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

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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 */H men. Actually, the */G women had 2.3 times as much pain as the */H men 12 months after the disc herniation (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 */H men. Actually, the */G women had 2.3 times as much pain as the */H 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; Yones 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 (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, 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
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 spine 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 12 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 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 ≤ 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 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 = 0.022; 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 ≤ 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 =...
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 [11C]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 N-glycosylation (Huang et al., 2012). 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 [11C]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).
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 modulation 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.

References