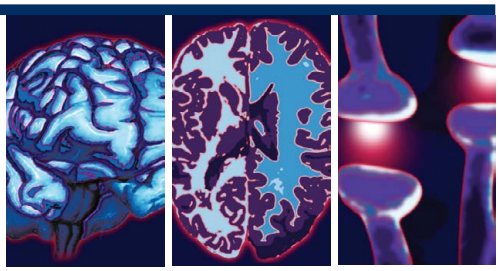

Is Cerebrospinal Fluid Fibroblast Growth Factor 19 (FGF19) a mood regulator?

Yanlong Liu[1,2,*], Dongsheng Yu[3,4,*], Xiaofang Wang[1], Xiaohua Tan[1], Xianming Luo[1], Hongzhen Fan[5], Yimin Kang[4], Jian Xiao[1], Xiaokun Li[†,1,2],Fan Wang[†,5,6]

ABSTRACT

Fibroblast growth factor 19 (FGF19) is a type of gut-derived postprandial hormone. FGF19 plays a key role in coordinating liver bile acid biosynthesis and gallbladder motility and acts as a regulator of metabolic homeostasis, including strengthening insulin sensitivity, decreasing triglyceride concentrations, and reducing body weight. In the present study, we investigated the presence of FGF19 in human cerebrospinal fluid (CSF) and investigated relationships with a cluster of characteristic emotions and behaviors. Eighty-three male subjects were recruited and participated in the study. The levels of CSF FGF19 were assayed by enzyme-linked immunosorbent assay, and FGF19 levels showed considerable interindividual variations, ranging from 80.54 to 1479.78 pg/ml. Pearson correlation analysis revealed no correlation between CSF FGF19 levels and age or Body Mass Index. Significant correlations were found between CSF FGF19 levels and Pittsburgh Sleep Quality Index scores, Suicide Attitude Questionnaire factors, Barratt Impulsiveness Scale scores, and Beck Depression Inventory scores, and CSF FGF19 levels independently affected Beck Depression Inventory scores. Our findings provide evidence of the presence of FGF19 in human CSF and the role of FGF19 in mood regulation in humans.

Keywords

FGF19, Cerebrospinal fluid, BDI, PSQI, BIS, SAS

Abbreviations

FGF19: Fibroblast growth factor 19, CSF: Cerebrospinal Fluid, BMI: Body Mass Index

Introduction

Fibroblast growth factors (FGFs) represent a large family of genes that encode proteins that are involved in cell growth and differentiation, embryonic development, angiogenesis, and wound healing [1]. FGF19, FGF21, and FGF23 are members of a gene subfamily with unique properties that are attributable to their structural resemblance and presumed "hormone-like" actions [2,3]. FGF19 is a kind of gut-derived postprandial hormone. As an atypical member of the FGF family, FGF19 functions as an endocrine hormone except regulating cell

[1]College of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou 325035, China

[2]Collaborative Innovation Center of Biomedicine, Wenzhou University, Wenzhou 325035, China

[3]Inner Mongolia Region Mental Health Center, Huhhot 010110, China

[4]Psychosomatic Medicine Research Division, Inner Mongolia Medical University, Huhhot 010110, China

[5]Beijing Hui-Long-Guan Hospital, Peking University, Beijing 100096, China

[6]Second Affiliated Hospital of Xinjiang Medical University, Urumqi 830028, China

*These authors contributed equally to this work.

[†]Author for correspondence: Fan Wang, M.D., Ph.D., Beijing Hui-Long-Guan Hospital, Peking University, Beijing, China. Tel: 86 10 62715511 ext. 6429; Fax: 86 10 62716285, email: FanWang@bjmu.edu.cn

Xiaokun Li, Ph.D., College of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou 325035, China. Tel: 86 10 62715511 ext. 6429; Fax: 86 10 62716285, email: xiaokunli@163.com

growth and differentiation. FGF19 plays a key role in coordinating liver bile acid biosynthesis and gallbladder motility and acts as a regulator of metabolic homeostasis, including strengthening insulin sensitivity, decreasing triglyceride concentrations, and reducing body weight. FGF19 is also related to coronary artery disease and renal function [4,5].

Of relevance to neuropsychiatry, the FGF system is critical for the induction of serotonergic and dopaminergic neurons and patterning of the cortex. The FGF system is also involved in hippocampal neurogenesis and might play a role in the effect of antidepressants [6,7]. Furthermore, recent evidence suggests that the FGF system is dysregulated in major depression. The expression of several FGF family transcripts have been shown to be altered in subjects with major depression, and these alterations are not attributable to antidepressant treatment [8].

FGF19 was originally identified in the human fetal brain, indicating that it plays an important role in brain development during embryogenesis [9]. Recent studies found that FGF21, one member of the family of endocrine FGFs, is detectable in human cerebrospinal fluid (CSF) [10]. Animal studies have shown that FGF21 may cross the blood-brain barrier (BBB) in mice and is a key mediator of the effects of mood stabilizers [11,12]. The existence of FGF19 in CSF and its potential role in the central nervous system remain unknown. In the present study, we measured the concentration of FGF19 in human CSF and investigated relationships with a cluster of characteristic emotions and behaviors.

Methods

■ Participant selection and sample collection

The subjects were originally recruited from several Chinese hospitals as a control group for a study that focused on nicotine dependence. Subjects, who were scheduled to undergo surgery for lower extremity injuries due to ligament damage without trauma below the knee without any medication, were recruited to extract CSF samples before spinal anesthesia. The study was approved by the Human Ethics Committee of Inner Mongolia Medical University. For each subject, written informed consent was obtained either directly from the participants or from their responsible guardians. Samples that were obtained during that study were reanalyzed in the present study. All of the subjects were male Chinese without a history of drug dependence or abuse (including alcohol and nicotine) according to self-report and confirmed by their next of kin. Subjects were excluded if they had a family history of psychiatric disorders or neurological diseases. Individuals with medical or other systemic or central nervous system diseases were also excluded. Each CSF sample was obtained in 0.5 ml fractions in polypropylene tubes at -80°C. The quantification of CSF FGF19 levels was performed using a commercial enzyme-linked immunosorbent assay (ELISA) measurement kit (Cloud-Clone, Houston, TX, USA) according to the manufacturer's instructions. Ten percent of each CSF sample was assayed in duplicate. The following scales were used in the present study: Pittsburgh Sleep Quality Index (PSQI), Suicide Attitude Questionnaire (SAQ), Barratt Impulsiveness Scale, version 11 (BIS11), Beck Depression Inventory (BDI-11), and Self-Rating Anxiety Scale (SAS). All of the scales were completed by the subjects by self-report one day before CSF extraction.

■ Statistical analysis

The results are expressed as mean ± standard deviation. Statistical correlation analysis was performed using parametric methods for continuous variables. Mediation analysis is one approach that is used to analyze a hypothesized causal chain, in which one variable influences a second variable that, in turn, affects a third variable. The second variable, M, is the mediator. It "mediates" the relationship between an independent variable, X, and an outcome, Y. Mediation analysis may disentangle direct effects that are part of an exposure effect, and indirect/mediated effects are part of an exposure effect that is mediated by a given cluster of potential mediators. In the present study, FGF19 level was identified as an independent variable. The PSQI total score, PSQI Component 7 score, and SAQ subscale F3 score were identified as potential mediators. The BDI score was considered a dependent variable of FGF19 levels. Values of $p < 0.05$ were considered statistically significant. All of the analyses were performed using SPSS 19.0 software, and mediation analysis was performed using the SOBEL test [13].

Results

■ FGF19 in human CSF and association with emotion- and behavior-related scores

FGF19 concentrations in CSF were assessed in samples from 83 male Chinese subjects. FGF19 levels showed considerable interindividual variations, ranging from 80.54 to 1479.78 pg/ml. Pearson correlation analyses revealed no correlation between CSF FGF19 levels and age or Body Mass Index (BMI). Significant correlations were found between CSF FGF19 levels and PSQI scores, suicide factors, BIS scores, and BDI scores (Table 1). We then performed Spearman correlations and found that CSF FGF19 levels were positively correlated with PSQI Component 7 scores (r = 0.331, p = 0.002), PSQI total scores (r = 0.274, p = 0.027), and BDI scores (r = 0.399, p = 0.0003) and negatively correlated with SAQ subscale F3 scores (r = -0.267, p = 0.017), SAQ subscale F4 scores (r = -0.284, p = 0.01), and BIS Plan subscale scores (r =- 0.22, p = 0.047). Correlations between BDI scores and other continuous variables were also found (PSQI Component 2 scores: r = 0.478, p = 0.000; PSQI Component 5 scores: r = 0.377, p = 0.001; PSQI Component 7 scores, r = 0.411, p = 0.000; PSQI total scores, r = 0.297, p = 0.009; SAQ subscale F3 scores: r = -0.349, p = 0.002; SAS scores, r = 0.404, p = 0.000; Table 1).

Table 1: Basic characteristics of all subjects and correlations between CSF FGF19 levels/BDI scores and other continuous variables (n = 83).

Variable	Mean ± SD	R_{FGF19}	p_{FGF19} [$]	R_{BDI}	p_{BDI} [#]
CSF FGF19 level (pg/ml)	244.74 ± 211.17	—	—	—	—
Age (years)	29.56 ± 9.49	0.009	0.933	0.095	0.406
Years of education (years)	13 ± 2.40	0.175	0.121	0.034	0.771
BMI	24.89 ± 4.17	-0.090	0.419	-0.028	0.807
PSQI-Component 1	0.48 ± 0.59	0.082	0.461	0.217	0.055
PSQI-Component 2	0.30 ± 0.56	0.206	0.067	0.478*	0.000*
PSQI-Component 3	0.72 ± 0.74	0.054	0.627	-0.155	0.172
PSQI-Component 4	0.07 ± 0.26	-0.014	0.897	0.100	0.383
PSQI-Component 5	0.36 ± 0.58	0.194	0.084	0.377*	0.001*
PSQI-Component 6	0.04 ± 0.19	-0.094	0.400	0.014	0.906
PSQI-Component 7	0.57 ± 0.75	0.331*	0.002*	0.411*	0.000*
PSQI-Total	2.49 ± 2.36	0.247*	0.027*	0.297*	0.009*
SAQ F1	3.28 ± 0.63	-0.072	0.526	0.054	0.645
SAQ F2	2.82 ± 0.60	0.007	0.948	-0.190	0.103
SAQ F3	2.85 ± 0.69	-0.267*	0.017*	-0.349*	0.002*
SAQ F4	3.19 ± 0.77	-0.284*	0.010*	-0.086	0.452
BIS Plan	25.57 ± 5.00	-0.220*	0.047*	-0.048	0.676
BIS Action	28.51 ± 7.01	-0.152	0.175	-0.076	0.513
BIS Cognition	25.13 ± 4.48	-0.135	0.225	-0.003	0.978
BIS Total	26.50 ± 4.57	-0.211	0.061	-0.051	0.660
BDI	1.30 ± 2.36	0.399*	0.000*	—	—
SAS	33.92 ± 3.65	0.186	0.103	0.404*	0.000*

The data are expressed as mean ± standard deviation. Comparisons between CSF FGF19 levels and continuous variables (sleep and negative emotion-related behavior scores) were made using Spearman correlation. *$p < 0.05$.

[$]Correlation between CSF FGF19 levels and other continuous variables

[#]Correlation between BDI scores and other continuous variables.

■ CSF FGF19 levels affected BDI scores independently

To explore the role of FGF19 in depression, simple mediation models were performed to estimate the effect of CSF FGF19 levels on BDI scores, with PSQI Component 7 scores, PSQI total scores, and SAQ subscale F3 scores set as mediators. These three variables were included because of their correlations with both CSF FGF19 levels and BDI scores. We tested the statistical significance of whether variable M (mediator: PSQI Component 7 scores, PSQI total scores, and SAQ subscale F3 scores) mediated the association between the independent variable, X (CSF FGF19 levels), and dependent variable, Y (BDI scores), using regression equations (Table 2). Whether the total effect of X on Y is significantly reduced upon the addition of a mediator to the model, the output mediation models were interpreted as the following: b(YX) is the total effect of the independent variable X on the dependent variable Y; b(MX) is the effect of the independent variable on the assumed mediator M; b(YM.X) is the effect of the mediator on the dependent variable, excluding the independent variable; b(YX.M) is the direct effect of the independent variable on the dependent variable, except the mediator. The indirect effect of X on Y through M was estimated. This analysis also generated a 95% confidence interval for the size of the indirect effect on the assumption that the sampling distribution of the effect is normal.

In the outputs of mediation, both SAQ subscale F3 scores and CSF FGF19 levels were associated with BDI scores (b[YM.X], t = -2.3891, p = 0.0194; b[YX.M], t = 3.1762, p = 0.0022). SAQ subscale F3 scores did not mediate the effects of CSF FGF19 levels that were associated with BDI scores (Z = 1.667, p = 0.096). CSF FGF19 levels had an effect on SAQ subscale F3 scores (t = -2.5236, p = 0.0137), PSQI total scores, and BDI scores, excluding PSQI total scores (b[MX], t = 2.344, p = 0.0218; b[YX.M], t = 3.3377, p = 0.0013). PSQI Component 7 scores were found to be a mediator when CSF FGF19 levels had

Table 2: The variable M mediated the association between the independent variable, X (CSF FGF19 level), and dependent variable, Y (BDI score).

M	Model	*t*	*p*	Indirect effect				
				Value	Low of 95% CI	Up of 95% CI	Z	*p*
SAQ subscale F3 score	b(YX)	3.8845	0.0002					
	b(MX)	-2.5236	0.0137					
	b(YM.X)	-2.3891	0.0194					
	b(YX.M)	3.1762	0.0022	0.0008	-0.0001	0.002	1.667	0.096
PSQI Component 7 score	b(YX)	3.821	0.0003					
	b(MX)	3.1365	0.0024					
	b(YM.X)	2.946	0.0043					
	b(YX.M)	2.7816	0.0068	0.0012	0.0001	0.0022	2.092	0.037
PSQI total score	b(YX)	3.9044	0.0002					
	b(MX)	2.344	0.0218					
	b(YM.X)	1.8741	0.0649					
	b(YX.M)	3.3377	0.0013	0.0006	-0.0002	0.0014	1.389	0.165

an effect on BDI scores (b[MX], t = 3.1365, p = 0.0024; b[YM.X], t = 2.946, p = 0.0043; Z = 2.092, p = 0.037), indicating that PSQI Component 7 scores may indirectly mediate the effect of CSF FGF19 levels on BDI scores. Additionally, CSF FGF19 levels affected BDI scores independently (b[YX.M], t = 2.7816, p = 0.0068).

Discussion

In the present study, we identified the presence of FGF19 in human CSF and observed a role for FGF19 in the regulation of human emotion and behaviors. FGF19 levels were shown to affect sleep and negative emotion-related behaviors in a group of male Chinese subjects. Correlation analysis showed that CSF FGF19 concentrations were positively correlated with PSQI Component 7 scores, PSQI total scores, and BDI scores and negatively correlated with SAQ subscale F3 scores, SAQ subscale F4 scores, and BIS Plan subscale scores. Regression analysis indicated that CSF FGF19 levels affected SAQ subscale F3 scores, PSQI total scores, and PSQI Component 7 scores independently. PSQI Component 7 scores were found to indirectly mediate the effect of CSF FGF19 levels on BDI scores, whereas SAQ subscale F3 scores and PSQI total scores did not have such an indirect effect.

Consistent with the growing number of FGFs that have been identified, the role of FGFs in the CNS has gained importance, expanding from their classic influence on development, neuronal repair, and neuronal protection to a possible role in learning and neuronal plasticity. During the past decade, accumulating evidence suggests the involvement of FGFs in psychiatric disorders [7]. Previous studies have shown that dysregulation of the FGF system in major depression involves FGF receptors and several FGF ligands [8]. Major depression is associated with a significant reduction of FGF transcript levels (including FGF1 and FGF2) and FGF receptors (FGFR2 and FGFR3) in fronto-cortical areas and upregulation of other FGFs (FGF9 and FGF12) in these brain areas compared with control and bipolar patients [14]. FGF21 is detectable in human CSF and a key mediator of the effects of mood-stabilizing agents [10,12,15]. In the present study, we found that CSF FGF19 levels affected SAQ subscale F3 scores, PSQI total scores, and PSQI Component 7 scores independently, and PSQI Component 7 scores mediated the effect of CSF FGF19 levels on BDI scores in normal Chinese males. FGF19 may regulate mood through signaling at FGFR1c, FGFR2c, and FGFR3c.

One limitation of the present study was that we did not recruit females because of the low number of female smokers in China. More samples, especially females, will be needed to confirm our findings. Another issue is that stress due to recent trauma may have some effects on the results. Furthermore, the anticipation of surgery could skew their mood self-ratings. Altogether, our findings provide evidence of the presence of FGF19 in human CSF and the role of FGF19 in mood regulation in humans. Thus, FGF19 may be a target for mood disorders. The role of FGF19 in disease condition should be investigated in future studies. In addition, more public

awareness should put on the roles of FGFs in mood regulation in humans.

Conflict of interest statement

The authors declare that they have no conflicts of interest (financial or otherwise) related to the data presented in this manuscript.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81100993, 81300311, 81571297, 81271475, 81500519 and 81560229), the Opening Project of Zhejiang Provincial Top Key Discipline of Pharmaceutical Sciences.

References

1. Beenken A, Mohammadi M. The FGF family: biology, pathophysiology and therapy. *Nat. Rev. Drug. Discov* 8(3), 235-53 (2009).
2. Itoh N. Hormone-like (endocrine) Fgfs: their evolutionary history and roles in development, metabolism, and disease. *Cell. Tissue. Res* 342(1), 01-11 (2010).
3. Long YC, Kharitonenkov A. Hormone-like fibroblast growth factors and metabolic regulation. *Biochim. Biophys. Acta* 1812(7), 791-795 (2011).
4. Fukumoto S. Actions and mode of actions of FGF19 subfamily members. *Endocr. J* 55(1), 23-31 (2008).
5. Beenken A, Mohammadi M. The structural biology of the FGF19 subfamily. *Adv. Exp. Med. Biol* 728(1), 1-24 (2012).
6. Turner CA, Watson SJ, Akil H. The fibroblast growth factor system and mood disorders. *Biol. Psychiatry* 59(12), 1128-1135 (2006).
7. Riva MA, Molteni R, Bedogni F, *et al.* Emerging role of the FGF system in psychiatric disorders. *Trends. Pharmacol. Sci* 26(5), 228-231 (2005).
8. Evans SJ, Choudary PV, Neal CR, *et al.* Dysregulation of the fibroblast growth factor system in major depression. *Proc. Natl. Acad. Sci. U S A* 101(43), 15506-15511 (2004).
9. Nishimura T, Utsunomiya Y, Hoshikawa M, *et al.* Structure and expression of a novel human FGF, FGF-19, expressed in the fetal brain. *Biochim. Biophys. Acta* 1444(1), 148-151 (1999).
10. Tan BK, Hallschmid M, Adya R, *et al.* Fibroblast growth factor 21 (FGF21) in human cerebrospinal fluid: relationship with plasma FGF21 and body adiposity. *Diabetes* 60(11), 2758-2762 (2011).
11. Hsuchou H, Pan W, Kastin AJ. The fasting polypeptide FGF21 can enter brain from blood. *Peptides* 28(12), 2382-2386 (2007).
12. Leng Y, Wang Z, Tsai LK, *et al.* FGF-21, a novel metabolic regulator, has a robust neuroprotective role and is markedly elevated in neurons by mood stabilizers. *Mol. Psychiatry* 20(2), 215-223 (2015).
13. Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behav. Res. Methods. Instrum. Comput* 36(4), 717-731 (2004).
14. He S, Zhang T, Hong B, *et al.* Decreased serum fibroblast growth factor - 2 levels in pre- and post-treatment patients with major depressive disorder. *Neurosci. Lett* 579(1), 168-172 (2014).
15. Tan BK, Sivakumar K, Bari MF, *et al.* Lower cerebrospinal fluid/plasma fibroblast growth factor 21 (FGF21) ratios and placental FGF21 production in gestational diabetes. *PLoS. One* 8(6), e65254 (2013).