Multiple Loci Modulate Opioid Therapy Response for Cancer Pain

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Abstract

Purpose: Patients treated with opioid drugs for cancer pain experience different relief responses, raising the possibility that genetic factors play a role in opioid therapy outcome. In this study, we tested the hypothesis that genetic variations may control individual response to opioid drugs in cancer patients.

Experimental Design: We tested 1 million single-nucleotide polymorphisms (SNP) in European cancer patients, selected in a first series, for extremely poor (pain relief ≤40%; n = 145) or good (pain relief ≥90%; n = 293) responses to opioid therapy using a DNA-pooling approach. Candidate SNPs identified by SNP-array were genotyped in individual samples constituting DNA pools as well as in a second series of 570 patients.

Results: Association analysis in 1,008 cancer patients identified eight SNPs significantly associated with pain relief at a statistical threshold of \( P < 1.0 \times 10^{-3} \), with rs12948783, upstream of the RHBDF2 gene, showing the best statistical association \( (P = 8.1 \times 10^{-3}) \). Functional annotation analysis of SNP-tagged genes suggested the involvement of genes acting on processes of the neurologic system.

Conclusion: Our results indicate that the identified SNP panel can modulate the response of cancer patients to opioid therapy and may provide a new tool for personalized therapy of cancer pain.

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Introduction

Pain is the most dreaded symptom in patients with malignant disease. Indeed, each year several millions of men and women of all ages suffer from some form of invasive cancer associated with pain requiring chronic opioid treatment. At present, opioids are virtually the only analgesics able to control moderate and severe cancer pain.

Although opioids are widely used to treat cancer pain, clinical studies indicate great variability among individuals with regard to the efficacy of these drugs and their side effects (1, 2). Many studies have investigated the potential genetic basis of individual variability in opioid drug response, but almost all have focused on a few candidate genes involved in opioid pharmacokinetics or pharmacodynamics, and results are not consistent among these, often small, studies (3, 4). For example, the catecholamines dopamine, epinephrine, and norepinephrine and their hydroxylated metabolites are substrates for the enzyme catechol-O-methyltransferase (COMT) encoded by the COMT gene, in which genetic variations have been associated with variations in pain perception and in doses of opioids required for pain treatment; however, discrepancies in the results from different association studies on COMT polymorphisms and pain perception have been reported, possibly due to the small size of the studies (reviewed in ref. 5).

The human mu 1 opioid receptor (OPRM1) gene, which is the main pharmacologic target of opioids, contains more than 100 polymorphisms, and these polymorphisms might affect opioid binding affinity to the receptor and, consequently, opioid-induced analgesia, tolerance, and dependence. However, a recent meta-analysis casts doubt on the clinical relevance of the OPRM1 118A>G polymorphism suggested by both preclinical and clinical studies as a candidate in personalized cancer pain therapy (6).

A complex genetics underlying interindividual differences in opioid response is expected in light of the biochemical and biological complexity of the drug response and of pain control. Indeed, opioid effects are governed by drug-related absorption, distribution, metabolism, intrinsic efficacy of the receptors involved, and by signal pathways downstream of the receptors. Because each of these steps in pain control is modulated by several genes, each of which can exist in allelic forms in the general population, a...
A genome-wide comprehensive analysis would be required to identify all genetic factors involved. In this study, we conducted the first genome-wide association study (GWAS) in cancer patients treated with opioids using a 1-million single-nucleotide polymorphism (SNP)-array to identify new genetic variations that modulate individual pain relief.

**Patients and Methods**

**Study design**

The European Pharmacogenetic Opioid Study (EPOS) represents a multicentric effort to examine symptoms, pharmacology, and pharmacogenomics related to the use of opioids. Recruitment criteria of patients included a regularly scheduled opioid treatment that was stable for at least 3 days. Opioids administered to more than 5% of patients included morphine, oxycodone, and fentanyl. The study includes 2,294 cancer patients treated with opioids for moderate or severe pain and recruited from 17 centers of 11 European countries. The participation of the Research Steering Committee from the European Association for Palliative Care (EAPC) facilitated international cooperation in the study.

The study collected demographic and personal data, as well as malignant diagnosis and opioid treatment details from cancer patients. Opioid doses were converted to equivalent total daily oral morphine doses (7). Pain was assessed by completing the Brief Pain Inventory (BPI; ref. 8). Functional status was measured by the Karnofsky performance status score. Further details about the EPOS series, including opioid doses and recruitments, have been reported (7). The study collected whole blood for genetic analysis and has established a biobank.

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**Pain relief phenotype and DNA pool construction**

The pain relief phenotype under study is semi-quantitative and determined based on the BPI, a robust and psychometric validated method to assess the subjective severity of pain. Pain relief is measured using an 11-point numerical rating scale, from 0%, representing "no pain relief" to 100% or "complete pain relief," that is, 0%, 10%, 20%, etc. (9).

For DNA pool preparation, we first defined the cancer patients as “good” or “poor” responders to opioid therapy, based on their pain relief phenotype score of 90% or more or 40% or less, respectively (Fig. 1). Selection was stratified by gender and country of origin, with a 1:2 matching of “poor” and “good” responders, to obtain a balanced representation of patients in the 2 groups. DNA pools of “good” (n = 293; mean pain relief = 94.1%) and “poor” (n = 145; mean pain relief = 27.0%) responders were created using equal amounts of fluorimetrically measured DNA from each sample.

**SNP genotyping**

SNP-array analysis of each of the 2 DNA pools was carried out using the HumanOmn1-Quad BeadChip (Illumina), which allows analysis of 1,140,419 genetic markers, including more than 20,000 markers in more than 300 important gene regions related to drug absorption, distribution, metabolism, and excretion; several of the SNPs map to opioid receptor or metabolism genes (http://www.illumina.com/products/humanomni1_quad_bead-chip_kits.ilmn). We carried out 12 replicas to verify genotype reproducibility and to estimate technical variability. Data were obtained as intensity signals, which were used to determine the allelic frequencies of each genetic marker.
and to reconstruct the number of chromosomes carrying each of the 2 possible alleles. Individual samples were genotyped using MassARRAY (Sequenom, Inc.), as described previously (10).

**Gene analysis**

To explore the role of the associated variants in the biological context, we mapped the best statistically associated SNPs in the DNA pools \( n = 486; P < 1.0 \times 10^{-5} \), using the Ensembl database to identify genes that are possibly involved in pain relief response, and analyzed the gene list for enrichment in ontologic annotation terms using the DAVID Functional Annotation Tool and Clustering (ref. 11; http://david.abcc.ncifcrf.gov/home.jsp).

**Statistical analyses**

Chromosome counts of the "poor" and "good" pain relief groups, from which DNA pools were prepared, were reconstructed based on allelic frequencies assessed in SNP-array hybridization, and the resulting \( 2 \times 2 \) contingency tables were tested by \( \chi^2 \) analysis. Association analyses between SNPs and the pain relief phenotype (categorical variable, "poor" or "good" responders, or quantitative variable) were carried out using PLINK software (12), which included analysis of the Hardy–Weinberg equilibrium (HWE), linkage disequilibrium between SNPs, and population-based associations between phenotypes and genotype/alleles (Supplementary Fig. S1).

<table>
<thead>
<tr>
<th>Table 1. Phenotypic characteristics of EPOS cancer patients examined for response to opioid therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject characteristics</strong></td>
</tr>
<tr>
<td><strong>No. of subjects</strong></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td><strong>Median age (range)</strong></td>
</tr>
<tr>
<td><strong>Country of origin</strong></td>
</tr>
<tr>
<td>CH</td>
</tr>
<tr>
<td>DE</td>
</tr>
<tr>
<td>DK</td>
</tr>
<tr>
<td>FI</td>
</tr>
<tr>
<td>GB</td>
</tr>
<tr>
<td>IS</td>
</tr>
<tr>
<td>IT</td>
</tr>
<tr>
<td>LT</td>
</tr>
<tr>
<td>NO</td>
</tr>
<tr>
<td>SE</td>
</tr>
<tr>
<td><strong>Pain relief (percentage score; mean ± SE)</strong></td>
</tr>
<tr>
<td><strong>Total oral morphine equivalent opioid dose (mg; mean ± SE)</strong></td>
</tr>
</tbody>
</table>

*Age in years.

Abbreviations: CH, Switzerland; DE, Germany; DK, Denmark; FI, Finland; GB, Great Britain; IS, Iceland; IT, Italy; LT, Lithuania; NO, Norway; SE, Sweden.

**Results**

The study population for which pain relief values were available included 1,907 patients from 4 ethnic groups: (i) Caucasian, including Hispanic (\( n = 1,859 \)), (ii) African (\( n = 13 \)), (iii) Oriental (\( n = 4 \)), and (iv) other (\( n = 29 \)), and from 11 European countries of origin. Patients included in the DNA pools constituted the first series (\( n = 438 \)), whereas the second series of 570 EPOS patients was selected based on pain relief phenotype score ≥90% or ≤50% to extend the results of the GWAS (Fig. 1). Table 1 lists the phenotypic characteristics of patients analyzed in the present study. The pain relief mean value was slightly higher in the second series (76.5 ± 1.1 vs. 71.9 ± 1.6; \( P = 0.014 \), on ANOVA), whereas no statistically significant differences were observed in the mean total oral morphine equivalent opioid dose (Table 1).

Preliminary analysis for factors that might confound the assessment of the pain relief phenotype revealed a weak effect of gender (\( P = 0.019 \)) and a strong effect of country of origin (\( P < 2.2 \times 10^{-16} \), on ANOVA; Supplementary Table S1). No statistically significant association between the pain relief phenotype and age of patients, ethnicity, or tumor type was found. Thus, we normalized the pain relief phenotype by the country mean value and adjusted analyses for gender as covariate.
Genes involved in neurologic system processes may modulate pain relief

In our search for genetic variations associated with individual pain relief, we analyzed the EPOS subjects showing the extreme phenotypic values of pain relief in order to reduce the bias in the subjective pain relief assessment of the borderline categories and to allow testing for genetic effects on response to pain therapy in the best candidate subjects (Fig. 1).

GWAS, using a DNA-pooling strategy and 1-million SNP-array, detected 173,851 informative SNPs, with overall minor allelic frequency more than 0.1 and coefficient of variation of allelic frequency of (SD/mean) more than 0.08. Statistical associations of these SNPs are visualized by Manhattan plot (Fig. 2). Analysis of chromosome counts obtained from allelic frequencies in DNA pools of "good" and "poor" responders identified 486 SNPs at the statistical threshold of \( P < 1.0 \times 10^{-3} \) (\( \chi^2 \) test; Fig. 2; Supplementary Fig. S1).

To explore the role of the associated variants in the biological context, we identified the genes tagged by these 486 SNPs. A list of 226 known genes, either near or containing a SNP, was obtained and analyzed using the Functional Annotation Tool (http://david.abcc.ncifcrf.gov/home.jsp), which identified as the best statistically significant gene ontologic annotation terms those linked to the nervous system, such as the "neurological system process," "transmission of nerve impulse," and "synaptic transmission." Notably, the best term was the "neurological system process," which included 31 genes (\( P = 3.5 \times 10^{-5} \); Table 2). Interestingly, 10 of these 31 genes were members of the olfactory receptor family. Use of Functional Annotation Clustering gave similar results, with the most highly significant cluster including the annotation terms "transmission of nerve impulse," "synaptic transmission," and "cell–cell signaling" (not shown).

A genetic profile controls pain relief

Among the 486 candidate SNPs identified by statistically significant differences (\( P < 1.0 \times 10^{-3} \), on \( \chi^2 \) test) between "poor" and "good" responders in chromosome counts, we selected 72 SNPs associated with the pain relief phenotype (at \( P < 1.0 \times 10^{-5} \) threshold value) for genotyping in individual samples constituting the DNA pools, and in the second series of 570 EPOS patients by MassARRAY (Table 1; Fig. 1). Six SNPs failed PCR or MassEXTEND primer design, and another 7 failed genotyping. Of the 59 genotyped SNPs, 2 showed significant deviation from the HWE (\( P < 1.0 \times 10^{-5} \)), reducing the number of markers to 57 SNPs.
Association analysis in the first series indicated that 54 of the 57 SNPs were significantly associated with the poor and good responder categories at \( P < 0.05 \) (logistic analysis), confirming the robustness of our DNA-pooling approach, which produces reliable results and is time- and cost-effective, in agreement with other reports (13, 14). SNP rs12948783, which maps to chromosome 17 upstream of the \( RHBDF2 \) gene, showed the strongest association \(( P = 1.1 \times 10^{-7})\).

Analysis of the total sample size of 1,008 cancer patients for the quantitative pain relief phenotype, allowing an increased statistical power of the study, pointed to 8 SNPs associated at the statistical threshold of \( P < 1.0 \times 10^{-3} \), with the strongest allelic association, again, for SNP rs12948783 \(( RHBDF2; P = 1.1 \times 10^{-6}; \) linear model; Table 3; Supplementary Fig. S1).

Genotype–phenotype analysis for rs12948783 SNP in 936 patients (72 genotypes were missing) showed that patients homozygous for the rare allele, that is, the AA genotype, experienced a low normalized pain relief value (mean ± SE, 80% ± 9%; \( n = 23 \)) that did not differ statistically from that of patients with the GA genotype (mean ± SE, 88.5% ± 3%; \( n = 243 \)). Comparison of the subjects carrying the rare allele (AA or GA genotypes, \( n = 266 \)) with patients carrying the common GG genotype (mean ± SE, 104% ± 1%; \( n = 670 \)) showed a highly statistically significant association \(( P = 8.1 \times 10^{-9})\) with patients carrying the GG genotype and with almost complete pain relief (Fig. 3).

**Table 2.** Gene ontologic analysis by DAVID of the 226 genes defined by the 486 SNPs most significantly \(( P < 0.001)\) associated with allelic imbalance in the SNP-array analysis of DNA pools from "poor" or "good" responders to opioid therapy for cancer pain

<table>
<thead>
<tr>
<th>Term</th>
<th>Gene count</th>
<th>( P^a )</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0050877 ~ neurological system process</td>
<td>31</td>
<td>( 3.5 \times 10^{-5} )</td>
<td>ABLIM1, OR8U9, AGTPBP1, SYT5, KCNA1, COL2A1, OR4C6, RAX2, OR4S2, ELOVL4, OR8K3, DMD, OR4C11, OR5AS1, KCNE1, OR5AP2, COL4A4, COL18A1, OR5R1, OR11L1, PCDH15, AFF2, NRXN1, OR5T3, ACCN1, SLC17A6, SBF2, ABAT, TBL1X, NCAN, PTENP1</td>
</tr>
<tr>
<td>GO:0007155 ~ cell adhesion</td>
<td>17</td>
<td>( 6.1 \times 10^{-3} )</td>
<td>COL18A1, PLXNC1, NRP1, C21orf29, NEL1, ACTN1, COL2A1, PCDH15, NRXN1, BTBD9, ITGB1L, CDH12, CD96, SORBS1, NCAN, JAM2, SPON1</td>
</tr>
<tr>
<td>GO:0022610 ~ biological adhesion</td>
<td>17</td>
<td>( 6.2 \times 10^{-3} )</td>
<td>COL18A1, PLXNC1, NRP1, C21orf29, NEL1, ACTN1, COL2A1, PCDH15, NRXN1, BTBD9, ITGB1L, CDH12, CD96, SORBS1, NCAN, JAM2, SPON1</td>
</tr>
<tr>
<td>GO:0019226 ~ transmission of nerve impulse</td>
<td>11</td>
<td>( 6.8 \times 10^{-3} )</td>
<td>COL4A4, ACCN1, SLC17A6, SYT5, AGTPBP1, SBF2, DMD, KCNA1, ABAT, NRXN1, NCAN</td>
</tr>
<tr>
<td>GO:0007268 ~ synaptic transmission</td>
<td>10</td>
<td>( 7.1 \times 10^{-3} )</td>
<td>COL4A4, ACCN1, SLC17A6, SYT5, AGTPBP1, SBF2, DMD, KCNA1, ABAT, NRXN1, NCAN</td>
</tr>
<tr>
<td>GO:0050890 ~ cognition</td>
<td>20</td>
<td>( 7.4 \times 10^{-3} )</td>
<td>COL18A1, ABLIM1, OR5R1, OR8U9, OR11L1, PCDH15, AFF2, COL2A1, OR4C6, OR5T3, RAX2, OR4S2, ELOVL4, OR8K3, OR4C11, OR5AS1, OR5AP2, KCNE1, TBL1X, PTENP1</td>
</tr>
</tbody>
</table>

\(^a\)Calculated by the DAVID functional annotation tool, using a modified Fisher’s exact test.

**Discussion**

Opioid efficacy for the treatment of pain in cancer patients varies greatly among individuals, suggesting a role for genetic factors in pain relief outcome. Unlike previous pharmacogenetic studies that have tested genetic variations of a few candidate genes in a small number of subjects, our present study represents the first use of genome-wide scanning in a large number of opioid-treated cancer patients to identify genetic variations that may explain differences in pain relief.

Pain relief, an inherently subjective parameter, was indeed the phenotype quantified through the BPI method in cancer patients included in the EPOS study; surprisingly, we found a significant correlation between pain relief and country of origin, but not with ethnicity. The association with country of origin might rest in subtle variations in phenotype definitions related to each country or in organizational differences between health care services in various European countries that introduce variability in selection of patients at EPOS recruitment, although the BPI questionnaire is officially recognized and recommended by EAPC and has been validated across cultures and languages. The lack of association between pain relief and ethnicity may be genuine, although the non-homogeneous distribution of patients into 4 ethnic groups (97.6% of patients were ethnically Caucasian) may undermine the statistical power to detect effects linked to ethnicity.
Table 3. List of 8 SNPs associated with the quantitative pain relief phenotype in 1,008 European cancer patients treated with opioids

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position (Mb)</th>
<th>Gene</th>
<th>Minor allele</th>
<th>Common allele</th>
<th>First and second combined series</th>
<th>First series</th>
<th>Second series</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs13421094</td>
<td>2</td>
<td>G/C</td>
<td>A</td>
<td>T</td>
<td>0.14</td>
<td>0.19</td>
<td>0.07</td>
</tr>
<tr>
<td>rs12211463</td>
<td>6</td>
<td>G/T</td>
<td>G</td>
<td>T</td>
<td>0.19</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>rs7757130</td>
<td>6</td>
<td>A/C</td>
<td>A</td>
<td>C</td>
<td>0.07</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>rs2473967</td>
<td>6</td>
<td>C/A</td>
<td>C</td>
<td>A</td>
<td>0.07</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>rs2884129</td>
<td>6</td>
<td>G/C</td>
<td>G</td>
<td>C</td>
<td>0.07</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>rs7104613</td>
<td>11</td>
<td>T/C</td>
<td>T</td>
<td>C</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>rs10413396</td>
<td>19</td>
<td>T/G</td>
<td>T</td>
<td>G</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

SNPs genotyped in individual cancer patients by MassARRAY and showing statistically significant association (P < 1.0 × 10⁻³) with pain relief; SNPs with statistically significant P-values obtained by χ² analysis on reconstructed chromosome numbers in DNA pools.

Figure 3. RHBDF2 on chromosome 17 is a major pain relief modifier locus in the EPOS series of opioid-treated cancer patients. Cancer patients carrying the rare allele (either the AA or GA genotype; n = 266) at SNP rs12948783 located in the 5'-proximal region of the RHBDF2 gene showed a statistically significant lower pain relief than patients with the common GG genotype (n = 670), who showed an almost complete response to opioid therapy (P = 8.1 × 10⁻⁹, on ANOVA). Dots and bars represent mean ± SE values of the normalized pain relief phenotype.

Analysis of candidate SNPs defined a set of 8 genetic markers associated with individual response to opioid therapy for cancer pain (Table 3). Of these 8 SNPs, the best statistically associated SNP maps at the 5'-proximal region, 1,892 base pairs (bp) from the transcriptional start site of the rhomboid 5 homolog 2 (Drosophila; RHBDF2) gene, of unknown function (Fig. 3). rs7104613 is located in the intron region of the spondin 1 extracellular matrix protein (SPON1) gene, which encodes a cell-adhesion protein that promotes the attachment of spinal cord and sensory neuron cells and the outgrowth of neurites in vitro. rs10413396 maps at −101 bp from the 5'-region of the zinc finger protein 235 (ZNF235) gene, which is located in a cluster of genes encoding members of regulatory proteins characterized by nucleic acid–binding zinc finger domains.

None of the genetic variations found in the present study mapped in the opioid receptor genes that were suggested by previous studies as candidates for individualized opioid treatment (reviewed in ref. 4). Instead, our results point to the involvement of genes related to transmission of nerve impulse, not opioid pharmacology, in mediating the main modulatory effect of genetic variability on the perception of pain. Nonetheless, it remains possible that opioid therapy outcome is the result of interaction of complex biological systems involved in both pathways, particularly considering that the biological function of some genes that we identified is, as yet, unknown or uncertain.

Our findings could represent the starting point to allow estimation of the probability of being a poor responder to opioid treatment, thus leading to personalized cancer pain therapy in which, for example, individuals at genetic risk of...
poor response would receive higher starting doses of opioids. Moreover, genes tagged by these polymorphisms may provide a target for new therapeutic strategies to control cancer pain perception.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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