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Abstract

Objective: To analyse the association between the rs4680 Catechol-O-Methyltransferase Val158Met polymorphism and to determine the association of this polymorphism with clinical, psychological and pain sensitivity variables in women with episodic or chronic migraine. **Methods**: Fifty women with episodic migraine, 50 with chronic migraine, and 50 matched healthy women participated. After amplifying Val158Met polymorphism by polymerase chain reaction, we assessed genotype frequencies and allele distributions. Participants were classified according to the Val158Met polymorphism genotype into Val/Val, Val/Met, or Met/Met. A headache diary was used for collecting migraine pain features. Disability was assessed with Migraine Disability Assessment Scale, trait/state anxiety levels with the State-Trait Anxiety Inventory, and depression/anxiety with the Hospital Anxiety and Depression Scale. Pressure pain thresholds (PPT) were bilaterally assessed over the temporalis, the upper trapezius, the second metacarpal and the tibialis anterior. **Results:** The distribution of rs4680 Val158Met genotype was not significantly different between women with/without migraine (P=0.157). No differences in migraine features were found depending on the Val158Met genotype. Women with the Met/Met genotype showed higher migraine-related disability than those with Val/Val or Val/Met genotype in both migraine groups (P<0.01). Women with chronic, but not episodic, migraine with the Met/Met genotype exhibited higher depressive and anxiety levels and lower PPTs than those with Val/Val or Val/Met genotype. **Conclusion**: The Val158Met rs4680 polymorphism does not appear to be involved in predisposition to suffer from migraine; however, this genetic factor may be involved in the phenotypic expression of chronic migraine, since anxiety, depression and widespread pressure pain sensitivity was greater in those women with chronic, but not episodic, migraine with the Met/Met genotype.

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Introduction

Migraine is a primary headache disorder with a worldwide prevalence of 11.6% (13.8% females/6.9% males) (1). In the last Global Burden of Disease Study, migraine and tension-type headache were found to be the second most prevalent pain conditions in the world (2). In fact, general costs of headaches in Europe (€13.8 billion) mainly account for migraine and tension type headache (3).

It is accepted that the pathophysiology of migraine is associated with abnormal neuronal excitability leading to cortical spreading depression and to central sensitization of trigemino-vascular pathways (4). There are several factors that could affect the pain processing. One of these factors is genetics. Different genetic epidemiological studies have investigated the familial aggregation in migraine and it seems that an hereditary component can be present in some migraine types, i.e. hemiplegic migraine (5,6).

The catechol-O-methyltransferase (COMT) gene is one of the potential genetic determinants in chronic pain (7). The COMT is an enzyme involved in the metabolic degradation of several neurotransmitters, e.g., dopamine, norepinephrine, or epinephrine (8). The activity of the COMT gene can be affected by different polymorphisms such as rs4680, rs6269, rs4633, or rs4818. It seems that the rs4680 genetic polymorphism due to a G→A substitution at codon 158 of this gene, leading to a valine (Val) to methionine (Met) substitution, will result in differences within COMT gene activity related to pain sensitivity. In fact, a valine (Val) allele at codon 158 results in a high-activity variant (Val/Val) whereas a methionine (Met) at this codon position (Val/Met, Met/Met) results in low-activity variants (9). It has been found that subjects with the Met/Met genotype exhibit higher pain sensitivity, that is, lower pain thresholds to different stimuli (10,11),

and different brain responses to painful stimuli (12) than those subjects with the Val/Val genotype, supporting a role of this gene in nociceptive pain processing.

There are several studies investigating the role of Val158Met polymorphisms in migraine; although the results are inconsistent. The most recent meta-analysis did not observe a significant association between the Val158Met polymorphism and migraine (13). Similarly, a recent study, not included in the abovementioned meta-analysis, did not also reveal differences in Val158Met polymorphism distribution between subjects with migraine and healthy controls (14). Based on current evidence, it would seem that Val158Met polymorphism (rs4680) is not associated to a higher risk of suffering from migraine. However, it should be noted that most studies did not differentiate between episodic and chronic migraine. Similarly, another study including subjects with chronic migraine did not also find an association of the rs4680 Val158Met polymorphism with this subgroup (15).

Although no differences in Val158Met polymorphism distribution would exist between individuals with and without migraine, there is evidence suggesting a genetic influence of the COMT enzyme in several aspects of different chronic pain conditions, e.g., related-fatigue and pressure pain sensitivity in breast cancer survivors (16) or mood disorders (anxiety and depression) in women with fibromyalgia (17). Therefore, it is possible that the Val158Met polymorphism can also influence some phenotypic aspects in patients with migraine. In line with this hypothesis, Park et al found that individuals with migraine carrying the Met allele experienced worse migraine-associated nausea and vomiting and higher pain intensity of migraine attacks than those with the Val allele (18). No previous study has investigated the role of the Val158Met polymorphism in clinical, psychological and pain sensitivity outcomes in women with migraine.

Therefore, the aims of the current study were: 1) to investigate the association of the Val158Met polymorphism in women with episodic or chronic migraine; and 2) to determine the relevance of the Val158Met polymorphism with clinical, psychological, and pain sensitivity variables in women with migraine.

Methods

Participants

One hundred and twenty consecutive women with migraine were recruited from a Headache Unit located in a tertiary university-based hospital. They were diagnosed following the third edition of International Headache Society (ICHD-III) criteria down to third-digit level (code 1.1, 1.3) by an experienced neurologist (19). Migraine features including location, quality of pain, years with disease, frequency and intensity of pain attacks, family history, and medication intake were collected. To be included, subjects had to describe typical pain features of migraine pain (unilateral location, pulsating pain, high intensity, and aggravation during physical activity) and associated symptoms including photophobia, phonophobia, mild nausea or vomiting (19).

Participants were excluded if they presented any of the following: 1) other primary or secondary headache, including medication overuse headache; 2) history of cervical or head trauma; 3) pregnancy; 4) history of cervical herniated disk or cervical osteoarthritis on medical records; 5) any systemic medical disease, e.g., rheumatoid arthritis, lupus erythematous; 6) comorbid fibromyalgia syndrome; 7) had received treatment including anesthetic blocks, botulinum toxin or physical therapy within the previous 6 months; or, 8) male gender. All participants were carefully interviewed for assesing their medical history. Further a medical exam, including neuro-imaging examination (MRI or CT) of the head, was performed in all patients in order to identify any exclusion criteria.

Age-matched healthy women without history of headache diagnosis and without reporting a headache pain attack during the previous year were also included. Exclusion criteria for the control group were the same as for the headache groups. All participants signed the informed consent form before their inclusion in the study. The local Ethics Committee of Hospital Rey Juan Carlos, Spain (HRJ 07/14) approved the study design.

DNA Collection and COMT Genotyping

Non-stimulated whole saliva samples were collected into collection tubes (passive drooling technique) according to standardized procedures. Saliva collections were made when participants were headache-free, or with a migraine intensity of less than 3 points (in those patients with high frequency of attacks). Immediately after collection, samples were centrifuged at 3000 rpm for 15min to obtain the cell sediment and they were stored at -20º C until the analysis. We prefer to use saliva instead of blood sampling because salivary collection is a non-invasive, stress-free and ethic suitable assessment method.

Laboratory technicians were blinded to the subject's condition. Genomic DNA was hence extracted from saliva cell sediments using the "Genomic DNA extraction and purification Kit" (Real Molecular Biology) following the manufacturer instructions. The single Val158Met (rs4680) nucleotide polymorphism was genotyped using a TaqMan® Drug Metabolism Genotyping Assays on a Real Time PCR ABI Prism 7000 Sequence Detection System (APPLIED BIOSYSTEM, USA) in the Genomic Unit at the Centro de Apoyo Tecnológico Universidad Rey Juan Carlos, Madrid (Spain). The 3 possible allelic variants were associated with different fluorescent dyes to proper identification of the different genotype forms: Val/Val (H/H), Val/Met (H/L), or Met/Met (L/L). The results are derived from a G→A substitution at the following sequence:

CCAGCGGATGGTGGATTTCGCTGGC [A/G] TGAAGGACAAGGTGTGCATGCCTGA

Migraine Features

A 4-weeks headache diary was used to register clinical features of migraine (20). The diary was used to calculate the following variables: 1, migraine intensity, calculated from the mean intensity of the days with s migraine attack as assessed with a 11-point numerical pain rate scale (21) (NPRS; 0: no pain, 10: maximum pain); 2, migraine frequency (days/month); and 3, migraine duration, calculated by dividing the total hours of the attack by the number of days with migraine (hours/attack).

Psychological and Disability Variables

The Hospital Anxiety and Depression Scale (HADS) was used to evaluate the levels of anxiety and depression. This questionnaire includes 7-items scored at a 4-points scale for assessing anxiety (HADS-A) and other 7-items for assessing depressive (HADS-D) symptoms (22). Both scales are considered reliable and valid for assessing anxiety (Cronbach's α: 0.83) and depression (Cronbach's α: 0.82) (23). In subjects with headache, the HADS has shown good internal consistency (Cronbach's α: 0.84) (24).

The State-Trait Anxiety Inventory (STAI) is a 40-items scale assessing separate dimensions of state anxiety (items l-20, STAI-S) and trait anxiety (items 21-40, STAI-T) (25,26). The STAI-S assesses relatively enduring symptoms of anxiety at a moment, and the STAI-T scale measures a stable propensity to experience anxiety and tendencies to perceive stressful situations as threatening. Both scales have exhibited good internal consistency (α: 0.89) and high reliability (ICC: 0.88) (27). Higher scores in both scales indicate greater levels of state or trait anxiety.

To assess the degree of related-disability in daily activities (work or school, family and social) caused by migraine, we used the Migraine Disability Assessment Scale (MIDAS) questionnaire. It consists of 5 questions related to days of partial or total loss within the last 3 months regarding 3 main activities: 1, paid work or school;

2, household chores; 3, family, social, or leisure activities (28). The final score comes from the sum of the missed days regarding the 3 activities.

Widespread Pressure Pain Sensitivity

The evaluation was held when patients were headache-free or, in those with high frequency of migraine, when the intensity of headache was less than 3 points on NPRS. Participants were asked to avoid any analgesic or muscle relaxant 24 hours prior to the examination. No change was made on the prophylactic treatment of the patients. All the participants attended a session for familiarization with the pressure test procedure over the wrist extensor muscles.

Pressure pain thresholds (PPTs), i.e. the minimal amount of pressure where a sensation of pressure changes to pain (29), were assessed with an electronic algometer (Somedic AB, Farsta, Sweden). The pressure was applied perpendicularly to the point at a rate of approximately 30 kPa/s. Participants were instructed to press the "stop button" when the sensation first changed from pressure to pain. The mean of 3 trials on each point was calculated and used for the main analysis. A 30sec resting period was allowed between trials for avoiding temporal summation (30). The reliability of pressure algometry has been found to be high (31,32).

To determine widespread pressure pain sensitivity, PPTs were bilaterally assessed over a trigeminal point (i.e. temporalis muscle), an extra-trigeminal point (i.e. C5/C6 joint), and two distant pain-free points (i.e. the second metacarpal and tibialis anterior muscle) by an assessor blinded to the individual's condition. The order of assessment was randomized between participants. Since no side-to-side differences were observed, mean of both sides were used in the analysis.

Sample Size Calculation

Sample size determination and calculations were based on detecting a moderate-large effect size of 0.7 on PPTs between migraine and healthy controls related to Val158Met genotype distribution, a 2-tailed test, with an alpha level (α) of 0.05, and a desired power (β) of 90%. This generated a sample size of, at least, 42 participants per group.

Statistical Analysis

Data were analyzed with the SPSS statistical package (22.0 Version). Results are expressed as mean and 95% confidence interval (95% CI). The Kolmogorov-Smirnov test showed that all quantitative variables showed a normal distribution of the data ($P > 0.05$). Comparisons of genotype distribution and allele frequency among groups were performed on raw frequencies using an extended chi-squared test (χ^2). A χ^2 analysis of the Hardy-Weinberg equilibrium for the genotypes was conducted to determine whether the allele frequencies were stable within all groups. A 2x2 analysis of variance ANOVA was used to compare clinical and psychological variables according to the Val158Met polymorphism genotype (Val/Val, Val/Met, Met/Met) in women with migraine (episodic, chronic). A 3x3 mixed-model ANOVA was used to investigate differences in PPTs over each point (temporalis, C5-C6 joint, second metacarpal, tibialis anterior) according to the Val158Met genotype (Val/Val, Val/Met, Met/Met) and group (episodic migraine, chronic migraine, healthy control). Post-hoc analyses comparisons were conducted with the Bonferroni test. The statistical analysis was conducted at a 95% confidence level. A P value less than 0.05 was considered statistically significant.

Results

One hundred and twenty (n=120) consecutive women presenting with headache were screened for eligibility criteria. Twenty (17%) were excluded for the following reasons: co-morbid headaches (n=7); previous head or neck trauma (n=6); receiving anaesthetic block in the past 3 months (n=5) or pregnancy (n=2). Finally, 50 women with chronic migraine (age: 43±12 years), 50 with episodic migraine (age: 42±13 years) satisfied all criteria, signed the informed consent, and agreed to participate. Further, 50 age-matched women without headache (age: 43±11 years) were also included. **Table 1** summarizes clinical, psychological and pain sensitivity data of the sample. Women with chronic migraine exhibited significant higher headache frequency ($P<0.001$) and higher migraine-related disability ($P=0.04$) than those with episodic migraine. Further, women with episodic or chronic migraine exhibited higher widespread pressure pain sensitivity ($P<0.001$) than healthy women, without differences between them ($P>0.9$).

Distribution of Val158Met Polymorphism in migraine

The genotype distributions in women with and without migraine did not deviate from those expected based on the Hardy-Weinberg equilibrium. The distribution of the Val158Met genotypes ($\chi^2=6.63$; $P=0.157$) was not significantly different among women with episodic or chronic migraine and healthy women (**table 2**).

Clinical and psychological measures and Val158Met Polymorphism

The mixed-model ANOVA did not reveal significant differences depending on the Val158Met polymorphism genotype (**table 3**) in both groups of migraine women for years with pain ($F=0.874$; $P=0.420$), migraine intensity ($F=0.172$; $P=0.842$), migraine frequency ($F=1.986$; $P=0.143$), and migraine duration ($F=0.308$; $P=0.736$).

Similarly, no significant differences depending on the Val158Met polymorphism genotype were either found (**table 4**) in both women with episodic or chronic migraine for STAI-T (F=0.340; P=0.712), and HADS-A (F=1.494; P=0.188). A significant group * Val158Met genotype interaction was observed for HADS-D (F=4.369; P=0.015) and STAT-S (F=3.219; P=0.045): women with chronic migraine, but not those with episodic migraine, carrying the Met/Met genotype showed higher depressive and anxiety state levels than those carrying the Val/Val (P=0.01) or Val/Met (P=0.04) genotype. Finally, significant differences based on the Val158Met polymorphism genotype for the MIDAS (F=7.078 P<0.001) were found in both migraine groups: women carrying the Met/Met genotype exhibited higher levels of related-disability than those with the Val/Val or the Val/Met genotype (P<0.01) in both episodic and chronic migraine groups (**table 4**).

Pressure pain sensitivity and Val158Met polymorphism

All patients with episodic migraine and 45 (90%) patients with chronic migraine were headache-free during PPT examination. The 3x3 mixed-model ANOVA revealed significant group*Val158Met polymorphism genotype interactions for PPTs over the temporalis muscle (F=3.714; P=0.025), the second metacarpal (F=3.641; P=0.024), and tibialis anterior (F=3.431; P=0.03), but not for the C5-C6 zygapophyseal joint (F=1.479; P=0.212). Women with chronic migraine with the Met/Met genotype showed lower PPT than women with chronic migraine with the Val/Met or Val/Val genotype (P<0.001). No significant differences existed in PPTs between women with chronic migraine with the Val/Val or Val/Met genotypes (P>0.5). **Table 5** shows PPT according to Val158Met polymorphism in women with episodic and chronic migraine and healthy women.

Discussion

The current study found no differences in the genotype distribution and allele frequency of the Val158Met polymorphism between those women with migraine, either episodic or chronic, and healthy women. Further, the presence of the Met/Met genotype was associated to higher levels of anxiety, depression, disability and greater widespread pressure hyperalgesia, in women with the chronic, but not episodic, form of the disease.

Val158Met polymorphism and migraine

We did not observe significant differences in the distribution of the Val158Met polymorphism between women with episodic or chronic migraine and healthy women, supporting the assumption that this polymorphism is not involved in a predisposition to suffer from migraine. Our results agree with a recent systematic review concluding that the Val158Met polymorphism was not associated with migraine risk (13). Additionally, Takigawa et al did not also observe differences in the presence of other haplotypes of the COMT gene, e.g., rs4633, rs6267, rs6270 between individuals with migraine and healthy people (14). Nevertheless, since the rs4680 Val158Met polymorphism has been associated, in some studies, to different conditions, e.g., fibromyalgia syndrome (33) or temporomandibular pain (34), it is possible that it could be associated to some particular pain conditions rather than to chronic pain in general. Furthermore, since migraine is comorbid with other chronic pain syndromes, i.e. fibromyalgia (35), we do not know if different subgroups of patients with migraine and co-morbid conditions would lead to different associations. Obviously, the fact that the rs4680 Val158Met polymorphism is not associated with migraine does not exclude the role of genetics in this headache form. Therefore, future studies investigating the role of other genetic components in migraine are guaranteed.

It has been previously that the Val158Met can be associated with worse clinical presentation of migraine. For instance, individuals with migraine carrying the Met allele experienced higher pain intensity and worse migraine-associated symptoms than those with Val allele (18). We observed that women with migraine, either episodic or chronic, with the Met/Met genotype exhibited higher migraine-related disability as assessed with the MIDAS than those with Val/Met or Val/Val genotype. Further, a Met/Met genotype was also associated with higher depressive and anxiety state levels, but only within the chronic migraine group, suggesting that the Val158Met polymorphism can play a role in different psychological aspects. In fact, our results agree with previous studies showing that the Met allele is associated with anxiety-related behaviors in healthy women (36), with higher stress responses after a whiplash injury (37), or with higher psychological distress in fibromyalgia syndrome (38). A potential explanation for these findings could be related to the fact that individuals carrying the Met/Met genotype had greater brain activation of the limbic region as response to emotionally challenging situations (39,40). Additionally, Met/Met carriers exhibited lower activation of the dorso-lateral pre-frontal cortex and cingulate cortex than Val/Val carriers (41). Therefore, it is also possible that individuals with the Met/Met genotype exhibit different cortical activation patterns than those carrying the Val/Val genotype.

Val158Met polymorphism and pain hyperexcitability

Another relevant finding of the current study is that women with chronic, but not episodic, migraine carrying the Met/Met genotype exhibited higher widespread pressure pain sensitivity than those with the Val/Val or Val/Met genotype. These findings would suggest that the Val158Met polymorphism could play a role within the nociceptive pain processing in the chronic form of the disease. A potential association of the Val158Met polymorphism with higher sensitivity to pressure pain has been previously observed in

children with chronic tension type headache (42) and women with fibromyalgia (43). Our study is the first reporting an association between the Val158Met polymorphism and widespread pressure pain sensitivity in chronic migraine. Several mechanisms could explain this association. For instance, a reduction within COMT gene activity associated with the Met allele at codon 158 of the Val158Met leads to a reduction in the content of enkephalins in some regions of the central nervous system associated with pain (9). This hypothesis would correlate with the presence of hyper-excitability of the central nervous system and of endogenous inhibitory pain pathways previously observed in adults with chronic migraine (44). In fact, migraine has been associated with a non-physiological production of some neuromodulators (45). Therefore, another potential mechanism may be an increase of catecholamine levels, which will promote stimulation of β2-adrenergic receptors in the central nervous system, associated with a reduced COMT gene activity (46). Since individuals with migraine exhibit hyper-excitability of the central nervous system, it is possible that the presence of the Met/Met genotype, in some predisposed subjects, could contribute to this process. In fact, this hypothesis is also suggested in subjects with fibromyalgia (47).

Limitations

Although the results of this study are informative, potential limitations should be considered. First, we included women with migraine and derived from a specialized tertiary hospital center. Therefore, our results should be not extrapolated to men and to other primary headaches such as tension-type headache. Second, it is possible that the study was underpowered for other outcomes different than PPTs. Therefore, a greater sample size including patients from the general population would be needed to further confirm these results. Third, we only investigated the rs4680 nucleotide of Val158Met polymorphism. Future studies should include a greater number of polymorphisms and

other genes to further clarify their potential role in the phenotypic expression of chronic migraine.

Conclusions

No differences were found in the genotype distribution and allele frequency of the Val158Met polymorphism between women with migraine, either episodic or chronic and healthy women. The presence of the Met/Met genotype was associated with higher related-disability in both episodic and chronic migraine, and with higher depressive and anxiety levels, and higher pressure pain hyperalgesia but only in the chronic migraine group. Our results suggest that the rs4680 Val158Met polymorphism may contribute to the altered nociceptive pain processing in women with chronic migraine and may contribute to the chronification process.

References

1. Woldeamanuel YW, Cowan RP. Migraine affects 1 in 10 people worldwide featuring recent rise: A systematic review and meta-analysis of community-based studies involving 6 million participants. *J Neurol Sci* 2017; 372: 307-315.
2. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; 388: 1545-602
3. Raggi A, Leonardi M. Burden and cost of neurological diseases: a European North-South comparison. *Acta Neurol Scand* 2015; 132: 16-22.
4. de Tommaso M, Sciruicchio V. Migraine and central sensitization: Clinical features, main co-morbidities and therapeutic perspectives. *Curr Rheumatol Rev* 2016; 12: 113-26
5. Gasparini CF, Sutherland HG, Griffiths L. Studies on the pathophysiology and genetic basis of migraine. *Curr Genomics* 2013; 14: 300-15.
6. Di Lorenzo C, Grieco GS, Santorelli F. Migraine headache: a review of the molecular genetics of a common disorder. *J Headache Pain* 2012; 13: 571-80.
7. Tammimäki A, Männistö PT. Catechol-O-methyltransferase gene polymorphism and chronic human pain: a systematic review and meta-analysis. *Pharmacogenet Genomics* 2012; 22: 673-91
8. Mannisto PT, Kaakkola S. Catechol-O-methyltransferase (COMT): biochemistry molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol Rev* 1999; 51: 593–628
9. Belfer I, Segall S. COMT genetic variants and pain. *Drugs Today* 2011; 47: 457-67

10. Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppe RA, Stohler CS, Goldman D: COMT val158 met Genotype affects μ-opioid neurotransmitter responses to a pain stressor. *Science* 2003; 299: 1240-1243
11. Diatchenko L, Slade GD, Nackley AG, Bhalang K, Sigurdsson A, Belfer I, Goldman D, Xu K, Shabalina SA, Shagin D, Max MB, Makarov SS, Maixner W. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum Mol Genet* 2005; 14: 135-143
12. Loggia ML, Jensen K, Gollub RL, Wasan AD, Edwards RR, Kong J.The catechol-O-methyltransferase (COMT) val158met polymorphism affects brain responses to repeated painful stimuli. *PLoS One* 2011; 6: e27764
13. Liao YJ, Jiang JR, Jin SQ. The association between COMT Val158Met polymorphism and migraine risk: A meta-analysis. *Cephalalgia* 2017; 37: 592-598
14. Takigawa H, Kowa H, Nakashima K. No associations between five polymorphisms in COMT gene and migraine. *Acta Neurol Scand* 2017; 135: 225-230
15. De Marchis ML, Barbanti P, Palmirotta R, Egeo G, Aurilia C, Fofi L, Piroso S, Ialongo C, Della-Morte D, D'Andrea G, Ferroni P, Guadagni F. Look beyond Catechol-O-Methyltransferase genotype for cathecolamines derangement in migraine: the BioBIM rs4818 and rs4680 polymorphisms study. *J Headache Pain* 2015; 16: 520.
16. Fernández-de-las-Peñas C, Fernández-Lao C, Cantarero-Villanueva I, Ambite-Quesada S, Rivas-Martínez I, del Moral-Avila R, Arroyo-Morales M. Catechol-O-methyltransferase genotype (Val158met) modulates cancer-related fatigue and

pain sensitivity in breast cancer survivors. *Breast Cancer Res Treat* 2012; 133: 405-12.

17. Fernández-de-las-Peñas C, Ambite-Quesada S, Gil-Crujera A, Cigarán-Méndez M, Peñacoba-Puente C Catechol-O-methyltransferase Val158Met polymorphism influence anxiety, depression, and disability, but not pressure pain sensitivity, in women with fibromyalgia syndrome. *J Pain* 2012; 13: 1068-74.
18. Park JW, Lee KS, Kim JS, Kim YI, Shin HE. Genetic contribution of Catechol-O-methyltransferase polymorphism in patients with migraine without aura. *J Clin Neurol 2007*; 3: 24-30
19. ICHD-III International Classification of Headache Disorders: Headache Classification Subcommittee of the International Headache Society, 3nd edition. *Cephalalgia* 2013; 33: 629-808
20. Phillip D, Lyngberg AC, Jensen R. Assessment of headache diagnosis: A comparative population study of a clinical interview with a diagnostic headache diary. *Cephalalgia* 2007; 27: 1-8
21. Jensen MP, Turner JA, Romano JM, Fisher L. Comparative reliability and validity of chronic pain intensity measures. *Pain* 1999; 83: 157-62
22. Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. *Acta Psychiatr Scand* 1983; 67: 361-370.
23. Bjelland I, Dahl AA, Tangen Haug T, Neckelmann D. The validity of The Hospital Anxiety and Depression Scale: an updated literature review. *J Psychosom Res* 2002; 52: 69-77.

24. Juang KD, Wang SJ, Lin CH, Fuh JL. Use of the Hospital Anxiety and Depression Scale as a screening tool for patients with headache. *Zhonghua Yi Xue Za Zhi (Taipei)* 1999; 62: 749-55
25. Spielberger CD. Manual for the State-Trait Anxiety Inventory STAI (Form Y). Palo Alto, CA: Mind Garden; 1983.
26. Spielberger CD. State-Trait Anxiety Inventory: a comprehensive bibliography. Palo Alto, CA: Consulting Psychologists Press; 1989.
27. Barnes LLB, Harp D, Jung WS. Reliability generalization of scores on the Spielberger State-Trait Anxiety Inventory. *Educ Psychol Meas* 2002; 62: 603-618
28. Stewart WF, Lipton RB, Kolodner K, Sawyer J, Lee C, Liberman JN. Validity of the Migraine Disability Assessment (MIDAS) score in com- parison to a diary-based measure in a population sample of migraine sufferers. *Pain* 2000;88:41–52.
29. Vanderweeen L, Oostendorp RB, Vaes P, Duquet W. Pressure algometry in manual therapy. *Man Ther* 1996; 1: 258–265
30. Nie H, Arendt-Nielsen L, Andersen H, Graven-Nielsen T. Temporal summation of pain evoked by mechanical stimulation in deep and superficial tissue. *J Pain* 2005; 6: 348-355
31. Walton DM, Macdermid JC, Nielson W, Teasell RW, Chiasson M, Brown L. Reliability, standard error, and minimum detectable change of clinical pressure pain threshold testing in people with and without acute neck pain. *J Orthop Sports Phys Ther* 2011; 41: 644-50

496 32. Chesterson LS, Sim J, Wright CC, Foster NE. Inter-rater reliability of algometry
497 in measuring pressure pain thresholds in healthy humans, using multiple raters.
498 *Clin J Pain* 2007; 23: 760-6
499 33. Gürsoy S, Erdal E, Herken H, Madenci E, Alasehirli , Erdal N. Significance of
500 the catechol-O-methyltransferase gene polymorphism in fibromyalgia syndrome.
501 *Rheumatol Int* 2003; 23: 104-107
502 34. Smith SB, Maixner DW, Greenspan JD, Dubner R, Fillingim RB, Ohrbach R,
503 Knott C, Slade GD, Bair E, Gibson DG, Zaykin DV, Weir BS, Maixner W,
504 Diatchenko L. Potential genetic risk factors for chronic TMD: genetic
505 associations from the OPPERA case control study. *J Pain* 2011; 12: T92-101
506 35. Giamberardino MA, Affaitati G, Martelletti P, Tana C, Negro A, Lapenna D et
507 al. Impact of migraine on fibromyalgia symptoms. *J Headache Pain* 2015; 17:
508 28.
509 36. Enoch MA, Xu K, Ferro E, Harris CR, Goldman D. Genetic origins of anxiety in
510 women: A role for a functional catechol-o-methyltransferase polymorphism.
511 *Psych Gen* 2003; 13: 33-41.
512 37. McLean SA, Diatchenko L, Lee YM, Swor RA, Domeier RM, Jones JS et al.
513 Catechol-o-methyltransferase haplotype predicts immediate musculoskeletal
514 neck pain and psychological symptoms after motor vehicle collision. *J Pain*
515 2011; 12: 101-107
516 38. Desmeules J, Piguet V, Besson M, Chabert J, Rapiti E, Rebsamen M, Rossier
517 MF, Curtin F, Dayer P, Cedraschi C. Psychological distress in fibromyalgia
518 patients: a role for catechol-O-methyl-transferase Val158met polymorphism.
519 *Health Psychol* 2012; 31: 242-9.

39. Drabant EM, Hariri AR, Meyer-Lindenberg A et al. Catechol-o-methyltransferase val158met genotype and neural mechanisms related to affective arousal and regulation. *Arch General Psychiatr* 2006; 63: 1396-1406
40. Smolka MN, Schumann G, Wrase J et al. Catechol-*o*-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *J Neuros* 2005; 25: 836-842
41. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, et al. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci USA 2001; 98: 6917-6922
42. Fernández-de-las-Peñas C, Ambite-Quesada S, Rivas-Martínez I, Ortega-Santiago R, de-la-Llave-Rincón AI, Fernández-Mayoralas DM et al. Genetic contribution of catechol-O-methyltransferase polymorphism (Val158Met) in children with chronic tension-type headache. *Pediatr Res* 2011; 70: 395-9.
43. Martínez-Jauand M, Sitges C, Rodríguez V, Picornell A, Ramon M, Buskila D, et al. Pain sensitivity in fibromyalgia is associated with catechol-O-methyltransferase (COMT) gene. *Eur J Pain* 2013; 17: 16-27
44. Mainero C, Louapre C. Migraine and inhibitory system - I can't hold it!. *Curr Pain Headache Rep* 2014; 18: 426
45. D'Andrea G, D'Arrigo A, Dalle Carbonare M, Leon A. Pathogenesis of migraine: role of neuromodulators. *Headache* 2012; 52: 1155-63.
46. Nackley AG, Tan KT, Fecho K, Flood P, Diatchenko L, Maixner W. Catechol-O-methyltransferase inhibition increases pain sensitivity through activation of both b2- and b3-adrenergic receptors. *Pain* 2007; 128: 199–208

47. Desmeules J, Chabert J, Rebsamen M, Rapiti E, Piguet V, Besson M et al. Central pain sensitization, Val158Met polymorphism, and emotional factors in fibromyalgia. *J Pain* 2014; 15: 129-35.