



Silent gonadotroph pituitary neuroendocrine tumor in a patient with tuberous sclerosis complex: evaluation of a possible molecular link

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Summary

Tuberous sclerosis complex (TSC) is an autosomal dominant multisystem hereditary cutaneous condition, characterized by multiple hamartomas. In rare cases, pituitary neuroendocrine tumors (PitNETs) have been described in patients with TSC, but the causal relationship between these two diseases is still under debate. TSC is mostly caused by mutations of two tumor suppressor genes, encoding for hamartin (TSC1) and tuberin (TSC2), controlling cell growth and proliferation. Here, we present the case of a 62-year-old Caucasian woman with TSC and a silent gonadotroph PitNET with suprasellar extension, treated with transsphenoidal endoscopic neurosurgery with complete resection. Therapeutic approaches based on mTOR signaling (i.e. everolimus) have been successfully used in patients with TSC and tested in non-functioning PitNET cellular models with promising results. Here, we observed a reduction of cell viability after an *in vitro* treatment of PitNET's derived primary cells with everolimus. TSC analysis retrieved no disease-associated variants with the exception of the heterozygous intronic variant c.4006-71C>T found in *TSC2*: the computational tools predicted a gain of a new splice site with consequent intron retention, not confirmed by an *in vitro* analysis of patient's lymphocyte-derived RNA. Further analyses are therefore needed to provide insights on the possible mechanisms involving the hamartin-tuberin complex in the pathogenesis of pituitary adenomas. However, our data further support previous observations of an antiproliferative effect of everolimus on PitNET.

Learning points:

- Pituitary neuroendocrine tumors (PitNET) in patients with tuberous sclerosis complex (TSC) are rare: only few cases have been reported in literature.
- Therapeutic approach related to mTOR signaling, such as everolimus, may be used in some patients with PitNETs as well as those with TSC.
- We reported a woman with both non-secreting PitNET and TSC; PitNET was surgically removed and classified as a silent gonadotroph tumor.
- Everolimus treatment in PitNET's-derived primary cells revealed a significant decrease in cell viability.
- Considering our case and available evidence, it is still unclear whether a PitNET is a part of TSC or just a coincidental tumor.



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Background

Tuberous sclerosis complex (TSC) is an autosomal dominant multisystem hereditary cutaneous condition, characterized by multiple hamartomas that can be associated to endocrine system alterations (1, 2). TSC is mostly caused by mutations of two tumor suppressor genes *TSC1* and *TSC2*, encoding for hamartin and tuberlin, respectively. In normal cells, hamartin and tuberlin form a molecular complex involved in intracellular signaling pathways controlling cell growth and proliferation, including the mammalian target of rapamycin (mTOR) cascade. Therapeutic approach related to mTOR signaling, such as everolimus, may be used in some patients (1, 2).

In nearly 10% of cases mutations are mosaic or intronic and a comprehensive genotype–phenotype correlation is still debatable. About two thirds of TSC cases are sporadic reflecting a high spontaneous mutation rate in these genes (3).

Some reports addressing PitNET in TSC patients suggest the possible involvement of pituitary gland alterations in the pathological process of TSC (4). The development of pituitary tumors by nearly half of adult Eker rats – i.e. spontaneous germline *TSC2* mutants – further supports this possible association (5). Up to now, however, no molecular evaluation of PitNET in patients with TSC has been performed to establish the weight of this link and whether PitNET should be considered a clinical manifestation of TSC is far from being clearly understood.

Case presentation

Here we present the case of a 62-year-old Caucasian woman whose diagnosis of TSC was made after the recognition of cognitive disability and neurobehavioral abnormalities of her only 32-year-old son, developed since childhood. She presented the typical TSC brain lesions at MRI (i.e. cortical tuber and right retinal hamartoma) without other typical TSC-related manifestations (e.g. renal, pulmonary, cardiac or skin lesions) and no family history of TSC. During the last 20 years she refused medical care, but in April 2012, she complained of headache: MRI scan showed a PitNET (size: 20×18×16 mm) with suprasellar extension, bilateral cavernous sinus invasion, shortened pituitary stalk and left optic nerve compression (Fig. 1A). Clinically, she had central obesity, moon face, congenital left blindness and a normal right visual field. A hormonal study revealed

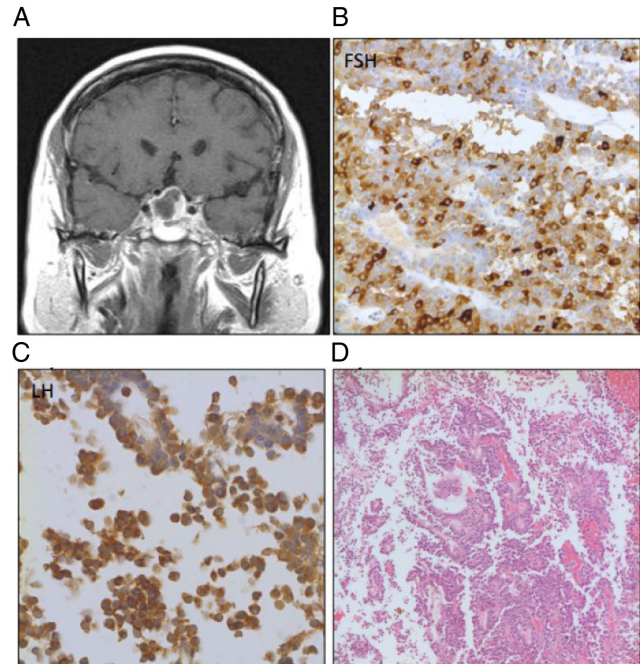


Figure 1

(A) Brain T1-weighted MRI after gadolinium injection: pituitary macroadenoma with suprasellar extension and bilateral cavernous sinus invasion; Immunohistochemical analysis of (B) FSH (20× magnification) and (C) LH (40× magnification) expression in the tissue slice of the silent gonadotroph PitNET (D) and standard hematoxylin-eosin staining.

normal thyroid and adrenal function, low gonadotropins (LH: 3.5 U/L, range: 11–61; FSH: 13.5 U/L, range: 35–150) and elevated prolactin (75.7–74–79 µg/L, range: 5–25, probably due to shortened pituitary stalk). After 4 months, she underwent transsphenoidal endoscopic neurosurgery with complete tumor resection. Histologic evaluation demonstrated a uniform PAS-positive basophilic adenoma with low proliferation index (MIB-1: <3%). Immunohistochemistry was positive for FSH and LH (Fig. 1B and C) and negative for ACTH, GH, prolactin and TSH. Clinical and histological findings were thus consistent with a silent gonadotroph PitNET.

Investigation

Neurosurgery is the first treatment choice for PitNET (6), especially for those non-functioning, which are still orphans of medical therapy. Reduction of cell viability as a consequence of an increased apoptosis has been reported after an *in vitro* treatment of non-secreting PitNET with everolimus and its possible use in patients has been suggested (7). Given the therapeutic potential of everolimus in TSC (1) and PitNETs (7) – both conditions

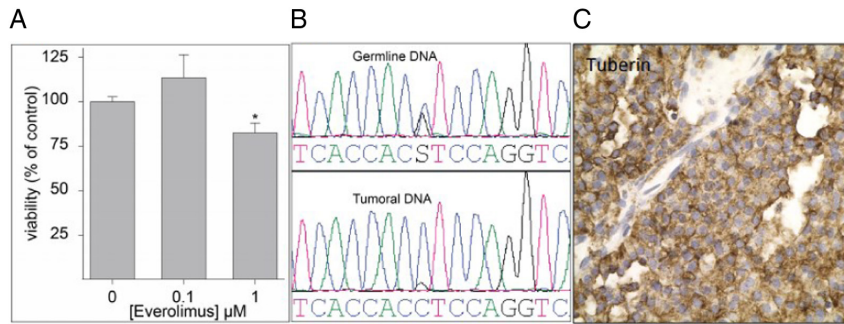


Figure 2

(A) Effects of everolimus treatment on pituitary tumor primary cells viability; (B) Electropherograms centered on the c.4006-71C>T variant both in germline (upper panel) and tumoral (lower panel) DNA; (C) immunohistochemical analysis of Tuberlin expression (40x magnification) in the tissue slice of the silent gonadotroph PitNET (D93F12, XP Rabbit mAb of Cell Signaling at 1:100 dilution after microwave antigen retrieval in 10mM citrate pH6.0).

present in our patient – primary cells derived from her pituitary adenoma were cultured *in vitro* (5000 cell/well in a 96 wells plate) and treated with everolimus (0.1 and 1 μM, kindly provided by Novartis) for evaluating its pharmacological effect. After 72-h treatment, the conversion of the tetrazolium dye MTT (Sigma-Aldrich) to formazan was ascertained following manufacturer protocol. After media removal and the addition of DMSO, the solution's absorbance was measured at 550 nm – background subtraction set at 620 nm – with a microplate reader (Victor3 V 1420 Multilabel 206 Counter, Perkin Elmer). Treatment with 1 μM induced a significant 20% decrease in cell viability ($P < 0.05$), confirming previous reported data (7) (Fig. 2A).

To search for the TSC disease-causative variant – and possibly associate it with pituitary adenoma development – patient's germline DNA was isolated from the peripheral blood and the genomic analysis of the entire *TSC1* (NM_000368.4) and *TSC2* (NM_000548.3) coding sequence and intronic boundaries was performed. The analysis retrieved no disease-associated variants with the exception of the heterozygous intronic variant c.4006-71C>T found in *TSC2* (Fig. 2B) and not present either in dbSNP or in ExAC databases. The computational tools MutationTaster and Human Splicing Finder both predicted a gain of a new splice site with consequent intron retention that was, however, not confirmed by an *in vitro* analysis of patient's lymphocyte-derived RNA. In addition, molecular analysis on archived paraffin-embedded pituitary tumoral tissues failed to identify both loss of heterozygosity in *TSC2* locus (Fig. 2B) and protein expression reduction (Fig. 2C), as expected if this would have been a loss-of-function mutation involved in the pathogenesis of this silent gonadotroph PitNET.

Treatment

We described the treatment in the intervention paragraph.

Outcome and follow-up

Pituitary imaging (with magnetic resonance) has been performed yearly, without evidence of a recurrent pituitary adenoma. The last visit was performed in May 2018.

Discussion

As far as we know, this is the first attempt to evaluate by a molecular approach the involvement of a TSC-related gene variant in the pathogenesis of the PitNET in a TSC patient. Considering the c.4006-71C>T variant causative for TSC – that is reasonable given the frequency of intronic mutations in TSC (i.e. 5%) (3) and its absence in the normal population – we could exclude the involvement of pituitary gland alterations in the pathological process of TSC, at least in this patient. On the other hand, however, until functional data on this variant has not been gathered, we cannot exclude possible alternative genetic mechanisms causative of TSC including mosaic mutations in *TSC1* regulatory regions – the mosaicism would easily explain the mild phenotype of our patient (3) – or an additional TSC causative gene. Nearly 10–15% of TSC patients lack, indeed, a conclusive molecular diagnosis (1, 2). Although relevant, our data are thus not conclusive for establishing unequivocally the causal nature of the association between TSC and PitNET that might only be a coincidence due to the relative high prevalence of pituitary tumors in the general population (8).

Although everolimus has been successfully tested in non-functioning and growth hormone-secreting PitNET cellular models (7, 9, 10), very few data are available on its use in PitNET patients. Everolimus has indeed been used for treating only two patients with pituitary carcinomas resistant to repeated resections, radiations and combined treatment with chemotherapeutic agents – i.e. Temozolomide and Capecitabine – with limited effects (11, 12). Our data corroborate the efficacy of everolimus in reducing cell viability in non-secreting PitNET and



support the need of further studies for demonstrating its efficacy and safety for treating this group of tumors.

Declaration of interest

Filippo Ceccato: PhD grant founded by Novartis. All other authors declare that they have no conflicts of interest that might be perceived as influencing the impartiality of the reported research.

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Patient consent

Written informed consent for publication of their clinical details and/or clinical images was obtained from the patient and is available for reviewers.

Author contribution statement

Daniela Regazzo, Marina Paola Gardiman, Marily Theodoropoulou performed the cell culture, *in vitro* study with everolimus, pituitary immunohistochemistry. Filippo Ceccato and Carla Scaroni were directly involved in the patient's clinical history and follow-up. Gianluca Occhi and Filippo Ceccato wrote the manuscript.

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