BMJ Open

Relationship between right-to-left shunt and migraine in patients with epilepsy

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-024144
Article Type:	Research
Date Submitted by the Author:	12-May-2018
Complete List of Authors:	Zhang, Lin Zhu, Xi Qiu, XIangmiao Li, Yajiao Chen, Yucheng Wang, Hui He, Shixu Lai, Wanlin Peng, Anjiao Ning, Mingming Chen, Lei; West China Hospital, Sichuan University,Chengdu, Sichuan, China,
Keywords:	Epilepsy < NEUROLOGY, Migraine < NEUROLOGY, Right-to-left shunt

Relationship between right-to-left shunt and migraine in patients with epilepsy

Lin Zhang,[1] Xi Zhu,[1] Xiangmiao Qiu,[1] Yajiao Li,[2] Yucheng Chen,[2] Hui Wang,[2] Shixu He,[1] Wanlin Lai,[1] Anjiao Peng,[1] Mingming Ning,[3] Lei Chen,[1]

[1]Department of Neurology, West China Hospital, Sichuan University, Chengdu, China

[2]Department of Cardiology, West China Hospital, Sichuan University, Chengdu, China

[3]Department of Neurology, Cardio-Neurology Clinic, Massachusetts General Hospital, Harvard Medical School, Boston, USA

Email for each author: Lin Zhang (zhanglinneur@163.com), Xi Zhu (zhu61900@163.com), Xiangmiao Qiu (qiuxmiao@126.com), Yajiao Li (liyajiao800@163.com), Yucheng Chen (chenyucheng2003@126.com), Hui Wang (dashu1985723@163.com), Shixu He (274464077@qq.com), Wanlin Lai (laiwl93@163.com), Anjiao Peng (peng_neurology@163.com), Mingming Ning (Ning@hms.harvard.edu), Lei Chen (leilei_25@126.com)

Key words：right-to-left shunt; epilepsy; migraine

Number of words (main text): 2189

Corresponding to: Prof Lei Chen, Department of Neurology, West China Hospital, Sichuan University, No. 37 Guoxue Road, Chengdu, Sichuan Province, 610041, China. Tel: 13258178634.E-mail: leilei_25@126.com

ABSTRACT

Objectives To investigate the relationship between right-to-left shunts (RLS) and migraine to account for the unexplained high prevalence of migraine in patients with epilepsy (PWE).

Design This is a cross-sectional study. The diagnosis and interview process of patients with migraine was based on the International Classification of Headache disorders in PWE. Participants underwent transthoracic echocardiography (TTE) with contrast medium to identify RLS. The highest number of microbubbles were recorded in the left atrium before the complete microbubble outflow of the right atrium. Moderate to large RLS was defined as the presence of 10 or more microbubbles.

Setting Single-center, 2015-2017.

Participants Patients with epilepsy.

Primary and secondary outcome measures Main outcome measures were the prevalence of migraine, RLS prevalence in PWE with and without migraine, and the prevalence of migraine in different degree of shunting.

Results Three hundred and thirty-nine participants with epilepsy who completed TTE were included in the analysis. The overall prevalence of migraine was 23.0%. Almost one-third of migraineurs have mild RLS and one-fifth of migraineurs have moderate to large RLS. Patients with mild RLS did not have a higher prevalence of migraine than those without RLS (26.3% vs 18.1%, p = 0.102), however, a higher prevalence of migraine was found in patients with moderate to large RLS (39.0% vs 18.1%, odds ratio = 2.90, 95% confidence interval = 1.41 to 5.98, p = 0.003). PWE with and without migraine had similar prevalence of mild RLS, however, patients with migraine had more moderate to large RLS (20.5% vs 9.6%, p=0.002). RLS, older age and female were factors predicting migraine prevalence.

Conclusions Almost one-fifth of migraineurs were correlated with moderate-to-large RLS which could be an underlying cause of migraine in epilepsy. PWE with migraine should be screened for RLS.

Strengths and limitations of this study

►Cohort study with data from a large number of patients with epilepsy.

►We firstly studied the relationship between the degree of shunts and migraine in patients with epilepsy.

►Transthoracic echocardiography with contrast medium was used as a tool to detect right-to-left shunt but not transoesophageal echocardiography.

INRODUCTION

Comorbidity of migraine is common in patients with epilepsy (PWE). The lifetime occurrence of migraine was up to 33.6% of PWE, representing an overall 52% increase relative to people without epilepsy.[1 2] Comorbid migraine may have an unfavourable effect on the prognosis of epilepsy in children and adults.[3 4] Evidence of genetics and animal experiments suggested similar pathophysiological mechanisms between migraine and familial epilepsy, such as genetic variants and abnormal neuronal excitability.[5-9] Despite these findings, the mechanisms underlying migraine in PWE are still controversial.

In the past 2 decades, right-to-left shunt (RLS), usually due to patent foramen ovale (PFO) and pulmonary arteriovenous shunts, have been found to be associated with migraine. Three-fold higher migraine with aura was observed in patients with PFO compared to people without PFO.[10] There are clinical trials showing that patients with PFO closure had a significantly greater reduction in headache days although responder rate defined as 50% reduction in migraine attacks is not different.[11 12] In addition, the meta-analyses showed a complete resolution of migraine post-PFO closure occurred in 54% of migraine-with-aura cases and in 39% of migraine-without-aura cases.[13 14] Therefore, PFO may be a fundamental cause in migraine. One of the possible mechanisms between the 2 conditions is that vasoactive chemicals triggering migraine bypass the pulmonary filter.[5] In addition, the presence of microemboli in patients with PFO is higher than in patients without PFO.[15] Animal experiments showed that microemboli can trigger the aura phenomenon by inducing cortical spreading depression (CSD).[7]

Similarly, PFO may also induced migraine by vasoactive chemicals and microemboli in PWE. Therefore, our main aim was to investigate the relationship

between RLS and migraine to account for the unexplained high prevalence of migraine in epilepsy. We also investigated other migraine predictive factors, for example, sex, age, familial epilepsy, duration of epilepsy and antiepileptic drugs (AEDs).

METHODS

Study design and patients

This is a cross-sectional study. Study populations were consecutively recruited between June 2015 and December 2017 from the Department of Neurology, West China Hospital, Sichuan University, China. Participants or legal guardians signed informed consent. Exclusion criteria were as follows: (1) age <3 years; (2) inaccurate communication because of language problems; (3) patients with one seizure (because high recurrence risk factors are controversial) and provoked seizures; (4) undetermined diagnosis of epilepsy; (5) clinical history of epilepsy including onset age of seizure, AEDs, familial history of epilepsy, seizure frequency in the last year and types of seizure were not recorded; (6) history of heart disease except RLS; (7) RLS confirmed by transthoracic echocardiography (TTE) at another hospital (because there may be differences between sonographers); and (8) informed consent not signed.

Definitions and collection of data

A definitive diagnosis and classification of epilepsy met the criteria proposed by the International League Against Epilepsy (ILAE) based on clinical history, seizure semiology, electroencephalography (EEG) and magnetic resonance imaging (MRI) findings.[16] For each patient, age, symptoms of seizure, onset age of seizure, AEDs, education status, seizure onset with visual symptoms, history of febrile seizure,

seizure frequency in the last year, familial history of epilepsy and types of seizure (focal onset, focal to bilateral tonic-clonic seizures, or generalized onset) were recorded.

The diagnosis and interview process for patients with migraine were based on the International Classification of Headache disorders (ICHD-3 beta).[17] The numbers of migraine attacks, estimated duration of migraine, location, quality, intensity (visual analogue scale, 0-10) and aura were recorded. Patients would not be classified as having migraine if only postictal and ictal migrainous headache occurred because ICHD-3 beta does not have related criteria. Every patient was diagnosed by 2 neurologists separately. If the diagnosis of epilepsy and migraine was inconsistent, a third senior neurologist was asked to discuss the case further.

For children, their parents (or the children themselves if they were old enough) were asked to give specific information about epilepsy and migraine. Medical records and seizure diaries during the last 12 months were reviewed to obtain information regarding the history of epilepsy and migraine, EEG and MRI.

Transthoracic Echocardiography

Participants underwent transthoracic echocardiography (TTE) with contrast medium and 1-5 MHz or 3-8MHz multiplane transducers in Philips IE33 to identify RLS; this was performed by 2 experienced sonographers who also analysed the videotapes together. Patients with other cardiac diseases (such as valvular heart diseases and cardiomyopathy) found by TTE were excluded. A microbubble bolus of agitated solution of 9 mL saline and 1 mL air was injected into one side of the patients' antecubital veins for increased sensitivity.[18 19] Patients were assessed for an RLS at rest and during Valsalva's manoeuvre and coughing. The highest number of

microbubbles were recorded in the left atrium before the complete microbubble outflow of the right atrium. If 3 or more microbubbles appeared, an RLS was diagnosed. The degree of shunting was defined as grade 0 if 0 to 2 microbubbles occurred, grade 1 (mild) if 3 to 9 microbubbles occurred, grade 2 (moderate) if 10 to 30 microbubbles occurred and grade 3 (large) if more than 30 microbubbles occurred.[20]

Data analysis

Data were collected using the same standard forms in Microsoft Excel (version 2013). Continuous variables were compared by ANOVA. Categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate. Bonferroni correction was used if necessary. Logistic regression analyses were used to determine whether some variables were migraine-predicting factors. For all statistical tests, $p<0.05$ was determined to be statistically significant. Statistics were performed using the SPSS statistical package (version 21.0).

Patient and public involvement

The development of the research question and outcome measures were informed by published literature on PFO and migraine. Patients' priorities, experience and preferences were not involved in designing the study. Patients who agreed to enrol to the study received TTE. The recruitment process was mentioned in the Study design and patients section. Two authors (Lin Zhang and Xi Zhu) disseminated the results to the study participants by interviews.

RESULTS

We carefully interviewed 532 patients with seizure (s) or (and) their parents. One hundred and sixty-one patients were excluded. The reasons for exclusion were showed in figure 1. Three hundred and thirty-nine participants with epilepsy who completed the interview process of migraine and TTE were included in the analysis. The details of migraine features according to the ICHD-3 beta criteria showed in the supplementary. The overall prevalence of migraine was 23.0% (table 1). A greater percentage of females had migraines than males (28.8% vs 17.2%, p = 0.022). Patients > 16 years seemed to have higher prevalence of migraine than those ≤ 16 years of age (25.7% vs 11.0%, p = 0.039). If the duration of epilepsy was > 10 years, patients were more likely to have migraines (34.6% vs 17.9%, p = 0.001). Patients with polytherapy may have more migraines (28.0% vs 19.0%, p = 0.052). Patients with first-degree and second-degree relatives with epilepsy may have not more migraines.

Almost one-third of migraineurs have mild RLS and one-fifth of migraineurs have moderate to large RLS (table 1 and table 2). Patients with mild RLS did not have a higher prevalence of migraine than those without RLS (26.3% vs 18.1%, odds ratio = 1.61, 95% confidence interval = 0.91 to 2.87, p = 0.102). In our cohort, 33.3% (26 of 78) of PWE with migraine and 28.0% (73 of 261) of PWE without migraine had mild RLS (p = 0.361).

Patients with moderate to large RLS had a higher prevalence of migraine than those without RLS (39.0% vs 18.1%, odds ratio = 2.90, 95% confidence interval = 1.41 to 5.98, p = 0.003, table 1 and table 2). Twenty-one percent (16 of 78) of PWE with migraine had moderate to large RLS; whereas only 10% (25 of 261) of PWE without migraine had moderate to large RLS (odds ratio = 2.44, 95% confidence interval = 1.23 to 4.84, p=0.002). After controlling the potential confounders, there are

significant differences of migraine prevalence between the groups with RLS grade 2-3 versus the group with RLS grade 0 (table 2). Therefore, moderate to large RLS but not mild RLS was associated with migraine in PWE.

We used a logistic regression analysis to identify the possible factors predicting migraine prevalence, such as sex, onset age, duration of epilepsy, current age, RLS and AEDs (table 3). RLS, older age and female were factors predicting migraine prevalence. Although patients with duration of epilepsy >10 years seemed to have higher migraine prevalence (table 1), the logistic regression analysis showed longer duration of epilepsy may be not a predictive factor.

PFO closure were performed in 7 patients. Four patients were followed for 6 month. We found that PFO closure full control migraine in 2 of 4 cases. Before PFO closure, the two patients have more than 11 migraine attacks in the last year. More interesting, the frequency of seizures in 3 patients with migraine after PFO closure decreased significantly.

DISCUSSION

This is the first study to evaluate the relationship between migraine and RLS in a large number of PWE. Our main findings were as follows: 1) Almost one-third of migraineurs have mild RLS and one-fifth of migraineurs have moderate to large RLS; 2) mild RLS did not have a higher prevalence of migraine than those without RLS; 3) moderate to large RLS was associated with migraine in PWE and 4) RLS, older age and female were factors predicting migraine prevalence in PWE.

The pathophysiological mechanisms of migraine in PWE were usually considered to be common genetic variants and abnormal neuronal excitability.[5-9] Our major hypothesis was that RLS may be one of pathophysiological mechanisms of migraine

in PWE. The results showed that mild RLS were not associated with migraine in PWE. Almost one-fifth of migraine were correlated with moderate-to-large RLS which could be an underlying cause of migraine in epilepsy. The mechanisms of the relationship between moderate to large RLS and migraine remains speculative. A previous study showed larger RLS tend to trigger migraine attacks because of the increased extent of the shunt.[21] Larger shunts allow harmful circulatory factors, such as vasoactive substances and microemboli which are eliminated by lung, to bypass the pulmonary filter and travel directly from the venous to the cerebral artery. PFO and higher rates of Doppler-detected cerebral microemboli have been reported to be associated.[22] In an animal experiment, air microemboli are able to trigger cortical spreading depression (CSD) and migraine aura often with transient microvascular occlusion which is insufficient to cause a permanent ischemic lesion.[7] In human, diagnostic microbubble injection also induced attacks of migraine with aura.[23] Therefore, microemboli may be a key role linking CSD, migraine and moderate to large RLS.

More intrersting, CSD increases neuronal excitability and triggers ictaform activity in neuronal tissues with partial impairment of inhibitory.[9] CSD also triggers migraine headache by activating neuronal Panx1 channels which contributes to neuronal hyperactivity in seizures.[24] [25] These may explain why migraine may have an unfavourable effect on the prognosis of epilepsy.[3] [4] Therefore, moderate to large RLS may be not only an underlying cause of migraine but also an adverse prognostic factors in PWE. Whether moderate to large RLS is an adverse prognostic factors in epilepsy should be also investigated.

We also investigated other migraine-predicting factors. Age showed effect on the migraine prevalence and logistic regression analysis also showed that age was a

predicting factor of migraine. There are some possible explanations for this finding. Previous studies found that PFO length and diameter increased with age which implied the shunts also increased with age.[26] Larger shunts are more likely to trigger migraine attacks.[21] In addition, more cases of migraine would be diagnosed if migraine occurred repeatedly as the duration of epilepsy became longer. Our results also support the hypothesis that age affected migraine prevalence.

The effect of PFO closure for migraine treatment still remain controversial, but a number of researches have proved to be effective, in particular in migraine with aura.[13 14] In our cohort, we also found PFO closure full control migraine in 2 of 4 cases. More interesting, the frequency of seizures in 3 patients with migraine after PFO closure decreased significantly. Although the effect of PFO closure in these cases needed to be explained with caution, it is worth investigating whether PFO closure improved migraine attacks and seizure outcomes in future.

This study has some limitations. First, transoesophageal echocardiography (TEE) is a gold standard diagnosis tool with a sensitivity and specificity of 80% to 100% for detection of an RLS.[18 19] The contrast TTE used by us has a lower sensitivity than TEE.[18] However, harmonic imaging improves sensitivity for the detection of RLS, ranging from 66% to 100%.[18 19 27 28] In addition, TTE is not inferior to TEE in the assessment of large shunts because TEE may miss large shunts detected by TTE.[28] In our study, moderate to large RLS are more likely to be associated with migraine, therefore, TTE may not be an inferior choice to TEE. Second, the population may be biased because a few patients did not complete TTE. However, there were no differences in demographic characteristics between patients who did or did not refuse TTE and the conclusions of our study should not be affected. Third, only Chinese were recruited, and this limits generalizability of results.

CONCLUSIONS

Moderate to large RLS but not mild RLS was associated with migraine. Based on these results, PWE with migraine should be screened for RLS. Whether moderate to large RLS is an adverse prognostic factors in epilepsy should be also investigated.

Acknowledgements No patients advisers involved in the study. We thank Dr. Qi Si for her help.

Contributors Lei Chen and Mingming Ning conceived and designed the work. Lin Zhang and Xi Zhu were involved in data collection, data analysis and interpretation. Lin Zhang drafted the manuscript. Lei Chen involved in critical revision of the article and final approval of the version to be published. Xiangmiao Qiu, Xiangmiao Qiu, Shixu He, Anjiao Peng, Wanlin Lai, Yajiao Li, Yucheng Chen, and Hui Wang were involved in the data collection and final approval of the version to be published. All authors have agreed to be accountable for all aspects of the work.

Funding Funding for this study was provided by China Association Against Epilepsy (No.2016005).

Competing interests None declared.

Patient consent Obtained.

Ethics approval The protocol was approved by the Ethics Committee of the West

China Hospital of Sichuan University.

Data sharing statement No additional data available.

REFERENCES

1. Keezer MR, Bauer PR, Ferrari MD, et al. The comorbid relationship between migraine and epilepsy: a systematic review and meta-analysis. Eur J Neurol 2015;22:1038-47.
2. Le H, Tfelt-Hansen P, Russell MB, et al. Co-morbidity of migraine with somatic disease in a large population-based study. Cephalalgia 2011;31:43-64.
3. Dragoumi P, Tzetzi O, Vargiami E, et al. Clinical course and seizure outcome of idiopathic childhood epilepsy: determinants of early and long-term prognosis. BMC Neurol 2013;13:206.
4. Velioglu SK, Boz C, Ozmenoglu M. The impact of migraine on epilepsy: a prospective prognosis study. Cephalalgia 2005;25:528-35.
5. Finocchi C, Del Sette M. Migraine with aura and patent foramen ovale: myth or reality? Neurol Sci 2015;36 Suppl 1:61-6.
6. Ludvigsson P, Hesdorffer D, Olafsson E, et al. Migraine with aura is a risk factor for unprovoked seizures in children. Ann Neurol 2006;59:210-3.
7. Nozari A, Dilekoz E, Sukhotinsky I, et al. Microemboli may link spreading depression, migraine aura, and patent foramen ovale. Ann Neurol 2010;67:221-9.
8. Winawer MR, Connors R. Evidence for a shared genetic susceptibility to migraine and epilepsy. Epilepsia 2013;54:288-95.

9. Eickhoff M, Kovac S, Shahabi P, et al. Spreading depression triggers ictaform activity in partially disinhibited neuronal tissues. Exp Neurol 2014;253:1-15.

10. Takagi H, Umemoto T. A meta-analysis of case-control studies of the association of migraine and patent foramen ovale. J Cardiol 2016;67:493-503.

11. Xing YQ, Guo YZ, Gao YS, et al. Effectiveness and Safety of Transcatheter Patent Foramen Ovale Closure for Migraine (EASTFORM) Trial. Sci Rep 2016;6:39081.

12. Tobis JM, Charles A, Silberstein SD, et al. Percutaneous Closure of Patent Foramen Ovale in Patients With Migraine: The PREMIUM Trial. J Am Coll Cardiol 2017;70:2766-74.

13. Kanwar SM, Noheria A, DeSimone CV, et al. Coincidental Impact of Transcatheter Patent Foramen Ovale Closure on Migraine with and without Aura - A Comprehensive Meta-Analysis. Clin Trials Regul Sci Cardiol 2016;15:7-13.

14. Shi YJ, Lv J, Han XT, et al. Migraine and percutaneous patent foramen ovale closure: a systematic review and meta-analysis. BMC Cardiovasc Disord 2017;17:203.

15. Shariat A, Yaghoubi E, Nemati R, et al. Microembolic signals in patients with cryptogenic stroke with or without patent foramen ovale. J Stroke Cerebrovasc Dis 2012;21:662-6.

16. Fisher RS, Cross JH, French JA, et al. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58:522-30.

17. Ettlin DA. The International Classification of Headache Disorders, 3rd edition

(beta version). Cephalalgia 2013;33:629-808.

18. Cotter PE, Martin PJ, Belham M. Improved sensitivity of transthoracic contrast echocardiography in the detection of right-to-left shunts. J Am Soc Echocardiogr 2010;23:578; author reply 78-9.
19. Ha JW, Shin MS, Kang S, et al. Enhanced detection of right-to-left shunt through patent foramen ovale by transthoracic contrast echocardiography using harmonic imaging. Am J Cardiol 2001;87:669-71, a11.
20. Mas JL, Arquizan C, Lamy C, et al. Recurrent cerebrovascular events associated with patent foramen ovale, atrial septal aneurysm, or both. N Engl J Med 2001;345:1740-6.
21. Tembl J, Lago A, Sevilla T, et al. Migraine, patent foramen ovale and migraine triggers. J Headache Pain 2007;8:7-12.
22. Rodriguez RA, Sinclair B, Weatherdon D, et al. Patent foramen ovale and brain microembolization during scoliosis surgery in adolescents. Spine (Phila Pa 1976) 2001;26:1719-21.
23. Dinia L, Roccatagliata L, Bonzano L, et al. Diffusion MRI during migraine with aura attack associated with diagnostic microbubbles injection in subjects with large PFO. Headache 2007;47:1455-6.
24. Karatas H, Erdener SE, Gursoy-Ozdemir Y, et al. Spreading depression triggers headache by activating neuronal Panx1 channels. Science 2013;339:1092-5.
25. Santiago MF, Veliskova J, Patel NK, et al. Targeting pannexin1 improves seizure outcome. PLoS One 2011;6:e25178.

26. McKenzie JA, Edwards WD, Hagler DJ. Anatomy of the patent foramen ovale for the interventionalist. Catheter Cardiovasc Interv 2009;73:821-6.

27. Clarke NR, Timperley J, Kelion AD, et al. Transthoracic echocardiography using second harmonic imaging with Valsalva manoeuvre for the detection of right to left shunts. Eur J Echocardiogr 2004;5:176-81.

28. Daniels C, Weytjens C, Cosyns B, et al. Second harmonic transthoracic echocardiography: the new reference screening method for the detection of patent foramen ovale. Eur J Echocardiogr 2004;5:449-52.

Table 1 Migraine prevalence in different groups

Characteristics	Sample	Migraine (%)	P
Male	169	29 (17.2)	0.022
Female	170	49 (28.8)	
Current age, years			0.039
≤16	82	12 (11.0)	
>16	257	66 (25.7)	
Duration of epilepsy, years			0.001
≤10	235	42 (17.9)	
>10	104	36 (34.6)	
Onset age, years			
≤16	183	39 (21.3)	0.421
>16	156	39 (25.0)	
Seizures onset with visual symptoms [a]	21	5 (23.8)	1.000 [b]
	293	69 (23.5)	
History of febrile seizures [a]	39	9 (23.1)	0.865
	259	63 (24.3)	
AEDs < or = 1	189	36 (19.0)	0.052
AEDs > or = 2	150	42 (28.0)	
Seizure types			0.319
Focal onset seizure	68	10 (14.7)	
Generalized onset seizure and FBTCS	227	58 (25.6)	
Undetermined	44	10 (22.7)	
Familial epilepsy	11	5 (45.5)	0.135 [b]

First-degree relatives	9	4 (44.4)	
Second-degree relatives	2	1 (50.0)	
No familial epilepsy	328	73 (22.3)	
RLS grade 1	99	26 (26.3)	0.102
RLS grade 2-3	41	16 (39.0)	0.003
RLS grade 1-3	140	42 (29.6)	0.010
RLS grade 0	199	36 (18.1)	

[a] Cases with uncertain history were excluded.

[b] Fisher's exact test

AEDs, antiepileptic drugs; FBTCS, focal to bilateral tonic-clonic seizures; RLS, right-to-left shunt.

Table 2 High prevalence of migraine in patients with moderate to large RLS

Characteristics	Right-to-left shunting			P
	Grade 2-3 (41)	Grade 1 (99)	Grade 0 (199)	
Male	17	52	100	0.413
Age, years	26.9 ± 11.7	27.6 ± 13.7	26.4 ± 13.2	0.769
Onset age, years	19.3 ± 11.2	17.9 ± 12.4	17.8 ± 12.3	0.779
Duration, years	8.1 ± 8.9	9.7 ± 9.6	8.7 ± 9.1	0.580
Familial epilepsy	2	2	7	0.648
Education, years				0.772
0-6	13	24	52	0.645
7-12	20	47	104	
≥13	8	28	43	
Seizure types				0.085
Focal onset	7	22	39	
Generalized onset seizure and FBTCS	32	69	126	
Undetermined	2	8	34	
Seizure frequency in the last year				0.170
0-12	27	71	142	
13-52	3	14	16	
>52	6	8	14	
Unknown	5	6	28	
AEDs < or = 1	25	47	117	0.139
AEDs > or = 2	16	52	82	

Antiepileptic drugs				0.382
Topiramate	10	21	35	
Valproate	8	45	74	
Levetiracetam	15	31	48	
Oxcarbazepine	12	34	48	
Migraine	16	26	36	0.010 [a]

The continuous data are presented as mean ± standard deviation.

[a] Bonferroni correction show statistical differences in grade 2-3 versus grade 0.

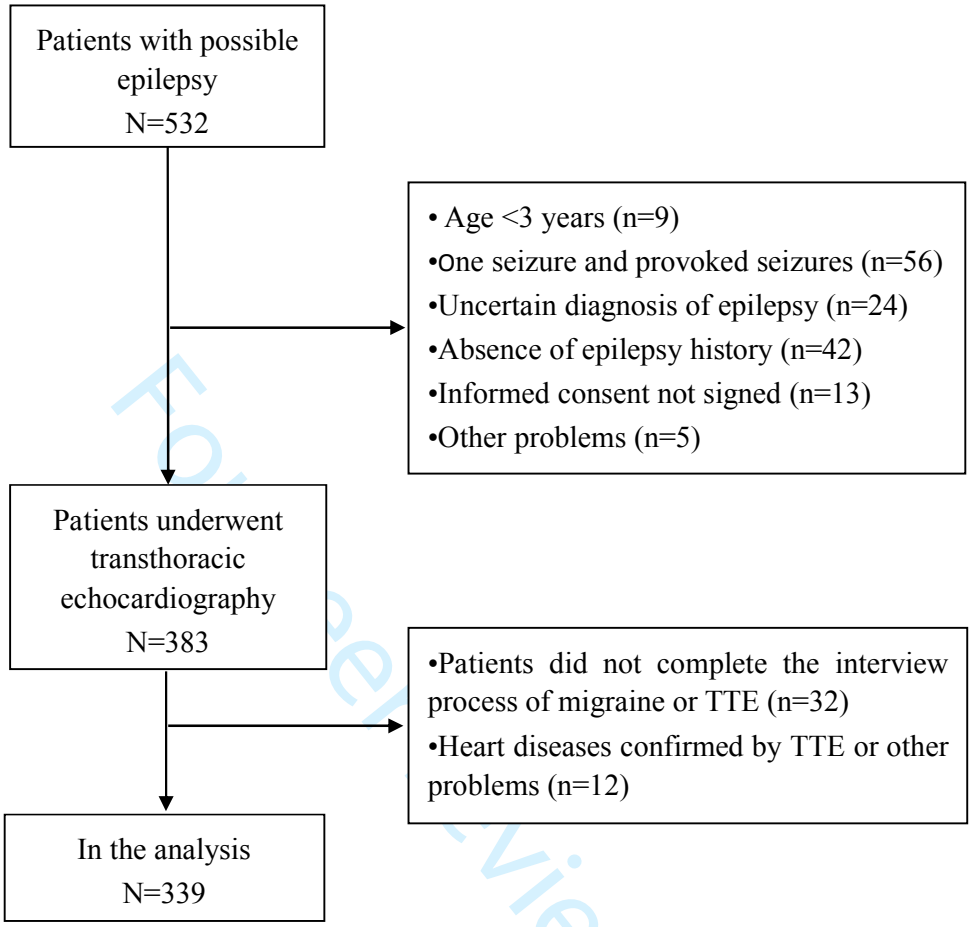
RLS, right-to-left shunt; FBTCS, focal to bilateral tonic-clonic seizures.

Table 3 Logistic regression analysis for migraine predictor

Characteristics	Odds ratio	95% confidence interval		P
		Lower	Upper	
Male	0.402	0.217	0.745	0.004
Age	1.038	1.010	1.067	0.007
Right-to-left shunt	2.544	1.392	4.650	0.002
Duration of epilepsy	1.023	0.987	1.061	0.214
Seizure frequency	1.082	0.811	1.443	0.593
Familial epilepsy	2.372	0.596	9.444	0.220
Valproate	1.300	0.752	2.248	0.347
Levetiracetam	0.914	0.483	1.731	0.782
Oxcarbazepine	0.741	0.386	1.420	0.366
Topiramate	1.639	0.806	3.333	0.173
Lamotrigine	0.551	0.142	2.146	0.390
Carbamazepine	0.768	0.373	1.582	0.474
Phenytoin	0.425	0.113	1.604	0.207
Phenobarbital	0.494	0.081	3.003	0.444

Figure legends

Figure 1. The flow diagram



sTable 1 Characteristics of migraine

Characteristics	N=78
Criteria	
Duration >2 hours (<18 years) or > 4 hours (≥ 18 years)[1]	78
At least 5 (without aura) attacks or 2 (with aura) attacks[1]	78
Headache has at least two of the following four characteristics	78
Location	
Left or Right[1]	46
Bilateral	32
Intensity[1,2]	
0-3	17
4-10[1]	61
Pulsating quality[1]	53
Aggravation by or causing avoidance of routine physical activity[1]	51
During headache at least one of the following	78
Nausea and/or vomiting[1]	62
Photophobia and Phonophbia[1]	55

[1]Diagnostic criteria of International Classification of Headache Disorders–3 criteria (beta)

[2]Visual analogue scale scores

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6
Bias	9	Describe any efforts to address potential sources of bias	*6,11*
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6,7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7,8
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7,8
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7,8
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9,10,11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

The relationship between right-to-left shunt and migraine in patients with epilepsy: a single-centre, cross-sectional study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-024144.R1
Article Type:	Research
Date Submitted by the Author:	07-Jul-2018
Complete List of Authors:	Zhang, Lin; Sichuan University West China Hospital, Department of Neurology Zhu, Xi; Sichuan University West China Hospital, Department of Neurology Qiu, XIangmiao; Sichuan University West China Hospital, Department of Neurology Li, Yajiao; Sichuan University West China Hospital, Department of Cardiology Chen, Yucheng; Sichuan University West China Hospital, Department of Cardiology Wang, Hui; Sichuan University West China Hospital, Department of Cardiology He, Shixu; Sichuan University West China Hospital, Department of Neurology Lai, Wanlin; Sichuan University West China Hospital, Department of Neurology Peng, Anjiao; Sichuan University West China Hospital, Department of Neurology Ning, Mingming; Cardio-Neurology Clinic, Massachusetts General Hospital, Harvard Medical School, Department of Neurology Chen, Lei; Sichuan University West China Medical Center, Department of Neurology
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Epidemiology
Keywords:	Epilepsy < NEUROLOGY, Migraine < NEUROLOGY, Right-to-left shunt

The relationship between right-to-left shunt and migraine in patients with epilepsy: a single-centre, cross-sectional study

Lin Zhang,[1] Xi Zhu,[1] Xiangmiao Qiu,[1] Yajiao Li,[2] Yucheng Chen,[2] Hui Wang,[2] Shixu He,[1] Wanlin Lai,[1] Anjiao Peng,[1] Mingming Ning,[3] Lei Chen [1]

[1]Department of Neurology, West China Hospital, Sichuan University, Chengdu, China

[2]Department of Cardiology, West China Hospital, Sichuan University, Chengdu, China

[3]Department of Neurology, Cardio-Neurology Clinic, Massachusetts General Hospital, Harvard Medical School, Boston, USA

Email for each author: Lin Zhang (zhanglinneur@163.com), Xi Zhu (zhu61900@163.com), Xiangmiao Qiu (qiuxmiao@126.com), Yajiao Li (liyajiao800@163.com), Yucheng Chen (chenyucheng2003@126.com), Hui Wang (dashu1985723@163.com), Shixu He (274464077@qq.com), Wanlin Lai (laiwl93@163.com), Anjiao Peng (peng_neurology@163.com), Mingming Ning (Ning@hms.harvard.edu), Lei Chen (leilei_25@126.com)

Key words：right-to-left shunt; epilepsy; migraine

Number of words (main text): 2656

Corresponding to: Prof Lei Chen, Department of Neurology, West China Hospital, Sichuan University, No. 37 Guoxue Road, Chengdu, Sichuan Province, 610041, China. Tel: 13258178634.E-mail: leilei_25@126.com

ABSTRACT

Objectives To investigate the relationship between right-to-left shunt and migraine to account for the unexplained high prevalence of migraine in patients with epilepsy.

Design This is a cross-sectional study. The diagnosis and interview process of patients with migraine was based on the International Classification of Headache Disorders-3 beta in patients with epilepsy. Participants underwent transthoracic echocardiography (TTE) with contrast medium to identify right-to-left shunt. The highest number of microbubbles were recorded in the left atrium before the complete microbubble outflow of the right atrium. A moderate-to-large shunt was defined as the presence of 10 or more microbubbles.

Setting Single-centre, 2015-2017.

Participants Patients with epilepsy.

Primary and secondary outcome measures The primary outcome measures were the prevalence of migraine; the prevalence of right-to-left shunt in patients with migraine and those without migraine; and the prevalence of migraine in different degrees of shunting.

Results Three hundred thirty-nine participants with epilepsy who completed TTE were included in the analysis. The overall prevalence of migraine was 23.0%. One third of the migraineurs had mild right-to-left shunt and one fifth of the migraineurs had moderate-to-large right-to-left shunt. Patients with mild shunt did not have a higher prevalence of migraine than those without shunt (26.3% vs. 18.1%, p = 0.102); however, a higher prevalence of migraine was found in patients with moderate-to-large shunt (39.0% vs. 18.1%, odds ratio = 2.90, 95% confidence interval = 1.41 to 5.98, p = 0.003). Patients with migraine and patients without migraine had similar prevalence of mild shunt; however, patients with migraine had more

moderate-to-large shunt (20.5% vs. 9.6%, p=0.002). Right-to-left shunt and female were factors predicting migraine prevalence.

Conclusions One fifth of migraineurs were correlated with moderate-to-large right-to-left shunt, which could be an underlying cause of migraine in epilepsy.

Strengths and limitations of this study

►Cohort study with data from a large number of patients with epilepsy.

►We first studied the relationship between the degree of shunt and migraine in patients with epilepsy.

►Transthoracic echocardiography with contrast medium was used as a tool to detect right-to-left shunt but not transoesophageal echocardiography.

INRODUCTION

The comorbidity of migraine is common in patients with epilepsy. The lifetime occurrence of migraine was as high as 33.6% in patients with epilepsy, representing an overall 52% increase relative to people without epilepsy.[1 2] Comorbid migraine may have an unfavourable effect on the prognosis of epilepsy in children and adults.[3 4] Evidence of genetics and animal experiments have suggested similar pathophysiological mechanisms between migraine and familial epilepsy, such as genetic variants and abnormal neuronal excitability.[5-9] Despite these findings, the mechanisms underlying migraine in patients with epilepsy are still controversial.

In the past 2 decades, right-to-left shunt, usually due to patent foramen ovale (PFO) and pulmonary arteriovenous shunt, have been found to be associated with migraine. A three-fold higher migraine with aura was observed in patients with right-to-left shunt compared to people without right-to-left shunt.[10] There are clinical trials showing that treatment for right-to-left shunt can significantly reduce headache days although the responder rate defined as a 50% reduction in migraine attacks is not different from baseline.[11-14] In addition, the meta-analyses showed that a complete resolution of migraine after treatment for right-to-left shunt occurred in 54% of migraine-with-aura cases and in 39% of migraine-without-aura cases.[15 16] Therefore, right-to-left shunt may be a fundamental cause of migraine. One of the possible mechanisms between the 2 conditions is that vasoactive chemicals triggering migraine bypass the pulmonary filter.[5] Right-to-left shunt may carry microemboli into the carotid circulation. Animal experiments showed that microemboli can trigger the aura phenomenon by inducing cortical spreading depression (CSD).[7]

Similarly, right-to-left shunt may also induce migraine by vasoactive chemicals and microemboli in patients with epilepsy. Therefore, our main aim was to investigate

the relationship between right-to-left shunt and migraine to account for the unexplained high prevalence of migraine in epilepsy. We also investigated other migraine predictive factors, for example, sex, age, familial epilepsy, duration of epilepsy and antiepileptic drugs (AEDs).

METHODS

Study design and patients

This is a cross-sectional study. Study populations were consecutively recruited between June 2015 and December 2017 from the Department of Neurology, West China Hospital, Sichuan University, China. Participants or legal guardians signed informed consent. The inclusion criteria were as follows: (1) patients with epilepsy or possible epilepsy (patients with possible epilepsy will be followed to establish the diagnosis of epilepsy); and (2) the clinical history and medical records of epilepsy can be obtained. The exclusion criteria were as follows: (1) age <3 years; (2) inaccurate communication because of language problems; (3) patients with one seizure (because high recurrence risk factors are controversial) and provoked seizures; (4) the diagnosis of epilepsy was still undetermined when we performed the data analysis; (5) the clinical history of epilepsy including onset age of seizure, AEDs, familial history of epilepsy, seizure frequency in the last year, and types of seizure were not recorded; (6) history of heart disease except right-to-left shunt; (7) right-to-left shunt confirmed by transthoracic echocardiography (TTE) at another hospital (because there may be differences between sonographers); (8) informed consent not signed; and (9) patients did not complete the interview process for the diagnosis of migraine.

Definitions and collection of data

A definitive diagnosis and classification of epilepsy met the criteria proposed by the International League Against Epilepsy (ILAE) based on the clinical history, seizure semiology, electroencephalography (EEG), and magnetic resonance imaging (MRI) findings.[17] For each patient, age, symptoms of seizure, onset age of seizure, AEDs, education status, seizure onset with visual symptoms, history of febrile seizure, seizure frequency in the last year, familial history of epilepsy, and types of seizure (focal onset, focal to bilateral tonic-clonic seizures, or generalized onset) were recorded.

The diagnosis and interview process for patients with migraine were based on the International Classification of Headache Disorders (ICHD-3 beta).[18] The number of migraine attacks, estimated duration of migraine, location, quality, intensity (visual analogue scale, 0-10), and aura were recorded. Patients would not be classified as having migraine if only postictal and ictal migrainous headache occurred because ICHD-3 beta does not have related criteria. Every patient was diagnosed by 2 neurologists separately. If the diagnosis of epilepsy and migraine was inconsistent, a third senior neurologist was asked to discuss the case further.

For children, their parents (or the children themselves if they were old enough) were asked to give specific information about epilepsy and migraine. Medical records and seizure diaries during the last 12 months were reviewed to obtain information regarding the history of epilepsy and migraine, EEG, and MRI.

Transthoracic Echocardiography

Participants underwent transthoracic echocardiography (TTE) with contrast medium and 1-5 MHz or 3-8MHz multiplane transducers in Philips IE33 to identify right-to-left shunt; this was performed by 2 experienced sonographers who also

analysed the videotapes together. Patients with other cardiac diseases (e.g., valvular heart disease and cardiomyopathy) found by TTE were excluded. A microbubble bolus of agitated solution of 9 mL saline and 1 mL air was injected into one side of the patients' antecubital veins for increased sensitivity.[19 20] Patients were assessed for right-to-left shunt at rest and during the Valsalva manoeuvre and coughing. The highest number of microbubbles were recorded in the left atrium before the complete microbubble outflow of the right atrium. If 3 or more microbubbles appeared, a right-to-left shunt was diagnosed. The degree of shunting was defined as grade 0 if 0 to 2 microbubbles occurred, grade 1 (mild) if 3 to 9 microbubbles occurred, grade 2 (moderate) if 10 to 30 microbubbles occurred and grade 3 (large) if more than 30 microbubbles occurred.[21]

Data analysis

Data were collected using the same standard forms in Microsoft Excel (version 2013). Continuous variables were compared by analysis of variance (ANOVA). Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Bonferroni correction was used if necessary. Logistic regression analyses were used to determine whether some variables were migraine-predicting factors. For all statistical tests, $p<0.05$ was determined to be statistically significant. Statistics were performed using the SPSS statistical package (version 21.0).

Patient and public involvement

The development of the research question and outcome measures were informed by published literature on PFO and migraine. Patients' priorities, experience and preferences were not involved in designing the study. Patients who agreed to enrol to

the study received TTE. The recruitment process was mentioned in the Study Design and Patients section. Two authors (Lin Zhang and Xi Zhu) disseminated the results to the study participants by interviews.

RESULTS

We carefully interviewed 532 patients with seizure (s) or (and) their parents. One hundred sixty-one patients were excluded. The reasons for exclusion are shown in figure 1. Three hundred thirty-nine participants with epilepsy who completed the interview process of migraine and TTE were included in the analysis. The details of migraine features according to the ICHD-3 beta criteria are shown in Supplementary file 1. The overall prevalence of migraine was 23.0% (table 1). A greater percentage of females had migraines than males (28.8% vs. 17.2%, p = 0.022). Patients > 16 years seemed to have a higher prevalence of migraine than those ≤ 16 years of age (25.7% vs. 11.0%, p = 0.039). If the duration of epilepsy was more than 10 years, patients were more likely to have migraines (34.6% vs. 17.9%, p = 0.001). Patients with polytherapy may have more migraines (28.0% vs. 19.0%, p = 0.052). Patients with first-degree and second-degree relatives with epilepsy, as well as a history of febrile seizures, may not have more migraines. Patients who have visual symptoms at the onset of seizures did not have more migraines than those without visual symptoms (data not shown).

One third of migraineurs have mild right-to-left shunt (table 1 and table 2). Patients with mild right-to-left shunt did not have a higher prevalence of migraine than those without right-to-left shunt (26.3% vs 18.1%, odds ratio = 1.61, 95% confidence interval = 0.91 to 2.87, p = 0.102). In our cohort, 33.3% (26 of 78) of patients with epilepsy and with migraine and 28.0% (73 of 261) of patients with epilepsy and

without migraine had mild right-to-left shunt (p = 0.361).

One fifth of migraineurs have moderate-to-large right-to-left shunt (table 1 and table 2). Patients with moderate-to-large right-to-left shunt had a higher prevalence of migraine than those without right-to-left shunt (39.0% vs. 18.1%, odds ratio = 2.90, 95% confidence interval = 1.41 to 5.98, p = 0.003). Twenty-one percent (16 of 78) of patients with epilepsy and with migraine had moderate-to-large right-to-left shunt; whereas only 10% (25 of 261) of patients with epilepsy and without migraine had moderate-to-large right-to-left shunt (odds ratio = 2.44, 95% confidence interval = 1.23 to 4.84, p=0.002). After controlling for potential confounders, there are significant differences of migraine prevalence between the groups with right-to-left shunt grade 2-3 versus the group with right-to-left shunt grade 0 (table 2). Therefore, moderate-to-large right-to-left shunt but not mild right-to-left shunt was associated with migraine in patients with epilepsy.

We used a logistic regression analysis to identify the possible factors predicting migraine prevalence, such as gender, duration of epilepsy, current age, right-to-left shunt and number of antiepileptic drugs (table 3). Right-to-left shunt and female were factors predicting migraine prevalence. Although patients with duration of epilepsy >10 years and older age have a higher migraine prevalence (table 1), the logistic regression analysis showed no significant statistical difference.

PFO closure were performed in 7 patients. Four patients were followed for 6 months. We found that PFO closure fully controlled migraine in 2 of 4 cases. Before PFO closure, the two patients have more than 11 migraine attacks in the last year. More interesting, the frequency of seizures in 3 patients with migraine after PFO closure decreased significantly.

DISCUSSION

This is the first study to evaluate the relationship between migraine and right-to-left shunt in a large number of patients with epilepsy. Our main findings were as follows: 1) one third of the migraineurs have mild right-to-left shunt and one fifth of the migraineurs have moderate-to-large right-to-left shunt; 2) patients with mild right-to-left shunt did not have a higher prevalence of migraine than those without right-to-left shunt; 3) moderate-to-large right-to-left shunt was associated with migraine in patients with epilepsy and 4) right-to-left shunt and female were factors predicting migraine prevalence in patients with epilepsy.

Migraine in patients without epilepsy has been proved to be associated with CSD and activation of the trigeminovascular system and its constituent neuropeptides.[22] In addition, recent studies also suggested the cortical excitatory/inhibitory imbalance due to neuronal and glial ion channels renders patients more vulnerable to an headache attack.[22] Migraine in patients with epilepsy is not only associated with the aforementioned mechanisms, but also is usually considered to be associated with abnormal neuronal excitability induced by genetic variants.[5-9] However, the high prevalence of migraine in patients with epilepsy cannot be fully explained by genetic variants. In the past 2 decades, right-to-left shunt have been found to be associated with migraine in patients without epilepsy. Our major hypothesis was that right-to-left shunt may also be one of the pathophysiological mechanisms of migraine in patients with epilepsy. The results showed that mild right-to-left shunt was not associated with migraine in patients with epilepsy. Almost one fifth of migraine were correlated with moderate-to-large right-to-left shunt, which could be an underlying cause of migraine in epilepsy. The mechanisms of the relationship between moderate-to-large right-to-left shunt and migraine remains speculative. A previous study showed that

larger right-to-left shunt tend to trigger migraine attacks because of the increased extent of the shunt.[23] Larger shunt allow harmful circulatory factors, such as vasoactive substances and microemboli which are eliminated by lung, to bypass the pulmonary filter and travel directly from the venous to the cerebral artery. Right-to-left shunt and higher rates of Doppler-detected cerebral microemboli have been reported to be associated.[24] In an animal experiment, air microemboli are able to trigger CSD and migraine aura often with transient microvascular occlusion that is insufficient to cause a permanent ischemic lesion.[7] In humans, diagnostic microbubble injection also induced attacks of migraine with aura.[25] Therefore, microemboli may be a key role linking CSD, migraine, and moderate-to-large shunt.

Interestingly, CSD increases neuronal excitability and triggers ictaform activity in neuronal tissues with partial impairment of inhibitory.[9] CSD also triggers migraine headache by activating neuronal Panx1 channels, which contributes to neuronal hyperactivity in seizures.[26 27] These findings may explain why migraine may have an unfavourable effect on the prognosis of epilepsy.[3 4] Therefore, moderate-to-large right-to-left shunt may not only be an underlying cause of migraine but also an adverse prognostic factor in patients with epilepsy. Whether moderate-to-large right-to-left shunt is an adverse prognostic factors in epilepsy should also be investigated.

The effect of PFO closure for migraine treatment still remains controversial. Randomised controlled trials (RCTs) looking at the effect of PFO closure on migraine were initially conducted. There are clinical trials showing that treatment for right-to-left shunt did not increase responder rate defined as 50% reduction in migraine attacks; however, it significantly reduced headache days.[11-14] Similarly to the stroke trials, they had initial negative RCTs. With better patient selection and longer

follow-up, subsequent trials may show more cessation of migraine headache.[28] In our cohort, we found that PFO closure fully controlled migraine in 2 of 4 cases. More interesting, the frequency of seizures in 3 patients with migraine after surgery decreased significantly. Although these cases need to be explained with caution, it is worth investigating in the future whether PFO closure improve migraine attacks and seizure outcomes. In addition, given the subjective nature of migraines and their vulnerability to bias and the placebo effect, perhaps a large, well-designed sham controlled trial with modern devices is required. If treatment for right-to-left shunt have been shown to be definitely effective, right-to-left shunt should be screened in patients with epilepsy and with migraine.

This study has some limitations. First, transoesophageal echocardiography (TEE) is a gold standard diagnostic tool with a sensitivity and specificity of 80% to 100% for the detection of a right-to-left shunt.[19 20] The contrast TTE used by us has a lower sensitivity than TEE.[19] However, harmonic imaging improves sensitivity for the detection of right-to-left shunt, ranging from 66% to 100%.[19 20 29 30] In addition, TTE is not inferior to TEE in the assessment of large shunt because TEE may miss large shunt detected by TTE.[30] In our study, moderate-to-large right-to-left shunt are more likely to be associated with migraine; therefore, TTE may not be an inferior choice to TEE. Second, the population may be biased because a few patients did not complete TTE. However, there were no differences in demographic characteristics between patients who did or did not refuse TTE and the conclusions of our study should not be affected. Third, only Chinese people were recruited, and this limits the generalizability of results.

CONCLUSIONS

Moderate-to-large right-to-left shunt but not mild right-to-left shunt were associated with migraine. On the basis of these results, patients with epilepsy and with migraine should be screened for a right-to-left shunt if treatment for a right-to-left shunt has been shown to be effective in migraine. Whether moderate-to-large right-to-left shunt is an adverse prognostic factor in epilepsy should be also investigated.

Acknowledgements No patient advisers were involved in the study. We thank Dr. Qi Si for her help.

Contributors Lei Chen and Mingming Ning conceived and designed the work. Lin Zhang and Xi Zhu were involved in data collection, data analysis and interpretation. Lin Zhang drafted the manuscript. Lei Chen involved in critical revision of the article and final approval of the version to be published. Xiangmiao Qiu, Xiangmiao Qiu, Shixu He, Anjiao Peng, Wanlin Lai, Yajiao Li, Yucheng Chen, and Hui Wang were involved in the data collection and final approval of the version to be published. All authors have agreed to be accountable for all aspects of the work.

Funding Funding for this study was provided by China Association Against Epilepsy (No.2016005).

Competing interests None declared.

Patient consent Obtained.

Ethics approval The protocol was approved by the Ethics Committee of the West

China Hospital of Sichuan University.

Data sharing statement No additional data available.

REFERENCES

1. Keezer MR, Bauer PR, Ferrari MD*, et al.* The comorbid relationship between migraine and epilepsy: a systematic review and meta-analysis. *Eur J Neurol* 2015;22:1038-47.
2. Le H, Tfelt-Hansen P, Russell MB*, et al.* Co-morbidity of migraine with somatic disease in a large population-based study. *Cephalalgia* 2011;31:43-64.
3. Dragoumi P, Tzetzi O, Vargiami E*, et al.* Clinical course and seizure outcome of idiopathic childhood epilepsy: determinants of early and long-term prognosis. *BMC Neurol* 2013;13:206.
4. Velioglu SK, Boz C, Ozmenoglu M. The impact of migraine on epilepsy: a prospective prognosis study. *Cephalalgia* 2005;25:528-35.
5. Finocchi C, Del Sette M. Migraine with aura and patent foramen ovale: myth or reality? *Neurol Sci* 2015;36 Suppl 1:61-6.
6. Ludvigsson P, Hesdorffer D, Olafsson E*, et al.* Migraine with aura is a risk factor for unprovoked seizures in children. *Ann Neurol* 2006;59:210-3.
7. Nozari A, Dilekoz E, Sukhotinsky I*, et al.* Microemboli may link spreading depression, migraine aura, and patent foramen ovale. *Ann Neurol* 2010;67:221-9.
8. Winawer MR, Connors R. Evidence for a shared genetic susceptibility to

migraine and epilepsy. *Epilepsia* 2013;54:288-95.

9. Eickhoff M, Kovac S, Shahabi P*, et al.* Spreading depression triggers ictaform activity in partially disinhibited neuronal tissues. *Exp Neurol* 2014;253:1-15.

10. Wammes-van der Heijden EA, Tijssen CC, Egberts AC. Right-to-left shunt and migraine: the strength of the relationship. *Cephalalgia* 2006;26:208-13.

11. Xing YQ, Guo YZ, Gao YS*, et al.* Effectiveness and Safety of Transcatheter Patent Foramen Ovale Closure for Migraine (EASTFORM) Trial. *Sci Rep* 2016;6:39081.

12. Tobis JM, Charles A, Silberstein SD*, et al.* Percutaneous Closure of Patent Foramen Ovale in Patients With Migraine: The PREMIUM Trial. *J Am Coll Cardiol* 2017;70:2766-74.

13. Dowson A, Mullen MJ, Peatfield R*, et al.* Migraine Intervention With STARFlex Technology (MIST) trial: a prospective, multicenter, double-blind, sham-controlled trial to evaluate the effectiveness of patent foramen ovale closure with STARFlex septal repair implant to resolve refractory migraine headache. *Circulation* 2008;117:1397-404.

14. Mattle HP, Evers S, Hildick-Smith D*, et al.* Percutaneous closure of patent foramen ovale in migraine with aura, a randomized controlled trial. *Eur Heart J* 2016;37:2029-36.

15. Kanwar SM, Noheria A, DeSimone CV*, et al.* Coincidental Impact of Transcatheter Patent Foramen Ovale Closure on Migraine with and without Aura - A Comprehensive Meta-Analysis. *Clin Trials Regul Sci Cardiol*

2016;15:7-13.

16. Shi YJ, Lv J, Han XT, *et al.* Migraine and percutaneous patent foramen ovale closure: a systematic review and meta-analysis. *BMC Cardiovasc Disord* 2017;17:203.

17. Fisher RS, Cross JH, French JA, *et al.* Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017;58:522-30.

18. Ettlin DA. The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia* 2013;33:629-808.

19. Cotter PE, Martin PJ, Belham M. Improved sensitivity of transthoracic contrast echocardiography in the detection of right-to-left shunts. *J Am Soc Echocardiogr* 2010;23:578; author reply 78-9.

20. Ha JW, Shin MS, Kang S, *et al.* Enhanced detection of right-to-left shunt through patent foramen ovale by transthoracic contrast echocardiography using harmonic imaging. *Am J Cardiol* 2001;87:669-71.

21. Mas JL, Arquizan C, Lamy C, *et al.* Recurrent cerebrovascular events associated with patent foramen ovale, atrial septal aneurysm, or both. *N Engl J Med* 2001;345:1740-6.

22. Pietrobon D, Moskowitz MA. Pathophysiology of migraine. *Annu Rev Physiol* 2013;75:365-91.

23. Tembl J, Lago A, Sevilla T, *et al.* Migraine, patent foramen ovale and

migraine triggers. *J Headache Pain* 2007;8:7-12.

24. Rodriguez RA, Sinclair B, Weatherdon D*, et al.* Patent foramen ovale and brain microembolization during scoliosis surgery in adolescents. *Spine (Phila Pa 1976)* 2001;26:1719-21.

25. Dinia L, Roccatagliata L, Bonzano L*, et al.* Diffusion MRI during migraine with aura attack associated with diagnostic microbubbles injection in subjects with large PFO. *Headache* 2007;47:1455-6.

26. Karatas H, Erdener SE, Gursoy-Ozdemir Y*, et al.* Spreading depression triggers headache by activating neuronal Panx1 channels. *Science* 2013;339:1092-5.

27. Santiago MF, Veliskova J, Patel NK*, et al.* Targeting pannexin1 improves seizure outcome. *PLoS One* 2011;6:e25178.

28. Mojadidi MK, Zaman MO, Elgendy IY*, et al.* Cryptogenic Stroke and Patent Foramen Ovale. *J Am Coll Cardiol* 2018;71:1035-43.

29. Clarke NR, Timperley J, Kelion AD*, et al.* Transthoracic echocardiography using second harmonic imaging with Valsalva manoeuvre for the detection of right to left shunts. *Eur J Echocardiogr* 2004;5:176-81.

30. Daniels C, Weytjens C, Cosyns B*, et al.* Second harmonic transthoracic echocardiography: the new reference screening method for the detection of patent foramen ovale. *Eur J Echocardiogr* 2004;5:449-52.

Table 1 Migraine prevalence in different groups

Characteristics	Sample	Migraine (%)	P values
Gender			0.022
Male	169	29 (17.2)	
Female	170	49 (28.8)	
Current age, years			0.039
≤16	82	12 (11.0)	
>16	257	66 (25.7)	
Duration of epilepsy, years			0.001
≤10	235	42 (17.9)	
>10	104	36 (34.6)	
Onset age, years			0.421
≤16	183	39 (21.3)	
>16	156	39 (25.0)	
Seizure frequency in the last year			0.256
0-12	246	56 (22.8)	
13-52	33	11 (33.3)	
>52	28	7 (25.0)	
Unknown	32	4 (12.5)	
Number of AEDs			0.052
< or = 1	189	36 (19.0)	
> or = 2	150	42 (28.0)	
AEDs			0.299
Topiramate	66	20 (30.3)	
Valproate	126	29 (23.0)	

Levetiracetam	94	26 (27.7)	
Oxcarbazepine	94	19 (20.2)	
Lamotrigine	27	2 (7.4)	
Carbamazepine	67	19 (28.4)	
Phenytoin	20	4 (20.0)	
Phenobarbital	12	2 (16.7)	
Seizure types			0.319
Focal onset seizure	68	10 (14.7)	
Generalized onset seizure and focal to bilateral tonic-clonic seizures	227	58 (25.6)	
Undetermined	44	10 (22.7)	
Familial epilepsy	11	5 (45.5)	0.135
First-degree relatives	9	4 (44.4)	
Second-degree relatives	2	1 (50.0)	
No familial epilepsy	328	73 (22.3)	
Right-to-left shunt			
Grade 1	99	26 (26.3)	0.102
Grade 2-3	41	16 (39.0)	0.003
Grade 1-3	140	42 (29.6)	0.010
Grade 0	199	36 (18.1)	

AEDs, antiepileptic drugs.

Table 2 High prevalence of migraine in patients with moderate to large right-to-left shunt

Characteristics	Right-to-left shunt			P
	Grade 2-3 (n=41)	Grade 1 (n=99)	Grade 0 (n=199)	
Male, n (%)	17 (41.5)	52 (52.5)	100 (50.3)	0.413
Age, years	26.9 ± 11.7	27.6 ± 13.7	26.4 ± 13.2	0.769
Onset age, years	19.3 ± 11.2	17.9 ± 12.4	17.8 ± 12.3	0.779
Duration, years	8.1 ± 8.9	9.7 ± 9.6	8.7 ± 9.1	0.580
Familial epilepsy, n (%)	2	2	7	0.648
Education, n (%)				0.772
0-6, years	13 (31.7)	24 (24.2)	52 (26.1)	
7-12, years	20 (48.8)	47 (47.5)	104 (52.3)	
≥13, years	8 (19.5)	28 (28.3)	43 (21.6)	
Seizure types, n (%)				0.085
Focal onset	7 (17.1)	22 (22.2)	39 (19.6)	
Generalized onset seizure and focal to bilateral tonic-clonic seizures	32 (78.0)	69 (69.7)	126 (63.3)	
Undetermined	2 (4.9)	8 (8.1)	34 (17.1)	
Seizure frequency in the last year, n (%)				0.284
0-12	27 (65.9)	71 (71.7)	148 (71.4)	
13-52	3 (7.3)	14 (14.1)	16 (8.0)	
>52	6 (14.6)	8 (8.1)	14 (7.0)	

Unknown	5 (12.2)	6 (6.1)	21 (14.1)	
Number of used AEDs				0.139
< or = 1, n (%)	25 (61.0)	47 (47.5)	117 (58.8)	
> or = 2, n (%)	16 (39.0)	52 (52.5)	82 (41.2)	
AEDs				0.735
Topiramate	10	21	35	
Valproate	8	45	74	
Levetiracetam	15	31	48	
Oxcarbazepine	12	34	48	
Lamotrigine	4	8	15	
Carbamazepine	8	28	31	
Phenytoin	1	6	13	
Phenobarbital	1	4	7	
Migraine, n (%)	16 (39.0)	26 (26.3)	36 (18.1)	0.010 [a]

The continuous data are presented as mean ± standard deviation.

[a] Bonferroni correction show statistical differences in grade 2-3 versus grade 0.

AEDs, antiepileptic drugs.

Table 3 Logistic regression analysis for migraine predictors

Characteristics	Odds ratio	95% confidence interval		P
		Lower	Upper	
Male	0.518	0.302	0.891	0.017
Current age, years	0.978	0.956	1.001	0.057
Right-to-left shunt	2.200	1.274	3.797	0.005
Duration of epilepsy, years	0.974	0.945	1.004	0.085
Familial epilepsy	2.828	0.765	10.453	0.119
Number of antiepileptic drugs > 1	1.354	0.784	2.340	0.277

Figure legends

Figure 1. The flow diagram

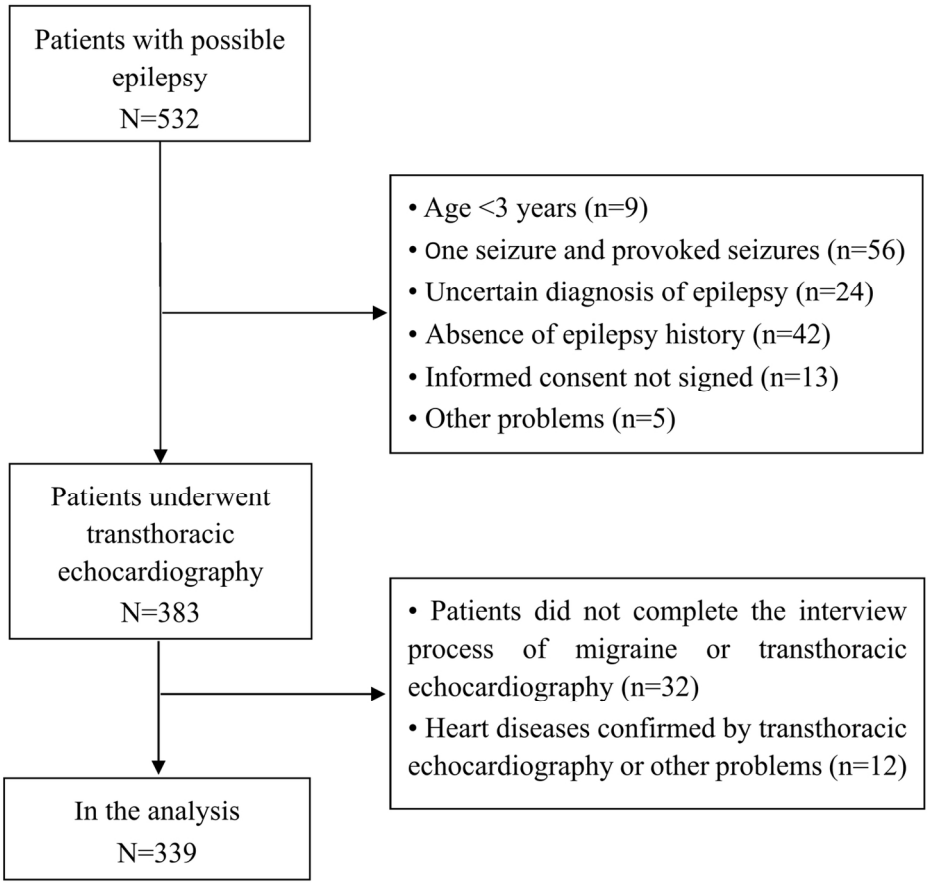


Figure 1. The flow diagram

136x127mm (300 x 300 DPI)

sTable 1 Characteristics of migraine

Characteristics	N=78
Criteria	
Duration >2 hours (<18 years) or > 4 hours (≥ 18 years)[1]	78
At least 5 (without aura) attacks or 2 (with aura) attacks[1]	78
Headache has at least two of the following four characteristics	78
Location	
Left or Right[1]	46
Bilateral	32
Intensity[1,2]	
0-3	17
4-10[1]	61
Pulsating quality[1]	53
Aggravation by or causing avoidance of routine physical activity[1]	51
During headache at least one of the following	78
Nausea and/or vomiting[1]	62
Photophobia and Phonophbia[1]	55

[1]Diagnostic criteria of International Classification of Headache Disorders–3 criteria (beta)

[2]Visual analogue scale scores

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6
Bias	9	Describe any efforts to address potential sources of bias	*6,11*
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6,7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7,8
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7,8
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7,8
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9,10,11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

The relationship between right-to-left shunt and migraine in patients with epilepsy: a single-centre, cross-sectional study in China

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-024144.R2
Article Type:	Research
Date Submitted by the Author:	28-Jul-2018
Complete List of Authors:	Zhang, Lin; Sichuan University West China Hospital, Department of Neurology Zhu, Xi; Sichuan University West China Hospital, Department of Neurology Qiu, XIangmiao; Sichuan University West China Hospital, Department of Neurology Li, Yajiao; Sichuan University West China Hospital, Department of Cardiology Chen, Yucheng; Sichuan University West China Hospital, Department of Cardiology Wang, Hui; Sichuan University West China Hospital, Department of Cardiology He, Shixu; Sichuan University West China Hospital, Department of Neurology Lai, Wanlin; Sichuan University West China Hospital, Department of Neurology Peng, Anjiao; Sichuan University West China Hospital, Department of Neurology Ning, Mingming; Cardio-Neurology Clinic, Massachusetts General Hospital, Harvard Medical School, Department of Neurology Chen, Lei; Sichuan University West China Medical Center, Department of Neurology
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Epidemiology
Keywords:	Epilepsy < NEUROLOGY, Migraine < NEUROLOGY, Right-to-left shunt

The relationship between right-to-left shunt and migraine in patients with epilepsy: a single-centre, cross-sectional study in China

Lin Zhang,[1] Xi Zhu,[1] Xiangmiao Qiu,[1] Yajiao Li,[2] Yucheng Chen,[2] Hui Wang,[2] Shixu He,[1] Wanlin Lai,[1] Anjiao Peng,[1] Mingming Ning,[3] Lei Chen [1]

[1]Department of Neurology, West China Hospital, Sichuan University, Chengdu, China

[2]Department of Cardiology, West China Hospital, Sichuan University, Chengdu, China

[3]Department of Neurology, Cardio-Neurology Clinic, Massachusetts General Hospital, Harvard Medical School, Boston, USA

Email for each author: Lin Zhang (zhanglinneur@163.com), Xi Zhu (zhu61900@163.com), Xiangmiao Qiu (qiuxmiao@126.com), Yajiao Li (liyajiao800@163.com), Yucheng Chen (chenyucheng2003@126.com), Hui Wang (dashu1985723@163.com), Shixu He (274464077@qq.com), Wanlin Lai (laiwl93@163.com), Anjiao Peng (peng_neurology@163.com), Mingming Ning (Ning@hms.harvard.edu), Lei Chen (leilei_25@126.com)

Key words：right-to-left shunt; epilepsy; migraine

Number of words (main text): 2644

Corresponding to: Prof Lei Chen, Department of Neurology, West China Hospital, Sichuan University, No. 37 Guoxue Road, Chengdu, Sichuan Province, 610041, China. Tel: 13258178634.E-mail: leilei_25@126.com

ABSTRACT

Objectives To investigate the relationship between right-to-left shunt and migraine to account for the unexplained high prevalence of migraine in patients with epilepsy.

Design This is a cross-sectional study. The diagnosis and interview process of patients with migraine was based on the International Classification of Headache Disorders-3 beta in patients with epilepsy. Participants underwent transthoracic echocardiography (TTE) with contrast medium to identify right-to-left shunt. The highest number of microbubbles were recorded in the left atrium before the complete microbubble outflow of the right atrium. A moderate-to-large shunt was defined as the presence of 10 or more microbubbles.

Setting A single-centre, cross-sectional study in China, 2015-2017.

Participants Patients with epilepsy.

Primary and secondary outcome measures The primary outcome measures were the prevalence of migraine; the prevalence of right-to-left shunt in patients with migraine and those without migraine; and the prevalence of migraine in different degrees of shunting.

Results Three hundred thirty-nine participants with epilepsy who completed TTE were included in the analysis. The overall prevalence of migraine was 23.0%. One third of the migraineurs had mild right-to-left shunt and one fifth of the migraineurs had moderate-to-large right-to-left shunt. Patients with mild shunt did not have a higher prevalence of migraine than those without shunt (26.3% vs. 18.1%, p = 0.102); however, a higher prevalence of migraine was found in patients with moderate-to-large shunt (39.0% vs. 18.1%, odds ratio = 2.90, 95% confidence interval = 1.41 to 5.98, p = 0.003). Patients with migraine and patients without migraine had similar prevalence of mild shunt; however, patients with migraine had more

moderate-to-large shunt (20.5% vs. 9.6%, p=0.002). Right-to-left shunt and female were factors predicting migraine prevalence.

Conclusions One fifth of migraineurs were correlated with moderate-to-large right-to-left shunt, which could be an underlying cause of migraine in epilepsy.

Strengths and limitations of this study

►Cohort study with data from a large number of patients with epilepsy.

►We first studied the relationship between the degree of shunt and migraine in patients with epilepsy.

►Transthoracic echocardiography with contrast medium was used as a tool to detect right-to-left shunt but not transoesophageal echocardiography.

INRODUCTION

The comorbidity of migraine is common in patients with epilepsy. The lifetime occurrence of migraine was as high as 33.6% in patients with epilepsy, representing an overall 52% increase relative to people without epilepsy.[1 2] Comorbid migraine may have an unfavourable effect on the prognosis of epilepsy in children and adults.[3 4] Evidence of genetics and animal experiments have suggested similar pathophysiological mechanisms between migraine and familial epilepsy, such as genetic variants and abnormal neuronal excitability.[5-9] Despite these findings, the mechanisms underlying migraine in patients with epilepsy are still controversial.

In the past 2 decades, right-to-left shunt, usually due to patent foramen ovale (PFO) and pulmonary arteriovenous shunt, have been found to be associated with migraine. A three-fold higher migraine with aura was observed in patients with right-to-left shunt compared to people without right-to-left shunt.[10] There are clinical trials showing that treatment for right-to-left shunt can significantly reduce headache days although the responder rate defined as a 50% reduction in migraine attacks is not different from baseline.[11-14] In addition, the meta-analyses showed that a complete resolution of migraine after treatment for right-to-left shunt occurred in 54% of migraine-with-aura cases and in 39% of migraine-without-aura cases.[15 16] Therefore, right-to-left shunt may be a fundamental cause of migraine. One of the possible mechanisms between the 2 conditions is that vasoactive chemicals triggering migraine bypass the pulmonary filter.[5] Right-to-left shunt may carry microemboli into the carotid circulation. Animal experiments showed that microemboli can trigger the aura phenomenon by inducing cortical spreading depression (CSD).[7]

Similarly, right-to-left shunt may also induce migraine by vasoactive chemicals and microemboli in patients with epilepsy. Therefore, our main aim was to investigate

the relationship between right-to-left shunt and migraine to account for the unexplained high prevalence of migraine in epilepsy. We also investigated other migraine predictive factors, for example, sex, age, familial epilepsy, duration of epilepsy and antiepileptic drugs (AEDs).

METHODS

Study design and patients

This is a cross-sectional study. Study populations were consecutively recruited between June 2015 and December 2017 from the Department of Neurology, West China Hospital, Sichuan University, China. Participants or legal guardians signed informed consent. The inclusion criteria were as follows: (1) patients with epilepsy or possible epilepsy (patients with possible epilepsy will be followed to establish the diagnosis of epilepsy); and (2) the clinical history and medical records of epilepsy can be obtained. The exclusion criteria were as follows: (1) age <3 years; (2) inaccurate communication because of language problems; (3) patients with one seizure (because high recurrence risk factors are controversial) and provoked seizures; (4) the diagnosis of epilepsy was still undetermined when we performed the data analysis; (5) the clinical history of epilepsy including onset age of seizure, AEDs, familial history of epilepsy, seizure frequency in the last year, and types of seizure were not recorded; (6) history of heart disease except right-to-left shunt; (7) right-to-left shunt confirmed by transthoracic echocardiography (TTE) at another hospital (because there may be differences between sonographers); (8) informed consent not signed; and (9) patients did not complete the interview process for the diagnosis of migraine.

Definitions and collection of data

A definitive diagnosis and classification of epilepsy met the criteria proposed by the International League Against Epilepsy (ILAE) based on the clinical history, seizure semiology, electroencephalography (EEG), and magnetic resonance imaging (MRI) findings.[17] For each patient, age, symptoms of seizure, onset age of seizure, AEDs, education status, seizure onset with visual symptoms, history of febrile seizure, seizure frequency in the last year, familial history of epilepsy, and types of seizure (focal onset, focal to bilateral tonic-clonic seizures, or generalized onset) were recorded.

The diagnosis and interview process for patients with migraine were based on the International Classification of Headache Disorders (ICHD-3 beta).[18] The number of migraine attacks, estimated duration of migraine, location, quality, intensity (visual analogue scale, 0-10), and aura were recorded. Patients would not be classified as having migraine if only postictal and ictal migrainous headache occurred because ICHD-3 beta does not have related criteria. Every patient was diagnosed by 2 neurologists separately. If the diagnosis of epilepsy and migraine was inconsistent, a third senior neurologist was asked to discuss the case further.

For children, their parents (or the children themselves if they were old enough) were asked to give specific information about epilepsy and migraine. Medical records and seizure diaries during the last 12 months were reviewed to obtain information regarding the history of epilepsy and migraine, EEG, and MRI.

Transthoracic Echocardiography

Participants underwent transthoracic echocardiography (TTE) with contrast medium and 1-5 MHz or 3-8MHz multiplane transducers in Philips IE33 to identify right-to-left shunt; this was performed by 2 experienced sonographers who also

analysed the videotapes together. Patients with other cardiac diseases (e.g., valvular heart disease and cardiomyopathy) found by TTE were excluded. A microbubble bolus of agitated solution of 9 mL saline and 1 mL air was injected into one side of the patients' antecubital veins for increased sensitivity.[19 20] Patients were assessed for right-to-left shunt at rest and during the Valsalva manoeuvre and coughing. The highest number of microbubbles were recorded in the left atrium before the complete microbubble outflow of the right atrium. If 3 or more microbubbles appeared, a right-to-left shunt was diagnosed. The degree of shunting was defined as grade 0 if 0 to 2 microbubbles occurred, grade 1 (mild) if 3 to 9 microbubbles occurred, grade 2 (moderate) if 10 to 30 microbubbles occurred and grade 3 (large) if more than 30 microbubbles occurred.[21]

Data analysis

Data were collected using the same standard forms in Microsoft Excel (version 2013). Continuous variables were compared by analysis of variance (ANOVA). Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Bonferroni correction was used if necessary. Logistic regression analyses were used to determine whether some variables were migraine-predicting factors. For all statistical tests, $p<0.05$ was determined to be statistically significant. Statistics were performed using the SPSS statistical package (version 21.0).

Patient and public involvement

The development of the research question and outcome measures were informed by published literature on PFO and migraine. Patients' priorities, experience and preferences were not involved in designing the study. Patients who agreed to enrol to

the study received TTE. Two authors (Lin Zhang and Xi Zhu) disseminated the results to the study participants by interviews.

RESULTS

We carefully interviewed 532 patients with seizure (s) or (and) their parents. One hundred sixty-one patients were excluded. The reasons for exclusion are shown in figure 1. Three hundred thirty-nine participants with epilepsy who completed the interview process of migraine and TTE were included in the analysis. The details of migraine features according to the ICHD-3 beta criteria are shown in Supplementary file 1. The overall prevalence of migraine was 23.0% (table 1). A greater percentage of females had migraines than males (28.8% vs. 17.2%, p = 0.022). Patients > 16 years seemed to have a higher prevalence of migraine than those ≤ 16 years of age (25.7% vs. 11.0%, p = 0.039). If the duration of epilepsy was more than 10 years, patients were more likely to have migraines (34.6% vs. 17.9%, p = 0.001). Patients with polytherapy may have more migraines (28.0% vs. 19.0%, p = 0.052). Patients with first-degree and second-degree relatives with epilepsy, as well as a history of febrile seizures, may not have more migraines. Patients who have visual symptoms at the onset of seizures did not have more migraines than those without visual symptoms (data not shown).

One third of migraineurs have mild right-to-left shunt (table 1 and table 2). Patients with mild right-to-left shunt did not have a higher prevalence of migraine than those without right-to-left shunt (26.3% vs 18.1%, odds ratio = 1.61, 95% confidence interval = 0.91 to 2.87, p = 0.102). In our cohort, 33.3% (26 of 78) of patients with epilepsy and with migraine and 28.0% (73 of 261) of patients with epilepsy and without migraine had mild right-to-left shunt (p = 0.361).

One fifth of migraineurs have moderate-to-large right-to-left shunt (table 1 and table 2). Patients with moderate-to-large right-to-left shunt had a higher prevalence of migraine than those without right-to-left shunt (39.0% vs. 18.1%, odds ratio = 2.90, 95% confidence interval = 1.41 to 5.98, p = 0.003). Twenty-one percent (16 of 78) of patients with epilepsy and with migraine had moderate-to-large right-to-left shunt; whereas only 10% (25 of 261) of patients with epilepsy and without migraine had moderate-to-large right-to-left shunt (odds ratio = 2.44, 95% confidence interval = 1.23 to 4.84, p=0.002). After controlling for potential confounders, there are significant differences of migraine prevalence between the groups with right-to-left shunt grade 2-3 versus the group with right-to-left shunt grade 0 (table 2). Therefore, moderate-to-large right-to-left shunt but not mild right-to-left shunt was associated with migraine in patients with epilepsy.

We used a logistic regression analysis to identify the possible factors predicting migraine prevalence, such as gender, duration of epilepsy, current age, right-to-left shunt and number of antiepileptic drugs (table 3). Right-to-left shunt and female were factors predicting migraine prevalence. Although patients with duration of epilepsy >10 years and older age have a higher migraine prevalence (table 1), the logistic regression analysis showed no significant statistical difference.

PFO closure were performed in 7 patients. Four patients were followed for 6 months. We found that PFO closure fully controlled migraine in 2 of 4 cases. Before PFO closure, the two patients have more than 11 migraine attacks in the last year. More interesting, the frequency of seizures in 3 patients with migraine after PFO closure decreased significantly.

DISCUSSION

This is the first study to evaluate the relationship between migraine and right-to-left shunt in a large number of patients with epilepsy. Our main findings were as follows: 1) one third of the migraineurs have mild right-to-left shunt and one fifth of the migraineurs have moderate-to-large right-to-left shunt; 2) patients with mild right-to-left shunt did not have a higher prevalence of migraine than those without right-to-left shunt; 3) moderate-to-large right-to-left shunt was associated with migraine in patients with epilepsy and 4) right-to-left shunt and female were factors predicting migraine prevalence in patients with epilepsy.

Migraine in patients without epilepsy has been proved to be associated with CSD and activation of the trigeminovascular system and its constituent neuropeptides.[22] In addition, recent studies also suggested the cortical excitatory/inhibitory imbalance due to neuronal and glial ion channels renders patients more vulnerable to an headache attack.[22] Migraine in patients with epilepsy is not only associated with the aforementioned mechanisms, but also is usually considered to be associated with abnormal neuronal excitability induced by genetic variants.[5-9] However, the high prevalence of migraine in patients with epilepsy cannot be fully explained by genetic variants. In the past 2 decades, right-to-left shunt have been found to be associated with migraine in patients without epilepsy. Our major hypothesis was that right-to-left shunt may also be one of the pathophysiological mechanisms of migraine in patients with epilepsy. The results showed that mild right-to-left shunt was not associated with migraine in patients with epilepsy. Almost one fifth of migraine were correlated with moderate-to-large right-to-left shunt, which could be an underlying cause of migraine in epilepsy. The mechanisms of the relationship between moderate-to-large right-to-left shunt and migraine remains speculative. A previous study showed that larger right-to-left shunt tend to trigger migraine attacks because of the increased

extent of the shunt.[23] Larger shunt allow harmful circulatory factors, such as vasoactive substances and microemboli which are eliminated by lung, to bypass the pulmonary filter and travel directly from the venous to the cerebral artery. Right-to-left shunt and higher rates of Doppler-detected cerebral microemboli have been reported to be associated.[24] In an animal experiment, air microemboli are able to trigger CSD and migraine aura often with transient microvascular occlusion that is insufficient to cause a permanent ischemic lesion.[7] In humans, diagnostic microbubble injection also induced attacks of migraine with aura.[25] Therefore, microemboli may be a key role linking CSD, migraine, and moderate-to-large shunt.

Interestingly, CSD increases neuronal excitability and triggers ictaform activity in neuronal tissues with partial impairment of inhibitory.[9] CSD also triggers migraine headache by activating neuronal Panx1 channels, which contributes to neuronal hyperactivity in seizures.[26 27] These findings may explain why migraine may have an unfavourable effect on the prognosis of epilepsy.[3 4] Therefore, moderate-to-large right-to-left shunt may not only be an underlying cause of migraine but also an adverse prognostic factor in patients with epilepsy. Whether moderate-to-large right-to-left shunt is an adverse prognostic factors in epilepsy should also be investigated.

The effect of PFO closure for migraine treatment still remains controversial. Randomised controlled trials (RCTs) looking at the effect of PFO closure on migraine were initially conducted. There are clinical trials showing that treatment for right-to-left shunt did not increase responder rate defined as 50% reduction in migraine attacks; however, it significantly reduced headache days.[11-14] Similarly to the stroke trials, they had initial negative RCTs. With better patient selection and longer follow-up, subsequent trials may show more cessation of migraine headache.[28] In our

cohort, we found that PFO closure fully controlled migraine in 2 of 4 cases. More interesting, the frequency of seizures in 3 patients with migraine after surgery decreased significantly. Although these cases need to be explained with caution, it is worth investigating in the future whether PFO closure improve migraine attacks and seizure outcomes. In addition, given the subjective nature of migraines and their vulnerability to bias and the placebo effect, perhaps a large, well-designed sham controlled trial with modern devices is required. If treatment for right-to-left shunt have been shown to be definitely effective, right-to-left shunt should be screened in patients with epilepsy and with migraine.

This study has some limitations. First, transoesophageal echocardiography (TEE) is a gold standard diagnostic tool with a sensitivity and specificity of 80% to 100% for the detection of a right-to-left shunt.[19 20] The contrast TTE used by us has a lower sensitivity than TEE.[19] However, harmonic imaging improves sensitivity for the detection of right-to-left shunt, ranging from 66% to 100%.[19 20 29 30] In addition, TTE is not inferior to TEE in the assessment of large shunt because TEE may miss large shunt detected by TTE.[30] In our study, moderate-to-large right-to-left shunt are more likely to be associated with migraine; therefore, TTE may not be an inferior choice to TEE. Second, the population may be biased because a few patients did not complete TTE. However, there were no differences in demographic characteristics between patients who did or did not refuse TTE and the conclusions of our study should not be affected. Third, only Chinese people were recruited, and this limits the generalizability of results.

CONCLUSIONS

Moderate-to-large right-to-left shunt but not mild right-to-left shunt were associated

with migraine. On the basis of these results, patients with epilepsy and with migraine should be screened for a right-to-left shunt if treatment for a right-to-left shunt has been shown to be effective in migraine. Whether moderate-to-large right-to-left shunt is an adverse prognostic factor in epilepsy should be also investigated.

Acknowledgements No patient advisers were involved in the study. We thank Dr. Qi Si for her help.

Contributors Lei Chen and Mingming Ning conceived and designed the work. Lin Zhang and Xi Zhu were involved in data collection, data analysis and interpretation. Lin Zhang drafted the manuscript. Lei Chen involved in critical revision of the article and final approval of the version to be published. Xiangmiao Qiu, Xiangmiao Qiu, Shixu He, Anjiao Peng, Wanlin Lai, Yajiao Li, Yucheng Chen, and Hui Wang were involved in the data collection and final approval of the version to be published. All authors have agreed to be accountable for all aspects of the work.

Funding Funding for this study was provided by China Association Against Epilepsy (No.2016005).

Competing interests None declared.

Patient consent Obtained.

Ethics approval The protocol was approved by the Ethics Committee of the West China Hospital of Sichuan University.

Data sharing statement No additional data available.

REFERENCES

1. Keezer MR, Bauer PR, Ferrari MD*, et al.* The comorbid relationship between migraine and epilepsy: a systematic review and meta-analysis. *Eur J Neurol* 2015;22:1038-47.
2. Le H, Tfelt-Hansen P, Russell MB*, et al.* Co-morbidity of migraine with somatic disease in a large population-based study. *Cephalalgia* 2011;31:43-64.
3. Dragoumi P, Tzetzi O, Vargiami E*, et al.* Clinical course and seizure outcome of idiopathic childhood epilepsy: determinants of early and long-term prognosis. *BMC Neurol* 2013;13:206.
4. Velioglu SK, Boz C, Ozmenoglu M. The impact of migraine on epilepsy: a prospective prognosis study. *Cephalalgia* 2005;25:528-35.
5. Finocchi C, Del Sette M. Migraine with aura and patent foramen ovale: myth or reality? *Neurol Sci* 2015;36 Suppl 1:61-6.
6. Ludvigsson P, Hesdorffer D, Olafsson E*, et al.* Migraine with aura is a risk factor for unprovoked seizures in children. *Ann Neurol* 2006;59:210-3.
7. Nozari A, Dilekoz E, Sukhotinsky I*, et al.* Microemboli may link spreading depression, migraine aura, and patent foramen ovale. *Ann Neurol* 2010;67:221-9.
8. Winawer MR, Connors R. Evidence for a shared genetic susceptibility to migraine and epilepsy. *Epilepsia* 2013;54:288-95.

9. Eickhoff M, Kovac S, Shahabi P, *et al.* Spreading depression triggers ictaform activity in partially disinhibited neuronal tissues. *Exp Neurol* 2014;253:1-15.

10. Wammes-van der Heijden EA, Tijssen CC, Egberts AC. Right-to-left shunt and migraine: the strength of the relationship. *Cephalalgia* 2006;26:208-13.

11. Xing YQ, Guo YZ, Gao YS, *et al.* Effectiveness and Safety of Transcatheter Patent Foramen Ovale Closure for Migraine (EASTFORM) Trial. *Sci Rep* 2016;6:39081.

12. Tobis JM, Charles A, Silberstein SD, *et al.* Percutaneous Closure of Patent Foramen Ovale in Patients With Migraine: The PREMIUM Trial. *J Am Coll Cardiol* 2017;70:2766-74.

13. Dowson A, Mullen MJ, Peatfield R, *et al.* Migraine Intervention With STARFlex Technology (MIST) trial: a prospective, multicenter, double-blind, sham-controlled trial to evaluate the effectiveness of patent foramen ovale closure with STARFlex septal repair implant to resolve refractory migraine headache. *Circulation* 2008;117:1397-404.

14. Mattle HP, Evers S, Hildick-Smith D, *et al.* Percutaneous closure of patent foramen ovale in migraine with aura, a randomized controlled trial. *Eur Heart J* 2016;37:2029-36.

15. Kanwar SM, Noheria A, DeSimone CV, *et al.* Coincidental Impact of Transcatheter Patent Foramen Ovale Closure on Migraine with and without Aura - A Comprehensive Meta-Analysis. *Clin Trials Regul Sci Cardiol* 2016;15:7-13.

16. Shi YJ, Lv J, Han XT, *et al.* Migraine and percutaneous patent foramen ovale closure: a systematic review and meta-analysis. *BMC Cardiovasc Disord* 2017;17:203.

17. Fisher RS, Cross JH, French JA, *et al.* Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017;58:522-30.

18. Ettlin DA. The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia* 2013;33:629-808.

19. Cotter PE, Martin PJ, Belham M. Improved sensitivity of transthoracic contrast echocardiography in the detection of right-to-left shunts. *J Am Soc Echocardiogr* 2010;23:578; author reply 78-9.

20. Ha JW, Shin MS, Kang S, *et al.* Enhanced detection of right-to-left shunt through patent foramen ovale by transthoracic contrast echocardiography using harmonic imaging. *Am J Cardiol* 2001;87:669-71.

21. Mas JL, Arquizan C, Lamy C, *et al.* Recurrent cerebrovascular events associated with patent foramen ovale, atrial septal aneurysm, or both. *N Engl J Med* 2001;345:1740-6.

22. Pietrobon D, Moskowitz MA. Pathophysiology of migraine. *Annu Rev Physiol* 2013;75:365-91.

23. Tembl J, Lago A, Sevilla T, *et al.* Migraine, patent foramen ovale and migraine triggers. *J Headache Pain* 2007;8:7-12.

24. Rodriguez RA, Sinclair B, Weatherdon D, *et al.* Patent foramen ovale and brain microembolization during scoliosis surgery in adolescents. *Spine (Phila Pa 1976)* 2001;26:1719-21.

25. Dinia L, Roccatagliata L, Bonzano L, *et al.* Diffusion MRI during migraine with aura attack associated with diagnostic microbubbles injection in subjects with large PFO. *Headache* 2007;47:1455-6.

26. Karatas H, Erdener SE, Gursoy-Ozdemir Y, *et al.* Spreading depression triggers headache by activating neuronal Panx1 channels. *Science* 2013;339:1092-5.

27. Santiago MF, Veliskova J, Patel NK, *et al.* Targeting pannexin1 improves seizure outcome. *PLoS One* 2011;6:e25178.

28. Mojadidi MK, Zaman MO, Elgendy IY, *et al.* Cryptogenic Stroke and Patent Foramen Ovale. *J Am Coll Cardiol* 2018;71:1035-43.

29. Clarke NR, Timperley J, Kelion AD, *et al.* Transthoracic echocardiography using second harmonic imaging with Valsalva manoeuvre for the detection of right to left shunts. *Eur J Echocardiogr* 2004;5:176-81.

30. Daniels C, Weytjens C, Cosyns B, *et al.* Second harmonic transthoracic echocardiography: the new reference screening method for the detection of patent foramen ovale. *Eur J Echocardiogr* 2004;5:449-52.

Table 1 Migraine prevalence in different groups

Characteristics	Sample	Migraine (%)	P values
Gender			0.022
Male	169	29 (17.2)	
Female	170	49 (28.8)	
Current age, years			0.039
≤16	82	12 (11.0)	
>16	257	66 (25.7)	
Duration of epilepsy, years			0.001
≤10	235	42 (17.9)	
>10	104	36 (34.6)	
Onset age, years			0.421
≤16	183	39 (21.3)	
>16	156	39 (25.0)	
Seizure frequency in the last year			0.256
0-12	246	56 (22.8)	
13-52	33	11 (33.3)	
>52	28	7 (25.0)	
Unknown	32	4 (12.5)	
Number of AEDs			0.052
< or = 1	189	36 (19.0)	
> or = 2	150	42 (28.0)	
AEDs			0.299
Topiramate	66	20 (30.3)	
Valproate	126	29 (23.0)	

Levetiracetam	94	26 (27.7)	
Oxcarbazepine	94	19 (20.2)	
Lamotrigine	27	2 (7.4)	
Carbamazepine	67	19 (28.4)	
Phenytoin	20	4 (20.0)	
Phenobarbital	12	2 (16.7)	
Seizure types			0.319
Focal onset seizure	68	10 (14.7)	
Generalized onset seizure and focal to bilateral tonic-clonic seizures	227	58 (25.6)	
Undetermined	44	10 (22.7)	
Familial epilepsy	11	5 (45.5)	0.135
First-degree relatives	9	4 (44.4)	
Second-degree relatives	2	1 (50.0)	
No familial epilepsy	328	73 (22.3)	
Right-to-left shunt			
Grade 1	99	26 (26.3)	0.102
Grade 2-3	41	16 (39.0)	0.003
Grade 1-3	140	42 (29.6)	0.010
Grade 0	199	36 (18.1)	

AEDs, antiepileptic drugs.

Table 2 High prevalence of migraine in patients with moderate to large right-to-left shunt

Characteristics	Right-to-left shunt			P
	Grade 2-3 (n=41)	Grade 1 (n=99)	Grade 0 (n=199)	
Male, n (%)	17 (41.5)	52 (52.5)	100 (50.3)	0.413
Age, years	26.9 ± 11.7	27.6 ± 13.7	26.4 ± 13.2	0.769
Onset age, years	19.3 ± 11.2	17.9 ± 12.4	17.8 ± 12.3	0.779
Duration, years	8.1 ± 8.9	9.7 ± 9.6	8.7 ± 9.1	0.580
Familial epilepsy, n (%)	2	2	7	0.648
Education, n (%)				0.772
0-6, years	13 (31.7)	24 (24.2)	52 (26.1)	
7-12, years	20 (48.8)	47 (47.5)	104 (52.3)	
≥13, years	8 (19.5)	28 (28.3)	43 (21.6)	
Seizure types, n (%)				0.085
Focal onset	7 (17.1)	22 (22.2)	39 (19.6)	
Generalized onset seizure and focal to bilateral tonic-clonic seizures	32 (78.0)	69 (69.7)	126 (63.3)	
Undetermined	2 (4.9)	8 (8.1)	34 (17.1)	
Seizure frequency in the last year, n (%)				0.284
0-12	27 (65.9)	71 (71.7)	148 (71.4)	
13-52	3 (7.3)	14 (14.1)	16 (8.0)	
>52	6 (14.6)	8 (8.1)	14 (7.0)	

Unknown	5 (12.2)	6 (6.1)	21 (14.1)	
Number of used AEDs				0.139
< or = 1, n (%)	25 (61.0)	47 (47.5)	117 (58.8)	
> or = 2, n (%)	16 (39.0)	52 (52.5)	82 (41.2)	
AEDs				0.735
Topiramate	10	21	35	
Valproate	8	45	74	
Levetiracetam	15	31	48	
Oxcarbazepine	12	34	48	
Lamotrigine	4	8	15	
Carbamazepine	8	28	31	
Phenytoin	1	6	13	
Phenobarbital	1	4	7	
Migraine, n (%)	16 (39.0)	26 (26.3)	36 (18.1)	0.010 [a]

The continuous data are presented as mean ± standard deviation.

[a] Bonferroni correction show statistical differences in grade 2-3 versus grade 0.

AEDs, antiepileptic drugs.

Table 3 Logistic regression analysis for migraine predictors

Characteristics	Odds ratio	95% confidence interval		P
		Lower	Upper	
Male	0.518	0.302	0.891	0.017
Current age, years	0.978	0.956	1.001	0.057
Right-to-left shunt	2.200	1.274	3.797	0.005
Duration of epilepsy, years	0.974	0.945	1.004	0.085
Familial epilepsy	2.828	0.765	10.453	0.119
Number of antiepileptic drugs > 1	1.354	0.784	2.340	0.277

Figure legends

Figure 1. The flow diagram

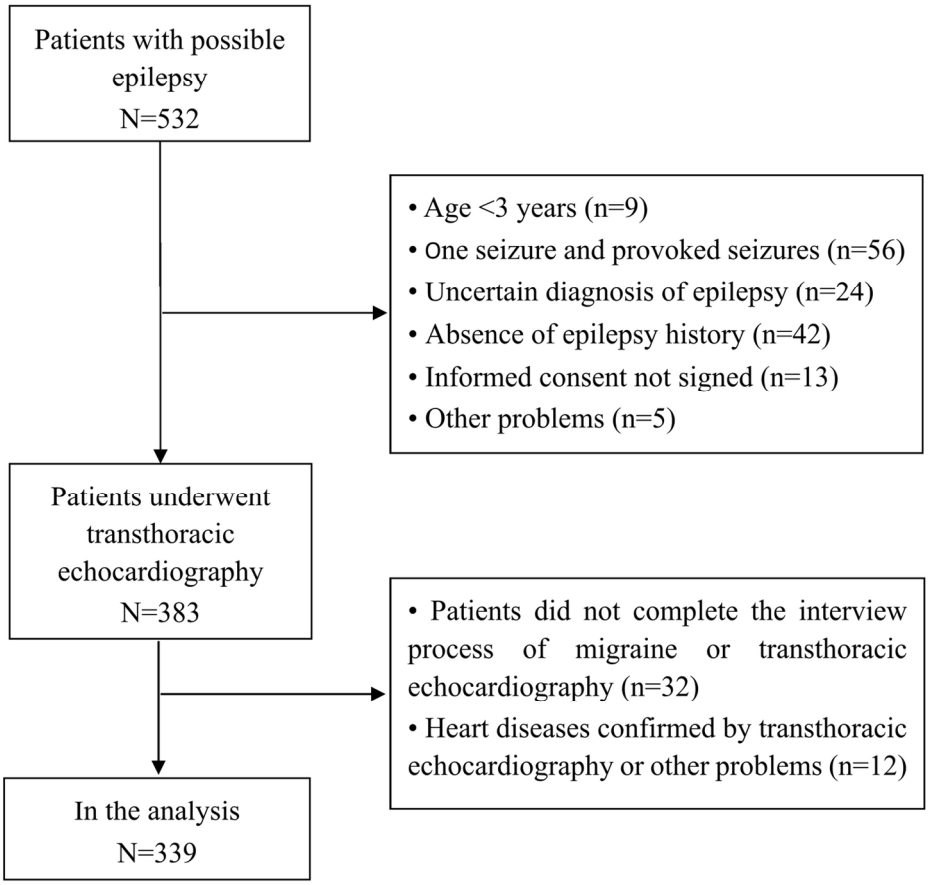


Figure 1. The flow diagram

136x127mm (300 x 300 DPI)

sTable 1 Characteristics of migraine

Characteristics	N=78
Criteria	
Duration >2 hours (<18 years) or > 4 hours (≥ 18 years)[1]	78
At least 5 (without aura) attacks or 2 (with aura) attacks[1]	78
Headache has at least two of the following four characteristics	78
Location	
Left or Right[1]	46
Bilateral	32
Intensity[1,2]	
0-3	17
4-10[1]	61
Pulsating quality[1]	53
Aggravation by or causing avoidance of routine physical activity[1]	51
During headache at least one of the following	78
Nausea and/or vomiting[1]	62
Photophobia and Phonophbia[1]	55

[1]Diagnostic criteria of International Classification of Headache Disorders–3 criteria (beta)

[2]Visual analogue scale scores

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6
Bias	9	Describe any efforts to address potential sources of bias	*6,11*
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6,7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7,8
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7,8
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7,8
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9,10,11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.