厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

Birt-Hogg-Dube (BHD)症候群の遺伝カウンセリングに関する研究

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研究要旨

バート・ホッグ・デュベ (Birt-Hogg-Dubé [BHD]) 症候群は常染色体優性遺伝性疾患である。責任遺伝子は17p11.2に存在し、14個のエクソンからなり、これまでに約40種類の変異が報告されている。遺伝形式が常染色体優性遺伝のため、発端者の確定診断がつくと同時に発症前診断を含めた対応が必要な血縁者が少なからず存在する。千葉大学病院遺伝子診療部で経験した4例のBHD症候群遺伝カウンセリング症例を検討した結果、2症例では発端者自身よりもむしろその次世代の方々への対応が求められた。しかし、発症前診断の必要性の有無をいつ、いかに伝えるか、発症前診断をいつの時点で実施すべきか、陽性結果が得られた場合の対応など、いずれも一律には実施できない状況にあることが明らかとなった。一方、遺伝性疾患における遺伝学的検査結果やきわめてプライベートな内容を含む遺伝カウンセリング記録の電子カルテ上の取り扱いについては施設毎に対応が異なっている、そこで臨床遺伝情報の取り扱いに関するアンケート調査を全国遺伝子医療部門連絡会議を通して実施した結果、過半数の施設では遺伝学的検査結果を電子カルテに掲載しているのに対し、遺伝カウンセリング記録の取り扱いについては慎重な施設が多かった。

A. 研究目的

Birt-Hogg-Dubé (BHD)症候群は常染色体優性遺伝形式をとり、浸透率も高いため、

1人の患者の確定診断がなされた場合はその血縁者への影響を考慮する必要がある。しがって、その遺伝子診断の実施に際して十分な遺伝カウンセリングを実施することが必要である。一方、遺伝カウンセリングの記録内容はきわめてプライベートな情報を含み、at risk の未発症者の将来を予見し得る発症前診断も行われる場合があり、カウンセリング記録や発症前遺伝子診断結果を診療録上、特に電子カルテにおいてどのように取り扱うべきかが問題となる。そこで本研究では

1)千葉大学病院遺伝子診療部で経験した BHD 症候群の遺伝カウンセリング症例を検討して本疾患の臨床遺伝医学的問題点を明らかにすること

2)遺伝性疾患の検査結果および遺伝カウンセリング記録の電子カルテ上の取り扱いに関する実態を調査すること

の2点を目的とした.

B. 研究方法

千葉大学病院遺伝子診療部で経験した4例（疑い症例1例を含む）の BHD 症候群の遺伝カウンセリング記録を検討した。

遺伝情報の取り扱いに関する実態調査は全国遺伝子医療部門連絡会議の維持会員（依頼当時 94 施設）の所属する施設を対象に郵送により送付して行った。主なアンケート項目の以下の7項目である.

(1)遺伝医療記録（遺伝カウンセリング記録、遺伝学的検査結果）と一般診療録を切り離しているか？

(2)遺伝学的検査はどの部署が責任を持って管理しているか？

(3)ゲノム薬理学情報はどのように扱っているか？

(4)電子カルテシステムを導入しているか？

(5)遺伝カウンセリング記録を電子カルテ上に掲載しているか？

(6)遺伝学的検査の結果を電子カルテ上に記載しているか？

(7)遺伝医療記録へのアクセス制限をかけているか？

（倫理面への配慮）

(ア)本研究におけるヒト血液・組織の使用については, 実施施設および関連施設のヒトゲノム・遺伝子解析研究に関する倫理審査承認を受けて施行している. 検体サンプリングは, 病院臨床倫理審査承認を受けたうえで主治医の協力体制のもと,患者各位から同意文書を得るシステムの利用規約を遵守している.

(イ)個人情報の取り扱いについては, 個人名が推定されるような記載はとっておらず, 各疾患においては年齢, 性別のみを記載している. また個人名が推測できるような発表形態は行わない.

(ウ)安全対策，環境保護対策については総合安全衛生機構の安全衛生管理マニュアルおよび大学環境 ISO に従い，年 1-2 回の査察と指導を受けている．

C. 研究結果

1)BHD 症候群遺伝カウンセリング症例の検討

千葉大学病院遺伝子診療部で遺伝カウンセリングを担当した 4 症例の男女比は 1:3、年齢は 32 歳~61 歳であった。各症例の遺伝カウンセリングの主たる目的は症例 1 では遺伝学的検査の説明、症例 2,3 は遺伝学的検査の説明に加えて、次世代のお子さん方への影響を心配され、その対応についての相談が主体であった。症例 4 では実母が BHD 疑いと言われた段階でその娘がネット検索などで自分への遺伝を心配して、直接来院されたケースであった。本例では最終的には実母の BHD 症候群の確定診断は得られなかった。

2)遺伝情報の取り扱いに関するアンケート調査

対象は遺伝子医療部門連絡会議維持会員 94 施設で 84 施設(89.3%)から回答を得た。遺伝医療記録（遺伝学的検査結果および遺伝カウンセリング記録）と一般診療録との区別については、「遺伝医療記録すべて切り離し」、「遺伝カウンセリング記録のみ切り離し」、「遺伝医療記録と一般診療記録は区別しない」、がそれぞれ 1/3 ずつであった。また、電子カルテ化している 72 施設のうち、40 施設（55.5%）において遺伝学的検査結果を電子カルテ上に掲載し、32 施設（44%）では遺伝カウンセリング記録の一部またはすべてを掲載していた。また 10 施設(14%)が遺伝医療記録にアクセス制限をかけていた。ゲノム薬理学情報は 58 施設(80%)で一般診療録扱いであった。フリーコメントにおいては遺伝学的検査結果の中央管理の重要性、電子カルテ上の遺伝医療情報の取り扱い方についての指針の必要性等の指摘があった。

D. 考察

遺伝学的検査で取りつかわれる情報は以下の理由で通常の臨床検査と異なる。

・ 生涯変化しないこと．
・ 血縁者間で一部共有されていること．
・ 血縁関係にある親族の遺伝型や表現型が比較的正確な確率で予測できること．
・ 非発症保因者（将来的に発症する可能性はほとんどないが，遺伝子変異を有しており，その変異を次世代に伝える可能性のある者）の診断ができる場合があること．
・ 発症する前に将来の発症をほぼ確実に予測することができる場合があること．
・ 出生前診断に利用できる場合があること

したがって、その実施にあたっては適切な遺伝カウンセリングが必要である。

遺伝カウンセリングは，疾患の遺伝学的関与について，その医学的影響，心理学的影響および家族への影響を人々が理解し，それに適応していくことを助けるプロセスである．このプロセスには）疾患の発生および再発の可能性を評価するための家族歴および病歴の解釈，2）遺伝現象，検査，マネージメント，予防，資源および研究についての教育，3）インフォームド・チョイス（十分な情報を得た上での自律的選択），およびリスクや状況への適応を促進するためのカウンセリング，などが含まれる

今回、我々が経験した 4 症例のうち 2 症例では発端者は御自分の今後のことよりもよりもむしろ次世代の方々への遺伝の有無そして遺伝している場合はいかに対応すべきかを心配して来談されていた。しかし、次世代の方々に遺伝性疾患であることをいつどのように伝えるべきか、その発症前遺伝子検査を行うべきか、行うとすればいつ行うか、などについてはケース毎の対応が必要と思われる。1 症例では発端者の確定診断がつく前からその娘さんがインターネットなどを通して得た情報をもとにご自分やそのお子さんのことを心配して来談されたが、最終的に発端者の確定診断が得られないケースであった。

電子カルテの普及に伴い、遺伝学的検査結果のうち、確定診断・除外診断目的で行われ

たものについてはその結果を電子カルテ上で共有する傾向になりつつある。しかし、遺伝カウンセリング記録の取り扱い方については意見が分かれる。診療科側からの「遺伝カウンセリング内容を知りたい」との要望は十分理解できるので、電子カル上に遺伝カウンセリング記録を何らかの形で掲載するべきと思われる。また、遺伝子診療部に限らず、すべての診療科の診療において家系図は必要であることは言うまでもないが、遺伝カウンセリングの場において初めて明かされるようなきわめてプライベートな情報を含む詳細な家系図の取り扱いは慎重であるべきである。

なお、千葉大学病院における遺伝医療情報の管理に対する基本的な考え方と現状を以下に述べる。遺伝子診療部を介して実施された確定診断・除外診断目的の遺伝学的検査結果は電子カルテ上に記載するが、解析結果の詳細については遺伝子診療部で長期的に中央管理することとしている。各診療科が独自のルートで実施した確定診断については各診療科に委ねる。発症前診断・保因者診断は直接遺伝子診療部に依頼される場合が多いので、カウンセリング記録および検査結果については遺伝子診療部において紙ベースで

保管する。診療科を通して依頼された発症前診断の結果の取り扱いについてはまだ結論が得られていない。
一方、遺伝カウンセリング記録については診療科担当医師からカウンセリングの具体的内容を知りたいとの要望に応え、400字程度の概要を電子カルテに掲載し、その他のきわめてプライベートな情報については遺伝子診療部で別保管するようにしている。

F. 健康危険情報
なし

G. 研究発表
<学会発表>
1) 国内
口頭発表
○野村文夫ほか:遺伝情報の取り扱いに関するアンケート結果－倫理問題検討委員会報告－第37回日本遺伝カウンセリング学会学術集会、川崎市、2013年6月

ポスター発表
○野村文夫ほか:千葉大学病院における遺伝カウンセリングロールプレイ実習. 第37回日本遺伝カウンセリング学会学術集会、川崎市、2013年6月

2) 海外
口頭発表
○Sawai S, Nomura F et al: Multiple genetic testing of 10 types of autosomal dominant spinocerebellar ataxias by multiple PCR and repeat-primed PCR. European Human Genetics Conferences 2013 Paris, France, 2013 June

<論文発表>
1) 原著
○ Nishimura M, Nomura F et al: Human apoprotein E resequencing by proteomic Analysis and its application to serotyping. PLoS One. 2014 Jan 14;9(1):e8
2) 著書
○野村文夫：遺伝学的検査. 福嶋義光監修. 遺伝医学やさしい系統講義. pp177-194. メディカル・サイエンス・インターナショナル、2013

H. 知的所有権の出願・取得状況（予定を含む
1 特許取得
該当なし
2 実用新案登録
該当なし
3 その他
該当なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

本邦におけるBirt-Hogg-Dube (BHD)症候群の疫学研究

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研究要旨
バート・ホッグ・デュベ (Birt-Hogg-Dubé [BHD]) 症候群は中高年に腎癌を発生する可能性が高い常染色体優性遺伝性疾患である．これまで当研究班および複数施設の医師・研究者から本邦におけるBHD症候群例が報告されてきたが，正確な患者数はいまだ把握されていない．当研究班では日本病理学会の後援を受けて平成26年3月に全国医療機関に大規模なアンケート調査を施行した．782施設に封書によるアンケートを送付し，185施設より回答を得た(回収率23.7%)．遺伝子検査によりBHD症候群と確定された症例は呼吸器疾患から14例，腎疾患から6例，皮膚疾患から1例であった．また遺伝子検査未施行であっても臨床的にBHD症候群と診断された症例が呼吸器疾患から9例，腎疾患から4例，皮膚疾患から3例であった．2014年現在，学会などのコンセンサスを得たBHD症候群の診断基準はまだ確立されておらず，診断後の診療も施設ごとにばらばらである．BHD症候群の患者と家族に対する診断基準および診療ガイドラインもの作成などを急ぐ必要がある．

A. 研究目的

本邦における BHD 症候群の罹患率はいまだ不明である．当研究班では 2014 年 3 月時点で累計 59 家系の BHD 症候群を遺伝子検査により確定した．家族性腫瘍のなかには必ずしも遺伝子検査を必要とせず臨床的な診断基準を満たせば確定する疾患も少なからず存在する．遺伝子検査は臨床遺伝専門医のいる医療機関で適切なカウンセリングのもとに施行すべきものであり，疾患情報の乏しいなかでは遺伝子検査を受けることに消極的な患者もいるため，日本人における BHD 症候群の症状や腎癌発症率などを把握することは大変重要である．正確な情報をもとに診断基準と診療ガイドラインを作成することが喫緊の課題であるため，全国的なアンケート調査を行うこととした．

B. 研究方法

BHD 症候群は呼吸器科・泌尿器科・皮膚科など多科の診療科にまたがる疾患のため，全診療科からの情報が得られる病理診断科・臨床検査科に対して第 1 回目のアンケートを施行した．アンケート内容に関しては事前に横浜市立大学・医学研究科の倫理審査承認を受け，日本病理学会の学術委員会に諮って作成した．日本病理学会認定施設あるいは登録施設に入っている 782 施設を対象に郵送でアンケート調査を行い，返信された情報を収集解析した．

具体的なアンケート項目は以下のとおりである．肺・腎臓・皮膚検体それぞれに関して，過去 20 年に質問に該当する項目ボックス□にチェックをいれる方式をとり，参考として年間の手術検体数の記入をお願いした．

1. 肺切除検体
1-A: 以下の診断名に該当する検体
□BHD 症候群疑い・BHD 症候群として矛盾しない．
□部位が典型的ではないブラ・ブレブ．
□LAM や子宮内膜症疑いなどと診断したが、非典型的な嚢胞性変化．
□診断困難な嚢胞性変化であったため、所見のみを記載．
□その他(自由記載)
1-B: 1-A で「BHD 症候群疑い・BHD 症候群として矛盾しない」を選択した場合
□既に遺伝子診断された BHD 症候群．
□遺伝子診断されていないが、症状から BHD 症候群(疑い例を含む)
□その他(自由記載)
1-C: 1-A で「BHD 症候群」以外の非典型症例ボックスを選択した場合
□その他の検査から BHD 症候群と診断された．
□その他の検査から BHD 症候群以外の疾患と診断された．
□経過不明
□その他(自由記載)
1-D: 年間肺切除検体例数(自由記載)

2. 腎手術検体
2-A: 以下の診断名に該当する検体
□BHD 症候群疑い・BHD 症候群として

矛盾しない腎癌・腎腫瘍
□多発性(異時性を含む)腎癌 and/or 家族性腎癌.
□嫌色素性腎細胞癌やオンコサイトーマなど多彩な組織からなる腎癌.
□多発性オンコサイトーシス.
□非腫瘍部にも上皮性胞巣が散見される腎腫瘍
□その他(自由記載)

2-B: 2-A で「BHD 症候群疑い・BHD 症候群として矛盾しない」を選択した場合
□既に遺伝子診断された BHD 症候群.
□遺伝子診断されていないが、症状から BHD 症候群(疑い例を含む)
□その他(自由記載)

2-C: 2-A で「BHD 症候群」以外の非典型症例ボックスを選択した場合
□その他の検査から BHD 症候群と診断された.
□その他の検査から BHD 症候群以外の疾患と診断された.
□経過不明
□その他(自由記載)

2-D: 年間腎切除検体例数(自由記載)

3. 皮膚手術検体

3-A: 以下の診断名に該当する検体
□BHD 症候群疑い・BHD 症候群として矛盾しない皮膚腫瘍・多発性皮膚丘疹
□BHD 症候群とされている (疑い例を含む) が、非特異的皮膚所見.
□背景不明の fibrofolliculoma, perifollicular fiboroma, trichodiscoma, acrochordon など.
□その他(自由記載)

3-B: 3-A で「BHD 症候群疑い・BHD 症候群として矛盾しない」を選択した場合
□既に遺伝子診断された BHD 症候群.
□遺伝子診断されていないが、症状から BHD 症候群(疑い例を含む)
□その他(自由記載)

3-C: 3-A で「BHD 症候群」以外の非典型症例ボックスを選択した場合
□その他の検査から BHD 症候群と診断された.
□その他の検査から BHD 症候群以外の疾患と診断された.
□経過不明
□その他(自由記載)

3-D: 年間皮膚生検検体例数(自由記載)

(倫理面への配慮)
(ア)本研究におけるプライバシー保護については横浜市立大学医学研究科においてヒトゲノム・遺伝子解析研究に関する倫理審査承認を受けて施行している. 検体サンプリングは, 病院臨床倫理審査承認を受けたうえで主治医の協力体制のもと,患者各位から同意文書を得るシステムの利用規約を遵守している.
(イ)個人情報の取り扱いについては, 個人名が推定されるような記載はとっておらず, 各疾患においては年齢, 性別のみを記載している. また個人名が推測できるような発表形態は行わない.
(ウ)安全対策, 環境保護対策については総合安全衛生機構の安全衛生管理マニュアルおよび大学環境 ISO に従い, 年 1-2 回の査察と指導を受けている.

C. 研究結果
782 施設に封書によるアンケートを送付し, 185 施設より回答を得た(回収率 23.7%). 遺伝子検査により BHD 症候群と確定された症例は呼吸器疾患から 14 例, 腎疾患から 6 例, 皮膚疾患から 1 例であった. また遺伝子検査未施行であっても臨床的に BHD 症候群と診断された症例が呼吸器疾患から 9 例, 腎疾患から 4 例, 皮膚疾患から 3 例であった.

肺病変に関しては, LAM や子宮内膜症疑いなどと診断したが非典型的な嚢胞性変化は 11 例, 診断困難な嚢胞性変化はであったため所見診断になったものは 16 例あり, いずれもそれ以上の精査に関する情報は得られなかった. BHD 症候群に関連する肺嚢胞の存在を知らなかったとの回答が 3 施設から寄せられた.

腎病変に関しては, 多発性(異時性を含む)腎癌 and/or 家族性腎癌が 24 例, 嫌色素性腎細胞癌やオンコサイトーマなど多彩な組織からなる腎癌が 22 例, 多発性オンコサイトーシスが 7 例あるとの情報が寄せられた.

皮膚病変に関しては, 背景不明の fibrofolliculoma, perifollicular fiboroma, trichodiscoma, acrochordon などが 33 例あるとの情報が寄せられた. BHD 症候群として矛盾しない皮膚腫瘍があるとされた 1 例および BHD 症候群とされている (疑い例を含む) が非特異的皮膚所見を有するとされた 3 例のアンケートには, いずれも腎腫瘍あるいは呼吸器症状を有している症例件数が記載されており, 発見の契機が必ずしも皮膚病変ではない可能性がある.

D. 考察
全国医療機関へのアンケート調査から, BHD 症候群の疾患概念がまだ医療関係者に十分に把握されていないことが改めて判った. また確定例の診断根拠が必ずしも遺伝子検査によるものではない症例が 16 例報告され, 診断基準が定まっていないため独自に診断をしている診療現場の実態が浮かび上がった. 今回病理診断医・臨床検査医を対象にしたアンケート調査では, 診断後の腎臓フォローに関する情報は得られなかった. 今後は呼吸器症状や皮膚症状で発見される

BHD 症候群患者が適切な腎臓フォローを受けているかどうかを調べ，診断することのメリットがきちんと患者さんと保因者家族に還元される診療ガイドラインを確立することが重要である．また切除検体の病理所見からBHD症候群の可能性が指摘されても，その後の検査や経過が診療医間で共有されていないことも分かった．多臓器に症状を発症する遺伝性疾患全般に言えることだが，患者と家族のプライバシー保護を守りながら診療情報を共有することで正しい診断と腎癌死・透析の減少につなげることが重要である．

F. 健康危険情報
なし

G. 研究発表
7-8 頁参照のこと

H. 知的所有権の出願・取得状況（予定を含む
1 特許取得
該当なし
2 実用新案登録
該当なし
3 その他
該当なし

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

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Ⅳ．研究成果の刊行物・別刷

OPEN ACCESS

Birt–Hogg–Dubé syndrome: clinicopathological features of the lung

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ABSTRACT

Birt–Hogg–Dubé syndrome (BHD) is an autosomal dominant inherited disorder characterised by fibrofolliculomas, renal tumours, pulmonary cysts and pneumothorax. The pulmonary cysts and repeated episodes of pneumothorax are the clinical hallmarks for discovering families affected by the syndrome. This disorder is caused by mutations in the gene coding for folliculin (FLCN). FLCN forms a complex with FLCN-interacting protein 1 (FNIP1) and FNIP2 (also known as FNIPL), and the complex cross-talks with signalling molecules such as 5′-AMP-activated protein kinase (AMPK) and the mammalian target of rapamycin (mTOR). Heterozygous *Flcn* knockout mice and rats with *Flcn* gene mutations develop renal cysts, adenomas and/or carcinomas. These findings suggest that FLCN functions as a tumour suppressor that inhibits renal carcinogenesis. However, the mechanisms of the formation of pulmonary cysts and pneumothorax associated with heterozygous mutations in *FLCN* are poorly understood. Resected lung specimens from patients with BHD are often misdiagnosed by pathologists as non-specific blebs or bullae or emphysema, and patients with BHD who have pulmonary cysts and repeated pneumothorax frequently do not receive appropriate medical investigations. This review discusses the clinical and pathological features of lungs of patients with BHD, focusing on the diagnostic pathology and possible mechanisms of cyst formation.

INTRODUCTION

Birt–Hogg–Dubé syndrome (BHD) is an inherited disorder characterised by multiple fibrofolliculomas, pulmonary cysts, pneumothorax and renal cysts and tumours. In 1977, Birt, Hogg and Dubé investigated members of a Canadian family who presented with thyroid cancers and found that some members of the kindred had fibrofollicular skin tumours that occurred in an inherited autosomal dominant pattern.[1] Although the familial disorder was later named the Birt–Hogg–Dubé syndrome, similar inherited skin tumours had been reported 2 years previously. The German dermatologists Hornstein and Knickenberg described a middle-aged woman and her brother who had multiple perifollicular fibromas and intestinal polyps.[2] They reported the detailed clinicopathological features of the siblings and suggested that the disorder was probably inherited from their father who had bilateral renal cysts and unilateral lung cysts in addition to skin tumours. Hornstein and Knickenberg called the systemic disorder 'a cutaneo-intestinal syndrome *sui generis*'.[2] The authors of both reports discussed the possibility of a distinctive hereditary disorder and referred to several reports on similar skin diseases that had been published before 1975.[1 2] Birt *et al* commented that the histopathological features of the skin tumours described by Hornstein and Knickenberg were different from those that they had characterised. However, dermatopathologists have subsequently concluded that the fibrofolliculomas, perifollicular fibromas, trichodiscomas and acrochordons seen in patients with BHD represent a spectrum of the same skin tumour. In a recent review, Happle insisted that the works by Hornstein and Knickenberg should be recognised and proposed that the syndrome should be renamed 'Hornstein–BHD'.[3] We would like to comment briefly on this issue. If the syndrome is renamed, we believe that Knickenberg should also be included. There is no justification for omitting the name of the woman physician who also contributed greatly to the series of studies in the 1970s. Both Birt *et al* and Hornstein and Knickenberg referred to previous studies reporting skin tumours associated with familial occurrence or systemic disorder. It is possible that other authors concerned with crediting the investigators responsible for first reporting on patients with clinical manifestations of this syndrome may have different priorities with regard to proper attribution.

In 2001, two groups of investigators found the chromosomal location of the gene responsible for BHD.[4 5] One year later, Nickerson *et al* delineated the susceptibility locus of the gene on chromosome 17p11.2.[6] The gene was called *BHD* at first. At present, the official symbol referring to the gene is called *FLCN* (*folliculin*) (OMIM: 607273). *FLCN* consists of 14 exons (NCBI Reference Sequence: NM_144997.5).

Among the characteristic manifestations of patients with BHD, renal cell carcinoma (RCC) is the most serious because of its poor prognosis. RCC generally occurs in adult members of a BHD-affected family and rarely affects infants and teenagers. Pulmonary cysts and pneumothorax are found occasionally in young adult patients aged 20–30 years. Skin tumours also develop around the age of 30 years, whereas RCC tends to affect middle-aged and elderly individuals with the syndrome.[7] It is therefore important to correctly diagnose patients who visit hospitals with repeated pneumothorax and those who have fibrofolliculomas in visible regions such as the neck and face (figure 1C,D). The pulmonary manifestations, however, are frequently misdiagnosed as spontaneous blebs, bullae or some other emphysematous condition.[8–10] The pathological features of the lung in patients with BHD have not been described in the major textbooks on pulmonary disease so pathologists still have problems distinguishing BHD-associated pulmonary cysts from blebs and

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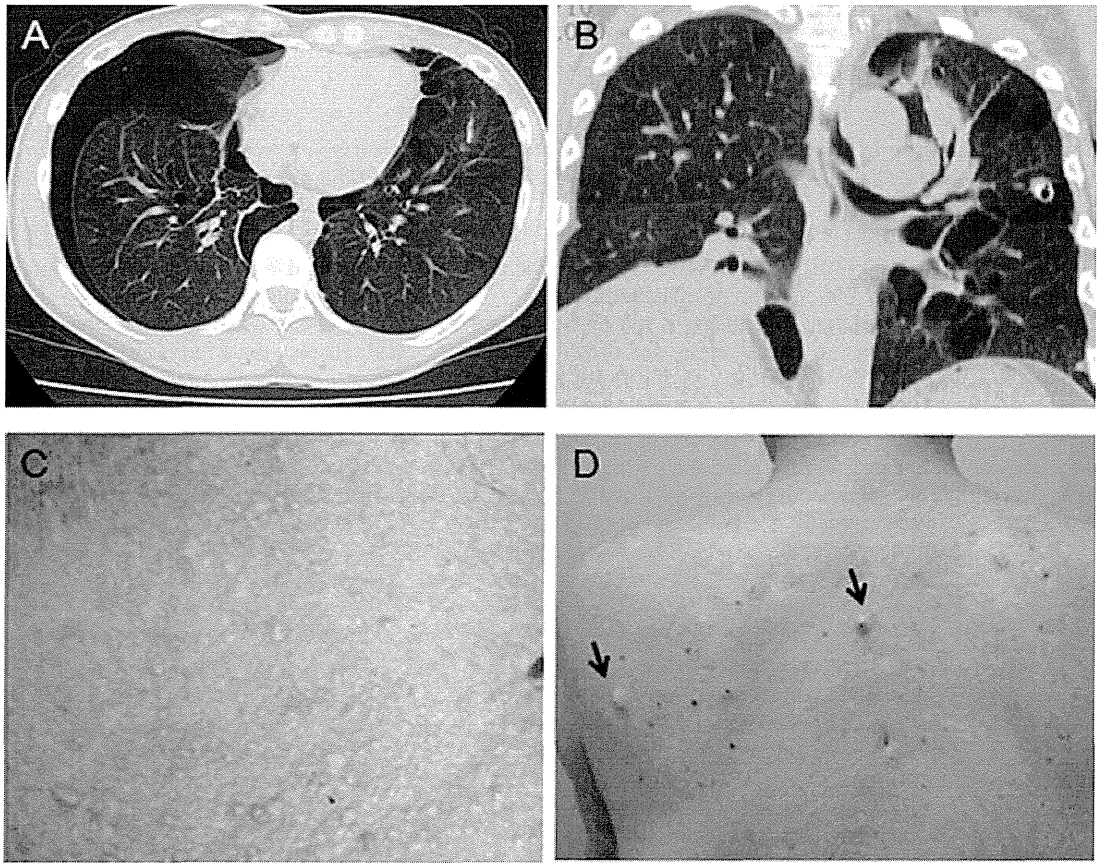

Figure 1 Thoracic CT scans and skin tumours of patients with Birt-Hogg-Dubé syndrome. (A) A 33-year-old woman who had a pneumothorax six times showing cysts localised in the perimediastinal subpleura. (B) A 53-year-old woman who had a pneumothorax twice showing most of the cysts in contact with the interlobular septum. (C) A 69-year-old woman with renal cell carcinoma who had multiple skin papules on the neck. (D) A 76-year-old man with a few flat-topped fibrous papules on the back (arrows).

bullae. The mechanisms of cyst development are largely unknown. In this review we describe in detail the pathological features of the pulmonary cysts and ruptured lesions found in lung tissue of patients with BHD. Diagnostic clues and recent advances in the molecular studies of FLCN-mediated signalling are also presented.

CLINICAL FEATURES OF PULMONARY CYSTS IN BHD

Epidemiological studies have demonstrated that multiple pulmonary cysts and multiple episodes of pneumothorax are frequently observed in patients with BHD.[11] Imaging studies using thoracic CT have found high rates of multiple pulmonary cysts in more than 80% of BHD-affected families.[11 12] In our practice, all 20 patients genetically diagnosed with BHD were found to have multiple pulmonary cysts on thoracic CT (figure 1A,B). Several differential diagnoses should be considered in patients who have a history of repeated pneumothorax, especially in women of reproductive age. Lymphangioleiomyomatosis (LAM) and pulmonary endometriosis should be included in the differential diagnosis. LAM tends to occur in women of reproductive age and about 30% of women with LAM have tuberous sclerosis complex (TSC),[13 14] which will be discussed later. Other genetic disorders such as α-antitrypsin deficiency and Marfan syndrome can also be considered.

Investigating the possible association between pulmonary BHD and chronic obstructive pulmonary disease (COPD), Cho *et al* compared single nucleotide polymorphisms (SNPs) of *FLCN*. None of those SNPs in patients with COPD were the same as the mutations previously reported for BHD, and there was no statistical association for four selected variants with the presence of COPD or emphysema-related phenotypes. Based on their results, they concluded that BHD and COPD may have distinct genetic causes.[15]

Thoracic CT yields findings that greatly aid in the diagnosis of BHD. Tobino *et al* summarised the detailed radiological findings of the pulmonary lesions in 12 patients with BHD.[16 17] Many pulmonary cysts occurred in individual patients; in a single patient with severe disease there were 407 pulmonary cysts bilaterally located in the medial and subpleural regions. The lower part of the middle zone was more frequently affected than the apex of the lung. Similar findings were reported by Ayo *et al* who reviewed five cases.[18] In our practice, we also observed similar radiological features in 19 patients with pulmonary cysts and/or pneumothorax who were diagnosed by genetic testing. Cysts occurring in the middle and lower lobes towards the mediastinum and intimate association of the cysts with interlobular septa and/or visceral pleura are therefore characteristic CT findings that aid in differentiating BHD lesions from LAM and other cystic pulmonary lesions.[17]

PATHOLOGICAL FEATURES OF PULMONARY CYSTS

Very little information on the microscopic findings of pulmonary cysts in BHD is available.[19–21] Some case reports of patients with BHD have described pathological findings that were not reliable because the lung specimens were inadequate.[8–10]

In these reports, BHD-associated pulmonary cysts were frequently confused with blebs, bullae or other cystic air space diseases. The pathological findings previously reported were based on ruptured lesions that caused pneumothorax. Only fragmented pleural walls were resected in these cases and detailed microscopic observation was not available. These specimens had elongated visceral pleurae with hyalinised stroma and, in some specimens, the tissue included true blebs or bullae with mesothelial invagination. It was inevitable that these specimens containing BHD-associated lung lesions were found to have non-specific blebs or bullae; however, physicians and pathologists should have been sceptical about the diagnosis because there were multiple bilateral cystic lesions, repeated clinical episodes and positive family histories. These cases should be carefully re-examined.

In unruptured pulmonary cysts associated with BHD, the cyst wall expands towards the visceral pleura and is partially incorporated into the parenchyma, interlobular septum and/or bronchovascular bundle (figure 2A). Enlarged cysts can also appear segmented by an alveolar wall and resemble a multicystic pneumatocoele and become deeply embedded in the interlobular septum (figure 2B).[19] The inner surface of the cyst is lined by alveolar cells immunostaining for epithelial markers and surfactant proteins (figure 2C). These epithelial cells do not show neoplastic proliferation or atypical morphology. They may be attenuated or not easily visible; however, cuboidal cells

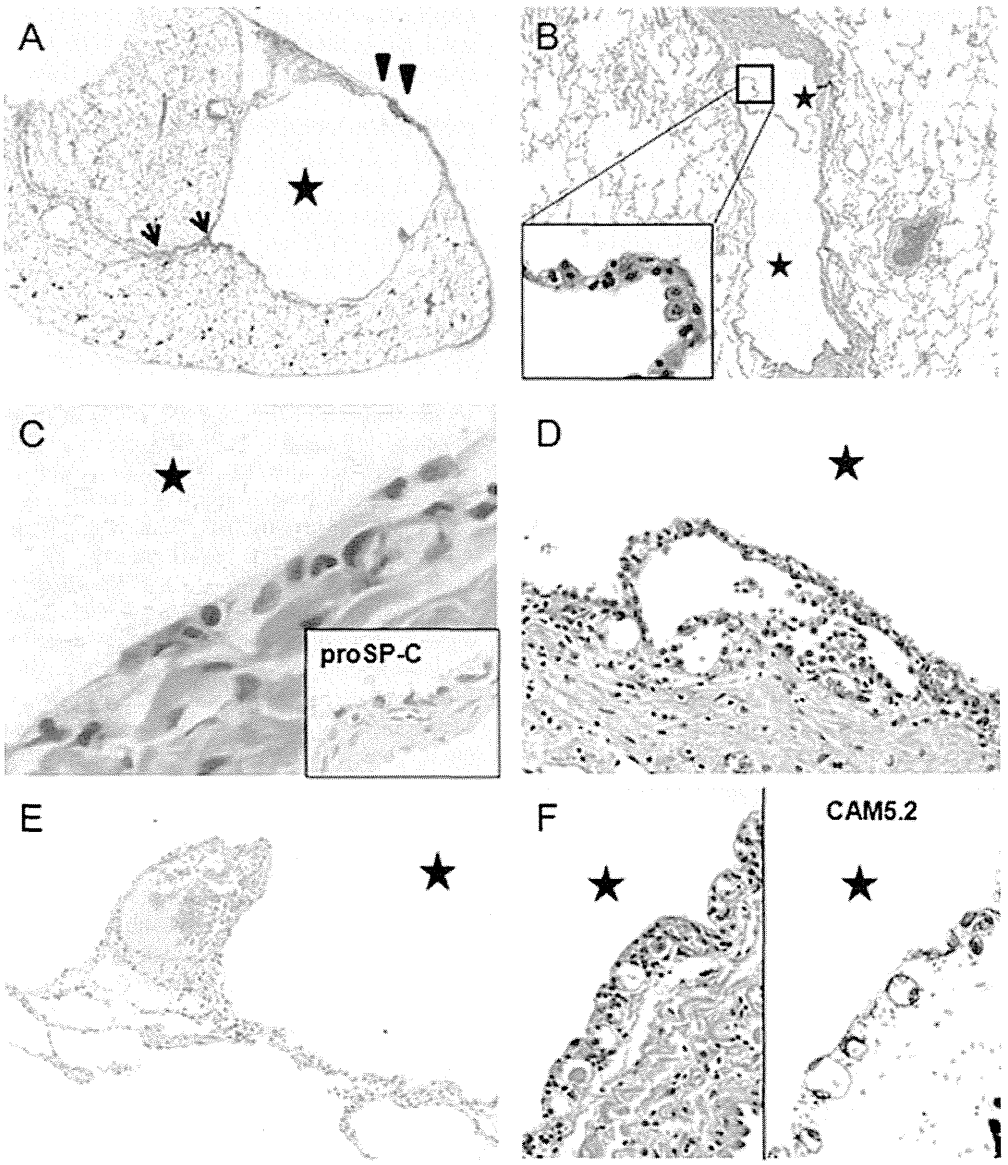

Figure 2 Histopathological features of lungs affected by Birt–Hogg–Dubé syndrome (BHD): H&E staining of resected lungs from patients diagnosed with BHD by genetic testing. Cysts are indicated by stars. (A) A resected cyst from a 41-year-old woman. The cyst wall partially incorporates the pleura (arrowheads) and interlobular septum (arrows). (B) A double-spaced microscopic cyst from the same patient. The inner surface is lined by pneumocytes (inset) and is partially embedded in an interlobular septum (reproduced from Koga *et al*[19] with permission of the publisher). (C) Innermost layer of a cyst: the lining cells show neither aberrant proliferation nor pleomorphic features. They are immunostained for proSP-C, suggesting pneumocytes. (D) A few alveoli abut on the innermost layer and are anastomosed to the cyst lining cells. (E) Finger-like protrusion of a vein into the cystic space. (F) Epithelial spores lining the inner layer of the cyst wall (left), which is highlighted by immunostaining for cytokeratin CAM5.2 (right).

resembling type II pneumocytes are often observed in the innermost layer. Sometimes a cyst wall is partially enveloped by another wall, demonstrating the unique pattern of 'an alveolus or a few alveoli within a cyst' (figure 2D). Some cysts have veins protruding into the cystic space (figure 2E). These characteristic findings are important microscopic clues for the pathological diagnosis.[20] Most of the epithelium lining the cyst appears to be friable. Some epithelial tissue exfoliates from the cyst wall and some remaining tissue appears thin; detailed evaluation shows unusual alveolar architecture (figure 2D) and epithelial cells budding in the walls of the cyst in a few cases (figure 2F). These complex structures are often associated with chronic inflammatory cell infiltration of various degrees, which suggests a possible development of cysts due to inflammation. We speculate that these might resemble hamartomatous cysts that expand very slowly, modified in their structures by inflammation, and sometimes rupture, causing pneumothorax.

In pneumothorax-associated ruptured cysts and cysts before rupture, the histopathology becomes complicated because of mechanical stress and tissue remodelling with associated inflammation. Pleural thickening, interstitial bleeding and hyalinisation alter the original architecture of the cysts. After rupture, mesothelial invagination and bleb formation are frequently observed (figure 3A,B). Cuboidal cells resembling type II pneumocytes tend to be more conspicuous at this stage. These metamorphosed pleurae demonstrate the non-specific histopathology common to blebs and interfere with the correct diagnosis. We therefore suggest that surgeons who perform pulmonary wedge resections in patients suspected of having BHD should also sample cystic areas that are not ruptured. Cystic alveoli and fusion of the epithelium of the cyst to the mesenchyme are diagnostic clues to BHD-associated lung lesions even at advanced stages with inflammatory modifications. The interlobular septum in contact with the cyst is sometimes oedematous (figure 3C, arrows), which may be a result of localised disturbance of the circulation and venous stasis. Other possible causes of oedema include FLCN insufficiency, which might lead to matrix remodeling,[22] or angiogenic factors that increase vascular permeability (see later).

Table 1 summarises the histopathological features that help to differentiate between BHD-associated cysts and idiopathic blebs and/or bullae. Small unruptured BHD cysts generally are free of signs of inflammation and fibrosis. Histopathological observations have indicated that BHD-associated cysts are initially located very close to the interlobular septa and/or are subpleural. Some cysts, if not all, become larger and prone to rupture, leading to pneumothorax. Ruptured cysts or modified cysts before rupture are accompanied by inflammation and become indistinguishable from blebs or bullae because of pleural fibrosis and hyalinisation. These metamorphosed cyst walls, however, may still retain some of the unique features mentioned above (figure 2D,F).[20] Modified BHD-associated pulmonary cysts may secondarily form blebs and/or bullae, but not vice versa.

Several disorders other than blebs/bullae should be considered in the differential diagnosis of BHD-associated cysts including emphysema, infectious lung diseases, LAM and thoracic endometriosis. Immunohistochemical staining using the monoclonal antibody HMB45 for melanoma-related antigen is helpful for differentiating between LAM- and BHD-associated cysts; however, in the early stages of LAM, HMB45-positive cells are inconspicuous. Female patients with BHD who have repeated episodes of pneumothorax are more likely to be carefully investigated than young male patients with BHD who tend to be misdiagnosed with idiopathic pneumothorax. Comprehensive information that includes clinical and family histories can alert the physician to the possibility of BHD. On the other hand, long-term smoking or long-term smoking-associated emphysematous disorder may mask the pulmonary microscopic characteristics of BHD, especially in elderly patients.

It should be noted that there is cystic lung disease of unexplained cause.[23] We also have had some patients who could not be diagnosed. These patients had family members with pneumothorax and/or histories of repeated episodes of pneumothorax. The radiological and histological features of the lungs of these patients were indistinguishable from the features of BHD-affected lungs; however, neither *FLCN* mutations nor intragenic deletions and duplications were identified in these patients. Investigations are needed to identify more specific diagnostic clues for diagnosing BHD in affected lungs and to understand the pathophysiology of non-BHD-associated pulmonary cysts that share some features with BHD-associated cysts.

SYMPTOMS ASSOCIATED WITH DYSFUNCTIONAL FLCN

FLCN is regarded as a tumour suppressor and its dysfunction leads to renal carcinogenesis. However, the role of FLCN in human pulmonary cysts is poorly understood. *Flcn*$^{+/-}$ rodent models for BHD are not good for demonstrating pulmonary cysts.[24 25] A lung-targeted *Flcn*-depleted animal model has not yet been developed. Sequence analysis of *FLCN* from microdissected fibrofolliculomas demonstrated a heterozygous pattern,[26] but *FLCN* in the pulmonary cysts of patients with BHD has not been characterised because the cells lining the cysts do not show neoplastic proliferation and the numbers of epithelial cells are too small for analysis. A histological study using in situ hybridisation reported that *FLCN* mRNA was detectable in stromal cells and type I pneumocytes in the lungs of patients with BHD.[27] In our immunohistochemical study, macrophages, pneumocytes and even the cells lining the cysts of BHD-affected lungs stained positive for FLCN,[19 20] whereas there were many unstained RCC cells in BHD-affected kidneys.[20] These data suggest that FLCN in pulmonary cysts probably displays haploinsufficiency.

Little is known about the metachronicity of pulmonary cyst development. Many cysts appear to remain unruptured because the frequency of pneumothorax in elderly patients with BHD is not as high as the frequency of pneumothorax in middle-aged patients. Thus, some cysts may evolve continually and rupture at different points in time. Alternatively, abnormal epithelial/mesenchymal interactions may weaken the extracellular matrix of the visceral pleura leading to a pneumothorax-prone condition. BHD-associated skin tumours demonstrate proliferation of mesenchymal cells in addition to extension of the follicular epithelium, indicating that dysfunctional FLCN affects both cell types. *FLCN* mRNA is detectable in both the epidermal and stromal components of the skin.[27] Additional studies are needed for better understanding of the budding and development of pulmonary cysts and the process of pneumothorax formation in patients with BHD.

Studies of BHD rodent models have elucidated the importance of FLCN in renal carcinogenesis. *Flcn*-null mice and rats are embryonic lethal, and *Flcn* heterozygous knockout mice and rats with *Flcn* mutations develop renal cell adenomas and RCC.[28 29] Nihon rats with heterozygous *Flcn* mutations develop renal cell adenomas at around 3–4 weeks which transform to RCC when the rats are 6 months old.[30] The carcinoma cells of RCC in this model frequently show loss of heterozygosity of *Flcn*. Somatic second hit mutations of *Flcn* have been

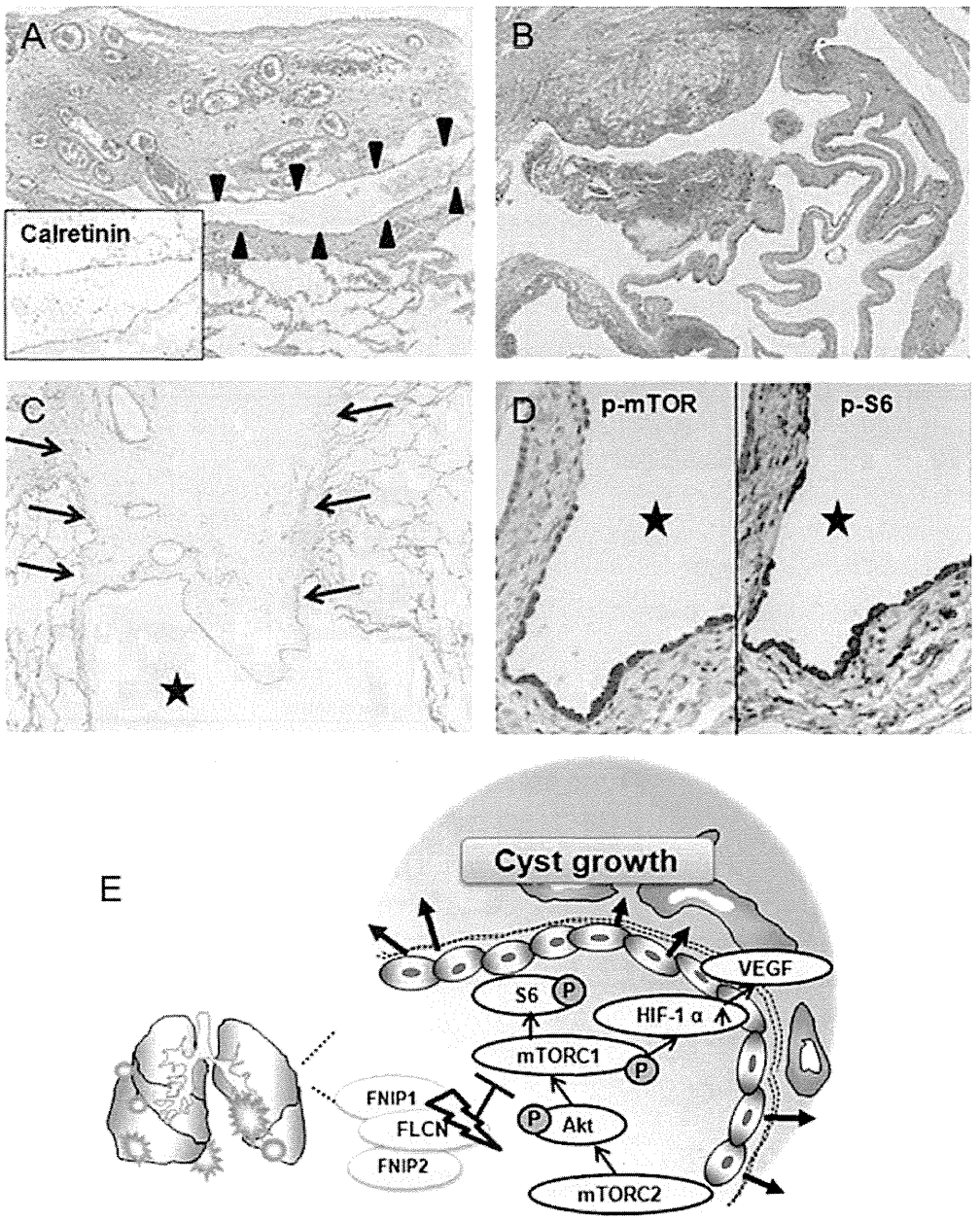

Figure 3 Histopathological features of ruptured cysts and hypothetical mechanism of cyst growth. (A) Ruptured visceral pleura thickened with fibrosis and inflammation. Mesothelial invagination (arrowheads) is observed. Inset shows the invaginated cells immunostained for calretinin. (B) Elongated cysts form a complicated structure. (C) Interlobular septum (arrows) associated with a cyst shows oedematous features. (D) The cyst lining epithelial cells are strongly immunostained for p-mammalian target of rapamycin (mTOR) (left) and p-S6 (right). (E) A hypothetical signalling pathway involved in pulmonary cyst growth. Folliculin insufficiency in cyst composing cells leads to mTOR activation and accelerates downstream molecules such as p-S6 and vascular endothelial growth factor (VEGF). FNIP, FLCN-interacting protein; HIF-1α, hypoxia-inducible factor 1α; mTORC1 and 2, mTOR complex 1 and 2.

found in RCC of patients with BHD.[31] The $BHD^{f/d}$/KSP-Cre mouse has kidney epithelium-specific depletion of *Flcn*, develops polycystic tubules within 2 weeks after birth and dies at 3 weeks from renal failure.[32] These results indicate that FLCN works as a tumour suppressor and also as a possible regulator of cystic remodelling.

In heterozygous *Flcn* knockout mice ($Flcn^{+/-}$ mice), adenocarcinoma and adenoma of the lung are detected sporadically.[24] A few human cases of low-grade atypical adenomatous hyperplasia and lung carcinomas have been reported,[7 18] and we also had a patient who developed bronchioloalveolar carcinoma with multiple pulmonary cysts (figure 4). There is a recent report of a 50-year-old man with BHD who was found to have a 12 mm histiocytoma in the lung plus multiple cysts and pneumothorax.[33] It is unknown whether the lung carcinoma and other types of lung tumours in patients with BHD are associated with FLCN haploinsufficiency. Long-term prospective studies are needed to elucidate whether neoplastic changes in the lung can occur.

Table 1 Comparison of the lung cystic lesions between Birt–Hogg–Dubé syndrome (BHD) and blebs/bullae

	BHD (primary cyst)	BHD (modified cyst)	Idiopathic blebs and bullae
Location	Lower lobes/perimediastinum	Lower lobes/perimediastinum	Upper lobes/apex
Number	A few to multiple	A few to multiple	One or a few
Basic structure	Alveolar cysts/partially fused to pleura	Elongated pleura/alveolus or a few alveoli within a cyst	Intrapleural and/or parenchymal air spaces/ destructed alveoli
Lining cells	Often attenuated/flattened respiratory epithelium	Often type II pneumocyte -like cuboidal cells	No or incomplete epithelial lining/destructed alveoli
Stroma	No fibrosis	Common fibrosis	Common fibrosis
Mesothelial invagination	No	Often	Often
Inflammation	None to mild	Mild to moderate	Moderate
Pneumothorax	Not yet	Often	Often

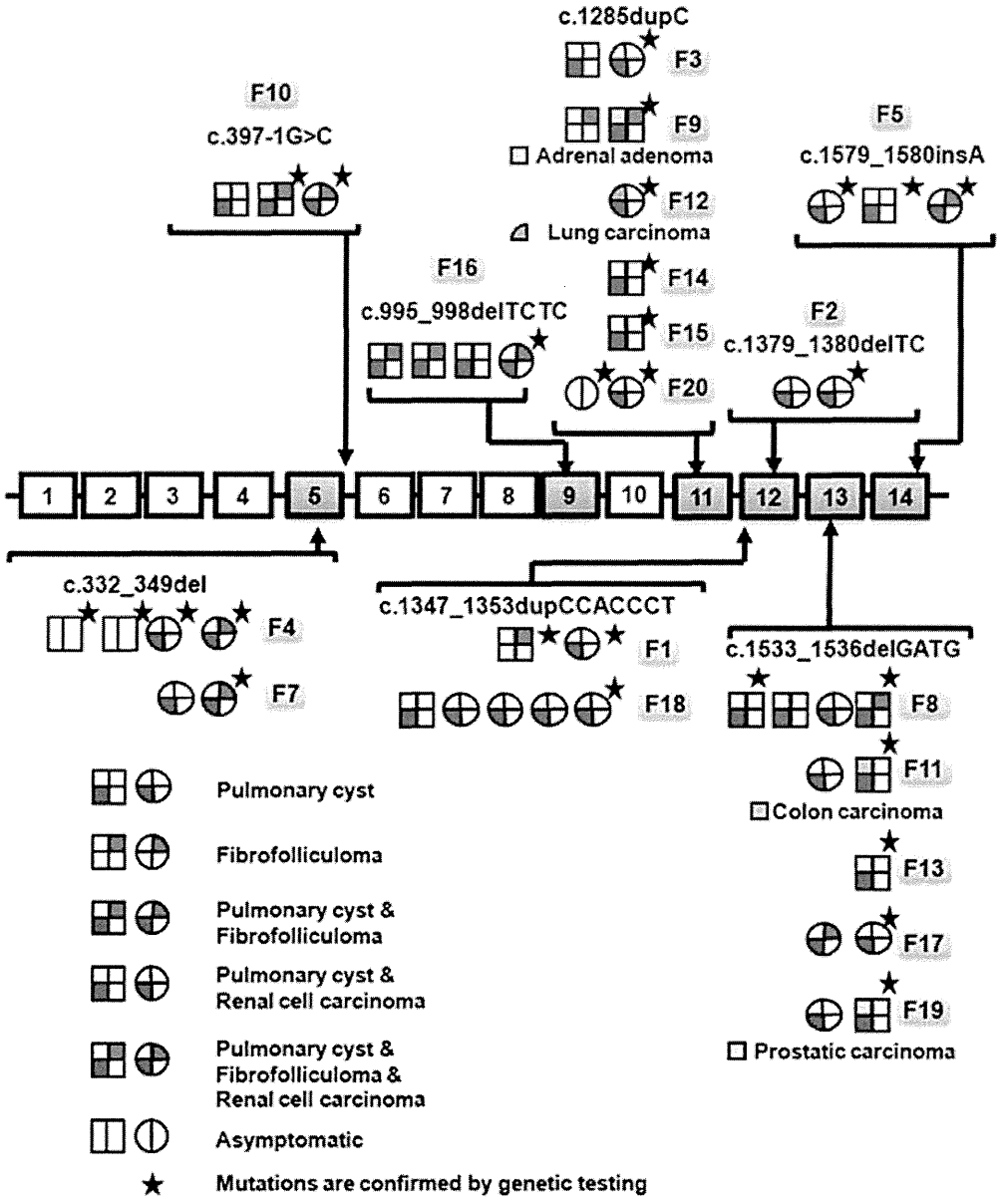

Figure 4 Schemes of mutation patterns of 19 Asian families with Birt–Hogg–Dubé syndrome (BHD). The responsible mutation sites of the 19 Asian families are indicated. F6 is excluded because genetic testing was done in another institute. F5 is Taiwanese and the others are Japanese families with BHD. The most frequent mutations are 4 bp deletion in exon 13 (c.1533_1536delGATG) and duplication of cytosine in exon 11 (c.1285dupC).

FLCN-ASSOCIATED SIGNALLING PATHWAYS

FLCN is involved in the mammalian target of rapamycin (mTOR) signalling pathway.[34] Recent studies revealed that FLCN forms a complex with two associated proteins. Baba *et al* identified the first binding protein of FLCN in 2006 and called it FLCN-interacting protein 1 (FNIP1). In 2008, they and other investigators identified a novel FNIP1 homologue, FNIP2 (also known as FNIPL).[35 36] The FLCN complex interacts with 5′-AMP-activated protein kinase (AMPK)[34–36] and regulates mTOR signalling. High levels of dysregulated mTOR activity are associated with several systemic syndromes including TSC. The complex of hamartin (TSC1) and tuberin (TSC2) interact with AMPK and mTOR, and mutations in TSC1 or TSC2 lead to uncontrolled cell growth.[37] TSC is associated with characteristic neoplasms such as neurofibromas in the skin, angiomyolipoma in the kidney and LAM in the lung. Other mTOR-associated hamartoma syndromes include Peutz–Jegers syndrome and Cowden's disease in which respective heterozygous mutations in *STK11/LKB1* and *PTEN* lead to hamartomatous colorectal polyps. BHD syndrome, TSC and the other mTOR-associated disorders have in common conditions such as multiple skin tumours and hereditary tumours and hamartomas which are associated with haploinsufficiency of the corresponding gene products. It is not completely understood whether the FLCN complex plays a role in mTOR signalling similar to the role of TSC1 and TSC2.

Both inhibitory and stimulatory effects of FLCN on mTOR complex 1 (mTORC1) have been seen, depending on the experimental design of the study,[24 28 32 35 36] which indicates that the function of FLCN in mTOR signalling may be dependent on context.[25] We have shown that the cells lining pulmonary cysts stain positively for phospho-mTOR and phospho-S6 expression (figure 3D).[20] We also found that hypoxia-inducible factor 1α and vascular endothelial growth factor (VEGF) were immunostained strongly in lung specimens from patients with BHD (unpublished observation). Although statistical validation of a sufficient number of patients is not yet available, the results of our histopathological investigations suggest that mTOR signalling is accelerated in the cells lining pulmonary cysts, and that downstream molecules such as S6 protein and VEGF contribute to the development of cysts under conditions of FLCN haploinsufficiency (figure 3E). Recent studies have elucidated the suppressor activity of FLCN in the regulation of transcription factor E3 and transforming growth factor β which are widely involved in tumorigenesis and apoptosis.[38 39] Further investigations of the molecular mechanisms involved in the formation of pulmonary cysts and rupture are needed.

FLCN MUTATIONS

In 2009, more than 50 germline mutations were reported from families affected by BHD[40] and, at present, over 100 have been reported. The C_8 tract in exon 11 is the most frequently affected site; duplication of cytosine (C_9) and deletion (C_7) have both been reported. A statistical association between mutations and clinical manifestations has not been clarified. Differential phenotypes are often observed in family members with BHD, and not all the members will develop RCC.[19] Painter *et al* reported that all 24 affected members of a Finnish kindred had multiple pulmonary cysts on thoracic CT.[41] They suggested that a 4 bp deletion in exon 4 (c.235_238delTCGG) causes pulmonary cysts with 100% penetrance. Toro *et al* investigated 189 patients with BHD with pulmonary cysts and pneumothorax and found that patients with *FLCN* mutations in exons 9 and 12 had pneumothorax more frequently than patients with *FLCN* mutations in other exons.[12] Schmidt *et al* suggested that patients with cytosine deletion in the C_8 tract of exon 11 (c.1285delC) may have a lower risk of developing RCC than individuals with cytosine duplication in the C_8 tract (c.1285dupC).[42] RCC tends to occur in elderly patients with BHD, thus long-term follow-up survey in age-matched groups is needed.

Although not all the kinships we studied underwent thoracic CT, our data indicate that a 4 bp deletion in exon 13 (c.1533_1536delGATG) causes pulmonary cysts with 100% penetrance (n=11 affected members from five different families; figure 4). Data are needed from more kinships to clarify statistical associations between the mutation patterns and clinical manifestations. According to the *FLCN* mutation online database (http://www.skingenedatabase.com/), the registered mutations are mainly from families in the USA. Kunogi *et al* summarised the mutations in Japanese pedigrees and compared them with those reported by the National Cancer Institute.[43] There were differences in the frequencies of mutation sites. For example, a 7 bp duplication in exon 12 (c.1347_1353dupCCACCCT)[19 43] and mutations of a splice acceptor site in intron 5[7 43] have not been reported from American and European pedigrees.[7] In addition, we found a few other unique mutations in Asian pedigrees.[20 44] Figure 4 depicts a summary of the mutations from 19 Asian families affected by BHD (18 Japanese and 1 Taiwanese) which we analysed between 2008 and 2012. A cytosine duplication or deletion in the C_8 tract of exon 11 (c.1285dupC or c.1285delC) is known to be the 'hot spot' in Western patients with BHD. Mutation analyses by us[20] and another Japanese group[43] have added new information; in Japanese patients with BHD, c.1347_1353dupCCACCCT in exon 12 and c.1533_1536delGATG in exon 13 are the 'hot spots'.

CONCLUSIONS AND FUTURE PROSPECTS

Around 10 years have passed since the discovery of *FLCN*.[6] Increasing numbers of case reports and reviews on BHD pedigrees have provided physicians with the diagnostic features of this inherited disorder.[45 46] However, there are still many patients who are misdiagnosed and thought to have spontaneous pneumothorax and sporadic RCC. Pneumothorax and chest pain are clinically important signs and symptoms and warrant a thorough medical examination,[47] and it is important to keep in mind that the onset of repeated pneumothorax generally starts at an earlier age than RCC in patients with BHD.[11] Pathologists should carefully distinguish pulmonary lesions associated with BHD from the lesions that occur in other emphysematous diseases. In this review we have presented the diagnostic histopathological features including the diagnostic puzzles of pulmonary cysts. Pathologists must remember that resected lesions are frequently modified by mechanical stress and post-rupture remodelling. In addition, it is not completely understood how pulmonary cysts develop under conditions of FLCN insufficiency. A better understanding of the molecular and cellular cross-talks and meta-analyses of patients with BHD are needed to develop a more precise diagnosis and better follow-up of affected families.

Key messages

- In un-ruptured pulmonary cysts associated with Birt-Hogg-Dubyndrome (BHD), the cyst wall expands toward the visceral pleura and is partially incorporated into the parenchyma, interlobular septum and/or bronchovascular bundle.
- The inner surface of the cyst is lined by alveolar cells that may be attenuated or not easily visible; however, cuboidal cells resembling type II pneumocytes are often observed.
- In pneumothorax-associated cysts, the histopathology becomes complicated because of mechanical stress and tissue remodeling with inflammation.

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