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CASE REPORT

Association of G472A allele of membrane bound catechol-*O*-methyltransferase gene with chronic post-sternotomy pain

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Abstract
Chronic persistent surgical pain (CPSP) is a complex disease with strong genetic component. The studies on revealed association of mutations in membrane bound catechol-*O*-methyltransferase gene with CPSP were reported indifferent ethnic populations across the globe. We identify that one out of four patients who underwent sternotomy procedure showed CPSP even after 3 months of surgery. The Mb.COMT gene sequence analysis revealed of the four patients, three patients had no mutation in Mb.COMT gene, while in one patient exhibited G472A mutation. Interestingly, this patient showed CPSP even after 90 days of surgery. The magnitude of the CPSP was evaluated with pain questionnaires' at the end of 3 months after discharge from the hospital. In this study 25% (1/4) showed presence G472A allele correlating with CPSP. Further the study suggested that evaluation of G472A allele of Mb.COMT gene in the patients undergoing sternotomy for monitoring pain in pre and post-surgical events.

Keywords Sternotomy · Post-surgical pain · COMT · Analgesia

Introduction

Chronic persistent surgical pain (CPSP) is a pain in the location of the surgery that persists for many months or even years and the incidence varies from 17 to 75% after open heart surgery with median sternotomy [1]. Apart from the environmental and clinical factors, the recent studies have shed lights on strong genetic components for development of CPSP. Here we study the G472A (valine158methionine) polymorphism in membrane bound catechol-o-methyltransferase gene in the patients who have underwent sternotomy procedure and exhibiting chronic persistent pain.

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Case report

Four adult Patients included who consented for surgery and genetic analysis of their body fluid were explained about the 10 cm numerical rating scale (NRS) [2] to indicate their pain perception, identifying 0 as no pain and 10 as worst imaginable pain. A standard anesthetic plan and post-operative pain regimen was instituted in all subjects. At baseline, we collected demographic data for age, gender, education level (high school obtained or not), and patients were questioned about their history of regular physical activity in adulthood (during a pain-free period). All our patients do not have any history suggestive of psychiatric condition necessitating the use of opioids.

On the day of surgery 5 ml of peripheral venous blood was collected from the patients for genetic analysis. The pure high molecular weight genomic DNA was extracted from the patient's blood using the standard protocol [3] and the DNA was quantified spectrophotometrically and analyzed by running in 1% agarose gel electrophoresis. The extracted DNA was used as template in the PCR experiments. The exon 4 of Membrane bound Catechol-o-methyltransferase gene primers were designed from the sequence obtained from NCBI data base further subjected to online primer 3 software and

the obtained sequences were synthesized at Sigma Pvt Ltd, Hyderabad.

The PCR amplification for exon 4 of Mb.COMT gene was performed using forward primer: 5′-GCAAGATCG TGGACGCCGTG-3′, reverse primer: 5′-CTTGTCCTTCAC GCCAGCGA-3′. The reaction mixture contained in a final volume of 50 µl consisted of 100 pmoles of each primer, 100 µmol of dNTPS mix, 10 mM Tris–Hcl (pH 8.8), 1.5 mM $MgCl_2$, 1 U of Taq DNA polymerase and 50 µg of genomic DNA. Amplification parameters included an initial denaturation step for 10 min at 65 °C in a master cycler gradient thermo cycler [3]. Amplified PCR products were sequenced and the sequence was deposited GenBank (http://www.ncbi.nlm.nih.gov). (Accession number: MF460457.1).Upon amplification PCR products showed 220 bp on 1% agarose gel electrophoresis (Fig. 1a). The sequence analysis showed homozygous mutation c.472 G>A resulted in p.Val158Met in the Mb.COMT enzyme (Fig. 1b, c). In the other three patients the pain subsided completely interestingly absence of G472A allele of Mb.COMT gene was noted.

A fixed post-operative analgesia regimen [paracetamol (1 g IV) and tramadol (50 mg IV) alternatively four times a day] with fentanyl (50 µg IV) as rescue analgesia for NRS score (NRS ≥ 4) was followed for all patients. Pain and its consequences were assessed by trained personnel, in face-to-face interviews, before surgery (at baseline) and during the first 4 days after surgery, and then over phone at 1, 2 and 3 months after surgery. To assess the magnitude of the pain, patients were asked to fill out a questionnaire about post-surgical pain [4].In the pain

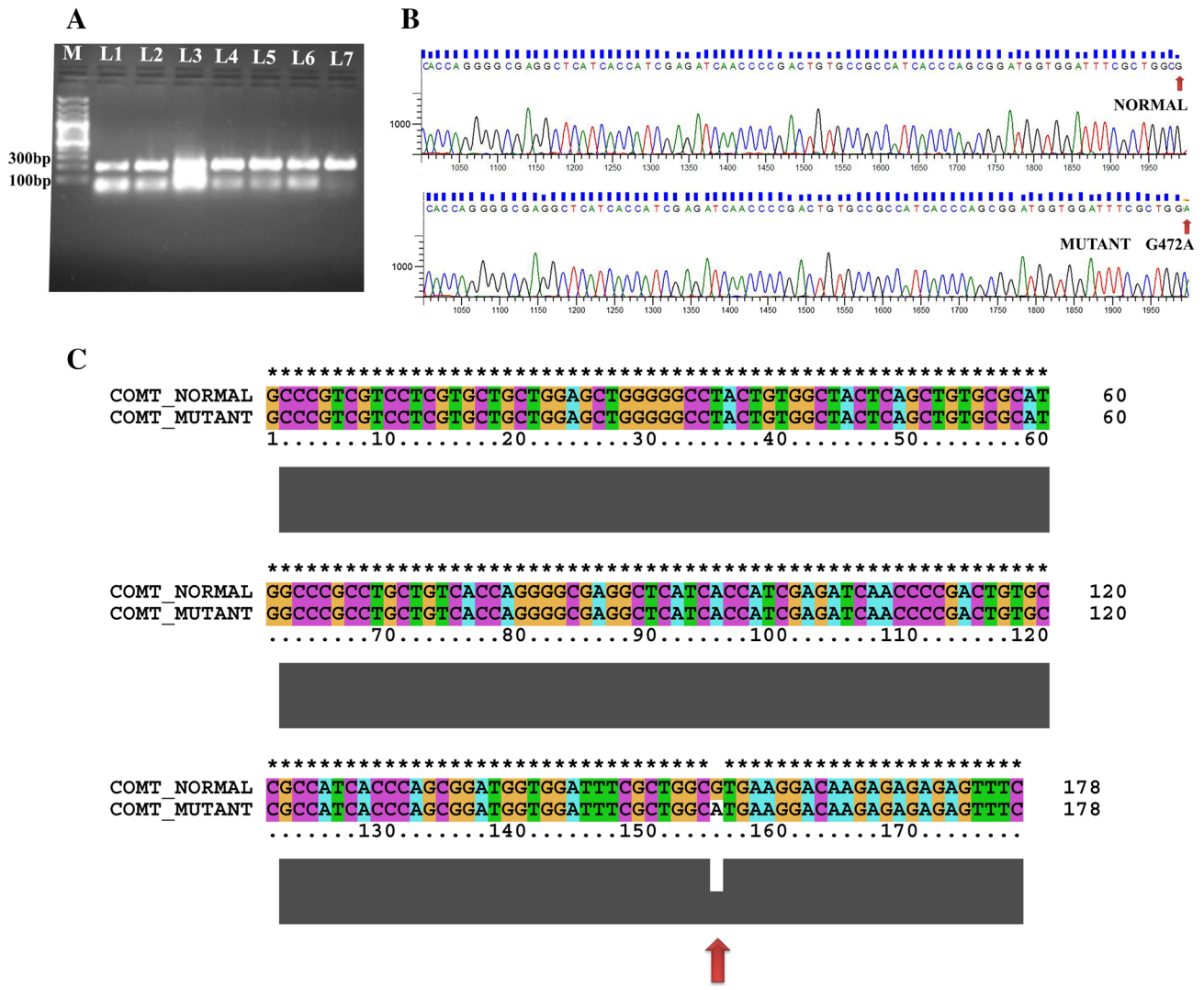

Fig. 1 **a**.The electrophoretogram showing PCR amplification of exons 4 of COMT gene. Lane M 100 bp ladder obtained from Merck Biosciences Pvt Ltd, India. Lane L1–L7 PCR amplified products of 220 bp of exon 4 of COMT gene. **b**. The Chromatogram portions **a** exon 4 of normal Mb.COMT gene sequencing, **b** exon 4 showing G472A mutation in Mb. COMT gene sequence. The red colored arrow designates the position of the mutation. **c** multiple sequence alignment of normal and mutant allele (G472A) Mb.COMT gene

questionnaires the patients were asked about the best and worst pain score in the preceding 1 week. In addition to this they were also enquired about location, characteristic of pain and need for analgesic use. The effect of pain on their sleep cycle and daily activity was also recorded [4]. Three months after post-surgery, one patient reported a characteristic of CPSP such as pain in the surgical site, which did not exist prior to surgery that turned out into a chronic pain after surgery and then persisted for at least 3 months (Table 1).

Discussion

Patients undergoing elective surgical procedure are broadly exposed to similar levels of tissue injury, nerve damage and nociceptive barrage, yet only a proportion of these develop a chronic pain state. Chronic persistent surgical pain is a pain in the location of the surgery that persists for many months or even years. There could be several possibilities of genetic polymorphism associated with clinical pain condition. Both ZFHX2 and PRDM12 are associated with hypoalgesic pain condition [5, 6]. Whereas P2X7 is present in cells of immune system and associated with a hypoalgesic pain condition [7]. In the present

Table 1 Demographic variables and patients characteristics

Characteristics	Patient 1	Patient 2		Patient 3	Patient 4
Age (years)	64	61		40	53
Gender	Male	Male		Female	Female
Weight (kg)	88	46		38	40
Height (cm)	163	150		147	152
Diagnosis	CAD	CAD		RMVD	RMVD
Surgical procedure	CABG	CABG		MVR	MVR
Post-operative events					
Surgical site infection	No	No	No		No
Sepsis	No	No	No		No
Re-bleeding/re operation	No	No	No		No
Hospital length of stay (days)	9	6	10		7
Pain scores in post-operative ward at each shift on NRS					
Day 1 (M/E/N)	2/2/4	2/2/4	5/4/8		1/1/3
Day 2 (M/E/N)	1/1/3	1/1/4	8/4/5		1/1/3
Day 3 (M/E/N)	0/0/3	2/2/3	6/4/6		1/1/4
McGill Pain Questionnaire at the end of 3rd month					
Have you experienced thoracic/sternotomy pain related to the surgery?	No	No	Yes		No
What was the intensity of the pain on the best day?	0/10	1/10	4/10		0/10
What was the intensity of the pain on the worst day?	2/10	2/10	8/10		2/10
What is the pattern of pain?	Rhythmic	Rhythmic	Steady		Transient
Where was the pain localized?	Non-localized	Non-localized	near sternal wound		Non-localized
How would you characterize the pain?	Aching	Aching	Aching and burning pain		Numbness at the site of incision
Have you used analgesics for the pain?	No	No	Yes		No
Has the pain interrupted your sleep?	No	No	Yes		No
Has the pain limited your daily activities?	No	No	Yes		No
Are you back to your normal activities?	Yes	Yes	No		Yes
Can you rate the quality of your health on a scale of 1—10?[a]	6/10	8/10	3/10		6/10
Mb.COMT(G472A) mutation	Wild type	Wild type	Mutant		Wild type

CAD coronary artery disease, *CABG* coronary artery bypass grafting, *RMVD* rheumatic mitral valve disease, *MVR* mitral valve replacement, *M* morning, *E* evening, *N* night, *NRS* numeric rating scale where 1 is no pain and 10 is worst pain imaginable

[a]Quality of health where 1 is very poor and 10 is excellent state of health, could not be better

study we have reported the best characterized SNP in Mb.COMT, a mutation expressed in nervous system which mediates pain perception by regulating the catecholamine level. Hence we evaluated the influence of the Mb.COMT G472A (Val158Met) mutations in postoperative cardiac patients. The main finding was that patients carrying the G472A allele of Mb.COMT gene experienced significantly increase in overall pain during the painful procedure, in comparison to patients with the normal Mb.COMT genotype. To our knowledge, this is the first study wherein we observed the impact of the Mb.COMT G472A (Val-158Met) mutation in one patient showing persistent (even after 3 months) chronic post-surgical sternotomy pain with NRS score of ≥ 8 at the end of 3 months. Zubieta et al. [8] demonstrated that Met/Met in Mb.COMT subjects were characterized by higher sensory and affective pain ratings; two other studies [9] found that individuals with the Met/Met Mb.COMT genotype were more susceptible to pain after repeated thermal stimuli. An American study opined no association is found between diverse genotypes of rs4680 and chronic pain, but the study was on lower abdominal hysterectomy [10]. However, a Dutch study demonstrated a significantly different mean pain score (as measured through NRS) across COMT genotype cluster [11], Although COMT genetic variant seems to be associated with mastectomy, the conclusions are different in the Ireland study has reported a trend between occurrence of CPSP and Val158 Met polymorphism after mastectomy which is statistically not significant ($P = 0.06$) [12]. In contrast, the American study [13] in 1000 women undergoing surgery for breast cancer found a weak association between COMT variant and experimental pain. However, this study is not designed to evaluate the development of CPSP in the post-operative period.

In the present study we have identified G472A genotype in Mb.COMT gene consistent with our hypothesis. Our study result shows higher NRS score in patient with the G472A (Val158Met) allele mutation, whereas the NRS score is reduced (< 2) in the patients with normal allele. In our study we realizes that the mean NRS scores generally high (NRS ≥ 8) (Table 1), which was an unacceptable pain during the painful procedures that should be properly addressed. On the other hand, the knowledge of patients with the G472A (Val158Met) genotype may be of value for procedural-related pain management. Ochroch et al. [14] assessed OPRM1 and COMT gene–gene interaction associated with postoperative pain and opioid consumption only up to third postoperative day of post-sternotomy pain, whereas the current study was carried out with an assessment period of 3 months after sternotomy procedure. This helped in rule out of acute pain caused due to surgery from that of chronic post-thoracotomy pain caused due to G472A mutation [14].

The results of our study suggest that patients carrying the G472A allele of Mb.COMT gene may benefit from a more individualized therapy in the event of pre-surgery to prevent recurrence of CPSP.

Conclusion

This pilot study put forth evaluation of G472A allele of Mb.COMT gene patients before undergoing open heart surgeries. This will give us a chance for reorientation of treatment to reduce the chronic persistent surgical pain exhibited by post-surgical patients.

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Compliance with ethical standards

Conflict of interest There are no financial or other relationships that might lead to a conflict of interest.

References

1. Kalso E, Mennander S, Tasmuth T, Nilsson E. Chronic post sternotomy pain. Acta Anaesthesiol Scand. 2001;45(8):935–9.
2. Downie WW, Leatham PA, Rhind VM, Wright V, Branco JA, Anderson JA. Studies with pain rating scales. Ann Rheum Dis. 1978;37(4):378–81.
3. Sambrook J, Russel WD. Molecular cloning. 3rd ed. New York: Cold Spring Harbor Laboratory; 2001.
4. Melzack R. The McGill Pain Questionnaire: major properties and scoring methods. Pain. 1975;1:277–99.
5. Habib AM, Matsuyama A, Okorokov AL, Santana-Varela S, Bras JT, Aloisi AM, et al. A novel human pain insensitivity disorder caused by a point mutation in ZFHX2. Brain. 2018;141(2):365–76.
6. Moss C, Srinivas SM, Sarveswaran N, Nahorski M, Gowda VK, Browne FM, et al. Mid-face Toddler Excoriation Syndrome (MiTES) can be caused by autosomal recessive biallelic mutations in a gene for congenital insensitivity to pain, PRDM12. Br J Dermatol. 2018. https://doi.org/10.1111/bjd.16893.
7. Foulkes T, Wood JN. Pain Genes. PLoS Genet. 2008;4(7):e1000086. https://doi.org/10.1371/journal.pgen.1000086.
8. Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, et al. COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science. 2003;299(5610):1240–3.
9. Stamer UM, Stuber F. Genetic factors in pain and its treatment. Curr Opin Anaesthesiol. 2007;20:478–484.
10. Kolesnikov Y, Gabovits B, Levin A, Veske A, Qin L, Dai F, et al. Chronic pain after lower abdominal surgery: do catechol-*O*-methyl transferase/opioid receptor μ-1 polymorphisms contribute? Mol Pain. 2013;9:19.

11. Ahlers SJ, Elens LL, van Gulik L, van Schaik RH, van Dongen EP, Bruins P, et al. The Val158Met polymorphism of the COMT gene is associated with increased pain sensitivity in morphine-treated patients undergoing a painful procedure after cardiac surgery. Br J Clin Pharmacol. 2013;75:1506–15.
12. Hickey OT, Nugent NF, Burke SM, Hafeez P, Mudrakouski AL, Shorten GD. Persistent pain after mastectomy with reconstruction. J Clin Anesth. 2011;23(6):482–8.
13. Kambur O, Kaunisto MA, Tikkanen E, Leal SM, Ripatti S, Kalso EA. Effect of catechol-o-methyltransferase-gene (COMT) variants on experimental and acute postoperative pain in 1,000 women undergoing surgery for breast cancer. Anesthesiology. 2013;119(6):1422–33.
14. Ochroch EA, Vachani A, Gottschalk A, Kanetsky Peter A. Natural variation in the m-opioid gene OPRM1 predicts increased pain on third day after thoracotomy. Clin J Pain. 2012;28:747–54.