Atypical tuberous sclerosis complex presenting as familial renal cell carcinoma with leiomyomatous stroma

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Abstract

We report an atypical tuberous sclerosis complex (TSC) phenotype presenting as familial multiple renal cell carcinomas (RCCs) with (angio)leiomyomatous stroma (RCCLS) (5/7 familial RCCs) on a background of multiple angiomyolipomas, hypopigmented skin macules, and absence of neurological anomalies. In the index case and three relatives, germline genetic testing identified a heterozygous TSC2 missense pathogenic variant [c.2714 G>A, (p.Arg905Gln)], a rare TSC-associated alteration which has previously been associated with a milder TSC phenotype. Whole-exome sequencing of five RCCs from the index case and one RCC from his mother demonstrated either unique tumour-specific deleterious second hits in TSC2 or significant allelic imbalance at the TSC2 gene locus (5/6 RCCs). This study confirms the key tumourigenic role of tumour-specific TSC2 second hits in TSC-associated RCCs and supports the notion that RCCLS may be strongly related to abnormalities of the mTOR pathway.

Keywords: tuberous sclerosis complex; renal cell carcinoma; TSC2; renal cell carcinoma with (angio)leiomyomatous stroma; renal angiomyo-adenomatous tumour

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder characterised by hyperplastic, dysplastic, and hamartomatous lesions in various tissues. Its frequency is estimated at 1 in 6000 live births, 65% of cases being sporadic [1]. Disease results most frequently from inactivating alterations of tumour suppressor genes TSC1 and TSC2, respectively, encoding proteins hamartin and tuberin, which normally regulate protein synthesis and cell growth via the mTOR pathway. Genetic alterations resulting in constitutively active mTORC1 signalling drive cell hyperplasia and dysplasia in TSC patients, with the development of variably severe phenotypes. The skin, brain, kidney, heart, and eyes are the organs most frequently involved [2].

We report the case of a 52-year-old man originally referred to the medical genetics service because of a personal and familial history of renal cancer. Our index patient first came to medical attention for a history of right flank pain appearing at age 33 and lasting over a year, with a subsequent computed tomography (CT) scan disclosing multiple bilateral kidney lesions, among which there were six right cortical lesions including a dominant 2 cm upper pole nodule. The latter lesion was resected in 2000.
and was diagnosed as a renal cell carcinoma with (angio)leiomyomatous stroma (RCCLS; Figure 1A). Follow-up CT scan in April 2008 at age 42 disclosed a growing 1.5 cm solid left kidney mass, along with ipsilateral concurrent smaller lesions, warranting a left partial nephrectomy which revealed four RCCLS tumours associated with one lymph node metastasis and background multifocal angioleiomyomas (mainly leiomyomatous; Figure 1B–D). A concurrent 1.5 cm right kidney solid nodule with an ipsilateral 1.5 cm cyst were also noted, while positron emission tomography and CT imaging revealed multiple bony lesions, stable up to this day, which are probably TSC-associated sclerotic bone lesions (SBL) (supplementary material, Figure S1) [3]. Seven infracentimetric ground glass pulmonary opacities were also noted at the time and remained stable in size, most likely corresponding to multifocal micronodular pneumocyte hyperplasia (supplementary material, Figure S2). In August 2008, a right partial nephrectomy led to a diagnosis of chromophobe-like renal cell carcinoma (RCC) (Figure 1E). Only small bilateral renal cysts were visualised 5 years later, on the 2013 follow up Magnetic Resonance Imaging (MRI). At age 49, in 2015, our patient displayed suspicious para-aortic lymphadenopathies on imaging for which he underwent an aorto-caval lymph node dissection disclosing concurrent RCC metastasis and follicular lymphoma in both nodes identified. As of 2016, at age 51, follow up MRI identified an enlarging left renal complex cystic 1.2 cm lesion with a 0.5 cm enhancing component, suspicious for RCC. The patient is thus currently being followed by the urology service, as well as by haematologists regarding his follicular lymphoma. Additionally, a 2012 chest CT identified fatty lesions most suggestive of TSC-associated myocardial fatty foci (MFF), further characterised by cardiac MRI in 2017 [4–6]. The proband’s personal history otherwise also includes three melanocytic nevi resected from his thorax, shoulder, and arm at age 40, a dysplastic junctional melanocytic nevus at age 41, as well as an intrastromal melanocytic nevus of the right bulbar

Figure 1. Multiple RCCs resected in the proband to date, alongside corresponding genetic alterations detected by WES. Four RCCLS all demonstrate a clear cell papillary growth pattern with focally aggregated stromal smooth muscle fibres (A, B1, C1, D1; H&E stains). Stromal smooth muscle cells are highlighted using leiomyomatous immunohistochemical markers such as desmin (B2), muscle-specific actin (C2), and smooth muscle actin (D3). All these RCCLS tumours displayed IHC positivity for CK7, CD10, CAIX, and vimentin (both epithelial and stromal positivity; data not shown). The largest among the left-sided RCCLS tumours reached 1.5 cm in size, displaying heterogeneous contrast uptake on computed tomography imaging (D2; 2008 CT Scan with contrast, nephrogenic phase). The right kidney lesion excised at age 42 corresponded to a chromophobe-like RCC composed of nests/tubules of cells with irregular nuclear membranes (‘raisinoid’ nuclei), perinuclear cytoplasmic clearing, granular eosinophilic cytoplasm, conspicuous cytoplasmic membranes, and thick eosinophilic septa separating cell groups (E).
conjunctiva with microcysts and a right arm lipoma both resected at age 43. On physical examination, numerous brachial and thoracic infracentimetric hypopigmented macules had been noted, accentuated under Wood’s lamp (supplementary material, Figure S3). The same cutaneous lesions were allegedly present in the proband’s sister and grandmother, in addition to being documented in his son. The index case also displayed prominent facial sebaceous glands on macroscopic clinical assessment.

Review of the family history showed the proband’s mother to have presented at age 60 with similar RCCLS tumour on a background of multifocal leiomyomatous angiomylipomas (AMLs) (Figure 2), followed by melanoma at 65, which metastasized and caused her demise at 67. The proband’s sister was diagnosed with RCC at age 43 (identified subsequently to the tumours in the index case), his maternal half-brother was known for epilepsy and died accidentally at age 21, and both the proband’s father and sister were known for renal cysts, while his son had two seizure episodes at the age of 4, with an unremarkable brain MRI at that time and at age 11. Following a diagnosis of tuberous sclerosis in his father, he was found to have hypopigmented macules and ash leaf lesions.

Material and methods

To confirm a diagnosis of TSC, we tested the proband for TSC1/TSC2 gene alterations via massive parallel sequencing of peripheral blood [Illumina (San Diego, CA, USA), GeneDx (Gaithersburg, MD, USA) proprietary targeted capture]. Additionally, using core biopsies of formalin-fixed, paraffin-embedded (FFPE) archival tissues, we performed whole-exome sequencing (WES) of all five resected RCCs from the index case, as well as the RCC resected in his mother, with corresponding FFPE normal tissue controls. Bioinformatic analysis of exome sequencing data was performed using our WES pipeline as previously described [7,8]. In brief, sequenced reads were trimmed and mapped to the reference genome (hg19) using trimmomatic (v.0.35) and Burroughs-Wheeler aligner (v.0.5.9), respectively [9]. After mapping, GATK [10] and Picard (http://broad-institute.github.io/picard/) were used to perform local realignment around small insertions and deletions (indels) and to mark read duplicates. Then, GATK was applied to assess capture efficiency and coverage for all samples. A mean coverage of 114.4X (ranging from 41.6X to 156.4X) was obtained for all consensus coding sequences (CCDS) and 98.1% and 95.8% of CCDS bases are covered by at least 5 and 20 reads, respectively (supplementary material, Table S1). Samtools (v. 0.1.18) [11] and ANNOVAR [12] were used for variants calling [single nucleotide variants (SNVs) and indels] and functional annotations, respectively [12]. To remove common variants and false positive calls, candidate mutations were subjected to several filtering steps and eliminated if they fulfilled any one of the following criteria: (i) genomic position of variant covered by <5X, (ii) <4
The index case’s germline was shown to harbour a heterozygous TSC2 c.2714 G>A, (p.Arg905Gln), (R905Q) missense pathogenic variant following massive parallel sequencing of peripheral blood (Illumina, GeneDx proprietary targeted capture), with confirmation via capillary sequencing. This mutation has been acknowledged to be pathogenic in various publications (detailed in the ‘Discussion’ section below) [15]. The proband’s son and sister were shown to be heterozygous for the same pathogenic germline variant, warranting regular clinical follow up with the neurology service, with this same mutation being also demonstrated in the proband’s mother by WES of non-neoplastic FFPE tissue. Among the RCCs sampled in both the index case and his mother, all except one displayed either unique tumour-specific deleterious second hits in TSC2, or significant AI at the TSC2 gene locus on chromosome 16 (Table 1 and supplementary material, Figure S4). As expected, all FFPE RCC samples harboured the mutation previously demonstrated in the proband’s blood. In addition, we detected no truncating mutations in an array of 114 cancer predisposition genes as collated by Rahman (supplementary material, Table S2) [16]. No significant alterations were detected among the array of less well-known genes previously observed to be mutated by Tyburczy et al in the context of TSC-associated RCCs secondary to TSC2 second hits (SDHB, RASA1, TACC3, PROS1, NPFFR2, XYLTI, GALNTL6, NUP214, GLUD2, USP34, MS4A7, NDE1, ZNF507, ZRANB1, PANXI, PHF20, PNIISR) [17]; nor were any TCEB1 gene mutations detected [18].

Discussion

While the commonest TSC clinical findings are usually cutaneous and neurological, our index case’s phenotype is atypical in that no significant neurological abnormalities were documented throughout various investigations he underwent through the years, and he presented no sign of epilepsy (bearing in mind that no dedicated brain imaging was performed in him). Regarding TSC cutaneous findings as they pertain to our patient, hypomelanotic macules were shown to be the major cutaneous finding at presentation in 28 of 68 TSC patients in a recent series [19] and are reported in over 90% of TSC patients [2]. Accordingly, a minimum of three hypomelanotic macules over 5 mm in diameter is considered a major criterion sufficient to evoke a possible diagnosis of TSC, according to the International Tuberous Sclerosis Complex Consensus Conference [20]. The proband studied here happened to display scores of smaller 1–2 mm hypopigmented macules (‘guttate’ or ‘confetti macules’, ‘white freckles’), which are seen

### Table 1. Tumour-specific TSC2 alterations in various RCCs as per WES

| RCC tumour | RCC histotype | TSC2 alteration on WES |
|------------|---------------|-----------------------|
| Mother’s RCC | RCCLS | c.T1670A: p.L557X (29% AF) stopgain |
| RCC #1 | RCCLS | c.G4537T: p.E1513X (32% AF) stopgain |
| RCC #2 | RCCLS | Undetected |
| RCC #3 | RCCLS | Allelic imbalance (75% AF) |
| RCC #4 | RCCLS | c.351_352del:p.G117 fs (27% AF) frameshift deletion |
| RCC #5 | Chromophobe-like | Allelic imbalance (72% AF) |

AF, Allelic frequency.
in under 5% of TSC infants and constitute a minor diagnostic criterion of the disease, but are thought to be the pigmented lesion most specific for TSC (supplementary material, Figure S3) [21]. Another typical cutaneous lesion present in our index case is prominent facial sebaceous glands, usually occurring after puberty in TSC patients [22]. Regarding melanocytic neoplasms as they were observed in the proband and his mother, there have been so far only exceptional reports hinting toward an association between these and TSC [23], which could correlate with the relatively significant 10% prevalence of mTOR pathway alterations in melanoma [24]. Similarly, follicular lymphoma has not been reported to be associated with TSC yet, although 10% of follicular lymphomas were reported to display aberrant mTOR pathway activation in one recent study [25,26]. Further probing for mTOR pathway dysregulation and possible TSC gene alterations in both melanocytic lesions and follicular lymphoma may be interesting in that regard. Other present findings significantly correlated with TSC include MFF, which have been reported to be highly specific and 33–50% sensitive for TSC in a limited number of studies [4–6], as well as SBL [3]. On the whole, the TSC phenotype in our index case appears somewhat milder than could be expected in TSC patients in general, with only one cutaneous major diagnostic criterion present in the form of multiple hypomelanotic macules, notable absence of neurological diagnostic criteria, and finally the presence of one other major diagnostic criterion in the form of multiple AMLs, the latter adopting a leiomyomatous-only histology. The attenuated phenotype observed in the family studied here echoes previous reports having associated the TSC2 R905Q mutation with milder TSC phenotypes [15].

The cutaneous findings mentioned above combined with a history of multiple renal tumours led to further investigations that uncovered an uncommon TSC-associated inherited variant in the proband and his family. Indeed, the TSC2 exon 23 c.2714 G > A, p.Arg905Gln (R905Q) missense mutation is a rare pathogenic variant seen in under 1% of TSC patients (approximately 45 published cases, including 25 within a single family). It has been documented to be a French Canadian founder mutation resulting in reduced tuberin function and characteristically mild TSC phenotypes with little intrafamilial phenotypic variability, as studied in 19 TSC families by Jansen et al in 2006 [15]. This specific TSC2 R905Q pathogenic germline variant occurs at a methylated CpG site through a C to T deamination reaction, and was shown to lead to tuberin instability via increased interaction with HERC1 ubiquitin ligase, which interaction would not be blocked by TSC1 as it is in wild-type patients, resulting in increased TSC2 ubiquitination [27].

In our proband’s family, TSC-related kidney neoplasms in the form of AML and RCCLS were observed across two generations. Regarding kidney lesions in general, modified smooth muscle cells of the renal capsule have the potential to engender smooth muscle outgrowths and neoplasms. For instance, lymphangioleiomyomatosis secondary to TSC2 mutations, arising most frequently in the lung, can also develop in the kidney [28]. In the context of tuberous sclerosis, AML is the most common renal neoplasm encountered, affecting 80% of patients, usually as of the age of 10 [29]. While exhibiting a standard AML immunophenotype, some of these lesions adopt an exclusively leiomyomatous histology [30,31], as seen in the proband and his mother. RCC appears earlier and often multifocally in TSC patients [32], often with concurrent AML [33], but at an incidence of approximately 2%, roughly comparable to that of the general population [34,35]. Interestingly, a TSC2 variant was first reported to be associated with multiple bilateral RCCs by Tyburczy et al in 2015 in two unrelated individuals [17], and it is the exact same variant in TSC2 (p.R905Q) we have detected in three related RCC-affected individuals including one patient with multiple bilateral tumours. Our findings thus strengthen the association between TSC and multiple bilateral RCCs. Regarding specific RCC histotypes, 30% of TSC-associated RCCs display prominent intrinsic smooth muscle proliferation and clear cell cytology, a histological aspect designated ‘renal cell carcinoma with (angio)leiomyomatous stroma’ (RCCLS), this RCC subtype being commoner in TSC patients as opposed to the general population [36]. This distinct association of TSC with RCCLS explains that, among RCCLS tumours in general, 35% have been reported to be associated with TSC (6/17 RCCLS tumours), half of which could represent the sentinel manifestation for a TSC diagnosis (3/6 RCCLS in Verkarre’s 2014 study) [37]. While RCCLS and clear cell papillary RCC are considered by the International Society for Urological Pathology to potentially represent the ends of one lesional spectrum, only RCCLS is diffusely positive for CD10, as was the case in both the proband and his mother (Figure 2) [31,36]. Of note, this same RCCLS subtype has been documented to display aberrant Succinate Dehydrogenase B (SDHB) negativity on immunohistochemistry, similarly to the succinate dehydrogenase-deficient RCC subtype, although in the absence of detectable deleterious SDHB genetic alterations that portend the development of SDHB-
deficient RCCs [38]. Indeed, a recent study linked SDHB IHC signal rarefaction in RCCLS to decreased mitochondrial density as assessed by electron microscopy [39]. As expected, no likely pathogenic or clearly pathogenic SDHB alterations were detected among our RCC samples studied via massive parallel sequencing. In non-TSC individuals, some RCCLS tumours have been shown to harbour TCEB1 mutations [18], this being followed by HIF-1α activation as is also observed in TSC-associated RCCLS [40]. As would be expected in TSC-affected relatives, the five RCCLS tumours that underwent WES displayed no TCEB1 mutation (either nonsense, canonical splice-site, missense, or coding indels). Most notably, none of the Y79C/S/F/N or A100P hot-spot mutations were detected. Otherwise, among all histotypes encountered in TSC-associated RCC, the most prevalent is a chromophobe-like RCC that shows phenotypic similarities to the eosinophilic variant of chromophobe RCC. This chromophobe-like RCC represents 60% of TSC-associated RCCs [36], and one of our proband’s tumours corresponded to that phenotype (Figure 1E).

The TSC2 R905Q pathogenic germline variant seems to have carried an increased RCC risk in the present index case, his mother as well as his sister, particularly regarding RCCLS, which has developed in the first two relatives, including five occurrences in our proband. Genetically speaking, TSC-associated RCC, the most prevalent is a chromophobe-like RCC that shows phenotypic similarities to the eosinophilic variant of chromophobe RCC. This chromophobe-like RCC represents 60% of TSC-associated RCCs [36], and one of our proband’s tumours corresponded to that phenotype (Figure 1E).

The TSC2 R905Q pathogenic germline variant seems to have carried an increased RCC risk in the present index case, his mother as well as his sister, particularly regarding RCCLS, which has developed in the first two relatives, including five occurrences in our proband. Genetically speaking, TSC-associated smooth muscle proliferations like AMLs (28/30 tumours) [32,41] as well as more aggressive smooth muscle-rich proliferations such as RCCLS tumours have been shown to result from second hits in TSC1/2, as shown by Tyburczy et al on 5/5 fresh frozen RCCs and 6/7 RCCs in total [17]. In the context of the same background TSC2 R905Q pathogenic germline variant, we have been able to reproduce this finding using WES in 5/6 different FFPE RCCs from the index case and his mother. TSC1/2 second hits seem even more pivotal as a tumourigenic mechanism in TSC considering that they have been detected in angiofibromas arising in TSC patients [42]. Our demonstration of unique tumour-specific TSC2 deleterious second hits/AI in multiple bilateral RCCs including a high proportion of RCCLS tumours supports the notion that RCCLS may be strongly related to abnormalities of the mTOR pathway [32]. The molecular driver could well be the same in both TSC-associated RCCs and their non-familial equivalents, and we hypothesize that mTOR pathway alterations are likely to play a significant role in the tumourigenesis of non-familial RCCLS as well. This mechanistic foray into RCCLS pathogenesis further emphasizes the relevance of a molecular classification of different RCCs. Additionally, we believe that the high prevalence of RCCLS tumours in our index case raises the question whether this RCC subtype should be included as a minor criterion for the diagnosis of TSC.

Our study’s limitations include our inability to retrieve, sequence, and review the histology of the sole RCC resected in the proband’s sister, which may have allowed us to show further similarities between this tumour and other RCCs we analysed. Given the relatively low population prevalence of TSC and of this particular TSC2 pathogenic germline variant more specifically, the number of patients studied herein is limited, which restricts one’s ability to make statistically powerful inferences pertaining to the whole of TSC patients with a similar genotype/phenotype (external validity).

This confirmatory study highlights the key tumourigenic role of tumour-specific TSC2 second hits in TSC-associated RCCs. The TSC2 R905Q along with other TSC variants may furthermore specifically lead to increased frequency of the RCC histotype known as RCCLS, as this neoplasm was observed to make up a notably large proportion of the renal malignancies resected in both our index case and his mother (5/6 neoplasms). In all events, one should stress the importance of recognizing this uncommon RCC histotype and of distinguishing it from clear cell papillary RCC, as it is becoming more and more apparent that RCCLS may present as a sentinel anomaly in TSC patients with atypical phenotypes [37]. These data further emphasize the relevance of a molecular classification of both familial and sporadic RCC, as well as the potential benefits of mutation-specific management and counselling for TSC patients.

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Author contributions statement

WDF and IB designed the study. IB, WDF, LRB, SF, MDA, ST, and NH collected data. WDF, IB and NH conceived experiments. IB and NH carried out experiments. IB, WDF, LRB, SF, and NH analysed and interpreted data. IB and LRB performed literature search. IB generated figures. IB and SF drafted the manuscript. All authors were involved in the
manuscript revision and had final approval of the submitted and published versions.

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**SUPPLEMENTARY MATERIAL ONLINE**

**Figure S1.** Multiple osseous hyperdense, sclerotic lesions (blue circles) are visualized on computed tomography imaging, having remained stable since first imaged 9 years ago, in keeping with TSC-associated sclerotic bone lesions (2015 chest, abdomen, and pelvis CT scan with contrast)

**Figure S2.** Upper lobes of the lungs display multiple ground glass nodular densities (blue circles) on computed tomography imaging, which have remained stable since first imaged 9 years ago, most likely corresponding to multifocal micronodular pneumocyte hyperplasia (MMPH) (2014 chest CT scan with contrast)

**Figure S3.** Numerous infracentimetric hypopigmented macules can be noted on the proband’s back. These lesions were accentuated under Wood’s lamp. The same cutaneous lesions were allegedly present in the proband’s sister and grandmother, in addition to being documented in his son

**Figure S4.** The BAF profiles on chromosome 16 are shown for two RCC samples with AI. Each point on the plot represents a heterozygote variant and the position-wise significant loci are in orange. The x-axis shows the genomic positions in Mb. The y-axis indicates either BAF (for all points as well as the red bars) or absolute deviation of BAF from 0.5 (only for the segments in blue). For each significant segment, there are two red bars showing the mean of the BAF (for BAF > 0.5 and BAF < 0.5) for that segment

**Table S1.** Sequencing coverage

**Table S2.** Variant filtering strategy and identification of *TSC2* mutations