

# Chronic tension-type facial pain- a pilot study on HTTLPR genetic polymorphisms\*

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## Abstract

**Background:** This study looked at the association between serotonin transporter gene variants and with chronic persistent tension-type facial pain. Known as triallelic 5-HTTLPR, the serotonin transporter gene-linked polymorphic region and its rs25531 polymorphism have been linked to alterations in pain perception.

**Patients and methods:** Genotype polymorphism analysis was carried out in 26 patients with chronic tension type facial pain unresponsive to or recurring after treatment with amitriptyline. Findings were compared to 33 asymptomatic age- and gender-matched controls. Blood serotonin levels in these two groups were also determined.

**Results:** Consistent with previous studies, patients with chronic pain had significantly lower blood serotonin compared to controls. In all genotypes, blood serotonin was low compared to controls and this difference was significant in patients homozygous for the L allele.

**Conclusion:** In this small pilot study, patients with the LL genotype seemed to have the best clinical outcome after three-year follow-up.

**Key words:** chronic, tension-type pain, facial pain, blood serotonin levels, 5-HTTLPR

## Introduction

Chronic tension-type headache and facial pain is due to central sensitisation in the thalamus caused by enhanced responsiveness of spinal dorsal horn neurons<sup>(1)</sup>. Distal projections of serotonergic neurons from the rostroventral medulla in the brainstem exert a descending tonic pain inhibition to the spinal cord<sup>(2,3)</sup>; serotonergic dysfunction thus enhances pain transmission. At synaptic level the serotonin transporter (5-HT transporter or 5-HTT) clears serotonin that has been released by the presynaptic neuron terminal and is the main mechanism controlling synaptic serotonin levels<sup>(4)</sup>. Reduction in serotonin re-uptake enhances its effect over a wider synaptic field<sup>(5)</sup>. Platelets are a convenient biochemical model for central serotonergic neurons since they share the same identical uptake protein<sup>(6,7)</sup>. Blood serotonin levels reflect intraplatelet serotonin, because 90 to 99% of blood serotonin is stored inside platelets<sup>(6,8,9)</sup>. Platelet serotonin levels in individuals with chronic tension-

type headache tend to be low<sup>(10)</sup>. Similarly, patients with chronic midfacial tension-type pain (CMFP) had significantly low whole blood serotonin levels compared to controls<sup>(11)</sup>.

The great majority of patients with CMFP are women<sup>(12)</sup>. In women with CMFP, treatment with low-dose amitriptyline significantly reduced pain frequency and intensity and this was associated with a non-significant reduction in blood serotonin. When taking the beta blocker pindolol in tandem with amitriptyline, blood serotonin was reduced even further to a significant extent while also significantly reducing pain frequency and intensity. In the presence of amitriptyline, pindolol acts as a partial agonist to the 5-HT 1A receptor on the somatodendritic area of serotonergic neurons in the brainstem. Stimulation of the 5-HT 1A receptor enhances peripheral serotonergic discharge in projection areas with enhanced inhibition of pain in the face<sup>(11)</sup>.

The serotonin uptake transporter protein (5-HTT) in platelets and in the central nervous system has an identical amino acid structure<sup>(13)</sup>. The human 5-HTT gene has been successfully cloned<sup>(14)</sup> and is one of a family of similar neurotransmitter-carrying transport proteins. It contains 630 amino acids with 12 transmembrane domains<sup>(15)</sup> and transports serotonin together with Na<sup>+</sup> and Cl<sup>-</sup> ions into the neuron while transporting K<sup>+</sup> ions outwards<sup>(5)</sup>. 5-HTT is coded by a single gene (SLC6A4) which is found on chromosome 17q 12<sup>(13)</sup>. The most studied polymorphism variant has a 43 base pair insertion/deletion in the 5'-regulatory promoter region of the gene. This is known as the 5-HTT linked polymorphic region or 5-HTTLPR and occurs in the population as two prevalent alleles<sup>(16)</sup>. The short variety is expressed with 14 amino acids (S-allele) and the long one with 16 amino acids (L-allele)<sup>(17)</sup>. Individuals with the S-allele have lower 5-HTT activity than those with the L-allele<sup>(17)</sup> and patients homozygous for the short form of 5-HTTLPR take up serotonin at about half the normal rate<sup>(18, 19)</sup>. Individuals may express LL, SS or LS genotypes. An Adenosine to Guanine substitution (rs25531) in the 5-HTT promoter region has also been described. In this single nucleotide polymorphism (SNP) the G-allele, which is found in phase with the L-allele, reduces the serotonin re-uptake of the L-allele to S-allele levels<sup>(20)</sup>. These two polymorphisms, when studied jointly, have been referred to as tri-allelic 5-HTTLPR.

Some alleles may be associated with a higher risk of developing chronic pain conditions<sup>(21)</sup>. Korean patients with chronic tension-type headache and analgesic-overuse have been demonstrated to have a relatively high incidence of the short allele<sup>(22)</sup>. Women with the short allele have been shown to have an increased susceptibility to depression and anxiety<sup>(23)</sup>. Individuals with the short allele have a reduced serotonin uptake protein activity in platelets<sup>(24)</sup>.

Tri-allelic 5-HTTLPR has been implicated in subjective pain perception<sup>(25)</sup>. For example, the short allele of the serotonin transporter gene has been associated with a negative emotional response to pain which is modulated at supraspinal level<sup>(26)</sup>. In a study on 67 healthy males and 60 healthy females using heat pain stimuli, carriers of the S-allele, with SS or LS genotypes had lower pain thresholds with an increased emotional reaction to pain<sup>(27)</sup>. Patients with trigeminal neuralgia have a higher prevalence of the 'SS' genotype than healthy age- and sex- matched controls<sup>(28)</sup>. There is a high incidence of depression in patients with chronic pain. In 277 patients with chronic pain, depression scores were significantly higher in patients with the 'SS' allele for 5-HTTLPR compared to those with the 'LL' allele<sup>(29)</sup>.

Considering that lower pain thresholds have been found in individuals with SS and SL genotypes and the fact that that women with CMFP had lower blood serotonin levels which were further reduced following the combination treatment of amitriptyline

Table 1. Study inclusion criteria.

Age 18 to 65 years
>15 pain days per month for >3 months
Bilateral, pressing or aching pain, affecting mid-face but may involve head
Normal ENT examination, fundoscopy, cranial nerves, blood pressure
Normal nasal endoscopy and CT sinuses/brain
Relapse of pain after initial response to amitriptyline or no response to analgesics or amitriptyline.

Table 2. Study exclusion criteria.

< 18 years of age or > 65 years
Rhinorrhoea, postnasal drip, hyposmia
Sinus surgery within 2 years
Previous facial or ophthalmic Herpes Zoster
Pregnancy
Barotrauma, Facial trauma
Temporomandibular joint dysfunction and pain of dental origin
Patients on antidepressants, hypnotics, beta-blockers, clopidrogel, aspirin (platelet activators)
> 1 attack migraine per month
Central Nervous System Degenerative disease or tumours
Substance or alcohol abuse
Hamilton Score >7 (clinical depression)
Medication overuse headache
CT mucosal thickening >3mm

and pindolol, this study investigated whether different serotonin transporter genotypes are associated with CMFP in women.

## Materials and Methods

Patients were recruited from a community based general otorhinolaryngology practice in Malta and blood samples were collected after informed consent was obtained and patients were given a full description of the study. Demographic and clinical data were stored in anonymised format for analysis. This study was approved by the University of Malta Ethics committee. Patients were recruited between 1 June 2014 and 30 November 2017.

The recruitment inclusion and exclusion criteria are listed in Tables 1 and 2; only patients between 18 and 65 years old were recruited. Pregnant women, patients with facial trauma or with pain due to changes in ambient pressure such as flying or diving were excluded. Patients with temporomandibular dysfunction with clicking, tenderness of the temporomandibular joint, or with dental pain related to thermal sensitivity or percussion of the teeth were excluded. A standard patient history was taken as shown in Table 3.

Table 3. Structured Clinical Interview for facial pain patients and their follow-up.

<b>Length of history</b>
<b>Site:</b> periorbital, paranasal, cheeks, frontal, vertex, occipital, temporal-unilateral, bilateral
<b>Quality:</b> pressing, aching, throbbing or other
<b>Duration,</b> frequency and intensity Presence of: photophobia, nausea, vomiting Presence of: nasal obstruction, postnasal drip, rhinorrhoea, hyposmia, cough, fever, halitosis, toothache, ear pressure, fatigue,
<b>Past history</b> of migraine, family history of migraine Past history of systemic illness (such as lung disease) Past history of atopy and skin test positivity Details of past of nasal surgery Cigarette smoke exposure Analgesic use –type and dose
<b>Nasal endoscopy:</b> normal, pus, polyps, mucosal oedema CT results: normal, rhinitis, sinusitis, anatomical abnormality
Final diagnosis

Chronic persistent mid-facial tension type pain was defined as bilateral symmetrical pressing tension-type pain according to International Headache Society criteria (IHS version III, 2013)<sup>(30)</sup> that persisted despite treatment with amitriptyline 10mg daily for 8 weeks or that recurred immediately on stopping amitriptyline.

Patients had an ENT examination, cranial nerve examination and fundoscopy to exclude papilloedema and their blood pressure was checked. Nasal endoscopy was carried out and those patients with intranasal pus or polyps were excluded. Those with oedematous mucosa were included since in previous local studies nasal mucosal oedema was a non-specific finding having no association with sinusitis<sup>(31, 32)</sup>. A computed tomogram of their brain and sinuses with coronal and axial cuts to exclude sinusitis and intracranial pathology was carried out. Computed tomography has for several years been considered the gold standard for evaluation of the paranasal sinuses<sup>(33)</sup>, especially when correlated with nasal endoscopy<sup>(34)</sup>. Patients with sinus mucosal thickening of over 3mm on the CT were excluded. Those patients with a history of psychiatric illness or those on any antidepressant, antipsychotic or hypnotic treatment were excluded since such individuals were suspected to have dysfunction of their serotonergic pathways and headaches have been associated with depression<sup>(35, 36)</sup>. As with previous studies<sup>11</sup> the Hamilton questionnaire was used to assess mood, feelings, insomnia, attitude towards work and somatic symptoms. Only those patients scoring up to 7 were included in this study, a Hamilton score of over 20 being strongly indicative of a depressive disorder<sup>(37)</sup>.

Individuals with medication-overuse headaches were excluded as it was determined that these constituted a particular group

that was difficult to treat and whose medication may have altered serotonin reuptake activity. Patients with substance or alcohol abuse were also excluded for the same reason. Five patients previously on antidepressants were ensured a 'washout' period of two weeks before starting other treatment. Any patients with intracranial tumours or neurological degenerative disease such as multiple sclerosis were excluded.

### Sampling and Laboratory analysis

#### Blood serotonin

Whole blood serotonin was estimated in 26 patients with persistent chronic tension-type facial pain and 33 healthy age- and gender-matched controls. Over ninety percent of blood serotonin is stored within platelets<sup>(6, 9, 38)</sup> with the remainder in the plasma. For 48 hours prior to the test, patients were instructed to avoid foods high in tryptophan (the metabolic precursor of serotonin) such as tea, coffee, nuts, avocado, pineapple, tomatoes, plums, eggplant and chocolate. This was done to enable maximal uptake of plasma serotonin into the platelets prior to the test so that when taken, the whole blood serotonin would more closely reflect intra-platelet serotonin. The dietary restrictions were also intended to avoid the variation possible in high-tryptophan diets, which have the potential to increase whole blood serotonin by up to 16%<sup>(38)</sup>. Those patients taking drugs that alter platelet activation, such as aspirin or clopidogrel, were excluded as activation releases serotonin into the plasma. Healthy controls were also required to avoid foods high in tryptophan for 2 days before their blood test.

Whole venous blood was collected at the St James Hospital laboratory, Malta using a pre-cooled heparinized plastic bottle and immediately frozen since serotonin levels are very sensitive to temperature. Samples were packed on dry ice in an insulated transport box, transported by courier to Biomnis laboratories in Lyon, France and analysed using High Performance Liquid Chromatography (HPLC) with electrochemical detection. Each HPLC analysis was preceded by internal standard calibration using standard solutions. Laboratory analysis was carried out by an observer with no knowledge of the headache condition of the patients or the controls.

To estimate intra-platelet serotonin a blood sample requires that it undergo immediate centrifugation to separate the plasma, which is then decanted off, from the platelet pellet. Centrifugation may disrupt platelets leading to release of serotonin into the plasma compartment while reducing platelet volume. Therefore, measurement of whole blood serotonin is preferable and more reliable<sup>(38)</sup> even if in this way one may not be able to differentiate between platelet and plasma values.

An information sheet was supplied to the patients and the corresponding author (AA) was on hand for guidance or questions.

Table 4. Sequence of primers used for the amplification of the 5-HTTLPR (BioneerR).

5-HTT-Forward	TCCTCCGCTTTGGCGCCTCT
5-HTT-Reverse	TGGGGGTTGCAGGGGAGATC

Patients were individually consented for entry into the study and were given the option to withdraw at any time.

**Genotyping**

DNA preparation. Blood samples from patients were collected in EDTA Vacutainer tubes. A volume of 200µl of blood was then used for DNA extraction using the Accuprep Genomic DNA extraction kit (BioneerR). The DNA was then collected in 40µL of elution buffer.

**Amplification of 5-HTTLPR**

For each sample the 5-HTT linked polymorphic region (5-HTTLPR) was amplified by Polymerase Chain Reaction (PCR) using the primers outlined in Table 4 (29). The reaction mixture consisted of 1XPCR buffer, MgCl2 dNTPs, Taq polymerase, forward and reverse primers and DNA. Samples were amplified on a PCR instrument (GeneAMP9700, Applied Biosystem) using the following thermal profile: 95°C for ten minutes, 30 cycles of 95°C for one minute, 62°C for one minute and 72°C for one minute and a final 72°C incubation for five minutes.

PCR products were then run in a 2.5% agarose gel stained with ethidium bromide which bound to the DNA. The DNA was run through the gel while a voltage of 200V was applied. After the run, the gel was visualised under Ultraviolet light.

The rate of DNA migration in the gel was dependent on the size of the DNA. Smaller fragments migrated faster down the gel than larger fragments. Depending on the 5-HTTLPR genotypes, SS, LS and LL DNA bands were observed.

As seen in Figure 1, LS was characterised by two bands, an upper one representing the L allele and a lower one representing the S allele. In comparison, an LL allele was represented by a single band that fell in line with the upper component of LS while SS was represented by a single band falling in line with the lower band of the LS.

**Sequencing of the rs25531 single nucleotide polymorphism (SNP)**

The rs25531 Adenosine to Guanine SNP was analysed by sequencing the 5-HTTLPR PCR product. The sequencing reaction was done using the BigDyeR Terminator v3.1 cycle sequencing kit (Applied Biosystems) and the forward primer as outlined in Table 4. The product was then run on the capillary electrophoresis genetic analyser 3130 (Applied Biosystem). Part of the sequencing data and the different results obtained for rs25531 are shown in Figure 2.

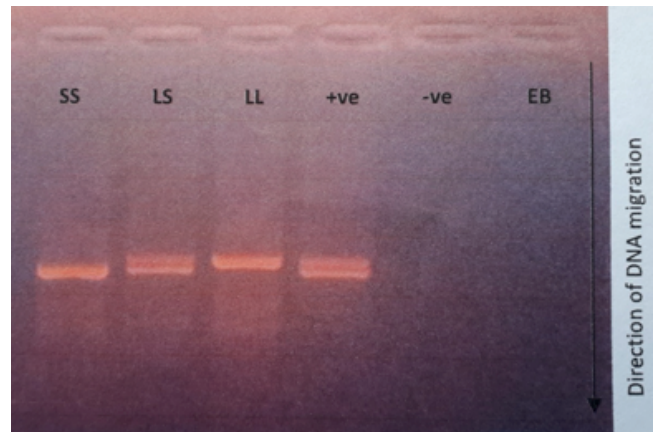


Figure 1. Agarose gel with DNA bands of the different genotypes (SS, LS and LL), positive control, negative control and extraction blank (EB) respectively. The lighter short DNA migrates further down than the heavier long one.

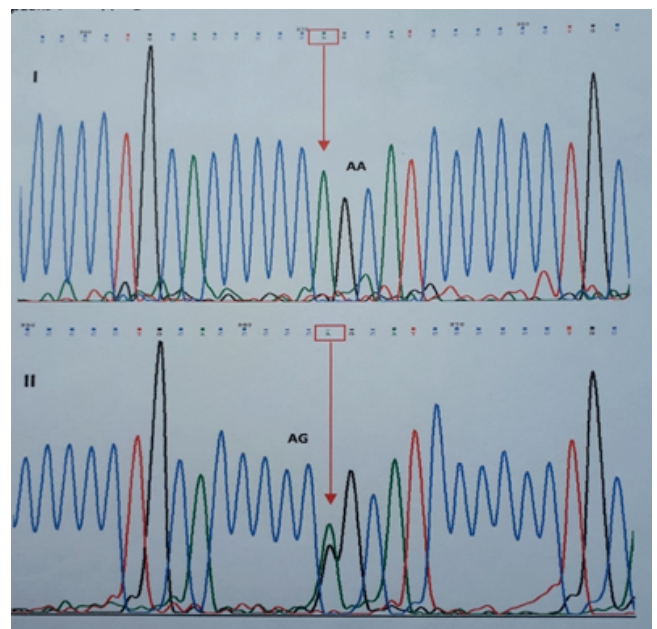


Figure 2. Section of the sequencing data surrounding the region of the Single Nucleotide Polymorphism rs25531. In diagram I (upper) the genotype AA is represented only by the presence of the Adenosine peak, in green. Diagram II (lower) shows the presence of the Adenosine (green) and Guanine (black) peaks which overlap.

**Results**

Twenty-six patients with chronic persistent mid-facial tension type pain (CMFP) satisfying the criteria laid out in table 1 were recruited. There were 23 women and 3 men. As with previous studies with facial pain (11,12), the overwhelming majority of patients were women and significant gender trends emerged during analysis when excluding male patients. It was therefore decided to report the results for the 23 women while excluding men.

Table 5. Clinical symptoms in 23 patients with the different genotypes. The symptom spectrum is similar in the three types.

Genotype	SS (n=4)	LS (n=13)	LL (n=6)
Mean pain Frequency (pain days per week)	5.3 ± 2.1 SD	5.4 ± 2.0 SD	6.7 ± 0.6 SD
Mean pain intensity (visual analogue score 1-10)	5.9 ± 2.0 SD	7.2 ± 1.9 SD	7.3 ± 0.6 SD

Table 6. Serotonin levels in female patients are lower than those in female controls (µg/L). The difference between patients and controls is statistically significant for those having the LL genotype (student's t test p<0.03).

Genotype	SS	LS	LL
Chronic Facial pain women(n=23)	162 ± 85 SD (n=4)	174 ± 63 SD (n=13)	143 ± 59 SD (n=6)*
Controls women (n=31)	212 ± 82 SD (n=7)	187 ± 41 SD (n=13)	199 ± 54 SD (n=11)*

Two female patients had concomitant migraine but they were included because they only had up to 1 attack monthly, within the criteria established in previous studies<sup>(39, 40)</sup>. The mean age of 23 female patients was 33.4 ± 10.2 standard deviation (SD) years, ranging from 20 to 39 years. They had a mean history of pain of 69 ± 51 months SD. Mean pain intensity based on visual analogue scale of 1 to 10 was 6.9 ± 1.8 SD. The mean pain frequency per week was 5.6 ± 1.8 SD pain days out of 7. Table 5 shows the mean pain frequency and intensity in the different genetic subgroups which were broadly similar and demonstrate the pain burden in these patients.

The 31 age-matched female controls were recruited from patients having elective surgery, on the basis that they had no history of chronic facial pain. Their mean age was 32.4 ± 11.1 SD years. The mean blood serotonin level in 23 female patients was 164 ± 64 µg/L SD. The mean blood serotonin level in controls was 197 ± 56 µg/L SD. This was significantly higher than patients (p=0.03, t test assuming unequal variances).

Table 6 compared mean blood serotonin level in the three genotypes of patients and controls. Chronic facial pain patients of all genotypes had a lower blood serotonin compared to controls. The blood serotonin in women with chronic pain having the SS genotype was less than in controls but was not statistically significant (p=0.19, t test assuming unequal variances). Similarly, women with pain having the LS genotype had a lower blood serotonin than control women with the same genotype but the difference was not statistically significant (p=0.27, t test assuming unequal variances). The difference in blood serotonin

Table 7. Number and percentage of women with chronic pain and female controls having the SS, LS or LL genotype (the difference is not significant, chi squared test p=0.6)

Genotype	SS	LS	LL
Chronic Facial pain (n=23)	4 (17%)	13 (56%)	6 (27%)
Healthy control women (n=31)	7 (22%)	13 (42%)	11 (36%)

Table 8. The percentage long-term significant clinical improvement in facial pain in the different genotypes after three-year follow-up. Patients with LL genotype did best but the statistical difference is non-significant, (chi squared test, p=0.1)

Genotype	SS	LS	LL
Patients clinically improved and % (n=9)	0 (0%)	5 (38%)	4 (67%)
Patients remaining the same (n=18)	4	8	2

levels between patients and controls in the LL genotype was significant (p=0.03).

Table 7 shows the numbers and percentages of female patients and female controls having LL, LS and SS genotypes. The genotype distribution in patients and controls was very similar. The presence of the polymorphism with Guanine replacing Adenosine (rs25531) was investigated as this polymorphism has been reported to affect function of the serotonin uptake protein. It was only present in two control individuals, one with the LL genotype and one with the LS genotype, so this did not seem relevant to the development of chronic facial pain in the Maltese population.

Patients were followed up for three years. Their outcome was classified into *significant clinical improvement*, where pain frequency or intensity was reduced by 50% or more according to previous studies<sup>(11)</sup>, or *same*, where pain continued without relief. From 6 women with the LL genotype, four (67%) were improved at three years. In the 13 women with the LS genotype, five were improved at three years (38%), while in those with the SS genotype, none were improved at three years since they all continued to have chronic pain (0% well, Table 8).

## Discussion

### Central sensitization and Diffuse Noxious Inhibitory Controls (DNIC)

Frequent nociceptive input from facial and cranial muscles in patients with infrequent episodic headache leads to second-order neuron sensitisation in the trigeminal subnucleus caudalis



and dorsal horn of the cervical spinal cord. As a result, patients subsequently develop frequent episodic tension-type headache. Continuous nociceptive stimulation in these patients sensitizes third order neurons in the thalamus and somatosensory cortex. Patients thus go on to develop chronic tension-type headache and exhibit hypersensitivity to stimuli in cephalic and extracephalic regions <sup>(41)</sup>.

In the sensitized state, the afferent A $\beta$ -fibres that normally inhibit nociceptive A $\delta$ - and C-fibres by presynaptic inhibition in the dorsal horn may instead stimulate ascending second-order neurons <sup>(42)</sup>. Moreover, the effect of A $\delta$ - and C-fibre stimulation is potentiated and the receptive fields of dorsal horn neurons are expanded <sup>(43)</sup>. Nociceptive input as well as its transmission to supraspinal structures would therefore be increased in central sensitization <sup>(44)</sup>.

Development of chronic pain such as seen in tension-type headache or fibromyalgia may therefore be due to a dysfunction in central pain inhibitory mechanisms. In so-called DNIC or diffuse noxious inhibitory controls, pain fibre stimulation is suppressed at the level of the spinal cord if multiple peripheral noxious stimuli converge. For example, in normal individuals subjected to heat stimulation at the thigh, further painful electrical stimuli at the forearm or head shows a higher pain threshold. This endogenous supraspinal pain modulation system is deficient in patients with chronic tension-type headache and their pain thresholds on further pain stimulation are significantly lower <sup>(45)</sup>. The term DNIC has recently been replaced by Conditioned Pain Modulation (CPM) <sup>(46)</sup>.

### Conditioned Pain Modulation (CPM)

Patients with chronic tension-type headache and fibromyalgia may be unable to activate their CPM <sup>(47,48)</sup> and the balance is shifted from pain inhibition towards pain facilitation. In a study on 191 healthy volunteers, carriers of the serotonin re-uptake protein long allele have been shown to have a significantly higher pain modulation compared to carriers of the short allele <sup>(49)</sup>. In a recent review of the literature, Hermans et al concluded that long allele carriers had better CPM <sup>(50)</sup>. The results of the current study indicate better long-term pain control in LL individuals.

There is an inherent difficulty with pain studies as conditions are not easily standardized <sup>(51)</sup>. Environmental influences, primarily stress, play a very important role in chronic tension-type pain. Hariri et al assessed the response to fearful stimuli using functional MRI and showed increased excitability of the emotion-processing areas of the brain in patients with the 'S' allele. This means that increased anxiety-related responses in these individuals may in turn, precipitate tension-type pain <sup>(52)</sup>.

It was not easy to recruit patients for this study as they had to

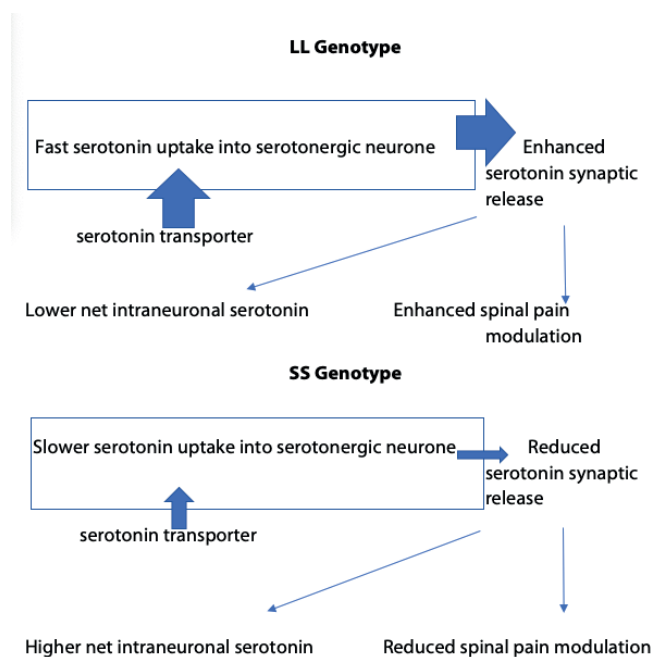


Figure 3. Diagram with hypothesis explaining intraneuronal serotonin levels in genetic subgroups.

satisfy the exhaustive list of exclusion criteria. Limited study numbers therefore have an impact on the study results and thus these need to be viewed with some caution.

The relative frequencies of the L and S alleles differ in various populations, with the L variant being twice as common in European compared to Asian populations <sup>(53,54)</sup>. The frequencies of SS, LS and LL alleles have been determined in a study on cord blood samples of 323 males and 307 females in Malta and were found to be 21.2%, 47.4% and 31.1% respectively - almost identical to the findings in this pilot study <sup>(55)</sup>. This supported the validity and accuracy of this pilot study even though the numbers were small.

Blood serotonin is almost exclusively platelet serotonin, which reflects intraneuronal levels <sup>(58)</sup>. Low blood serotonin in patients thus reflects low intraneuronal serotonin in the central nervous system. Patients with all genotypes had a lower blood serotonin than in matched controls. This difference was significant in those having the LL genotype ( $p=0.03$ ).

Explanation for this can be extrapolated from a previous investigation by the same authors where serial blood serotonin samples were taken in a randomized controlled study on 62 patients with CMFP <sup>(56)</sup>. Here patients were divided into three groups: a control group having placebo, a second group treated with 10mg amitriptyline daily and a third group receiving the combination treatment of amitriptyline 10mg with pindolol 10mg daily.

In the control group blood serotonin levels remained the same over 8 weeks while pain frequency and intensity remained

the same. In the amitriptyline group, serotonin levels went down but not significantly, while pain scores at the same time significantly decreased. In the amitriptyline with pindolol group, serotonin was significantly reduced and this was also associated with a very significant reduction in pain scores. Therefore the third group in the randomized trial with the most effective pain control showed the largest reduction in blood serotonin <sup>(11)</sup>.

It was likely that pindolol enhanced serotonin release from serotonergic neurons via its action on the 5-HT<sub>1A</sub> receptors thus resulting in high synaptic serotonin and enhanced pain control. Less net serotonin would have been eventually left inside the neuron, and this is reflected in lower blood serotonin levels in the platelet model (Figure 3).

There is a trend (Table 6) for blood serotonin to be reduced in patients with SS and LS genotypes compared to controls but this reduction is not significant. Blood serotonin in LL patients is significantly lower than controls. In LL patients the low blood serotonin compared to the other two groups is thought to represent a drop in intraneuronal serotonin due to distal synaptic release resulting in better pain control.

It is hypothesised that LL patients show the best pain control because they have a more efficient serotonin uptake and release. The high serotonin turnover gives clinical success despite low blood/neuronal serotonin because their intraneuronal serotonin would be more efficiently released and then taken up by a fully functioning serotonin transporter.

Patients whose pain did not respond to amitriptyline were given a four-week course of pindolol as adjunct, based on experience in other studies <sup>(11)</sup>. Two patients reported vivid dreams and discontinued their treatment. A variety of drugs including carbamazepine, gabapentin, pregabalin or citalopram were used with patients not responding to amitriptyline, with varying degrees of success.

In view of the costs of treatment with relatively expensive drugs, genotyping may help identify patients who are more likely to respond in the long term. In a clinical practice setting, genetic and serotonin tests as described above involve taking a blood sample that costs around 60 euro per test and a result is

obtained within one week. Good history-taking and long-term follow-up are important.

## Conclusions

The incidence of 'SS', LS and 'LL' genotypes of the serotonin transporter gene-linked polymorphic region (HTTLPR) in patients with chronic persistent tension-type facial pain is very similar to that in matched pain-free controls. Clinical data on long-term outcomes suggests a better prognosis for patients with the LL genotype, although patient numbers are small and results have to be interpreted with caution. Patients with the SS or LS genotype seem to be more likely to need treatments other than first-line amitriptyline. As with previous studies, patients with chronic facial pain have lower blood serotonin compared to healthy controls. This is a consistent finding regardless of genotype.

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## Authorship contribution

AMA: study design, patient recruitment, examination, testing, analysis; RM: study design, analysis.

## Conflict of interest

No conflict of interest reported.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data and materials

Not applicable.

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