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Abstract

Objectives: The aims of this study were: 1, to investigate the association between the rs4680 Val158Met polymorphism in frequent episodic (FETTH) and chronic (CTTH) tension-type headache; and 2, to analyse the association between the rs4680 Val158Met polymorphism with clinical, psychological, or psychophysical variables. **Methods**: Fifty women with FETTH, 50 with CTTH, and 50 matched healthy women participated. After amplifying Val158Met polymorphism by polymerase chain reaction, the genotype frequencies and allele distributions based on restriction fragment length polymorphism were assessed. Participants were classified according to the Val158Met polymorphism rs4680 genotype (Val/Val, Val/Met, or Met/Met). A headache diary collected clinical features. Disability (Headache Disability Inventory), sleep quality (Pittsburgh Sleep Quality Index), and depression/anxiety levels (Hospital Anxiety and Depression Scale) were also assessed. Pressure pain thresholds (PPT) were assessed bilaterally over the temporalis, upper trapezius, second metacarpal, and tibialis anterior by a blinded assessor. **Results:** The distribution of rs4680 Val158Met genotype was not significantly different between women with/without headache (P=0.796). No differences in headache features, disability, anxiety, and sleep quality were observed depending on the rs4680 Val158Met genotype. Women with CTTH, but not FETTH, carrying the Met/Met genotype had lower widespread PPTs and higher depressive symptoms than those with Val/Val or Val/Met genotype (P<0.05). **Conclusion:** The Val158Met polymorphism (rs4680) does not appear to be involved in predisposition to suffer from tension-type headache; however, this genetic factor may be involved in the pathogenesis expression of CTTH, as greater pressure pain sensitivity and higher depressive levels were found in CTTH carrying the Met/Met genotype.

Key word: Catechol-O-methyltransferase gene, tension-type headache, pressure pain.

Catechol-O-Methyltransferase (COMT) rs4680 Val158Met Polymorphism is Associated with Widespread Pressure Pain Sensitivity and Depression in Women with Chronic, but not Episodic, Tension Type Headache

Introduction

Tension type headache (TTH) is the most common headache disorder with a global annual prevalence of 42% in the general population.[1] In the last Global Burden of Disease Study, headache (mostly including TTH and migraine) was found to be the second most prevalent pain condition in the world.[2] Similarly, the general costs of headache, most related to migraine and TTH, in Europe in 2010 were €13.8 billion.[3]

Although the pathophysiology of this condition is not completely understood, it seems clear that individuals with TTH exhibit an altered nociceptive pain processing.[4] There are several factors that could affect the pain processing in humans. One of these factors is genetics. Some genetic epidemiological studies have investigated the familial aggregation in some headaches. In fact, a hereditary component seems to be clear in migraine;[5,6] however, this topic has been less investigated in TTH, and mostly focused in the chronic form. For instance, first-degree relatives of patients with chronic tension type headache (CTTH) had a 3.1-fold increased risk also of experiencing CTTH.[7,8]

The catechol-O-methyltransferase (COMT) gene is considered one potential genetic determinant in chronic pain syndromes.[9] The COMT is an enzyme generally involved in the metabolic degradation of several neurotransmitters, e.g., dopamine, norepinephrine, or epinephrine.[10] The activity of the COMT gene is affected by different polymorphisms (i.e., rs4680, rs6269, rs4633, or rs4818), being the rs4680 the COMT polymorphism the most investigated in the literature in relation to chronic pain.[9] It has been observed 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, results in different COMT gene activity related to pain sensitivity. In fact, a valine (Val) at codon 158 results in a heat-stable, high-activity variant (Val/Val), whereas a methionine (Met) at the same

position (Val/Met or Met/Met) results in heat-labile, low-activity variants.[11] It has been found that subjects with Met/Met genotype exhibit higher pain sensitivity[12,13] and different brain responses to painful stimuli[14] than those with the Val/Val genotype, supporting that this polymorphism could play a relevant role in nociceptive processing.

Although the COMT gene may have a potential role in the development of some chronic pain conditions, particularly fibromyalgia syndrome, its role in localized pain syndromes, such as headaches, is controversial.[15] In fact, most studies investigating the role of the Val158Met polymorphism in primary headaches have been conducted in migraine. Few published studies have investigated the role of Val158Met polymorphism in patients with episodic and chronic TTH.[16,17] Previous studies did not find significant differences in the distribution of the Val158Met polymorphism between subjects with TTH and healthy controls.[16,17] Similarly, a study in children with CTTH did not also find significant differences in the distribution of the Val158Met polymorphism between children with or without CTTH.[18] Therefore, based on current results the Val158Met polymorphism (rs4680) seems to be not associated to a higher risk of developing TTH; however, most previous studies included adults with episodic, but not chronic, TTH.

Although no significant differences in Val158Met polymorphism distribution would exist between individuals with and without TTH, there is evidence suggesting a genetic influence of this polymorphism in several clinical and neuro-physiological variables on different pain conditions, e.g., related-fatigue and localized pressure pain sensitivity in breast cancer survivors,[19] or mood disorders in fibromyalgia syndrome.[20] Therefore, it would be possible that the rs4680 Val158Met polymorphism could potentially influence some aspects of nociceptive processing in TTH. There is evidence supporting an hyper-excitability of the central nervous system in individuals with TTH.[4] This excitability of

the nervous system is characterized by hyperalgesia to different noxious stimuli, e.g., thermal, pressure, electrical. Some meta-analyses have observed that primary headaches mostly TTH and migraine, exhibit hyperalgesia to pressure pain as their main clinical manifestation of central sensitization.[21,22] Additionally, it sems that emotional factors may also influence central nervous system excitability found in individuals with TTH. Among these factors, depression, anxiety, and sleep disorders can play a relevant role in the sensitization process since they are able of triggering hyperalgesic responses.[23] The only study investigating the role of the Val158Met polymorphism in pain processing in primary headaches was conducted in children and found that children with CTTH with the Met/Met genotype showed higher pressure pain sensitivity than children carrying the Val/Val or the Val/Met genotype.[18] No study has previously investigated the role of rs4680 Val158Met polymorphism in the phenotypic expression and altered nociceptive processing in adults with TTH.

The aims of the current study were: 1) to evaluate the association between the rs4680 Val158Met polymorphism in adults with frequent episodic (FETTH) or CTTH; and 2) to analyze the association between the rs4680 Val158Met polymorphism with clinical, psychological and psychophysical variables (sensitivity to pressure pain) in adults with FETTH or CTTH. The hypotheses of this study were: 1, the presence of the rs4680 Val158Met polymorphism will be associated with CTTH, but not FETTH; 2, the presence of the Met/Met allele will be associated with worse clinical, psychological and psychophysical variables in both FETTH and CTTH.

Methods

Participants

Consecutive individuals with headache recruited from an university-based hospital between January 2016 and January 2018 were screened for possible eligibility criteria.

Tension type headache was diagnosed according to the International Classification of Headache Disorders criteria, third edition (ICHD3 beta 2013) down to third-digit level (code 2.2, 2.3) by a neurologist expert in headache.[24] The neurologist performed a face-to-face interview followed by a neurological examination. To be included, participants had to describe typical pain features of TTH including bilateral location, pressing and tightening pain, moderate-intense intensity, and no aggravation of pain during physical activity. In participants with high frequency of attacks, no more than 5 days/month with headache could meet criteria for migraine.[24] Participants were excluded if presented: 1, other primary/secondary headache; 2, medication overuse headache as defined by the ICHD-III;[24] 3, history of neck or head trauma; 4, pregnancy; 5, cervical herniated disk; 6, any systemic degenerative disease; 7, fibromyalgia syndrome; 8, receiving any treatment including anesthetic blocks, botulinum toxin or physical therapy within the previous 6 months; or, 9, male gender.

Age-matched healthy subjects without history of headache diagnosis and without reporting a headache pain attack the previous year were also included. Exclusion criteria for the control group was the same as for the headache group. All participants read and signed the written consent form prior to their inclusion in the study. The local Ethics Committee of the Universidad Rey Juan Carlos (URJC 23/2015) approved the study.

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 with participants seated and relaxed and between 9-11 am on days when were they were headache-free, or with a headache intensity of less than 3 points (in those patients with high frequency of attacks). Participants were asked not to eat or drink or chew gum for 1 hour before the collection. 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 halotypes 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

Headache Clinical Features

A 4-weeks headache diary was used to register clinical features of the headache.[25] The headache diary was used to calculate the following variables: 1) headache intensity, calculated from the mean intensity of the days with headache as assessed with a 11-points numerical pain rate scale[26] (NPRS; 0: no pain, 10: maximum pain); 2) headache frequency, calculated by dividing the number of days with headache by the number of weeks (days/week); and 3) headache duration, calculated by dividing the total hours of headache by the number of days with headache (hours/day).

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.[27] Both subscales are considered reliable and valid for assessing anxiety (Cronbach's α: 0.83) and depression (Cronbach's α: 0.82).[28] In individuals with headache, the HADS has shown good internal consistency (Cronbach's α: 0.83-0.84).[29]

The Headache Disability Inventory (HDI) was designed to assess the burden of headache using 25 items inquiring about the perceived impact of headache on emotional (HDI-E, 13-items, score 0-52) or physical (HDI-P, 12-items, score 0-48) functioning.[30] A greater score suggests a greater burden or related-disability of headache. The HDI has exhibited good short (HDI-E: 0.93, HDI-P: 0.95) and long (HID-E:0.76, HDI-P: 0.83) stability.[31]

The Pittsburgh Sleep Quality Index (PSQI) was used for evaluating the quality of sleep over a 1-month period by including 19 self-perceived questions and 5 questions answered by bed or roommates.[32] Item use varying response categories recording usual bed time, usual wake time, number of actual hours slept and number of minutes to fall asleep. Score from all answers is transformed into a global score (0-21) where a higher score indicates worse sleep quality.[33] The PSQI has shown good internal consistency and test-retest reliability.[34]

Psychophysical Variables

It seems that the main manifestation of the altered pain processing in individuals with TTH is the presence of sensitivity to pressure pain.[4] It is generally considered that pressure hyperalgesia in painful areas, e.g., those related to trigemino-cervical nucleus caudalis, is more associated to localized sensitization; whereas the presence of pressure hyperalgesia in distant pain-free areas, i.e., lower extremity, is associated to widespread central sensitization.

Pressure pain thresholds (PPTs), i.e., the minimal amount of pressure where a sensation of pressure first changes to pain,[35] were assessed with an electronic algometer (Somedic AB, Farsta, Sweden). The pressure was applied perpendicularly to the point at an approximately rate of 30 kPa/s via a $1cm^2$ probe. Participants were instructed to press the "stop button" of the algometer 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.[36] The reliability of pressure algometry has been found to be high.[37,38]

The evaluation was held when patients were headache-free or, in those with high frequency of headache, it was permitted when the headache intensity was less than 3/10 points on a NPRS. Only 5% of the patients experienced pain during PPTs assessment. 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.

To determine pressure pain sensitivity at localized and remote (or non-localized), PPTs were bilaterally assessed over the temporalis muscle (i.e. trigeminal point), C5/C6 zygapophyseal joint (i.e. extra-trigeminal point), the second metacarpal and the tibialis anterior muscle (i.e. distant pain-free points) 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 TTH and healthy controls accordingly to Val158Met

polymorphism 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 outcomes according to the Val158Met polymorphism genotype (Val/Val, Val/Met, Met/Met) in women with headache (FETTH, CTTH). A 3x3 ANOVA test was used to investigate differences in PPTs over each point (temporalis, C5-C6 zygapophyseal joint, second metacarpal, tibialis anterior) according to the Val158Met polymorphism genotype (Val/Val, Val/Met, Met/Met) and by group (FETTH, CTTH, or controls). Post-hoc analyses comparisons were conducted with the Tukey test. The standardized mean difference (SMD: between-group differences/pooled standard deviation) to enable comparison of effect sizes in those variables significantly different between groups accordingly to Val158Met polymorphism distribution. Values were considered as trivial when range from 0.0 to 0.2, small from 0.2 to 0.49, moderate from 0.5 to 0.79, and large when greater than 0.8. The statistical analysis was conducted at a 95% confidence level, and a P value < 0.05 was considered statistically significant.

Results

One hundred and thirty (n=130) consecutive women presenting with headaches between January 2016 and 2018 were screened for eligibility criteria. Thirty (23%) were

excluded for the following reasons: migraine (n=14), previous whiplash (n=6), hemi-cranial headache (n=5), and pregnancy (n=5). Finally, 50 women with CTTH (mean age: 47±11 years), 50 with FETTH (mean age: 48±10 years) satisfied all criteria, signed the informed consent and agreed to participate. Further, 50 age-matched women without headache (mean: 48±11 years old) were also included. **Table 1** summarizes clinical, psychological and psychophysical data of the sample. Women with CTTH exhibited significant longer headache duration (P=0.045), higher headache frequency (P<0.001), higher physical (P=0.04) or emotional (P=0.01) burden of headache, and higher levels of depression (P=0.007) than those with FETTH. Similarly, women with CTTH and FETTH exhibited higher widespread pressure pain sensitivity (P<0.001) as compared with healthy women, without differences between them (P>0.9).

Distribution of rs4680 Val158Met Polymorphism in the COMT gene

The genotype distribution in women with and without headache did not deviate from those expected based on the Hardy-Weinberg equilibrium. The distribution of the Val158Met rs4680 genotypes (χ^2=1.670; P=0.796) or alleles (χ^2=0.880; P=0.644) was not significantly different between women with TTH and healthy women (**table 2**).

Clinical and psychological measures and rs4680 Val158Met Polymorphism

The mixed-model ANOVA did not reveal significant differences depending on the Val158Met polymorphism genotype (**table 3**) in both headache groups for years with headache (F=0.224; P=0.800), headache intensity (F=0.572; P=0.566), headache frequency (F=0.134; P=0.875) and headache duration (F=0.030; P=0.970). Additionally, no significant differences in prophylactic medication intake were found depending on the Val158Met polymorphism genotype (P=0.764).

Similarly, no significant differences depending on the Val158Met polymorphism genotype were either found (**table 4**) in both women with FETTH and CTTH for HDI-P

(F=1.026; P=0.362), HDI-E (F=0.548; P=0.580), PSQI (F=0.814; P=0.446), or HADS-A (F=1.529; P=0.199). A significant group * Val158Met genotype interaction (F=3.352; P=0.03) was observed for HADS-D: women with CTTH, but not those with FETTH, carrying the Met/Met genotype exhibited higher depressive levels than those with the Val/ Val (P=0.01; SMD: 1.01) or Val/Met (P=0.045; SMD: 0.51) genotype.

Pressure pain sensitivity and rs4680 Val158Met polymorphism

The 3x3 mixed-model ANOVA revealed significant group*Val158Met polymorphism genotype interactions for PPTs over all points (temporalis: F=3.887, P= 0.023; C5-C6 zygapophyseal joint: F=4.306, P=0.02; second metacarpal: F=4.492, P=0.018; tibialis anterior: F=4.656, P=0.015). Women with CTTH, but not with FETTH, carrying the Met/Met genotype showed significant (P<0.01) lower PPTs (i.e., greater pressure pain sensitivity) at localized and remote (or non-localized) sites than the women with CTTH carrying the Val/Met (0.62<SMD< 0.83) or Val/Val genotype (0.82<SMD<1.05). No significant differences in PPTs were observed between women with CTTH carrying the Val/Val or Val/Met genotype (P>0.8). **Table 5** shows PPT according to Val158Met polymorphism genotype in women with FETTH, CTTH and healthy women.

Discussion

The current study did not find differences in the genotype distribution and allele frequency of the rs4680 Val158Met polymorphism among women with CTTH, FETTH and healthy women. Additionally, the presence of the Met/Met genotype was associated to greater pressure pain hyperalgesia at localized and remote (or non-localized) sites and depressive symptoms in women with CTTH, but not in those with FETTH or healthy controls. No association between the rs4680 Val158Met polymorphism and clinical or psychological variables was either found.

rs4680 Val158Met polymorphism in tension-type headache

No significant differences were observed in the distribution of the rs4680 Val158Met polymorphism between patients and healthy controls, supporting the assumption that this polymorphism does not appear to be involved in predisposition to suffer from TTH. Current results are similar to those previously reported in adults[16,17] and children[18] with CTTH. Our study is the first reporting the lack of this association in individuals with FETTH. In accordance with our results, the Val158Met polymorphism (rs4680) has not been associated with neuropathic pain,[39] widespread pain,[40] or musculoskeletal pain.[41] Similarly, the rs4680 Val158Met polymorphism has not been either associated to the presence of migraine.[42] The current results are supported by Takigawa et al finding of no significant differences in the presence of other haplotypes of the COMT gene, e.g., rs4633, rs6267, rs6270, between individuals with migraine, TTH or healthy people,[17] supporting that the COMT rs4680 Val158Met polymorphism seems to be not associated to TTH. Nevertheless, since the rs4680 Val158Met polymorphism has been associated, in some studies, to conditions such as fibromyalgia syndrome[43] or temporomandibular pain,[44] it is possible that this genotype may be associated to particular pain conditions rather than to chronic pain syndromes in general. Further, since TTH is comorbid with other pain syndromes, i.e., fibromyalgia syndrome,[45,46] we do not know if different subgroups of subjects with primary headaches and co-morbid conditions would lead to different associations. Finally, the fact that the rs4680 Val158Met polymorphism is not associated with TTH does not negate the role of genetics in this headache. For instance, some authors have suggested that the 5-HTT-gene-linked polymorphic gene could be involved in CTTH.[47] Therefore, future studies investigating the role of other genetic components in TTH are guaranteed.

rs4680 Val158Met polymorphism and pressure pain sensitivity

The most relevant finding of this study is that we found that women with CTTH, but not FETTH, carrying the Met/Met genotype exhibited greater pressure sensitivity at localized and remote (non-localized) sites, suggesting widespread pressure hyperalgesia 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 CTTH. The association of the rs4680 Val158Met polymorphism with greater pressure pain hyperalgesia has been also previously reported in children with CTTH,[18] breast cancer survivors,[19] and women with fibromyalgia.[48] Previous studies observed that patients with the Met/Met genotype exhibited higher localized, but not widespread, pressure pain sensitivity than those with the other genotypes.[18,19] Our study is the first reporting an association between rs4680 Val158Met polymorphism and pressure pain sensitivity at localized and remote sites in TTH, particularly the chronic form.

Determining the mechanisms involved in the relationship between the Val158Met polymorphism and pressure pain hypersensitivity in adults with CTTH are beyond the scope of this study; however, a few hypotheses can be proposed. First, a reduction in the COMT activity associated with the Met allele at codon 158 of the Val158Met leads to a reduction in the content of enkephalins in certain regions of the central nervous system associated with pain.[9] This hypothesis would correlate with the dysfunctional state of endogenous inhibitory pain pathways previously observed in adults with CTTH.[49,50] Another mechanism may be that reduced COMT activity would result in elevated levels of catecholamimes, e.g., epinephrine, which promote the production of persistent pain states via stimulation of β2-adrenergic receptors in the central nervous system.[51] Since subjects with CTTH have hyper-excitability of the central nervous system, it is possible

that the presence of the Met/Met genotype, in predisposed individuals, could contribute to central sensitization. This hypothesis has been also suggested for fibromyalgia.[52]

rs4680 Val158Met polymorphism and depressive levels

We also found that women with CTTH carrying the Met/Met genotype showed higher depressive symptoms than women with CTTH carrying the other genotypes, suggesting that the rs4680 Val158Met polymorphism can play a potential role in mood disorders. In agreement with our results, the Met allele was also associated with greater levels of depression in fibromyalgia syndrome women.[20] A potential neurophysiological link between mood disorders and the COMT gene may be the fact that subjects carrying the Met/Met genotype exhibit greater brain activation of the limbic region as response to emotionally challenging situations.[53,54] In addition, Met/Met carriers exhibited lower activation of the dorso-lateral pre-frontal cortex and cingulate cortex than those Val/Val carriers.[55] Therefore, it is possible that subjects carrying the Met/Met genotype would exhibit different cortical activation patterns than those carrying the Val/Val or Val/Met genotype. Nevertheless, recent meta-analyses have concluded that the association of the Val158Met polymorphism and depression, particularly mayor depressive disorder, is more complex than expected and several cofounders, such as gender or ethnicity can be present[56,57] Future studies are needed to determine if the association between the rs4680 Val158Met polymorphism and depression is related to the pain condition rather than to other factors.

Limitations

Although the results of this study are promising, we should recognize potential limitations. First, we included women with TTH and derived from a specialized tertiary hospital center. Therefore, our results should be not extrapolated to men with TTH, and to other primary headaches such as migraine. Second, a greater sample size including

patients from the general population are now needed to confirm these results. Third, we only investigated the rs4680 nucleotide of the Val158Met polymorphism. Future studies should include a greater number of nucleotides and other genes to further clarify their potential role in the phenotypic expression of TTH.

Conclusions

We found no differences in the genotype distribution and allele frequency of the rs4680 Val158Met polymorphism between women with FETTH, CTTH, and healthy controls. The presence of the Met/Met genotype was associated to higher pressure pain hyperalgesia at localized and remote (non-localized) sites and depressive symptoms in individuals with CTTH, but not FETTH, suggesting that the Val158Met polymorphism may contribute to the altered nociceptive pain processing in CTTH. Future studies are needed to further elucidate the relevance of this relationship as a possible contributor to the development of CTTH.

Conflict of Interest Statement

The Author(s) declare(s) that there is no conflict of interest.

Author Contributions

All authors contributed to the study concept and design. CFdlP, SAQ, and MPC did the main analysis and interpretation of data. All authors contributed to draft the report. AGM, AGP, JAP and LAN provided administrative, technical, and material support. LAN supervised the study. All authors revised the text for intellectual content and have read and approved the final version of the manuscript.

Key Findings

1. This study found that the genotype distribution of the Val158Met polymorphism (rs4680) was similar between women with FETTH, CTTH, and healthy controls.

2. The presence of the Met/Met genotype of the Val158Met polymorphism was associated to pressure pain hyperalgesia and depressive symptoms in CTTH, but not FETTH.
3. It is possible that the rs4680 Val158Met polymorphism may contribute to the altered nociceptive pain processing in individuals with CTTH.

References

1. Ferrante T, Manzoni GC, Russo M, Camarda C, Taga A, Veronesi L et al. Prevalence of tension-type headache in adult general population: the PACE study and review of the literature. Neurol Sci 2013; 34: S137-8.
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, Fernández-de-las-Peñas C. Tension type headache. Curr Rheumatol Rev 2016; 12: 1-13.
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. Ostergaard S, Russell MB, Bendtsen L, Olesen J. Comparison of first degree relatives and spouses of people with chronic tension headache. BMJ 1997; 14: 1092-1093
8. Russell MB, Ostergaard S, Bendtsen L, Olesen J.Familial occurrence of chronic tension-type headache. Cephalalgia 1999; 19: 207-10.
9. 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

10. 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
11. Belfer I, Segall S. COMT genetic variants and pain. Drugs Today 2011; 47: 457-67
12. 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
13. 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
14. 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
15. Tammimäki A, Männistö PT. Catechol-O-methyltransferase gene polymorphism and chronic human pain: a systematic review and meta-analysis. Pharmacogenet Genomics 2012; 22: 673-9
16. Hagen K, Pettersen E, Stovner LJ, Skorpen F, Zwart JA. The association between headache and Val158Met polymorphism in the catechol-O-methyltransferase gene: the HUNT study. J Headache Pain 2006; 7: 70–74
17. Takigawa H, Kowa H, Nakashima K. No associations between five polymorphisms in COMT gene and migraine. Acta Neurol Scand 2017; 135: 225-230

18. Fernández-de-las-Peñas C, Ambite-Quesada S, Rivas-Martínez I, Ortega-Santiago R, de-la-Llave-Rincón AI, Fernández-Mayoralas DM, Pareja JA. Genetic contribution of catechol-O-methyltransferase polymorphism (Val158Met) in children with chronic tension-type headache. Pediatr Res 2011; 70: 395-9.
19. 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.
20. 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.
21. Andersen S, Petersen MW, Svendsen AS, Gazerani P. Pressure pain thresholds assessed over temporalis, masseter, and frontalis muscles in healthy individuals, patients with tension-type headache, and those with migraine: a systematic review. Pain 2015; 156: 1409-23.
22. Castien RF, van der Wouden JC, De Hertogh W. Pressure pain thresholds over the cranio-cervical region in headache: a systematic review and meta-analysis. J Headache Pain 2018; 19: 9.
23. Cathcart S, Petkov J, Winefield AH, Lushington K, Rolan P. Central mechanisms of stress-induced headache. Cephalalgia 2010; 30: 285-95

24. ICHD-III International Classification of Headache Disorders: Headache Classification Subcommittee of the International Headache Society, 3nd edition Beta. Cephalalgia 2013; 33: 629-808.
25. 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
26. Jensen MP, Turner JA, Romano JM, Fisher L. Comparative reliability and validity of chronic pain intensity measures. Pain 1999; 83: 157-62
27. Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand1983; 67: 361-370.
28. 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.
29. 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
30. Jacobson GP, Ramadan NM, Norris L, Newman CW. The Henry Ford Hospital Headache Disability Inventory. Neurology 1994; 44; 837-42
31. Jacobson GP, Ramadan NM, Norris L, Newman CW. Headache disability inventory (HDI): short-term test-retest reliability and spouse perceptions. Headache 1995;35: 534-9
32. Cole JC, Dubois D, Kosinski M. Use of patient-reported sleep measures in clinical trials of pain treatment: a literature review and synthesis of current sleep measures and a conceptual model of sleep disturbance in pain. Clin Ther 2007; 29: 2580-2588.

33. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989; 28: 193-213
34. Carpenter JS, Andrykowski MA. Psychometric evaluation of the Pittsburgh Sleep Quality Index. J Psychosom Res. 1998; 45: 5-13.
35. Vanderweeen L, Oostendorp RB, Vaes P, Duquet W. Pressure algometry in manual therapy. Man Ther 1996; 1: 258–265
36. 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
37. 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
38. Chesterson LS, Sim J, Wright CC, Foster NE. Inter-rater reliability of algometry in measuring pressure pain thresholds in healthy humans, using multiple raters. Clin J Pain 2007; 23: 760-6
39. Armero P, Muriel C, Santos J, Sanchez-Montero FJ, Rodriguez RE, Gonzalez-Sarmiento R. COMT (Val158Met) polymorphism is not associated to neuropathic pain in a Spanish population. Eur J Pain 2005; 9: 229-232
40. Nicholl BI, Holliday KL, Macfarlane GJ, Thomson W, Davies KA, O'Neill TW, Bartfai G, Boonen S, Casanueva F, Finn JD, Forti G, Giwercman A, Huhtaniemi IT, Kula K, Punab M, Silman AJ, Vanderschueren D, Wu FC, McBeth J; European Male Ageing Study Group. No evidence for a role of the catechol-O-

methyltransferase pain sensitivity haplotypes in chronic widespread pain. Ann Rheum Dis 2010; 69: 2009-12

41. Hagen K, Pettersen E, Stovner LJ, Skorpen F, Zwart JA. No association between chronic musculoskeletal complaints and Val158Met polymorphism in the Catechol-O-methyltransferase gene: The HUNT study. BMC Musculoskeletal Disorders 2006; 7: 40
42. Liao YJ, Jiang JR, Jin SQ. The association between COMT Val158Met polymorphism and migraine risk: A meta-analysis. Cephalalgia. 2017; 37: 592-598
43. Gürsoy S, Erdal E, Herken H, Madenci E, Alasehirli , Erdal N. Significance of the catechol-O-methyltransferase gene polymorphism in fibromyalgia syndrome. Rheumatol Int 2003; 23: 104-107
44. Smith SB, Maixner DW, Greenspan JD, Dubner R, Fillingim RB, Ohrbach R, Knott C, Slade GD, Bair E, Gibson DG, Zaykin DV, Weir BS, Maixner W, Diatchenko L. Potential genetic risk factors for chronic TMD: genetic associations from the OPPERA case control study. J Pain 2011; 12: T92-101
45. Kanaan RA, Lepine JP, Wessely SC. The association or otherwise of the functional somatic syndromes. Psychosom Med 2007; 69: 855-9
46. Schoenen J. Tension-type headache and fibromyalgia: what's common, what's different? Neurological Sciences 2004; 25: S157-9
47. Park JW, Kim JS, Lee HK, Kim YI, Lee KS. Serotonin transporter polymorphism and harm avoidance personality in chronic tension-type headache. Headache 2004; 44:1005-1009

48. Martínez-Jauand M, Sitges C, Rodríguez V, Picornell A, Ramon M, Buskila D, Montoya P. Pain sensitivity in fibromyalgia is associated with catechol-O-methyltransferase (COMT) gene. Eur J Pain 2013; 17: 16-27
49. Pielstickera A, Haagc G, Zaudigh M, Lautenbachera S. Impairment of pain inhibition in chronic tension-type headache. Pain 2005; 118: 215-23
50. Sandrini G, Rossi P, Milanov I, Serrao M, Cecchini AP, Nappi G. Abnormal modulatory influence of diffuse noxious inhibitory controls in migraine and chronic tension-type headache patients. Cephalalgia 2006; 26: 782-9
51. 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
52. 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.
53. 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
54. 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
55. 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

56. Klein M, Schmoeger M, Kasper S, Schosser A. Meta-analysis of the COMT Val158Met polymorphism in major depressive disorder: the role of gender. World J Biol Psychiatry 2016; 17: 147-58.

57. Wang M, Ma Y, Yuan W, Su K, Li MD. Meta-Analysis of the COMT Val158Met Polymorphism in major depressive disorder: Effect of ethnicity. J Neuroimmune Pharmacol 2016; 11: 434-45.

Table 1: Clinical, psychological and psychophysical data of women with tension type headache (TTH) and healthy women

	Frequent Episodic TTH (n=50)	**Chronic TTH (n=50)**	**Healthy Control (n=50)**
Age (years)	47 (43-51)	49 (45-53)	48 (44-52)
Years with pain	7.5 (6.0-9.0)	7.7 (5.7-9.7)	------
Headache intensity (NPRS, 0-10)	6.4 (6.0-6.8)	7.1 (6.7-7.5)	------
Headache duration (hours/attack) *	7.1 (6.0-8.2)	8.8 (7.7-9.9)	------
Headache frequency (days/months) *	9.2 (8.4-10.0)	26.4 (25.6-27.2)	------
Prophylactic treatment (amitriptyline)	10 (20%)	13 (26%)	------
HDI-E (0-52) *	20.4 (17.6-23.2)	26.5 (22.5-30.5)	------
HDI-P (0-48) *	23.7 (21.2-26.2)	27.9 (24.1-31.7)	------
PSQI (0-21)	8.1 (7.0-9.2)	9.8 (8.3-11.3)	------
HADS-A (0-21) *	13.1 (12.1-14.1)	10.4 (9.1-11.7)	------
HADS-D (0-21) *	8.3 (7.3-9.3)	10.7 (9.3-12.1)	------
Pressure Pain Thresholds (kPa)			
Temporalis [#]	209.2 (190.8-227.6)	201.5 (177.8-225.2)	275.3 (258.1-292.5)
C5-C6 zygapophyseal joint [#]	184.1 (166.0-202.2)	188.8 (169.7-207.9)	236.4 (221.3-251.5)
Second metacarpal [#]	274.7 (255.5-293.9)	271.0 (240.9-301.1)	370.1 (342.7-397.5)
Tibialis anterior [#]	403.5 (361.7-445.2)	421.0 (386.8-455.4)	518.7 (489.2-548.2)

HDI: Headache Disability Inventory (P: Physical, E: Emotional); PSQI: Pittsburgh Sleep Quality Index; HADS: Hospital Anxiety and Depression Scale (D: Depression, A: Anxiety),

* Significant differences between frequent episodic and chronic tension type headache (student t-test, $P<0.05$)

[#] Significant differences between both groups of tension type headache and healthy controls (ANOVA test, $P<0.001$)

Table 2: Distribution of the Val158Met Genotypes and Alleles of the Catechol-O-Methyltransferase Gene in Women with and without Tension Type Headache (TTH)

	Frequent Episodic TTH (n=50)	**Chronic TTH (n=50)**	**Healthy Control (n=50)**
Genotypes			
H/H (Val/Val)	20 (40%)	16 (32%)	21 (42%)
H/L (Val/Met)	21 (42%)	25 (50%)	19 (38%)
L/L (Met/Met)	9 (18%)	9 (18%)	10 (20%)
Alleles			
Val	61 (61%)	57 (57%)	61 (61%)
Met	39 (39%)	43 (43%)	39 (39%)

Table 3: Differences in Headache Clinical Outcomes in Women with Tension Type Headache Depending on the Val158Met Polymorphism in the Catechol-O-Methyltransferase Gene

	Years with Pain	Intensity (NPRS, 0-10)	Duration (hours/attack)	Frequency (days/month)
Women with the H/H (Val/Val) genotype				
FETTH (n=20)	7.3 (4.3-10.3)	6.6 (5.8-7.4)	7.3 (5.4-9.2)	9.0 (7.7-10.3)
CTTH (n=16)	7.7 (4.3-11.1)	7.0 (6.2-7.8)	9.1 (8.0-10.2)	26.5 (25.1-27.9)
Women with the H/L (Val/Met) genotype				
FETTH (n=21)	7.1 (4.1-10.1)	6.4 (5.7-7.1)	6.7 (4.9-8.5)	9.7 (8.4-11.0)
CTTH (n=25)	7.9 (5.2-10.6)	7.4 (6.7-8.1)	8.6 (7.0-10.2)	26.6 (25.4-27.8)
Women with the L/L (Met/Met) genotype				
FETTH (n=9)	9.1 (5.5-12.7)	6.8 (5.7-7.9)	7.3 (5.6-9.0)	8.4 (6.4-10.4)
CTTH (n=9)	7.4 (4.8-10.0)	6.9 (5.8-8.0)	8.7 (6.9-10.5)	25.8 (23.8-27.8)

Values are expressed as mean (95% confidence interval); FETTH: Frequent episodic tension type headache; CTTH: Chronic tension type headache

Table 4: Differences in Anxiety, Depression, Sleep Quality and Headache Burden in Women with Tension Type Headache Depending on the Val158Met Polymorphism in the Catechol-O-Methyltransferase Gene

	HDI-E (0-52)	HDI-P (0-48)	PSQI (0-21)	HADS-A (0-21)	HADS-D (0-21) *
Women with the H/H (Val/Val) genotype					
FETTH (n=20)	21.4 (15.8-27.0)	24.8 (19.7-29.9)	8.4 (6.3-10.5)	12.2 (10.5-13.9)	8.7 (7.0-10.4)
CTTH (n=16)	24.8 (18.5-31.1)	25.3 (19.6-31.0)	8.5 (6.2-10.8)	9.8 (7.9-11.7)	8.3 (6.4-10.2)
Women with the H/L (Val/Met) genotype					
FETTH (n=21)	19.5 (14.1-24.9)	21.4 (16.5-26.3)	7.8 (5.7-9.9)	13.3 (11.6-15.0)	8.1 (6.5-9.7)
CTTH (n=25)	27.1 (22.2-32.0)	29.2 (24.7-33.7)	10.1 (8.3-11.9)	11.5 (9.9-13.1)	11.2 (9.6-12.8)
Women with the L/L (Met/Met) genotype					
FETTH (n=9)	20.4 (12.2-28.6)	26.7 (19.1-34.3)	8.4 (6.4-10.4)	14.8 (12.2-17.4)	7.6 (6.0-9.2)
CTTH (n=9)	30.9 (22.6-39.2)	31.1 (23.6-38.6)	11.6 (8.3-14.9)	8.4 (6.0-10.8)	13.3 (10.8-15.8)

Values are expressed as mean (95% confidence interval);

FETTH: Frequent episodic tension type headache; CTTH: Chronic tension type headache; HDI: Headache Disability Inventory (P: Physical, E: Emotional); PSQI: Pittsburgh Sleep Quality Index; HADS: Hospital Anxiety and Depression Scale (D: Depression, A: Anxiety),

* Significant higher HADS-D score in Met/Met genotype group as compared to Val/Val and Val/Met genotypes (2-way ANOVA test) within the CTTH group

Table 5: Differences in Pressure Pain Thresholds (kPa) in Women with and without Tension Type Headache Depending on the Val158Met Polymorphism in the Catechol-O-Methyltransferase Gene

	Temporalis*	**C5-C6 joint***	**Second metacarpal***	**Tibialis anterior***
Women with the H/H (Val/Val) genotype				
Healthy Controls (n=21)	288.2 (258.4-318.0)	237.6 (211.1-264.1)	375.3 (335.4-415.2)	531.9 (477.9-585.9)
FETTH (n=20)	220.4 (189.8-251.0)	181.1 (153.8-208.4)	279.5 (238.6-320.4)	396.8 (341.4-452.2)
CTTH (n=16)	233.2 (209.0-257.4)	202.7 (172.2-233.2)	280.8 (245.2-316.4)	423.1 (381.2-465.0)
Women with the H/L (Val/Met) genotype				
Healthy Controls (n=19)	264.4 (233.1-295.7)	235.4 (207.5-263.3)	364.6 (322.7-406.5)	524.2 (467.4-581.0)
FETTH (n=21)	202.4 (172.6-232.2)	188.2 (161.6-214.8)	273.8 (233.9-313.7)	411.3 (357.2-465.4)
CTTH (n=25)	201.0 (183.7-218.3)	196.3 (171.9-220.7)	282.6 (246.1-319.1)	426.5 (376.9-476.2)
Women with the L/L (Met/Met) genotype				
Healthy Controls (n=10)	268.6 (225.4-311.8)	235.5 (196.9-274.1)	369.6 (321.8-417.4)	480.3 (422.0-538.6)
FETTH (n=9)	200.4 (154.9-245.9)	181.4 (150.8-212.0)	266.6 (225.7-307.5)	400.1 (337.6-462.6)
CTTH (n=9)	146.7 (121.2-172.2)	143.6 (118.9-168.3)	221.3 (190.4-252.2)	329.0 (286.5-371.5)

Values are expressed as mean (95% confidence interval); FETTH: Frequent episodic tension type headache; CTTH: Chronic tension type headache * Significant lower PPTs in Met/Met genotype group as compared to Val/Val and Val/Met genotypes (3-way ANOVA test) within the CTTH group