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Cephalalgia

Vagally Mediated Heart Rate Variability in Headache Patients – A Systematic Review and Meta-Analysis

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Abstract

Objective: Vagal nerve activity – indexed by heart rate variability (HRV) – has been linked to altered pain processing and inflammation, both of which may underpin headache disorders and lead to cardiovascular disease (CVD). Here we examined the evidence for differences in parasympathetic (vagal) activity indexed by time- and frequency-domain measures of HRV in patients with headache disorders compared to healthy controls (HCs).

Methods: A systematic review and meta-analysis was conducted on studies investigating group differences on resting-state, vagally-mediated HRV (vmHRV) including time- (root-mean-square of successive R-R-interval differences (RMSSD)) and frequency- (high-frequency HRV) domain measures. Studies eligible for inclusion were identified by a systematic search of the literature, based on the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) statement.

Results: 7 studies reporting a total of 10 comparisons of patients with headache disorders (HF-HRV n=67, RMSSD n=122) and HCs (HF-HRV n=64, RMSSD n=125) were eligible for inclusion. Random-effects meta-analysis revealed a significant main effect on RMSSD ($Z = 2.03$, $p = 0.04$; Hedges' $g = -0.63$; 95% CI [-1.24, -0.02]; $k = 6$) and similar pooled effect size estimates for HF-HRV when breathing was controlled ($g = -0.30$; 95% CI [-0.69; 0.10]) but not when breathing was not controlled ($g = 0.02$; 95% CI [-0.69; 0.74]). Controlling for breathing had no effect on RMSSD.

Conclusion: vmHRV is reduced in patients with headache disorders, findings associated with a medium effect size. Suggestions for future research in this area are provided, emphasizing a need to investigate the impact of headache disorders and commonly comorbid conditions – including mental disorders – as well as the investigation of the risk for CVD in migraine in particular. We further emphasize the need for large scaled studies to investigate HRV as a mechanism mediating the association of migraine and CVD.

Keywords: *Primary Headache Disorders, Migraine, Tension Type Headache, Heart Rate Variability, Parasympathetic Activity, Meta-Analysis*

1. INTRODUCTION

Autonomic nervous system (ANS) dysfunction has been linked to common headache disorders including migraine (1,2,3) and tension type headache (TTH, 4,5). The association between ANS dysfunction and migraine, in particular, is well documented within the literature. Heart rate variability (HRV) is the beat-to-beat variation in the heart rate and is a surrogate measure of parasympathetic (vagal) activity. Parasympathetic modulation of the heart rate is fast (timescale in the order of milliseconds) while sympathetic effects are much slower (6,7). Therefore, high-frequency (HF) HRV and time-domain measures reflecting these fast changes (i.e., the root-mean-square of successive R-R-interval differences, RMSSD) provide a readily available, surrogate measure of vagal-activity. Here we conducted a systematic review and meta-analysis to provide an objective and up-to-date assessment of this literature, and to clarify the association between headache and vagal function, as assessed by HRV. While most studies have emphasized a role for sympathetic hyperactivity in headache disorders, examining a potential role for vagal parasympathetic-activity may provide additional insights into the mechanism underlying headache disorders.

Importantly, there is evidence that migraine with aura (MwA) is associated with increased risk of major CVD, myocardial infarction, ischemic stroke, and death due to ischemic CVD (8). Other studies found, that both MwA and migraine without aura (MwoA) are associated with CVD and risk factors for CVD (9,10). A recent systematic review found that migraine is associated with a twofold-increased risk of ischaemic stroke, which is only apparent among people who have MwA (11). Therefore, understanding the role of HRV in headache disorders might promote a deeper understanding of the increased risk for CVD in headache patients.

Vagal impairment – as indexed by HRV – may further lead to heightened inflammatory processes, which have also been associated with migraine (12). In particular, impaired vagal efferent activity may lead to excessive inflammation via failure to inhibit release of pro-inflammatory cytokines, reflecting a poorly functioning anti-inflammatory reflex (13-18). These inflammatory processes may cause prolonged, ongoing excitation of primary nociceptive neurons leading to chronic painful conditions. Recently, we were able to show that vagally-mediated HRV (vmHRV) predicts increased levels of inflammation four years later (19), providing evidence for the anti-

inflammatory reflex in humans. Vagal-nociceptive networks are also involved in pain processing (20), and decreased vagal-activity leads to greater somatic and visceral input via the spinothalamic track. Such loss of sensory integration due to decreased vagal activity may result in greater affective processing of nociceptive information that results in overstraining adaptive capabilities (21).

Lower vmHRV is reported in a variety of chronic painful conditions, such as chronic neck pain (22), chronic pelvic pain (23), complex regional pain (24), fibromyalgia (25,26) and the irritable bowel syndrome (27). In light of this work, the vagus nerve is widely perceived as a promising target for therapeutic interventions in the treatment of chronic pain. An important area involved in descending inhibitory modulation of pain is the periaqueductal grey (PAG). It has been shown that stimulation of the ventral PAG increases HRV and decreases pain in humans with chronic pain (28). Given that this pathway is distinct from dorsal PAG stimulation, it has been suggested that analgesia with deep brain stimulation in chronic pain is associated with increased vagal parasympathetic activity (28). Considering these anatomical connections, the prominent role of the vagus nerve in pain processing, and promising findings on vagus nerve stimulation in subjects with chronic pain (29,30) – including migraine (31) and cluster headaches (32) – is slowly gaining recognition. Resting vagal-activity in headache patients might be more than a simple marker of ANS dysfunction; it may actually be a major contributor to the condition itself. Recently, we were able to show (21), a negative correlation of vmHRV and pain in multivariate adjusted analysis. Most interestingly – and deserving special notice – while individuals with chronic pain reported lower vagal activity, the correlation of pain severity and vmHRV was only present in respondents without chronic pain, suggesting that the descending inhibitory pathway via the vagus nerve is disturbed in persons with chronic pain (21).

Chronic reductions in HRV are also associated with a variety of comorbidities that are, themselves associated with headache disorders. While decreased HRV increases risk for morbidity, especially CVD, and is an independent risk factor for mortality (33,34), headache disorders are also associated with comorbid conditions such as depression and anxiety (35,36), which are also associated with reductions in HRV (37,38). The lifetime prevalence of major depression is approximately three times higher in patients with migraine and in persons with severe headache disorders (39). Depression without CVD is associated with reduced HRV (37,40). While the

underlying mechanisms in the association of migraine and psychiatric comorbidity remain unclear (41), vmHRV may provide a valuable tool to further investigate the association of migraine, CVD, and comorbid depression. The present systematic review and meta-analysis, reviews the current evidence on differences in resting-baseline vmHRV addressing if vmHRV is reduced in headache patients in comparison to healthy controls (HCs).

2. METHODS

2.1 Systematic Literature Search

A systematic search of the literature, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (42) was employed. *PubMed*, *PsycNET/PsycINFO*, *CINAHL Plus*, and *Web of Science* (WOS) databases were searched using the search terms "*heart rate variability*" and "*headache*" OR "*migraine*" (*see* Appendix A, for search strategy by database). The number of initial hits was recorded and additionally, a hand search (i.e., *Google*, *Google Scholar* and other sources) was performed. Reference lists of included studies were also checked for additional studies eligible for inclusion. After removing duplicates, abstracts of all articles were screened based on pre-defined inclusion criteria. Studies were included if they reported (i) an empirical investigation in (ii) humans and (iii) recorded HRV in (iv) headache patients compared to HCs. All titles meeting the inclusion criteria were retrieved and reviewed in full-text. Empirical investigations were defined as studies involving active data collection in human subjects. Reviews, meta-analysis, comments, or single-case reports were excluded. Animal studies and studies using a computational modeling approach (i.e., virtual data) were also excluded. The number of studies meeting the pre-specified inclusion criteria, number of studies excluded, and reasons for exclusion were recorded (Figure 1).

2.2 Data Extraction

Guidelines from the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (43) were used to define the HRV measurements included for analysis. Only components reflecting primarily vagal cardiac modulation were included. These included the root-mean-square of successive R-R-interval differences (RMSSD) or any spectral measure in the high frequency (HF) range of 0.15 – 0.4Hz (natural

log transformed (lnHF), normalized (HFnu) or expressed as absolute power in ms^2 (HFP)). Authors who reported HRV but who did not report RMSSD or HF-HRV, or did not provide sufficient quantitative data (e.g., only a graphical display), were contacted to request the necessary information to derive effect size estimates and confidence limits on the selected indices of HRV. Studies that reported multiple groups of headache patients were included as long as they reported findings against HCs, while studies that compared different groups of headache patients only were excluded. When multiple groups of headache patients (e.g., MwoA and MwA) were reported, each group was compared to the same group of HCs.

2.3 Meta-Analysis

Descriptive statistics (means and standard deviations (SD)) of time- (RMSSD) and frequency- (HF-HRV) domain measures of vagally-mediated HRV (vmHRV) from HRV recordings were extracted for each group. In case descriptive statistics were reported other than as mean and SD, data was imputed if possible (44,45). Where longitudinal or pre-post data were reported, only baseline resting HRV was included to minimize confounding effects by experimental manipulation and conflation of effect size estimates. True effect estimates were computed as adjusted standardized mean differences (Hedge's g). We undertook meta-analyses using both fixed-effect and random-effects models. When results of both analyses are consistent (with confidence intervals (CI) of fixed-effect analyses being included within that of the random-effects analysis), the results from random-effects models are reported, as it better conveys the variability of data. However, when fixed-effect and random-effect models give different results, results of fixed effects analyses are reported, as they provide a more reliable estimate of the true effect (46). Possible sources of heterogeneity or inconsistency among trials in the magnitude or direction of effects were investigated. Heterogeneity was assessed using the standard I^2 index, Chi-Square, and Tau^2 tests (47). Bias was examined using a funnel plot of effect size against standard error for asymmetry. Meta-analytic computations were performed using RevMan (*Version 5.3.4, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014*). Risk of bias was also assessed in regards to reporting of comorbid conditions, medication intake at the time of testing, other restrictions prior to the recording of HRV (i.e., smoking, alcohol), and sample homogeneity including age, gender, headache diagnosis, headache history and frequency reported. Furthermore,

meta-regression on potential covariates was conducted if sufficient data was available. These covariates include headache diagnosis, headache history (duration of disorder), symptom/attack frequency, age differences, gender differences, differences in the length of HRV recording and control for respiration.

3. RESULTS

3.1 Selection of Studies

The systematic search of the literature revealed a total of 77 abstracts (after removing duplicates) that were screened for eligibility of inclusion within the present meta-analysis. A total of 18 studies were considered eligible for inclusion and retrieved in full-text (if possible). Of the 77 abstracts evaluated, 7 studies (48-54) yielding a total of 10 comparisons (HF-HRV k = 4, n = 67 headache patients and n = 64 healthy controls; RMSSD: k = 6, n = 122 headache patients and n = 125 healthy controls) were included in the meta-analysis. The systematic literature search and reasons for exclusion of studies are illustrated in Figure 1.

< Insert Figure 1 >

3.2 Included Studies by Clinical Etiology

Sample characteristics of included studies are summarized in Table 1. The majority of studies investigated patients with migraine. Nilsen and colleagues (49) investigated differences in HRV and spontaneous baroreflex sensitivity in female migraine patients (n = 16) compared to HCs (n = 14). They recorded HRV for 24 hours and 10 min of paced breathing. To avoid effect size inflation by including multiple measures from the same participants, only HRV indices derived from the 24-hour measurement period was used. Long-term recordings were also obtained by Tabata and colleagues (51) who investigated differences in the circadian rhythm (48-hour recordings of HRV) in 27 patients with migraine and 24 HCs. The authors only report values derived from the Midline Estimating Statistic of Rhythms (MESOR) variable, a measure derived from cosinor analysis of long term recordings. There were no significant differences between headache patients and HCs. We included mean and SD of RMSSD from

MESOR analysis for meta-analysis. The study by Vollono et al. (52) reported HRV data in different sleep stages and wakefulness and found a significant reduction of RMSSD, HF total power, and NN50 in patients with migraine compared to HCs during waking rest. The authors compare headache patients to a larger sample of HCs and age and sex-matched HCs, of which the later comparison was used for meta-analysis. Sanya and colleagues (53) assessed the autonomic regulation of the heart and peripheral blood vessels in migraine patients (n = 30) and HCs (n = 30) during baroreflex stimulation by oscillatory neck suction. Analysis of 5 min baseline recording (paced breathing), revealed no significant difference on measures of HF-HRV between patients and HCs. Thomsen and colleagues (54) compared cardiovascular response in 50 participants (n = 27 MwoA and n = 23 MwA) and HCs (n = 30), undergoing a head-up tilt test, a cold-pressor test, and a Valsalva maneuver. Data reported from the normal breathing baseline recording (150 consecutive heart beats, outside a migraine attack) expressed as MSD index (mean successive difference of the numeric values of the R-R intervals) were extracted and subjected to meta-analysis. The authors reported no significant difference between headache patients and HCs within this condition.

< Insert Table 1 >

Gass and Glaros (48) investigated whether HRV measures taken at rest from patients with headache disorders (n = 21; TTH, migraine, and mixed) differed significantly from those without headaches (n = 19). They reported that headache subjects had less variability in all HRV time domain measures, increased sympathetic nervous system (SNS) activity and decreased parasympathetic nervous system (PNS) activity compared to HCs. However, significant differences between groups were only reported for pNN50 and NN50 indices of HRV – both measures of vmHRV. RMMSD and HF-HRV derived from 5-minute recordings of HRV were subjected to the meta-analysis. Furthermore, one study by Tubani and colleagues (50) investigated differences in 24-hour recordings of HRV in patients with cluster headache (n = 8) compared to HCs (n = 8). Results revealed significant differences on low-frequency HRV (LF-HRV) but not HF-HRV. Reported HF-HRV values for the entire length of

the recording (24-hour) were subjected to meta-analysis. Details on extracted HRV values and conditions are provided in Table 2.

< Insert Table 2 >

3.3 Meta-Analysis Main Effect

First, we aimed to identify an overall effect of headache disorder on HRV, followed by subsequent analysis that aimed to investigate differences between clinical etiologies (diagnosis). Random-effect meta-analysis, on RMSSD, revealed that headache patients (n = 122) displayed significantly lower HRV compared to HCs (n = 125) (Z = 2.03, $p = 0.04$) (g = -0.63; 95% CI [-1.24, -0.02]; k = 6) indicating lower vagal activity in headache patients. CIs of fixed-effect models (Z = 4.35, $p < 0.00001$; Hedges' g = -0.58; 95% CI [-0.85, -0.32]; k = 6) were included in those of random-effect models, thus random-effects are illustrated in Figure 2 (negative effect estimates reflect lower RMSSD in headache patients).

< Insert Figure 2 >

Random-effect meta-analyses on HF-HRV revealed no significant difference (Z = 1.26, $p = 0.21$) between headache patients (n = 67) and HCs (n = 64) (Hedges' g = -0.22; 95% CI [-0.57, 0.12]; k = 4). As the CI of the fixed-effect model (Z = 1.26, $p = 0.21$; Hedges' g = -0.22; 95% CI [-0.57, 0.12]; k = 4) was included in that of the random-effects model, results of random-effects are illustrated (Figure 3; negative effect estimates reflect lower HF in headache patients).

< Insert Figure 3 >

Significant heterogeneity was observed for RMSSD (*see test results in* Figure 2) and visual examination of funnel plots for HF (Figure 4a) and RMSSD (Figure 4b) revealed considerable asymmetry for RMSSD, indicating

publication bias. Substantial heterogeneity was assumed if I^2 was greater than 50%, indicating that 50% of the variability in the outcome cannot be explained by sampling variation.

< Insert Figure 4 >

As we assumed, that the significant effect on RMSSD might relate to the considerable asymmetry of included studies, in particular the unlikely large effect reported by Tabata et al. (51), and given that this effect estimate was derived from a different analytical approach (MESOR-analysis) we excluded the study in a secondary analysis (leave-one-out meta-analysis). When excluding the study (*see outlier in* Figure 4), the significant main effect on RMSSD remained in fixed-effect meta-analysis ($Z = 2.33$, $p = 0.02$; Hedges' $g = -0.34$; 95% CI [-0.63, -0.05]; $k = 5$) (CIs of fixed-effect not included in those of random-effect models). Thus, we conclude, that the main effect reported for RMSSD is likely driven by study heterogeneity, but remains significant when controlling for such bias.

3.4 Risk of Bias and Meta-Regression

Risk of bias assessment (Table 3), revealed large risk of bias regarding the reporting of comorbid conditions (i.e., depression, anxiety), medication intake, and other restrictions prior to the examination (i.e., smoking, alcohol) that have substantial effects on HRV. Furthermore, reported samples from studies showed large heterogeneity in age, gender, headache history and symptom frequency.

< Insert Table 3 >

We performed a series of meta-regressions and sub-group analysis to identify potential covariates of the observed main effect found. There were not enough studies to address differences between studies recording HRV during uncontrolled (free) breathing and paced breathing using meta-regression on HF but RMSSD (Table 2). In a

sub-group analysis on HF, studies that controlled or recorded breathing showed a significant greater pooled effect estimate for HF (g = -0.30; 95% CI [-0.69; 0.10]), compared to studies using uncontrolled conditions (g = 0.02; 95% CI [-0.69; 0.74]). Controlled breathing was not a significant covariate of RMSSD (β = 0.822, SE = 0.637; 95% CI [-0.427; 0.637]; *p = 0.197*). Further sub-groups analysis on etiology, indicated lower RMSSD in migraine patients (g = -0.93; 95% CI [-1.69; -0.18]) compared to a sample of patients with a variety of headache disorders, including migraine and TTH (g = -0.48; 95% CI [-0.48; 0.15]). Furthermore, patients with cluster headache showed increased HF (g = -0.48; 95% CI [-0.65; 1.41]) and patients with migraine decreased HF (g = -0.18; 95% CI [-0.63; 0.27]). No other covariate yielded a significant influence on the effects reported.

4. DISCUSSION

Research on differences in resting state vagal activity in headache patients may have important implications for better understanding the elevated risk for CVD in these individuals. The present meta-analysis is the first to summarize the current evidence on vagal activity as indexed by vmHRV in headache patients. Meta-analysis revealed a significant and sizeable main effect on RMSSD (Hedges' g = -0.63) and similar effect-size estimates for HF-HRV when breathing was controlled (Hedges' g = -0.30). The effect for both selected indices of vmHRV revealed similar findings, indicating decreased vagal activity in headache patients.

While our initial analysis indicated significant differences between headache patients and healthy controls on RMSSD only, sub-group analysis showed an effect of respiratory control on the reported effects for HF-HRV, but not RMSSD. Our findings demonstrate that studies controlling for respiration reported larger effect sizes, than those that did not control for respiration. Thus, the results for RMSSD and HF-HRV are consistent when respiration is controlled. The fact that respiration had a greater effect on HF-HRV than on RMSSD is not surprising. Our group (55) as well others have shown that RMSSD is less affected by respiration than other indices of HRV including spectrally derived HF HRV (56). HRV indices derived from frequency domain analysis provide information of different quality and detail compared to time domain analysis (57). While RMSSD and HF are highly correlated (58), it has been suggested that time domain parameters can be estimated with less bias and considerably smaller variability as compared with frequency domain parameters (59, p. 290).

While we selected the two most prominent and frequently used parameters of vagally-mediated HRV (RMSSD and HF) for the present analysis, other indices reflecting vagal activity such as the NN50 or pNN50 component are of interest. Three studies by Gass and Glaros (48), Tabata (51) and Volono et al. (52) reported these and found significantly lower NN50 and pNN50 in headache patients compared to healthy controls in line with our findings of lower vagal activity indexed by RMSSD and HF.

Of the four studies reporting RMSSD, only two studies initially reported significant differences between headache patients and healthy controls (HCs) (49,52), while three found no significant differences on the selected indices of vagal activity (48,51,54). One study that reported significant differences found RMSSD to be greater in headache patients (49), and one found RMSSD to be lower in headache patients (52). Except for one study (52), none of the studies on HF-HRV initially reported significant differences between headache patients and HCs (48,50,53). This further highlights the utility of meta-analysis to provide a more objective assessment of the extant literature.

Results from sub-group analysis on diagnosis of headache disorder revealed lower vagal-activity in migraineuers compared to mixed samples of patients with a variety of headache disorders, including migraine and TTH, and patients with cluster headache. These findings provide first evidence for a unique association of migraine and vagal activity, lending further support for our hypothesis that reduced vagal activity may provide a physiological pathway linking migraine and CVD. However, it is important to note that there is an insufficient data to determine robust differences in vagal activity comparing different diagnosis of primary headache disorders. Regarding a potential pathway linking HRV and CVD in headache patients, the distinction between different headache disorders is crucial, as different diagnosis are associated with different risk factors for CVD (60), and it is proposed that different mechanism underlie the elevated CVD risk in MwA in comparison to other headache types (61,62). In general, symptom history and frequency – both important diagnostic qualities – are inadequately reported in most of the included studies, and as a result we did not have sufficient data to perform meta-regression on symptom/attack frequency. In fact only two studies provided adequate reporting of headache frequency (49,52) and headache history (52,53). We analyzed the reported mean *duration* of headache symptoms as a potential covariate on RMSSD but found no significant differences. Given that, in most cases, studies on migraine patients

did not distinguish between migraine with and without aura symptoms, we were not able to address the potential impact of migraine aura. Critically, past research demonstrates that the association of migraine and CVD might depend on the presence of aura symptoms (63). One of the included studies explicitly addressed differences in migraine patients with and without aura (54), but found no significant differences. Given that we were not able to address differences in vagal activity between MwoA and MwA using meta-analysis, future studies on differences between the two etiologies are encouraged. Thus, future studies are needed to (i) compare different diagnosis of primary headache disorders, (ii) investigate the association of symptom severity (i.e., frequency, intensity, duration of attacks) and vmHRV, as well as (iii) the (longitudinal) association of headache history and vmHRV, and (iv) address differences in migraine with and migraine without aura.

While we found age not to be a significant covariate of HF-HRV, interpretations should be drawn with caution given the relatively small mean differences on age between samples (Table 1) included in the present analysis. HRV decreases with age (64-67) and future studies should investigate age as important covariate – in particular linking the age of onset of symptoms and age at time of HRV recording. We were unable to explore potential gender differences in HRV that have recently been reported (68-72) as included studies only reported mixed (women and men) samples. Lastly, we compared short- *(< 1 hour*) with long-term recordings (*24 or 48 hours*) using meta-regression, but found no significant differences. It is noted that guidelines for the measurement of HRV (45) suggest that spectral analysis of 24-hour long-term HRV (where spectral estimates are calculated over long data epochs that are not likely to be stationary) may not accurately reflect autonomic modulation, which may be better captured by estimates based on shorter data epochs. Future studies should take this into account. Furthermore, no study recording HRV for at least 24 hours (49-51) addressed potential differences between awake and sleep periods. The data by Vollono et al (52), shows, that such analyses may be important to address the involvement of the vagus nerve, as during nighttime, movement artifacts are minimized and the contribution of sympathetic influences is low, in particular when further exploring sleep-related headache.

The present systematic review and meta-analysis faces several limitations that need to be addressed in future research, especially, given the availability of primary research on this topic. Overall, this analysis was performed on a relatively small data set, including only 7 studies, all with high risk of bias (Table 3). We were not

able to retrieve one full-text of a study conducted in Poland (73), which compared 40 headache patients to 62 HCs. One study conducted in children and adolescents (74) with potential data of interest, that also reviewed the existing literature without taking a meta-analytical approach, did not report the respective indices of interest (HF-HRV or RMSSD) and we were not able to obtain the data. Two studies published in 1993 by the same group of authors, investigating ANS function in patients with TTH (75) and migraine (76) did not report sufficient baseline data (i.e., only graphical display instead of reporting exact means and SDs) on vagal indices of HRV, and again, we were not able to obtain the data. Another three studies, two by Zigelmann et al. (77,78) and one by Appel et al. (79) were insufficient in terms of reporting data of interest, and we were unable to contact the authors. Pierangeli and colleagues (80) explored differences in cardiovascular responses to the tilt test and Valsalva maneuver in HCs compared to patients with MwoA and MwA, but only provide data on HF as graphical display and we were unable to contact the authors.. Within a commentary to a paper by Kurth et al. (10), Perciaccante et al. (81) report some preliminary findings on HRV differences in headache patients compared to HCs. However again, we were not able to obtain the necessary data to include the study in the present meta-analysis. The study by Shechter et al. (82) reported RR variation but no indices of vmHRV, and we were not able to obtain the data. We also requested details on the HRV measures taken in the study by Aygül et al. (83) that only reported RR variation but not values for HF and RMSSD, but did not receive a response from the authors. Studies that illustrate but insufficiently report findings on vagal indices of HRV are inconsistent, reporting lower parasympathetic activity in headache patients compared to healthy controls (73) or no differences (74-76,79). Given the exclusion of these 11 studies (73-83) with potential data of interest, the present analysis is limited and not completely reflective of the existing evidence.

In addition, it is critical to note, that all included studies carry a potential high risk of bias (Table 3). In general, most studies failed to report medication in sufficient detail, an important issue considering well-known effects on HRV (37,84). Furthermore, most studies failed to control for frequent comorbid conditions described in the context of headache disorders (i.e. anxiety and depression) that are also linked with HRV. We also found large methodological differences between studies regarding the recording of HRV. Specifically, some studies asked participants to avoid exercise, smoking or alcohol consumption prior to the recording of HRV, while others did not

control for such influences. We addressed publication bias and study-heterogeneity excluding one study on RMSSD (38), reporting an unlikely large effect and found the main-effect for RMSSD to hold.

Lower vagal activity, indexed by vmHRV, is associated with an increased risk for CVD. Furthermore, lower vmHRV is associated with greater inflammation and less inhibitory control in pain processing. Chronic pain patients show lower HRV and vagus nerve stimulation in chronic pain patients (including migraine and cluster headache) may lead to pain release and a simultaneous increase in HRV. Therefore, reduced vmHRV may reflect an important mechanism underlying headache disorders and an increased risk for CVD in headache patients. Three potential models (61) may underpin the link between headache and CVD: (i) the frequent experience of headache, may lead to life-style changes such as decreased physical activity, altered smoking habits or an unhealthy diet – all of which may reduce vmHRV – that elevate CVD risk; (ii): an unfavorable CVD risk profile is associated with reduced vmHRV, which could subsequently lead to the development of headache; (iii) genetic or environmental causes common to both headache and an unfavorable CVD risk profile may lead to reductions in vmHRV. Here we emphasize how vmHRV may relate to headache disorders and CVD, however future research is needed to determine whether vmHRV is actually a mediating factor of headache disorders and its relationship to CVD. Future studies are needed to: (i) carefully distinguish between different diagnosis based on established criteria, (ii) examine homogenous samples of patients of the same age, gender, with the same diagnosis, and headache characteristics (i.e., frequency, intensity, headache history), that (iii) control for comorbid conditions and (iv) determine the impact of medication on vagal activity, in addition to primary headache disorders and comorbid conditions.

5. CONCLUSION

We demonstrate here that vmHRV is reduced in patients with primary headache disorders, findings associated with a large effect size. Future studies are needed to further examine the association between headache disorders - and migraine in particular – vmHRV, common comorbid conditions and risk for CVD. We emphasize the necessity of future large scaled studies to further investigate these pathways.

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** indicates that the study was included in the meta-analysis*

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Appendix A – Search Strategy by Database

PubMed: (((headache) OR migraine)) AND heart rate variability: 63 hits; PsycNET/PsycINFO: (Any Field:(headache) OR Any Field:(migraine)) AND (Any Field:(heart rate variability)): 0 hits; CINAHL Plus: heart rate variability AND (migraine OR headache): 17 hits; WOS: (TITLE: heart rate variability) AND (TITLE: (migraine) OR TITLE: (headache)): 13 hits

Figure Captions and Legends

Figure 1. Systematic Literature Search Flow Chart; *k: number of included comparisons on respective measure of vmHRV*

Figure 2. Random-Effect Meta-Analysis Main Effect Forrest Plot for RMSSD

Figure 3. Random-Effect Meta-Analysis Main Effect Forrest Plot for HF-HRV

Figure 4. Funnel Plot for (a) RMSSD and (b) HF-HRV; *SMD: standardized mean difference; SE: standard error*