

## ORAL PRESENTATIONS

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### FAMILIAL BASILAR-TYPE MIGRAINE ASSOCIATED WITH A NEW MUTATION IN THE *ATP1A2* GENE

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**Background** Basilar-type Migraine (BM) and Familial Hemiplegic Migraine (FHM) are phenotypically similar subtypes of migraine with aura, differentiated only by motor symptoms, which characterize FHM. The latter is thus far the only migraine subtype in which mutations have been found in the *CACNA1A* and *ATP1A2* genes.

**Methods** We looked for mutations in *CACNA1A* and *ATP1A2* in members of a family with BM. Screening was performed by direct sequencing on blood genomic DNA.

**Results** No mutations were found in *CACNA1A*. However, we identified a novel point mutation in exon 12 of the *ATP1A2* gene resulting in replacement of arginine 548 with histidine (R548H). Four individuals of this family carried the mutation, which was absent in 400 control chromosomes and in 174 chromosomes from unrelated migraineurs. "A posteriori" genotyping was consistent with linkage to the FHM2 locus.

**Conclusions** In this study we report a novel mutation in the *ATP1A2* gene (R548H) in a family with Basilar-type Migraine, a subtype of migraine with aura phenotypically close to FHM. This is the first report of an *ATP1A2* gene mutation in a form of migraine other than FHM. This finding suggests that BM and FHM may be allelic disorders.

### THE BLINK REFLEX IN CHRONIC MIGRAINE

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**Objectives** Activation of the trigeminovascular system as well as of brainstem trigeminal nuclei are thought to play an important role in migraine. The aim of this study was to investigate the habituation phenomenon of the blink reflex in "chronic migraine".

**Patients and methods** An EMG device with a specific habituation test program was used to elicit and record blink reflex responses on both the right and left sides, and to randomly repeat the stimulations at different time intervals in order to induce habituation. We studied 15 patients suffering from "chronic migraine" (diagnosed according to the IHS classification criteria) and 15 control subjects.

**Results** Whereas the R1 and R2 latencies, amplitudes and areas in the basal assessment were similar in patients and control subjects, the blink reflex habituation responses were markedly reduced in patients with "chronic migraine". In these patients, the differences between the R2 areas, obtained when stimuli were delivered at subsequent time intervals ranging between 10–5, 5–4, 4–3 and 3–2 seconds, were statistically different ( $p < 0.001$ ) from those of controls subjects.

**Conclusions** Our data suggest that the brainstem pathways involved in the blink reflex may be activated in chronic migraine, probably through mechanisms that involve dopaminergic function.

### CUTANEOUS ALLODYNIA AND CEREBROSPINAL FLUID GLUTAMATE LEVELS IN CHRONIC MIGRAINE PATIENTS

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**Introduction** Cutaneous allodynia has been described during migraine attack. Repeated measurements of mechanical and thermal pain thresholds of periorbital and forearm skin areas verified the occurrence of cutaneous allodynia during migraine in 79% of migraine patients. Cutaneous allodynia occurred either solely within the referred pain area on the ipsilateral head, or within and outside the ipsilateral head [1, 2]. Allodynia is considered a manifestation of central sensitization, but it is not routinely evaluated during clinical interviews even though its therapeutic implications are known [3]. In particular, no studies have been performed on cutaneous allodynia in patients with chronic migraine (CM) and its biochemical correlates have never been investigated.

**Patients** Fifty consecutive CM patients diagnosed according to ICHD-II Classification were included in the study and evaluated using a semi-structured questionnaire. Thirty CM patients underwent lumbar puncture, and measurements of glutamate were performed using HPLC.

**Results** Forty-eight (96%) CM patients reported allodynia. Twenty-seven (54%) had cephalic, 18 (36%) had both cephalic and extracephalic, and 3 (6%) exhibited pure extracephalic allodynia. Scalp allodynia was ipsilateral to the predominant headache side in the majority of patients and occurred at the height of the headache. Extracephalic allodynia of the upper extremities occurred in 8 (16%), and of the toes in 2 (4%). Truncal allodynia occurred in 3 (6%). Correlation was found between the duration of headache, and in particular, chronic headache and allodynia. The subgroup of patients with cutaneous allodynia exhibited higher CSF glutamate levels than healthy control subjects, all without evidence of cutaneous allodynia ( $p < 0.01$ ). CSF glutamate levels were greater in patients with both cephalic and extracephalic allodynia than in those with only cephalic or extracephalic allodynia.

**Conclusions** The present study supports the occurrence of detectable allodynia in the majority of CM patients. This is associated with higher glutamate levels, which can be considered the biochemical counterpart of central sensitization of second-order and third-order neurons in chronic migraine patients.

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### MODULATION VIA NOP1 RECEPTOR OF CGRP AND NITRIC OXIDE RELEASE IN AN IN VITRO MODEL OF TRIGEMINAL NEURON ACTIVATION

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**Background** Nitric oxide (NO) and calcitonin gene-related peptide (CGRP) are important mediators of vasodilation of blood vessels and are also algogenic substances. Both mediators are released from trigeminal ganglion neurons; recently NO was indicated as one of the molecules most involved in sensitization and thus in chronic headache. Nociceptin is a relatively novel peptide, which interacts with the NOP<sub>1</sub>

(ORL1) receptor, but does not bind classical opioid receptors; nociceptin produces *in vivo* supraspinal analgesic effects and is involved in antagonism of allodynia and hyperalgesia of noxious stimuli. The aim of our study was to investigate which mechanisms are involved in release of NO and CGRP, using the model of trigeminal ganglion neurons in culture, and how nociceptin could modulate this secretion.

**Methods** Trigeminal ganglion neurons in culture were prepared as described in previous studies. Briefly, ganglia from 6–7-day-old rats were quickly removed and digested by collagenase and trypsin, and finally neurons were collected through Percoll spin centrifugation. For plating we used from 120 to 150x10<sup>3</sup> cells per dish. IL-1 $\beta$  was used as proinflammatory stimulus. Immunocytochemical analysis against neurofilament 200 was performed to demonstrate purified neurons in culture. First, we evaluated the trend in IL-1 $\beta$ -induced release of CGRP and NO from cultures of trigeminal neurons and which concentration of IL-1 was more effective. Afterward, we evaluated CGRP and NO release by stimulation of the NOP1 receptor with a potent agonist, such as nociceptin-NH<sub>2</sub>, in two experimental conditions: (1) trigeminal neurons pre-treated with IL-1 $\beta$ , and (2) without pre-treatment.

**Results** At 24–48 hours, IL-1 $\beta$  induced maximal release of CGRP and NO; these findings confirm the data in the literature for CGRP and show a similar release pattern for NO. Nociceptin shows different modulation of NO and CGRP release in the two experimental conditions.

**Conclusions** Nociceptin modulates the secretion of inflammatory mediators in an *in vitro* model of trigeminal neuron activation.

## POSSIBLE ASSOCIATION BETWEEN MIGRAINE WITH ATYPICAL FEATURES OF AURA AND PATENT FORAMEN OVALE

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Atrial septal defect and patent foramen ovale (PFO) are present in approximately 25% of patients at autopsy and 45% of patients with cryptogenic stroke. Recent reports suggest an increased prevalence of PFO in migraine with aura (MA) patients and show that PFO closure reduces attack frequency. Transcranial doppler (TCD) is a very sensitive tool for detecting microembolic signals in the cerebral circulation after injection of saline solution mixed with air in cases of right-to-left shunt. Most of the studies in the literature are retrospective assessments and evaluate the modification of attack frequency after PFO closure in MA patients and migraine patients without (MO) aura. We set out to evaluate whether PFO is more prevalent among patients with atypical aura features, consisting of prolonged aura and/or interictal aura. MA patients who referred to a headache unit in the last year underwent contrast-enhanced TCD. The diagnosis of MA was made in accordance with the revised International Headache Society criteria (ICHD-II). Each patient underwent a structured interview in which we investigated family history, cardiocerebrovascular risk factors, comorbidity, and in detail, the clinical features of aura and headache. All the patients also underwent thrombophilic screening (antithrombin III, anticoagulant protein C, functional protein S, resistance to activated protein C, homocysteine, lupus anticoagulant and fibrinogen). We studied 65 subjects (48 females, mean age 35.2 $\pm$ 11.7 years; 17 males, mean age 37 $\pm$ 7.7 years). Forty-one (63.1%) of these patients fulfilled the criteria for typical aura and 24 (39.9%) for atypical aura. PFO was more prevalent in the subjects with atypical aura features than in those presenting typical aura (79.17% vs. 46.3%; OR 4.4; CI 1.4–14.0). When we compared the two aura groups (typical and atypical), no significant differences emerged in the thrombophilic markers. Similarly, no significant differ-

ences were found in these markers between the atypical aura subjects with patent foramen ovale *versus* atypical aura subjects without patent foramen ovale, and *versus* all typical aura patients. Our preliminary results strengthen the possibility that PFO is most frequently associated with atypical aura features, but the underlying pathophysiology of this association is not clear.

## CLUSTER HEADACHE IN ELDERLY PATIENTS

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**Introduction** Cluster Headache (CH) is commonly considered a disorder of young men. We report 18 patients with CH over 65 years of age.

**Patients and methods** We analysed the clinical charts of 7336 consecutive patients referred to our Headache Centre from 1995 to 2004. The elderly patients diagnosed with CH underwent re-evaluation and follow-up visits.

**Results** We identified 140 patients with CH, 18 of whom were over age 65. This group of geriatric CH patients consisted of 11 females and 7 males. Most of the patients suffered from episodic CH (12 cases, 7 females and 5 males), while the remaining six cases (4 females and 2 males) were affected by chronic CH. The age at onset occurred in the decade 35–44 for three males, in the decade 45–54 for one female, in the decade 55–64 for four females and one male, in the decade 65–74 for four females and one male, and in the decade 75–84 for two females and two males. Only the three cases with an early age at onset had several cluster periods, occurring every 1 to 2 years, but less frequently after age 60. The patients with an older age at onset had sporadic active periods. In four patients only two bouts occurred, with remissions ranging from 5 to 28 years, while four other patients had only one cluster period until now. The characteristics of the pain and its manner of occurrence were similar to those reported in the younger population, only the autonomic features seemed to be less prominent.

**Discussion** Our cases demonstrate that CH, although commonly considered a young male disorder, may also begin in geriatric age. Moreover, with regard to onset of CH, we found a significantly higher number of female patients above age 55. To our knowledge, we report the most numerous population of elderly patients with CH and the patient with the oldest age of onset of cluster headache (an 84-year-old woman) published in the literature to date.

**Conclusions** The onset of CH seems to be independent of the patients' period of life, even if the average age of onset peaks towards the third decade. Apparently peculiar to the female distribution, an increased frequency appears to occur in middle-aged and elderly patients. In the geriatric CH group, females represented the majority of cases, in contrast with the evident male preponderance in the previous decades.

## LONGITUDINAL UPDATE ON QUALITY OF LIFE, BODILY PAIN, ANXIETY AND DEPRESSION IN PRIMARY HEADACHE

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In this preliminary study, the time course of quality of life (QoL), bodily pain, anxiety, and depression were documented in sixteen headache outpatients (3 men and 13 women, mean age 29.31 years, range 21–62) at the baseline visit, and at 3- and 6-month follow-up visits. During the study, patients received only symptomatic treatment (simple or combination analgesics and triptans). No psychological therapies were introduced. QoL was assessed using the medical outcomes study Short Form

Health Survey (SF-36), a generic, self-administered instrument, which has been widely used in several chronic disorders [1]. The McGill-Melzack Pain Assessment Questionnaire was used to assess the quality and intensity of bodily pain [2]. The Beck Depression Inventory (BDI) was applied for assessment of depression [3]. Finally, the State-Trait Anxiety Inventory, form Y [4] was used to assess anxiety traits. In 7 of 16 patients (43.75%), QoL was globally restored to the baseline visit level three months after recruitment. Nearly identical prevalences were observed at the 3-month and 6-month follow-up visits. The patients' perception of bodily pain showed only marginal changes or remained stable at the two follow-up visits. Depression was a common finding at the baseline visit (75% had a BDI score indicating mild to moderate or moderate to severe depression). At the six-month follow-up, in 6 of 16 patients (37.5%) a degree in reduction of the depressive disorder was observed. The percentage of patients with anxiety traits (81.2%) also showed a declining trend in 12 of 16 patients (75%). The results of this preliminary report highlight the importance of health status evaluation as an essential step in the longitudinal assessment of patients with headache attending a headache centre.

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## NEONATAL HYPERACTIVITY AND MIGRAINE RISK: A 15-YEAR FOLLOW-UP STUDY

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**Background** From birth, human beings have individual emotional and behavioural characteristics, in particular, they have different reactivity. This is a characteristic strongly linked to the concept of temperament [1], and some authors considered it as an early temperamental trait [2]. Some infants (15%) presented a number of behavioural and physical peculiarities, in particular, they showed a low threshold of sensory stimulation and a lack of habituation to stimuli: the hyperactive (HPR) neonates [3]. Recent studies suggested that a low threshold of neurophysiological reactions and a lack of habituation are the principal abnormalities of sensory processing in migraineurs [4].

**Objective** To evaluate, after at least 15 years, the prevalence of primary headache in HPR infants.

**Subjects and methods** In 2003, we contacted 100 children (60 M, 40 F, mean age=17.5 years): 50 with at least 2 symptoms of HPR, 50 without; the groups were similar for age and gender. All infants were visit-

ed between 1 and 18 months of life at the Puericulture Institute of "La Sapienza". We administered a revised headache questionnaire [5], using chi-square test.

**Results** Twenty-one (43.2%) HPR infants suffered from migraine (69% were coded 1.6.1) vs. 5 (10.3%) of the control group (25% were 1.6.1) ( $p<0.05$ ); 19 (37.8%) suffered from TTH (57% were 2.4.1) vs. 15 (30.7%) of the control group (83% were 2.4.1).

**Discussion** The HPR neonates showed an "amplified" response to environmental stimuli [3] that was correlated with a real variation of brain electrical activity, i.e., an increased amplitude of evoked potentials [6]. Both evoked potential and transcranial magnetic stimulation studies showed that the cerebral cortex, and possibly subcortical structures, are dysfunctioning in migraine. These electrophysiologic abnormalities, whose hallmark is deficient habituation, tend to normalise just before and during an attack [4]. From these electrophysiologic data and from the greater incidence of migraine in our experimental group, we can hypothesize that neonatal hyperactivity represents the first step in CNS hypersensitivity which, during childhood, could manifest itself as migraine. It is also true that a reduced ability to ignore novel or repeated stimuli influences behaviour and cognition [7], and that certain personality traits are assumed to predispose to the development of particular diseases [8]. This is important for prevention, treatment and recovery from the disease.

**Conclusions** HPR, a part of negative emotionality, already proved to be related to psychosomatic disorders [9] of temperament, is an important risk factor for developing migraine, but not other primary headaches.

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