Research Article

FGFR2 gene polymorphism rs2981582 is associated with non-functioning pituitary adenomas in Chinese Han population: a case–control study

Bin Zhu1,2, Juan Wang3, Lingling Qin4, Lei Wang5, Yanfei Zheng1, Lei Zhang6 and Wei Wang1

1 School of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 100029, China; 2 Department of Pharmacy, Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, China; 3 Education Research Evaluation Center, Beijing University of Chinese Medicine, Beijing 100029, China; 4 Technology Department, Beijing University of Chinese Medicine, Beijing 100029, China; 5 Department of Endocrinology, Third Affiliated Hospital of Beijing University of Chinese Medicine, Beijing 100029, China; 6 Department of Pharmacy, Beijing Shijitan Hospital, Capital Medical University, Beijing 100050, China

Correspondence: Wei Wang (wangwei26960@126.com)

The association of the fibroblast growth factor receptor 2 gene (FGFR2) polymorphism rs2981582 with breast cancer has been extensively studied, whereas the role of this polymorphism in non-functioning pituitary adenoma (NFPA) has not been elucidated. We thus investigated a potential association of rs2981582 with NFPA. A total of 79 patients and 142 healthy control participants were enrolled in our study. DNA of the participants was extracted from peripheral blood samples and genotyped by using the MassARRAY method. We found that the AA genotype was associated with a higher risk of developing NFPA (OR = 1.743, 95%CI: 1.151–2.64, P=0.008). After adjusting for risk factors, significant difference was still observed between the two groups (OR = 1.862, 95%CI: 1.172–2.957, P=0.008). Moreover, under the assumptions of the recessive model (OR = 3.051, 95%CI: 1.403–6.635, P=0.005) and the additive model (AG: OR = 0.329, 95%CI: 0.144–0.755, P=0.009; AA: OR = 0.326, 95%CI: 0.141–0.757, P=0.009), rs2981582 was associated with an increased risk of NFPA. Our results proved that FGFR2 rs2981582 AA genotype was associated with a higher risk of NFPA. The recessive model and additive model also showed increased the risk of NFPA.

Introduction

Non-functioning pituitary adenomas (NFPAs) are benign pituitary neoplasms that arise from adenohypophyseal cells and are not associated with any clinical syndrome related to hormone overproduction from the tumor [1,2]. NFPAs constitute 15–37% of all pituitary adenomas and have a prevalence of 70–220 per million in the population [3]. It is the most common type of adult pituitary macroadenoma, and its most frequent clinical symptoms are headache, visual defects, and hypopituitarism [4-6]. As the clinical manifestations of hormonal hypersecretion are absent, the diagnosis of such patients is always delayed [7].

Several studies have explored the relationships between single nucleotide polymorphism (SNP) sites and pituitary adenoma (PA) in general by large genome-wide association studies (GWAS) [8-10]. However, no studies examined the relationship of SNPs with NFPA. Fibroblast growth factor receptor 2 (FGFR2) is a member of the FGFR family of tyrosine kinase receptors. The FGFR2 gene is located on chromosome 10q26 and contains 22 exons [11,12]. FGFR2 participates in the process of tumorigenesis by mediating mitogenic and survival signals as well as by promoting invasiveness and angiogenesis [13]. Variants of the FGFR2 polymorphism rs2981582, which was first identified by Easton et al. in a large-scale GWAS, were most commonly reported to be associated with breast cancer or prostate cancer [14,15]. Glebauskiene et al. observed that the FGFR2 rs2981582 G/G genotype was observed less frequently in the non-invasive PA subgroup than in healthy controls, whereas the G/A genotype was more frequently
observed in the non-invasive PA subgroup than either in control group or invasive PA subgroup [16]. Nevertheless, given that the relationship between rs2981582 variants and specifically NFPA is still unknown, we sought to assess the impact of FGFR2 rs2981582 on sporadic NFPA and determine a possible association of rs2981582 variants with NFPA risk in Chinese population in a case–control study.

Materials and methods
Patient recruitment
Participants were consecutively recruited from June to December in 2017 among inpatients in the Beijing Tiantan Hospital. The inclusion criteria were as follows: (1) diagnosis of PA in patients confirmed via magnetic resonance imaging (MRI); (2) normal laboratory tests for the growth hormones prolactin and adrenocorticotropic hormone; (3) absence of other serious diseases. In the control group, age, gender, symptoms, physical examinations, and medical history were recorded. Control group participants were enrolled among healthy individuals after examination that did not reveal any disease or family history of related diseases. Patients with severe hepatic, renal, or cardiac impairment, or any serious illness were excluded. The study was performed according to the guidelines of the Declaration of Helsinki, and was approved by the Ethics Committee of the Beijing Tiantan Hospital. A signed informed consent was obtained from all patients or from their direct relatives.

Genotyping
Blood samples of each participant were collected into tubes containing ethylenediaminetetraacetic acid and stored at c −80°C until use. Genomic DNA was extracted from the whole blood using a commercial kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. DNA quantity was examined by a NanoDrop2000 spectrophotometer (Thermo Fisher, Waltham, MA, U.S.A.). Polymerase chain reaction (PCR) primer pairs used to amplify FGFR2 rs2981582 were as follows: ACTGCTGCGGTTCCTAAG (forward); CACTCATGCCCCACTTAATG (reverse). Genotyping was performed by using time-of-flight mass spectrometry on a MassARRAY iPLEX platform (Sequenom, San Diego, CA, U.S.A.) by Bio Miao Biological Technology (Beijing, China). The average genotype call rate for this SNP was >98%.

Statistical analysis
All statistical analyses were performed by SPSS (version 17.0, SPSS Inc., Chicago, U.S.A.). The Hardy–Weinberg equilibrium (HWE) was assessed by using a χ² test and a P-value <0.05 was considered to indicate significant disequilibrium. Differences in genotypic frequencies between groups were calculated by the Pearson’s χ² test or Mann–Whitney U test. Odds ratio (OR) values with 95% CI were calculated. Logistic regression analysis was used to evaluate the contribution of genetic and non-genetic factors to NFAP risk. Patients’ gender, age, bodyweight, smoking, and drinking habits were treated as risk factors to be adjusted in the analysis.

Results
Characteristics of the study participants
A total of 79 patients (38 female) with a mean age of 41.37 ± 11.63 years and bodyweight 72.26 ± 13 kg were enrolled in this hospital-based case–control study. All patients were Chinese and with confirmed diagnosis of NFPA. Of them, 24.5% had a history of smoking and 13.9% were drinking alcohol. The control group enrolled 142 healthy persons (73 female) with a mean age of 42.12 ± 11.63 years and bodyweight 69.04 ± 14.35 kg. Nearly 17.6% and 16.8% of control group participants smoked and consumed alcoholic beverage, respectively. No deviation from the HWE was observed (HWE = 0.236, P > 0.05). The minor allele frequency was 0.3455. Demographic characteristics of the participants are given in Table 1.

Genetic association analysis
Genotype and allele frequencies of the FGFR2 gene polymorphisms in patients and healthy controls are shown in Table 2. Significant differences were observed between patients and healthy controls in the distribution of AA, GA, and GG genotypes (P = 0.018). In addition, there was also a significant difference in the recessive model (P = 0.007). To analyze the influence of gender on the development of NFPA, we also divided all participants into males and females. There were no significant differences in the frequencies of AA, GA, and GG between patients and healthy controls in males or females considered separately. However, we found that there was a difference in the recessive model (AA vs. GA + GG) in females (Table 3).
Table 1 The basic characteristic of the enrolled patients

|                  | Control          | Case            |
|------------------|------------------|-----------------|
| Gender (M/F)     | 142 (69/73)      | 79 (41/38)      |
| Age (y)          | 42.12±11.63      | 41.37±11.63     |
| Bodyweight (kg)  | 69.04±14.35      | 72.26±13        |
| Smoking (%)      |                  |                 |
| Yes              | 17.6%            | 24.5%           |
| No               | 82.4%            | 75.9%           |
| Drinking (%)     |                  |                 |
| Yes              | 16.8%            | 13.9%           |
| No               | 83.2%            | 86.1%           |

Table 2 The genotype of FGFR2 rs2981582 polymorphism in case and control group

| rs2981582 | Control       | Case       | P    |
|-----------|---------------|------------|------|
| AA        | 13            | 18         | 0.018|
| GA        | 62            | 28         |      |
| GG        | 68            | 31         |      |
| A         | 88            | 64         | 0.027|
| G         | 198           | 90         |      |
| AA + AG vs. GG | 75/68 | 46/31 | 0.32 |
| AA vs. AG + GG | 13/130 | 18/59 | 0.007|

Table 3 The genotype of FGFR2 rs2981582 polymorphism with different gender

|       | Male |          |      |       | Female |          |      |       |
|-------|------|----------|------|-------|--------|----------|------|-------|
|       | Case | Control  | P    |       | Case   | Control  | P    |       |
| AA    | 9    | 7        | 0.223|       | 9      | 6        | 0.057|
| GA    | 16   | 32       |      |       | 12     | 30       |      |       |
| GG    | 16   | 31       |      |       | 15     | 37       |      |       |
| A     | 34   | 46       | 0.247|       | 30     | 42       | 0.067|
| G     | 48   | 94       |      |       | 42     | 104      |      |       |
| AA + AG vs. GG | 25/16 | 39/31 | 0.691|       | 21/15 | 36/37   | 0.419|
| AA vs. AG + GG | 9/32  | 7/65    | 0.099|       | 9/27  | 6/67     | 0.035|

Table 4 The logistic regression analysis of rs2981582 SNP genotype with the morbidity of NFPA

| rs2981582 | OR   | 95%CI       | P    | OR*  | 95%CI*   | P*   |
|-----------|------|-------------|------|------|----------|------|
| GG        | Reference |           |      | Reference |        |      |
| AA        | 1.743 | 1.151–2.64  | 0.008| 1.862 | 1.172–2.957 | 0.008|
| GA        | 0.5684 | 0.309–1.046 | 0.069| 0.6539 | 0.3365–1.27 | 0.021|

* Adjusting for gender, age, bodyweight, smoking, and drinking. Significant associations are marked in bold.

The logistic regression analysis of rs2981582 SNP genotype between patients and healthy controls was also performed. We found that the AA allele significantly correlated with NFPA morbidity (OR = 1.743, 95%CI: 1.151–2.64, P=0.008) even after adjusting for gender, age, bodyweight, smoking, or drinking habits (OR = 1.862, 95%CI: 1.172–2.957, P=0.008) (Table 4). In addition, the effects of genetic models (dominant, recessive, and additive models) on NFPA risk were also observed between two groups. We did not found any significant associations of rs2981582 with NFPA in the dominant model (OR = 1.345, 95%CI: 0.7674–2.359, P=0.300). However, rs2981582 had a significant impact in the recessive model (OR = 3.051, 95%CI: 1.403–6.635, P=0.005) and the additive model (AG: OR = 0.329, 95%CI: 0.144–0.755, P=0.009; AA: OR = 0.326, 95%CI: 0.141–0.757, P=0.009), even after adjusting for gender, age, bodyweight, smoking, or drinking habits (Table 5).
Table 5 The logistic regression analysis of the dominant model, recessive model, and additive model in case and control group

| rs2981582   | OR   | 95% CI        | P    | OR*  | 95% CI* | P*  |
|-------------|------|---------------|------|------|---------|-----|
| Dominant model (GA + AA vs. GG) | 1.345 | 0.7674–2.359 | 0.300 | 1.622 | 0.8784–2.995 | 0.122 |
| Recessive model (AA vs. GA + GG) | 3.051 | 1.403–6.635 | 0.005 | 3.153 | 1.329–7.481 | 0.009 |
| Additive model | | | | | | |
| AG          | 0.329 | 0.144–0.755 | 0.009 | 0.325 | 0.141–0.749 | 0.008 |
| AA          | 0.326 | 0.141–0.757 | 0.009 | 0.310 | 0.133–0.722 | 0.007 |

*Adjusting for gender, age, bodyweight, smoking, and drinking. Significant associations are marked in bold.

**Discussion**

In the absence of clinical manifestations of hormonal hypersecretion, NFPA s are usually found when they are large enough to exert pressure on surrounding structures [17]. Until now, there are no effective drugs available for NFPA, and surgical resection remains the first-line treatment [18]. However, a complete resection is achieved only in 40–50% of the cases, and at least 10–20% of completely resected tumors recur after 5–10 years [5]. As a consequence, the long-term survival of individuals with NFPA is compromised.

NFPA emergence involves a complex combination of genetic, environmental, and lifestyle factors. Inherited susceptibility makes an important contribution to cancer development [19]. Identifying and understanding the functional importance of genetic alterations is crucial to extend our understanding of NFPA. Although FGFR2 polymorphism rs2981582 has been extensively evaluated in breast and prostate cancers, the role of this polymorphism in NFPA patients has not been elucidated yet [11,20–22]. Our results have demonstrated that FGFR2 rs2981582 AA genotype or the recessive model was associated with a higher risk of NFPA. To the best of our knowledge, this is the first study of the relationship between FGFR2 polymorphism rs2981582 and NFPA.

The relationship of FGFR2 rs2981582 polymorphism with PA was also reported by Glebauskiene et al. [16]. However, there were several differences between our study and theirs. First, the enrolled patients were different. Our study only enrolled NFPA patients, whereas Glebauskiene et al. enrolled patients with PA in general. Second, neither of the three rs2981582 genotypes had statistically different prevalence in the overall PA group compared with that in controls in their Caucasian population, although in a more detailed analysis, FGFR2 rs2981582 G/G genotype was less prevalent in non-invasive PA cases than in healthy controls [16]. In contrast, in our study in Chinese population, we found that individuals with rs2981582 AA genotype may have a higher NFPA risk. In addition, by using binomial logistic regression analysis, we showed that rs2981582 had a significant effect in the recessive and additive models.

Although our results are promising and might be useful in helping to stratify patients, they should be considered as preliminary ones and further research is necessary. There were also some limitations that may not be ignored. First, as the mechanism of NFPA is dramatically complex, and we only focused on a polymorphism in one gene, this cannot completely explain the morbidity. Furthermore, the function of FGFR2 in NFPA is still unclear, and more experimental data on this subject are required. Second, as the sample size was limited in our study, several indices bordered the level of statistical significance. In this regard, larger cohorts of patients and healthy controls will be needed in future studies. Third, although we chose the control subjects strictly from the individuals deemed to be healthy by necessary examinations, they did not undergo MRI diagnostics to fully exclude the PA risk. Thus, in future, even stricter inclusion and exclusion criteria should be adopted.

**Conclusion**

In summary, we for the first time showed an association of FGFR2 polymorphism rs2981582 with NFPA. However, this result needs to be replicated in a larger sample of patients, and functional studies will be necessary to investigate whether and how this polymorphism might affect NFPA pathogenesis.

**Competing Interests**

The authors declare that there are no competing interests associated with the manuscript.
References

1 Huang, W. and Molitch, M.E. (2018) Management of nonfunctioning pituitary adenomas (NFAs): observation. *Pituitary* 21, 162–167, https://doi.org/10.1007/s11102-017-0856-0
2 Greenman, Y. and Stern, N. (2015) Optimal management of non-functioning pituitary adenomas. *Endocrine* 50, 51–55, https://doi.org/10.1007/s12020-015-0685-8
3 Tampourlou, M., Fountas, A., Ntali, G. and Karavitaki, N. (2016) Mortality in patients with non-functioning pituitary adenoma. *Pituitary* 21, 203–207, https://doi.org/10.1007/s11102-018-0863-9
4 Levy, M.J., Robertson, I.J., Khalil, N., Vitello, S., Reddy, N., Bhake, R. et al. (2018) Long-term follow up of a large prospective cohort of patients with non-functioning pituitary adenomas: the outcome of a conservative management policy. *Clin. Endocrinol.* 89, 354–359, https://doi.org/10.1111/cen.13791
5 Delgado-Lopez, P.D., Pi-Barrio, J., Duenas-Polo, M.T., Pascual-Llorente, M. and Gordon-Bolanos, M.C. (2018) Recurrent non-functioning pituitary adenomas: a review on the new pathological classification, management guidelines and treatment options. *Clin. Transl. Oncol.* 20, 1233–1245, https://doi.org/10.1007/s12994-018-1868-6
6 Iglesias, P., Rodriguez, B.V. and Diez, J.J. (2018) Non-functioning pituitary adenomas: growth and aggressiveness. *Endocrine* 55, 164-165-x, https://doi.org/10.1007/s12020-018-1840-7
7 Oystese, K.A., Evang, J.A. and Bollerslev, J. (2016) Non-functioning pituitary adenomas: the outcome of a conservative management policy. *Endocrinologist* 26, 477–491, https://doi.org/10.2217/epi.15.140
8 Ye, Z., Li, Z., Wang, Y., Mao, Y., Shen, M., Zhang, Q. et al. (2015) Common variants at 10p12.31, 10q21.1 and 13q12.13 are associated with sporadic pituitary adenoma. *Nat. Genet.* 47, 793–797, https://doi.org/10.1038/ng.3322
9 Kho, S.K., Pendek, R., Nickolov, R., Luccio-Camelo, D.C., Newton, T.L., Massie, A. et al. (2009) Genome-wide scan identifies novel modifier loci of acromegalic phenotypes for isolated familial somatotropinoma. *Endocr. Relat. Cancer* 16, 1057–1063, https://doi.org/10.1677/ERC-08-0287
10 Bi, W.L., Larsen, A.G. and Dunn, I.F. (2018) Genomic alterations in sporadic pituitary tumors. *Curr. Neur. Neurosci. Rep.* 18, 4, https://doi.org/10.1007/s11910-018-0811-0
11 Xia, P., Li, B., Geng, T., Deng, Z., Chang, D. et al. (2015) FGFR2 gene polymorphisms are associated with breast cancer risk in the Han Chinese population. *Am. J. Cancer Res.* 5, 1854–1861
12 Haworth, K.E., Farrell, W.E., Emes, R.D., Ismail, K.M., Carroll, W.D., Hubball, E. et al. (2014) Methylation of the FGFR2 gene is associated with high birth weight centile in humans. *EpiGenomics* 6, 477–491, https://doi.org/10.2217/epi.14.40
13 Terwisscha, V., Bakker, S.C., Khan, R.S. and Kas, M.J. (2013) Fibroblast growth factors in neurodevelopment and psychopathology. *Neuroscientist* 19, 479–494, https://doi.org/10.1177/1078754412472339
14 Easton, D.F., Pooley, K.A., Dunning, A.M., Pharoah, P.D., Thompson, D., Ballinger, D.G. et al. (2007) Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature* 447, 1087–1093, https://doi.org/10.1038/nature05887
15 Siddiqui, S., Chattopadhyay, S., Akhtar, M.S., Najm, M.Z., Deo, S.V., Shukla, N.K. et al. (2014) A study on genetic variants of Fibroblast growth factor receptor 2 (FGFR2) and the risk of breast cancer from North India. *PLoS One* 9, e110426, https://doi.org/10.1371/journal.pone.0110426
16 Gledauskiene, B., Vilkieviciute, A., Liukteviciene, R., Jakstiene, S., Kriauciuniene, L., Zemaitiene, R. et al. (2017) Association of FGFR2 rs2981582, SIRT1 rs12773836, STAT3 rs744166 gene polymorphisms with pituitary adenoma. *Oncol. Lett.* 13, 3087–3099, https://doi.org/10.3892/ol.2017.5840
17 Minniti, G., Flickinger, J., Tolou, B. and Paolini, S. (2018) Management of nonfunctioning pituitary tumors: radiotherapy. *Pituitary* 21, 154–161, https://doi.org/10.1007/s11102-018-0866-8
18 Ntali, G. and Wass, J.A. (2018) Epidemiology, clinical presentation and diagnosis of non-functioning pituitary adenomas. *Pituitary* 21, 111–118, https://doi.org/10.1007/s11102-018-0869-3
19 Köber, P., Boresowicz, J., Rusetskia, N., Maksymowicz, M., Goryca, K., Kunicki, J. et al. (2018) DNA methylation profiling in nonfunctioning pituitary adenomas. *Mol Cell. Endocrinol.* 473, 194–204, https://doi.org/10.1016/j.mce.2018.01.020
20 Lei, H. and Deng, C.X. (2017) Fibroblast growth factor receptor 2 signaling in breast cancer. *Int. J. Biol. Sci.* 13, 1163–1171, https://doi.org/10.7150/ijbs.20792

© 2018 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution License 4.0 (CC BY).
21 Yang, Y.B., Zhao, Z.X., Huang, W., Liu, H., Tan, Y.L. and Wang, W.M. (2016) Association between fibroblast growth factor receptor-2 gene polymorphism and risk of breast cancer in Chinese populations: A HuGE review and meta-analysis. J. Cancer Res. Ther. 12, 543–549, https://doi.org/10.4103/0973-1482.148715

22 Parker, B.C., Engels, M., Annala, M. and Zhang, W. (2014) Emergence of FGFR family gene fusions as therapeutic targets in a wide spectrum of solid tumours. J. Pathol. 232, 4–15, https://doi.org/10.1002/path.4297