Cerebral cortical dimensions in headache sufferers aged 50-66 years: a population-based imaging study in the Nord-Trøndelag Health Study (HUNT-MRI)

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Background

In recent years several magnetic resonance imaging (MRI) studies have reported differences in cortical morphology between headache patients and healthy controls (Table 1). The current criteria for the primary headache conditions, such as migraine and tension-type headache (TTH), include no such descriptions of macroscopic anatomical cortical changes in the brain. If such changes exist, they could give valuable insight into the pathophysiology and mechanisms of headache.

Most previous MRI studies on cortical morphology and headache have been clinic-based with a case-control design and relatively small sample sizes, and the majority investigated volume or thickness of the cortex of migraineurs. Reduced cortical grey matter volume in regions linked to affective pain processing, such as the anterior cingulate cortex (ACC), insula and various regions in the prefrontal cortex (PFC) are the most consistent findings[8]. Studies of other headache types such as TTH[46], medication overuse headache[41] and cluster headache[1] have yielded similar results. To the present authors' knowledge only one study[36] has examined cortical surface area in headache sufferers with migraine, demonstrating both increased and decreased surface area in several cortical regions in the frontal and temporal lobes.

Many MRI studies[1; 5; 7; 24; 25; 29; 30; 33; 35; 37-41; 43-48; 54] exploring brain morphology and headache have used voxel-based morphometry (VBM) where differences in volume or density of grey matter are investigated. Other studies have

used surface-based morphometry, such as FreeSurfer[6; 11; 12; 19; 24; 25; 34; 36; 42; 50; 58], which provides separate measures of cortical thickness and surface area. Both methods are fully automated enabling fast processing of large datasets. To facilitate comparison to previous studies, the present study used both VBM to examine cortical volume and FreeSurfer to examine cortical thickness and surface area.

The aim of the present study was to investigate cerebral cortical morphology in relationship to headache in a large population-based sample. Both migraine and TTH diagnoses were available as well as data on frequency of attacks and evolution of headache. Based on a review of the results of previous studies, summarized in Table 1, we hypothesized that headache sufferers, regardless of type, would have decreased cortical grey matter, i.e. volume, thickness or surface area, in the ACC, PFC and insula. In addition, exploratory analyses on cortical volume, thickness and surface area across the cerebral cortical mantle were performed.

Methods

Cohort

The large population-based Nord-Trøndelag Health Surveys (HUNT) were conducted in 1984 to 1986 (HUNT1), 1995 to 1997 (HUNT2) and 2006 to 2008 (HUNT3). Health related data from individuals aged ≥20 years in the county of Nord-Trøndelag, Norway,

were collected with questionnaires and various supplementary investigations (e.g. blood samples, blood pressure).

As part of HUNT3 a group of 1494 individuals were invited to participate in a neuroimaging study (HUNT-MRI). Participants were eligible for inclusion if they were between 50 and 65 years at the time of consent, had previously participated in HUNT1, HUNT2 and HUNT3, and lived maximally 45 minutes away by car or public transport from Levanger hospital where the scanning was performed. At the time of scanning 18 individuals had turned 66 years. Exclusion criteria were restricted to standard safety contraindication to MRI, i.e. pacemaker, severe claustrophobia or body weight above 150 kg. Between the 21st of July 2007 and the 10th of December 2009, 1006 individuals (530 women) underwent brain imaging with a standardized MRI protocol. The mean time between answering the questionnaire in HUNT3 and being scanned was 1.2 years. Details about the recruitment of participants to the HUNT-MRI study and the imaging procedure have been published previously[22; 23]. A separate analysis of the HUNT-MRI participants showed that these were not widely different from the general population, with the possible exception of somewhat reduced cardiovascular risk[23].

Headache diagnoses

Participants in the HUNT3 survey were classified as either headache sufferers or headache non-sufferers based on their answers ("yes/no") to the opening screening

question of the headache questionnaire, “Have you suffered from headache during the last 12 months?”. The accuracy of being a headache sufferer was evaluated and showed a sensitivity of 88% and a specificity of 86%[20]. Headache sufferers were further categorized into the three mutually exclusive headache categories migraine, TTH ≥1 day per month and unclassified headache. The migraine and TTH diagnoses were based on the criteria of the 2nd edition of the International Classification of Headache Disorders (ICHD-II). The classification and accuracy of the questionnaire-based diagnoses have been described previously[20]. For migraine, the sensitivity was 51% and the specificity was 95% and for TTH the sensitivity was 96% and the specificity was 69%. Headache sufferers not fulfilling the criteria of either migraine or TTH were categorized as having unclassified headache. In the present study no analyses were performed on this group alone. In addition, the headache sufferers categorized themselves into one of four groups according to number of headache attacks per month (<1 day; 1-6 days; 7-14 days; >14 days). To ensure sufficiently sized groups a dichotomization was performed with the cut off at 7 days which resulted in the two groups headache <7 days/month and headache ≥7 days/month.

Since the participants in the HUNT-MRI population had participated in both HUNT2 and HUNT3 it was possible to describe four headache trajectories based on the evolution of their headache: previous headache (headache in HUNT2 but no headache in HUNT3), new onset headache (no headache in HUNT2 but headache in HUNT3),

stable headache (headache in both HUNT2 and HUNT3) and stable non-suffering (headache in neither HUNT2 nor HUNT3). The last group was used as a control group in all analyses, to ensure that controls were mostly headache free over a long period.

MRI scanning

All imaging was performed on the same 1.5 T General Electric Signa HDx 1.5 T MRI scanner equipped with an eight-channel head coil and software version pre-14.0M (GE Healthcare). No scanner updates were performed during the time of scanning. All participants underwent the same MRI protocol. In the present study the Alzheimer Disease Neuroimaging Initiate (ADNI) volume, which is a T1-weighted volume (TR = 10.2 ms, TE = 4.1 ms, FOV = 240 mm, slice thickness = 1.2 mm, gap = 0 mm, matrix size = 192 x 192, flip angle = 10°), was used.

Voxel-based morphometry (VBM)

The T1-weighted volumes were first corrected for inhomogeneities using the N4 algorithm[53], and thereafter segmented with SPM12 with default options, except that bias field estimation was disabled. A brain mask was constructed by summing the three tissue probability masks (grey matter + white matter + cerebrospinal fluid) from the

segmentation and thresholding by 0.05. This brain mask was used to skull-strip the T1-weighted images.

The ANTS toolkit version 2.1.0 (http://stnava.github.io/ANTs/) was used to normalize the images to standard space. First a study-specific template was formed by dividing the subjects into four age groups, 50-54, 55-59, 60-64 and 65-66 years, and randomly selecting 4 males and 4 females with Fazekas = 0 and no gross pathology from each age group, giving a total of 32 scans as basis for the template. Next the template was formed by using the "antsMultivariateTemplateConstruction" script on the 32 skull-stripped T1-weighted images.

Since white matter hyperintensities appear hypointense in T1W images and may affect the normalization[49], a lesion-filling method in the FMRIB Software Library was used to mask hypointense regions with intensities similar to normal appearing white matter[4]. The skull-stripped and lesion-filled T1-weighted images were warped to the study-specific template using "antsRegistration" with a symmetric image normalization transform[3] and a cross correlation metric. This resulted in a nonlinear transform between each subject`s native space and the study specific template space. To bring the image data into Montreal Neurological Institute (MNI) space, an additional transform between the study-specific template and the MNI 152 template was computed using "antsRegistration" and the same settings as described previously. Combining the "native space to study specific template space" and the "study specific template to

MNI" transforms produced a single transform from native to MNI space. A MNI template with 1.5 mm isotropic resolution was used to reduce the size of the dataset and the memory requirements in the statistical analysis.

The grey matter images were normalized to MNI space using the combined transform described above and multiplied by the Jacobian giving "modulated" grey matter maps in MNI space. To limit the analyses only to grey matter, a grey matter mask was constructed from the mean of all grey matter segments in MNI space and thresholded by $P<0.05$. This mask was used in the VBM statistical analyses described below. Since volume and shape of subcortical structures in the present population have been published previously[27], the present study focused on only the cerebral cortex. Before statistical analysis, the maps were smoothed by an 8 mm full-width half maximum Gaussian filter. This was similar to most previous studies[1; 21; 24; 25; 30; 35; 37; 38; 40; 41; 44; 47; 48].

Surface-based morphometry (SBM)

Estimation of cortical thickness and surface area was performed on the T1 weighted volumes using the FreeSurfer image analysis suite, version 5.3 (http://surfer.nmr.mgh.harvard.edu/). The technical details of cortical reconstruction with FreeSurfer are described elsewhere[9; 10; 14-18]. Matching of cortical geometry across subjects is achieved by registration to a spherical atlas based on individual

cortical folding patterns. Cortical thickness and surface area estimates were obtained as described in previous publications[14; 55]. The two cerebral hemispheres were processed separately, and cortical thickness and surface area were estimated in more than 160 000 vertices across the cortical mantle. In order to facilitate comparison to previous studies[12; 24; 25; 34; 36; 42; 50] the surfaces were smoothed with a full-width-half-maximum Gaussian kernel of 10 mm. The statistical model is described below.

Statistics

For both the VBM and FreeSurfer analyses, the eight different headache groups (headache in HUNT3, migraine in HUNT3, TTH in HUNT3, headache <7 days/month, headache ≥7 days/month, previous headache, new onset headache and stable headache) were compared one-on-one to the control group (headache in neither HUNT2 nor HUNT3). Age (continuous) and sex (binary) were included as covariates in all analyses. In addition, the analyses were rerun twice, firstly, with the Hospital Anxiety and Depression Scale (HADS) score added as a covariate and secondly, with correction for having muscle/joint pain the last year. With regard to the hypothesis the three groups headache in HUNT3, migraine in HUNT3 and TTH in HUNT3 were compared to the controls.

The VBM image statistics were done using non-parametric permutation-based inference implemented in the PALM program (v. alpha-1.05)[57]. Correction for multiple comparisons were performed with the family-wise error (FWE) rate method, and a corrected significance threshold of $P<0.05$ was used in all analyses. The tail approximation and 500 permutations were used to speed up the calculations with negligible impact on accuracy[56].

All statistical analyses of the FreeSurfer data were performed within the MATLAB software suite 2011b (MATLAB and Statistics Toolbox Release 2011b. The MathWorks, Inc., Natick, Massachusetts, US). A general linear model was fitted for each vertex across the cortical mantle, with cortical surface area or cortical thickness as dependent variable, headache status as independent variable and age and sex as covariates. The appropriate contrast vectors were set to test for a relationship between headache status and cortical morphology. The hemispheres were analyzed separately, and cortical maps of *P*-values (*P*-maps) were generated. To correct for multiple comparisons, the *P*-maps were thresholded to yield an expected false discovery rate (FDR) of 5%. In addition, cortical maps of Cohen's *d* values for the analyses of headache sufferers in HUNT3 vs the controls with a smoothing of 10 mm full-width-half-maximum Gaussian kernel were generated.

Differences in basic characteristics between the headache and control groups were analysed in SPSS version 21 and thresholded at $P<0.05$ (two-tailed). Age and sex

differences were assessed with an Analysis of Variance (ANOVA) and a chi-square test respectively. Differences in level of education, smoking and having muscle/joint pain were examined with binary logistics regression corrected for age and sex. Analysis of Co-Variance (ANCOVA), with age and sex as covariates, was used to assess differences in body mass index (BMI), hospital anxiety and depression scale (HADS) score and systolic and diastolic blood pressure.

Ethical approval

The study was approved by the Norwegian Data Inspectorate, the Norwegian Board of Health, and the Regional Committee for ethics in Medical Research. All participants gave their informed, written consent.

Results

Exclusion of participants and characteristics of the present population

Of the 1006 participants in HUNT-MRI, 44 individuals were excluded from the present analyses because of cortical brain pathology influencing morphology (e.g. tumours, multiple sclerosis, cortical infarctions, lacunar infarctions, traumatic contusions, postoperative changes or arachnoid cysts). Furthermore, MRI data from 50 individuals were not included in the analyses owing to poor image quality (mostly motion artefacts)

or other errors in the image data acquisition incompatible with the software algorithms. Of the remaining 912 individuals, 782 had answered the headache questionnaire in HUNT3 and 705 had answered the headache questionnaires in both HUNT2 and HUNT3.

Figure 1 summarizes the participation and exclusion of the participants and Table 2 shows the number of individuals in the different headache groups and basic demographic and health-related characteristics. Compared to the controls a significantly higher percentage of women and individuals suffering from muscle/joint pain were found in all headache groups except for the new onset headache group. Those with migraine, headache <7 days/month or stable headache were also significantly younger than the controls. In addition, those suffering from headache, except those with previous headache, had significantly higher HADS scores than the controls. BMI, blood pressure, daily smoking and level of education were similar among the groups.

A priori hypothesis

Individuals suffering from headache, migraine or TTH in HUNT3, did not show a significant decrease in cortical volume (VBM), thickness (FreeSurfer) or surface area (FreeSurfer) in ACC, PFC or insula compared to the controls.

Exploratory analyses

The exploratory VBM analyses showed no differences in cortical volume between any of the headache groups and the controls. This was also true when the analyses were corrected for HADS scores or having muscle/joint pain. The Cohen's *d* maps of the VBM-based cortical volume analyses of headache sufferers in HUNT3 vs the controls showed values in the range of -0.3–0.3 where the large majority of the values were in the range of -0.2–0.2 (Figure 2).

Similar to the VBM analyses, the exploratory FreeSurfer analyses showed no differences in cortical thickness or surface area between any of the headache groups and the controls across the entire cortical mantle. This was also true when the analyses were corrected for HADS scores or having muscle/joint pain. The Cohen's *d* maps of the FreeSurfer-based cortical thickness and surface area analyses of headache sufferers in HUNT3 vs the controls showed values in the range of -0.3–0.3 where the large majority of the values were in the range of -0.2–0.2 (Figure 3).

Discussion

The present study failed to confirm our hypothesis that headache sufferers, migraine and TTH included, would have a decrease in grey matter in the ACC, PFC and insula. Likewise, the exploratory analyses across the cerebral cortical mantle showed no difference in cortical volume, thickness or surface area between any of the headache groups and those not suffering from headache. Thus, neither evolution of headache,

frequency of attacks nor type of headache was associated with differences in cortical morphology.

There are several strengths of the present study. Firstly, the participants were randomly drawn among individuals attending a large longitudinal epidemiological study (HUNT) in the general population and there were no major group differences in socioeconomic status, smoking, BMI or blood pressure. Secondly, headache sufferers were categorized into different headache categories allowing for investigation of associations between different types of headache and cortical differences. Thirdly, all scans were performed on the same scanner with no scanner updates during the study. Fourthly, both VBM and FreeSurfer were applied, facilitating comparison to previous studies. Fifthly, before running the analyses a precise hypothesis based on previous findings was formulated. In addition, exploratory analyses were performed. Sixthly, data on headache status in HUNT2 and HUNT3 allowed selection of individuals with presumably no headache complaints over several years as controls. Lastly, compared to the previous studies this study was superior in terms of number of participants.

An important limitation in the present study is the relatively long time interval from the participants answered the headache questionnaire (1995-1997 in HUNT2 and 2006-2008 in HUNT3) to when they were scanned (2007-2009). It has previously been reported that morphological changes can both arise and recede within a year[33; 52]. Although this effect cannot be ruled out it seems unlikely that the headache had

improved or increased dramatically in the majority during the time from the HUNT3 questionnaire to the scanning (mean 1.2 years). Furthermore, as the evolution of the participant's headache was based on data from only two time points, caution must be taken when interpreting these specific analyses. Also, we had no information on whether the participants were scanned during an attack or interictally. Lastly, estimating the headache status with a questionnaire is inferior to a clinical interview. However, the headache criteria were validated[20] showing acceptable accuracy. The migraine diagnosis was highly specific but had lower sensitivity. This relationship was opposite for the TTH diagnosis, probably classifying some true migraineurs as having TTH. Such misclassification will diminish rather than increase differences between the groups.

In contrast to several previous VBM and SBM studies the present analyses showed no structural difference in the cerebral cortex between headache sufferers and non-sufferers. Nearly all significant findings in previous VBM studies demonstrated a decrease in cortical grey matter, and most frequently in ACC, PFC and insula. Studies based on FreeSurfer on the other hand, have reported both thicker and thinner cortex in several brain regions in those with headache, but with no clear association to ACC, PFC or insula (Table 1). Taking the present results into consideration, there is little evidence for an association between headache status and cortical thickness in these three brain regions. One other study has examined cortical surface area in headache sufferers[36]

and found migraineurs to have regions of both larger and smaller surfaces in the frontal and temporal lobes. None of these findings were replicated in the present study. This could be due to the fact that the previous study used a liberal significance threshold of $P<0.01$ with a cluster extent of 100 mm^2, whereas we used a threshold of $P<0.05$ FDR corrected.

Perhaps the most important difference between the present study and the previous ones was the design. We included individuals from the general population whereas most of the others conducted research on patients drawn from tertiary clinics. There is an increased likelihood that individuals with multiple conditions will seek medical care compared to those with only one condition[13]. Therefore, it cannot be ruled out that a confounder could explain the different results. Previously anxiety and depression have been shown to be associated to headache and to differences in brain morphology similar to those found in headache samples[2; 28]. In the present study headache sufferers had higher HADS scores than the controls, but correction for HADS did not affect the results. However, it should be pointed out that the HADS scores were generally low and maybe a higher degree of anxiety/depression is needed to affect brain morphology. Similarly, headache sufferers had more muscle/joint pain but correction for this did not affect the results.

Alternatively, the difference in results between the present and several previous studies may be due to patients from tertiary clinics being more severely affected by their

headache than individuals participating in population-based studies. However, if this were true, one would expect to find a dose-response effect between headache suffering and morphology. In the present study, no association between cerebral morphology and frequency of headache attacks was found.

The participants in the present study were somewhat older, i.e. 50-66 years, than the participants in the other studies, most of whom were in their thirties or forties. The prevalence of headache is known to peak in the thirties and forties[28]. However, the prevalence of migraine and headache in the present population were 9% and 31% respectively[26] and thus not widely different from the prevalence in the general population[51]. One could speculate that some of the individuals classified as controls in the present analyses had suffered from headache earlier in their lives. However, since the control group had not suffered from headache during HUNT2, such misclassification would probably only be applicable to a few individuals and not affect the results. Since the present study was based on middle-aged and elderly individuals and individuals with presumably long-lasting headache complaints was identified, the present results give no indication that suffering from headache for many years have effects on cortical morphology.

Most previous VBM studies used either the FWE correction, a cluster-based threshold or a stringent significance level ($P<0.001$) without correction for multiple comparison, whereas in previous FreeSurfer studies the FDR and Monte Carlo

corrections were frequently used (Table 1). Seven previous studies used more than one statistical threshold[1; 7; 36-38; 40; 43], and five of these[1; 7; 36-38] reported no or very few significant findings when correcting for multiple comparisons (e.g. FDR or FWE). Cluster-based thresholds and uncorrected tests are considered to be too sensitive and increase the risk of type I errors[32]. When performing a large number of tests, as is the case in voxel and surface based MRI studies, FWE or FDR corrections should be used[32]. However, FWE correction can be too stringent when analyzing small samples. Since the number of individuals in the present study was quite high, FWE correction was applicable[32]. The present VBM analyses were carried out using the ANTS-SyN toolbox and not the often-used SPM DARTEL toolbox. It has previously been shown that these two approaches give similar results and are the highest ranked VBM registration methods[31]. If anything, our approach is reported to be slightly better in terms of normalization. As the present study resembled previous studies with regard to level of smoothing, the discrepancy in findings is probably not caused by this.

The effect size maps had Cohen's *d* values mostly in the range of -0.2–0.2. At a power level of 0.8 and a probability level of 0.05 (two-sided), a sample size per group of minimum 394 individuals would be needed to draw conclusions on the association between headache and cortical morphology. Effect sizes of 0.3 and 0.5 would require minimum groups sizes of 176 and 64 respectively. Since the number of individuals in the present effect size analysis was 283 (headache sufferers) and 309 (controls), we lack

the power to detect small to very small differences, but we can conclude that having headache in the general population has no medium to large effects on cortical volume, thickness or surface area.

There is now a sizeable literature on headache and cortical morphology, but the results are mixed. We suggest that future studies should investigate the relationship between brain morphology and headache in population-based samples to avoid selection bias which is more likely to be present in clinic-based studies. Furthermore, studies should be based on a high number of cases and controls to provide sufficient statistical power to discover potentially small to very small differences in cortical morphology.

This large population-based imaging study implementing both VBM and SBM failed to confirm our hypothesis that headache sufferers would have a decrease in cortical grey matter in ACC, PFC and insula. In the exploratory analyses neither evolution of headache, frequency of attacks nor type of headache was associated to cerebral cortical morphology. In the general population aged 50-66 years there are probably no or only small differences in cerebral cortical volume, thickness or surface between those with and without headache.

Conflict of interest

The authors declare that there is no conflict of interest.

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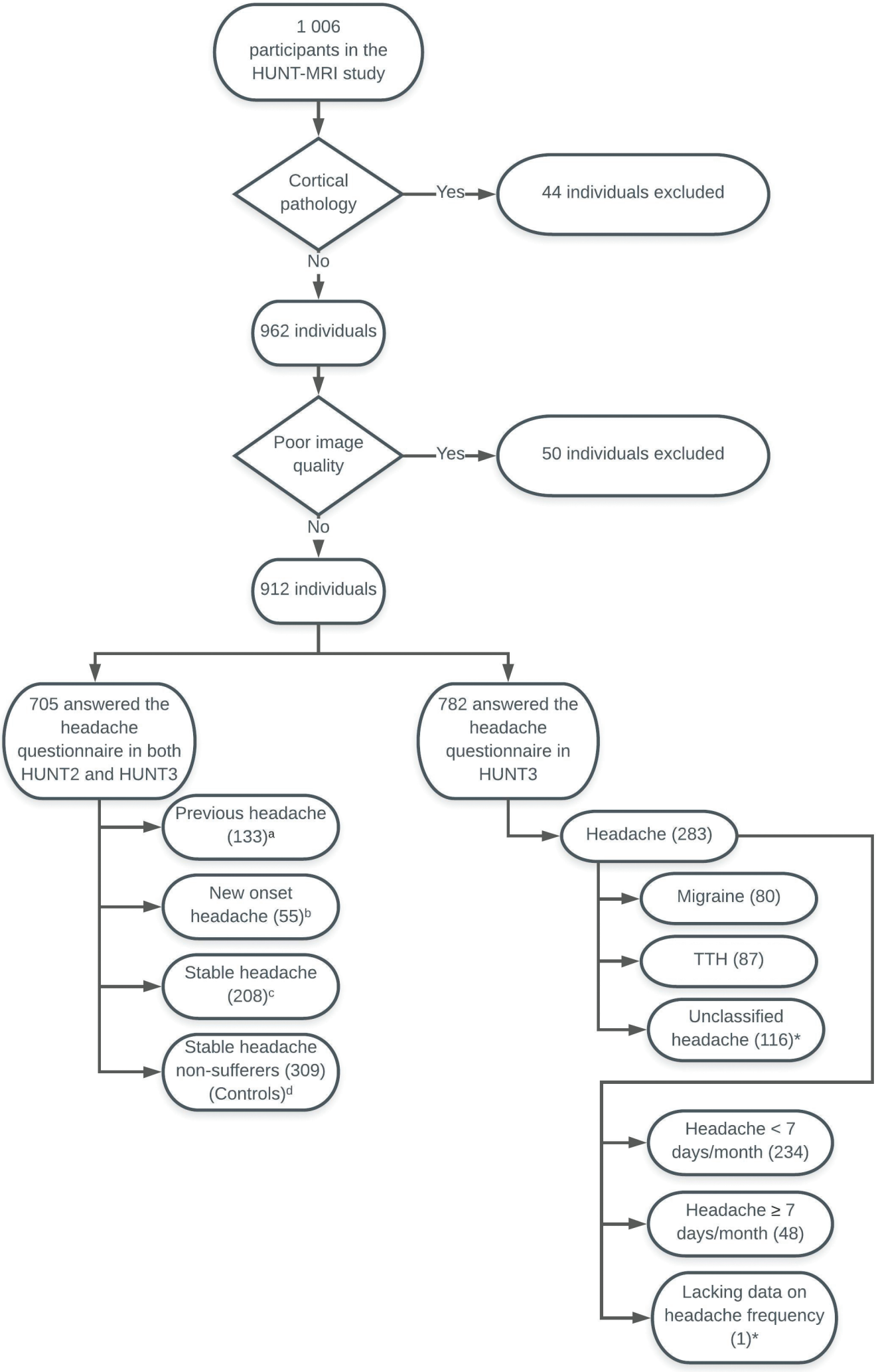
Figure legends

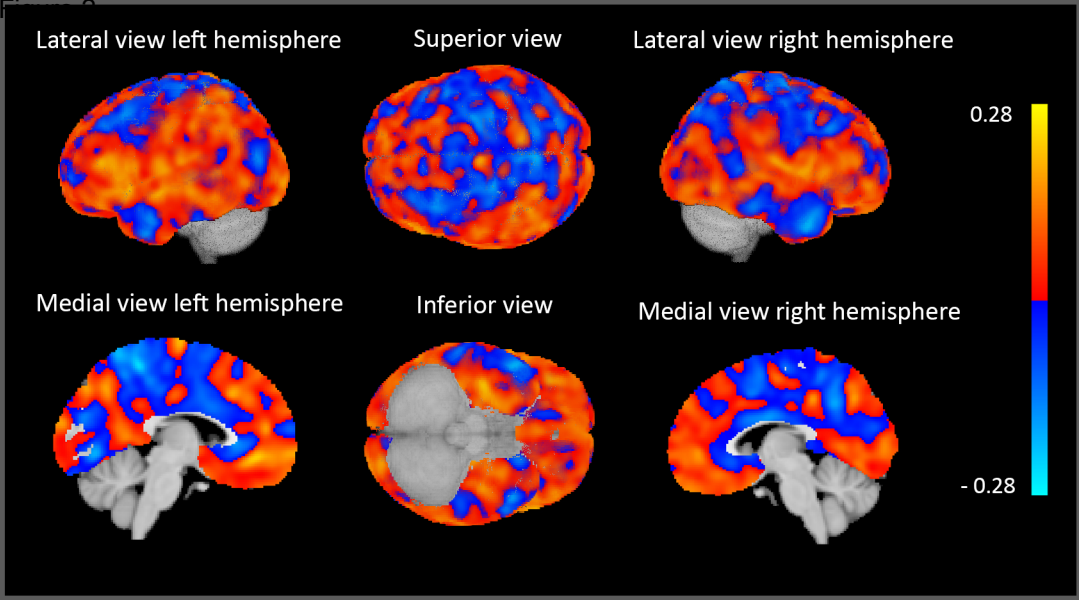
Figure 1. Participation and exclusion of individuals in the present study

Figure 2. Maps and graphical distribution of Cohen's *d* values based on the VBM analyses comparing cortical volume between those suffering from headache in HUNT3 and those not suffering from headache in neither HUNT2 nor HUNT3. Differences were small and not statistically significant in any of the cortical areas.

Figure 3. Cohen's *d* (effect size) maps comparing cortical thickness and surface area in those suffering from headache in HUNT3 to those not suffering from headache in neither HUNT2 nor HUNT3. Differences were small and not statistically significant in any of the cortical areas. The maps were smoothed with a full-width-half-maximum Gaussian kernel of 10 mm.

Figure 1


1 006 participants in the HUNT-MRI study
Cortical pathology
Yes
44 individuals excluded
No
962 individuals
Poor image quality
Yes
50 individuals excluded
No
912 individuals
705 answered the headache questionnaire in both HUNT2 and HUNT3
782 answered the headache questionnaire in HUNT3
Previous headache (133)a
New onset headache (55)b
Stable headache (208)c
Stable headache non-sufferers (309) (Controls)d
Headache (283)
Migraine (80)
TTH (87)
Unclassified headache (116)*
Headache < 7 days/month (234)
Headache ≥ 7 days/month (48)
Lacking data on headache frequency (1)*


Lateral view left hemisphere
Superior view
Lateral view right hemisphere
0.28
Medial view left hemisphere
Inferior view
Medial view right hemisphere
- 0.28

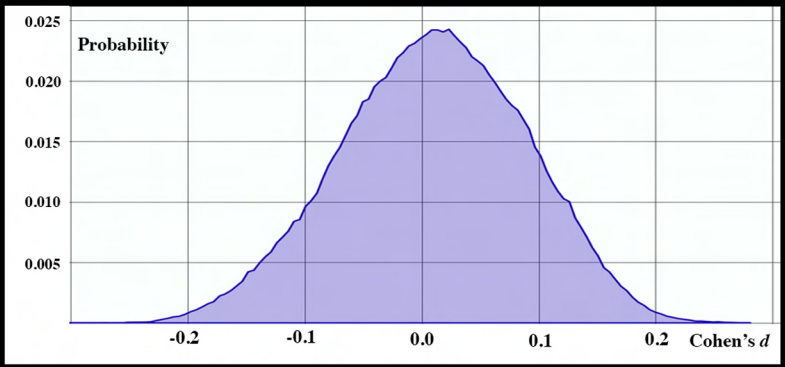
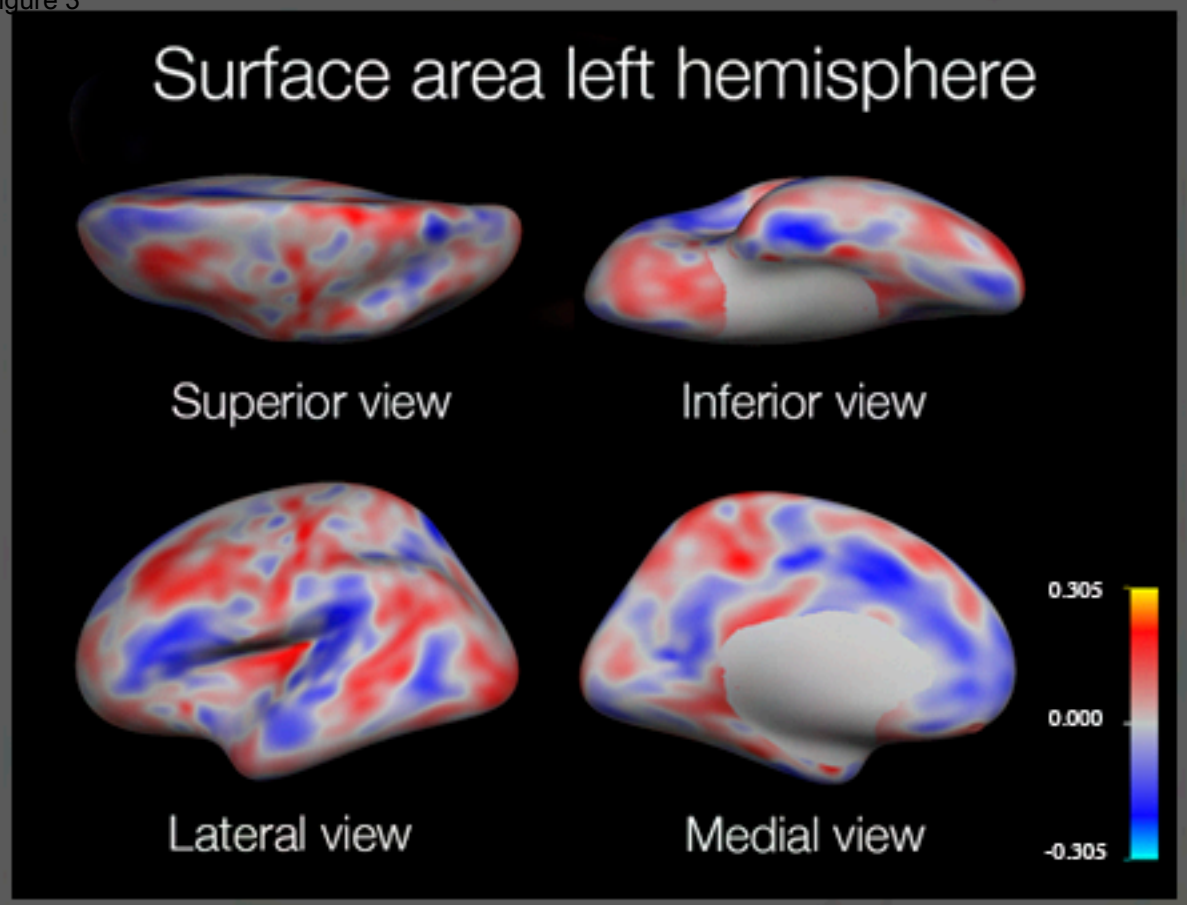
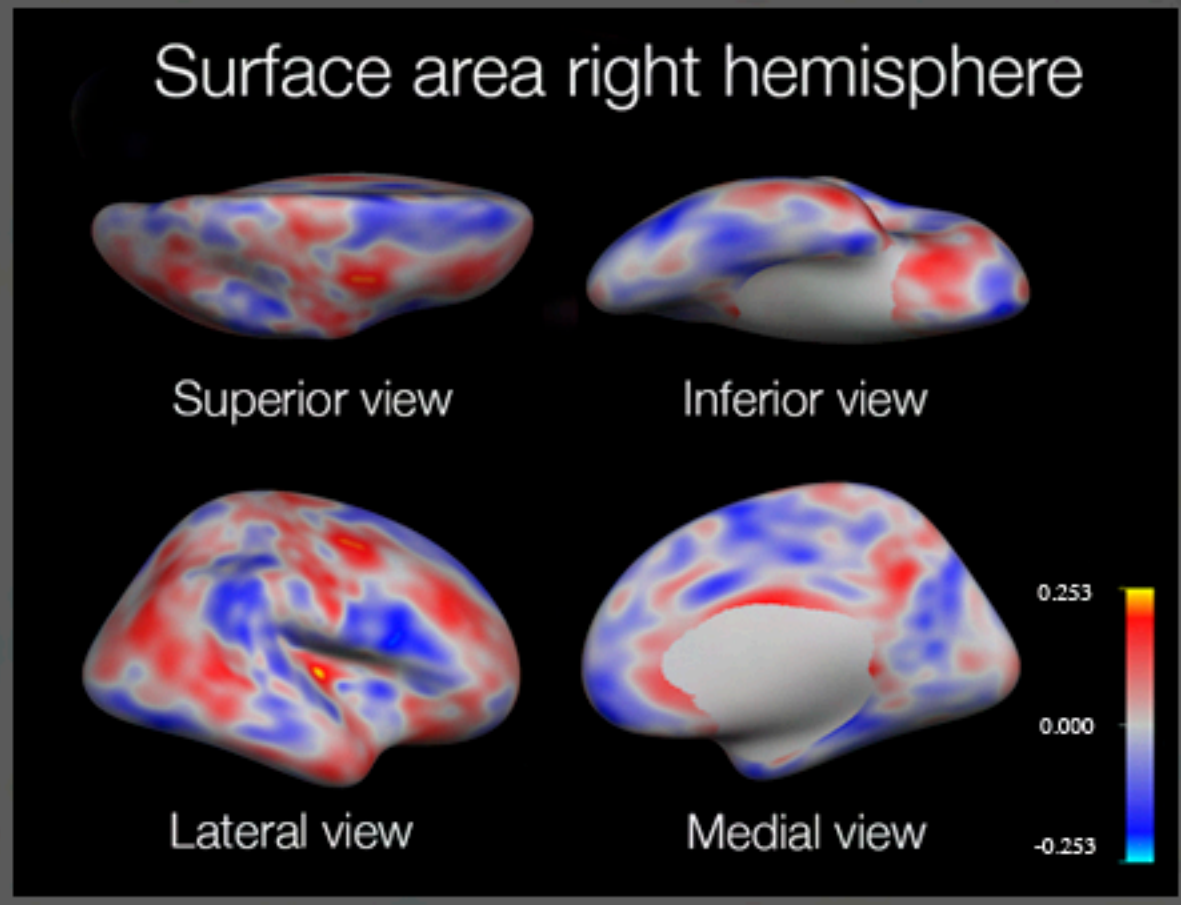
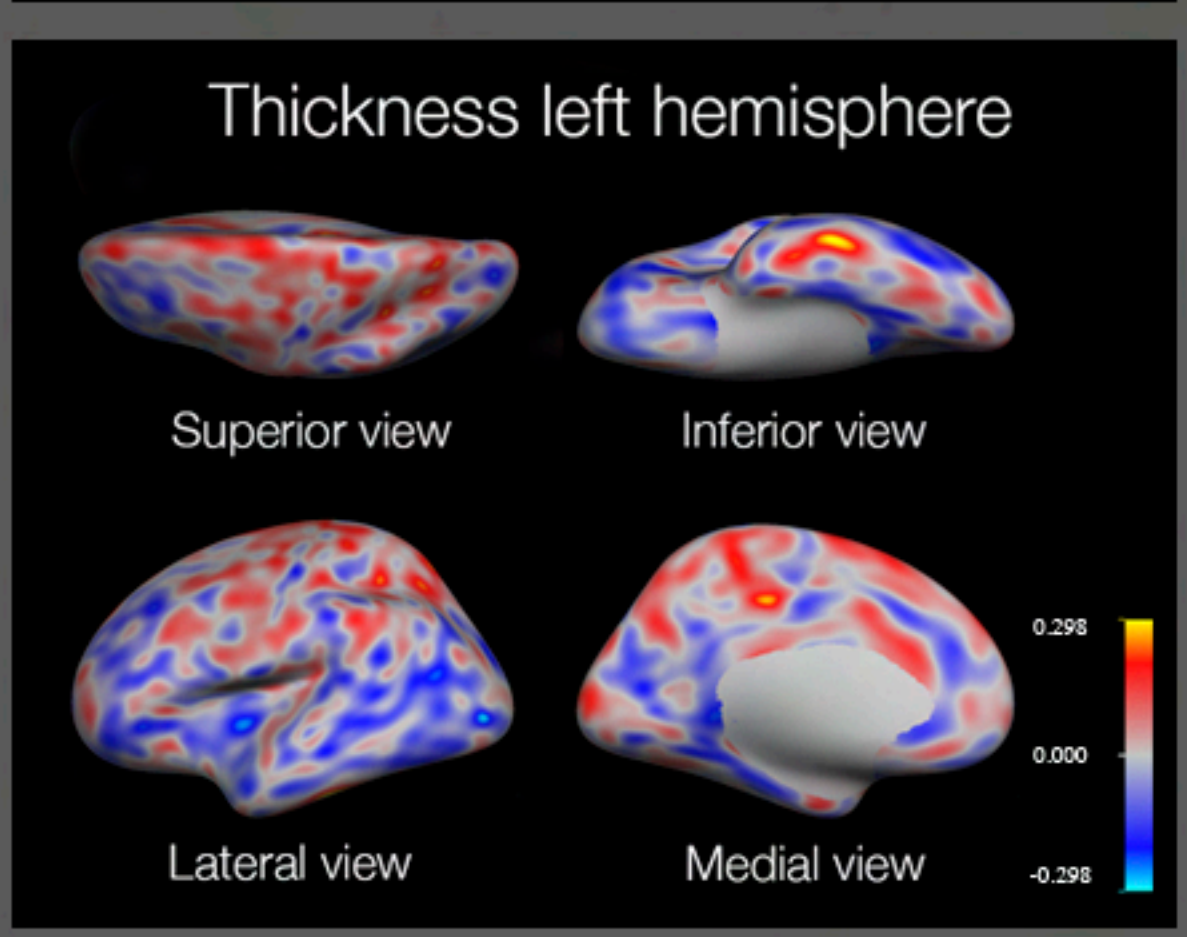

Histogram
Probability
0.025
0.020
0.015
0.010
0.005
-0.2
-0.1
0.0
0.1
0.2
Cohen's d

Figure 3


Surface area left hemisphere
Superior view
Inferior view
Lateral view
Medial view
0.305
0.000
-0.305


Surface area right hemisphere
Superior view
Inferior view
Lateral view
Medial view
0.253
0.000
-0.253


Thickness left hemisphere
Superior view
Inferior view
Lateral view
Medial view
0.298
0.000
-0.298

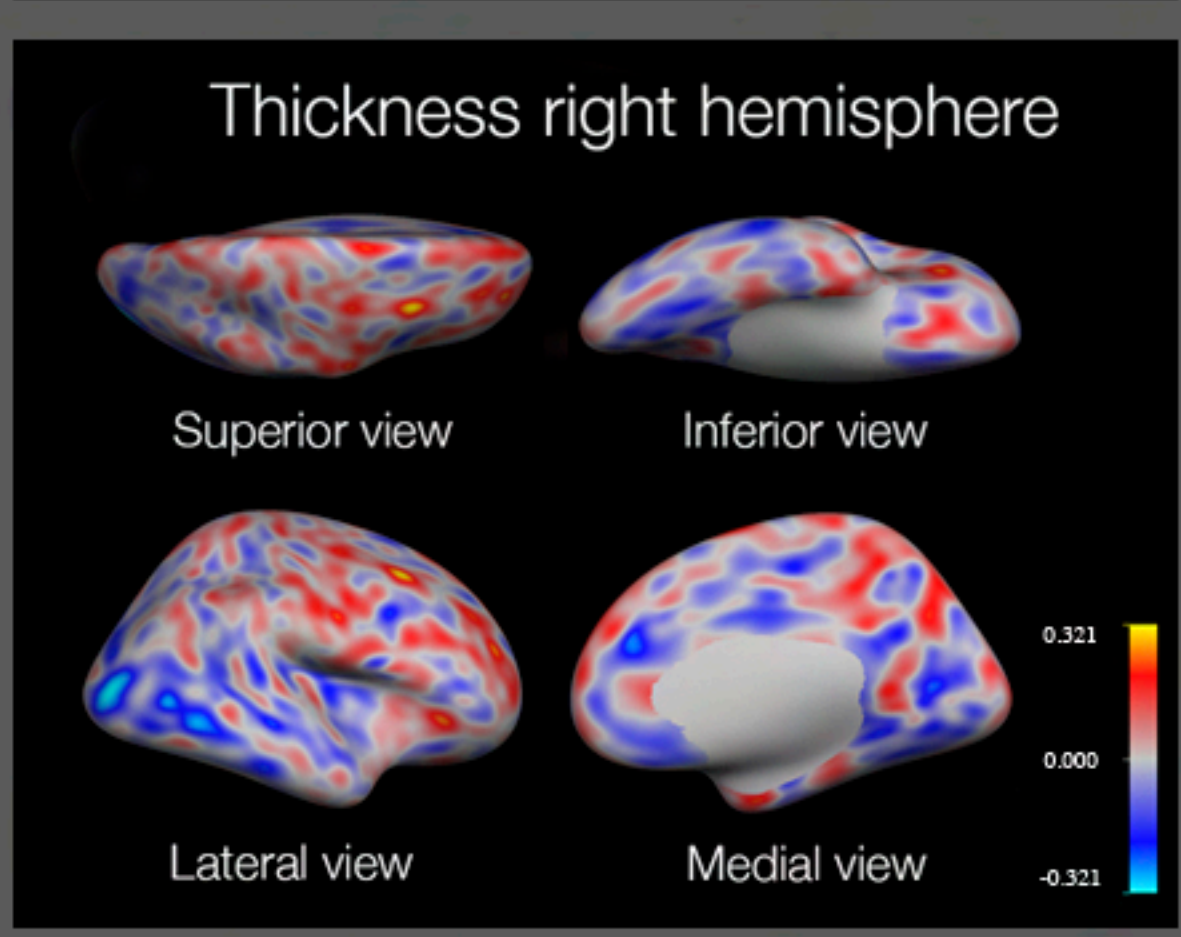

Thickness right hemisphere
Superior view
Inferior view
Lateral view
Medial view
0.321
0.000
-0.321

Table 1. Cerebral cortical regions associated to headache suffering in previous MRI studies investigating the whole cortical mantle*. Increase (↑) or decrease (↓) in cortical volume/thickness are marked by arrows.

	Author of study**	Cerebral cortical region																														
		Anterior cingulate cortex	Posterior cingulate cortex	Insula	Postcentral gyrus/somatosensory cortex	Precentral gyrus	Paracentral lobule	Motor/premotor cortex	Prefrontal cortex	Dorsolateral prefrontal cortex	Medial prefrontal cortex	Orbitofrontal cortex	Superior frontal gyrus	Middle frontal gyrus	Inferior frontal gyrus	Superior parietal cortex	Inferior parietal cortex	Parietal operculum	Superior temporal gyrus	Middle temporal gyrus	Inferior temporal gyrus	Temporal pole	Angular gyrus	Temporo-occipital incisure	Occipital lobe	Inferior occipital gyrus	Precuneus	Cuneus	Visual cortex V1	Visual cortex V2	Supramarginal gyrus	Parahippocampus	Fusiform
Thickness of cortex	**FreeSurfer**																															
	Datta (2011) a														↑																	
	Hougaard (2014) b													↑																		
	Hougaard (2016) b				↑									↑																		
	Kim (2014) c				↑																↑											
	Maleki (2012) c																							↑								
	Messina (2013) a					↓							↓	↑																		
	Messina (2013) d					↓							↓	↑																		
	Yu (2016) e	↓	↓	↓	↓	↓				↓		↓		↓			↓			↓	↓				↓			↓	↓			
	Seifert et al. (2012) f					↓																	↓									
	Riederer (2017) c													↓																		
	Chong (2018) c					↓							↓	↓		↓	↓											↓			↓		
Volume of cortex	**FreeSurfer**																															
	Maleki (2012) c	↓	↓	↓																												
	Ha (2019) e	↓			↓																											
	Meyer (2017) e													↑					↓						↓							↓
	Voxel-based morphometry																															
	Celle (2018) ***																															
	Coppola (2015) j			↑													↓		↓		↓	↓										
	Coppola (2017) g																															
	Coppola (2017) h											↓								↓		↓							↓	↓		
	Hougaard (2014) i																															
	Hougaard (2016) i																															
	Jin (2013) b	↓									↓														↓							
	Kim (2008) f	↓	↓	↓		↓		↓	↓			↓	↓	↓	↓	↓	↓												↓			
	Liu (2013) g				↓		↓					↓	↓			↓	↓										↓				↓		
	Matharu (2003) g																															
	Messina (2018) j														↑				↑	↑	↑				↑		↑					
	Neeb (2017) g																															
	Neeb (2017) f									↓																						
	Rocca (2014) g	↓										↓			↓					↓												
	Rocca (2006) a	↓				↓							↓	↓	↓					↓	↓											
	Rocca (2006) j	↓				↓							↓	↓	↓				↓	↓	↓											
	Schmidt-Wilcke (2008) g																															
	Schmitz (Aug 2008) f													↓			↓															
	Schmitz (July 2008) f												↓				↓															
	Valfre et al. (2008) k	↓		↓		↓								↓	↓			↓	↓													
	Schmidt-Wilcke (2005) j	↓	↓	↓								↓																				↓	
	Absinta (2012) g																															
	Absinta (2012) j		↓	↓		↓								↓			↓			↓												
	Naegel (2014) f	↓	↓	↓	↓							↓	↓							↑	↓											
	Naegel (2014) g			↑																↓												
	Chanraud (2014) a																															
	Riederer (2012) a	↓	↑	↓							↓	↓			↓												↓					↑
	Obermann (2009) g	↓								↓																						
	Obermann (2014) f	↓		↓												↓				↓	↓									↓		
	Obermann (2014) j	↓		↓						↓						↓			↓	↓	↓					↓				↓		

Migraine Tension-type headache Cluster headache Medication overuse headache Other types of headaches

a $P<0.05$ false discovery rate correction; b $P<0.05$ cluster-based threshold; c $P<0.05$ Monte-Carlo correction; d $P<0.01$ cluster-based threshold; e $P<0.05$ uncorrected; f $P<0.001$ cluster-based threshold; g $P<0.05$ threshold-free cluster enhancement; h $P<0.05$ family-wise error correction; i $P<0.005$

uncorrected; ĵ $P<0.001$ uncorrected;

* Results based on region-of-interest analyses were not included in the table

** Some of the studies are listed more than once because of implementation of more than one significance threshold

*** Full text not available. The authors reported in the abstract that the results were corrected for multiple comparisons

Table 2

Table 2. Basic characteristics of the present headache population.

Variables	Headache status								
	Headache in HUNT3[a]	Migraine in HUNT3[a]	TTH in HUNT3[a]	Headache < 7 days/month in HUNT3[a]	Headache ≥ 7 days/month in HUNT3[a]	Previous headache in HUNT2[b]	New onset headache in HUNT3[b]	Stable headache in HUNT2 and HUNT3[b]	Controls (no headache in HUNT2 and HUNT3)[b]
	n=283	**n=80**	**n=87**	**n=234**	**n=48**	**n=133**	**n=55**	**n=208**	**n=309**
Demographics									
Women (n [%])[1]	175 [61.8]***	60 [75]***	50 [57.5]**	142 [60.7]***	32 [66.7]**	79 [59.4]***	28 [50.9]	135 [64.9]***	124 [40.1]
Age (mean [SD])[2]	58.0 [4.2]	57.4 [4.3]*	58.1 [4.1]	57.9 [4.3]*	58.9 [3.8]	58.7 [4.1]	58.4 [4.7]	57.8 [4.1]*	58.7 [4.1]
Education > 12 years (n [%])[3]	86 [30.4]	27 [33.8]	28 [32.2]	73 [31.2]	13 [27.1]	46 [34.6]	14 [25.5]	69 [33.2]	111 [35.9]
Health-related									
BMI (mean [SD])[4]	26.9 [4.0]	26.7 [4.1]	27.1 [4.4]	26.8 [4.0]	27.2 [3.7]	26.9 [3.7]	27.2 [4.2]	26.6 [3.9]	27.1 [3.6]
SBP (mean [SD])[4]	131.9 [17.9]	131.3 [18.6]	132.4 [18.1]	132.0 [17.2]	131.0 [19.0]	132 [17.0]	135.0 [19.3]	131.3 [18.0]	130.8 [16.1]
DBP (mean [SD])[4]	76.5 [11.8]	74.6 [12.1]	77.4 [11.1]	76.8 [11.6]	75.0 [13.2]	75.0 [10.1]	78.4 [12.7]	75.7 [11.9]	75.2 [10.1]
Daily smoking (n [%])[3]	49 [17.3]	16 [20.0]	14 [16.1]	44 [18.8]	5 [10.4]	19 [14.3]	11 [20.0]	36 [17.3]	44 [14.2]
HADS total (mean [SD])[4]	7.8 [5.8]***	7.9 [5.8]**	7.6 [5.7]**	7.4 [5.6]**	10.1 [6.4]***	6.2 [4.9]	7.6 [5.7]*	8.0 [6.0]***	5.9 [4.8]
Muscle/joint pain last 12 months (n [%])[3]	183 [64.7]***	57 [71.3]***	52 [59.8]**	145 [62.0]***	37 [77.1]***	81 [60.9]**	22 [40]	149 [71.6]***	135 [43.7]
Painkillers ≥ 1/week for headache relief (n [%])	147 [51.9]	52 [65.0]	48 [55.2]	109 [46.6]	37 [77.1]	9 [6.8]	20 [36.4]	120 [57.7]	n/a

* P<0.05 (compared to the controls)
** P<0.01 (compared to the controls)
*** P<0.001 (compared to the controls)

[1] Chi-square test; [2] Analysis of Variance; [3] Binary logistic regression corrected for age and sex; [4] Analysis of Co-Variance corrected for age and sex

n=Number of individuals
SD=Standard deviation
BMI=Body Mass Index
SBP=Systolic Blood Pressure
DBP=Diastolic Blood Pressure
HADS=Hospital Anxiety and Depression Scale

[a] These groups were based on information from the HUNT3 study
[b] These groups were based on information from the HUNT2 and the HUNT3 studies