JNNP Online First, published on October 6, 2014 as 10.1136/jnnp-2014-308813

RESEARCH PAPER

TRPV1, CGRP and SP in scalp arteries of patients suffering from chronic migraine

Marina Del Fiacco,[1] Marina Quartu,[1] Marianna Boi,[1] M Pina Serra,[1] Tiziana Melis,[1] Riccardo Boccaletti,[2] Elliot Shevel,[3] Carlo Cianchetti[4]

[1]Dipartimento di Scienze Biomediche, Sezione di Citomorfologia, University of Cagliari, Cagliari, Italy
[2]Divisione Neurochirurgia, Azienda Ospedaliera, Sassari, Italy
[3]Headache Clinic, Johannesburg, South Africa
[4]Neuropsichiatria Infantile, Azienda Ospedaliero-Universitaria, University of Cagliari, Cagliari, Italy

Correspondence to
Professor Carlo Cianchetti, Neuropsichiatria Infantile, Azienda Ospedaliero-Universitaria, University of Cagliari, Cagliari I-09124, Italy; cianchet@unica.it

Received 3 July 2014
Revised 28 August 2014
Accepted 7 September 2014

ABSTRACT

Objective The transient receptor potential vanilloid type-1 receptor (TRPV1) and the neuropeptides calcitonin gene related peptide (CGRP) and substance P (SP) appear to be differently involved in migraine pain. A role of neurovascular scalp structures is also suggested by several data. We performed a quantitative study of TRPV1-like immunoreactive (LI), CGRP-LI and SP-LI innervation of scalp arterial samples from patients affected with chronic migraine (CM).

Methods Short segments of scalp arteries were collected from 17 participants undergoing vascular surgery for treatment-resistant CM and from 6 controls who underwent neurosurgery for various indications. The immunoreactivity of the arterial innervation to TRPV1, CGRP, SP and to the pan-neuronal marker protein gene product 9.5 (PGP9.5) was examined. Immunoreactive nerve fibres in vessel cross-sections were quantified by computerised image analysis.

Results A significant increase of TRPV1-LI nerve fibres was found in the arterial wall from CM compared with control patients ($p<0.05$), while no significant difference was found for CGRP and SP.

Conclusions This study yields the first evidence for the existence of a TRPV1-LI innervation in human scalp arteries and provides the first quantitative assessment of the TRPV1-LI, CGRP-LI and SP-LI innervation of those vessels. The increase of TRPV1-LI periarterial nociceptive fibres of scalp arteries may represent, at least in some participants, a structural condition favouring CM (and possibly migraine), for example, by causing a higher sensitivity to algogenic agents.

INTRODUCTION

The transient receptor potential vanilloid type-1 receptor (TRPV1) and the neuropeptides calcitonin gene-related peptide (CGRP) and substance P (SP) appear to be differently involved in migraine pain.[1 2] Migraine pathogenesis classically implies central[3] and neurovascular intracranial structures.[5] However, a possible role of the neurovascular scalp structures is also suggested by several data recently reviewed,[6] and the ligature of scalp arteries has been reported as beneficial in chronic migraine (CM).[7] The availability of arterial specimens coming from such a surgical practice prompted us to study by immunohistochemistry the presence of TRPV1, CGRP and SP in them. Our results indicate a significantly increased number of TRPV1-like immunoreactive (LI) nerve fibres in the wall of scalp arteries from patients suffering from CM compared with controls.

To cite: Del Fiacco M, Quartu M, Boi M, *et al*. *J Neurol Neurosurg Psychiatry* Published Online First: [please include Day Month Year] doi:10.1136/jnnp-2014-308813

MATERIALS AND METHODS

Sampling

Short segments (about 5 mm long) of the superficial temporal (STA) and occipital (OA) scalp arteries were collected from 17 patients with CM undergoing vascular surgery as described by Shevel.[7] Inclusion criteria were diagnosis of CM according to the International Classification of Headache Disorders (ICHD-2)[8] and absence of any concomitant disorder contraindicating the operation. Groups of 3–4 consecutive patients with CM undergoing surgery on the same day or on the following day were included, and the samples were sent by express courier from the site of collection, Johannesburg. This caused long intervals in collecting samples, requiring the possibility of having more patients to operate in close proximity. Recruitment began in January 2009 and ended in February 2013. Scalp arterial segments from non-migraine patients were collected at surgery for intracranial pathologies in Sassari and used as controls. The collected segments belonged to the distal part of the artery, separated by the scalp incision to access the skull and destined to obliteration.

Table 1 shows the clinical characteristics of the patients. Improvement after surgery was evaluated by the patient according a 100 point numeric rating with a follow-up of at least 3 months.

Ethics approval

The sampling and handling of human specimens conformed to the programme approved by the Ethics Committee of the Azienda Ospedaliero-Universitaria (National Health System) of Sassari, in compliance with the principles enunciated in the Declaration of Helsinki, and the patients gave their informed consent to the surgery and to the use of specimens in the study.

Technical procedures

In Johannesburg and Sassari, specimens were collected at surgery and immediately fixed by immersion in 4% freshly prepared phosphate-buffered formaldehyde, pH 7.3, for 4–6 h at 4°C, and rinsed overnight in 0.1 M phosphate buffer (PB), pH 7.3, containing 10% sucrose.

Soon on arrival in the laboratory of Cagliari, all samples were cryostat cut transversely at 12 µm. Sections were collected on chrome alum-gelatin coated slides, stored at −80°C and processed within 3 months. The avidin–biotin–peroxidase complex (ABC) immunohistochemical technique was applied for the TRPV1 receptor, the neuropeptides CGRP

Table 1 List of specimens and clinical features of the patients

Case no.	Age	Gender	Pathology	Duration of migraine (years)	Specimen	Headache attack at surgery	Improvement >50% at follow-up
Chronic migraine							
1	31	F	Chronic migraine	12	STA	Yes	Yes
2	37	M		7	STA	No	Yes
3	47	F		4	STA	Yes	Yes
4	48	F		23	STA	No	No
5	24	F		4	OA	Yes	Yes
6	46	M		10	STA	Yes	No
7	48	F		19	STA	No	No
8	21	F		8	STA	Yes	Yes
9	54	F		40	STA	Yes	No
10	40	F		26	STA	Yes	Yes
11	51	F		34	STA	Yes	Yes
12	38	F		8	STA	Yes	Yes
13	27	F		14	STA	No	Yes
14	42	F		21	OA	No	Yes
15	59	M		42	STA	Yes	Yes
16	64	M		30	STA	Yes	Yes
17	22	F		5	STA	Yes	No
Control							
18	34	M	Meningioma	–	STA	No	
19	69	F	Middle cerebral art. aneurysm	–	STA	No	
20	55	M	Meningioma	–	STA	No	
21	61	F	Cavernous angioma	–	STA	No	
22	40	F	Clinoidal meningioma	–	STA	No	
23	59	M	Pituitary macroadenoma	–	STA	No	

F, female; M, male; OA, occipital artery; STA, superficial temporal artery.

and SP, and the pan-neuronal marker protein gene product 9.5 (PGP9.5). The latter, considered the best immunohistological marker for neuronal processes at all levels of the central and peripheral nervous system, was used to label the whole vessel innervation so as to quantify the TRPV1-LI, CGRP-LI and SP-LI nerve fibres in relation to the total innervation. Endogenous peroxidase activity was blocked with 0.1% phenylhydrazine in phosphate-buffered saline (PBS) containing 0.2% Triton X-100 (PBS-T). The following antisera were used as primary antibody: rabbit anti-TRPV1 (Thermo Scientific), raised against a 16 amino acid peptide near the carboxy terminus of human TRPV1, diluted 1:500; rabbit anti CGRP (Chemicon), raised against the whole molecule of human origin, diluted 1:1000; guinea-pig anti-SP (AbCam), raised against a synthetic peptide corresponding to amino acids 1–11 of rat SP, diluted 1:2000, guinea-pig anti PGP9.5 (Neuromics), raised against the amino acid sequence GASSEDTLLKDAAKVCR of the rat molecule, diluted 1:300. Biotin-conjugated goat anti-rabbit and anti-guinea-pig sera (Vector), both diluted 1:400, were used as the secondary antiserum. The immunoreaction was revealed with ABC (BioSpa Div.), diluted 1:250, followed by incubation with a solution of 0.1 M PB, pH 7.3, containing 0.05% 3–3′-diaminobenzidine (Sigma), 0.04% nickel ammonium sulfate and 0.01% hydrogen peroxide. Incubations with the primary antiserum were carried out overnight at 4°C. Incubations with the secondary antiserum and ABC lasted 60 and 30 min, respectively, and were performed at room temperature. All antisera and the ABC were diluted in PBS-T. Negative control preparations were obtained by incubating tissue sections in parallel with either PBS-T alone or with the primary antiserum preabsorbed with an excess of the corresponding peptide antigen. Alternate vessel sections were stained with modified Mayer's haematoxylin for morphological evaluation. Slides were dehydrated, cover-slipped and observed with an Olympus BX61 microscope. Digital images were captured with a ColorViewII Olympus camera.

Immunoreactive nerve fibres in the vessel sections were quantified by computerised image analysis (Olympus AnalySIS software) by an observer unaware of sample origin. The total length in μm of the immunostained fibre segments detectable in each entire cross-section of the vessel wall was captured using a ×20 objective magnification. The density of the positive innervation for each examined substance was calculated in three sections of each specimen as the ratio of such value to the relevant section area in μm^2. Density values obtained for PGP9.5 were further used to normalise those relevant to TRPV1, CGRP and SP in each section.

After the first collection of eight CM (2 different dispatches) and three control samples (3 different dispatches), a first blind evaluation of the results was performed by the laboratory staff (MQ, MB, MPS TM); at least three persons gave the evaluation of each sample and the mean of read values was used for the calculation. Later, the other two groups of CM (5 and 4 samples, respectively) and controls (1 and 2 samples) were studied with the same criteria.

Statistics

Non-parametric Mann-Whitney test (GraphPad Prism 5.00) was used for comparison between samples, and Pearson's test for the correlations.

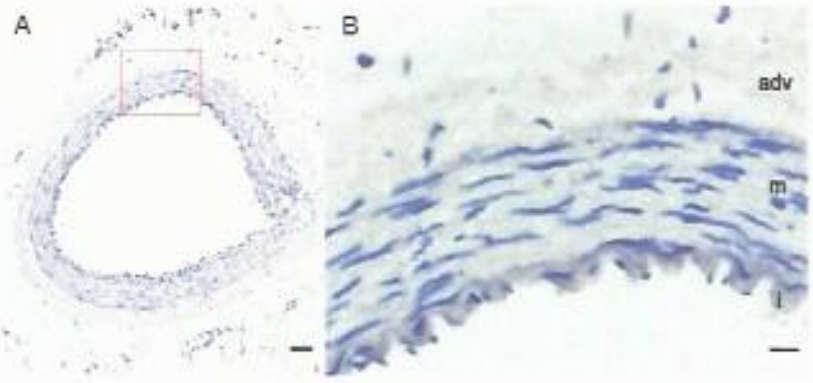

Figure 1 Histological aspect of the sections examined as exemplified in a sample of superficial temporal artery from a chronic migraine patient, after Mayer's haematoxylin staining. (A) whole section; (B) higher magnification of the field squared in A. i, tunica intima; m, tunica media; adv, tunica adventitia. Scale bars: A=250 mm; B=20 mm.

RESULTS

Histological analysis proved the good preservation of the structural tissue organisation of the collected specimens (figure 1). TRPV1-LI, CGRP-LI, SP-LI and PGP9.5-LI material was detected in varicose nerve fibres localised to the depth of the tunica adventitia, at the boundary with the tunica media of the vessel wall (figure 2). In addition, small nerve bundles displaying immunoreactivity for the examined molecules could be seen in the immediate surrounding area (eg, see figure 2C for CGRP-LI). Density of stained nerve fibres differed for each marker and varied among specimens (figures 2 and 3).

Values of mean density normalised to PGP9.5 in superficial temporal artery (STA) samples (15 patients with CM vs 6 controls) were (figure 3): (a) for TRPV1, CM specimens 0.60±0.51 (range 0.18–1.83) versus controls 0.22±0.17 (range 0.09–0.49), Mann-Whitney U=18.0, p=0.039; (b) for CGRP, CM 0.60 ±0.80 (range 0,10–2.5) versus controls 0.69+0.86 (range 0.23–1.52), Mann-Whitney U=35.0, p=0.28; (c) for SP, CM 0.18

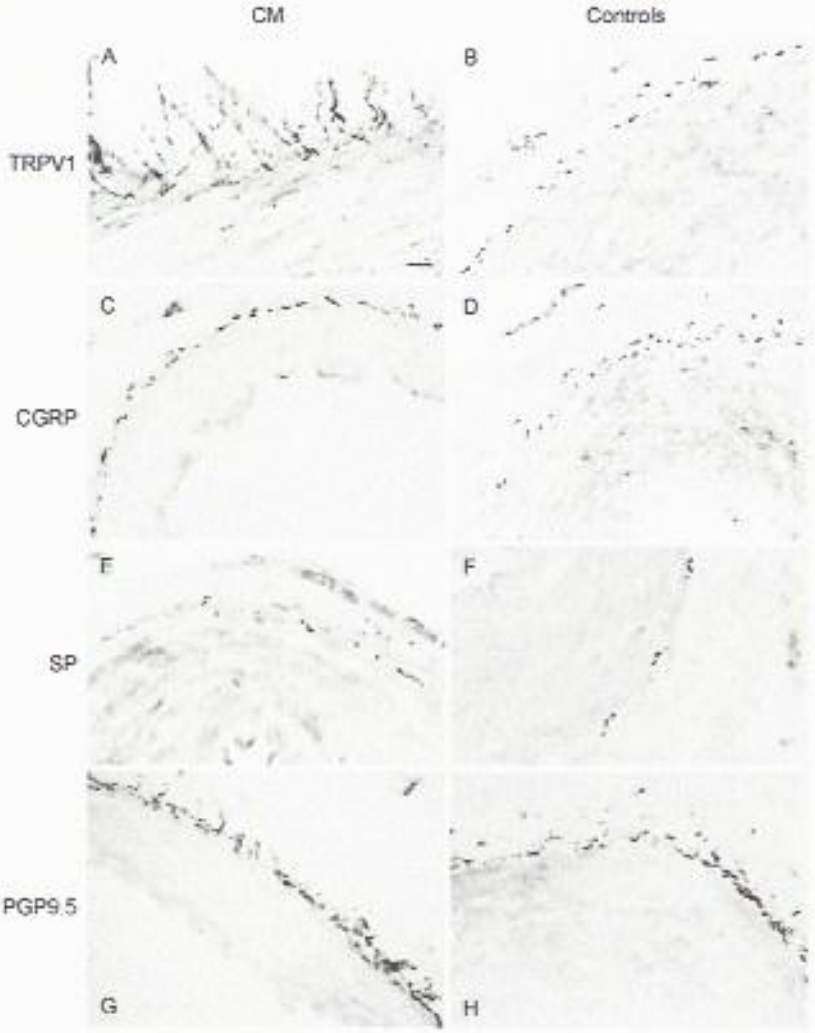

Figure 2 Nerve fibres immunoreactive to TRPV1 (A and B), CGRP (C and D), SP (E and F) and PGP9.5 (G and H) are localised to the depth of the tunica adventitia, at the boundary with the tunica media of the vessel wall. A higher density of positive fibres can be appreciated in CM samples (A, C, E and G) compared to controls (B, D, F and H). Scale bar in A=B–H=25 µm. CM, chronic migraine; CGRP, calcitonin gene-related peptide; PGP9.5, protein gene product 9.5; SP, substance P; TRPV1, transient receptor potential vanilloid type-1 receptor.

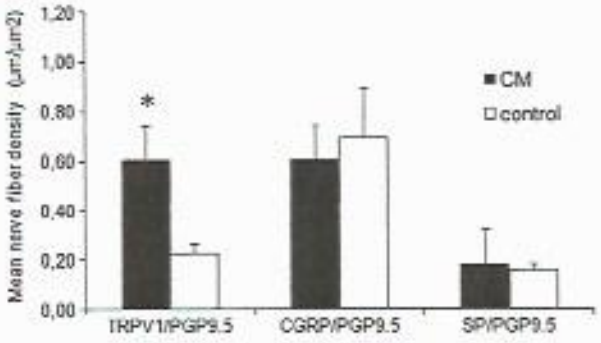

Figure 3 Density of TRPV1-LI, CGRP-LI, SP-LI innervation normalised to that of PGP9.5 in specimens from superficial temporal arteries of 15 sufferers (black columns) and 6 controls (open columns). Each column represents the average of the innervation quantified in three cross sections of each specimen of the relevant group. Bars indicate the standard errors. Differences between the two groups, as analysed by Mann-Whitney test, are statistically significant only for TRPV1/PGP9.5 (asterisk; p=0.033). CGRP, calcitonin gene-related peptide, CM, chronic migraine; LI, like immunoreactive; PGP9.5, protein gene product 9.5; SP, substance P; TRPV1, transient receptor potential vanilloid type-1 receptor.

±0.02 (range 0.03–0.80) versus controls 0.16±0.10 (range 0.07–0.24), Mann-Whitney U=36.0, p=0.31. The 2 OAs had TRPV1/PGP9.5 values in the range of the STAs (figure 4); with their inclusion in the STA CM group, the normalised density of TRPV1 positive innervation remained statistically different between the two groups (Mann-Whitney U=20.0, p=0.033).

Figure 4 shows the distribution of normalised TRPV1 mean density values in patients with CM and controls: two control samples show lower values than any other CM, and seven CM samples show values exceeding the highest values of controls.

Of the 17 patients with CM, 12 improved more than 50% after the operation, but 5 did not (table 1). The specimens of 2

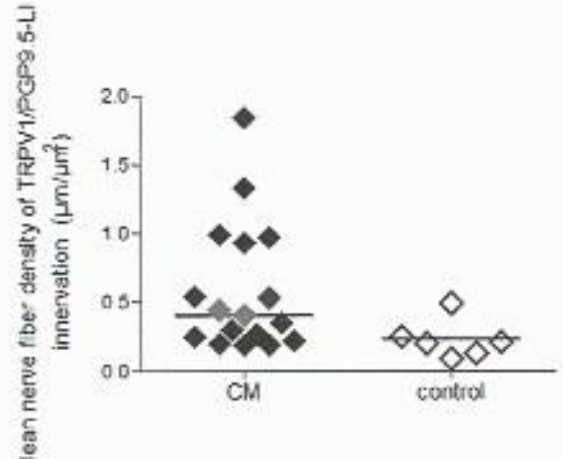

Figure 4 Density of TRPV1-LI innervation normalised to that of PGP9.5 in specimens from CM sufferers (black diamonds for the 15 superficial temporal arteries; grey diamonds for the 2 occipital arteries) and controls (open diamonds). Each scatter plot represents the mean density values of the innervation quantified in three cross sections of each specimen of the relevant group. The horizontal lines represent the average of the innervation values. CM, chronic migraine; LI, like immunoreactive; PGP9.5, protein gene product 9.5; TRPV1, transient receptor potential vanilloid type-1 receptor.

of these 5 had TRPV1/PGP9.5 values higher than the mean of the whole group, while the other 3 had lower. Mean TRPV1/PGP9.5 values were 0.59±0.52 in the 12 improved and 0.57±0.37 in the 5 not, mean CGRP/PGP9.5 values were 0.67 ±0.64 vs 0.35±0.30 and mean SP/PGP9.5 values were 0.20 ±0.23 vs 0.12±0.13; all differences were non-statistically significant.

Comparison of the normalised mean density in the 12 patients with CM under migraine attack at surgery (mean density values: 0.56 for TRPV1/PGP9.5; 0.66 for CGRP/PGP9.5; 0.20 for SP/PGP9.5) with those in the five patients with CM who were not under attack at surgery (mean density values: 0.63 for TRPV1/PGP9.5; 0.40 for CGRP/PGP9.5; 0.10 for SP/PGP9.5) resulted in no statistically significant difference between the two groups for any marker.

The increase of TRPV1/PGP9.5 did not correlate with age (for CM r=0.046; for controls r=0.230) nor with duration of migraine (r=0.268); TRPV1/PGP9.5 values did not differ by gender (F 0.60±0.54; M 0.59±0.42). Moreover, the values obtained in the samples collected in different periods were randomly distributed.

DISCUSSION

To the best our knowledge, this is the first study reporting evidence of the occurrence and evaluation of a TRPV1-LI innervation in human scalp arteries, providing a quantitative assessment of the TRPV1-LI, CGRP-LI and SP-LI innervation in those vessels, and examining the presence of TRPV1-LI, CGRP-LI and SP-LI in scalp arteries of patients with CM. Previous studies have shown the presence of CGRP and SP in nerve fibres of scalp arteries of normal participants[9 10] and that they elicit there a potent vasodilator response.[11] In agreement with Jansen Olesen et al,[10] we found a higher density of CGRP-LI than of SP-LI vessel innervation.

Our main result is that, compared with controls, the TRPV1-LI innervation of scalp arteries is frequently increased in CM sufferers. TRPV1-LI innervation does not show changes in relation to the presence or not of migraine at sampling and did not correlate with the duration of migraine. Of the CM STA samples, 8 of 15 showed TRPV1/PGP9.5 values in the range of the controls (figure 4). Moreover, 5 of the 17 patients with CM did not improve after the operation, although 2 of them had TRPV1/PGP9.5 values higher than the mean of the CM group. These results could suggest a different pathophysiology in these CM cases, including a more relevant role of central mechanisms, and, peripherally, the possible participation of another receptor of the TRP family (eg, the ankyrin type TRPA1).[12]

In front of the increase of the membrane receptor TRPV1-LI nerve fibres, we did not find a significant variation of CGRP-LI and SP-LI nerve fibres in arterial samples of patients with CM, indicating no significant variations of these peptides in the nerve fibres. CGRP has a definite role in migraine[3]: besides the fact that it can trigger an attack[13] and that the block of its receptor relieves migraine attacks,[14] CGRP is reported to be increased in blood from patients with migraine[7 15] and CM.[16] The increase in blood suggests an increased release of CGRP, which is favoured by the activation of TRPV1[17] and could be higher in the presence of a higher number of vanilloid receptors in nerve fibres. The lack of CGRP (and SP) variations in nerves fibres could therefore be due to an equilibrium or compensation between a probable neuronal hyperproduction and an increased release. Although no experimental data are available in this respect, a reduction of CGRP in the STA nerve fibres in case of

an inflammatory condition, where the vasodilatation suggests an increased release of CGRP has been reported.[18]

The involvement of TRPV1 in migraine has been suggested by genetic[19] and clinical[20–22] reports, although contrasted by a study on rats.[23] TRPV1 appears to be implicated in the antinociceptive mechanism of botulinum toxin A,[24 25] which is effective in CM[26] and its activation, as said above, elicits CGRP exocytosis.[17]

The increase of TRPV1-LI innervation in scalp arteries may be a structural condition favouring migraine or CM. It may cause a higher sensitivity to noxious agents. Although a role of central mechanisms in migraine is strongly supported by several data,[4] the sharing of a peripheral mechanism mainly acting on scalp arteries is also reliable.[6] Pressure painful scalp arteries are present in a large percentage of migrainous participants, during and in the interval between the migraine attacks.[27] This suggests a susceptibility to pain of such structures in migraine. All these data support the hypothesis of a peripheral vulnerability that, in synergy with a central dysfunction, can predispose to and cause the attacks.

Acknowledgements MPS gratefully acknowledges financial support from Fondazione Banco di Sardegna.

Contributors All authors have made substantial contributions to this work. CC, MDF and MQ prevalently to study design, laboratory analysis, manuscript preparation and revision; ES, RB, MB, MPS and TM prevalently to sampling and laboratory data collection and analysis. All authors critically revised the manuscript and approved the submitted version.

Competing interests None.

Patient consent Obtained.

Ethics approval Ethics Committee of the AOU of Sassari (National Health System).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Meents JE, Neeb L, Reuter U. TRPV1 in migraine pathophysiology. *Trends Mol Med* 2010;16:153–9.
2 Tsuyasu L, Kowwa H, Takeshima T, *et al*. Increased plasma substance P and CGRP levels, and high ACE activity in migraineurs during headache free periods. *Pain* 2007;128:209–14.
3 Ho TW, Edvinsson L, Goadsby PJ. CGRP and its receptors provide new insights into migraine pathophysiology. *Nat Rev Neurol* 2010;6:573–82.
4 Akerman S, Holland PR, Goadsby PJ. Diencephalic and brainstem mechanisms in migraine. *Nat Rev Neurosci* 2011;12:570–84.
5 Olesen J, Burstein R, Ashina M, *et al*. Origin of pain in migraine: evidence for peripheral sensitisation. *Lancet Neurol* 2009;8:679–90.
6 Cianchetti C. The role of neurovascular scalp structures in migraine. *Cephalalgia* 2012;32:778–84.
7 Shevel E. Vascular surgery for chronic migraine. *Therapy* 2007;4:451–6.
8 International Headache Society. The international classification of headache disorders. 2nd edition. *Cephalalgia* 2004;24(Suppl 1):9–160.
9 Jansen I, Uddman R, Hocherman M, *et al*. Localization and effects of neuropeptide Y, vasoactive intestinal polypeptide, substance P, and calcitonin gene-related peptide in human temporal arteries. *Ann Neurol* 1986;20:496–501.
10 Jansen Olesen I, Gulbenkian S, Valença A, *et al*. The peptidergic innervation of the human superficial temporal artery: immunohistochemistry, ultrastructure, and vasomotility. *Peptides* 1995;16:775–87.
11 Brain SD, Williams TJ, Tippins JR, *et al*. Calcitonin gene-related peptide is a potent vasodilator. *Nature* 1985;313:54–6.
12 Benemei S, De Cesaris F, Fusi C, *et al*. TRPA1 and other TRP channels in migraine. *J Headache Pain* 2013;14:71.
13 Lassen LH, Haderslev PA, Jacobsen VB, *et al*. CGRP may play a causative role in migraine. *Cephalalgia* 2002;22:54–61.
14 Edvinsson L. CGRP blockers in migraine therapy: where do they act? *Br J Pharmacol* 2008;155:967–9.
15 Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann Neurol* 1990;28:183–7.
16 Cernuda-Morollón E, Larrosa D, Ramón C, *et al*. Interictal increase of CGRP levels in peripheral blood as a biomarker for chronic migraine. *Neurology* 2013;81:1191–6.
17 Meng J, Ovsepian SV, Wang J, *et al*. Activation of TRPV1 mediates calcitonin gene-related peptide release, which excites trigeminal sensory neurons and is attenuated by a retargeted botulinum toxin with anti-nociceptive potential. *J Neurosci* 2009;29:4981–92.
18 Saldanha G, Longo J, Hunt G, *et al*. Decreased CGRP, but preserved Ink A immunoreactivity in nerve fibres in inflamed human superficial temporal arteries. *J Neurol Neurosurg Psychiatry* 1999;66:390–2.
19 Carreno O, Corominas R, Fernández-Morales J, *et al*. SNP variants within the vanilloid TRPV1 and TRPV3 receptor genes are associated with migraine in the Spanish population. *Am J Med Genet B Neuropsychiatr Genet* 2012;159B:94–103.
20 Fusco BM, Barzoi G, Agrò F. Repeated intranasal capsaicin applications to treat chronic migraine. *Br J Anaesth* 2003;90:812.
21 Cianchetti C. Capsaicin jelly against migraine pain. *Int J Clin Pract* 2010; 64:457–9.
22 Evans MS, Cheng X, Jeffry JA, *et al*. Sumatriptan inhibits TRPV1 channels in trigeminal neurons. *Headache* 2012;52:773–84.
23 Summ O, Holland PR, Akerman S, *et al*. TRPV1 receptor blockade is ineffective in different in vivo models of migraine. *Cephalalgia* 2011;31:172–80.
24 Gazerani P, Pedersen NS, Staahl C, *et al*. Subcutaneous Botulinum toxin type A reduces capsaicin-induced trigeminal pain and vasomotor reactions in human skin. *Pain* 2009;141:60–9.
25 Shimizu T, Shibata M, Toriumi H, *et al*. Reduction of TRPV1 expression in the trigeminal system by botulinum neurotoxin type-A. *Neurobiol Dis* 2012;48:367–78.
26 Dodick DW, Turkel CC, DeGryse RE, *et al*. OnabotulinumtoxinA for treatment of chronic migraine: pooled results from the double-blind, randomized, placebo-controlled phases of the PREEMPT clinical program. *Headache* 2010;50:921–36.
27 Cianchetti C, Lecca MR, Serra MC, *et al*. Painful scalp arteries in migraine. *J Neurol* 2010;257:1642–8.

TRPV1, CGRP and SP in scalp arteries of patients suffering from chronic migraine

Marina Del Fiacco, Marina Quartu, Marianna Boi, et al.

J Neurol Neurosurg Psychiatry published online October 6, 2014
doi: 10.1136/jnnp-2014-308813