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ORIGINAL ARTICLE

Association of PTTG1 polymorphism rs1895320, rs2910200 and rs6882742 with non-functioning pituitary adenomas in Chinese Han population: a case-control study

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Abstract

Due to absence of clinical manifestations of hormonal hyper secretion, the treatment of Nonfunctioning pituitary adenoma (NFPA) was always delayed. PTTG1 was reported to be overexpressed in most of pituitary tumors, however, the polymorphism of PTTG1 rs1895320, rs2910200 and rs6882742 with NFPA were still not fully elucidated in NFPA. Thus, a hospital based case control study which included 79 patients and 142 healthy control participants were conducted. DNA was extracted from peripheral blood samples and genotyped by Mass Array methods. In addition, a meta-analysis of rs2910200 was also employed to further testify the conclusion. Significant difference were observed between patients and healthy controls under rs2910200 locus between allelic genotype ($p = 0.0219$). However, no other significant difference was observed in rs1895329 and rs6882742. In addition, a logistic regression analysis showed that the dominant model of rs2910200 were closely correlated with the NFPA susceptibility (OR = 1.951, 95% CI:1.075–3.542, $p = 0.028$). While no significant difference was observed in the rs1895320 and rs6882742 under dominant model, recessive model and additive model The meta-analysis results showed that the dominant model and heterozygote model can significantly increase the risk of PA ($p = 0.007$, OR = 1.57, 95% CI:1.14–2.18; $p = 0.009$, OR = 1.57, 95% CI:1.12–2.19). Whereas no significant difference were observed under the homozygous model and recessive model. In conclusion, the polymorphism of PTTG1 rs2910200 dominant model and T allelic might increase the risk of NFPA.

Keywords Non functioning pituitary adenoma · Tumor · PTTG1 · Polymorphism · Genotype

Introduction

Pituitary adenoma is one of the most common intracranial and neuroendocrine neoplasms, with its prevalence reported as up to 16.7%, and an incidence rate of 2.7–3.13 per 100,000 population(Liu et al. 2018; Wildemberg et al. 2018). Nonfunction pituitary adenoma (NFPA), as one type of adult pituitary macro adenomas, which constitute 15–37% of all pituitary adenomas(Tampourlou et al. 2018). The clinical manifestation of NFPA varies, from being completely asymptomatic to serious pituitary dysfunction and visual field compromise(Ntali and Wass 2018). For the absence of clinical symptoms of hormonal hyper secretion, the NFPA was not diagnosed until symptoms appear. When the adenoma grows to compress adjacent structures, it is no longer asymptomatic and visual function often becomes acutely damaged(Chanson et al. 2015). Thus, it may be helpful in the early detecting of NFPA if newly diagnosis biomarkers were discovered (Fig. 1).

The pathogenesis of NFPA is complex. However, more and more studies had reported that genetic mutations involving overexpression of oncogenes, inactivation of tumor suppressor genes had play a vital role during the tumor genesis(Yagnik et al. 2017; Zlatkute et al. 2017). Single nucleotide polymorphisms (SNPs) which discovered by Large

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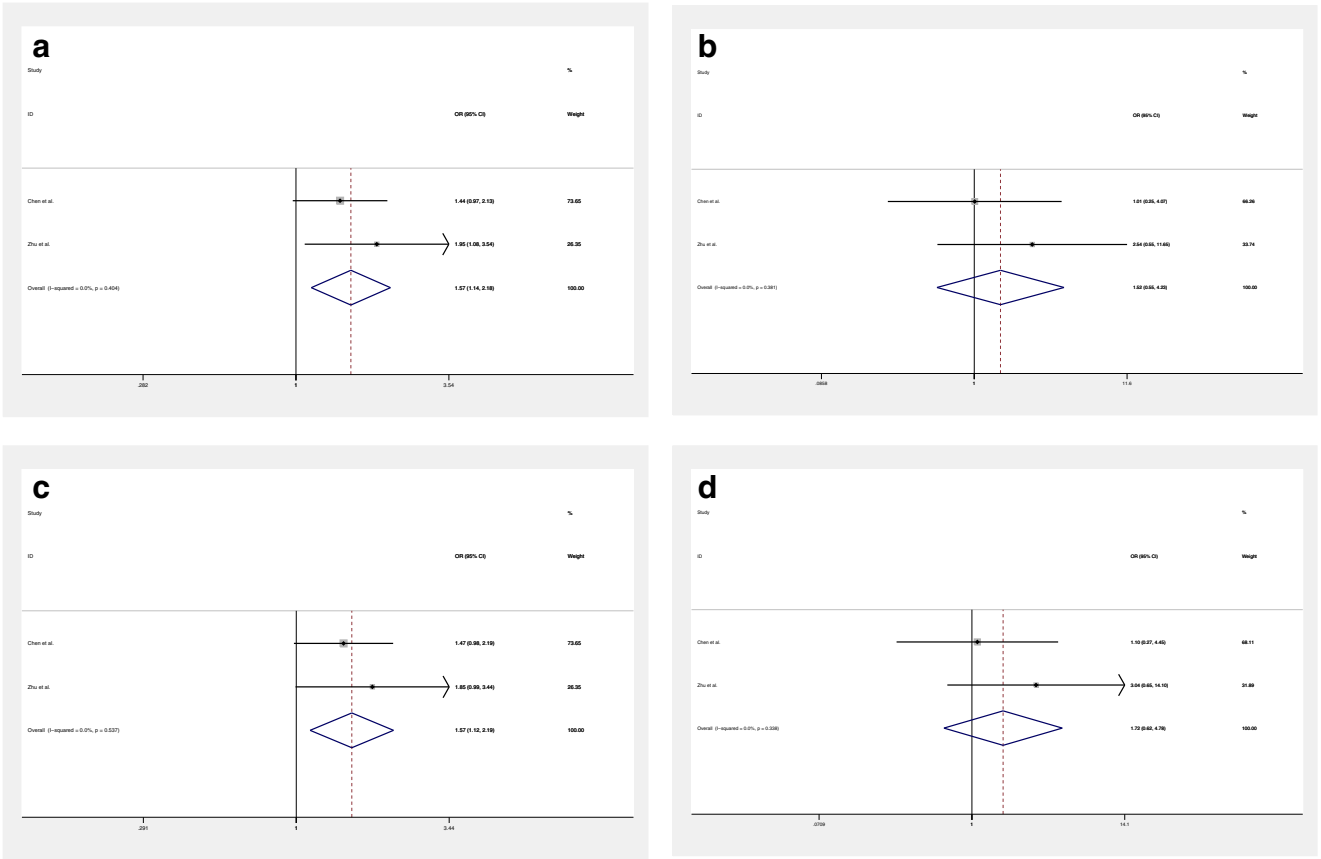

Fig. 1 The forest plot of PTTG1 polymorphism rs2910200 under different genetic models. Panel A was the dominant model (CT+ TT vs. CC); Panel B was the recessive model (TT vs. CT + CC); Panel C was the heterozygote model (CT vs. CC); Panel D was the homozygote model (TT vs. CC)

Genome Wide Association Studies (GWAS) had been reported to be associated with genetic basis of this disease(Ye et al. 2015). Pituitary tumor-transforming gene-1 (PTTG1), also known as securin, encodes a regulatory protein that participates in cellular pathways involving cell division, chromosome stability, and DNA repair (Tong and Eigler 2009). It is first isolated from rat pituitary tumor cells in 1997 (Pei and Melmed 1997) and is a vital component that inhibits the separase activity and prevents the separation of premature chromosome(Romero et al. 2018).A number of studies focused on exploring the relationship of PTTG1 with various kinds of tumor development (Xu et al. 2018; Yeganeh et al. 2017). Zhang et al. reported that PTTG1 mRNA was overexpressed in more than 90% of all types of pituitary tumors (Zhang et al. 1999) While Xu et al. had identified that PTTG1 as an independent prognostic biomarker for gastric cancer (Xu et al. 2016). Whereas little was conducted to study the association of genetic alterations with the risk of NFPA.

Given that the relationship between PTTG1 variants and specifically NFPA is still unknown, we sought to assess the impact of PTTG1 variants on sporadic NFPA and determine a possible association of PTTG1 variants with NFPA risk in Chinese population in a case-control study. In this study, three PTTG1 gen polymorphism rs1895320, rs2910200 and rs6882742 were observed in northern China with NFPA susceptibility in order to identify target locus and reliable biomarkers to predict NFPA risk.

Materials and methods

Patients recruitment

A hospital-based case–control design was used in the present study. Patients were consecutively recruited from June to December in 2017 among inpatients in Beijing Tiantan Hospital. Histopathologic analyses and follow-up data after surgery were recorded for each patient. Tumor size was measured as the maximum diameter on MRI. Age and sex matched controls group were enrolled from healthy examination individuals without a familial history of related diseases. All participants' age, gender, symptoms, physical examinations and medical history were recorded. Written informed consent was obtained from all participants in the study and

the study was approved by the Ethics Committee of Beijing Tiantan Hospital.

The inclusion criteria was as follows: (1) Patients determined and confirmed PA via magnetic resonance imaging (MRI); (2) laboratory tests for the growth hormones prolactin and adrenocorticotropic hormone levels were within normal range; (3) No other brain or other localization tumors.

Exclusion criteria: patients with severe liver, renal or cardiac impairment, and serious illness were excluded.

Genotyping

Peripheral samples of each participant were collected in tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) and stored at −80 °C until use. Genomic DNA was extracted from whole blood leukocytes using a commercially kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). The quantity was examined by NanoDrop2000 spectrophotometer (Thermo Fisher, Waltham, MA, USA). Polymerase chain reaction (PCR) primer pairs used to amplify were showed in Table 1. The PCR cycling conditions were 2 min at 94 °C followed by 45 cycles of 20 s at 94 °C, 30 s at56°C (MMP1), and 60 s at 72 °C, and with a final extension at 72 °C for 3 min. Genotyping analysis was performed by time-of-flight mass spectrometry on a MassARRAY iPLEX platform (Sequenom, San Diego, CA, USA) in Bio Miao Biological Technology (Beijing). The average genotype call rate for the SNP was >98%.

Meta-analysis

Studies published by 10 July, 2018 were included by a systematic literature search in PubMed, Embase, Web of Knowledge, China National Knowledge Infrastructure (CNKI) and WanFang Data. We searched all publications related to association studies and checked the reference lists of the identified studies for additional studies.

Statistical analysis

All statistical analyses were performed using SPSS software (version 17.0, SPSS Inc., Chicago, USA). Hardy-Weinberg equilibrium (HWE) was assessed by using χ^2 test. Differences in genotypic frequencies between groups were calculated by the Pearson's chi-square test or Mann-Whitney U test. Odds ratios (OR) with 95% CI were calculated. Logistic regression analysis was used to evaluate the contribution of genetic and non-genetic factors to NFAP risk..

For meta-analysis, the odds ratio (OR) and 95% confidence interval (95% CI) were calculated to assess the relationship between PTTG1 polymorphism and NFPA susceptibility. Pooled ORs were obtained from combination of single study under recessive model (TT vs. CT + CC) and dominant model (TT + CT vs. CC). Heterogeneity was evaluated by Q statistic and I^2 statistic. The significance of the pooled ORs was assessed by Z-test, where $p < 0.05$ indicated statistically significant..

Results

The characteristic of the study

A total of 79 patients (38 female) with mean age 41.37 ± 11.63 and 142 healthy controls (73 female) with mean age 42.12 ± 11.63 were enrolled in this hospital-based case and control study. The mean body weight in NFPA group were 72.26 ± 13 and 69.04 ± 14.35 in control group. All patients were Chinese and had accepted the surgical resection of tumor. The tumor diameter in NFPA group were 28.93 ± 11.28. Of all patients, 24.5% were with a history of smoking and 13.9% were drinking while nearly 17.6% and 16.8% people with a history of smoking and drinking history in control group. Deviation from Hardy–Weinberg equilibrium was not observed (HWE = 0.236, $p > 0.05$). The minor allele frequencies (MAF) was 0.8085, 0.6212 and 0.2598 for rs1895320, rs2910200 and rs6882742 respectively. The demographics characteristics were in Table 2.

Genetics association analysis

The frequencies of genotype and alleles of the PTTG1 gen polymorphisms in patients and healthy controls were showed in Table 3. Significant difference was observed between patients and healthy controls under rs2910200 locus between allelic genotype ($p = 0.0219$), whereas no other significant difference were observed under different genotype among rs1895320 and rs6882742 ($p > 0.05$).

To further analyze the influence of different genetic model to NFPA risk, a logistic regression analysis was

Table 1 The primers of the Polymerase chain reaction

SNP_ID	Forward	Reverse
rs1895320	GGAAGTTACTGAATCTCTGC	TAAACTTGACCTCTCACCCC
rs2910200	TTGGCATCATCCTACGTAGC	TCTGAGTCTCCTCCACTAC
rs6882742	TCCTGACTGTTAGCTCCTAC	CAACTCTGTAAACCTTCTGC

Table 2 The basic characteristic of the enrolled patients

	Control	Case
Gender(M/F)	142(69/73)	79(41/38)
Age	42.12 ± 11.63	41.37 ± 11.63
Bodyweight	69.04 ± 14.35	72.26 ± 13
Tumor Diameter	–	28.93 ± 11.28
Tumor Character*(N)	–	
1	–	44
2	–	22
3	–	9
Smoking(%)		
Yes	17.6%	24.5%
No	82.4%	75.9%
Drinking(%)		
Yes	16.8%	13.9%
No	83.2%	86.1%

*Tumor character represent the tumor tissue characteristic during operation

1 represent tumor tissues soft

2 represent tumor tissues medium

3 represent tumor tissues solid

employed. Our results showed that the dominant model of rs2910200 was significant correlated with NFPA susceptibility (OR = 1.951, 95%CI:1.075–3.542, $p = 0.028$). Nevertheless, there were no significant difference under the recessive model and additive model between the 2 group. In addition, no significant differences were observed in the rs1895320 and rs6882742 under dominant model, recessive model and additive model ($p > 0.05$) (Table 4).

Patients were divided into 2 groups according to the pathological examination (the hormone immunohistochemistry), however, no significant differences were observed in rs1895320, rs2910200 and rs6882742 between the two groups ($p > 0.05$) (Table 5).

Meta-analysis results

Only one article which focus on the relationship of PTTG1 rs2910200 polymorphism with PA were found in our literature searching. Thus we combined the literature results with our results together to conduct a meta-analysis. The results showed that the dominant model and heterozygote model can significantly increase the risk of PA ($p = 0.007$, OR = 1.57, 95% CI:1.14–2.18; $p = 0.009$, OR = 1.57, 95% CI:1.12–2.19). Whereas no significant difference were observed under the homozygous model and recessive model.

Table 3 The genotype of PTTG1 polymorphism (rs1895320, rs2910200 and rs6882742) in patient and healthy control group. Significant associations are marked in bold

	Case	Control	OR	95% CI	*P*
rs1895320					
CC	2	3	Reference		0.717
CT	29	44	0.682	0.106–4.405	
TT	56	96	0.875	0.142–5.397	
Allelic					
C	24	50	Reference		
T	132	236	0.8582	0.5045–1.46	0.5969
rs2910200					
CC	47	107	Reference	Reference	0.075
CT	26	32	1.85	0.994–3.441	
TT	4	3	3.035	0.654–14.099	
Allelic					
C	120	246	Reference		
T	34	38	1.834	1.1–3.059	**0.0219**
rs6882742					
CC	4	6	Reference		0.933
CT	22	39	0.846	0.215–3.326	
TT	52	98	0.796	0.215–2.947	
C	30	51	Reference		
T	126	235	1.097	0.6653–1.809	0.702

Discussion

PTTG1 is located in chromosome 5q33.3 and contains five exons and four introns, its mRNA is 1.3 kb with an open reading frame of 609 nucleotides encoding a securin protein (Chen et al. 2011). It is overexpressed in a variety of endocrine-related tumors, especially pituitary, thyroid, breast, ovarian, and uterine tumors (Vlotides et al. 2007). As a recently discovered gene with carcinogenic characteristics, PTTG1 serves as a proto-oncogene and promotes cell-cycle progression, sustains chromosomal stability, and modulates transformation in vitro and tumor genesis in vivo (Ramaswamy et al. 2003; Ren and Jin 2017). Kim et al suggested that PTTG may also induce the secretion of basic fibroblast growth factor (bFGF) which is necessary for tumor growth (Kim et al. 2006).

In the present study, we evaluated the relationships between the SNPs of PTTG1 gen and NFAP in Chinese Han population. Our results showed that rs2910200 in the intron area have been identified as susceptible loci for NFPA. Mutations in this gene are known to be correlated with the development of NFPA; however, this particular SNP has not been described previously as a NFPA marker in the literature. The mechanistic role of this polymorphism sites is still worth further exploring.

Table 4 The logistic regression analysis of the dominant model, recessive model and additive model with the morbidity of NFPA among PTTG1 polymorphism of rs1895320, rs2910200 and rs6882742. Significant associations are marked in bold

	rs1895320			rs2910200			rs6882742		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Dominant model	1.228	0.201–7.510	0.824	1.951	1.075–3.542	**0.028**	0.810	0.222–2.962	0.75
Recessive model	0.802	0.439–1.468	0.475	0.394	0.086–1.807	0.231	1.089	0.605–1.961	0.777
Additive model	Reference			Reference			Reference		
CT	0.682	0.106–4.405	0.687	1.85	0.994–3.441	0.052	0.846	0.215–3.326	0.811
TT	0.875	0.142–5.397	0.886	3.035	0.654–14.099	0.165	0.796	0.215–2.947	0.733

Till now only one research reported by Chen et al. showed that the PTTG1 polymorphism (rs1895320, rs2910200 and rs6882742) were not correlated with the risk of pituitary adenoma (Chen et al. 2011). However, polymorphism of PTTG1 with NFPA were not fully studied. Our results showed that rs2910200 was strongly correlated with NFAP risk between the allelic model and the dominant model, which were quite different with Chen's. To further evaluate the difference, a meta-analysis of 2 studies were employed. The results showed that the dominant model and heterozygote model were a risk factor of PA.

Due to absence of clinical manifestations of hormonal hypersecretion, patients may not raise attention until the cancer tissues are large enough to cause serious headache or visual impairment. Till now, none of effective drugs are available for NFPA and surgical resection remains the first-line treatment (Ntali and Wass 2018). However, 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(Delgado-Lopez et al. 2018). As a result, the long-term survival of these patients may be compromised. How to made an early diagnosis to avoid possible harmful effect is a quite difficult problem. A SNP may influenced the mRNA folding by influencing splicing, processing, or translational control and regulation. Thus genetic mutation may play an important role in the early detecting of complex diseases. Ye et al. had found rs2359536, rs10828088, rs10763170 and rs17083838 were genetic susceptibility loci for sporadic pituitary adenoma by a GWAS (Ye et al. 2015). Thus our results might be useful in helping diagnosis of NFPA patients.

Some limitations still exist. Such as the quantity of the enrolled patients were not enough, some index in our research were nearly at the edge of the significance. Meantime, we did not conduct a cranial imaging for participants in the control group and this may influence the accuracy of our results. In addition, we only found one research deal with PA in the meta analysis, this may cause some publication bias and finally influence the overall results. Last, the mechanism of NFPA is extremely complex, and we had only focused on the PTTG1 polymorphism, this alone cannot explain the morbidity.

Table 5 The genotype of rs1895320, rs2910200 and rs6882742 in patients that divided according to hormone IHC in tumor tissues

	Negative			Positive			*P*
	CC	CT	TT	CC	CT	TT	
rs1895320	2	14	36	0	8	17	0.572
rs2910200	15	9	0	34	16	2	0.065
rs6882742	1	8	16	3	16	33	0.946

Conclusion

In conclusion, our study had for the first time showed an association of PTTG1 polymorphism rs2910200 with NFPA. However, In future the results should be replicated in a larger cohort study and functional studies will also be necessary to investigate whether and how the polymorphism might involve in the pathogenesis of NFPA.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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