both *SCN9A* and *COMT* polymorphisms were included in the regression models.

Finally, our results suggest that *SCN9A* rs6746030 polymorphism may be associated with pain intensity in patients suffering from symptomatic disc herniation. This finding has limited clinical utility but supports the functional character of the investigated missense variant. Genetic polymorphism may be responsible in part for interpersonal variability in intensity of pain triggered by similar stimuli. However, further studies and meta-analyses will finally verify the significance of *SCN9A* rs6746030 polymorphism in pain sensitivity and intensity.

References

1 Andersson GB. Epidemiological features of chronic low-back pain. Lancet 1999;354(9178):581–5.

2 Jensen MC, Brant-Zawadzki MN, Obuchowski N, et al. Magnetic resonance imaging of the lumbar spine in people without back pain. N Engl J Med 1994;331(2):69–73.

3 Kovacs FM, Arana E, Royuela A, et al. Disc degeneration and chronic low back pain: An association which becomes nonsignificant when endplate changes and disc contour are taken into account. Neuroradiology 2014;56(1):25–33.

4 Seaman DR, Cleveland C 3rd. Spinal pain syndromes: Nociceptive, neuropathic, and psychologic mechanisms. J Manipulative Physiol Ther 1999;22 (7):458–72.

5 Baron R, Binder A, Attal N, et al. Neuropathic low back pain in clinical practice. Eur J Pain 2016;20 (6):861–73.

6 Lee M, Tracey I. Neuro-genetics of persistent pain. Curr Opin Neurobiol 2013;23(1):127–32.

7 Raymond CK, Castle J, Garrett-Engele P, et al. Expression of alternatively spliced sodium channel alpha-subunit genes. Unique splicing patterns are observed in dorsal root ganglia. J Biol Chem 2004; 279(44):46234–41.

8 Drenth JP, Waxman SG. Mutations in sodium-channel gene SCN9A cause a spectrum of human genetic pain disorders. J Clin Invest 2007;11 :3603–9.

9 Reimann F, Cox JJ, Belfer I, et al. Pain perception is altered by a nucleotide polymorphism in SCN9A. Proc Natl Acad Sci U S A 2010;107(11):5148–53.

10 Rut M, Machoy-Mokrzyńska A, Ręcławowicz D, et al. Influence of variation in the catechol-O-methyltransferase gene on the clinical outcome after lumbar spine surgery for one-level symptomatic disc disease: A report on 176 cases. Acta Neurochir (Wien) 2014;156(2):245–52.

11 Finnerup NB, Attal N, Haroutounian S, et al. Pharmacotherapy for neuropathic pain in adults: A systematic review and meta-analysis. Lancet Neurol 2015;14(2):162–73.

12 Dib-Hajj SD, Black JA, Waxman SG. Voltage-gated sodium channels: Therapeutic targets for pain. Pain Med 2009;10(7):1260–9.

13 Dib-Hajj SD, Yang Y, Black JA, Waxman SG. The Na(V)1.7 sodium channel: From molecule to man. Nat Rev Neurosci 2013;14(1):49–62.

14 Estacion M, Harty TP, Choi JS, et al. A sodium channel gene SCN9A polymorphism that increases nociceptor excitability. Ann Neurol 2009;66 (6):862–6.

15 Valdes AM, Arden NK, Vaughn FL, et al. Role of the Nav1.7 R1150W amino acid change in susceptibility to symptomatic knee osteoarthritis and multiple regional pain. Arthritis Care Res (Hoboken) 2011;63 (3):440–4.

16 Holliday KL, Thomson W, Neogi T, et al. The non-synonymous SNP, R1150W, in SCN9A is not associated with chronic widespread pain susceptibility. Mol Pain 2012;8:72.

17 Landmark T, Romundstad P, Dale O, Borchgrevink PC, Kaasa S. Estimating the prevalence of chronic pain: Validation of recall against longitudinal reporting (the HUNT pain study). Pain 2012;153 (7):1368–73.

18 Reeder JE, Byler TK, Foster DC, et al. Polymorphism in the SCN9A voltage-gated sodium channel gene associated with interstitial cystitis/ bladder pain syndrome. Urology 2013;81 (1):210.e1–4.

19 Duan G, Xiang G, Guo S, et al. Analysis of SCN9A for prediction of postoperative pain in female patients undergoing gynecological laparoscopic surgery. Pain Phys 2016;19:E151–62.

20 Duan G, Guo S, Zhang Y, et al. The effect of SCN9A variation on basal pain sensitivity in the general population: An experimental study in young women. J Pain 2015;16(10):971–80.