Prosjektleder Johannes Gjerstad 26.01.13

NY BEHANDLING AV LUMBAGO OG ISJIASSMERTE 2008/2/0296

SLUTTRAPPORT

Sammendrag i et populærvitenskapelig og lett forståelig språk, og publikasjonsliste

Bakgrunn og målsetting: Utvikling av langvarige korsrygg- og isjiaspassmerter er trolig forårsaket av kompliserte neuro-biologiske mekanismer. Formålet med dette prosjektet har derfor vært å frambringe mer kunnskap om disse mekanismene og å undersøke hvorfor noen blir kronikere.

Metode og gjennomføring: Smerte og funksjon er målt med spørreskjemaene VAS, McGill og Oswestry. I tillegg er det tatt blodprøver, prolapsprøver, serumprøver, samt MR bilder av pasientene. Totalt ca 260 pasienter isjiaspasienter er blitt fulgt over 1 år. Samtlige pasienter er blitt genotypet med hensyn på en rekke genvarianter. Pasientene har vært undersøkt etter både 6 uker, 6 mnd og 12 mnd. Vi har hatt bare ca 10 % drop-outs (noe som er unikt i denne typen studier). Prosjektet har pga det krevende analysearbeidet og den totale arbeidsmengden blitt forlenget fra 3 til 4 år.

Resultater: Våre data viser at pasienter som er predisponert for økt frisetting av inflammatoriske stoffer i mellomvirvelskiven spesielt (MMP1), og for økt immunrespons generelt (HLA), i større grad enn andre utvikler langvarig korsryggs- og isjiassmerte. Videre har våre data vist at korsrygg- og isjiaspasienter predisponert for høye verdier av stresshormoner (COMT), og redusert aktivitet i hjernens smertereguleringssystem (OPRM1) er viktig for utvikling av langvarige smerter etter skiveprolaps. Vi har derfor avdekket konkrete biologiske faktorer som bidrar til smerteutviklingen.

Vitenskapelig betydning: Samlet støtter våre data hypotesen om at individuell genetiske varianter i gener som koder for MMP1[1], HLA[2], COMT[3] og OPRM1[4] i interaksjon med andre faktorer spesielt kjønn, er viktige for den enkelte ryggpasients smerteutvikling. Kunnskap om dette er definitivt viktig for vår forståelse av denne type pasienters plager – og for å forebygge og behandle denne typen lidelser. Totalt 4 vitenskapelige artikler i vel renomerte internasjonale tidsskrifter er blitt publisert i 2012 og 2013.

Videre planer: Prosjektet videreføres. Flere intervensjoner kan være aktuelle. Videre planlegger vi å øke antallet pasienter i årene som kommer. Avhengig av finansiering vil vi også vurdere å kalle inn igjen pasientene etter 5 år for å se hvordan det har gått.

Publikasjonsliste

1. L.M. Jacobsen, E. Schistad, A. Storesund, L.M. Pedersen, A. Espeland, L.J. Rygh, C. Røe and J. Gjerstad. The MMP1 rs1799750 2G allele is associated with increased low back pain, sciatica and disability after lumbar disc herniation. The Clinical Journal of Pain, In press.

2. C.A. Dominguez, M. Kalliomäki, U. Gunnarsson, A. Moen, G. Sandblom, I. Kockum, E. Lavant, T. Olsson, F. Nyberg, L.J. Rygh, C. Røe, J. Gjerstad, T. Gordh and F. Piehl. The DQB1*03:02 HLA haplotype is associated with increased risk of chronic pain after inguinal hernia surgery and lumbar disc herniation. Pain, In press.

3. L.M. Jacobsen, E. Schistad, A. Storesund, L.M. Pedersen, L.J. Rygh, C. Røe and J. Gjerstad. The COMT rs4680 met-allele contributes to long lasting low back pain, sciatica and disability after lumbar disc herniation. European Journal of Pain, 16: 1064-1069, 2012.

4. M.B. Olsen, L.M. Jacobsen, E.I. Schistad, L.M. Pedersen, L.J. Rygh, C. Røe and J. Gjerstad. Pain intensity the first year after lumbar disc herniation is associated with the A118G polymorphism in the opioid receptor mu 1 gene: evidence of a sex and genotype interaction. The Journal of Neuroscience, 32: 9831-9834, 2012.

ORIGINAL ARTICLE

The MMP1 rs1799750 2G Allele is Associated With Increased Low Back Pain, Sciatica, and Disability After Lumbar Disk Herniation

Line M. Jacobsen, PhD, ~~MSc,~~ Elina I. Schistad, MD,† Anette Storesund, MSc,‡ Linda M. Pedersen, PhD,*† Ansgar Espeland, MD, PhD,§‖ Lars J. Rygh, MD, PhD,‡ Cecilie Røe, MD, PhD,†¶ and Johannes Gjerstad, PhD*#*

Objectives: Previous studies indicate that genetic variants in genes encoding proteins like matrix metalloproteinase (MMP) enzymes may affect degeneration of the intervertebral disk. One such genetic variant is a single nucleotide polymorphism insertion in the promoter region of the *MMP1* gene, that is, the MMP1 rs1799750 2G allele, which increases the MMP1 expression in vitro. In this study, we examine if the MMP1 rs1799750 2G allele might be associated with disk degeneration and clinical outcome after lumbar disk herniation.

Materials and Methods: A total of 260 patients with lumbar disk herniation and sciatic pain were included in this study and genotyped for the MMP1 rs1799750 2G allele.

Results: The present data showed no differences in the frequency of the MMP1 2G allele in patients recently diagnosed with disk herniation compared with healthy controls. Moreover, in the patients, the MMP1 2G allele was not directly related to the disk degeneration. However, our data demonstrated that the MMP1 2G allele was associated with both pain and disability, that is, increased visual analog scale score, McGill pain questionnaire score, and Oswestry Disability Index score. Clearly, the patients homozygous for the 2G allele had more pain and reduced function compared with those carrying the 1G allele.

Discussions: Our findings suggest that the MMP1 rs1799750 2G/2G genotype may contribute to low back pain, sciatica, and disability after lumbar disk herniation.

Key Words: matrix metalloproteinase-1 (MMP1), single nucleotide polymorphism (SNP), disk herniation, sciatica, low back pain

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Discogenic low back pain and sciatica affects about 5% of the population and is one of the leading causes of work absenteeism and disability pensioning.[1,2] Although the etiology of disk degeneration and its clinical symptoms is poorly understood, genetic susceptibility may be important.[3,4] Previous studies have suggested that genetic variants in genes encoding proteins like matrix metalloproteinase (MMP) enzymes, affecting the extracellular matrix of the intervertebral disk, may contribute to the development of degenerative disk disease (DDD) and subsequent clinical symptoms.[5,6]

The most abundant component of the extracellular matrix of the intervertebral disk is the collagen fibers. These fibers provide tissue tension. In particular, the annulus fibrosus and nucleus pulposus are rich in type I and II fibrillar collagen.[7] MMP1 is the main collagenase that degrades these collagen fibers. Hence, degradation of the extracellular matrix of the intervertebral disk by MMP1 collagenase may promote disk degeneration leading to discogenic low back pain and sciatica.

Under normal physiological conditions, the expression of MMPs is low to ensure stabile tissue turnover. During pathologic disk degeneration, however, this balance is disrupted and matrix catabolism increases.[8] Thus, genetic variants within genes encoding MMP enzymes may influence MMP expression and thereby lead to matrix degradation. One genetic variant that appears to be important is a single nucleotide polymorphism (SNP) at position −1607 in the MMP1 promoter region (rs1799750). This SNP, which causes a guanine insertion/deletion, has been shown to increase MMP1 expression and matrix degradation.[9] The insertion of guanine creates a binding site for Ets transcription factors adjacent to the activating protein-1 site, promoting increased MMP1 transcription. In fact, the guanine insertion (2G) variant has been shown to enhance transcription by as much as 30-folds in vitro.[9]

Recent data show that this functional SNP may be associated with pain conditions like temporomandibular joint disorder (TMJD).[10] No previous studies have, however, addressed the relationship between the MMP1 rs1799750 SNP and pain and disability after disk herniation. Our hypothesis was that the MMP1 rs1799750 SNP might contribute to individual differences in low back pain, sciatica, and disability after lumbar disk herniation.

MATERIALS AND METHODS

Patients

A total of 260 patients with lumbar disk herniation and sciatic pain, all European-white, were recruited from Oslo University Hospital, Ullevaal, Norway and Haukeland University Hospital, Norway during the period from 2007 to 2009. The number of drop outs was 24 (9%).

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From the *National Institute of Occupational Health; #Department of Molecular Biosciences; ¶Faculty of Medicine, University of Oslo; †Department of Physical Medicine and Rehabilitation, Oslo University Hospital, Ullevaal; Departments of ‡Anesthesiology and Intensive Care; §Radiology, Haukeland University Hospital, Oslo; and ‖Department of Surgical Sciences, University of Bergen, Bergen, Norway.
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Reprints: Line M. Jacobsen, PhD, MSc, National Institute of Occupational Health, Pb 8149 Dep., Oslo 0033, Norway (e-mail: line.jacobsen@stami.no).

Inclusion criteria were: age between 18 and 60 years, lumbar disk herniation on magnetic resonance imaging (MRI) with corresponding sciatica pain, and positive straight leg raising test. Exclusion criteria were: lumbar spinal stenosis, previous surgery for herniated disk at the same level or fusion at any level in lumbar spine, generalized musculoskeletal pain, inflammatory rheumatic disease, diabetic polyneuropathy, cardiovascular disease (NYHA III and IV), cancer, psychiatric disease, alcohol or drug abuse, completion of another surgery within 1 month, pregnancy, poor DNA quality on blood sample, non-European-white ethnicity, or poor Norwegian language. For patient-control comparison, patients were matched (1:1) regarding age, sex, and smoking status with pain-free patients without a history of back disease collected from the general health survey Nord-Trøndelag Health Study (HUNT)—a population with <3% nonwhites.[11] For characteristics of patients/controls, see Tables 1 and 2.

The study was approved by the Norwegian Regional Committee for Medical Research Ethics and the Norwegian Social Science Data Services. Patients were included after signing a written informed consent and were told that they could withdraw from the study at any time without giving any reason.

Clinical Procedure

The newly diagnosed patients were followed up at 6 weeks and 12 months after inclusion: 54% received conservative treatment and 46% had surgical treatment. Conservative treatment involved medication, activity guidance in the acute phase of sciatica, and advices and physical therapy for back muscles in case of spontaneous regression of sciatica. Surgical treatment was given to patients with severe radiating pain and the majority of these patients underwent microdiscectomy. At inclusion, all patients underwent a standardized neurological examination including assessment of sensory and motor function and tendon reflexes of the lower limbs and MRI. At 6 weeks and 12 months of follow-up, the neurological examination was repeated. If their pain was persistent at 12-month follow-up, MRI was repeated.

DNA Extraction and SNP Genotyping

Genomic DNA was extracted from whole blood cells using a commercial DNA isolation kit (Qiagen, Hilden, Germany). SNP genotyping was carried out using a predesigned TaqMan assay (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations. Approximately 10 ng genomic DNA was amplified in a 5 μL reaction mixture in a 384-well plate containing 1× universal TaqMan master mix and 1× assay mix, the latter containing the respective primers and MGB-probes. The probes were labeled with the reporter dye FAM or VIC to distinguish between the 2 alleles. After initial denaturation and enzyme activation at 95°C for 10 minutes, the reaction mixture was subjected to 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. As previously described, the reactions were performed on an ABI 7900HT sequence detection system.[12,13] Negative controls were included in every run. Genotypes were determined using the SDS 2.2 software (Applied Biosystems). In 3 patient samples and 7 HUNT control samples, the genotypes could not be determined and they were therefore excluded from the analysis. Genotyping quality was tested by regenotyping at least 10% of the samples and the concordance rate was 100%.

TABLE 1. Characteristics of Patients and Controls

	Patients	Controls
Mean age (minimum-maximum)	41 (18-60)	41 (19-60)
Sex, male/female (%)	137/120 (53/47)	131/122 (52/48)
Current smoker, yes/no (%)	93/164 (36/64)	91/162 (36/64)

TABLE 2. Characteristics of Patients Grouped by MMP1 rs1799750 Genotype

	1G/1G + 1G/2G	2G/2G	Group Differences (*P*)
Mean age (minimum-maximum)	41 (18-60)	41 (20-59)	0.657*
Sex, male/female (%)	108/92 (54/46)	29/28 (51/49)	0.677†
Current smoker, yes/no (%)	76/124 (38/62)	17/40 (30/70)	0.247†
Treatment, conservative/surgery (%)	105/95 (52/48)	34/23 (60/40)	0.339†

*Students *t* test.
†Pearson χ^2.

Outcome Measures

The degree of disk degeneration at each lumbar level on the MRI was graded on the basis of Schneiderman's classification (0 to 3); established by Schneiderman et al[14] and revised by Jim et al.[15] All the MRI scans were rated by 2 independent experienced physicians blinded to the clinical history and genetic results. Differences were reviewed by both the physicians and settled by consensus. A total DDD score was calculated by summation of the scores for each of the 5 lumbar levels. All patients were asked to rate their pain intensity in rest during the last week on a 10-cm visual analog scale (VAS) with end points, "no pain" and "worst possible pain." The validated Norwegian version of McGill pain questionnaire (MPQ) was used to measure the total components of the pain experience.[16] The validated Norwegian version of Oswestry Disability Index (ODI) was used to assess problems with physical function related to low back pain, 10 items scored on a 6-point Likert scale.[17]

Protocol

First, to determine how common the MMP1 rs1799750 SNP was in the disk herniation patients versus the healthy Norwegian population, we compared the frequency of the MMP1 SNP among the newly diagnosed patients and healthy, pain-free controls from the general health survey HUNT. Next, to explore if this SNP was associated with degeneration of the disk at the time the patients were referred to the hospitals, the degree of disk degeneration at inclusion was examined with regard to MMP1 genotypes. Finally, we investigated if this SNP might contribute to individual differences in pain and disability development.

Statistical Analysis

All data are shown as mean ± SEM. MMP1 genotypes were grouped into 1G/1G + 1G/2G and 2G/2G. No deviation from Hardy-Weinberg equilibrium was observed

in the control group. First, the frequencies of the MMP1 genotypes in patients versus controls were analyzed using a χ^2 test, whereas the degree of disk degeneration at inclusion was compared regarding the genotype status by a Student *t* test. Next, VAS score, MPQ score, and ODI measurements over time were compared regarding MMP1 genotypes by repeated measure analysis of variance (ANOVA) (within-patients/between-patients effects). Missing values were replaced for the repeated measure ANOVA (series mean). When sphericity assumption was not met, a Greenhouse-Geisser correction was applied. Potential confounding effects of the covariates age, sex, smoking status, and treatment were checked for and statistically significant covariates were kept in the final repeated measure ANOVA (Table 3). Finally, post hoc comparisons at each time point were performed by Student *t* tests. Statistical analyses were performed using the SPSS (version 17) statistical package (SPSS Inc., Chichago, IL). A *P*-value >0.05 was chosen as the level of statistical significance.

RESULTS

The data showed no clear differences in the frequency of the MMP1 2G allele in the recently diagnosed patients versus healthy controls (Table 4). Moreover, the MRI data at inclusion, graded by Schneiderman's classification, showed that this SNP was not directly related to the total disk degeneration; mean total DDD (minimum-maximum) were 6 (2 to 11) in the 1G/1G + 1G/2G genotype group when compared with 5.9 (2 to 11) for the 2G/2G genotype group (Fig. 1). Thus, we did not show any relationship between MMP1 genotype and disk degeneration. However, the analyses demonstrated that the MMP1 2G allele was important with regard to the clinical outcomes. Interestingly, the present findings showed that the MMP1 2G allele was associated with a significant increase in low back pain, sciatica, and disability after lumbar disk herniation (VAS score $P = 0.003$, MPQ score $P = 0.001$, ODI score $P = 0.030$, between-patients, and repeated measures ANOVA) (Fig. 2). No significant associations were, however, found for the clinical outcomes over time, that is, from inclusion to 12 months, with regard to the MMP1 genotype (VAS score $P = 0.210$, MPQ score $P = 0.210$, ODI score $P = 0.087$, within-patients, and repeated measures ANOVA). Thus, although there were no differences over time within the genotype groups, the 2G patients had higher VAS/MPQ/ODI scores than the other patients. Post hoc comparisons revealed significant differences among the genotype groups for VAS and MPQ at 6 weeks and 12 months and for ODI at 12 months (Table 5). No clear sex-dependent relationship between MMP1 genotype and clinical outcomes were found in the present study.

TABLE 3. Significance of Covariates in Repeated Measure ANOVA

	Covariates	Within-Patients Effect (*P*)	Between-Patients Effect (*P*)	Included in Final Analysis
VAS	Age	0.132	0.607	No
	Sex	0.854	0.757	No
	Smoking	0.995	0.015	Yes
	Treatment	0.000	0.051	Yes
MPQ	Age	0.181	0.159	No
	Sex	0.641	0.969	No
	Smoking	0.497	0.008	Yes
	Treatment	0.002	0.975	Yes
ODI	Age	0.152	0.008	Yes
	Sex	0.876	0.168	No
	Smoking	0.824	0.022	Yes
	Treatment	0.000	0.072	Yes

MPQ indicates McGill pain questionnaire; ODI, Oswestry Disability Index; VAS, visual analog scale.

TABLE 4. MMP1 rs1799750 Genotype Distribution

	1G/1G + 1G/2G	2G/2G	*P**
Patients	200	57	0.819
Controls	199	54	

*Pearson χ^2.

DISCUSSION

For the first time, we present data demonstrating that the MMP1 rs1799750 SNP, which increases the expression of MMP1 and thereby promote degradation of the extracellular matrix of the intervertebral disk, is associated with increased low back pain, sciatica, and disability after lumbar disk herniation.

Multiple factors may, however, contribute to the degeneration of the disk and subsequent development of low back pain and sciatica. These factors may include age-related changes and the history of lifetime physical activity.[18] Nevertheless, genetics are assumed to play a partial role in this pathogenesis.[3,4] This might involve genes affecting the structure of the disk, genes affecting the inflammatory process after disk herniation, or genes affecting the interindividual nociceptive modulation important for the pain experience.

Matrix degradation is assumed to be important for the process leading to disk degeneration, but also the proinflammatory process after herniation, which could explain the more pronounced pain experience reported by the patients homozygous for the 2G allele. Moreover, matrix degradation has previously been linked to painful tissue

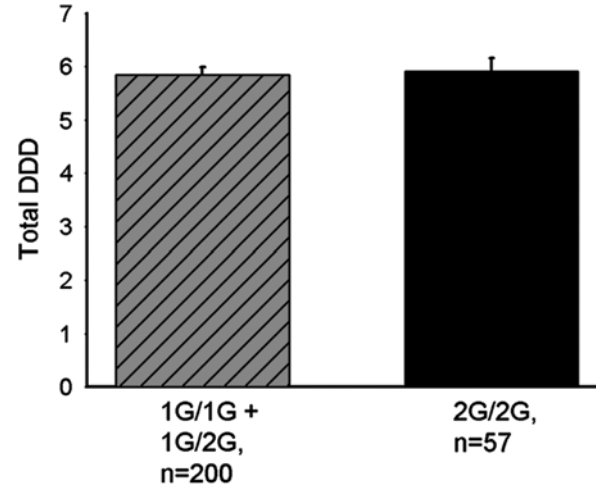

FIGURE 1. Total degenerative disk disease (DDD) score grouped by MMP1 genotypes. Data are given as mean ± SEM.

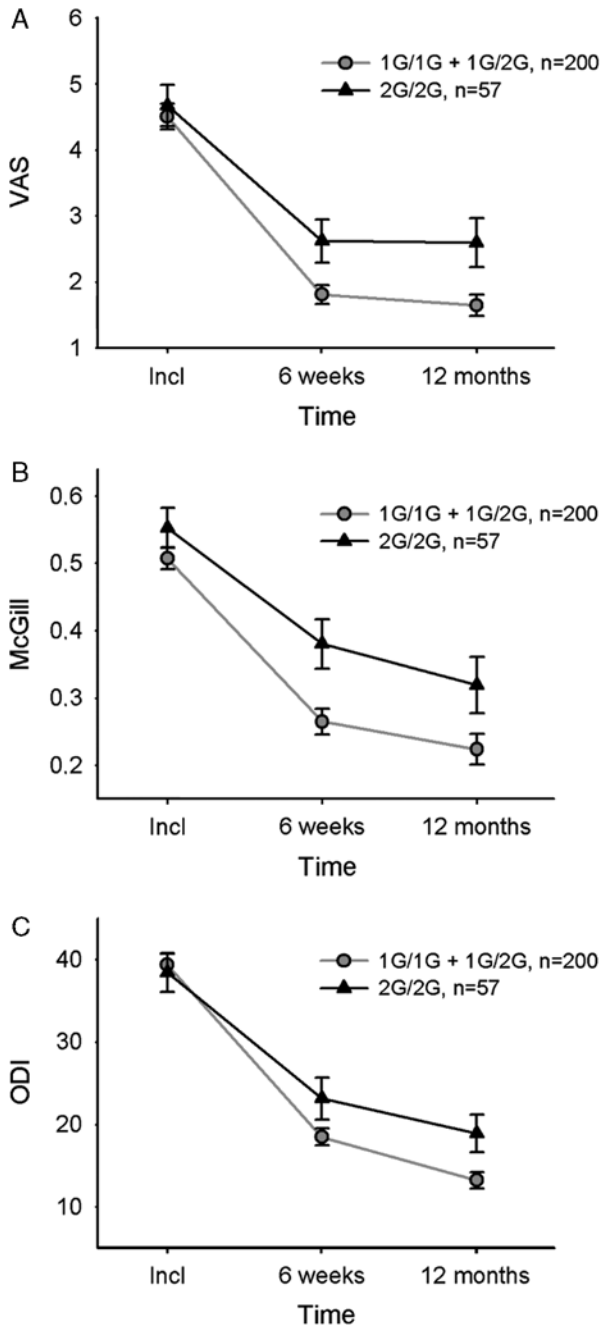

FIGURE 2. Pain and disability following disc herniation grouped by MMP1 genotypes. A, Visual analog scale (VAS) score over time; (B) McGill pain questionnaire (MPQ) score over time; (C) Oswestry Disability Index (ODI) score over time. Data are given as mean ± SEM.

degenerative diseases such as arthritis and neuropathic pain.[19–21] In line with these findings, our data indicate that the MMP1 genotype could affect the development of persistent low back pain and sciatica as well. MMP inhibitors are now also in clinical trials for the treatment of neuropathic pain and multiple sclerosis.[19]

Previous studies have shown that MMP1 degrades collagens but may also degrade aggrecans.[22] Therefore, expression of the *MMP1* gene is assumed to promote tissue degeneration and might also cause pain. Consistent with these data, the MMP1 2G allele has earlier been associated with painful degenerative inflammatory conditions such as TMJD and periodontitis.[10,23,24] However, we did not show any link between MMP1 2G allele and changes visible on MRI. Notably, the 2G allele has also been suggested to protect against disk degeneration in an Asian study population.[7]

TABLE 5. Outcome Comparisons Regarding MMP1 rs1799750 Genotype at Each Time Point

	VAS (*P*)	MPQ (*P*)	ODI (*P*)
Inclusion	0.645	0.182	0.737
6 wk	0.004	0.001	0.032
12 mo	0.004	0.005	0.005

Bonferroni corrected level of significance: $P \leq 0.016$.
MPQ indicates McGill pain questionnaire; ODI, Oswestry Disability Index; VAS, visual analog scale.

Taken together, these data show that the relationship between genetics, degenerative diseases, and clinical outcome may be complicated. Moreover, the divergent results might be caused by the fact that this MMP1 SNP generally displays large ethnic variations; previously reported 2G allele frequencies in white population have been 47% when compared with 69% in Asians.[7,25,26] Hence, the data from the Asian population cannot be directly compared with the data from our European-white population, where we observed that the 2G/2G genotype was associated with poorer pain prognosis.

It has recently been suggested that β-estradiol affect MMP1 expression through the relaxin-signaling pathway in joint fibrocartilage of TMJD, accompanied by loss of collagen.[27] Moreover, earlier data show that women are more prone to developing temporomandibular pain and degeneration, indicating that the effects of MMP1 might be linked to sex.[28] Therefore, it might be possible that the effect of the 2G allele of the MMP1 SNP partly might be sex dependent. However, when examining our outcome variables, that is, VAS, MPQ, and ODI, no sex-dependent relationship between MMP1 genotypes and clinical outcomes were found in the present study.

In conclusion, our findings suggest that the MMP1 2G/2G genotype may contribute to low back pain, sciatica, and disability after lumbar disk herniation.

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The DQB1*03:02 HLA haplotype is associated with increased risk of chronic pain after inguinal hernia surgery and lumbar disc herniation

Cecilia A. Dominguez [a,*], Maija Kalliomäki [b,c], Ulf Gunnarsson [d], Aurora Moen [e,f], Gabriel Sandblom [d], Ingrid Kockum [a], Ewa Lavant [g], Tomas Olsson [a], Fred Nyberg [h], Lars Jørgen Rygh [i], Cecilie Røe [j], Johannes Gjerstad [e,f,1], Torsten Gordh [b,1], Fredrik Piehl [a,1]

[a] Neuroimmunology Unit, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
[b] Department of Surgical Sciences, Anaesthesiology/Pain research, Uppsala University, Uppsala, Sweden
[c] Department of Anaesthesiology, University of Tammerfors, Tampere, Finland
[d] Department of Clinical Science, Intervention and Technology (Surgery), Karolinska Institutet, Huddinge, Sweden
[e] National Institute of Occupational Health, Oslo, Norway
[f] Department of Molecular Biosciences, University of Oslo, Norway
[g] Department of Biomedical Laboratory Science, Faculty of Health and Society, Malmö University/Labmedicine Skåne, Clinical Chemistry, Malmö, Sweden
[h] Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden
[i] Department of Anesthesiology and Intensive Care, Haukeland University Hospital, Bergen, Norway
[j] Department of Physical Medicine and Rehabilitation, Oslo University Hospital, Ullevaal, Norway

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Lumbar disc hernia

ABSTRACT

Neuropathic pain conditions are common after nerve injuries and are suggested to be regulated in part by genetic factors. We have previously demonstrated a strong genetic influence of the rat major histocompatibility complex on development of neuropathic pain behavior after peripheral nerve injury. In order to study if the corresponding human leukocyte antigen complex (HLA) also influences susceptibility to pain, we performed an association study in patients that had undergone surgery for inguinal hernia ($n = 189$). One group had developed a chronic pain state following the surgical procedure, while the control group had undergone the same type of operation, without any persistent pain. HLA DRB1genotyping revealed a significantly increased proportion of patients in the pain group carrying DRB1*04 compared to patients in the pain-free group. Additional typing of the DQB1 gene further strengthened the association; carriers of the DQB1*03:02 allele together with DRB1*04 displayed an increased risk of postsurgery pain with an odds risk of 3.16 (1.61-6.22) compared to noncarriers. This finding was subsequently replicated in the clinical material of patients with lumbar disc herniation ($n = 258$), where carriers of the DQB1*03:02 allele displayed a slower recovery and increased pain. In conclusion, we here for the first time demonstrate that there is an HLA-dependent risk of developing pain after surgery or lumbar disc herniation; mediated by the DRB1*04 – DQB1*03:02 haplotype. Further experimental and clinical studies are needed to fine-map the HLA effect and to address underlying mechanisms.

1. Introduction

Persistent pain following surgery is a relatively common phenomenon that affects, to some degree, about 20% of the population that undergoes surgical procedures [23]. Most of the patients suffer neuropathic pain, a phenomenon characterized by areas of sensory loss, in combination with allodynia and hyperalgesia [22,23]. The underlying mechanisms leading to neuropathic pain are still obscure, and large individual differences in the susceptibility and perception of pain make it complex to study. However, both clinical and preclinical studies have demonstrated that some of these individual differences are mediated by genetic heterogeneity [5,8,27,34,35]. Similar mechanisms may be of relevance also in persistent pain after lumbar disc herniation.

In a previous study we demonstrated that the rat major histocompatibility complex (MHC; also called RT1 in the rat) regulates the degree of neuropathic pain-like behaviour after nerve injury induced by an ischemic sciatic nerve lesion [10]. Inbred Dark-Agouti

* Corresponding author. Address: Neuroimmunology Unit, CMM L8:05, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden. Tel.: +46 8 51776041; fax: +46 8 51770885.
E-mail address: cecilia.dominguez@ki.se (C.A. Dominguez).
[1] These authors contributed equally to this work.

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(DA) rats were found to display only a short period of pain-like behaviour after nerve lesion, whereas inbred Piebald Virol Glaxo (PVG) rats demonstrated increased pain sensitivity for many weeks. By studying MHC congenic strains on these 2 strain backgrounds as well as an F2 intercross between PVG and congenic PVG rats carrying the MHC of the DA strain, a major part of this strain difference could be explained by differences in the MHC complex.

The MHC comprises more than 200 genes, many of which are related to important immune functions such as antigen presentation. Activation of the immune system may be involved in the development and maintenance of pain through activation of glial cells and by the release of proinflammatory mediators. Prior studies have found an association between the MHC, such as human leukocyte antigen (HLA) in man, and certain chronic pain conditions [2,7,24,39]. Moreover, earlier data show that persistent postoperative pain after surgery for inguinal hernia [1,3,6,21] is observed in a sub-group of 5–20% of patients. The pain mechanism in this sub-group with long-lasting pain has been identified to mainly be neuropathic, with allodynia and hyperalgesia in the inner part of thighs or groin, presumably due to more or less unavoidable lesions to sensory nerves during surgical procedures [22].

The aim of the present study was to examine whether the HLA is associated with risk of developing a neuropathic pain condition. The main part of this study was based on inguinal surgery patients selected from the Swedish Hernia Register that collects information from patients operated on for inguinal hernia in the county of Uppsala. The patients were divided into one pain-free group and one containing patients that had developed long-lasting pain after the same type of surgical procedure. In this material we demonstrate that individuals carrying the HLA haplotype DRB1*04 – DQB1*03:02 have increased risk of developing persistent postoperative pain after inguinal surgery. The association of DQB1*03:02 to increased pain is then replicated in a separate cohort of patients from Norway with lumbar disc herniation.

2. Materials and methods

2.1. Study population

Inguinal hernia cohort: 200 individuals were initially invited to participate in the study by Kalliomäki et al., 2009 [22] and were selected from the Swedish Hernia Register based on results from a pain questionnaire previously reported [22].The included patients (aged 21-85 years) had undergone inguinal hernia surgery during the period 1998-2004 and had answered the Ingual Pain Questionnnaire 6 months after the surgery, as well as undergone a clinical examination by a physician specializing in pain medicine [21]. Due to reasons such as incoherent answers in the questionnaire and/or the clinical examination, withdrawn consent, or development of new inguinal hernias, a total of 11 patients were excluded, leaving 189 patients (179 male, 10 female) for the association study, of which 94 had developed long-lasting pain, and matching numbers, 95 were pain-free when investigated at least 3 years following surgery. For further details about the patients, see Kalliomäki et al. [22]. HLA DRB1, DQA1, and DQB1 allele status was compared to a control population selected from the Swedish national population register for a multiple sclerosis (MS) incidence study ($n = 213$) [16] and the Diabetes Incidence Study in Sweden ($n = 423$) [13,19]. The MS controls had to answer a standardized questionnaire by mail or telephone.

The replication cohort consisted of patients with lumbar disc herniation that has been described in detail previously [37]. In brief, patients aged 18–60 years with lumbar disc herniation verifyed by magnetic resonance imaging (MRI) and sciatic pain were recruited from Oslo University Hospital and Haukeland University Hospital, Norway, during the period 2007–2009. A total of 258 patients were initially recruited, 6 of which decided not to participate at inclusion, and 21 were lost during follow-up. At inclusion, all patients underwent a standardized clinical examination with assessment of sensory and motor functions and tendon reflexes of the lower limbs as well as an MRI scan. At 6 weeks and 12 months the clinical examination was repeated, while the 6-month follow-up consisted of a telephone interview and a written questionnaire. Forty-two percent had conservative treatment and the remaining underwent surgery. Pain ratings consisted of the visual analogue scale (VAS) and the validated Norwegian version of the McGill questionnaire [41]. The validated Norwegian version of the Oswestry Disability Index (ODI) [15] was used to assess problems with physical function related to low back pain.

In both cohorts the sampling of clinical data was completed before the genotyping of the patients was performed. All included subjects had given informed consent and the study procedures were approved by the regional ethics board for research (Regionala Etikprövningsnämnden and the Norwegian Regional Committee for Medical Research Ethics and the Norwegian Social Science Data Services, respectively).

2.2. HLA typing

Genomic DNA was extracted from blood samples and genotyped for HLA-DRB1 with a sequence-specific primer (SSP) DR low-resolution kit by allele-specific polymerase chain reaction (PCR) amplification (PCR-SSP) [36]. Further typing of HLA-DQA1 and DQB1 was carried out by a method optimized for capillary electrophoresis as previously described [28], with the exception of a DNA concentration of 1 ng/μL and inclusion of a DQB1*06:02 specific primer. The control set selected from the Swedish national population register was genotyped for DRB1 as previously described [13]. The second set of controls from the Diabetes Incidence Study in Sweden was genotyped with PCR amplification followed by dot blot hybridizations for DQA1and DQB1 [13,19].

DQB1*03:02 status in the replication cohort was imputed from single-nucleotide polymorphisms (SNPs) tagging the DQB1*03:02 allele. Thus, we screened for SNPs in the MHC region on chromosome 6 that could be used for tagging the DQB1*03:02 allele using SNP markers run in the Human660-Quad Illumina chip in 440 Swedish controls and 515 MS cases in a genome-wide association study analysis of MS [40]. HLA genotypes had been imputed for DQB1 in this data set using the HLA*IMP software [9,29] as described [40]. We first screened for markers with high linkage disequilibrium (LD) (r^2) with DQB1*03:02; among these SNPs we choose SNPs that were available as TaqMan assays (Applied Biosystems, Foster City, CA, USA). Three SNPs were identified that, in combination, had both high sensitivity and specificity for DQB1*03:02 (Table 1).

The patients in the Norwegian cohort were genotyped for these 3 SNPs (rs927312, rs3916765, and rs2395185) using the TaqMan allelic discrimination method. As previously described [37], the probes were labelled with the reporter dye FAM or VIC (Applied Biosystems) to distinguish between the 2 alleles. The reactions were performed on an ABI 7900HT sequence detection system (Applied Biosystems). Negative controls containing water instead of DNA were included in every run. Genotypes were determined using the SDS 2.2 software (Applied Biosystems). Approximately 10% of the samples were re-genotyped and the concordance rate was 100%.

2.3. Statistics

The allele frequency in the Swedish cohorts was analyzed, and the difference in distribution between the groups was tested with χ^2 test and corrected for multiple comparisons with Bonferroni correction. Haldane correction was used when the allele frequency

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Table 1
Three SNPs were identified that in combination had both high sensitivity and specificity for DQB1*03:02 (rs927312, rs3916765 and rs2395185) and were used for genotyping of the Norwegian cohort using the TaqMan allelic discrimination method.

Imputed DQB1*03:02	RS9275312	RS3916765	RS2395185	Number of individuals	Tagged DQB1*03:02	Sensitivity	Specificity
POS POS	GG	AA	AA	8	POS POS	1.0000	1.0000
POS POS	GG	AG	AA	6	POS POS		
POS NEG	GA	AA	AC	6	POS NEG	0.9958	0.9945
POS NEG	GA	AG	AA	24	POS NEG		
POS NEG	GA	AG	AC	135	POS NEG		
POS NEG	GA	GG	AA	10	POS NEG		
POS NEG	GA	GG	AC	64	POS NEG		
POS NEG	AA	AG	CC	1	NEG NEG		
NEG NEG	AA	AG	AC	10	NEG NEG	0.9944	0.9961
NEG NEG	AA	AG	CC	37	NEG NEG		
NEG NEG	AA	GG	AA	16	NEG NEG		
NEG NEG	AA	GG	AC	147	NEG NEG		
NEG NEG	AA	GG	CC	419	NEG NEG		
NEG NEG	GA	AG	CC	4	NEG NEG		
NEG NEG	GA	GG	AC	14	NEG NEG		
NEG NEG	GA	GG	CC	58	NEG NEG		
NEG NEG	GG	GG	CC	2	NEG NEG		
NEG NEG	GA	AG	AC	3	POS NEG		
NEG NEG	GA	GG	AA	1	POS NEG		

SNP, single-nucleotide polymorphism; POS, positive; NEG, negative.

was 0. The correlation between DRB1*04 and DQB1*03:02 was analyzed with UNPHASED −3.0.13, using an expectation-maximization algorithm [11].

In the Norwegian cohort, VAS back pain score, McGill sensory score, and ODI measurements over time were compared regarding the DQB1*03:02 genotype by repeated-measures analysis of variance (rm ANOVA) and subsequent post hoc Student's *t*-test at 12 months. When sphericity assumption was not met, a Greenhouse-Geiser correction was applied in the ANOVA. As previously described [37], separate analyses were performed to check for potential effects of the covariates: age, smoking status, and treatment. Covariates with $P \leqslant 0.1$ were kept in the final rm ANOVA model. Statistical analyses were performed using the statistical package PASW statistics 18 (SPSS Inc, Chicago, IL, USA). A *P*-value <0.05 was chosen as the level of statistical significance.

3. Results

Initially, HLA DRB1 genotyping was performed. The distributions of detected alleles in the pain group and pain-free group after inguinal surgery are shown in Table 2. A statistically significant difference in the frequency of DRB1*04 alleles was detected, with an allele frequency of 24% in the pain group and 12% in the pain-free group ($P < 0.05$). The frequency of DRB1*04 alleles in the population control group was in between these 2 groups (19%), suggesting enrichment for DRB1*04 in the pain group and depletion in the pain-free group, respectively (Fig. 1).

Further genotyping of DQA1 and DQB1 showed a significantly increased proportion of DQB1*03:02 -positive individuals (20%) in the pain group ($P < 0.01$) compared to in the pain-free group (7%; Table 3). Also, in this instance the frequency of DQB1*03:02 in population controls (14%) was in between that found in the pain and pain-free groups, respectively (Fig. 2). No significant differences between groups were detected for DQA1 (data not shown).

An analysis of the frequency of DRB1*04 and DQB1*03:02 as homozygotes or heterozygotes demonstrates that carriage of one allele is enough to give a significant difference between the pain and pain-free group ($P < 0.001$, odds ratio = 3.08 for DRB1*04 heterozygotes; $P < 0.003$, odd ratio = 3.24 for DQB1*03:02 heterozygotes).

As expected, an LD analysis shows that DRB1*04 and DQB1*03:02 is in strong LD (D′ 1, $r^2 = 0.56$), and carriers of the DQB1*03:02 – DRB1*04 haplotype displayed increased risk of postsurgery pain with an odds ratio of 3.16 (1.61-6.22) compared to noncarriers. In an attempt to distinguish which of the 2 alleles is

Table 2
HLA-DRB1 allele frequency among pain and pain-free subjects.

HLA-DRB1	Pain		Pain-free		OR (95% CI)	*P*-value	P_c-value
	n	Allele frequency (%)	n	Allele frequency (%)			
01[a]	27	14	25	13	1.11 (0.62-1.99)	NS	NS
03[a]	19	10	33	17	0.53 (0.29-0.98)	NS	NS
04[a,b]	45[b]	24[b]	23[b]	12[b]	2.28 (1.32-3.96)[b]	0.004[b]	0.05[b]
07[a]	15	8	16	8	0.94 (0.45-1.97)	NS	NS
08[a]	11	6	4	2	2.89 (0.90-9.24)	NS	NS
09[a]	2	1	4	2	0.5 (0.09-2.76)	NS	NS
011[a]	3	2	7	4	0.42 (0.11-1.66)	NS	NS
012[a]	4	2	6	3	0.67 (0.19-2.40)	NS	NS
013[a]	28	15	27	14	1.06 (0.60-1.87)	NS	NS
014[a]	7	4	6	3	1.19 (0.39-3.60)	NS	NS
015[a]	27	14	36	19	0.72 (0.42-1.24)	NS	NS
016[a]	0	0	3	2	0.25(0.03-2.25)[a]	NS	NS

HLA, human leukocyte antigen complex; OR, odds ratio; CI, confidence interval; NS, not significant; P_c, *P* values corrected for multiple testing.
[a] Haldane correction.
[b] Significant difference in allele frequency between pain and pain-free subjects. Analyzed with χ^2 test and corrected for multiple comparisons with Bonferroni posttest.

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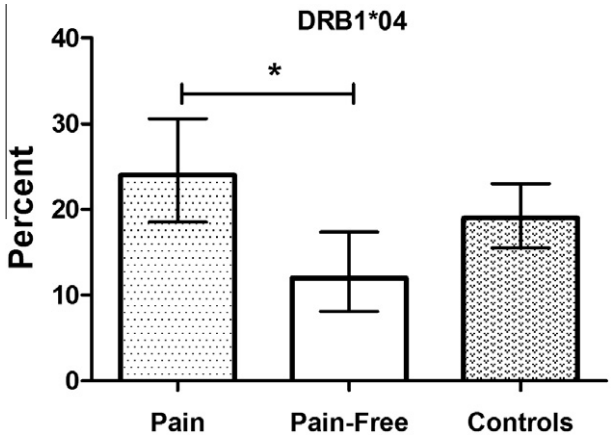

Fig. 1. DRB1*04 allele frequency in pain, pain-free, and control subjects. χ^2 test followed by correction for multiple comparisons with Bonferroni posttest indicates that there is a significant difference in DRB1*04 allele frequency between pain and pain-free subjects ($^*P > 0.05$). There was no significant difference between the control subjects and pain or pain-free subjects.

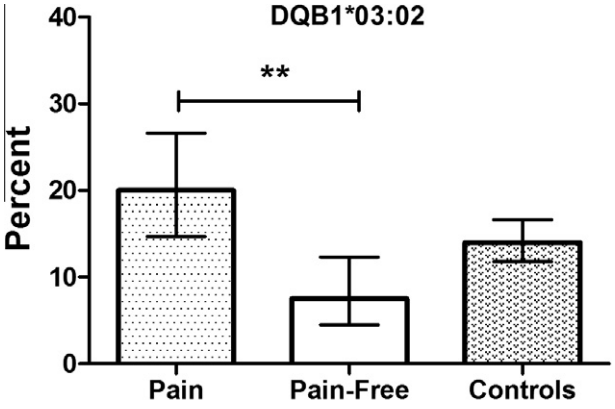

Fig. 2. DQB1*03:02 allele frequency in pain, pain-free, and control subjects. χ^2 test followed by correction for multiple comparisons with Bonferroni posttest indicates that there is a significant difference in DQB1*03:02 allele frequency between pain and pain-free subjects ($^{**}P > 0.01$). There was no significant difference between the control subjects and pain or pain-free subjects.

responsible for the genetic association, a stratification analysis was carried out. All individuals with DQB1*03:02 also had DRB1*04. However, in a minority of cases, DRB1*04 was associated with DQB1*03:01, but without significant differences in distribution between the pain and pain-free groups ($P > 0.98$), indicating that the association most likely is dependent on DQB1*03:02.

Next, in order to replicate the association between the DQB1*03:02 and persistent neuropathic pain, we determined DQB1*03:02 status in a well-characterized cohort of patients with sciatic pain due to MRI-verified lumbar disc herniation. The characteristics of the cohort stratified by DQB1*03:02 status are shown in Table 4. The frequency of DQB1*03:02 in the Norwegian patients (21%) corresponded to the frequency in the Swedish control materials (19%). In accordance with the data presented above, these analyses (Fig. 3) showed that the DQB1*03:02 */pos genotype, that is, carriers of at least one DQB1*03:02 allele, were associated with increased pain and slower functional recovery after lumbar disc herniation over the prospective follow-up period (VAS score $P = 0.018$, McGill sensory score $P = 0.362$, ODI score $P = 0.025$). Moreover, the DQB1*03:02 */pos genotype was also associated with a trend for more pain and statistical significance for reduced function at last follow-up, that is, at the 1-year study visit (VAS $P = 0.127$, McGill $P = 0.083$, and ODI $P = 0.046$). Mean ± SEM values at 12 months are listed in Table 5. An overview of the covariates is given in Table 6.

Table 4
Characteristics of lumbar disc hernia patients grouped by DQB1*03:02 status (DQB1*03:02-positive homo- and heterozygotes vs DQB1*03:02-negative patients).

	*/Pos $n = 52$	Neg/neg $n = 192$
Gender, men/women (%)	22/30 (42/58)	111/81 (58/42)
Mean age (min-max)	40 (19–59)	42 (22–60)
Current smoker, yes/no (%)	21/31 (40/60)	68/124 (35/65)
Treatment, surgery/conservative (%)	29/23 (56/44)	111/81 (58/42)

4. Discussion

The present study was carried out as an attempt to provide replication in the clinical setting of our previous experimental findings in rats, where the MHC was demonstrated to regulate neuropathic pain-like behavior after injury to the peripheral nervous system. In humans, the HLA is divided into 3 major classes, I, II and III, where the first 2 include genes encoding the class I and II molecules that are of vital importance for antigen presentation. In different animal models of neuropathic pain, an increase in MHC class II in the spinal cord after peripheral nerve lesions has been demonstrated, and mice lacking MHC class II exhibit decreased allodynia after peripheral nerve injury [4,38,42]. In addition, accumulating evidence suggests that adaptive immune reactions are involved in the regulation of pain [12,17,44]. However, it is still unclear if anti-

Table 3
HLA-DQB1 allele frequency among pain and pain-free subjects.

HLA-DQB1	Pain		Pain-free		OR (95% CI)	*P*-value	P_c-value
	n	Allele frequency (%)	n	Allele frequency (%)			
02[a]	26	15	38	22	0.64 (0.37-1.11)	NS	NS
04[a]	10	6	4	2	2.62 (0.81-8.53)	NS	NS
05[a]	29	17	33	19	0.87 (0.50-1.50)	NS	NS
03:01[a]	17	10	22	13	0.76 (0.39-1.48)	NS	NS
03:02[a,b]	35[b]	20[b]	13[b]	7[b]	3.16 (1.61-6.22)[b]	0.0009[b]	0.01
03:03[a]	8	5	9	5	0.89 (0.34-2.37)	NS	NS
03:04[a]	1	1	0	0	2.03(0.18-22.6)[a]	NS	NS
06:01[a]	4	2	0	0	5.52(0.60-44.8)[a]	NS	NS
06:02[a]	21	12	32	18	0.62 (0.34-1.12)	NS	NS
06:03[a]	14	8	17	10	0.82 (0.39-1.72)	NS	NS
06:04[a]	7	4	6	3	1.19 (0.39-3.61)	NS	NS

HLA, human leukocyte antigen complex; OR, odds ratio; CI, confidence interval; NS, not significant; P_c, *P* values corrected for multiple testing.
[a] Haldane correction.
[b] Significant difference in allele frequency between pain and pain free subjects. Analyzed with χ^2 test and corrected for multiple comparisons with Bonferroni posttest.

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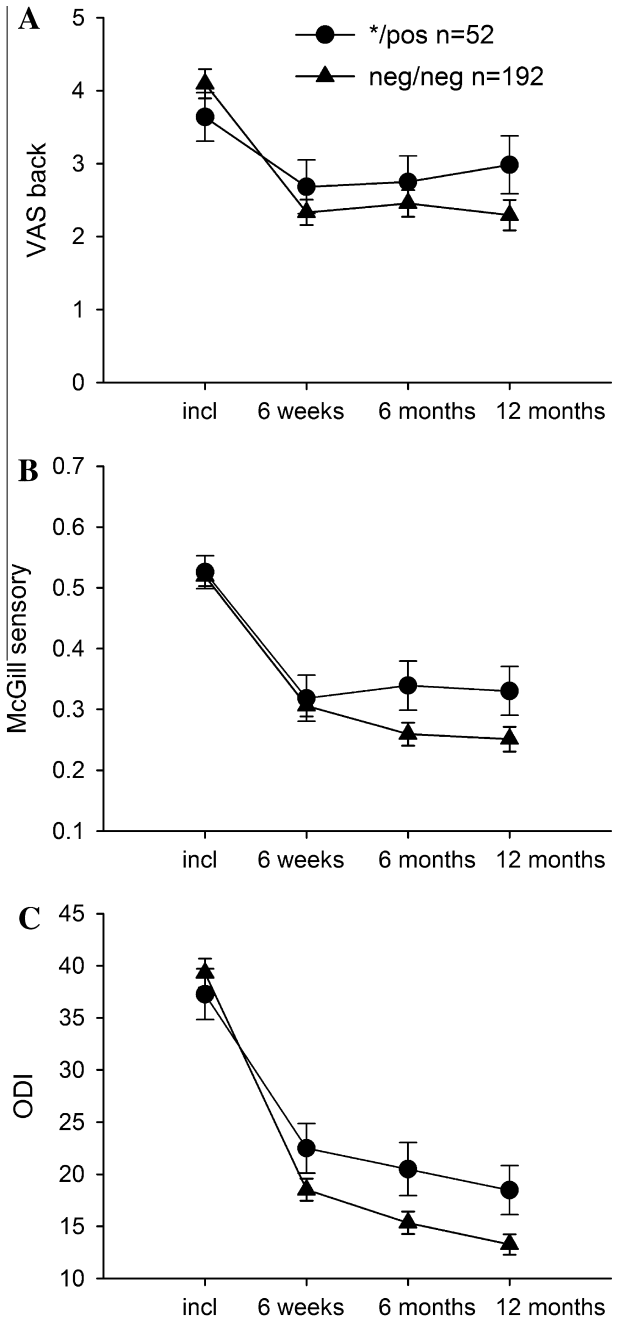

Fig. 3. The time course for outcome measures grouped by DQB1*03:02 status following disc herniation. (A) Visual analogue scale (VAS) back score ($P = 0.018$ within-subjects effect repeated-measures (rm) analysis of variance (ANOVA), $P = 0.127$ unpaired Student's t-test at 12 months); (B) McGill sensory score ($P = 0.362$ within-subjects effect rm ANOVA, $P = 0.083$, unpaired Student's t-test at 12 months). (C) Oswestry Disability Index (ODI) score ($P = 0.025$ within-subjects effect rm ANOVA, $P = 0.046$ unpaired Student's t-test at 12 months). Data are given as means ± SEM.

Table 5
Pain and disability ratings at 12 months in DQB1*03:02-positive homo- and heterozygotes vs DQB1*03:02-negative patients.

	VAS back	McGill sensory	ODI
*/Positive	2.98 ± 0.40	0.33 ± 0.04	18.48 ± 2.37
Negative/negative	2.29 ± 0.21	0.25 ± 0.02	13.27 ± 0.98

Note: Pain ratings consisted of the visual analog scale (VAS) and the validated Norwegian version of the McGill questionnaire. The validated Norwegian version of the Oswestry Disability Index (ODI) reflects problems with physical function related to radiating low back pain. Mean ± SEM values are shown.

gen-dependent reactions are relevant in the setting of human disease [14,18,33,44]. MHC is also associated with a number of complex diseases like MS and rheumatoid arthritis, in which pain symptoms often arise. The disease regulatory effect in autoimmune diseases is believed mainly to be caused by variability in the class II region, but with additional influences from other MHC regions [30–32].

In humans, the genes within the HLA class II region are denoted HLA-DR, -DQ, and -DP; where the letter D indicates that they encode the class II genes, and R, Q, and P the family [25]. A few previous studies have addressed a possible genetic association of the HLA to different pain conditions, showing association both to HLA-DR and -DQ [2,7,24,39]. Initially, in the cohort studied here, DRB1 allele distribution was determined, showing a significant association for DRB1*04 to risk of pain after inguinal surgery with an odds ratio of 2.28. Interestingly, in one of the prior studies, DQB1*03:02 was associated with complex regional pain disorder in a Dutch cohort [7], which was replicated in a recent study from the same group [43]. We therefore determined DQB1 and DQA1 frequency in our material and found that DQB1*03:02 is associated with risk of pain after hernia surgery with an odds ratio of 3.16. No association was observed in DQA1. Further analysis showed that heterozygote carriage is enough to give association for the DRB1*04 – DQB1*03:02 haplotype. As expected from prior studies, we also found that the DRB1*04 and DQB1*03:02 are in strong LD. Interestingly, this HLA haplotype is known to be associated with autoimmune diseases such as type I diabetes [26]. In addition, a small prior study found association of DRB1*04 to fibromyalgia [2].

In most cases, genetic studies in human diseases require very large numbers of patients due to the low effect conferred by individual genes and heterogeneity of the clinical sample under study. Arguably, the situation is different when studying the HLA, which often is inherited en bloc, with strong LD between the genes, and where HLA-dependent effects in autoimmune disease initially were defined in relatively small cohorts of patients and controls (see, e.g., [20]). Also, in this study, well-characterized homogenous material of patients having undergone the same surgical procedure (open technique or laparoscopy) was studied. The pain mechanism relevant for long-lasting pain after inguinal surgery is believed to depend on the damage of sensory nerves during surgical procedures [22], making it a relevant correlate to our previous findings in the rat. Although the allelic variation is species specific, due to the fact that we do see an interaction of the MHC/HLA in both rat and humans, we believe that it is reasonable to speculate on a mechanism that is present across species.

In addition, to the best of our knowledge, development of inguinal hernia has not been shown to depend on HLA, thus implying that the DRB1*04 – DQB1*03:02 haplotype is associated with risk of postsurgery pain and not to inguinal hernia. The notion that DQB1*03:02 status indeed is a genetic determinant for neuropathic pain is also supported by our finding that neuropathic pain symptoms are more severe and long lasting in homo-/heterozygous DQB1*03:02-positive patients with sciatic neuralgia following lumbar disc herniation. Taken together, these observations suggest that the DQB1*03:02-mediated effect can be of more generic relevance in conditions of peripheral nerve injury.

In conclusion, we here provide strong evidence supporting the notion of an HLA-dependent effect on the risk for developing chronic pain after peripheral nerve injury, thus replicating our previous findings in the rat. We believe this finding to be of clinical importance since it may be used to stratify for risk in situations of elective procedures, but also that further mechanistic dissection may unravel pathways that can be targeted therapeutically. Thus, for example, early immune modulatory treatments in DQB1*03:02-positive patients may prove to be more effective than in patients lacking this genetic susceptibility marker.

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Table 6
Overview of covariates included in the statistical analysis and the 3 outcome measures: VAS back, McGill Sensory and ODI.

Covariates	Repeated-measures ANOVA		
	Within-subjects effects *P*-values	Between-subjects effects *P*-values	Included in final model Yes/no
VAS back			
Age	0.055	0.424	Yes
Gender	0.900	0.643	No
Smoking	0.402	0.054	Yes
Treatment	0.000	0.085	Yes
McGill sensory			
Age	0.488	0.264	No
Gender	0.090	0.254	Yes
Smoking	0.851	0.044	Yes
Treatment	0.000	0.020	Yes
ODI			
Age	0.195	0.045	Yes
Gender	0.766	0.325	No
Smoking	0.652	0.003	Yes
Treatment	0.000	0.611	Yes

VAS, visual analogue scale; ODI, Oswestry Disability Index; ANOVA, analysis of variance.
Covariates with a *P* value $\leqslant 0.1$ were included in the final model.

Conflicts of interest statement

The authors state that there are no conflicts of interests. The present work was supported by EXTRA funds from the Norwegian Foundation for Health and Rehabilitation and the Norwegian Research Council.

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ORIGINAL ARTICLE

The COMT rs4680 Met allele contributes to long-lasting low back pain, sciatica and disability after lumbar disc herniation

L.M. Jacobsen[1], E.I. Schistad[2], A. Storesund[3], L.M. Pedersen[1,2], L.J. Rygh[3], C. Røe[2,4], J. Gjerstad[1,5]

1 National Institute of Occupational Health, Oslo, Norway
2 Department of Physical Medicine and Rehabilitation, Oslo University Hospital, Ullevål, Norway
3 Department of Anesthesiology and Intensive Care, Haukeland University Hospital, Bergen, Norway
4 Faculty of Medicine, University of Oslo, Oslo, Norway
5 Department of Molecular Biosciences, University of Oslo, Oslo, Norway

Correspondence
Line Melå Jacobsen
E-mail: line.jacobsen@stami.no

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Abstract

Background: The COMT enzyme metabolizes catecholamines and thus modulates adrenergic, noradrenergic and dopaminergic signaling. A functional polymorphism in the gene encoding this enzyme, i.e. the COMT Val158Met SNP that reduces enzyme activity, has previously been linked to pain sensitivity.
Methods: We examined if the COMT Val158Met SNP could contribute to discogenic subacute low back pain and sciatica by comparing the frequency of the Val158Met genotypes of degenerative disc disease patients with healthy controls. Moreover, we examined if this SNP could predict the clinical outcome, i.e. the progression of pain and disability.
Results: The present data demonstrated that there were no differences in COMT genotype frequencies between the newly diagnosed patients and controls. Analysis of pain and disability in the patients over time revealed, however, a significant or border-line significant increase in McGill sensory score and Oswestry Disability Index (ODI) score for individuals with COMT Met/Met genotype. Furthermore, significant associations between the COMT Met-allele and VAS activity score, McGill sensory score and ODI score were observed in the patients 6 months after inclusion.
Discussion: Although the Val158Met SNP was not a risk factor for disc herniation, patients with Met/Met had more pain and slower recovery than those with Val/Met, which in turn also had more pain and slower recovery than those with Val/Val suggesting the SNP contributes to the progression of the symptoms of disc herniation.
Conclusion: We conclude that the functional COMT Val158Met SNP contributes to long lasting low back pain, sciatica and disability after lumbar disc herniation.

1. Introduction

Catechol-O-methyltransferase (COMT) is an enzyme that metabolizes catecholamines and thus modulates adrenergic, noradrenergic and dopaminergic signalling. The COMT gene contains the functional single-nucleotide polymorphism (SNP) rs4680, also known as COMT Val158Met that causes a substitution of valine (Val) to methionine (Met) at codon 158. The substitution affects enzyme activity where individuals homozygous for the Met allele have a 3–4 times reduced enzyme activity compared to those homozygous for the Val-allele (Lotta et al., 1995). The alleles are co-dominant so the Val/Met genotype shows an enzyme activity halfway between the homozygous genotypes (Baekken et al., 2008).

During the past decade, it has been shown that this functional SNP may be associated with fibromyalgia (Gursoy et al., 2003), migraine (Emin Erdal et al., 2001) as well as sensitivity to experimental pain (Zubieta et al., 2003; Diatchenko et al., 2006). These data and observations showing that haplotypes including this SNP have been associated with increased experimental pain as well as enhanced pain ratings after surgery, suggest that the genetic variability in the gene encoding COMT may be important for development of hyperalgesia (Diatchenko et al., 2006; George et al., 2008). Hence, it is a reason to believe that the COMT Val158Met SNP might affect nociceptive modulation and contribute to the development of persistent pain.

No previous studies have addressed the relationship of the COMT Val158Met SNP with development and progression of discogenic low back pain and sciatica. Therefore, we here compare the frequency of the COMT SNP rs4680 genotypes among newly diagnosed patients and healthy controls to examine whether this SNP could be a factor that contribute to discogenic subacute low back pain and sciatica. Moreover, to investigate whether this SNP could explain the clinical outcome regarding the progression of the pain and disability, we compared the scores of pain intensity and functional disability of the COMT SNP rs4680 genotypes.

2. Methods

2.1 Subjects

A total of 258 subjects with lumbar disc herniation and sciatic pain, all European-Caucasian, were recruited from Oslo University Hospital, Ullevaal, Norway and Haukeland University Hospital, Norway during the period of 2007–2009. The number of dropouts was 29 subjects (11%). Inclusion criteria were age between 18 and 60 years, lumbar disc herniation on magnetic resonance imaging (MRI) with corresponding sciatica pain and positive straight leg raising test. Exclusion criteria were lumbar spinal stenosis, previous surgery for herniated disc at the same level or fusion at any level in lumbar spine, generalized musculoskeletal pain, inflammatory rheumatic disease, diabetic polyneuropathy, cardiovascular disease (NYHA III and IV), cancer, psychiatric disease, alcohol or drug abuse, completion of another surgery within 1 month, pregnancy, poor DNA quality on blood sample, non-European-Caucasian ethnicity or poor Norwegian language. For case-control analysis, cases were matched (1:1) regarding age, gender and smoking status with pain-free subjects without a history of back disease collected from the general health survey Nord-Trøndelag Health Study (HUNT) – a population with less than 3% non-Caucasians (Holmen et al., 2003). For characteristics of cases/controls, see Tables 1 and 2. All participants received written information and signed an informed consent form. The study was approved by the Norwegian Regional Committee for Medical Research Ethics and the Norwegian Social Science Data Services.

Table 1 Characteristics of cases and controls.

	Cases	Controls
Mean age (min-max)	41 (18–60)	41 (19–60)
Sex, male/female (%)	138/120 (53/47)	129/120 (52/48)
Current smoker, yes/no (%)	94/164 (36/64)	92/157 (37/63)

2.2 DNA extraction and SNP genotyping

Genomic DNA was extracted from whole blood cells using a commercial DNA isolation kit (Qiagen, Hilden, Germany). SNP genotyping was carried out using a pre-designed TaqMan assay (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's recommendations. Approximately 10 ng genomic DNA was amplified in a 5 μL reaction mixture in a 384-well plate containing 1x universal TaqMan master mix and 1x assay mix, the latter containing the respective primers and MGB-probes. The probes were labeled with the reporter dye FAM or VIC to distinguish between the two alleles. After initial denaturation and enzyme activation at 95 °C for 10 min, the reaction mixture was subjected to 40 cycles of 95 °C

Table 2 Characteristics of cases grouped by COMT Val158Met genotypes.

	Val/Val	Val/Met	Met/Met
Mean age (min-max)	42 (20–59)	42 (18–59)	39 (19–60)
Sex, male/female (%)	28/31 (47/53)	60/51 (54/46)	50/38 (57/43)
Current smoker, yes/no (%)	25/34 (42/58)	39/72 (35/65)	31/57 (35/65)
Treatment, conservative/surgery (%)	28/31 (47/53)	68/43 (61/39)	45/43 (51/49)

for 15 s and 60 °C for 1 min. The reactions were performed on an ABI 7900HT sequence detection system. Negative controls were included in every run. Genotypes were determined using the SDS 2.2 software (Applied Biosystems, Foster City, CA, USA). In nine HUNT control samples, the genotypes could not be determined and they were therefore excluded from the analysis. Genotyping quality was tested by re-genotyping at least 10% of the samples and the concordance rate was 100%.

2.3 Clinical procedure

The newly diagnosed patients were after inclusion followed up at 6 weeks, 6 months and 12 months. Fifty-five percent received conservative treatment and 45% received surgical treatment. At inclusion, all patients underwent a standardized neurologic examination including assessment of sensory and motor function and tendon reflexes of the lower limbs as well as an MRI scan. At 6 weeks follow-up, the neurologic examination was repeated, while at 6 months follow-up, patients reported their back condition and work status by a telephone interview and answered a questionnaire by mail. At 12 months follow-up, patients underwent the same examination as by inclusion, and if their pain was persistent, an MRI scan was repeated.

2.4 Clinical measures

All patients were asked to rate their pain intensity in activity during the last week on a 10-cm visual analogue scale (VAS) with endpoints 'no pain' and 'worst possible pain'. The validated Norwegian version of McGill pain questionnaire was used to measure the sensory components of the pain experience (Strand and Wisnes, 1991). The validated Norwegian version of Oswestry Disability Index (ODI) was used to assess problems with physical function related to low back pain, 10 domains scored on a 6-point Likert scale with separate wording for each domain (Grotle et al., 2003).

2.5 Statistical analysis

The data are shown as means ± standard error of the mean (SEM). No deviation from the Hardy–Weinberg equilibrium was observed in the control group. First, the frequencies of the COMT genotypes in patients and controls were analyzed using a chi-square test. Next, VAS activity score, McGill sensory score and ODI measurements over time were compared regarding COMT genotypes Val/Val versus Met/Met by repeated measure analysis of variance (ANOVA). Missing values were replaced for the repeated measure ANOVA (series mean).When sphericity assumption was not met, a Greenhouse-Geisser correction was applied. Separate analyses were performed to check for potential confounding effects of the covariates age, gender, smoking status and treatment, respectively. Statistically significant covariates were kept in the final model. Finally, VAS activity score, McGill sensory score and ODI score 6 months after disc herniation were examined regarding all three COMT genotypes by a one-way ANOVA and Tukey honestly significant difference (HSD) post hoc comparisons. Statistical analyses were performed using the SPSS (version 17) statistical package (SPSS Inc, Chicago, IL, USA). A p-value less than 0.05 was chosen as the level of statistical significance.

Table 3 COMT Val158Met genotype distribution.

	Val/Val	Val/Met	Met/Met	p-value[a]
Cases (%)	59 (23)	111 (43)	88 (34)	0.143
Controls (%)	41 (17)	108 (43)	100 (40)	

[a]Pearson chi-square.

3. Results

The examination of the frequency of COMT genotypes in patients compared with controls showed that this SNP was not important for the occurrence of discogenic low back pain and sciatica at inclusion (Table 3).

Moreover, analysis of the clinical measures over time in the patients revealed no clear associations between VAS activity score and Met/Met versus Val/Val genotype, but a significant and borderline significant increase in McGill sensory score and ODI score was observed for individuals with Met/Met genotype (VAS activity score $p = 0.13$, McGill sensory score $p = 0.017$ and ODI score $p = 0.060$, repeated measures ANOVA, Met/Met vs. Val/Val; Fig. 1). Smoking status was a significant covariate in the analysis of VAS activity score and McGill sensory score, whereas age was a significant covariate in the analysis of ODI score. The repeated measure ANOVA also indicated an association between the clinical measures at 6 months after inclusion and Met/Met versus Val/Val genotype. Further analyses at this time point demonstrated significant associations between all three clinical measures and the COMT Met allele (VAS activity score $p = 0.028$, McGill sensory score $p = 0.023$, ODI score $p = 0.037$, one-way ANOVA, Met/Met, Val/Met and Val/Val; Fig. 1).

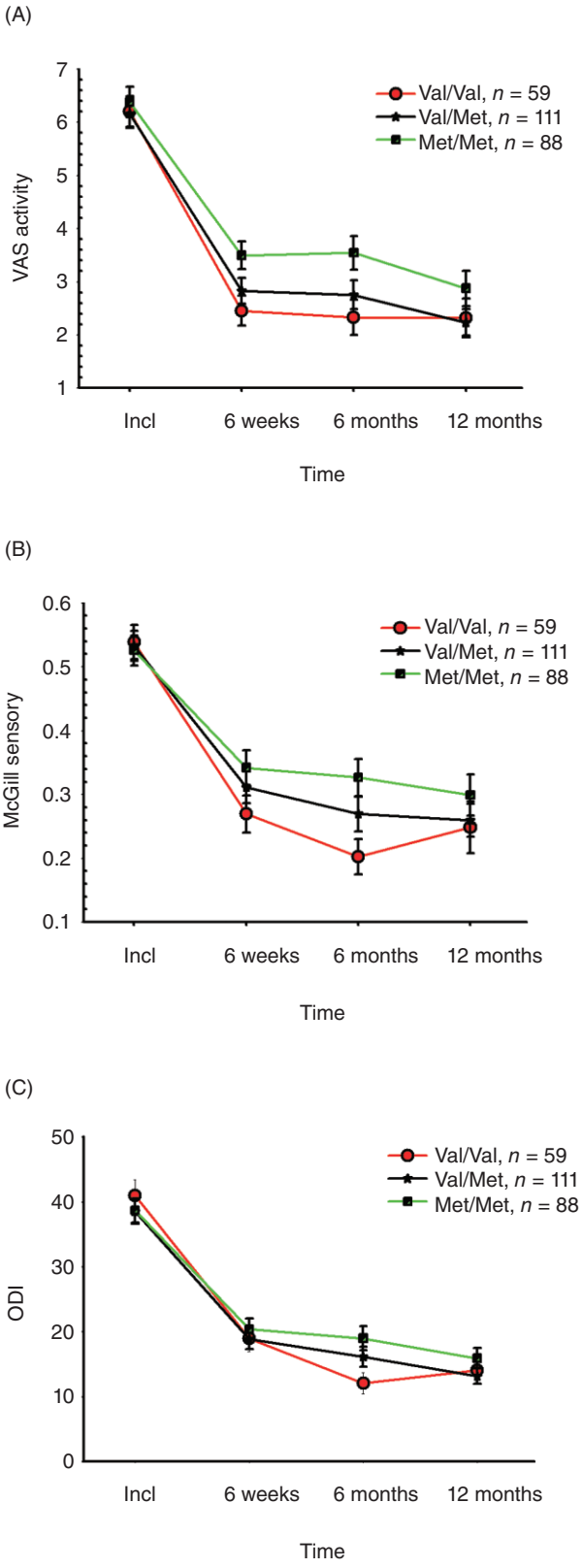

Figure 1 The time course for outcome measures grouped by genotypes Met/Met (low COMT activity), Val/Met (medium COMT activity) and Val/Val (high COMT activity) following disc herniation. (A) The time course of VAS activity score following disc herniation; (B) The time course of McGill sensory score following disc herniation; (C) The time course of ODI score following disc herniation. Data are given as means ± SEM.

The pain and disability after 6 months was allele dependent for all outcome measures where the Met/Met group (low COMT activity) reported relatively pronounced pain and disability, the Val/Met group reported a less-pronounced pain and disability and the Val/Val group (high COMT activity) reported the lowest pain and disability. The following means ± SEM outcome scores at 6 months were observed; VAS activity Met/Met ($n = 86$) 3.54 ± 0.32, Val/Met ($n = 100$) 2.75 ± 0.27 and Val/Val ($n = 55$) 2.33 ± 0.33, McGill sensory Met/Met ($n = 80$) 0.33 ± 0.03, Val/Met ($n = 94$) 0.27 ± 0.03 and Val/Val ($n = 48$) 0.20 ± 0.03 and ODI Met/Met ($n = 77$) 18.98 ± 1.83, Val/Met ($n = 94$) 16.13 ± 1.52 and Val/Val ($n = 49$) 12.08 ± 1.61.

In addition, post hoc comparisons also revealed significant differences between Met/Met and Val/Val genotypes for both VAS activity, McGill sensory and ODI at 6 months after inclusion (VAS activity score $p = 0.030$, McGill sensory score $p = 0.018$ and ODI score $p = 0.028$ between Met/Met and Val/Val genotypes, Tukey HSD).

4. Discussion

Degenerative disc disease characterized by disc herniation, low back pain and sciatica is a common, painful disorder affecting about 5% of the population and one of the leading causes for work disability (Heliovaara et al., 1987; Hansson and Hansson, 2007). Although the aetiology of disc herniation and clinical symptoms is poorly understood, genetic susceptibility may be one of the contributing factors (Battie et al., 1995; Hestbaek et al., 2004). The individual progression of the disease may also, at least in part, be explained by genetic polymorphisms.

Previous data have suggested that genetic polymorphisms in genes encoding inflammatory cytokines important for the inflammation possibly caused by disc herniation, may be associated with low back pain and sciatic pain (Noponen-Hietala et al., 2005; Karppinen et al., 2008). Moreover, long-lasting pain states is a multidimensional experience involving numerous components, not only stimulation of the spinal nerve roots. Here, we have presented data demonstrating

that the COMT Val158Met SNP may also be important for progression of discogenic low back pain and sciatic pain.

In the present study, we focused on the pain development and functional disability relevant for the clinical situation of the patients: VAS activity, McGill sensory and ODI. Our results indicated that although the Val158Met SNP is perhaps not a risk factor for disc herniation, it might contribute to the progression of the symptoms, i.e., pain and functional disability possibly occurring in the follow-up period. Hence, the presented data support the hypothesis that the COMT Val158Met SNP may affect nociceptive modulation and contribute to the development of persistent pain.

Multiple factors may contribute to the degeneration of a disc and the subsequent development of sciatic pain. Genetics are assumed to play a partial role in this pathogenesis (Battie et al., 1995; Hestbaek et al., 2004). This might involve genes affecting the structure of the discs, genes affecting the inflammation process after disc herniation, but also genes affecting the inter-individual nociceptive modulation important for the pain experience. The COMT gene is believed to affect nociceptive modulation due to the COMT enzyme's influence on epinephrine, norepinephrine and dopamine level and earlier data have shown that the Val/Val, Val/Met and Met/Met genotypes predict high, intermediate and low enzyme activity, respectively (Lotta et al., 1995).

The effect of reduced COMT enzyme activity on nociception has been studied in numerous animal and human pain models. Earlier animal data have demonstrated that low COMT activity increase peripheral pain sensitivity (Nackley et al., 2007). However, low COMT activity attenuated spinal nociceptive activity and central sensitization (Jacobsen et al., 2010). Thus, the effect of low COMT activity may be complex. Regarding the human pain models, these often report an association between Met alleles producing low enzyme activity and pain hypersensitivity (Zubieta et al., 2003; Diatchenko et al., 2006). In addition, previous associations between this SNP and clinical efficacy of opioid treatment for cancer pain have also been suggested (Rakvag et al., 2005; Reyes-Gibby et al., 2007). Still, the role of the COMT enzyme polymorphism is controversial (Kim et al., 2004; Klepstad et al., 2011) and previous studies have failed to find an association between the Val158Met SNP and persistent musculoskeletal complaints in the general population (Hagen et al., 2006). Moreover only a non-significant trend towards better improvement of VAS back pain and ODI after surgical treatment for disc disease patients with Met/Met genotype has been reported (Dai et al., 2010).

These discrepancies might be due to different pain models involving different mechanisms. Furthermore, the divergent effects of reduced COMT activity in different parts of the sensory system counteracting each other could also contribute to the conflicting findings in previous studies. Recent animal data, for example, have demonstrated that inhibition of COMT increases nocifensive responses through $\beta_{2/3}$-adrenoceptors highly expressed in peripheral tissue (Nackley et al., 2007), whereas we observed the opposite effect at the spinal level, i.e., reduced dorsal horn nociceptive signaling (Jacobsen et al., 2010). This might be due to the engagement of different receptor systems suggesting that the pronociceptive effect of low COMT activity in peripheral tissue partly may be counteracted by the antinociceptive effect in the spinal cord. Altogether, this illustrates the divergent effects catecholamines exert on nociception and pain sensitivity.

We conclude that the COMT Val158Met SNP affects nociceptive modulation and contributes to the progression of discogenic low back pain, sciatica and reduced function after disc herniation.

Author contributions

L.M.J. contributed to the study design, performed the data interpretation and analysis and wrote the manuscript. E.I.S. contributed to the study design, data acquisition, data interpretation and analysis and the drafting of the manuscript. A.S. contributed to the data acquisition and data analysis. L.M.P. contributed to the study design and data analysis. L.J.R. contributed to the study design, data interpretation and the drafting of the manuscript. C.R. contributed to the study design, data interpretation and the drafting of the manuscript. J.G. conceived the study, participated in the study design, data interpretation and analysis, the drafting of the manuscript and oversaw the execution of the project. All authors discussed the results and commented on the manuscript.

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Brief Communications

Pain Intensity the First Year after Lumbar Disc Herniation Is Associated with the A118G Polymorphism in the Opioid Receptor Mu 1 Gene: Evidence of a Sex and Genotype Interaction

Maria Belland Olsen,[1] Line Melå Jacobsen,[1] Elina Iordanova Schistad,[2] Linda Margareth Pedersen,[1,2] Lars Jørgen Rygh,[3] Cecilie Røe,[2] and Johannes Gjerstad[1,4]

[1]National Institute of Occupational Health, 0033 Oslo, Norway, [2]Department of Physical Medicine and Rehabilitation, Oslo University Hospital, Ullevaal, N-0424 Oslo, Norway, [3]Department of Anesthesiology and Intensive Care, Haukeland University Hospital, N-5021 Bergen, Norway, and [4]Department of Molecular Biosciences, University of Oslo, N-0316 Oslo, Norway

Earlier studies have shown that the single nucleotide polymorphism (SNP) A118G (rs1799971) in the opioid receptor mu 1 (OPRM1) gene may affect pain sensitivity. In the present study we investigated whether the A118G SNP could predict clinical outcome regarding progression of pain intensity and disability in patients with low back pain and sciatica after lumbar disc herniation. Patients ($n = 258$) with lumbar disc herniation and sciatic pain, all European-Caucasian, were recruited from two hospitals in Norway. Pain and disability were rated on a visual analog scale (VAS), by McGill Sensory Questionnaire and by Oswestry Disability Index (ODI) over a 12 months period. The data revealed a significant interaction between sex and A118G genotype regarding the pain intensity during the 12 months (VAS, $p = 0.002$; McGill, $p = 0.021$; ODI, $p = 0.205$, repeated-measures ANOVA). We found that */G women had a slower recovery rate than the */G men. Actually, the */G women had 2.3 times as much pain as the */G men 12 months after the disc herniation (VAS, $p = 0.043$, one-way ANOVA; $p = 0.035$, Tukey HSD). In contrast, the A/A women and A/A men seemed to have almost exactly the same recovery rate. The present data suggest that OPRM1 G allele increases the pain intensity in women, but has a protective effect in men the first year after disc herniation.

Introduction

Many factors may contribute to the development of low back pain and sciatica. These include age related changes, body weight, smoking and occupational loading (Miranda et al., 2002; Younes et al., 2006; Samartzis et al., 2011). Moreover, psychosocial aspects as well as genetic variability may affect the risk of long-term low back pain and sciatica (Jacobsen et al., 2012).

One important genetic factor that may increase the risk of persistent low back pain and sciatica is the single nucleotide polymorphism (SNP) A118G, rs1799971, in the opioid receptor mu 1 (OPRM1) gene. This SNP leads to a substitution of asparagine (Asn) to aspartic acid (Asp) at amino acid 40 and therefore removal of a putative *N*-linked glycosylation site in the receptor (Bergen et al., 1997; Bond et al., 1998). Recent data show that the equivalent A112G SNP in the brain of mice leads to reduced OPRM1 *N*-glycosylation and similarly that the human A118G SNP causes decreased *N*-glycosylation and reduced stability of the receptor in cell cultures (Huang et al., 2012).

Among individuals free of clinical pain it has been suggested that 118G allele carriers, in particular men, have higher pressure pain thresholds than 118A carriers (Fillingim et al., 2005). Carriers of the 118G allele may also have lower cortical responses to experimental pain stimuli (Lötsch et al., 2006). However, in contrast, the women carrying the 118G allele seem to report more pain than the women homozygous for the 118A the first 24 h after a cesarean operation (Sia et al., 2008; Tan et al., 2009). In addition, evidence exist that carriers of the OPRM1 118G allele may require higher doses of morphine in the early postoperative period (Klepstad et al., 2004; Chou et al., 2006; Hayashida et al., 2008).

Consistent with these findings, the effect of the opioid agonists have also been linked to sex and strain in animal experiments (Baamonde et al., 1989; Vendruscolo et al., 2004). Moreover, in mice, the OPRM1 G allele, depending on sex, may reduce μ-opioid receptor expression in some brain regions (Wang et al., 2012). In addition, earlier data suggest that the density of the μ-opioid receptor may be different in the male and female human brain (Zubieta et al., 1999). Hence, we hypothesized that the

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Correspondence should be addressed to Maria Belland Olsen, National Institute of Occupational Health, Pb 8149 Dep., 0033 Oslo, Norway. E-mail: maria.b.olsen@stami.no.

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Table 1. Characteristics of patients grouped by sex and OPRM1 A118G genotype

	Women */G ($n = 23$)	Men */G ($n = 41$)	Women A/A ($n = 94$)	Men A/A ($n = 94$)
Mean age (min-max)	43 (26–58)	41 (24–57)	41 (18–59)	41 (19–60)
Current smoker, yes/no (%)	9/14 (39/61)	13/28 (32/68)	31/63 (33/67)	39/55 (41/59)
Treatment, conservative/surgery (%)	9/14 (39/61)	18/23 (44/56)	44/50 (47/53)	35/59 (37/63)

Min, minimum; max, maximum.

Table 2. Significance of covariates

		Repeated-measures ANOVA		
Outcome measure	Covariates	Within-subjects effects, *p* values	Between-subjects effects, *p* values	Included in final model, yes/no
VAS	Age	0.844	0.002	Yes
	Smoking	0.697	0.924	No
	Treatment	0.000	0.250	Yes
McGill	Age	0.428	0.019	Yes
	Smoking	0.343	0.086	Yes
	Treatment	0.000	0.003	Yes
ODI	Age	0.417	0.003	Yes
	Smoking	0.070	0.150	Yes
	Treatment	0.000	0.150	Yes

The table gives an overview of the association between covariates and the three outcome measures: VAS, McGill, and ODI. Covariates with a p value ≤ 0.1 were included in the final model.

OPRM1 A118G SNP may have different effects in men and women as well. In the present study we demonstrate that the pain after lumbar disc herniation is dependent on a sex and OPRM1 A118G genotype interaction.

Materials and Methods

Subjects. Patients with lumbar disc herniation and sciatic pain were recruited from Oslo University Hospital, Ullevaal, Norway and Haukeland University Hospital, Norway, during the period of 2007–2009 (Table 1). Inclusion criteria were: age between 18 and 60 years, confirmed lumbar disc herniation by magnetic resonance imaging (MRI) with corresponding sciatic pain and positive Straight Leg Raising (SLR) test. Further exclusion criteria were: lumbar spinal stenosis, previous surgery for herniated disc at the same level or fusion at any level in lumbar spine, generalized musculoskeletal pain, inflammatory rheumatic disease, diabetic polyneuropathy, cardiovascular disease (NYHA III and IV), cancer, psychiatric disease, neurological disease, alcohol or drug abuse, completion of another surgery within 1 month, pregnancy, nondetectable genotype, non-European-Caucasian ethnicity or poor Norwegian language. A total of 258 patients were included in the present study. However, at inclusion, 6 patients changed their mind and did not want to participate, which gave us data from 252 patients. In addition, 21 patients (8%) dropped out during the follow-up.

All participants received written information and signed an informed consent form. The study was approved by the Norwegian Regional Committee for Medical Research Ethics and the Norwegian Social Science Data Services.

Clinical procedure. After inclusion, the newly diagnosed patients had a follow-up at 6 weeks, 6 months and 12 months. Conservative treatment was received by 42% and surgical treatment received by 58%. At the time of inclusion, all patients underwent a standardized clinical examination including assessment of sensory and motor function and tendon reflexes of the lower limbs as well as an MRI scan. At 6 weeks follow-up, the clinical examination was repeated, while at 6 months follow-up, patients reported their back condition by a telephone interview and answered questionnaires by mail. At 12 months follow-up, patients underwent the same examination as by inclusion, and if their pain was persistent, an MRI scan was repeated. The sampling of the clinical data was completed before the genotyping of the patients was performed.

Clinical measures. All patients were asked to rate their pain intensity in activity during the last week on a 10 cm visual analog scale (VAS) with endpoints "no pain" and "worst possible pain." The validated Norwegian version of the McGill questionnaire was used to measure the sensory components of the pain experience (Strand and Wisnes, 1991). The validated Norwegian version of the Oswestry Disability Index (ODI) (Grotle et al., 2003) was used to assess problems with physical function related to low back pain.

Genotyping. Blood samples were drawn and genomic DNA was extracted from whole blood cells using FlexiGene DNA isolation kit (Qiagen). SNP genotyping was performed using predesigned TaqMan SNP genotyping assays (Applied Biosystems). Approximately 10 ng of genomic DNA was amplified in a 5 μl reaction mixture in a 384-well plate containing 1× TaqMan genotyping master mix (Applied Biosystems) and 1× assay mix, the latter containing the respective primers and probes. The probes were labeled with the reporter dye FAM or VIC to distinguish between the two alleles. After initial denaturation and enzyme activation at 95°C for 10 min, the reaction mixture was subjected to 60 cycles of 95°C for 15 s and 60°C for 1 min. The reactions were performed on an ABI 7900HT sequence detection system. Negative controls containing water instead of DNA were included in every run. Genotypes were determined using the SDS 2.2 software (Applied Biosystems). Approximately 10% of the samples were re-genotyped and the concordance rate was 100%.

Data evaluation and statistics. The data are shown as means ± SEM. VAS activity score, McGill sensory score and ODI measurements over time were compared regarding sex and OPRM1 genotypes with the groups; women */G, men */G, women AA and men AA by repeated measures ANOVA, within-subjects effect. When sphericity assumption was not met, a Greenhouse-Geiser correction was applied. Separate analyses were performed to check for potential effects of covariates age, smoking status and treatment. Covariates with $p \leq 0.1$ were kept in the final model (Table 2). Finally, VAS activity score, McGill sensory score and ODI score at 12 months were examined regarding the four sex/genotype groups by a one-way ANOVA and Tukey honestly significant difference (HSD) *post hoc* comparison. Statistical analyses were performed using the statistical package PASW statistics 18 (SPSS). A p value <0.05 was chosen as the level of statistical significance.

Results

The present material of the 252 patients consisted of 94 homozygous A/A, 20 heterozygous A/G and 3 homozygous G/G among the females, and 94 homozygous A/A, 40 heterozygous A/G and 1 homozygous GG among the males. The allele frequency of the G allele was therefore 13%, which is in accordance with previous reports from Caucasian populations (Klepstad et al., 2004).

As expected, we observed a clear decrease in pain and disability over time the first year after the disc herniation (VAS $p = 0.000$, McGill $p = 0.000$, ODI $p = 0.000$, repeated-measures ANOVA). From inclusion to 6 weeks, a distinct reduction in pain was observed, whereas a less pronounced reduction in pain intensity was observed from 6 weeks to 6 and 12 months.

Interestingly, however, our data showed that the decrease in pain and disability, i.e., the recovery after disc herniation, may be affected by both sex and the OPRM1 A118G SNP. A significant interaction between sex and genotype regarding the pain experience over time were observed (VAS, $p = 0.002$; McGill, $p = 0.021$; ODI, $p = 0.205$, repeated-measures ANOVA, women */G, men */G, women A/A and men A/A, including covariates smoke, treatment and age with $p \leq 0.1$).

The genotype */G seemed to be associated with more pain in women, but to protect the men from pain after lumbar disc herniation (Fig. 1). Wild-type A/A women and men reported similar pain ratings. Hence, the women carrying */G alleles appeared to have a slower recovery than the */G men.

The analysis of main outcome, i.e., pain and disability at 12 months, showed a significant association between sex and genotype regarding the pain experience (VAS, $p = 0.043$; McGill, $p =$

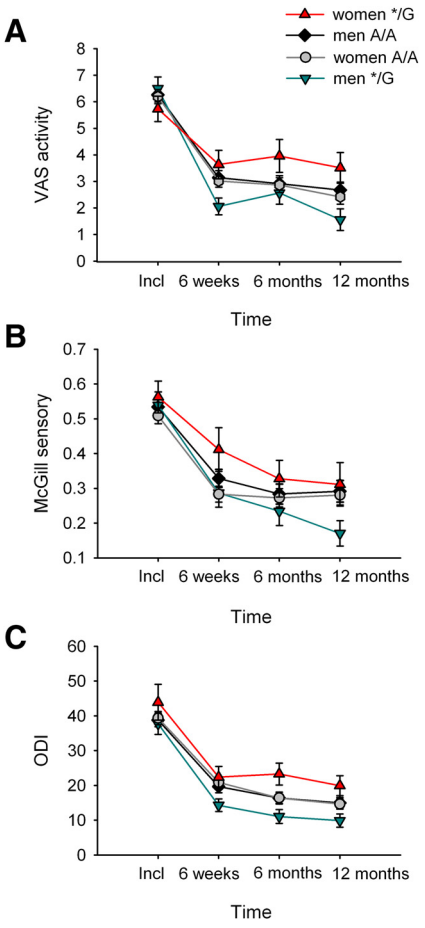

Figure 1. The time course for outcome measures grouped by sex and A118G genotypes following disc herniation. ***A***, VAS activity score ($p = 0.002$, rm ANOVA; $p = 0.043$ one-way ANOVA at 12 months). ***B***, McGill sensory score ($p = 0.021$, rm ANOVA; $p = 0.103$, one-way ANOVA at 12 months). ***C***, ODI score ($p = 0.205$, rm ANOVA; $p = 0.057$, one-way ANOVA at 12 months). Data are given as means ± SEM.

Table 3. Pain and disability ratings at 12 months

	VAS activity	McGill sensory	ODI
Women */G	3.51 ± 0.58	0.31 ± 0.06	19.92 ± 2.87
Men */G	1.56 ± 0.41	0.17 ± 0.04	9.89 ± 1.88
Women A/A	2.42 ± 0.27	0.28 ± 0.03	14.66 ± 1.47
Men A/A	2.67 ± 0.30	0.29 ± 0.03	15.00 ± 1.59

The table shows the 12 month VAS, McGill, and ODI scorings for the patients grouped by sex and A118G genotype. Mean ± SEM values are shown. *G or A allele.

0.103; ODI, $p = 0.057$, one-way ANOVA, women */G, men */G, women A/A and men AA). Mean ± SEM values at 12 months are listed in Table 3.

The *post hoc* comparison further confirmed that the */G women had more pain than the */G men (VAS, $p = 0.035$, Tukey HSD). However, the wild-type men and women seemed to have the same pain level (VAS, $p = 0.993$, Tukey HSD). The women carrying the 118G allele had, 12 months after the disc herniation, 2.3, 1.8 and 2.0 times higher VAS, McGill, and ODI scores respectively than the men with the same genotype.

Discussion

For the first time we demonstrate an interaction between sex and OPRM1 A118G genotype regarding recovery of low back pain and sciatica. Clearly, women with the */G genotype reported more pain than the */G men 12 months after the disc herniation. However, women and men with homozygote A/A alleles had almost exactly the same recovery rate regarding the pain intensity. Hence, our data indicated that the OPRM1 118G allele affected the clinical outcome after a disc herniation and that the */G women had a slower recovery than the */G men.

Our study support the earlier observation that female sciatic patients may have a slower recovery and a poorer one-year outcome than male sciatic patients (Peul et al., 2008). However, here we have extended these findings and demonstrated that the pain also is related to a sex-specific genetic factor. As presented in this study, women carrying the 118G allele had a mean VAS pain score 2.3 times higher than men with the same genotype 12 months after the lumbar disc herniation. Earlier data show that women carrying the 118G allele may have increased basal level of cortisol (Bart et al., 2006), consistent with a higher report of pain. Together these findings suggest that the high pain intensity in women compared with men in the low back pain and sciatic patients 12 months after the lumbar disc herniation may be related to the 118G substitution.

The present data are consistent with the observations of more pain in women carrying the 118G allele 24 h after a cesarean operation (Sia et al., 2008; Tan et al., 2009) and with carriers of the 118G allele, in particular males, having higher pressure pain thresholds (Fillingim et al., 2005). Interestingly, Fillingim and colleagues reported that */G women might be more sensitive to heat pain than A/A women and that the */G men might be less sensitive to heat pain than the A/A men. Moreover, sex-specific effects regarding the A118G SNP and reward effects of stimulants have been found. For example, women carrying the 118G allele have reported attenuated reward effects of nicotine (Ray et al., 2006). Also, female rats, homozygote for the 112G allele, an equivalent to the 118G allele in humans, have shown diminished reward properties of morphine (Mague et al., 2009).

At the molecular level, consistent with our observations of more pain in */G women, a 1.5–2.5-fold reduced mRNA expression of the OPRM1 has been found in human brain tissues of 118G carriers and a further tenfold reduction in protein levels has been found in cell cultures (Zhang et al., 2005). However, the molecular phenotype of the OPRM1 A118G seems to be region specific. For example, data from humans obtained by harvesting brain tissue postmortem have demonstrated that 118G allele carriers have a decreased receptor signaling efficacy in response to DAMGO in the secondary somatosensory cortex (Oertel et al., 2009). Moreover, positron emission tomography (PET) data based on the OPRM1 ligand tracer [^{11}C]carfentanil have suggested that smokers carrying the 118G allele may have lower levels of receptor binding potential in the amygdala, thalamus, and anterior cingulate cortex (Ray et al., 2011).

The Asn to Asp amino acid exchange results in reduced OPRM1 *N*-glycosylation (Huang et al., 2012). *N*-glycosylation, which has been suggested to be region-specific (Huang et al., 2008), plays a part in many cellular processes like receptor folding, sorting, expression, and ligand binding. As the level and type of *N*-glycosylation is found to differ in men and women (Knezević et al., 2009; Stanta et al., 2010; Ding et al., 2011), this mechanism has been proposed as a possible explanation for the region- and sex-specific differences observed for the OPRM1 A112G expression in the mouse brain (Wang et al., 2012). Hence, and in accordance with the data in the present study, it is tempting to speculate that lack of *N*-glycosylation, as a consequence of the amino acid exchange in the μ-opioid receptor, also may give sex-specific effects with regards to pain sensitivity in patients.

In conclusion, the present data demonstrate that the OPRM1 118G allele is associated with increased pain intensity in women, but reduced pain intensity in men the first year after a disc herniation. This finding strongly support the hypothesis that the OPRM1 118G allele may influence the endogenous pain modulatory system differently depending on sex, which also might be relevant for the understanding of the mechanisms underlying development of persistent low back pain and sciatica.

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