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· *OPRM1* c.118A>G AND *COMT* p.Val158Met ON PAIN SUSCEPTIBILITY ·

A META-ANALYSIS

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Abbreviations

CI - Confidence Interval

ED - Emergency Department

FEN - Fentanyl

HWE - Hardy-Weinberg Equilibrium

HYDROM - Hydromorphone

MAF - Minor Allele Frequency

MEP – Meperidine

M6G – Morphine-6-Glucoronide

NSAID - Non Steroidal Anti-Inflammatory Drug

MOR - Morphine

OXY - Oxycodone

NRS - Numeric Rating Scale

SMD - Standardized Mean Difference

SNPs - Single Nucleotide Polymorphisms

TRAM - Tramadol

VAS - Visual Analogue Scale

Abstract

The c.118A>G on *OPRM1* and the p.Val158Met on *COMT* are two common single nucleotide polymorphisms (SNPs) that have been associated with pain responses in numerous studies. However, a clear consensus is still missing. The primary objective was to determine the effect of the risk-alleles of both c.118A>G and p.Val158Met on pain intensity. The secondary objective was to determine the effect of the risk-allele dosage on the same parameter.

A meta-analysis was designed. A web search was performed using the PubMed database with the keywords "*OPRM1* A118G pain" and "*COMT* Val158Met pain". Association studies enrolling adult patients with any type of health condition with acute or chronic pain were included. The main outcome measure was pain intensity. A standardized mean difference (SMD) with 95% confidence interval using a random effect model was used to analyze data.

Compared to AA genotype, carriers of the G allele of *OPRM1*, reported higher pain intensity albeit non-significant (SMD=0.25, 95% CI [-0.05 – 0.55]). Allele dosage analysis showed no significant difference (SMD=0.10, 95% CI [-0.12 – 0.32]). The same pattern of results was found regarding the pain intensity variation after pharmacological pain management. Concerning the p.Val158Met, the pooled data indicate that both the presence (SMD=0.26, 95% CI [-0.03 – 0.55]) and the homozygosity for the Met allele (SMD=0.39, 95% CI [-0.05 – 0.83]) were associated with higher pain intensity although non-significant. Additionally, no significant differences were found for pain variation scores after drug-induced pain control.

In conclusion, both the c.118A>G and the p.Val158Met appear to be associated with pain intensity, with carriers of the risk-allele reporting higher pain intensity scores. Replication studies that use similar and clearly defined samples and outcomes are warranted.

Keywords Pain, Single Nucleotide Polymorphism, Humans, *COMT*, p.Val158Met, *OPRM1,* c.118A>G

Resumo

Cerca de metade da população mundial irá reportar dor pelo menos uma vez em algum momento da sua vida. Vários estudos têm documentado que tanto a susceptibilidade à dor como a eficácia da analgesia é sujeita a influência de fatores genéticos. Vários genes candidatos e os seus polimorfismos têm sido estudados em animais e humanos. Dois dos polimorfismos mais estudados são o c.118A>G do gene *OPRM1* e o p.Val158Met do gene *COMT*. As consequências funcionais de ambos estão bem documentadas, sendo que o alelo menos frequente de ambos tem sido associado níveis reduzidos de mRNA e/ou proteína e a níveis reduzidos de atividade biológica. Estas consequências traduzem-se na clínica por uma menor latência, um limiar mais baixo e uma sensibilidade à dor aumentada. Embora estes resultados sejam promissores, não foram replicados em alguns estudos. Desconhece-se, portanto, qual o efeito destas variantes genéticas nas respostas de dor em adultos no contexto clínico.

Assim, os objetivos definidos para este estudo foram: 1) determinar o efeito da presença do alelo menos frequente dos polimorfismos c.118A>G (*OPRM1*) e p.Val158Met (*COMT*) na intensidade da dor e na variação da intensidade da dor após controlo farmacológico e 2) avaliar o efeito da homozigotia para os alelos menos frequentes dos referidos polimorfismos nos mesmos parâmetros.

Para atingir os objectivos, foi delineada uma meta-análise de estudos de associação que avaliassem a intensidade ou a variação dos *scores* de dor após o seu controlo farmacológico. Foram incluídos estudos que recrutem adultos com qualquer tipo de patologia, com dor aguda ou crónica, sob analgesia ou não. Estudos experimentais envolvendo humanos ou animais foram excluídos. Estudos em que não estava disponível informação específica sobre os polimorfismos em análise, incluindo haplotipos, foram também excluídos. Foi realizada uma pesquisa eletrónica na base de dados "*PubMed*" com as palavras-chave "*OPRM1* A118G pain" e "*COMT* Val158Met pain", separadamente. Esta pesquisa foi complementada pela revisão manual da bibliografia dos estudos mais relevantes. A seleção dos textos seguiu um metodologia de três níveis: (1) leitura do título e avaliação da sua relevância; (2) leitura do resumo e (3) leitura do texto completo. Para cada publicação foram registadas as seguintes informações: primeiro autor, ano de publicação, etnia, diagnóstico, equilíbrio de Hardy-Weinberg, analgesia e *scores* de dor. A análise estatística dos dados foi realizada com o *Review Manager 5.3* para MacOSX. A heterogeneidade foi avaliada usando a análise estatística Q e pelo cálculo do I^2. O cálculo da diferença de médias padronizada e do intervalo de confiança a 95% foi usado para analisar as diferenças na intensidade da dor entre os grupos de genótipos.

Foram incluídos 29 estudos originais: 20 sobre o c.118A>G (*OPRM1*) e 9 sobre o p.Val158Met (*COMT*). A maioria dos estudos recrutou adultos caucasianos submetidos a cirurgia ou em tratamento oncológico. Relativamente ao polimorfismo c.118A>G (*OPRM1*), verificou-se uma diferença não significativa (IC 95% [-0.05 – 0.55], p=0.10) entre os doentes AA e os portadores do alelo menos frequente, c.118G, com os homozigóticos para o alelo *wild type* a reportar níveis de dor mais baixos. A homozigotia para o alelo G não conferiu risco adicional para uma maior suscetibilidade à dor (SMD = 0.07, IC 95% [-0.06 – 0.19], p=0.28). A análise do efeito do polimorfismo p.Val158Met (*COMT*) revelou que, tanto os

portadores (SMD = 0.26, IC 95% [-0.03 – 0.55], *p*=0.08) como os homozigóticos (SMD = 0.39, IC 95% [-0.05 – 0.83], *p*=0.09) para o alelo Met apresentam uma tendência não significativa para reportar níveis mais elevados de intensidade de dor. O mesmo padrão de resultados foi encontrado para a variação na intensidade de dor após o seu controlo farmacológico.

A influência de ambos os polimorfismos em análise nas respostas de dor é controversa. Os resultados da meta-análise apontam para a tendência teórica defendida, isto é, o alelo menos frequente está associado a intensidade de dor mais elevada. Todavia, os estudos como conjunto são heterogéneos e as diferenças observadas não são significativas. Embora sejam necessárias investigações adicionais para clarificar a relação entre estes dois polimorfismos e a intensidade de dor, é provável que o seu efeito seja pequeno e influenciado por várias co-variáveis. Atualmente, a genotipagem isolada dos polimorfismos c.118A>G (*OPRM1*) e p.Val158Met (*COMT*) continua a ter um valor mais científico do que clínico.

Introduction

Up to 50% of the world population will experience pain at any given moment[1]. Although pain medical therapy has greatly evolved, it is still difficult to achieve an appropriate pain control[2].

Both susceptibility to feel pain and analgesia efficacy are issues related to high inter individual variability[3] and genetic influence has been proved both in experimental and clinical pain[1]. Dissection of genetic influence in pain trait is complex, yet hereditary studies have shown that up to ¾ of the pain trait is explained by genetic factors. Several candidate pain susceptibility genes have been described in mice[4] and some studies were replicated in human populations.

Many strong opioid analgesics produce its effect by activating the μ_1 opioid receptor (OPRM1) in a manner similar to morphine[5]. Several variants of the *OPRM1* gene have been described[6], but the most common is the change of adenine to guanine in the 118th position, c.118A>G[7]. Also, cathecol-O-methyltranferase (COMT) is an enzyme that catalyses the degradation of the catecholamines, such as epinephrine and dopamine, that have an important role in nociception and analgesia responses[8]. A change in the protein from valine to methionine in the 158th position, p.Val158Met, has been associated with lower levels in COMT activity and is one of the most common polymorphisms included in association studies.

Although being still raw, accumulating evidence suggests an association between the c.118A>G and p.Val158Met SNPs (in *OPRM1* and *COMT* genes, respectively) and pain

responses. However, no summarized or systematic data about these polymorphisms and clinical pain susceptibility is available.

The main question of this study was: are pain responses in the clinical context associated to the specific genetic variations on both the *OPRM1* and *COMT* genes? Given the fact that every gene is composed of two alleles, a second question was raised: are pain responses associated with allele dosage?

In order to find the answer to these questions, a meta-analysis of published clinical studies was designed. Studies examining the effect of genetic variation on clinical pain conditions provide information that is not easily obtained in an experimental context. All relevant studies involving the c.118A>G and p.Val158Met SNPs and its association to pain susceptibility were reviewed and its evidence was summarized in the present work.

This research report is organized in two main parts: the background and the empirical study. In the first part, the theoretical background for studying the influence of both the c.118A>G and p.Val158Met SNPs on pain responses is presented. Functional consequences of the polymorphisms and human experimental studies are reviewed. The second part presents the empirical study. The objectives are enunciated and the methods of the research and data analysis are explained. Further on this second part, meta-analysis' results are presented, summarized and discussed according to the state of art. Limitations of the study are highlighted. The report is concluded with an overall view of the research and puts forward the implications for both the clinical practice and scientific research.

Chapter 1

Background

1.1. The μ opioid receptor and c.118A>G polymorphism

The c.118A>G SNP (rs1799971) leads to a change in the amino acid asparagine (N) to aspartic acid (D) at position 40 (p.N40D) of the extracellular region of the receptor. An experiment with healthy volunteers emphasized that the relevance of the c.118A>G when studying the role of the *OPRM1* in pain field[9].

Functional effects of the c.118A>G polymorphism on the mRNA and protein levels have been reported. In a mouse model, lower levels of mRNA were observed in several brain regions related to pain. Also, a decrease in protein size and levels in the thalamus were found in mice homozyguous for the G allele[10]. In transfected Chinese hamster ovary cells, protein levels were 10-fold lower in the c.118G variant. These results were replicated in human autopsy brain tissues, in which mRNA from A allele samples was 1.5–2.5-fold more abundant, comparing to those of the G allele carriers[11]. A modified binding activity is also reported: GG-mice exhibit a decreased specific receptor binding in the thalamus and, consequently, a deficient antinociceptive response after morphine administration[10]. However, these data fails replication in different publications[12,13].

Human experimental studies have shown that carriers of the G allele exhibited significant shortening of latency to pain perception and, consequently, a higher sensitivity in a model of cold pain sensitivity[14,15]. However, contradictory data is presented in an experimental model of thermal, mechanical and ischemic pain, enrolling 167 multiethnic healthy volunteers. In

this study carriers of the G allele had significantly higher pressure pain thresholds, i.e., lower sensitivity to pressure pain when comparing to the subjects harboring the wild type variant[16]. These results were later replicated by Lötsch and collaborators[9]. However replication of the association between the c.118A>G SNP and pain responses failed in various studies[17].

1.2. The COMT and p.Val158Met polymorphism

The p.Val158Met SNP (rs4680) is due to a substitution of valine to methionine at codon 158 of the *COMT* gene. This transition causes a three-to-four-fold reduction of wild types in COMT enzyme activity[18] and in protein content, but not in mRNA expression levels[19]. The monoamines are the main substrate of COMT and play a crucial role in the modulation of pain. Knockout mice for *COMT* gene exhibit a profound increase in pain sensitivity[20,21]. Replication of these results in healthy human volunteers revealed that low concentrations of dopamine contributes to the development of pain symptoms[22]. Indeed, agonists of the dopaminergic receptors have been found to reduce heat pain sensitivity, increase the threshold to mechanical stimuli and to diminish the responses of dopaminergic neurons to nociceptive stimuli[23].

Zubieta *et al*[24] suggested that a reduced COMT enzymatic activity that leads to an enhanced activation of dopaminergic neurotransmission, with lower endogenous levels of enkephalins and thus more tendency to experience exaggerated pain sensitivity. The experiment proved that individuals that are homozygous to the Met allele are likely to report higher pain ratings than did the homozygous carriers of the Val allele. These results were later confirmed in two other experimental studies with healthy volunteers[20,25]. However, other studies found no association between the p.Val158Met and pain responses[26,27].

1.3. Rationale for this review

No systematic review concerning the two most common SNPs of the *OPRM1* and *COMT* genes (c.118A>G and p.Val158Met, respectively) and its probability to be associated with higher pain intensity scores in the clinical setting has been performed so far. This type of study is needed to clarify which factors are important in the clinical context and to test whether pain control algorithms should include genetic testing to tailor pain medication.

Chapter 2

Objectives

The main question of this study is: are pain responses in the clinical context associated to the specific variants c.118A>G of *OPRM1* and p.Val158Met of *COMT*? In order to clarify the question, primary and secondary objectives were formulated.

Primary objectives:

1. To determine the effect of the presence of the risk-alleles G and Met from *OPRM1* and *COMT* genes, respectively, on clinical pain severity.
2. To determine the effect of the presence of the risk-alleles G and Met from *OPRM1* and *COMT* genes, respectively, on pain intensity scores variation after a pain management intervention.

Secondary objectives:

3. To evaluate the effect of the homozygosity for the risk-allele G and Met from *OPRM1* and *COMT* genes, respectively, on clinical pain severity.
4. To evaluate the effect of the homozygosity for the risk-allele G and Met from *OPRM1* and *COMT* genes, respectively, on pain intensity scores variation after a pain management intervention.

Chapter 3

Methodology

In this chapter criteria for inclusion of studies will be presented, as well as the web search methodology, data collection and analysis processes.

This study was approved by the Faculty of Medicine, University of Coimbra (Portugal) institutional review board.

3.1. Criteria for inclusion of studies in this review

Type of studies Clinical association studies assessing pain severity or pain intensity scores variation after pain control were considered. Human and animal experimental studies were excluded.

Type of participants Patients older than 18 years old with any type of health condition, with acute or chronic pain and with or without analgesia were considered. Studies focused in healthy volunteers were excluded.

Type of association The interest of the present review has focused in any comparison between the possible genotypes and/or its combination and the pain intensity scores. Studies in which the variants c.118A>G or p.Val158Met were not included or specific data were not reported, including haplotypes, were excluded.

Type of outcome measures and phenotypes Pain data were included if measured either by a Numeric Rating Scale (NRS) or a Visual Analogue Scale (VAS). Pain responses examined are pain severity and pain intensity variation. *Pain severity* is defined as the pain intensity score, measured by a NRS or a VAS in one specific time point or regarding a past period of time (ex: last 24 hours). *Pain intensity variation* is defined by the change in pain intensity scores, measured before and after a pain management intervention. Different populations were included in the same analysis due to the small number of studies concerning one type of population.

3.2. Search methods for identification of studies

Electronic search A PubMed data base search was performed throughout April 2015 with the keywords combinations "OPRM1 A118G pain" and "COMT Val158Met pain". The search for each variant was performed separately. The selection of the papers to be included in the present meta-analysis followed a three-step methodology: (1) reading of the title and evaluation of its relevance, (2) reading of the abstract and (3) reading of the full text. In stages (1) and (2) efforts were made to aim more for sensitivity than specificity, i.e., we wished to be more inclusive than exclusive. Review authors were not blinded to author, Institution, journal, or results of a study for its assessment. Final decisions were made in agreement between two authors (DC and MG).

Manual search References from the included studies were manually reviewed in order to identify additional titles. Reading of the abstract was performed to do a pre-selection and finally the full text was read in order to decide its inclusion.

3.3. Data collection process and extracted items

We created a data extraction sheet that allowed the review of the entire study and its independent samples. Data were extracted by one author (DC) and checked twice. For each paper, the following data was extracted: first author, year of publication, ethnic group, diagnostic, genotype frequencies in agreement with the Hardy-Weinberg Equilibrium (HWE), presence of analgesia and pain intensity ratings. We made attempts to contact authors of the study if data were missing or needed to be clarified. When the contact was not reciprocated or the author was unable to provide the requested data, the study was excluded from the review.

3.4. Statistical analysis

Pain data scores were all converted to a 0-10 point scale as for the most of the studies this was the preferred form of reporting pain data. For the majority of the papers, it was necessary to calculate combined mean and standard deviation for the group of carriers of the risk-allele (AG/GG - *OPRM1* - and Val/Met/Met/Met - *COMT*). The re-calculation was performed following the guidelines of the Cochrane Handbook. Also, mean and standard deviation were estimated from median and range according to Hozo and collaborators[28].

The statistical analysis was performed using the Review Manager 5.3 software for MacOSX. Heterogeneity of the sets of the included studies was assessed using the Q statistics and by calculation the I^2 ($I^2 > 50\%$ equals significant heterogeneity and $I^2 < 25\%$ equals insignificant heterogeneity). Standardized mean differences (SMD) and 95% confidence intervals (CI) were calculated for all samples. The use of the pooled standard deviation in the SMD accounts for the variability in pain measurement scales by placing the differences on the same comparable scale. Standardized mean differences were pooled using inverse variance methods to generate a summarized standardized mean differences and the corresponding 95% confidence interval. Independent samples were weighted by the inverse of the study variance

so that those with lower variance received more weight. Data were analyzed within the random effects and fixed-effects frameworks, but because significant heterogeneity among studies was often found, we report the results from random effects model only. The significance was tested using Z statistics. Forest plots were generated to better comprehend the standardized mean differences for each study and for the global analysis as well as the corresponding 95% confidence interval.

For each SNP the analysis was divided according the pain responses of interest, i.e., *pain severity* and *pain intensity variation* (see **Type of outcome measures and phenotypes** for definitions). Also, for each pain response, a primary and a secondary analysis – in line with the objectives enunciated – was performed. On the primary analysis, wild-type and carriers of the risk-allele for both SNPs were compared. On the secondary analysis, wild-type and homozigosity for the risk-allele was compared.

Chapter 4

Results

This chapter begins with the presentation of the results of the web search for both polymorphisms under analysis. Later, for each SNP, characteristics of the samples and the pooled data are presented.

4.1. Results of the web search

For the c.118A>G (*OPRM1* gene), a total of 180 titles were screened. Abstracts of 48 papers were judged for its relevance. Thirty-one full texts were included in the final screening panel prior to inclusion. Four additional articles were found through manual search. Fifteen studies were excluded upon full-text reading because they did not address the subject (n=8), it was impossible to extract data (n=4) and it was an experimental study (n=3). The final corpus of the review regarding the *OPRM1* c.118A>G yielded 20 papers (Figure 1a).

For the p.Val158Met, a total of 178 titles and 59 abstracts were screened for its relevance. Forty-nine full texts were included in the final screening panel prior to inclusion. One study was found through manual search. Exclusion upon full-text reading was done because they did not address the subject (n=18), it was impossible to extract data (n=9) and it was a duplicate paper (n=1). Another study was excluded because it reported data of the same study as Reyes-Gibby[29]. The final corpus of the review concerning the p.Val158Met yielded 9 papers (Figure 1b).

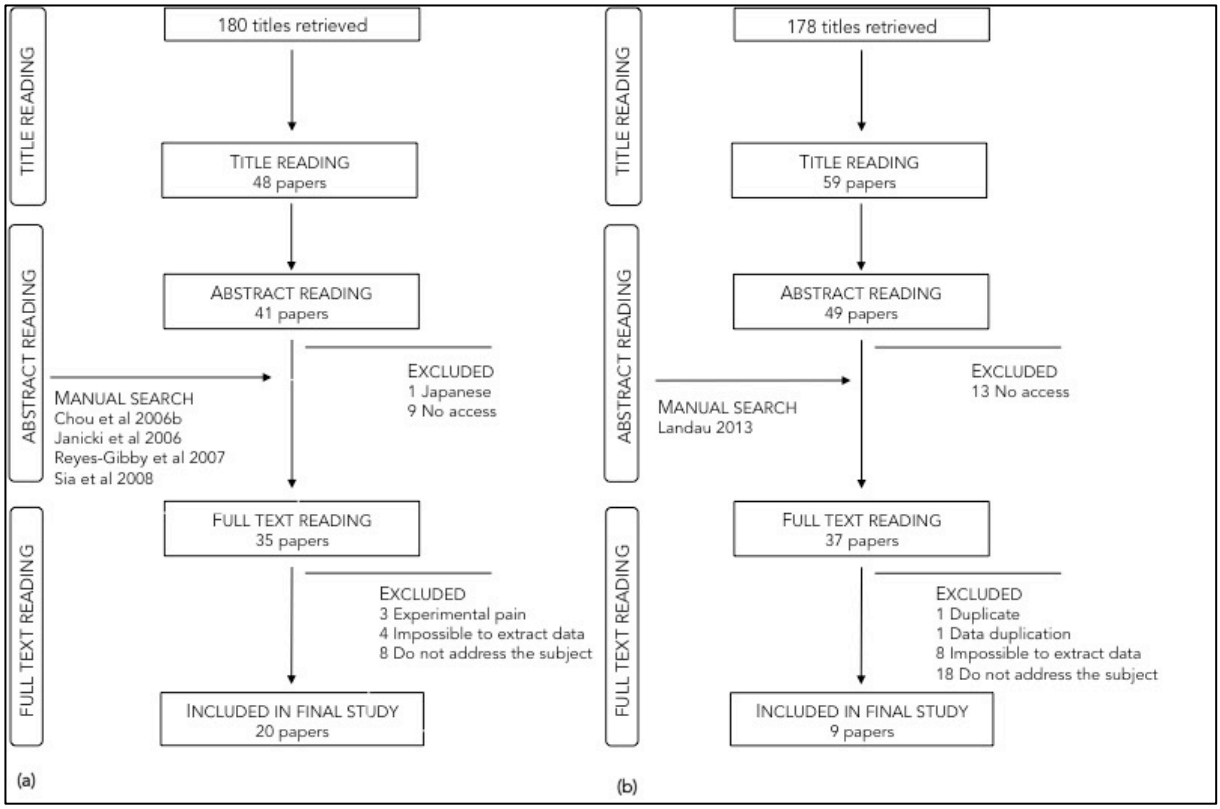

Figure 1 Flow diagram for (a): the c.118A>G variant of *OPRM1* gene. (b): the p.Val158Metvariant of *COMT* gene.

4.2. The influence of the c.118A>G SNP of *OPRM1* gene on pain responses

Description of the included studies Table 1 shows the general characteristics of the independent samples from the 20 papers included. Papers were published between 2004 and 2015 and enrolled 5044 patients older than 18 years old. The number of participants per study ranged between 50 and 993. Ten studies were performed in Caucasian patients; six studies enrolled Asian patients and three recruited a mixed-ethnic sample. Fourteen studies reported that their samples were in Hardy Weinberg Equilibrium (HWE). The minor allele frequency (MAF) exhibited a high variation among the studies. Caucasians samples reported a MAF between [0.10 – 0.171]; Asian samples' MAF varied between [0.245 – 0.438]. The type of health conditions was diverse. Most of the studies were conducted in surgical patients (visceral and non-visceral surgery) and in cancer patients. All but three samples were under analgesia, mostly opioid drugs. The majority of the studies registered the pain intensity scores

at 24 hours of hospitalization. Only three studies reported on pain intensity variation after a pain management intervention.

Pain severity versus c.118A>G *OPRM1* polymorphism Seventeen studies reported data that could be incorporated into pain severity analysis. The final analysis yields 29 independents samples, enrolling 6,348 participants. Of these, 4,010 are homozygous AA and 2,338 are G carriers. For the AA patients, pain intensity scores varied between [1.6±1.1 - 6.3±1.5]. The carriers of the G allele reported pain intensity between [1.3±0.8 - 7.3±1.2].

Table 1 General characteristics of the independent samples retrieved from the included studies on the c.118A>G polymorphism *of OPRM1 gene*

Study	n	Type of population		Genotype information				Analgesia	Pain intensity scores
		Race	Condition	AA	AG/GG	MAF	HWE		
[1] KLEPSTAD 2004[30]	99	Caucasian	Cancer	0.788	0.212	0.104	no data	MOR	Pain scores in last 24h, NRS AA=1.9±1.5 *G =2.9±1.2
[2] JANICKI 2006[31]	101	Caucasian Black	Surgery	0.693	0.307	0.158	yes	MOR	Pain scores at 24h, NRS AA=3.3±0.3 *G =3.3±0.4
[3] CHOU 2006a[32]	120	Asian	Surgery	0.617	0.383	0.245	no data	MOR	Pain scores at 24h, VAS AA=2.2±1.1 *G =2.2±0.9
[4] CHOU 2006b[33] (1)	80	Asian	Surgery	0.538	0.463	0.344	no data	MOR	Pain scores at 0.5h, VAS AA=5.8±2.8 *G =6.4±2.3
[4] CHOU 2006b[33] (2)	80	Asian	Surgery	0.538	0.463	0.344	no data	MOR	Pain scores at 01h, VAS AA=5.3±2.4 *G =5.0±1.9
[4] CHOU 2006b[33] (3)	80	Asian	Surgery	0.538	0.463	0.344	no data	MOR	Pain scores at 1.5h, VAS AA=4.3±2.2 *G =4.1±1.8
[4] CHOU 2006b[33] (4)	80	Asian	Surgery	0.538	0.463	0.344	no data	MOR	Pain scores at 06h, VAS AA=3.8±1.8 *G =3.7±1.4
[4] CHOU 2006b[33] (5)	80	Asian	Surgery	0.538	0.463	0.344	no data	MOR	Pain scores at 24h, VAS AA=2.8±1.2 *G =2.8±0.8
[4] CHOU 2006b[33] (6)	80	Asian	Surgery	0.538	0.463	0.344	no data	MOR	Pain scores at 48h, VAS AA=2.1±0.9 *G =2.1±0.9
[5] CAMPA 2008[34]	138	Caucasian	Cancer	0.768	0.232	0.152	no data	MOR	Δ pain intensity, NRS AA =3.7±1.7 *G =1.9±1.7
[6] REYES-GIBBY 2007[29]	207	Caucasian	Cancer	0.512	0.155	0.152	yes	MOR	Pain scores in last 24h, NRS AA=3.6±2.6 *G =3.4±1.9
[7] SIA 2008[35]	585	Asian	Surgery	0.463	0.537	0.340	yes	MOR	Pain scores at 24h, VAS AA=2.8±3.3 *G =3.5±3.6
[8] FUKUDA 2009[15]	280	Asian	Surgery	0.307	0.693	0.438	yes	FEN	Pain scores at 24h, VAS AA=2.7±0.7 *G =2.5±0.8

Table 1 *continuation*

[9] ZHANG 2010[36] (1)	165	Asian	Surgery	0.485	0.515	0.324	yes	FEN	Pain scores post-op, VAS AA=5.8±1.4 *G =5.5±1.7
[9] ZHANG 2010[36] (2)	165	Asian	Surgery	0.485	0.515	0.324	yes	FEN	Pain scores at 24h, VAS AA=2.1±0.9 *G =2.3±0.7
[10] ZHANG 2011[37]	174	Asian	Surgery	0.494	0.506	0.313	yes	FEN	Pain scores post-op, VAS AA=6.2±1.3 *G =6.1±1.1
[11] ZWISLER 2012[38]	266	Caucasian	Surgery	0.823	0.177	0.100	yes	OXY	Pain scores at 24h, NRS AA=1.6±1.1 *G =1.3±0.8
[12] MENON 2012[39]	153	Caucasian	Chronic migraine	0.778	0.222	0.118	yes	No	Pain score, NRS AA=6.3±1.5 *G =7.3±1.2
[13] BOSWELL 2013[40]	158	Caucasian	Surgery	0.829	0.171	0.171	yes	HYDROM	Pain scores D3 postop, VAS AA= 4.4±2.4 *G = 4.3±2.0
[14] LANDAU 2013[41]	98	Caucasian Asian Other	Labour	0.602	0.398	0.224	yes	FEN	Δ pain intensity NRS AA=3.2±2.0 *G =3.5±2.3
[15] LIAO 2013[42] (1)	97	Asian	Surgery	0.433	0.567	0.356	yes	FEN	Pain scores at 24h, VAS AA= 2.0±4.2 *G = 1.9±4.2
[15] LIAO 2013[42] (2)	97	Asian	Surgery	0.433	0.567	0.356	yes	FEN	Pain scores at 48h, VAS AA= 1.9±3.4 *G = 1.9±3.4
[16] KOLESNIKOV 2013[43]	102	Caucasian	Surgery	0.824	0.176	0.100	yes	Non-op	Pain score AA= 3.5±1.3 *G = 5.4±2.1
[17] XIA 2014[44]	163	Caucasian Black	Acute pain at ED	0.736	0.264	0.144	no data	HYDROM	Δ pain intensity, NRS AA=5.3±0.8 *G =6.5±0.7
[18] CAJANUS 2014[45] (1)	993	Caucasian	Surgery	0.635	0.365	0.200	yes	OXY	Pain scores rest, NRS AA= 2.1±2.5 *G = 2.6±2.7
[18] CAJANUS 2014[45] (2)	993	Caucasian	Surgery	0.635	0.365	0.200	yes	OXY	Pain scores mov, NRS AA= 5.0±1.4 *G = 5.1±1.5
[19] HASVIK 2014[46] (1) male patients	50	Caucasian	Disc Herniation	0.720	0.280	no data	no data	No	Pain baseline scores, NRS AA= 6.1±2.7 *G =5.7±3.1
[19] HASVIK 2014[46] (2) male patients	50	Caucasian	Disc Herniation	0.720	0.280	no data	no data	No	Pain at 1 year scores, NRS AA= 3.5±3.1 *G =1.5±5.4
[19] HASVIK 2014[46] (3) female patients	68	Caucasian	Disc Herniation	0.868	0.132	no data	no data	No	Pain baseline scores, NRS AA= 5.7±2.7 *G =5.2±2.5
[19] HASVIK 2014[46] (4) female patients	68	Caucasian	Disc Herniation	0.868	0.132	no data	no data	No	Pain at 1 year scores, NRS AA= 3.1±2.9 *G =5.4±2.3
[20]LINNSTAEDT 2015[47] (1) female patients	575	Caucasian	Musculo-skeletal pain	0.769	0.230	no data	yes	No	Pain scores at 6 weeks, NRS AA=4.2±0.2 *G =4.0±0.2
[20]LINNSTAEDT 2015[47] (2) male patients	373	Caucasian	Musculo-skeletal pain	0.780	0.220	no data	yes	No	Pain scores at 6 weeks, NRS AA=3.1±0.1 *G =4.0±0.2

*G: carriers of the G allele (AG and GG patients); MAF: Minor Allele Frequency, HWE: Hardy-Weinberg Equilibrium, MOR: Morphine, FEN: Fentanyl, OXY: Oxycodone, TRAM: Tramadol, HYDROM: Hydromorphone, MEP: Meperidine, ED: Emergency Department, VAS: Visual Analogue Scale, NRS: Numeric Rating Scale

Primary analysis The primary analysis focused on the effect of the presence of the G allele against wild type genotype on pain intensity scores (AG/GG versus AA). Significant heterogeneity is present across the studies (I^2=96%, Chi^2=755.82, p<0.00001). The meta-analysis showed differences between the AA and the G carriers with a non-significant SMD of 0.25 (95% CI [-0.05 – 0.55], p=0.10). However, significant differences between AA and G

carriers are present at the sample level[35,39,43,45,47,48]. All but the male sample from Linnstaedt study[47] showed that G carriers report higher pain intensity scores compared to the AA patients (Figure 2).

Study or Subgroup	AG/GG Mean	AG/GG SD	AG/GG Total	AA Mean	AA SD	AA Total	Weight	Std. Mean Difference IV, Random, 95% CI
Boswell 2012	4.3	2	27	4.4	2.4	131	3.5%	-0.04 [-0.46, 0.37]
Cajanus 2014 (1)	2.6	2.7	362	2.1	2.5	631	3.7%	0.19 [0.06, 0.32]
Cajanus 2014 (2)	5.1	1.5	362	5	1.4	631	3.7%	0.07 [-0.06, 0.20]
Chou 2006a	2.2	0.9	46	2.2	1.1	74	3.5%	0.00 [-0.37, 0.37]
Chou 2006b (1)	6.4	2.3	37	5.8	2.8	43	3.4%	0.23 [-0.21, 0.67]
Chou 2006b (2)	5	1.9	37	5.3	2.4	43	3.4%	-0.14 [-0.58, 0.30]
Chou 2006b (3)	4.1	1.8	37	4.3	2.2	43	3.4%	-0.10 [-0.54, 0.34]
Chou 2006b (4)	3.7	1.4	37	3.8	1.8	43	3.4%	-0.06 [-0.50, 0.38]
Chou 2006b (5)	2.8	0.8	37	2.8	1.2	43	3.4%	0.00 [-0.44, 0.44]
Chou 2006b (6)	2.1	0.9	37	2.1	0.9	43	3.4%	0.00 [-0.44, 0.44]
Fukuda 2009	2.5	0.8	194	2.7	0.7	86	3.6%	-0.26 [-0.51, -0.00]
Hasvik 2014 (1)	5.7	3.1	14	6.1	2.7	36	3.2%	-0.14 [-0.76, 0.48]
Hasvik 2014 (2)	1.5	5.4	14	3.5	3.1	36	3.2%	-0.51 [-1.14, 0.12]
Hasvik 2014 (3)	5.2	2.5	9	5.7	2.7	59	3.1%	-0.18 [-0.89, 0.52]
Hasvik 2014 (4)	5.4	2.3	9	3.1	29	59	3.1%	0.08 [-0.62, 0.79]
Janicki 2006	3.3	0.4	31	3.3	0.3	70	3.5%	0.00 [-0.42, 0.42]
Klepstad 2004	2.9	1.2	21	1.9	1.5	78	3.4%	0.69 [0.20, 1.18]
Kolesnikov 2013	5.4	2.1	18	3.5	1.3	84	3.3%	1.29 [0.75, 1.83]
Liao 2013 (1)	1.9	4.2	55	2	4.2	42	3.5%	-0.02 [-0.43, 0.38]
Liao 2013 (2)	1.9	3.4	55	1.9	3.4	42	3.5%	0.00 [-0.40, 0.40]
Linnstaedt 2015 (1)	4	0.2	132	4.2	0.2	442	3.6%	-1.00 [-1.20, -0.80]
Linnstaedt 2015 (2)	4	0.2	82	3.1	0.1	290	3.3%	6.98 [6.42, 7.54]
Menon 2012	7.3	1.2	34	6.3	1.5	119	3.5%	0.69 [0.30, 1.08]
Reyes-Gibby 2007	3.4	1.9	32	3.6	2.6	106	3.5%	-0.08 [-0.48, 0.31]
Sia 2008	3.5	3.6	314	2.8	3.3	271	3.7%	0.20 [0.04, 0.36]
Zhang 2010a (1)	5.5	1.7	85	5.8	1.4	80	3.6%	-0.19 [-0.50, 0.11]
Zhang 2010a (2)	2.3	0.7	85	2.1	0.9	80	3.6%	0.25 [-0.06, 0.55]
Zhang 2011	6.1	1.1	88	6.2	1.3	86	3.6%	-0.08 [-0.38, 0.21]
Zwisler 2012	1.3	0.8	47	1.6	1.1	219	3.6%	-0.28 [-0.60, 0.03]
Total (95% CI)			**2338**			**4010**	**100.0%**	**0.25 [-0.05, 0.55]**

Heterogeneity: Tau² = 0.62; Chi² = 755.82, df = 28 (P < 0.00001); I² = 96%
Test for overall effect: Z = 1.63 (P = 0.10)

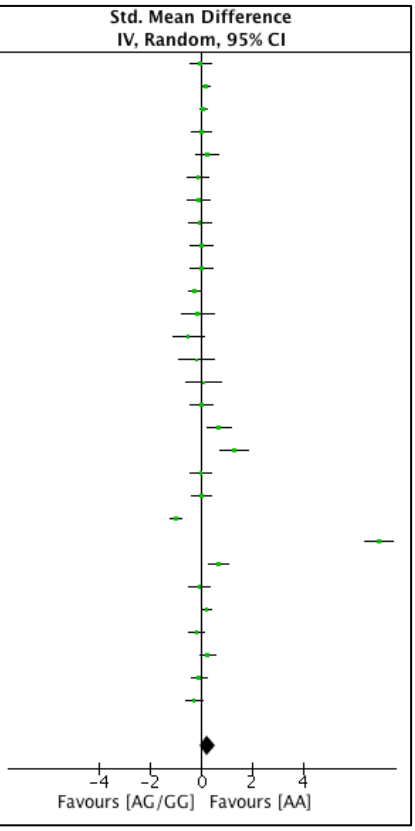

Figure 2 Standardized Means Differences for each independent sample, forest plot and overall results for the meta-analysis on the influence of the G allele on pain intensity scores.

Secondary analysis The secondary analysis focused on the effect of the homozygosity for the G allele against wild type genotype on pain intensity scores (GG versus AA). Seven studies[15,31,38,40,46,47] were excluded from this analysis because there were no specific pain data regarding the GG group. The GG patients exhibited non-significant higher pain intensity scores (random effects model: SMD=0.07, 95% CI [-0.06 – 0.19], p=0.28) compared to wild type homozygous. However, significant differences between AA and GG are present at the sample level[35,43], with AA patients exhibiting a lower pain score compared to GG patients (appendix 1).

Pain intensity variation versus c.118A>G *OPRM1* polymorphism Three studies examined the variation in pain intensity after a pain management intervention. Of the 399 patients enrolled, 285 were genotyped as wild type and 114 are carriers of the minor allele. In AA patients, the decrease in pain intensity scores varied between [3.2±2.0 - 5.3±0.8]. In G carriers, the highest decrease in pain intensity scores reported was 6.5±0.7 and the lowest 1.9±1.7.

Primary analysis The primary analysis focused on the effect of the presence of the G allele against wild type genotype on pain intensity variation (AG/GG versus AA). The results indicate a non-significant tendency to higher pain variation for the AA genotype at 0.21, 95% CI [-1.27 – 1.69]. However, contradictory differences at the sample level were detected[34,44]. Campa and collaborators[34] show that AA patients experience a higher pain decrease comparing to c.118G patients (3.7±1.7 *vs*. 1.9±1.7) after morphine therapy. However, in a sample with hydromorphone analgesia c.118G patients experienced a better pain relief (6.5±0.7 *vs*. 5.3±0.8) (Appendix 2).

Secondary analysis The secondary analysis focused on the effect of the homozygosity for the G allele against wild type genotype on pain intensity variation (GG versus AA). No significant differences were present for this analysis. Only one study[34] showed that AA patients experience a better pain decrease score than GG patients (3.7±1.7 *vs*. 1.8±1.8) (Appendix 3).

4.3. The influence of the p.Val158Met SNP of *COMT* gene on pain responses

Description of the independent samples from the included studies Table 2 shows the general characteristics of the 26 independent samples included. Papers were published between 2007 and 2014. The included studies reported on 1,264 patients with a number of participants per study ranging between 93 and 241. All but two studies were performed in Caucasian patients. Six samples were in HWE. The MAF varied between [0.441 – 0.545]. Most of the studies were conducted in surgical and in cancer patients. Most of the papers analyzed their samples in different stages of the health condition. Five samples reported on pain intensity variation after a pain management intervention.

Table 2 General characteristics of the independent samples retrieved from the included studies on the p.Val158Metpolymorphism of *COMT gene*

Study	n	Type of population		Genotype information				Analgesia	Pain intensity scores
		Race	Condition	ValVal	*Met	MAF	HWE		
[1] REYES-GIBBY 2007[29]	207	Caucasian	Cancer	0.213	0.787	0.441	yes	MOR	Pain scores in last 24h, NRS ValVal=3.9±2.2 *Met=3.6±2.5
[2] FERNANDEZ-DE-LAS-PEÑAS 2012a[49]	100	Caucasian	Fibromyalgia	0.310	0.690	0.465	no data	NSAID	Pain scores last week, NRS ValVal=6.9±1.2 *Met=6.8±1.1
[3] FERNANDEZ-DE-LAS-PEÑAS 2012b[50] (1)	128	Caucasian	Cancer	0.266	0.734	0.484	no data	no data	Neck Pain scores, VAS ValVal=3.8±2.1 *Met=5.8±2.3
[3] FERNANDEZ-DE-LAS-PEÑAS 2012b[50] (2)	128	Caucasian	Cancer	0.266	0.734	0.484	no data	no data	Shoulder Pain scores, VAS ValVal=3.8±2.8 *Met=3.9±2.7
[4] JACOBSEN 2012[51]	241	Caucasian	Lumbar Disc Herniation	0.228	0.772	0.564	yes	no data	Pain scores 6M after, VAS ValVal=2.3±0.3 *Met=3.1±0.5
[5] OMAIR 2012[52] (1)	93	Caucasian	Low Back Pain	0.215	0.785	0.460	yes	no data	Baseline Pain scores, VAS ValVal=6.1±1.4 *Met=6.2±1.4
[5] OMAIR 2012[52] (2)	93	Caucasian	Low Back Pain	0.215	0.785	0.460	yes	no data	Follow-up Pain scores, VAS ValVal=4.5±2.9 *Met=3.6±2.5
[5] OMAIR 2012[52] (3)	93	Caucasian	Low Back Pain	0.215	0.785	0.460	yes	no data	Δ Pain scores, VAS ValVal=1.7±3.1 *Met=2.7±2.6
[6] AHLERS 2013[53] (1)	117	Caucasian African Asian	Surgery	0.256	0.744	0.462	yes	MOR	Overall Pain scores, NRS ValVal=1.2±0.2 *Met=1.7±0.2
[6] AHLERS 2013[53] (2)	117	Caucasian African Asian	Surgery	0.256	0.744	0.462	yes	MOR	Highest Pain scores, NRS ValVal=1.7±1.7 *Met=2.9±2.5
[6] AHLERS 2013[53] (3)	117	Caucasian African Asian	Surgery	0.256	0.744	0.462	yes	MOR	Pain scores at rest, NRS ValVal=1.6±1.8 *Met=1.6±1.8
[6] AHLERS 2013[53] (4)	117	Caucasian African Asian	Surgery	0.256	0.744	0.462	yes	MOR	Pain scores before proc, NRS ValVal=0.9±1.4 *Met=1.5±1.7

[6] AHLERS 2013[53] (5)	117	Caucasian African Asian	Surgery	0.256	0.744	0.462	yes	MOR	Pain scores during proc, NRS ValVal=1.7±1.7 *Met=2.9±2.5
[6] AHLERS 2013[53] (6)	117	Caucasian African Asian	Surgery	0.256	0.744	0.462	yes	MOR	Pain scores after proc, NRS ValVal=1.0±1.2 *Met=1.3±1.7
[7] LANDAU 2013[41]	102	Caucasian Asian Other	Labour	0.220	0.780	0.455	yes	FEN	Δ Pain intensity, NRS ValVal=3.5±2.2 *Met=3.2±2.2
[8] KOLESNIKOV 2013[43]	102	Caucasian	Surgery	0.245	0.755	0.490	yes	Non-op	Pain scores, NRS ValVal=3.8±1.2 *Met=3.8±1.6
[9] RUT 2014[54] (1)	176	Caucasian	Surgery	0.261	0.739	0.490	yes	no data	Total Pain score pre-op, VAS ValVal=3.4±0.9 *Met=3.0±1.3
[9] RUT 2014[54] (2)	176	Caucasian	Surgery	0.261	0.739	0.490	yes	no data	Total Pain score post-op, VAS ValVal=2.0±1.5 *Met=1.7±1.6
[9] RUT 2014[54] (3)	176	Caucasian	Surgery	0.261	0.739	0.490	yes	no data	Δ Total Pain score, VAS ValVal=1.4±1.7 *Met=1.3±1.8
[9] RUT 2014[54] (4)	176	Caucasian	Surgery	0.261	0.739	0.490	yes	no data	Back Pain score pre-op, VAS ValVal=7.4±2.1 *Met=6.4±2.8
[9] RUT 2014[54] (5)	176	Caucasian	Surgery	0.261	0.739	0.490	yes	no data	Back Pain score post-op, VAS ValVal=4.1±2.5 *Met=4.6±2.9
[9] RUT 2014[54] (6)	176	Caucasian	Surgery	0.261	0.739	0.490	yes	no data	Δ Back Pain score, VAS ValVal=3.3±2.6 *Met=1.9±2.9
[9] RUT 2014[54] (7)	176	Caucasian	Surgery	0.261	0.739	0.490	yes	no data	Legs Pain score pre-op, VAS ValVal= 7.3±2.1 *Met= 6.9±2.7
[9] RUT 2014[54] (8)	176	Caucasian	Surgery	0.261	0.739	0.490	yes	no data	Legs Pain score post-op, VAS ValVal= 4.1±2.9 *Met= 3.9±3.3
[9] RUT 2014[54] (9)	176	Caucasian	Surgery	0.261	0.739	0.490	yes	no data	Δ Legs Pain score, VAS ValVal= 3.2±3.4 *Met= 2.9±3.7

*Met: carriers of the Met allele (Val/Met and Met/Met); MAF: Minor Allele Frequency, HWE: Hardy-Weinber Equilibrium, MOR: Morphine, NSAID: Non Steroidal Anti-Inflammatory Drug, VAS: Visual Analogue Scale, NRS: Numeric Rating Scale.

Pain severity versus p.Val158Met *COMT* polymorphism The final analysis yields 20 independents samples, enrolling 2,850 participants. Of these, 719 are homozygous Val/Val and 2,131 are Met carriers. For the Val/Val patients, pain intensity scores varied between [0.9±1.4 – 7.4±2.1]. The carriers of the Met allele reported pain intensity between [1.3±1.7 – 6.9±1.7].

Primary analysis The primary analysis focused on the effect of the presence of the Met allele against wild type genotype on pain intensity scores (Val/Met&Met/Met versus Val/Val). Significant heterogeneity is present across the studies (I^2=91%, p<0.00001). The SMD was non-significant at 0.26 (95% CI [-0.03 – 0.55]). However, significant differences

between Val/Val and Met carriers are present at the sample level[50,51,53,54]. All but one[54] show that the carriers of the Met allele report higher pain intensity scores, when compared to the Val/Val patients (Figure 3).

Secondary analysis The secondary analysis focused on the effect of the homozygosity for the Met allele against wild type genotype on pain intensity scores (Met/Met and Val/Val). There was a SMD of 0.39 favouring the Val/Val patients with lower pain intensity scores (95% CI [-0.05 – 0.83]), albeit non-significant. However, significant differences between Val/Val and Met/Met are present at the sample level[50,51,53,54]. All but three samples from Rut study[54] indicate that Met/Met is associated with higher pain intensity scores (Appendix 4).

Study or Subgroup	ValMet / MetMet Mean	SD	Total	ValVal Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Ahlers 2013 (1)	1.7	0.2	87	1.2	0.2	30	4.7%	2.48 [1.96, 3.01]
Ahlers 2013 (2)	2.9	2.5	87	1.7	1.7	30	5.0%	0.51 [0.09, 0.93]
Ahlers 2013 (3)	1.6	1.8	87	1.6	1.8	30	5.0%	0.00 [-0.41, 0.41]
Ahlers 2013 (4)	1.5	1.7	87	0.9	1.4	30	5.0%	0.37 [-0.05, 0.78]
Ahlers 2013 (5)	2.9	2.5	87	1.7	1.7	30	5.0%	0.51 [0.09, 0.93]
Ahlers 2013 (6)	1.3	1.7	87	1	1.2	30	5.0%	0.19 [-0.23, 0.60]
Fernández-de-las-Peñas 2012a	6.8	1.1	69	6.9	1.2	31	4.9%	-0.09 [-0.51, 0.34]
Fernández-de-las-Peñas 2012b (1)	5.8	2.3	94	3.8	2.1	34	5.0%	0.88 [0.48, 1.29]
Fernández-de-las-Peñas 2012b (2)	3.9	2.7	94	3.8	2.8	34	5.0%	0.04 [-0.36, 0.43]
Jacobsen 2012	3.1	0.5	186	2.3	0.3	55	5.1%	1.72 [1.39, 2.06]
Kolesnikov 2013	3.8	1.6	77	3.8	1.2	25	4.9%	0.00 [-0.45, 0.45]
Omair 2012 (1)	6.2	1.4	73	6.1	1.4	20	4.8%	0.07 [-0.42, 0.57]
Omair 2012 (2)	3.6	2.5	73	4.5	2.9	20	4.8%	-0.34 [-0.84, 0.15]
Reyes-Gibby 2007	3.6	2.5	163	3.9	2.2	44	5.2%	-0.12 [-0.46, 0.21]
Rut 2014 (1)	3	1.3	130	3.4	0.9	46	5.1%	-0.33 [-0.67, 0.01]
Rut 2014 (2)	1.7	1.6	130	2	1.5	46	5.1%	-0.19 [-0.53, 0.15]
Rut 2014 (4)	6.4	2.8	130	7.4	2.1	46	5.1%	-0.38 [-0.72, -0.04]
Rut 2014 (5)	4.6	2.9	130	4.1	2.5	46	5.1%	0.18 [-0.16, 0.51]
Rut 2014 (7)	6.9	2.7	130	7.3	2.1	46	5.1%	-0.16 [-0.49, 0.18]
Rut 2014 (8)	3.9	3.3	130	4.1	2.9	46	5.1%	-0.06 [-0.40, 0.27]
Total (95% CI)			**2131**			**719**	**100.0%**	**0.26 [-0.03, 0.55]**

Heterogeneity: Tau² = 0.39; Chi² = 210.84, df = 19 (P < 0.00001); I² = 91%
Test for overall effect: Z = 1.74 (P = 0.08)

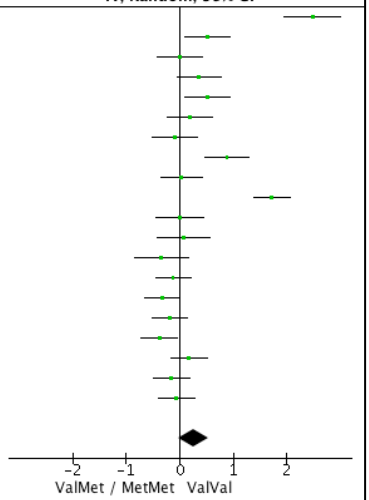

Figure 3 Standardized Mean Differences for each independent sample, forest plot and overall results for the meta-analysis on the influence of the Met allele on pain intensity scores.

Pain intensity variation versus p.Val158Met *COMT* polymorphism Five independent samples reported data on pain intensity variation after a pain management intervention. The analysis was performed with 621 patients. Of the patients enrolled in this model, 158 were genotyped as wild type and 463 are carriers of the minor allele. In Val/Val patients, the

decrease in pain intensity scores varied between [1.4±1.7 - 3.3±2.6] and for Met carriers between [1.3±1.8 – 2.9±3.7].

Primary analysis The primary analysis focused on the effect of the presence of the Met allele against wild type genotype on pain intensity variation (Val/Met&Met/Met versus Val/Val). The SMD was non-significant at -0.13 (95% CI [-0.31 – 0.005]). Only the post-operative decrease in pain intensity on the back in patients submitted to lumbar spine surgery was significant, with Met carriers exhibiting a lower pain decrease than Val/Val patients (1.9±2.9 *vs*. 3.3±2.6, SMD=0.49, 95% CI [0.15 to 0.83], $p<0.05$) (Appendix 5).

Secondary analysis The secondary analysis focused on the effect of the homozygosis for the Met allele against wild type genotype on pain intensity variation (Met/Met versus Val/Val). No significant differences were found for this analysis. Only one independent sample showed that Val/Val patients experience a better pain management than Met/Met patients (3.3±2.6 *vs*. 1.7±2.9) (Appendix 6).

Chapter 5

Discussion

In this chapter, the findings will be summarized and discussed in the light of the state of art. Also, theoretical issues and implications will be pointed out.

Summary of main results Twenty-nine studies were included, twenty reported data on the c.118A>G of *OPRM1* gene and nine on the p.Val158Met *COMT* variants. Most of the studies measured pain scores in a given time point and only a few studies examined pain intensity variation after a pain management intervention. The majority reported data on Caucasian patients submitted to surgery or being treated for cancer and were in accordance with the principle of the HWE. Data was highly heterogeneous among studies.

Regarding the c.118A>G polymorphism, the meta-analysis showed a non-significant difference of 0.25 (CI 95% -0.05 to 0.55) between the AA patients and those carrying the G allele, with the homozygous for the wild type variant reporting a lower pain intensity score. Also, homozygosity for G allele did not confer increased pain intensity. Albeit, significant differences were evident at the sample level in a quite remarkable number of studies. All but one indicated that G carriers are more prone to report higher pain intensity scores or a lower decrease in pain intensity after pain management (Figure 2).

When analyzing the *COMT* p.Val158Met polymorphism, pooled data showed differences between the genotypes ranging from -0.03 and 0.55 units with a non significant SMD of 0.26,

favoring the Val/Val patients with a lower pain intensity score. Homozygosity for the Met allele was not associated to increased pain intensity. However, significant differences were found in some samples. All but three sample analyses suggested that the carriers of the Met allele are likely to have high intensity pain scores and a lower decrease after drug-induced pain control (Figure 3).

Theoretical issues At the functional level, there is evidence that the *OPRM1* c.118A>G influences pain mechanisms. It is documented that G variant yields 10 times less binding sites when comparing to the wild type[11] and that the SNP reduces the potency of the active M6G metabolite of morphine in humans[55]. This may explain why AA patients experience a more effective continuing endogenous pain inhibition and that G carriers do not present the same results[14,35,37]. Indeed, clinical trials reported that GG patients complain of more severe pain and consume higher doses of opioid analgesics to achieve adequate pain relief[56]. Reyes-Gibby and collaborators[29] estimated, in a cancer sample, that GG and AG patients required higher morphine doses compared to AA patients (93% and 18%, respectively). However, contradictory data indicates that the G carriers are less sensitive to nociceptive stimuli[9] and exhibit a reduced pupil constriction[55], probably because an enhanced endogenous opioid system is occurring in G carriers[57]. Replication of the association between the *OPRM1* c.118A>G and pain sensitivity failed in experimental pain sensitivity[37,58] and in clinical post-operative pain[32,33,37,59].

It has been proposed that the reduced COMT activity caused by the Met allele is associated with a reduced encephalin content in pain brain regions and with high levels of cathecolamines, which promotes persistent pain through beta-adrenergic central and peripheral stimulation[21]. Yet the p.Val158Met showed a marginal and non-significant relation with pain sensitivity in experimental[21,60] and clinical studies[61–63]. Still, in a model of opioid

induced hyperalgesia, the Met allele was associated to higher pain intensity scores[25]. Also, the Met allele is considered a risk factor in multiple sclerosis[64] and fibromyalgia[65,66] patients for reporting high pain scores. Indeed, p.Val158Met polymorphism may be more relevant in temporal summation of pain[67] and differences in pain ratings may be more expressed in individuals where the inhibitory descendent pain system is already challenged, as it is the case of chronic pain patients[25]. However, the presence of the risk-allele was associated to a better response to morphine in chronic low back pain[68] and surgical patients[69]. The opposite direction was found in patients with migraine treated with triptans[68].

Clearly, the influence of both of polymorphisms under review is controversial. In the present meta-analysis although a number of studies recruited large samples, there was a wide variability among the studies concerning this item.

Reviews concerning the two polymorphisms also document contradictory data. No consistent association was either found in a meta-analysis of eight studies that addressed the influence of c.118A>G on pain, opioid dosing and opioid side effects. Only weak association with less nausea and higher opioid doses in GG patients was reported[17]. The effect of p.Val158Met depends on the pain condition, i.e., it was found that the Met allele confers additional risk for patients with fibromyalgia and chronic widespread pain, but not for migraine or chronic musculoskeletal pain[70].

Our results show that, on average, the effects of both c.118A>G and p.Val158Met pointed toward the expected direction but effects sizes were small, non-significant and showed wide 95% CI. Also, some statistical significant differences at sample level were from studies that did not report HWE. If samples were not in HWE conclusions cannot be made from these studies, due to the fact that the disequilibrium would account for the results rather than the association.

Even though additional studies are needed to clarify the role of these two polymorphisms, its effect size will probably remain small and influenced by a number of co-variates. Therefore, c.118A>G *OPRM1* and p.Val158Met *COMT* genotyping continues to be of more scientific rather than of clinical interest, discouraging personalized pain therapy based on the genotypes concerning these variants.

Limitations of this review The results from this study are prone to several confounders including, but not limited to the patients having different pain modalities, different co-medications and different intensities of other co-symptoms. However, in clinical studies, such confounders are inherent features of the patient population and it may be difficult to overcome them.

Although it was interesting to know if the number of risk-alleles contributed to a higher pain susceptibility, we were not able to conduct this analysis. Also, we were not able to conduct subgroup analysis to test whether sex, ethnicity and health conditions influenced the results somehow. In addition, a regression model analysis would be helpful to determine if genotype is a potential co-variate in pain ratings, but we were not able to conduct this analysis.

Conclusion

At any given moment, half of the world population will experience pain. Two common polymorphisms on the *OPRM1* and the *COMT* genes have been associated to pain phenotypes; the c.118A>G and the p.Val158Met, respectively. Meta-analysis comparing the presence of the risk-allele against the wild type for both the c.118A>G and the p.Val158Met variants showed results in the expected direction, albeit non-significant. The homozygosity for the risk-allele did not confer additional pain susceptibility. Although we believe that the risk-alleles appear to be associated with higher pain intensity scores, the extent of benefit may not be as large as we would expect and it is influenced by various factors.

Implications for clinical practice The results of this review are applicable to adult patients mainly submitted to a surgery or diagnosed with cancer. Whether the same results would be identified in healthy volunteers is uncertain. This review cannot make any summary statement regarding the influence of both the *OPRM1* c.118A>G and the *COMT* p.Val158Met polymorphisms on pain intensity, as the studies are highly heterogeneous and of borderline statistical significance.

Although we believe that the two polymorphisms are linked to pain susceptibility, the degree of influence may not be significant. Available evidence does not support the use of genotyping of the two variants under analysis to tailor pharmacological pain management.

Implications for research There are numerous areas under this topic that requires further research before definitive statements can be made. To begin with, studies with clearly characterized samples, outcome measures and end-points are needed, so that the size effect and its significance can be better estimated and the applicability can be more properly addressed.

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Appendixes

Appendix 1

Study or Subgroup	GG Mean	GG SD	GG Total	AA Mean	AA SD	AA Total	Weight	Std. Mean Difference IV, Random, 95% CI
Cajanus 2014 (1)	2.8	2.6	35	2.1	2.5	631	9.9%	0.28 [-0.06, 0.62]
Cajanus 2014 (2)	5.1	1.6	35	5	1.4	631	9.9%	0.07 [-0.27, 0.41]
Chou 2006a	2.2	1	32	2.2	1.1	74	7.3%	0.00 [-0.41, 0.41]
Chou 2006b (1)	6.2	2.5	18	5.8	2.8	43	4.5%	0.15 [-0.41, 0.70]
Chou 2006b (2)	5.2	2.1	18	5.3	2.4	43	4.5%	-0.04 [-0.59, 0.51]
Chou 2006b (3)	4.4	2	18	4.3	2.2	43	4.5%	0.05 [-0.50, 0.60]
Chou 2006b (4)	3.8	1.4	18	3.8	1.8	43	4.5%	0.00 [-0.55, 0.55]
Chou 2006b (5)	2.9	1	18	2.8	1.2	43	4.5%	0.09 [-0.46, 0.64]
Chou 2006b (6)	1.9	0.8	18	2.1	0.9	43	4.5%	-0.23 [-0.78, 0.33]
Klepstad 2004	2	1.2	4	1.9	1.5	78	1.5%	0.07 [-0.94, 1.07]
Kolesnikov 2013	6	0.1	3	3.5	1.3	84	1.1%	1.93 [0.74, 3.12]
Liao 2013 (1)	1.9	4.2	14	2	4.2	42	3.8%	-0.02 [-0.63, 0.58]
Liao 2013 (2)	1.9	3.7	14	1.9	3.4	42	3.8%	0.00 [-0.60, 0.60]
Reyes-Gibby 2007	2	1.2	10	3.6	2.6	106	3.3%	-0.63 [-1.28, 0.02]
Sia 2008	3.7	4	80	2.8	3.3	271	14.9%	0.26 [0.01, 0.51]
Zhang 2010a (1)	5.7	1.7	22	5.8	1.4	80	5.9%	-0.07 [-0.54, 0.40]
Zhang 2010a (2)	2.2	0.8	22	2.1	0.9	80	5.9%	0.11 [-0.36, 0.58]
Zhang 2011	2	0.7	21	2.1	0.8	86	5.8%	-0.13 [-0.60, 0.35]
Total (95% CI)			**400**			**2463**	**100.0%**	**0.07 [-0.06, 0.19]**

Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 20.09$, df = 17 (P = 0.27); $I^2 = 15\%$
Test for overall effect: Z = 1.08 (P = 0.28)

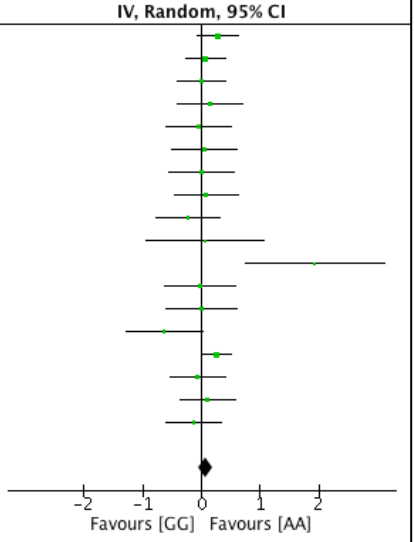
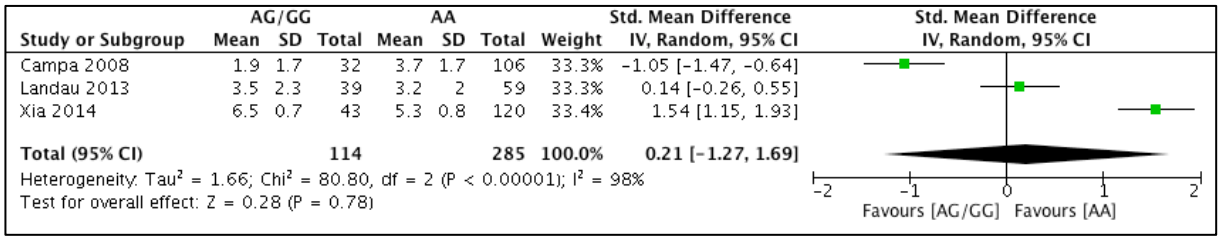


Figure A1. Relative SMD for each independent sample, forest plot, overall results of the meta-analysis between pain intensity and GG *versus* AA genotype carriers.

Appendix 2

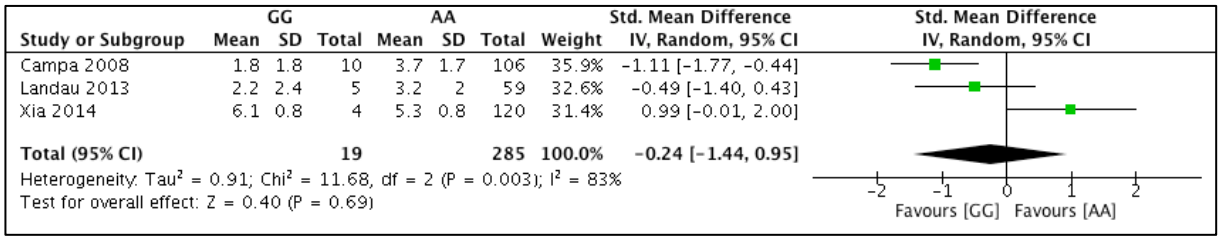


Study or Subgroup	AG/GG Mean	AG/GG SD	AG/GG Total	AA Mean	AA SD	AA Total	Weight	Std. Mean Difference IV, Random, 95% CI
Campa 2008	1.9	1.7	32	3.7	1.7	106	33.3%	-1.05 [-1.47, -0.64]
Landau 2013	3.5	2.3	39	3.2	2	59	33.3%	0.14 [-0.26, 0.55]
Xia 2014	6.5	0.7	43	5.3	0.8	120	33.4%	1.54 [1.15, 1.93]
Total (95% CI)			**114**			**285**	**100.0%**	**0.21 [-1.27, 1.69]**

Heterogeneity: $Tau^2 = 1.66$; $Chi^2 = 80.80$, df = 2 ($P < 0.00001$); $I^2 = 98\%$
Test for overall effect: Z = 0.28 (P = 0.78)

Figure A2. Relative SMD for each independent sample, forest plot, overall results of the meta-analysis between pain intensity variation and AA *versus* *G carriers.

Appendix 3



Study or Subgroup	GG Mean	GG SD	GG Total	AA Mean	AA SD	AA Total	Weight	Std. Mean Difference IV, Random, 95% CI
Campa 2008	1.8	1.8	10	3.7	1.7	106	35.9%	-1.11 [-1.77, -0.44]
Landau 2013	2.2	2.4	5	3.2	2	59	32.6%	-0.49 [-1.40, 0.43]
Xia 2014	6.1	0.8	4	5.3	0.8	120	31.4%	0.99 [-0.01, 2.00]
Total (95% CI)			**19**			**285**	**100.0%**	**-0.24 [-1.44, 0.95]**

Heterogeneity: Tau² = 0.91; Chi² = 11.68, df = 2 (P = 0.003); I² = 83%
Test for overall effect: Z = 0.40 (P = 0.69)

Figure A3. Relative SMD for each independent sample, forest plot, overall results of the meta-analysis between pain intensity variation and AA *versus* GG genotype carriers.

Appendix 4

Study or Subgroup	MetMet Mean	MetMet SD	MetMet Total	ValVal Mean	ValVal SD	ValVal Total	Weight	Std. Mean Difference IV, Random, 95% CI
Ahlers 2013 (1)	1.9	0.2	21	1.2	0.2	30	4.4%	3.45 [2.55, 4.34]
Ahlers 2013 (2)	3.4	2.8	21	1.7	1.7	30	4.9%	0.76 [0.18, 1.33]
Ahlers 2013 (3)	1.8	2	21	1.6	1.8	30	5.0%	0.10 [-0.45, 0.66]
Ahlers 2013 (4)	1.4	1.8	21	0.9	1.4	30	5.0%	0.31 [-0.25, 0.87]
Ahlers 2013 (5)	3.4	2.8	21	1.7	1.7	30	4.9%	0.76 [0.18, 1.33]
Ahlers 2013 (6)	1.7	2.1	21	1	1.2	30	5.0%	0.42 [-0.14, 0.99]
Fernández-de-las-Peñas 2012a	6.9	1.1	24	6.9	1.2	31	5.0%	0.00 [-0.53, 0.53]
Fernández-de-las-Peñas 2012b (1)	6.7	2.2	30	3.8	2.1	34	5.0%	1.33 [0.79, 1.88]
Fernández-de-las-Peñas 2012b (2)	4.2	3	30	3.8	2.8	34	5.1%	0.14 [-0.36, 0.63]
Jacobsen 2012	3.5	0.3	86	2.3	0.3	55	4.9%	3.98 [3.40, 4.56]
Kolesnikov 2013	3.3	1.7	22	3.8	1.2	25	4.9%	-0.34 [-0.92, 0.24]
Omair 2012 (1)	5.9	1.4	27	6.1	1.4	20	4.9%	-0.14 [-0.72, 0.44]
Omair 2012 (2)	4.3	1.8	27	4.5	2.9	20	4.9%	-0.08 [-0.66, 0.49]
Reyes-Gibby 2007	3.5	2.3	67	3.9	2.2	44	5.2%	-0.18 [-0.56, 0.21]
Rut 2014 (1)	2.7	1.5	43	3.4	0.9	46	5.1%	-0.57 [-0.99, -0.14]
Rut 2014 (2)	1.3	1.3	43	2	1.5	46	5.1%	-0.49 [-0.92, -0.07]
Rut 2014 (4)	6	2.1	43	7.4	2.1	46	5.1%	-0.66 [-1.09, -0.23]
Rut 2014 (5)	4.3	2.5	43	4.1	2.9	46	5.1%	0.07 [-0.34, 0.49]
Rut 2014 (7)	6.4	3.3	43	7.3	2.1	46	5.1%	-0.32 [-0.74, 0.09]
Rut 2014 (8)	3.5	2.7	43	4.1	2.9	46	5.1%	-0.21 [-0.63, 0.20]
Total (95% CI)			**697**			**719**	**100.0%**	**0.39 [-0.05, 0.83]**

Heterogeneity: Tau² = 0.95; Chi² = 296.63, df = 19 (P < 0.00001); I² = 94%
Test for overall effect: Z = 1.72 (P = 0.09)

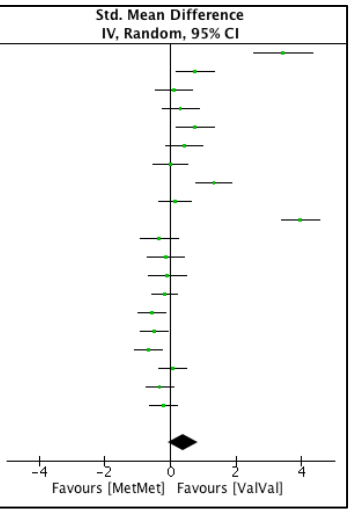
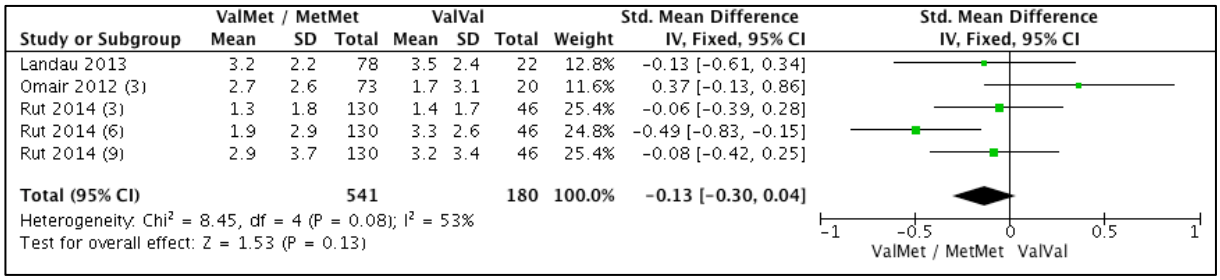


Figure A4. Relative SMD for each independent sample, forest plot, overall results of the meta-analysis between pain intensity and Val/Val *versus* Met/Met genotype carriers.

Appendix 5



Study or Subgroup	ValMet / MetMet Mean	SD	Total	ValVal Mean	SD	Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
Landau 2013	3.2	2.2	78	3.5	2.4	22	12.8%	-0.13 [-0.61, 0.34]
Omair 2012 (3)	2.7	2.6	73	1.7	3.1	20	11.6%	0.37 [-0.13, 0.86]
Rut 2014 (3)	1.3	1.8	130	1.4	1.7	46	25.4%	-0.06 [-0.39, 0.28]
Rut 2014 (6)	1.9	2.9	130	3.3	2.6	46	24.8%	-0.49 [-0.83, -0.15]
Rut 2014 (9)	2.9	3.7	130	3.2	3.4	46	25.4%	-0.08 [-0.42, 0.25]
Total (95% CI)			**541**			**180**	**100.0%**	**-0.13 [-0.30, 0.04]**

Heterogeneity: $Chi^2 = 8.45$, df = 4 (P = 0.08); $I^2 = 53\%$
Test for overall effect: Z = 1.53 (P = 0.13)

Figure A5. Relative SMD for each independent sample, forest plot, overall results of the meta-analysis between pain intensity variation and Val/Val *versus* Val/Met / Met/Met genotype carriers.

Appendix 6

Study or Subgroup	MetMet Mean	MetMet SD	MetMet Total	ValVal Mean	ValVal SD	ValVal Total	Weight	Std. Mean Difference IV, Random, 95% CI
Landau 2013	2.4	1.8	31	3.5	2.4	22	16.2%	-0.52 [-1.08, 0.03]
Omair 2012 (3)	1.8	2.5	27	1.7	3.1	20	15.3%	0.04 [-0.54, 0.61]
Rut 2014 (3)	1.5	1.7	43	1.4	1.7	46	23.0%	0.06 [-0.36, 0.47]
Rut 2014 (6)	1.7	2.9	43	3.3	2.6	46	22.5%	-0.58 [-1.00, -0.15]
Rut 2014 (9)	2.9	3.2	43	3.2	3.4	46	23.0%	-0.09 [-0.51, 0.33]
Total (95% CI)			**187**			**180**	**100.0%**	**-0.22 [-0.49, 0.06]**

Heterogeneity: Tau² = 0.04; Chi² = 6.70, df = 4 (P = 0.15); I² = 40%
Test for overall effect: Z = 1.56 (P = 0.12)

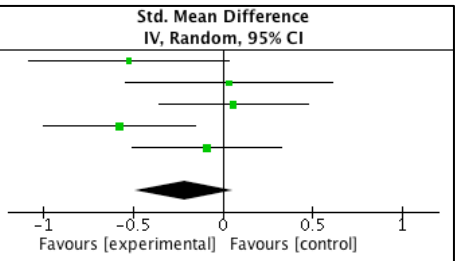


Figure A6. Relative SMD for each independent sample, forest plot, overall results of the meta-analysis between pain intensity variation and Val/Val *versus* Met/Met genotype carriers.