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SHORT COMMUNICATION

Birt–Hogg–Dubé syndrome: novel *FLCN* frameshift deletion in daughter and father with renal cell carcinomas

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Abstract Germline mutation of the *FLCN* gene causes Birt–Hogg–Dubé syndrome (BHD), a rare autosomal dominant condition characterized by skin fibrofolliculomas, lung cysts, spontaneous pneumothorax and renal tumours. We identified a hitherto unreported pathogenic *FLCN* frameshift deletion c.563delT (p.Phe188Serfs*35) in a family of a 46-year-old woman presented with macrohematuria due to bilateral chromophobe renal carcinomas. A heritable renal cancer was suspected due to the bilaterality of the tumour and as the father of this woman had suffered from renal cancer. Initially, however, BHD was overlooked by the medical team despite the highly suggestive clinical presentation. We assume that BHD is underdiagnosed, at least partially, due to low awareness of this variable condition and to insufficient use of appropriate genetic testing. Our study indicates that BHD and *FLCN* testing should be routinely considered in patients with positive family or personal history of renal tumours. In addition, we demonstrate how patients and their families can play a driving role in initiating genetic diagnosis, presymptomatic testing of at-risk relatives, targeted disease management, and genetic counselling of rare diseases such as BHD.

Keywords Birt–Hogg–Dubé syndrome · BHD · Folliculin · *FLCN* · Haploinsufficiency · Inherited kidney cancer

Introduction

Birt–Hogg–Dubé syndrome (BHD, OMIM #135150) is a rare autosomal dominant condition [1] characterized by skin fibrofolliculomas [2], lung cysts with associated spontaneous pneumothorax [3] and renal tumours [4]. The disease is caused by germline mutation of the *FLCN* gene at 17p11.2 (OMIM *607273), a tumour suppressor gene [5] encoding folliculin [6], whose function remains largely elusive despite intense research [7]. Since 2002, when the first mutations in the *FLCN* gene were reported [6, 8], only about 120 *FLCN* mutations have been published in BHD patients (HGMD Professional version 2015.2 and LOVD version May 2015). In this study, we report on a novel germline *FLCN* frameshift deletion. Moreover, we emphasize the importance of appropriate counselling and genetic testing in patients with clinical features of BHD.

Patients and methods

Clinical data and medical history

A 46-year-old German woman (index patient) presented with macrohematuria but no other complaint to her primary physician. Since hematuria increased after a week, she was referred to an urologist. Ultrasonography showed a mass in

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the right kidney. Subsequent computer tomography (CT) of the abdomen, thorax and pelvis presented inhomogeneous masses in both kidneys (6.6 × 5.4 cm right and 2.1 × 1.8 cm left) being highly suspicious of renal cancer with no indication of metastases (Fig. 1a, b). Both masses were removed by surgery. Pathology showed two morphologically similar but not identical well differentiated tumours, both signed out as eosinophilic variants of bilateral chromophobe renal carcinomas (Fig. 1c, d). Tumour, lymph node, and metastatic (TNM) stages were pT2aN0M0, G1, R0 and pT1aN0M0, G1, R0 at the right and left sides, respectively. In addition, thoracic CT revealed several small lung cysts, especially in the basilar region (Fig. 1e, g). Two years after the initial diagnosis of renal cancer, the index patient started with the recommended follow-up [4]. Magnetic resonance imaging (MRI) of the abdomen and additional CT of the thorax did not show any metastases, whereas the number and size of lung cysts remained stable. So far, the index patient has not developed any obvious fibrofolliculomas detectable in a clinical examination by an experienced dermatologist.

As the father of the index patient had kidney surgery a decade earlier, the husband of the index patient, an advanced nurse practitioner in the oncology field, suspected heritable renal cancer. After consulting the literature, he asked for a genetic counselling because of

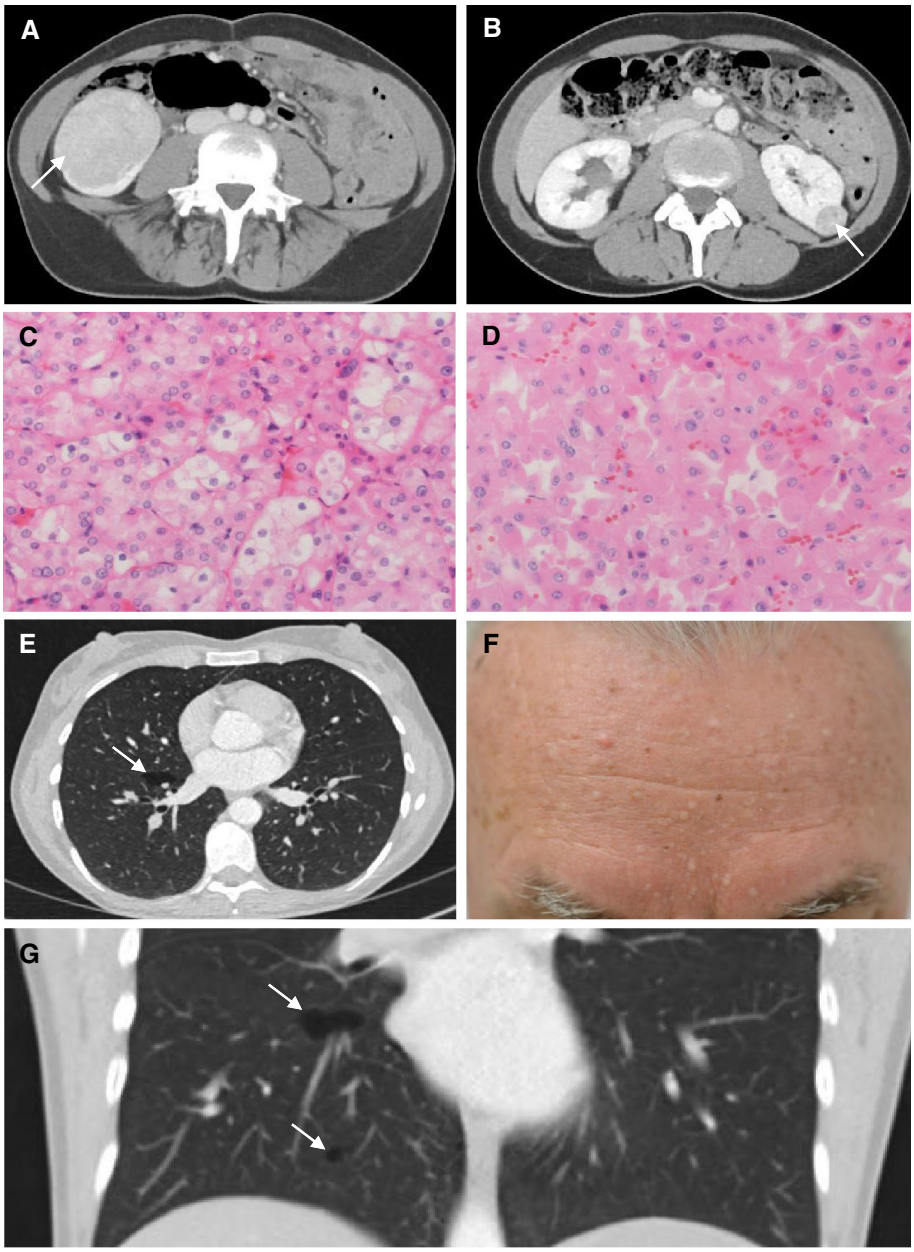

Fig. 1 Clinical manifestations of BHD in the index patient (**a**–**e** and **g**) and her father (**f**). **a** and **b** Abdominal CT showing renal tumors of the *right* **a** and *left* **b** kidneys, as indicated by *white arrows*. **c**, **d** Representative histopathology of the *right* **c** and *left* **d** renal tumors, revealing eosinophilic variants of chromophobe renal cell carcinomas (×40, H & E staining). **e**, **g** Thoracic CT showing lung cysts, as exemplified by *white arrows*. **f** Dome-shaped papules on the face of the index patient's father classified as fibrofolliculomas by skin biopsy

suspected BHD. The presence of familial BHD was supported by the chromophobe histology of the bilateral tumours and by lung cysts in the index patient as well as by the fact that her father had developed multiple of the typical dome-shaped papules at his face and neck in the last years, one of which was classified as fibrofolliculoma by skin biopsy (Fig. 1f). Moreover, the father had kidney surgery due to bilateral renal cancer but at the time of surgery he was not aware of the underlying disease. The pathology reports stated two clear cell renal cell carcinomas on the left side as well as multiple renal cell carcinomas and adenomas on the right side without specification of their histological types. The father also had suffered from skin basal cell carcinoma and at the age of 36 years from rectal adenocarcinoma (not investigated for Lynch syndrome). So far, no lung cysts have been detected in the father and neither he nor the index patient has had a history of pneumothorax. After appropriate genetic counselling, the index patient as well as her father and her two adult sons opted for the molecular genetic testing of the *FLCN* gene and provided informed consent.

Genetic testing

Genomic DNA was isolated from peripheral blood leukocytes. All coding exons (exons 4–14), and flanking intronic sequences of the *FLCN* gene were amplified by polymerase chain reaction and sequenced exon-by-exon using Sanger sequencing under routine conditions (details are available upon request). Detected sequence variant was verified by repeated sequencing on newly amplified PCR products. The control data set included publicly available whole exome sequences (WES) of ~60,000 unrelated individuals collected by the Exome Aggregation Consortium (ExAC, Cambridge, MA, http://exac.broadinstitute.org/gene/ENSG00000154803, accessed May 2015).

Transcript analysis

Total RNA was extracted from PAXgene-stabilized peripheral whole blood using the PAXgene blood RNA kit (Qiagen, Hilden, Germany; www.qiagen.com) according to the manufacturer's instructions. Semi-quantitative sequence analysis was performed as described elsewhere [9]. Accordingly, reverse transcription (RT)-PCR was performed to amplify complementary DNA (cDNA) fragments using the Qiagen OneStep RT-PCR kit (Qiagen, Hilden, Germany; www.qiagen.com) and cDNA-specific primers flanking the mutated exon. RT-PCR products were purified by ExoSAP-IT treatment (USB Corporation, Cleveland, OH; www.usbweb.com) and subsequently sequenced in both directions on an ABI PRISM 3100 Genetic Analyzer with POP-6 polymer using BigDye terminator v1.1 (Applied Biosystems, Zug, Switzerland; www.appliedbiosystems.com).

Results and discussion

In the index case, genetic testing revealed a heterozygous 1-bp deletion in exon 6 of the *FLCN* gene (NM_144997.5: c.563delT, p.Phe188Serfs*35; Fig. 2), which has not been previously reported and was not detected in ~60,000 unrelated individuals (~120,000 alleles in the ExAC database). This hitherto unreported deletion induces a frameshift in the normal open reading frame of the *FLCN* gene and the new reading frame of 35 codons ends in a premature termination codon (PTC). Due to PTC, the mutant transcript (mRNA) produced might be targeted for nonsense-mediated mRNA decay (NMD), which leads to the degradation of PTC-containing transcripts and hence results in functional haploinsufficiency [9]. Indeed, in PAXgene-stabilized blood samples semi-quantitative sequencing confirmed not only the presence of PTC-containing transcripts but also revealed reduced mutant transcript level in comparison to genomic DNA (gDNA with relative mutant allele frequency of 50 %), suggesting incomplete NMD (Fig. 2). Consequently, this novel frameshift deletion leading to PTC and NMD is most probably the reason for the clinical manifestation of BHD in the index patient and her father. In the affected tissues, however, the efficiency/extent of NMD remains to be elucidated.

In addition, genetic testing revealed the *FLCN* mutation c.563delT in the affected father and both still asymptomatic sons of the index patient (note that the sons are only 20 and 21 years old; Fig. 3). As the youngest patient with BHD in whom renal tumour has been reported was 20 years of age [10], both sons will start with appropriate tumour screening in the next future. The identification of the familial *FLCN* mutation enables the (presymptomatic) testing of other family members with a priori risk for BHD, allowing for targeted disease management and genetic counselling as well.

The husband of the index patient informed the family (first-degree relatives and siblings of the index patient's father and their descendants) by an appropriate letter (approved by a medical geneticist) with details on the clinical appearance, practical impact, and inheritance of BHD. Moreover, the letter indicated that testing of the familial *FLCN* mutation c.563delT is possible and that family doctor may receive additional information but BHD requires expert management in specialised genetic clinics. Six months later, the patient's father heard about lung problems, which turned out to be a spontaneous pneumothorax based on multiple lung cysts, in one of his 1st

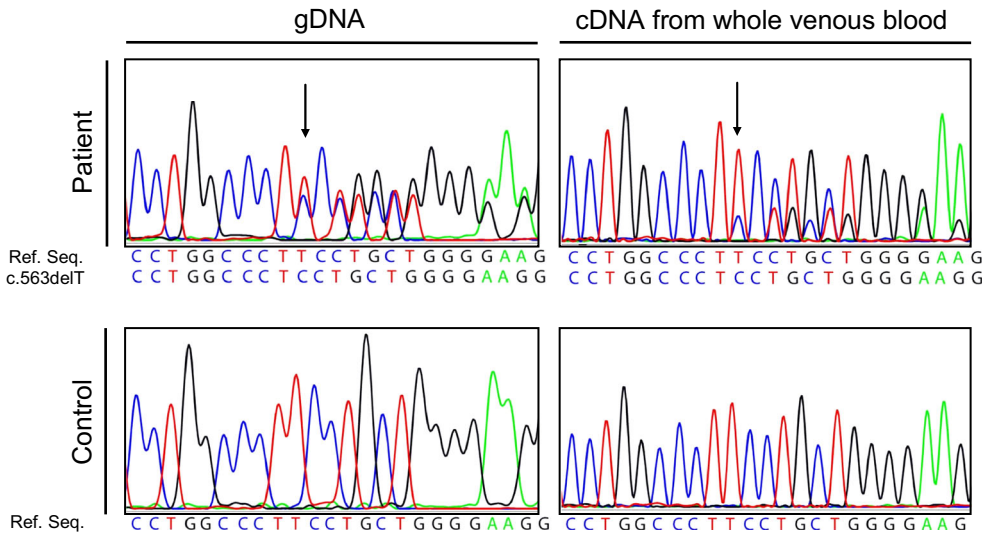

Fig. 2 Electropherograms showing the partial forward (5′ → 3′) sequence of the *FLCN* gene flanking the heterozygous deletion c.563delT (arrow) in the index patient (Patient) compared with a control individual (Control) at both genomic DNA (gDNA) and transcript (cDNA) levels. *Note* sequence positions where the sequence of the mutant (c.563delT) allele differs from the sequence of the wild-type (Ref. Seq.) allele: The cDNA sequence of the mutant allele resulted in reduced electropherogram peak heights in comparison with the corresponding gDNA sequence, demonstrating incomplete nonsense-mediated mRNA decay of the PTC-containing mutant transcript (p.Phe188Serfs*35) in leukocytes

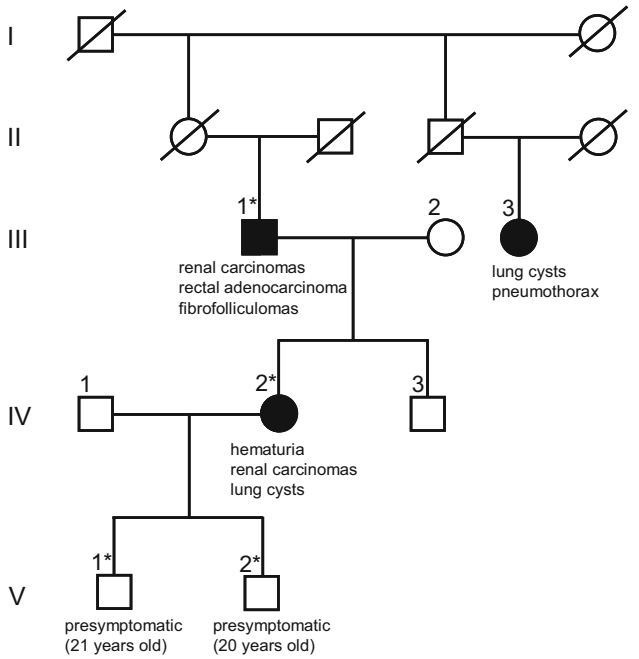

Fig. 3 Five-generation partial pedigree of the family of the index patient. Males and females are represented by *rectangles* and *circles*, respectively; individuals are numbered (1–3) per generation (*I–V*). *Asterisk* indicates the confirmed heterozygous presence of the *FLCN* deletion c.563delT (p.Phe188Serfs*35) in the index patient (*IV.2*), her father (*III.1*), and both sons (*V.1* and *V.2*). In her 1st cousin once removed (*III.3*) as well as in her asymptomatic mother (*III.2*), brother (*IV.3*), and husband (*IV.1*) no genetic testing was performed. *Black symbols* indicate individuals with clinical manifestations as given below the corresponding symbols

grade cousins (62-year-old female relative, Fig. 3). Thanks to the letter, a potential relation to BHD was recognised and the cousin is now in the decision process regarding genetic testing.

Unlike renal tumours in patients with other inherited kidney cancer syndromes [11], renal tumour pathology of the index patient and her father differed and also varied to some degree within these two individuals. This is consistent with the finding of Pavlovich et al. [12], who found that tumours of different histological types were detected in the same patient (in four of ten cases) and that tumour histology was not clearly concordant within affected families.

The index patient's father suffered from a rectal carcinoma at a remarkably young age of 36 years. A possible association between BHD and colon cancer has been reported elsewhere [13, 14]. While Zbar et al. [15] only found a statistically not significant higher risk for colonic tumours in BHD patients, Nahorski et al. [16] hypothesised that different mutations in *FLCN* might be associated with differing risks of colorectal cancer since they found a significantly higher risk of colorectal cancer in patients with an exon 11 mononucleotide tract mutation (c.1285dupC) compared to patients with a mutation in exon 6 (c.610_611delGCinsTA). These authors also suggested that *FLCN* gene mutations might represent rather a passenger than a driver mutation in colorectal cancer.

Skin basal cell carcinomas have been reported in BHD patients [17]. So far, however, a causal relationship has not

been proved. The occurrence of basal cell carcinoma in the index patient's father can also be explained by his heightened sun exposure as a carpenter. While he developed multiple fibrofolliculomas in the past 30 years (Fig. 1f), his daughter (the index patient) has not shown obvious sign of it. This observation can be explained by the facts that phenotype varies within BHD families with the same *FLCN* germline mutation and, although fibrofolliculomas are common in *FLCN* mutation carriers (>50 %), some of the BHD patients do not show cutaneous manifestations even beyond the age of 40 years [10, 18–20].

Thanks to sufficient information, the index patient's father was able to link the "lung problems" of his cousin to BHD. Indeed, spontaneous pneumothorax and multiple lung cysts in the 62-year-old cousin can be indicative of BHD. If the cousin decides for molecular genetic testing of the familial *FLCN* deletion and this mutation can be detected, a further branch of the large family should be informed about the risk of BHD and about the possibility of targeted genetic testing. However, our experience with the first round of information showed that some of the informed relatives hesitated to seriously deal with the risk to be affected by BHD. Despite the fact that the information letter emphasised the advantages of screening for renal cancer and restrained from some high-risk activities (such as scuba diving due to risk for spontaneous pneumothorax), informed relatives had a rather fatalistic view of the situation in the beginning, not least due to the fact that a disease-causing *FLCN* mutation as such cannot be corrected/reversed.

BHD patients and individuals with risk for BHD may have manifold questions and concerns [21]. For a helpful answer, appropriate counselling and information on this condition are required. As BHD is rare, however, there is low awareness of this condition. Our study demonstrates that even medical professionals need more awareness of BHD as the medical team in charge failed to think of this syndrome despite the highly suggestive clinical presentation. In fact, without the efforts of the patient's husband the diagnosis BHD would have been missed, with potential medical consequences for the family. In addition to original articles and reviews [22], the websites of GeneReviews (www.ncbi.nlm.nih.gov/books/NBK1522), OMIM (omim.org/entry/135150), Orphanet (www.orpha.net), and the BHD foundation (www.bhdsyndrome.org) as well as facebook users provide scientific and basic information not only for medical professionals but also for BHD patients and their relatives. The facebook or other online discussion groups can answer individual questions timely.

Our study suggests that *FLCN* analysis should be part of genetic testing in patients with suspected renal cancer, especially in cases with syndromic features of BHD. We believe that BHD has been underdiagnosed due to low awareness of this variable condition and to insufficient use of appropriate genetic testing. Considering that in the ExAC database (exac.broadinstitute.org/gene/ENSG00000154803) at least ~20 most likely pathogenic *FLCN* mutations (such as frameshift, stop, and splice site mutations) are listed for ~60,000 unrelated individuals, the genetic predisposition for BHD might be more frequent (up to ~1/3000) than one could suspect from the fact that only about 120 *FLCN* mutations have been described in the HGMD and LOVD databases (cf. the prevalence of BHD is estimated at 1/200,000 but the exact incidence is unknown; www.orpha.net). As our study demonstrates, adequate information gathering by patients and/or their relatives (in our case by the husband of the index patient) can significantly contribute to the successful, genetically confirmed diagnosis of BHD.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical standard Prior to molecular genetic testing, all tested individuals underwent pre- and post-test genetic counselling and signed an informed consent for genetic testing.

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