Endokrynologia Polska
DOI: 10.5603/EP.a2020.0073
Volume/Tom 72; Number/Numer 2/2021
ISSN 0423–104X, e-ISSN 2299–8306

Is there a common cause for paediatric Cushing's disease?

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Abstract

Introduction: According to recent literature, somatic mutations in the ubiquitin-specific protease 8 (*USP8*) gene are the most common changes in patients with Cushing's disease (CD). Data on the frequency of these mutations in the paediatric population are limited. The aim of the presented study was to determine the frequency of the *USP8* gene mutations in a group of paediatric patients with CD treated at the Children's Memorial Health Institute (CMHI).

Material and methods: Eighteen patients (nine females) with CD were treated at CMHI, Warsaw, Poland between 1993 and 2019. All patients underwent transsphenoidal surgery (TSS) as a primary treatment for CD. The average age of all patients at TSS was 13.10 years (5.42–17.25). DNA was extracted from formalin-fixed paraffin-embedded resected tumour tissue. Sanger sequencing was performed on DNA sequence corresponding to the exon 14 of USP8 gene.

Results: The mean age at diagnosis of CD was 13.08 years, and the average duration of symptoms before diagnosis was 2.96 years. All patients were operated at CMHI by the same neurosurgeon. Fifteen out of 18 patients (83.33%) had initial biochemical remission after a single TSS procedure (post-operative serum cortisol < 1.8 μg/dL). The result of genetic testing was negative for all samples at the hotspot area of the *USP8* gene.

Conclusion: The current retrospective study demonstrates that mutations in the *USP8* gene may not be as common a cause of paediatric Cushing's disease, as previously reported. **(Endokrynol Pol 2021; 72 (2): 104–107)**

Key words: *Cushing's disease; transsphenoidal surgery; USP8 gene mutations; molecular background*

Introduction

Paediatric Cushing's disease (CD) is characterised by growth retardation with concomitant weight gain as well as other classic clinical features of CD (skin changes, psychiatric disorders, decreased bone mineral density, weakness, and others) caused by excessive secretion of adrenocorticotropic hormone (ACTH) [1–3]. The first-line treatment is transsphenoidal surgical resection (TSS) of the pituitary adenoma [4–6].

According to recent literature, somatic variants in the *USP8* gene encoding ubiquitin-specific protease 8 (USP8), clustered into a hotspot region overlapping with the 14-3-3 binding motif, are the most important in CD pathogenesis. The frequency of these variants is estimated to be 31–63%, but the performed studies have mainly concerned adult patients [7–11]. The effect of this mutation is increased deubiquitination of epidermal growth factor and thus increased induction of proopiomelanocortin transcription and secretion of ACTH [7]. Literature data on the clinical significance of the presence of mutations in the *USP8* gene in children are limited. There has only been one study on children, made by Faucz et al. on a group of 42 children aged 6.1–18.7 years with CD, indicating a frequency of somatic variants in the *USP8* gene of around 30% [8]. At the same time, published results indicate a different course of disease in children than in adults, with an increased risk of recurrence in the group of patients with a variant in the *USP8* hotspot [8]. The need for additional research is emphasised to verify the observed correlation. Hayashi et al. also showed that tumours with *USP8* variants have more type 5 somatostatin receptors that can be a therapeutic target with specific somatostatin analogues such as pasireotide [11].

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Material and methods

Eighteen patients (nine females) with diagnosed CD were treated at the Children's Memorial Health Institute (CMHI), Warsaw, Poland between 1993 and 2019. All patients underwent TSS as a primary treatment for CD (all patients were operated by the same neurosurgeon at CMHI). The average age of all patients at TSS was 13.10 years (5.42–17.25). The Institutional Bioethical Commission (10/KBE/2019) approved this study. Written informed consent from the parents and assent from the minor patients were obtained.

After excluding 12 patients (from 30 patients with CD) because of lack of patient's consent for genetic testing and/or lack of inability to obtain patient's consent or lack of material because surgery was outside CMHI, our study was performed on 22 tumour samples from 18 patients. Two samples from TSS2 were analysed. An additional two samples were from TSS1 of the same patient. Genetic testing was performed at the National Institutes of Health, Bethesda, MD. The tumour samples were anonymised so that no personal data were revealed to the centre carrying out the tests. The genetic material was resected tumour tissue secured in the form of paraffin blocks. The obtained anthropometric parameters were presented as standard deviations (SDS) for BMI using the LMS method [12, 13]. Z-score values were calculated from the formula:

$$z - score(x) = \frac{\left(\frac{X}{M}\right)^{L} - 1}{LxS}$$

The L, M, and S values were taken from the tables of the reference system for a given age and sex [14, 15].

Cortisol levels at night were defined as the mean values of two measurements, respectively: cortisol level at 00.00 H and 00.30 H. High 24-h urine free cortisol was defined as free urine cortisol more than 80 μg/m^2 [16]. The disease onset was defined as the moment when the first symptom of the disease occurred.

DNA was extracted from microdissected tumour tissues with the Pinpoint™ Slide Isolation System (Zymo, Irvine, CA). DNA was extracted after confirming that the selected cut of the tissue originated exclusively from the corticotroph adenoma, to exclude the possibility of mixing the tumour DNA sample with DNA from normal cells.

Somatic DNA encompassing the exon 14 hotspot region of the USP8 gene (amino acids 718 to 721) was amplified by end-point PCR (GoTaq Green Master Mix — M7123, Promega, Durham, NC) using the following primers: forward, 5′-CTTGACCCAATCACTGGAAC-3′ and reverse, 5′- TTACTGTTGGCTTCCTCTTCTC-3′. PCR product was purified (ExoSAP-IT™ Express — 75001.1.ML, Thermofisher — Waltham, MA) and direct bidirectional sequencing (BigDye Terminator 3.1 Cycle Sequencing Kit — 4337456, Applied Biosystems, Foster City, California) using a 3500xL Genetic Analyzer (Applied Biosystems) was performed. Sequences were analysed using Geneious Prime software v.2019.0.4 (Biomatters, San Diego, CA).

Results

Patient characteristics

In our group of 18 patients there was no sex predominance (Tab. 1). The mean age at diagnosis of CD was 13.08 years, and the average duration of symptoms before diagnosis was 2.96 years. The mean midnight serum cortisol and 24-hour urinary free cortisol (UFC) levels were 23.17 μg/dL and 692.43 μg/24 hours, respectively. In every patient 24-h urine free cortisol was increased [16]. Two of the 18 patients (11.12%) presented with evidence of cavernous sinus invasion (in one patient cavernous sinus invasion was detected after TSS2). In one patient (5.56%) the tumour penetrated the cavernous sinus and did not infiltrate its walls but dislocated the sinus.

Transsphenoidal pituitary surgery

All patients were operated at CMHI by the same neurosurgeon. Fifteen out of 18 patients (83.33%) had initial biochemical remission after a single TSS procedure (post-operative serum cortisol < 1.8 μg/dL [17]). One of three patients who did not have biochemical remission after TSS1 had successful hypophysectomy one year after TSS1. The overall rate of remission following TSS was 88.9%. In one patient biochemical remission was achieved after radiotherapy.

Pituitary histology

Histopathological examination confirmed corticotroph adenoma in 16/18 (88.9%) patients. Histopathological examinations of two patients revealed focal corticotroph hyperplasia and normal pituitary gland, respectively.

Table 1. *Data of analysed group of patients*

	Average	Min	Max
Age at diagnosis [years]	13.08	5.5	17.3
Time from first symptom to diagnosis [years]	2.92	0.8	9.00
Female sex n (%)	50.0		
BMI [kg/m^2]	24.27	19.53	31.50
BMI SDS	1.43	–0.30	2.48
Midnight plasma cortisol [μg/dL]	23.17	9.1	52.14
24-hour UFC [μg/24 h]	692.43	199.3	2263.2
Max. morning serum ACTH	131.23	49	536
Invasion to cavernous sinus, n (%)	2/18 (11.12%)		

ACTH — adrenocorticotropic hormone; BMI — body mass index; BMI SDS — body mass index standard deviation score; UFC — 24-hour urinary free cortisol

Follow-up

During a mean of 3.37 years. (0.17–8.33) of follow-up at CMHI, 15 patients (83.3%) were in remission, and one patient (5.56%) had recurrence of the disease. One patient died one month after TSS2 as a result of postoperative complications, and one patient had a persistent disease after TSS1 and TSS2 and was undergoing radiotherapy at latest follow-up.

Frequency of USP8 gene mutations

None of the samples showed any pathogenic variants at the known *USP8* hotspot region. One rare synonymous variant (c.2154C>T / p.Ser718= / rs1261832527 / / 0.0007%) was identified in the hotspot area. Surrounding the *USP8* hotspot area a new missense variant (predict as benign by PolyPhen in silico tool — http://genetics.bwh.harvard.edu/pph2/) was also identified: the variant p.Thr723Ile is formed by a transition from a C to a T at position 2168 (c.2168C>T).

Discussion

Somatic variants in the ubiquitin 8 specific protease gene have recently been identified as the most common changes in patients with CD. Including the studies of Reincke et al. (17 patients aged 33–56 yrs) [9], Perez-Rivas et al. (145 patients aged 7–76 yrs) [7], Ma et al. (120 patients aged 26–46 yrs) [10], and Albani et al. (34 patients aged 39–61 yrs) [18], the total frequency of *USP8* hotspot variants varies between 35 and 63%. Until now the paediatric population has not been extensively assessed, and its real frequency remains uncertain. In a recent study by Perez-Rivas et al. the frequency of *USP8* variants was lower in children than in adults (17% *vs*. 36%) [7]. In the biggest and the only paediatric study, the frequency of genetic changes in *USP8* gene was 31% [8]. In the present study, we did not find any pathogenic variants within the known USP8 hotspot region, comprising the amino acids 718 to 721. It is possible that a different population in the presented study could have influenced the results.

From the two variants identified in exon 14 in the presented study one was synonymous and within the hotspot region (p.Ser718=) and another was located nearby (p.Thr723Ile). Both were somatic. Because the first one is synonymous it is probably not related to the phenotype; however, without functional analysis, a creation of a splice site, for example, cannot be ruled out. For the p.Thr723Ile, even though an *in silico* tool predicted it as benign, the variant is very close to the hotspot, and the amino acid is highly conserved. However, so far, all disease-causing variants are located in the hotspot region, and position 723 does not seem to affect the 14-3-3 binding motif; so probably this variant also does not contribute to the formation of the corticotropinoma. Despite the low possibility of those two variants cause Cushing's disease, in the absence of functional studies is difficult to know if they are somehow responsible for the development of Cushing's disease in those patients. Additional functional tests should be performed in those two variants to rule them out as Cushing's disease drivers. Unfortunately, there were no conditions to perform such tests in the present work.

The identification of a molecular pathogenesis of CD is important because identified mutations create the opportunity to use new treatment targets. The results of our study imply the need for further research into other possible causes of CD in children. Among the possible somatic changes found in patients with CD are mutations in the following genes: *GNAS1*, *TP53*, *NR3C1* (Nuclear Receptor Subfamily 3 Group C Member 1), *NR0B1* (Nuclear receptor subfamily 0 group B member 1), *Brg1* (brahma-related gene 1), and *HDAC2* (Histone Deacetylase 2) [19–22]. Moreover, transcription factors associated with progenitor proliferation and differentiation (such as *TPIT*, *PROP1*, *LHX3*, *LHX4*, or *HESX1*) as well as somatic mutations in genes that cause syndromes associated with pituitary adenomas (*MEN1, PRKAR1A, AIP*) can be associated with molecular changes resulting in CD [23, 24]. However, the frequency of mutations in the above-mentioned genes is very low, and only a few literature reports add them to the range of potential factors in CD. In a study by Williamson et al. a variant in the *GNAS* was identified only in 2/32 ACTH-secreting adenomas [19]. Riminucci et al. reported a case report of an 11-year-old girl with CD with activating mutation in the *GNAS* gene. The girl did not achieve remission after TSS but only after radiotherapy [25].

Among the germline variants in patients with CD there are some genetic changes in genes such as *MEN1*, *CDKN1B* (MEN4), *AIP*, *DICER1*, and *CABLES1*; however, they are not responsible for a significant number of CD cases. Stratakis et al. studied a group of 74 children with CD, and germline changes in *MEN1* were detected in only two patients with positive family history with MEN1 [26]. The disease in these patients was recurrent or difficult to treat [26].

According to the literature, the *USP8* mutational status could predict remission and/or recurrence in patients with CD [18]. Our objective was also to correlate the presence of somatic *USP8* mutations with the rate of recurrence after successful TSS. Unfortunately, the negative results obtained prevent us from making such an analysis and from comparing between groups with and without a variant in the *USP8* gene.

Conclusions

The presented retrospective study demonstrates that variants in the *USP8* gene may not be a common cause of paediatric Cushing's disease in the cohort population. The knowledge about molecular background of corticotroph adenomas is still not entirely known. Future genetic research is essential to determine the pathology of Cushing's disease.

Acknowledgments

The authors would like to thank all patients who took part in the research.

Declaration of interest statement

C.A.S. holds a patent on the PRKAR1A, PDE11A, and GPR101 genes and/or their function, and his laboratory has received research funding from Pfizer Inc. F.R.F., who holds a patent on the GPR101 gene and/or its function.

The other authors declare that there is no conflict of interest.

Grants or other funding sources

This work was supported in part by the Intramural Research Program, *Eunice Kennedy Shriver* National Institute of Child Health & Human Development (NICHD), National Institutes of Health.

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