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USP8 Gene Expression in Sporadic Pituitary Adenomas

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Abstract

Purpose

In sporadic pituitary adenomas the role of Ubiquitin-specific protease 8 (USP8) is not clearly defined. Although mutations in USP8 gene are known to cause corticotroph adenomas, whether changes in expression of USP8 in other pituitary adenomas have not been clarified, yet. In this study we addressed the changes in USP8 gene expression levels in pituitary adenomas relative to non-adenomatous brain tissue.

Methods

USP8 gene expression analysis was performed on a total of 43 tissue samples from human pituitary adenomas and on 16 tissue samples from non-pituitary brain tissues (control group). Adenomatous tissues and control tissues were assessed for quantification of RNA expression of USP8.The levels of USP8 gene expression were determined relative to those in control group.

Results

USP8 gene expression levels in pituitary adenomas (PA) were 3.7 times higher than the levels in control brain tissues (CBT) ($p = 0.002$). Levels of USP8 expression in secertory PA's were significantly higher in comparison to the levels in CBT ($p = 0.002$).

Consclusions

Present findings support that USP8 gene expression levels may contribute to pitutary tumorigenesis and hormonogenesis.

Introduction

Pituitary tumors are among common intracranial neoplasms with increasing prevelance of 14–22 % based on post-mortem and radiologic series [1]. They are notorious for being generally benign. Nevertheless their clinical impacts, which may stem from their localisation, size or hormonal activity, cannot be disregarded

Majority of pituitary tumors are known to be sporadic [2]. Genetic mechanisms contributing to pituitary tumorigenesis have not been completely unravelled, yet. To date, only a limited number of somatic mutations, including those of GNAS and ubiquitin-specific protease 8 (USP8) genes, have been identified in pituitary adenomas [3, 4].

Ubiquitin-specific protease 8 (USP8) is a deubiquinitase involved in various cellular processes and, altered USP8 gene expression has been shown in various cancers [5–9]. This alteration in gene expression, hence the USP8 enzyme level, is hold responsible for tumorogenesis and tumor progression [9–11]. As

USP8 acts by removing conjugated ubiquitin from proteins and decreasing protein degredation, it is also involved in reversal of ubiquination, in other words downregulation, of epidermal growth factor receptor (EGFR) [12–16]. This function of USP8 may make an important contribution to tumorigenesis.

In human, the gene for USP8 is located on chromosome 15 [17]. Mouse models showed a good level of USP8 expression in brain [13]. However data on expression of USP8 in normal human tissues is limited. Available data showed low region specifity of USP gene, while it was detected nearly in all tissues [18, 19]. Based on the data from the available samples, which were collected and analyzed from multiple human post mortem tissues, normal human brain showed wide distribution and high expression of USP8 gene [19].

Although somatic mutations of USP8 in corticotropinomas have been determined previously, its role in other types of pituitary adenomas has yet to be determined. To our knowledge, whether its expression changes during pituitary tumorigenesis has not been determined, yet. Herein we aimed to investigate whether pituritary adenomas show a change in expression levels of USP8 and if available how this change affects the clinic.

Material And Methods

Ubiquitin-specific protease 8 gene expression analysis was performed on a total of 43 tissue samples from human pituitary adenomas (7 non-secretory, 36 hormone secreting) and on 16 tissue samples from non-pituitary brain tissues (control group). Family history of a pituitary adenoma was excluded for each case in the study group. Preoperative basal pituitary hormone levels and pituitary MRI findings were also obtained for cases with pituitary adenoma. In the control group absence of a pituitary mass was confirmed radiologically prior to surgery. Tissues from pituitary adenomas were obtained during pituitary surgery of the adenomas, whereas control brain tissues were obtained from temporal lobe parenchyma during surgery of the patients for medial temporal sclerosis. Demographic and hormonal characteristics of the group with pituitary adenoma are represented in Table 1.

The ethical committee of the Bakirkoy Dr. Sadi Konuk Training and Research Hospital, approved the study and informed consents were provided for investigations.

RNA extraction and real-time quantitative PCR (RT-qPCR)

The tissue samples were stored at -80° C after being formalin fixed and embedded in paraffin blocks immediately after resection. RNA was extracted from tissue and serum samples using a Trizol reagent (Invitrogen, Carlsbad, CA, USA) to obtain the total RNA according to the manufacturer's recommendations. Complementary DNA (cDNA) was transcribed from 100 ng of total RNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), in a total volume of 20 µL. Reverse transcription (RT) master mix contained the following: 10 × RT buffer, 25 × dNTP (deoxynucleotide) mix (100 mM), 10 × RT random primers, MultiScribe™ reverse transcriptase, RNase inhibitor, and nuclease-free water. The RT reaction was performed in a Thermocycler (Eppendorf,

Hamburg, Germany) in the following conditions: 5 min at 25°C, followed by 60 min at 42°C, then the samples were heated to 70°C for 5 min (27). The relative expression of USP8 was assessed using TaqMan® probes (Applied Biosystems) for the studied gene (Hs00987105_g1), with ACTB (Hs99999903_m1) as the reference gene. The procedure was performed in an Applied Biosystems 7500 Fast Real-Time PCR System, for 40 cycles. The PCR mixture was as follows: cDNA (1–100 ng), 20 × TaqMan® Gene Expression Assay, 2 × TaqMan® Gene Expression Master Mix, and RNase-free water, in a total volume of 20 µL. Gene expression levels were quantified using 7500 Fast Real-Time Sequence detection system Software (Applied Biosystems, Foster City, CA). Gene expression was defined based on the threshold cycle (Ct), and ACTB was used as a reference gene that acts as an internal reference to normalize the RNA expression, which was calculated as $2 - \Delta\Delta CT$.

Data were statistically analyzed with the SPSS 15.0 package program. The results are presented as medians and interquartile ranges [IQR]. The Kruskal-Wallis test was used to compare the medians between the multiple groups. The Mann-Whitney U test was used to compare two independent variables. Pearson's correlation coefficient was used for the calculation of associations between variables. The χ^2 test was used when necessary. $P < 0.05$ was considered statistically significant.

Results

USP8 gene expression levels in pituitary adenomas (PA) were 3.7 [IQR: 1.1–50.2] times higher than the levels in control brain tissues (CBT) ($p = 0.002$) (Fig. 1). Although the USP8 gene expression levels in secretory PA's were higher than the levels in non-secretory PA's, the difference was not statistically significant (for secretory PA's 4.2 [IQR: 1.2–50.6] and for non-secretory PA's 1.9 [IQR: 0.6–42.6], $p = 0.6$). Levels of USP8 gene expression in secertory PA's were significantly higher in comparison to the levels in CBT ($p = 0.002$), expression levels in non-secretory PA's were not statistifically different from those in CBT ($p = 0.09$) (Fig. 2).

When the secretory PA's were stratified by their hormone secretion, again there was not a difference between the USP8 expression levels (for FSH/LH secreting PA's: 17.9 [IQR: 1-3.8], for PRL secreting PA's: 8.9 [IQR: 2.03–2.15], for ACTH secreting PA's: 3.4 [IQR: 1.5–16.6], and for GH secreting PA's: 2.5 [IQR: 0.6–50.1], $p = 0.6$). In all pituitary adenomas USP8 expression levels did not show any correlation with hormone levels, preoperative adenoma size, KNOSP and Hardy classification and, postoperative residual status (Data is not shown here). Of the secretory PA's there was not a correlation between ACTH levels and USP8 expression in corticotrop adenomas ($p = 0.3$).

Discussion

In the presented study the expression levels of USP8 gene were significantly higher in tissue samples taken from the pituitary adenomas in comparison to the levels in tissue samples taken from the temporal lobe parenchyma. After stratification of pituitary adenomas by their functional status, secretory PA's had higher USP8 gene expression levels in comparison to non-adenomatous brain tissue. When further

analyzed, there were not statistically significant differences between secretory and non-secretory PA's and, between non-secretory PA and non-adenomatous brain tissue. We did not detect a relationship between USP8 expression levels and any feature of corticotroph adenomas.

Ubiquitin is a protein which is expressed in eukaryotic cells and selectively labels protein structures to be degraded [20]. Ubiquination is a posttranslational process which adds ubiquitin and therefore regulates protein degradation, DNA repair and, endocytosis and lysosomal functions in the cell [21]. However this process of ubiquination is not without control and is limited by deubiquinatinases which remove ubiquitin from the substrate proteins [22]. Ubiquitin-specific protease 8 (USP8) is among these deubiquinatinases, which limit protein degradation. By removing the ubiquitin from epithelial growth factor receptor (EGFR), USP8 prevents degradation of EGFR and ultimately causes increased EGFR, which subsequently promotes cell cycle progression and increased proliferation [23]. USP8 expression level has been related to various cancers including cholangiocarcinomas, cervical squamous carcinoma and have been depicted as a potential target for resistant non-small cell lung carcinomas [24–26]. Gain of function mutations of USP8 have been shown to be involved in tumorigenesis of corticotropinomas [27, 28]. However it is still uncertain whether, without a mutation, USP8 expression levels contribute to pituitary tumorigenesis like they did in other cancers.

In the current study USP8 gene expression levels were significantly, 3.7 [IQR: 1.1–50.2] times, higher in tissue samples taken from the pituitary adenomas ($p = 0.002$). Theodoropoulou et al. previously proposed that deubiquitination related to USP8 mutation led to decreased downregulation of EGFR and sustained epithelial growth factor (EGF) activity, which in turn not only increased cell proliferation but also increased hormone, ACTH, secretion [23]. Our study shows that USP8 may be involved in not only corticotropinomas but all pituitary adenomas. Of course certain mutations may cause differentiation to a certain pituitary adenoma subgroup, however increased USP8 levels may also facilitate pituitary tumorigenesis as a whole.

As epithelial growth factor (EGF) functions in also pituitary hormone secretion it is not surprising that increased EGF activity secondary to USP8 gain of function mutations lead enhanced ACTH secretion [23, 29]. We could not detect a relationship between USP8 levels and ACTH secretion, this may be due to small sample size and also mutated USP8 not wild type may be responsible of increased ACTH. In the current study secretory PA's had a median of 4.2 times higher USP8 expression levels in comparison to non-adenomatous brain tissue. On the other hand USP8 expression in non-secretory pituitary adenomas did not show a statistically significant difference from those in secretory pituitary adenomas and non-pituitary tissue. We had relatively small number of non-secretory pituitary adenoma tissues, which may underestimate the role of USP8 function in pituitary hormonogenesis. Based on relatively increased USP8 expression in secretory adenomas we propose that USP8 may have an additional role in pituitary hormone secretion.

Of course this study was not without certain limitations. Small number of non secretory adenoma samples may have limited the findings. Addittionally due to ethical issues we could not get samples from

normal pituitary tissues, therefore we compared expression levels with non-adenomatous brain tissue, which were taken during surgery performed for nonpituitary pathologies.

In conclusion changes in USP8 gene expression levels may contribute to pitutary tumorigenesis and hormonogenesis. Our findings may shed some light into pathonogenesis of sporadic pituitary adenomas.

Declarations

Acknowledgments Informed consent was obtained from the patients.

Declaration of interest : The authors declare that they have no conflict of interest.

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References

1. Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML, et al. The prevalence of pituitary adenomas. Cancer. 2004;101(3):613-9. doi: https://doi.org/10.1002/cncr.20412.

2. Caimari F, Korbonits M. Novel Genetic Causes of Pituitary Adenomas. Clinical Cancer Research. 2016;22(20):5030-42. doi: 10.1158/1078-0432.ccr-16-0452.

3. Bi WL, Horowitz P, Greenwald NF, Abedalthagafi M, Agarwalla PK, Gibson WJ, et al. Landscape of Genomic Alterations in Pituitary Adenomas. Clinical Cancer Research. 2017;23(7):1841-51. doi: 10.1158/1078-0432.ccr-16-0790.

4. Song Z-J, Reitman ZJ, Ma Z-Y, Chen J-H, Zhang Q-L, Shou X-F, et al. The genome-wide mutational landscape of pituitary adenomas. Cell research. 2016;26(11):1255-9.

5. Sun J, Shen D, Gao Y, Zheng Y, Zhao L, Maa M, et al. Down-Regulation of USP8 Suppresses HER-3 Positive Gastric Cancer Cells Proliferation. OncoTargets and therapy. 2020;13:7973.

6. Jing X, Chen Y, Chen Y, Shi G, Lv S, Cheng N, et al. Down-regulation of USP8 Inhibits Cholangiocarcinoma Cell Proliferation and Invasion. Cancer Management and Research. 2020;12:2185.

7. Shin S, Kim K, Kim H-R, Ylaya K, Do S-I, Hewitt SM, et al. Deubiquitylation and stabilization of Notch1 intracellular domain by ubiquitin-specific protease 8 enhance tumorigenesis in breast cancer. Cell Death & Differentiation. 2020;27(4):1341-54.

8. Qiu H, Kong J, Cheng Y, Li G. The expression of ubiquitin-specific peptidase 8 and its prognostic role in patients with breast cancer. Journal of Cellular Biochemistry. 2018;119(12):10051-8.

9. Yan M, Zhao C, Wei N, Wu X, Cui J, Xing Y. High expression of ubiquitin-specific protease 8 (USP8) is associated with poor prognosis in patients with cervical squamous cell carcinoma. Medical science monitor: international medical journal of experimental and clinical research. 2018;24:4934.

10. Hussain S, Zhang Y, Galardy P. DUBs and cancer: the role of deubiquitinating enzymes as oncogenes, non-oncogenes and tumor suppressors. Cell cycle. 2009;8(11):1688-97.

11. Byun S, Lee S-Y, Lee J, Jeong C-H, Farrand L, Lim S, et al. USP8 is a novel target for overcoming gefitinib resistance in lung cancer. Clinical cancer research. 2013;19(14):3894-904.

12. Kato M, Miyazawa K, Kitamura N. A deubiquitinating enzyme UBPY interacts with the Src homology 3 domain of Hrs-binding protein via a novel binding motif PX (V/I)(D/N) RXXKP. Journal of Biological Chemistry. 2000;275(48):37481-7.

13. Gnesutta N, Ceriani M, Innocenti M, Mauri I, Zippel R, Sturani E, et al. Cloning and characterization of mouse UBPy, a deubiquitinating enzyme that interacts with the Ras guanine nucleotide exchange factor CDC25Mm/Ras-GRF1. Journal of Biological Chemistry. 2001;276(42):39448-54.

14. Row PE, Prior IA, McCullough J, Clague MJ, Urbé S. The ubiquitin isopeptidase UBPY regulates endosomal ubiquitin dynamics and is essential for receptor down-regulation. Journal of Biological Chemistry. 2006;281(18):12618-24.

15. Mizuno E, Iura T, Mukai A, Yoshimori T, Kitamura N, Komada M. Regulation of epidermal growth factor receptor down-regulation by UBPY-mediated deubiquitination at endosomes. Molecular biology of the cell. 2005;16(11):5163-74.

16. Alwan HA, van Leeuwen JE. UBPY-mediated epidermal growth factor receptor (EGFR) de-ubiquitination promotes EGFR degradation. Journal of Biological Chemistry. 2007;282(3):1658-69.

17. Berruti G, Martegani E. Ubiquitin specific peptidase 8. 2013.

18. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Tissue-based map of the human proteome. Science. 2015;347(6220).

19. : https://www.proteinatlas.org/ENSG00000138592-USP8 Accessed.

20. S.SrinivasanZ.Nawaz. 35 - Molecular Biology of Estrogen Receptor Action. In: Donald W. Pfaff APA, Anne M. Etgen, Susan E. Fahrbach, Robert T. Rubin, editor. Hormones, Brain and Behavior (Second Edition). Academic Press; 2009. p. 1187-220.

21. Kulathu Y, Komander D. Atypical ubiquitylation - the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. Nature reviews Molecular cell biology. 2012;13(8):508-23. doi: 10.1038/nrm3394.

22. Reyes-Turcu FE, Ventii KH, Wilkinson KD. Regulation and Cellular Roles of Ubiquitin-Specific Deubiquitinating Enzymes. Annual Review of Biochemistry. 2009;78(1):363-97. doi: 10.1146/annurev.biochem.78.082307.091526.

23. Theodoropoulou M, Reincke M, Fassnacht M, Komada M. Decoding the genetic basis of Cushing's disease: USP8 in the spotlight. European journal of endocrinology. 2015;173(4):M73-83. doi: 10.1530/eje-15-0320.

24. Jing X, Chen Y, Chen Y, Shi G, Lv S, Cheng N, et al. Down-regulation of USP8 Inhibits Cholangiocarcinoma Cell Proliferation and Invasion. Cancer Manag Res. 2020;12:2185-94. doi: 10.2147/cmar.s234586.

25. Yan M, Zhao C, Wei N, Wu X, Cui J, Xing Y. High Expression of Ubiquitin-Specific Protease 8 (USP8) Is Associated with Poor Prognosis in Patients with Cervical Squamous Cell Carcinoma. Medical science monitor : international medical journal of experimental and clinical research. 2018;24:4934-43. doi: 10.12659/msm.909235.

26. Byun S, Lee SY, Lee J, Jeong CH, Farrand L, Lim S, et al. USP8 is a novel target for overcoming gefitinib resistance in lung cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2013;19(14):3894-904. doi: 10.1158/1078-0432.ccr-12-3696.

27. Reincke M, Sbiera S, Hayakawa A, Theodoropoulou M, Osswald A, Beuschlein F, et al. Mutations in the deubiquitinase gene USP8 cause Cushing's disease. Nature Genetics. 2015;47(1):31-8. doi: 10.1038/ng.3166.

28. Ma Z-Y, Song Z-J, Chen J-H, Wang Y-F, Li S-Q, Zhou L-F, et al. Recurrent gain-of-function USP8 mutations in Cushing's disease. Cell Research. 2015;25(3):306-17. doi: 10.1038/cr.2015.20.

29. Renner U, Pagotto U, Arzt E, Stalla GK. Autocrine and paracrine roles of polypeptide growth factors, cytokines and vasogenic substances in normal and tumorous pituitary function and growth: a review. European journal of endocrinology. 1996;135(5):515-32. doi: 10.1530/eje.0.1350515.

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

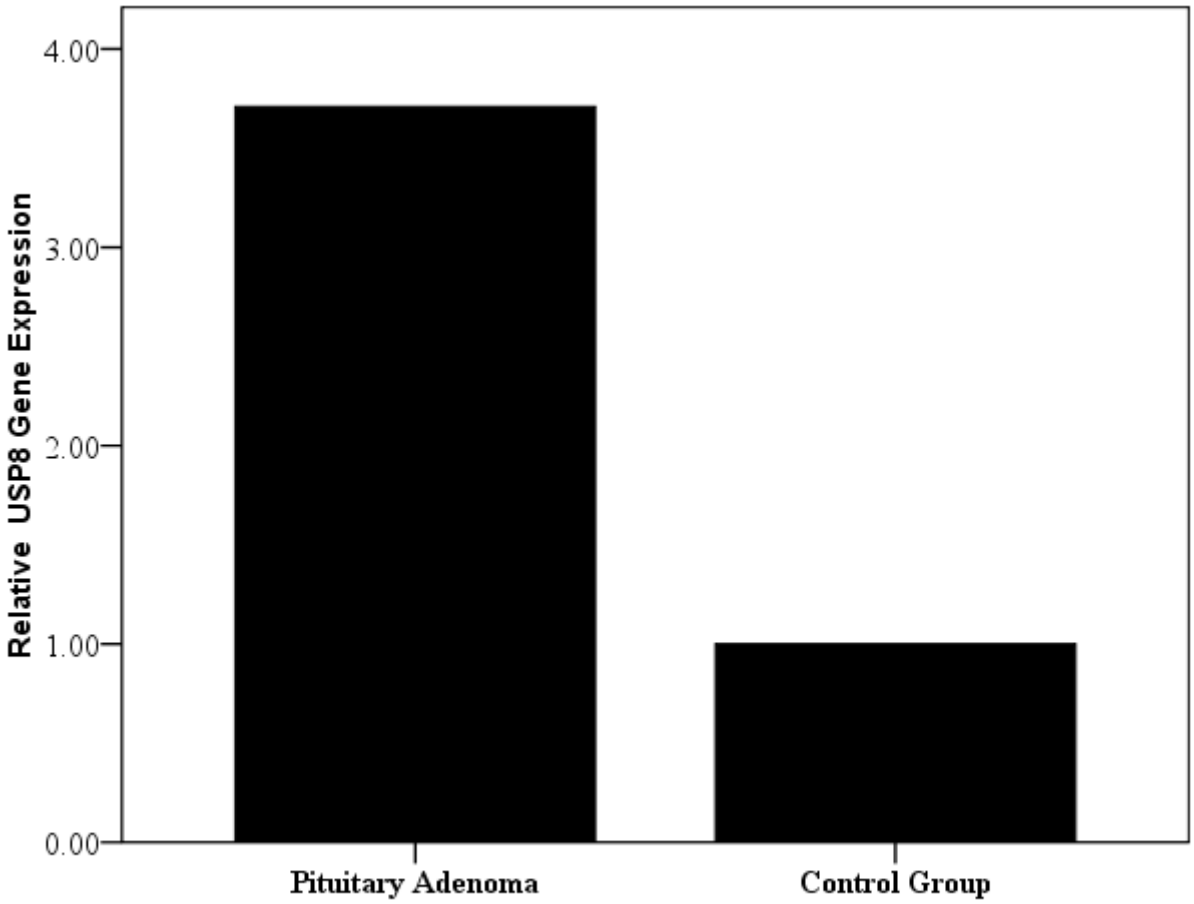

Figure 1

Relative expression levels of USP8 in pituitary adenomas and non-adenomatous brain tissue (control group)

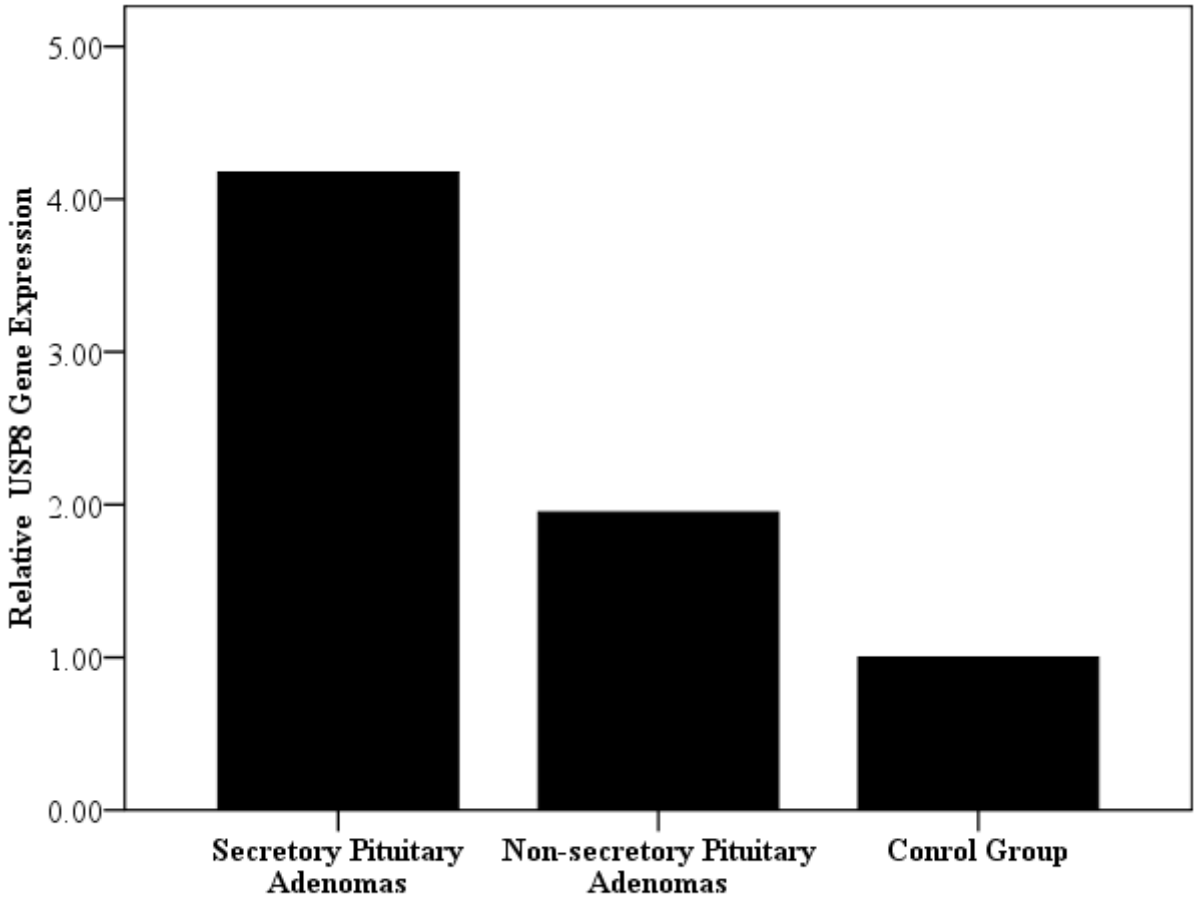

Figure 2

Relative expression levels of USP8 in secretory, nosecratory pituitary adenomas and, non-adenomatous brain tissue (control group)

Supplementary Files

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- Table1.docx