表1　VHL 病の臨床診断基準

VHL 病の家族歴が明らかである場合
中枢神経系血管芽腫，網膜血管腫，腎癌，褐色細胞腫，膵臓の病気（膵嚢胞・膵神経内分泌腫瘍），精巣上体嚢胞腺腫があることが診断されている．
VHL 病の家族歴がはっきりしない場合
・中枢神経系血管芽腫あるいは網膜血管腫を複数個（2 個以上）発症
・中枢神経系血管芽腫または網膜血管腫と以下にのべる病気がある
腎癌
褐色細胞腫
膵臓の病気（膵嚢胞・膵臓の神経内分泌腫瘍）
精巣上体嚢胞腺腫

表2　VHL 病の臨床的病型と調査における症例数

分類	腎癌	褐色細胞腫	網膜血管腫	中枢神経系血管芽腫	症例数
VHL 病 1 型	+	−	+	+	
VHL 病 2 型 A	−	+	+	+	31
VHL 病 2 型 B	+	+	+	+	20
VHL 病 2 型 C	−	+	−	−	11

図1　VHL 病褐色細胞腫の発症年齢分布

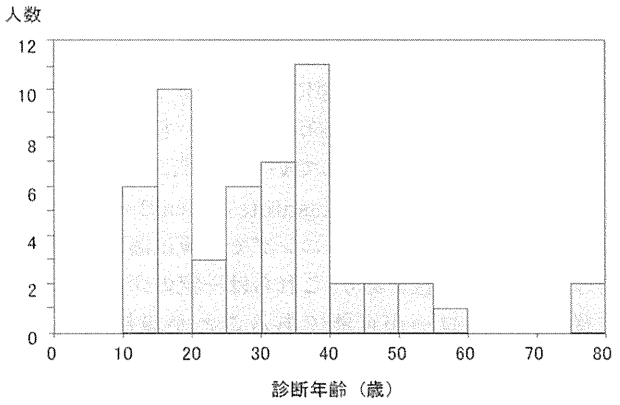
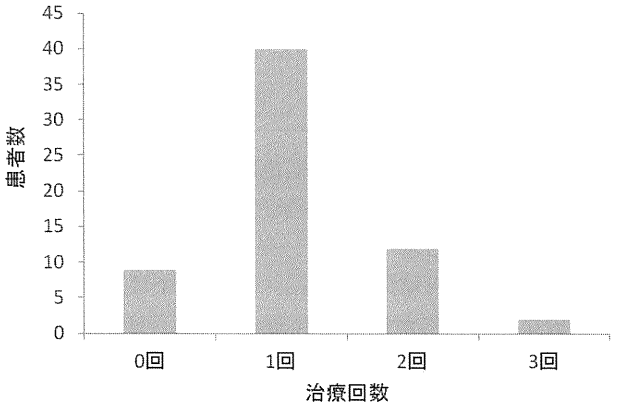


図2　VHL 病褐色細胞腫の治療回数別患者数



育施設及び教育関連施設の専門医施設長に対して，消化器内科は，日本膵臓学会員に調査を依頼した．本調査は，日本泌尿器科学会および日本膵臓病学会の学術委員会の許諾を得て，その他の関連学会は事務局の了解を得て行った．一次調査として，VHL 病の診断治療経験の有無を調査し，その結果，診療経験有りと回答のあった医師（240 名）に対し，二次調査として疾患ごとの個別調査項目を提示しアンケート調査を行った．二次調査の回答率は 70.4% であった．褐色細胞腫に関する調査項目は，発症年齢，性別，居住県，治療内容，副腎不全の有無，予後情報等を集積した．本疫学調査研究は高知大学医学部の倫理委員会にて審査，承認を得た．

結　果

1）発症病態調査の結果

VHL 病の診断治療の経験があった各科専門医師より回答された今回の調査で VHL 病褐色細胞腫の発症者数は 62 例で，VHL 病全登録患者 409 例の 15.4% に発症がみられた．この発症頻度は欧米報告とほぼ同様であった．発症平均年齢 29.7 ± 2.0 歳，中央値 31.5 歳，発症年齢は 10 歳から 75 歳までと幅広く，15〜20 歳と，35〜40 歳に二峰性の発症ピークを示した（図 1）．両側副腎褐色細胞腫が 26 例（41.9%），副腎外発症のいわゆる傍神経節腫（paraganglioma）が 8 例（12.9%）であった．性別では男性，女性患者がともに 31 例ずつで，性差はなかった．転移が確認された悪性例が 4 例（6.5%）あったが，これは欧米報告（1.6〜3.6%）よりやや高頻度であった[1)4)5)]．図 1 上で悪性例は 20 歳代，50 歳代の発症頻度が低い年代で発症がみられた．

2）治療内容と予後についての調査結果

褐色細胞腫に対する手術回数は，1 回のみが 65%，複数回（2, 3 回）のものが 19.3% であった（図 2）．4 回以上手術を受けた症例はなかった．

術後のステロイド補充療法については，両側例 26 例中 14 例（56%）で手術後にステロイド補充が行われていた．一方残りの 12 例では補充は不要で，8 例が機能温存手術

表3　VHL病褐色細胞腫の外科的治療とステロイド補充療法について

	ステロイド補充療法あり	ステロイド補充療法なし
両側副腎摘除	5	0
一側副腎摘除＋対側副腎部分切除・腫瘍核出術	2	7
一側副腎摘除	1	27
両側副腎部分切除・腫瘍核出術	0	1
一側副腎部分切除・腫瘍核出術	0	3

施行，3例は片側のみの手術で対側は経過観察がなされていた（表3）．

死亡例については褐色細胞腫に関連した死亡例は5例で，その内訳は悪性褐色細胞腫で転移によるもの4例，手術後ステロイド補充を行い経過観察中に重症感染症（胆嚢炎）を併発したもの1例であった．褐色細胞腫発作による循環器系の致死的合併症を起こした症例は今回みられなかった．

調査施行時点で経過観察中の症例については，10例で経過観察が行われており，このうち4例は降圧剤投与が行われていた．一方残りの6例（2型A，4例，2型B，2例）は，無症候性，非機能性と考えられ，無治療で経過観察がなされていた．

考　察

VHL病のうち2型家系で褐色細胞腫が好発することが，欧米での中心的な施設や研究グループによる疫学的解析報告から知られてきた[1)2)]．これらは数十例規模の症例を集積した解析結果である．一方本邦では，これまで単発的な症例報告が散見されるのみで，VHL病のまとまった疫学調査はなされておらず，日本人でのVHL病患者における褐色細胞腫の頻度，発症経過や治療法，予後などの詳細はまったく不明であった．そこで今回我々は，厚生労働科学研究費補助金難治性疾患克服研究事業の研究奨励疾患として，平成21～23年度にかけて本邦のVHL病の全国疫学調査を行い，さらにそこで集計されたVHL病に伴う褐色細胞腫62例について臨床的特徴を解析した．

過去の成瀬班で詳細な褐色細胞腫の研究がなされ，「褐色細胞腫診療指針2010年度版」が発表されているが[6)]，その報告書では2例にのみにVHL病の合併する褐色細胞腫が報告されていた．本研究との間の重複については不明であり，本研究では別の褐色細胞腫の症例を調査した可能性が高いと考えられた[7)]．

従来の欧米例の報告ではVHL患者内での褐色細胞腫症例の頻度は10～20％で，診断年齢は3～60歳，性差はなく，両側副腎例が約50％，副腎外発症が14～20％，悪性転化例1.6～3.6％などが知られている[1)4)5)8)]．本調査では本邦における褐色細胞腫の発症年齢は最若年で10歳，発症年齢の平均年齢29.7±2.0歳，中央値31.5歳であることが明らかとなった．また，興味深いことは15～19歳と35～39歳の2つの発症診断のピークを示した（図1）．2型Aは10～19歳に発症が多い傾向があり，2型Bは35～39歳に発症が多い傾向があったが，はっきりとした有意差は認めなかった．このような特徴は網膜血管芽腫，膵神経内分泌腫瘍にも類似する傾向があった．これらの特徴は全体としては欧米と類似した傾向で，その中で本邦例では診断年齢が10～75歳とやや高年齢側に偏っている点，また悪性例の頻度が本邦では6.4％とやや高い傾向が観察された．

遺伝性褐色細胞腫の原因疾患としてはVHL病，Pheochromocytomas and Paragangliomas syndromes，Mutiple Endocrine Neoplasia type2等の疾患がある[9)]．その中ではVHL病の褐色細胞腫の特徴の一つに，孤発例や多発性内分泌腺腫症（MEN）等に合併した症例と比較して，よりホルモン活性が低く臨床症状が軽いものの頻度が高いことが欧米例で知られている[4)10)～12)]．米国National Cancer Institute/National Institute of Healthのデータでは，VHL家系のスクリーニングで新規に診断された場合35％が非機能性であり，これらは一定のプロトコールに従ってフォローが可能であることが報告されている[2)4)5)10)]．今回の集計からも，10人の経過観察症例中6人が非機能性と考えられ無治療でフォローされていた．これらについてのカテコールアミンの検査値や腫瘍サイズ，観察期間等の細かな情報は今回集計できていないが，欧米例と同様にホルモン活性の低い症例が存在することを示すと考えられた．

今回，VHL病における褐色細胞腫の病態の複雑な点が明らかとなった．カテコールアミン分泌能による機能性か非機能性かの判断や，機能性の場合の早期手術による副腎機能の温存の可能性が示唆された．これらの結果に基づき，VHL病患者におけるCTなどの画像検査による診断と経過観察の開始年齢は10歳が妥当と考えた[9)]．

今回のアンケート調査では中枢神経系血管芽種と網膜血管腫の発症中央値は約28歳，腎癌の発症中央値は約35歳，膵神経内分泌胞腫の発症中央値は34歳であることも判明した．褐色細胞腫の発症中央値は約31.5歳と発症年齢の中央値に差があるため，治療を受ける時期がずれことが考えられる[13)]．それぞれの腫瘍を早期に発見できれば，腎癌や褐色細胞腫では腹腔鏡手術，さらに腎癌にはラジオ波焼灼という手段もあるため，より侵襲の少ない治療を受けることが可能と考えられる．

VHL病に伴う褐色細胞腫は本来非常に頻度が低く，本邦ではこれまで単一の症例が散発的に報告されてきたのが実情である．今回初めて全国規模での集積が行われ，その臨床的特徴がある程度明らかになった．欧米の中心的な機関での報告も，今回の集計とほぼ同様の50～60例程度の解析結果であり[3)~5)]，今後さらに全国調査を継続し症例を集積し，本邦例の特徴，欧米例との差異を明らかにしていくことが肝要と考えられる．

今回，厚生労働省難治疾患克服研究事業，平成21年度「褐色細胞腫の実態調査と診断指針の作成に関する研究」および平成22年度「褐色細胞腫の診断および治療法の推進に関する研究」の報告書の内容を検討したが，その中にはVHLに関連する褐色細胞腫の記載は2例のみであった．そのため我々の調査結果は「褐色細胞腫の実態調査と診断指針の作成に関する研究」とは重複が少なくほぼ別の対象を調査している可能性が示唆された．今後，褐色細胞腫の実態調査と診断指針も参考にしてVHL病の調査を進めて診療指針も改変する必要があると考えられた[14)]．

結　論

我々は平成21～23年度にかけてVHL病に伴う褐色細胞腫とその治療内容について国内泌尿器科，脳神経外科，眼科，膵臓内科の各専門医を対象に厚生労働科学研究費補助金難治疾患克服研究事業の研究奨励疾患として本邦で初めて全国疫学調査を行った．その結果ではVHL病で発症する褐色細胞腫の発症の特徴はほぼ欧米と同様であり，若年発症で且つ一生涯発症し，発症頻度15％で，若年例，多発性異時性に発症，両側例，悪性例等，多彩な特徴が明らかとなった．

本研究は，厚生労働科学研究費補助金難治性疾患克服研究事業による助成を受けて行われた．本アンケート調査に協力頂いた全国の泌尿器科，脳神経外科，眼科，消化器内科，その他診療科の先生方に感謝いたします．

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CLINICAL STATUS OF VON HIPPEL-LINDAU DISEASE ASSOCIATED PHEOCHROMOCYTOMA IN JAPAN: A NATIONAL EPIDEMIOLOGIC SURVEY

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Abstract:

(Purpose) To understand the current clinical status of pheochromocytoma (Pheo) in patients with von Hippel-Lindau disease (VHL) in Japan.

(Patients and methods) We picked up and summarized Pheos from a nationwide epidemiologic survey for VHL disease based on the epidemiologic study program for incurable disease by the Japanese Ministry of Health, Labour and Welfare. The details of the survey included age of onset, sex, living area, treatment modalities, functional status of the adrenal gland after surgical treatment, and patient outcome.

(Results) The incidence rate of Pheo in VHL disease in Japan was 15.1% (62/409). Males and females were equally affected. The mean and median ages of onset were 29.7 and 31.5 years, respectively. The age of onset was distributed between 10 and 75 years and presented two large peaks between 15–20 and 35–40 years. Twenty-six (41.9%) bilateral cases, 8 (12.9%) paragangliomas, and 4 (6.4%) malignant cases were found. Forty-one (65%) patients underwent surgical resection once and 13 (9%) underwent 2 or 3 times surgeries whereas six (10%) nonfunctional cases were surveyed without surgical treatment. Fourteen of 26 bilateral Pheos (56%) received steroid replacement therapy following surgery. Four cases died from metastases of malignant Pheos and one from a severe infection during steroid replacement therapy. None of the patients died of cardiovascular complication due to Pheo crisis.

(Conclusion) It is concluded that Pheos in VHL disease developed from a relatively young age and was associated with 15% of all patients, including a small ratio of malignant cases. More than 40% of cases suffered bilateral adrenal tumors. The clinical features in Japan appear to be similar to those in the Western countries according to the current survey.

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Keywords: von Hippel-Lindau disease, pheochromocytoma, nationwide epidemiological survey

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von Hippel-Lindau（VHL）病における網膜血管腫発症の全国疫学調査結果

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Epidemiological Investigation of Retinal Angioma of von Hippel-Lindau Disease in Japan

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過去における欧米の文献では，von Hippel-Lindau（VHL）病に一定の割合で網膜血管腫が発症することが知られている．しかし，わが国では正確な疫学調査がされておらず，VHL病患者の網膜血管腫の頻度や病態は明らかではない．平成21年から23年にかけて，筆者らはVHL病に合併する網膜血管腫について，国内脳神経外科，眼科，泌尿器科，膵臓病内科の各専門医を対象に疫学調査を行った．その結果，VHL病患者の網膜血管腫の発症数は140名で，VHL病全患者の34%に合併していた．男女比は1：1で，発症年齢は5～68歳で，平均値28.5歳であった．患者分布は北海道，太平洋沿岸から瀬戸内海地域に帯状に多い傾向にあった．治療に関しては網膜光凝固術を施行されている症例が最も多く，ついで冷凍凝固術が施行されていた．抗vascular endothelial growth factor（VEGF）抗体硝子体注射など新たな治療に取り組む施設もあった．

Previous reports demonstrate that retinal angioma is observed in a certain percentage of patients with von Hippel-Lindau disease (VHL) patients in Europe and the United States. However, because no epidemiological investigation has yet been conducted in Japan, the frequency and conditions of retinal angioma remain obscure in Japan. From 2009 to 2011, we conducted an epidemiological investigation using questionnaires for neurosurgeons, ophthalmologists, urologists and physicians specialized in pancreatic diseases. Of 409 VHL patients, 140 had retinal angioma, a frequency of 34%. The ratio between males and females was 1；the mean (range) age at the diagnosis was 28.5 (5～68) years. Geographically, distribution of patients is likely to be in a belt-shaped pattern along the coast of Hokkaido, from the Pacific Ocean to the Inland Sea. Most of the patients received laser photocoagulation. New therapeutic approaches, such as intravitreal injection of anti-vascular endothelial growth factor (VEGF) antibody, were tried in some institutions.

〔Atarashii Ganka (Journal of the Eye) 28(12)：1773～1775，2011〕

Key words：フォン・ヒッペル・リンドウ病，網膜血管腫，疫学調査，治療．von Hippel-Lindau disease, retinal angioma, epidemiological investigation, therapy.

はじめに

von Hippel-Lindau（VHL）病は，染色体3番短腕に原因遺伝子が存在する常染色体優性遺伝性疾患である．欧米では発症頻度は3万6千人に1人，または100万人に1家系の発症であるとされる．中枢神経系，内耳，網膜，副腎，腎臓，膵臓，精巣上体，子宮などの多数の臓器に腫瘍，嚢胞を発症するとされる．10歳未満という幼少児期から70歳までの長期間にわたって発症し[1,2)]，治療は各臓器の合計で平均5回以上の手術に及ぶ患者も多い．その結果，多くの後遺症を残すためquality of life（QOL）の悪い難治性疾患とされる．

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欧米では過去にVHL病の詳細な病態調査が行われている[3,4]が，わが国では大規模な病態調査はまったくなされていなかった．特に網膜病変は幼児期から発症するため，早期からの経過観察が必要とされるが，調査結果に基づく診療のガイドラインとなるものはわが国には存在しなかった．今回，筆者らは平成21年から23年にかけて厚生労働省難治疾患克服研究事業研究奨励疾患の一つとして，全国の脳神経外科，眼科，泌尿器科，膵臓病の専門医を対象に疫学調査を行うことにより，日本におけるVHL病網膜血管腫の現状を把握したので報告する．

I　研究対象および方法

平成21年から23年にかけて厚生労働省難治疾患克服研究事業研究奨励疾患としてVHL病に合併する網膜血管腫について，国内の脳神経外科（1,141名），眼科（1,149名），泌尿器科（1,200名），膵臓病内科（1,055名）の各専門医を対象に疫学調査を行った．全国の専門医に対して，VHL病の診断治療経験の有無を調査した．VHL病患者を診療していると回答のあった医師に対して，調査項目を提示してアンケート調査を行った．網膜血管腫についての調査項目は性別，発症年齢，現在の居住県，治療法と，視力障害と視野障害の有無，死亡情報などであった．治療法と治療回数に関するアンケートでは，各患者について，10回分の治療を報告していただき，その何回目にどの治療を行ったかを記載してもらった．これらの疫学調査は高知大学医学部の倫理委員会審査で許可を得て匿名調査で行った．回収された結果から，わが国の網膜血管腫の実態を把握することにより経過観察，診断・治療指針のアルゴリズムを作成した．

II　結　　果

1．発症病態の調査結果

アンケートの回収率は全体で約50%であった．VHL病の診断治療の経験があった各科専門医師より回答されたVHL病網膜血管腫の発症数は140名で，VHL病全患者409名の34%に合併していた．男性：女性は70：70で性差はなかった．発症年齢は5～68歳で発症年齢の平均値28.5歳，中央値28歳であった．発症年齢は小児から高齢者まで幅広いが，15歳から35歳までの若年発症が多かった（図1）．患者の分布は，北海道，太平洋沿岸から瀬戸内海地域にかけて帯状に広がって分布する傾向がみられた（図2）．死亡例については，VHL全体と網膜血管腫で明らかな差はなかった．

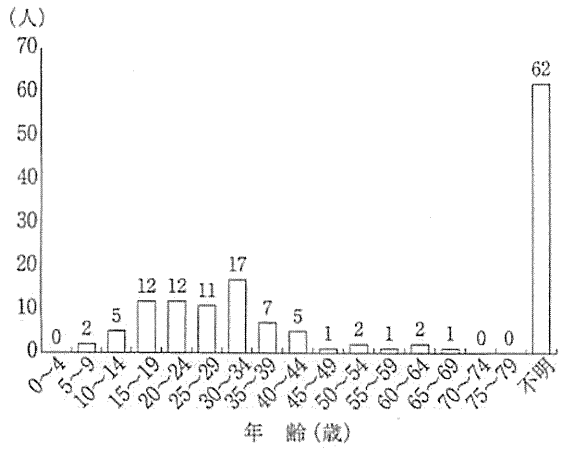

図1　発症年齢分布

2．治療内容についての調査結果

治療についてはレーザー治療が最も多く行われていた．レーザー治療についで冷凍凝固術が施行されていた．抗vascular endothelial growth factor（VEGF）抗体硝子体注射，光線力学的療法あるいは硝子体手術が施行された症例もあった

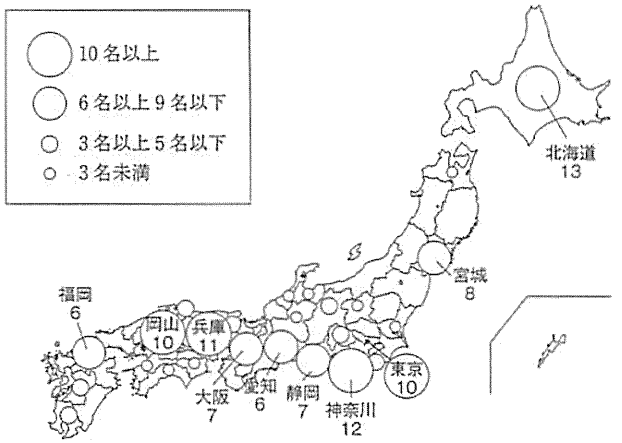

図2　地域分布

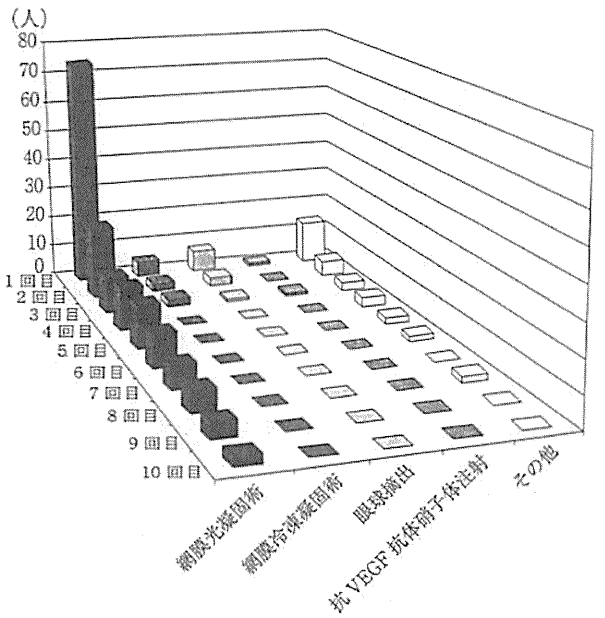

図3　治療法と治療回数

表1　VHL病網膜血管腫診療ガイドラインの要約

1) 可能であれば新生児より経過観察を開始する.
2) 眼底検査により診断するが，蛍光眼底造影検査などの補助検査も重要である.
3) 治療の基本は網膜光凝固であり合併症に対して手術を行う．傍視神経乳頭型では網膜光凝固が不可能な場合もある．その場合には抗VEGF抗体硝子体注射や光線力学的療法を考慮する.

(図3).

3. 経過観察，診断・治療指針のアルゴリズム

発症病態の調査結果と治療内容についての調査結果に基づき，VHL病患者の早期経過観察による診断と治療の指針とアルゴリズムを作成した[5]．その要約を表1に示す.

III 考　　察

平成21年から23年にかけて厚生労働省難治疾患克服研究事業研究奨励疾患としてVHL病に合併する網膜血管腫について，国内の脳神経外科，眼科，泌尿器科，膵臓病内科の各専門医を対象に疫学調査を行った．今回の調査結果から，わが国のVHL病における網膜血管腫の合併率は34%であることが判明した．白色人種での調査結果による海外の既報では40〜70%とされている[4,6]．VHL病の各病態の発症頻度についての報告は白色人種のみでなされており，黄色人種では網膜血管腫の発症頻度の報告は初めてである．今回の結果は，白色人種と比較し，日本人では網膜血管腫の発症頻度はやや低かった．しかし，性差はなく，青壮年期に発症する傾向については，海外の既報と同様の結果であった[4,6]．分布に関し，アンケート調査では「現在の居住県」を尋ねており，必ずしも発症県ではないことにも注意を払う必要がある．また，人口の多い地域により多くの患者が分布する傾向にあり，人口当たりに換算し，地域差を検討する必要もあると考えられた.

VHL病の網膜血管腫に対する治療として，VHL病以外の血管腫でも第一選択である網膜光凝固術が最も行われていた．今回の調査の結果，抗VEGF療法や光線力学的療法など新たな治療に取り組む施設もあった．網膜血管腫の組織学検討からVEGFをはじめとする種々の血管増殖因子が網膜血管腫の発生に関与する可能性が示唆されている[7]．欧米では，傍視神経乳頭型に対し抗VEGF抗体硝子体注射を含む抗VEGF療法[8,9]や光線力学的療法[10]を試み，一定の効果が得られた報告がある．今回の調査では治療法選択に関する詳細な情報は得られていないが，黄斑部に影響を与えている網膜血管腫に施行されたと考えられる．今後の詳細な調査と多施設での検討が期待される．筆者らが昨年作成した網膜血管腫の診療アルゴリズムでも傍視神経乳頭型の網膜血管腫で網膜光凝固術が不可能な症例には抗VEGF抗体硝子体注射や光線力学的療法を考慮するとした[5]．今回の調査結果により，本アルゴリズムの妥当性が示され，今後のVHL診療に役立つものと考えられた.

死亡例にVHL病全体と網膜血管腫で特に差がなかったことから，網膜血管腫の合併の有無が生命予後に与える影響は少ないと考えられた．しかし，本調査で得られた範囲では23名の患者で片眼もしくは両眼が失明していたことから，視力障害，視野障害という観点からQOLは著しく障害されていると考えられる．今回の調査結果から，5歳で診断された症例がいることが判明した．アルゴリズムにも記載しているように[5]，家族歴がある場合は可能であれば新生児より眼底検査を行うことにより早期発見・早期治療が可能となり，視力・視野障害の進行予防に役立つと考えられる.

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家族性に腎がんを発症する VHL病に関して教えてください

腎がんの大部分は散発性に発症します．しかし，家族性・遺伝性の腎腫瘍症候群は全体の2～3%とまれですが複数知られており，そのなかでvon Hippel-Lindau(VHL)病は最も頻度が高く代表的な疾患です．VHL病は常染色体優性遺伝性で，VHLがん抑制遺伝子の遺伝的変異により生来腫瘍ができやすい体質をもち，腎がん以外では，中枢神経系の血管芽腫，網膜血管腫，膵の嚢胞・神経内分泌腫瘍，腎嚢胞，褐色細胞腫，精巣上体嚢胞腺腫などを発症します．腎がんはVHL患者の25～50%にみられ，20歳以後に同時性あるいは異時性に多発傾向があります．病理組織型は淡明細胞型でその特性は非遺伝性の孤発例と差がないと考えられ，治療法も病変を手術的に切除するのが基本ですが，生涯にわたり発症を繰り返す可能性が高いので，正常腎機能を可能な限り温存する核出術や部分切除術などが勧められます．

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解　説

VHL病は，**常染色体優性遺伝性**の疾患で，複数の臓器に**血管に富む腫瘍あるいは嚢胞性病変**を多発する．代表的な発症病変を表1に示す．このうち，褐色細胞腫の発症がない家系(VHL1型家系と呼ばれ，家系全体の約80%)と，好発する家系(2型家系，約20%)が知られており，後者では80～90%の患者で褐色細胞腫が発症する．VHL病以外では，家族性乳頭状腎がん，遺伝性平滑筋腫症・腎がん，Birt-Hogg-Dubé病などの遺伝性腎がん症候群がまれであるが知られている．

VHL遺伝子およびその機能

VHL遺伝子は**がん抑制遺伝子**(tumor suppressor gene)に分類され，Knudsonが提唱した2-hitの機構で2つのアレル(allele)に変異が起こりその機能が消失し，細胞の腫瘍化が始まると考えられる．VHL家系患者では，遺伝的変異(germline mutation)により，出生時にすでに片側のVHL遺伝子の不活性化が起こっており(1-hit)，その後対立アレルに体細胞変異(somatic mutation)が起こることで(2-hit)，遺伝子機能が完全に消失する．一方，散発例の淡明細胞型腎がんでもVHL遺伝子の高頻度(50～80%)の変異，不活性化が検出されるが，この場合には2回の体細胞変異が起きている．臨床的にVHL病と診断された家系患者においては80～90%でVHL遺伝子の遺伝的変異が検出できるので，この変異を指標とした遺伝子診断(DNA test)が行われている．また，VHL患者の約20%はその患者から疾患が始まる，いわゆるnew mutationである．

VHL遺伝子は3つのexonより構成され，ヒトでは染色体3p25.3上の約13,000bpの領域に存在し，そこから全長約4.5kbのmRNAが転写される．mRNAのタンパク翻訳領域は639塩基であるが，アミノ酸1番と54番の2ヵ所のメチオニンより翻訳が開始され，213と160アミノ酸(それぞれ約

表1　VHL病の主な病変と発症年齢・頻度

臓　器	病　変	発症年齢(歳)	頻　度(%)
網　膜	血管腫	1～67	40～70
中枢神経系	血管芽腫	9～78	60～80
小　脳			44～72
脳　幹			10～25
脊　髄			13～50
内　耳	内耳リンパ嚢腫	12～50	11～16
膵	嚢　胞	13～80	17～61
	神経内分泌腫瘍	16～68	8～17
腎	嚢　胞	15～	60～80
	淡明細胞腎がん	20～60	25～50
副腎，パラガングリオン	褐色細胞腫	3～60	10～20
精巣上体(男性)	嚢腫腺腫	思春期以降	25～60
子宮広間膜(女性)	嚢腫腺腫	16～46	～10

30kdと19kdのサイズ)の2種類のVHLタンパクが作られ，両者ともに腫瘍抑制機能を示す．

VHLタンパク(pVHL)はElongin C, Elongin B, CUL2, RBX1と結合し，E3 ubiquitin ligase複合体を形成し，HIF prolyl hydroxylase (HPH)により翻訳後修飾(プロリン残基の水酸化)をうけたHIF(hypoxia-inducible factor)(低酸素誘導因子)αをポリユビキチン化する．ユビキチン化されたHIFαタンパクは，その後26S proteasomeで速やかに分解される．HPHは正常酸素圧下では活性化してHIFの分解を誘導するが，一方低酸素状態や一酸化窒素(NO)，活性酸素(ROS)，コバルトの存在下ではHPHに抑制がかかり，HIFαの蓄積・転写促進が進む．

HIFにより転写促進される遺伝子はこれまでに100個以上が知られており，①血管新生(VEGF, PDGFBなど)，②アシドーシス補正(CA9など)，③グルコースの取り込み・嫌気的解糖系の促進，クエン酸回路の抑制(GLUT1, LDHなど)，④細胞の接着性低下，運動性・転移能促進，マトリックスの再構成(TGFα, CXCR4, MET, MMP2, CTSD, FN1, VCAM1など)が知られている．一方，VHLが不活性化した細胞では，正常酸素圧状態においてもHIFαの分解ができず，HIFは上記遺伝子群を非生理的・恒常的に発現させ，細胞の腫瘍化に結びついていることが想定されている．さらにVHLタンパクは，①神経細胞のアポトーシス抑制と褐色細胞腫の発生機構，②fibronectin (FN1), type IV collagenとの結合と細胞外マトリックスの構成調節，③細胞のprimary ciliaの形成と嚢胞形成，など多様な機能にかかわることが明らかになりつつある．

診断基準

VHL病の家族歴が明らかな場合，表1の典型的な腫瘍病変が1つでも発症すれば診断できる．一方，家族歴がない場合は，①中枢神経系あるいは網膜の血管芽腫が複数個発症，②中枢神経系あるいは網膜の血管芽腫が1ヵ所とほかの典型的な腫瘍病変発症で臨床的に診断する．さらに，VHL遺伝子診断で変異が確認できれば確実である．

治療法，スクリーニング

発症する腫瘍の治療法は基本的に散発例のものと同様であるが，VHL病では，より若年層から複数臓器に病変を繰り返すので，患者のQOLおよび罹患臓器の機能を可能な限り温存しつつ病変のコントロールを行うことが肝要である．腎では15歳すぎからダイナミックCTによるスクリーニングを開始し，腫瘍がない場合には，～1x/3年で画像フォローを行う．腫瘍がある場合は，サイズがおおむね2cmになった時点で外科的治療を考慮

する．核出術，部分切除，ラジオ波焼却術など残腎機能を温存する術式がまず推奨される．腎嚢胞は症状がない場合は経過観察でよいが，腫瘍切除術に際しては，筆者らは切除ないし電気メスによる焼却処置を同時に行っている．腎がん以外では膵神経内分泌腫瘍と，まれに褐色細胞腫が悪性所見を示すので留意する．わが国におけるVHL病の研究班が組織され，診療ガイドラインが間もなく上梓される予定である．

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RESEARCH ARTICLES

Copy Number Profiling in Von Hippel-Lindau Disease Renal Cell Carcinoma

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Germline mutations in the *VHL* tumor suppressor gene cause von Hippel-Lindau (VHL) disease and somatic *VHL* mutations occur in the majority of clear cell renal cell carcinoma (cRCC). To compare copy number abnormalities (CNAs) between cRCC from VHL patients and sporadic cRCC cases without detectable somatic *VHL* mutations, we analyzed 34 cRCC with Affymetrix 250K arrays. To increase the power of the study, we then combined our results with those of a previously published study and compared CNAs in VHL and non-VHL related cRCC using the genomic identification of significant targets in cancer (GISTIC) program. In VHL, cRCC GISTIC analysis identified four statistically significant regions of copy number gain and four statistically significant regions of deletion that occurred in >10% of tumors analyzed. Sporadic cRCC without detectable *VHL* mutations had, on average, more copy number abnormalities than VHL cRCC though the most common regions of loss/gain (e.g., 3p and 14q loss and 5q gain) were present in both tumor sets. However, CNAs on chromosome arms 7p (gain) and 8p (loss) were only detected in VHL RCC. Although individual copy number abnormality peaks contained clear candidate cancer genes in some cases (e.g., the 3p loss peak in VHL cRCC contained only six genes including *VHL*), most peaks contained many genes. To date, only a minority of the candidate genes included in these peaks have been analyzed for mutation or epigenetic inactivation in cRCC but *TNFRSF10C* and *DUSP4* map to the 8p region deleted in VHL cRCC and *TP53* and *HIF2A* (*EPAS1*) mapped to CNA loss and gain peaks (chromosomes 17 and 2, respectively) detected in sporadic *VHL* wild-type cRCC.

INTRODUCTION

Von Hippel-Lindau (VHL) disease is a dominantly inherited familial cancer syndrome characterized by the development of retinal and central nervous system hemangioblastomas, clear cell renal cell carcinoma (cRCC), phaeochromocytoma, and pancreatic tumors. VHL disease is a rare disorder with a birth incidence of ~1 in 36,000 (Maher et al., 1990a,b; Kaelin, 2007) whereas RCC accounts for 2–3% of all cancers. Investigations of the molecular basis of VHL disease have provided seminal insights into the pathogenesis of sporadic RCC. Statistical analysis of the age incidence curves for RCC in VHL disease and sporadic renal cell carcinoma were compatible with a single rate-limiting step mutation model for VHL disease and a two rate limiting mutation model for sporadic RCC (Maher et al., 1990a,b). Subsequently, (a) VHL disease was shown to result from inactivating mutations in the *VHL* tumor suppressor gene (TSG) and RCC from patients with VHL disease demonstrated somatic inactivation of the wild-type allele (Latif et al., 1993; Prowse et al., 1997) and (b) most sporadic clear cell RCC (the most

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common form of RCC) were found to harbor biallelic *VHL* TSG inactivation (Foster et al., 1994; Gnarra et al., 1994; Banks et al., 2006). Biallelic inactivation of the *VHL* TSG is a critical and early event in the pathogenesis of cRCC in VHL disease and in many sporadic cRCC; however, additional genetic and epigenetic events are required for the development of cRCC. Recently high resolution genome wide copy number analysis and high throughput sequencing of candidate genes have been employed to delineate the "post-*VHL* inactivation events" that occur in the development of sporadic RCC (Beroukhim et al., 2009; Dalgliesh et al., 2010; Tan et al., 2010). However, RCC is clinically and histopathologically heterogeneous. Familial RCC, such as those seen in patients with VHL disease, provide an opportunity to investigate a more homogeneous group of cancers. Only one previous study (Beroukhim et al., 2009) has reported high resolution copy number analysis of cRCC from patients with VHL disease.

To further define the role of large-scale copy number abnormalities in cRCC tumorigenesis in VHL disease and in *VHL*-wild-type sporadic cRCC, we analyzed tumor DNA for copy number abnormalities from 21 cRCC from VHL disease patients and 13 sporadic cRCC without evidence of somatic *VHL* inactivation and undertook an in silico analysis using the genomic identification of significant targets in cancer (GISTIC) program of our own results and those previously published by Beroukhim et al. (2009).

MATERIALS AND METHODS

Tumor Samples

Genomic DNA was extracted from primary renal cancers and cell lines by standard methods, and stored at -80°C. Three groups of renal cancers were investigated: (a) 21 clear cell RCC from 18 patients with von Hippel-Lindau disease, (b) 13 sporadic clear cell RCC without evidence of somatic *VHL* mutations or promoter methylation [details of mutation and methylation analyses have been reported previously (McRonald et al., 2009)]. In addition, normal constitutional DNA from two VHL disease patients was analyzed by SNP arrays. Ethical approval for collection of clinical material was obtained from the South Birmingham Ethics Committee and relevant local ethics committees. DNA concentrations were measured with Nanodrop model ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Copy Number Analysis

Experiments were performed according to standard protocols for Affymetrix GeneChip Mapping 250K Sty arrays (Gene Chip Mapping 500K Assay Manual, P/N 701930 Rev2., Affymetrix Santa Clara, CA). Genotype analysis was performed using Affymetrix Genotyping Console version 4.0 with the default settings. QC call rates of the 34 cRCC samples ranged from 87.9% to 98.7%. The array signal intensity CEL files of the 34 cRCC and 268 hapmap samples (www.hapmap.org/downloads/raw_data/affy500k/) were analyzed together using dchip (Li and Wong, 2001) with invariant set normalization and the PM/MM difference model. SNP-level raw log2 ratios relative to the average of the hapmap samples were exported from dchip. Data within copy number variation regions (Affymetrix Mapping250K_Sty Annotations release 29, July 2009) were removed. Raw log2 ratios were centered to a median of zero and segmented using GLAD (Hupe et al., 2004) with the HaarSeg algorithm (Ben-Yaacov and Eldar, 2008). GISTIC analysis (Beroukhim et al., 2007) was performed using GenePattern public server (Reich et al., 2006) with the default settings of amplifications threshold of 0.1, deletions threshold of 0.1, join segment size of 4, and qv threshold of 0.25. SNP, gene, and cytogenetic band locations are based on the hg18 (March, 2006) genome build (http://genome.ucsc.edu). Raw log2 ratio data of previously published cRCC samples (Beroukhim et al., 2009) were kindly provided by Dr. Rameen Beroukhim.

RESULTS

GISTIC Analysis of Copy Number Analysis Data

The GISTIC software program was developed to distinguish "driver" (functionally important) copy number alterations (CNAs) from associated "passenger alterations." Thus, the GISTIC method aims to identify genomic regions that are aberrant more often than would be expected by chance and to give greater weight to high amplitude events (e.g., amplifications or homozygous deletions) that are less likely to represent random events (Beroukhim et al., 2007). GISTIC calculates (a) a G score that takes into account the frequency and the amplitude of the CNAs and (b) a q value to that reflects the probability that the specific CNA results from chance fluctuation (based on the overall pattern of CNAs across the genome and taking into account multiple-

hypothesis testing and possible false-discovery). We considered all events with q values <0.25 to be statistically significant.

Comparison of GISTIC Copy Number Analysis in VHL and Sporadic Non-VHL cRCC

To most effectively compare the GISTIC copy number profiles of *VHL* cRCC tumors with sporadic *VHL* wild-type (*VHL*-wt) cRCC, we combined our data on 34 cRCC with that previously reported by Beroukhim et al. (2009), who analyzed 36 primary tumors from 12 patients with VHL disease and nine sporadic *VHL*-wt cRCC using the same the Sty I (250K) single nucleotide polymorphism (SNP) arrays used in our study. Thus, in total, copy number analysis data was available for 57 VHL disease cRCC and 22 sporadic *VHL*-wt cRCC.

GISTIC copy number analysis in VHL cRCC

GISTIC analysis of the combined data set of VHL cRCC revealed four statistically significant peaks for copy number gains: on chromosome 2 (21% of tumors; peak at 2q31.1), 5 (56%; 5q34), 7 (18%; 7p14.1), and 12 (11%; 12q12) (Table 1 and Fig. 1). The peaks on chromosomes 7 and 12 were wide (~15.9 Mb and ~9.1 Mb, respectively) and contained large numbers of genes (862 and 695, respectively). However, the peaks on chromosomes 2 and 5 contained smaller numbers of genes (~ 2.8 Mb and 131 genes and ~1.85 Mb and 133 genes, respectively).

GISTIC analysis identified five statistically significant peaks for deletions: on chromosomes 3 (86%; 3p25.3), 4 (14%; 4q28.3), 8 (21%; 8p21.2), 12 (5%; 12q12), and 14 (25%; 14q23.3). The chromosome 3 peak (at 3p25.3) contained only six genes including the *VHL* TSG. The next most significant peak on chromosome 14 contained 67 genes whereas those on chromosomes 4 and 8 contained >200 genes (297 and 220 genes, respectively). The chromosome 12 peak was narrow and did not contain any known genes (the closest was *KIF21A*).

The median number of significant events (gain or loss) per VHL disease tumor was 2 (range: 0–7) (Fig. 3). The most common early event was 3p loss (present in 9/10 tumors with a single gain/loss event), followed by 5q gain (of 18 tumors with only two events all had 3p loss and 16 had 5q gain). The other changes were all most commonly seen in tumors with three or more changes though 2q gain was present in two tumors with only two changes and a 12q deletion, though rare, was present as the only change in one tumor.

To identify potential candidate tumor suppressor or oncogenic genes in areas of copy loss and gain we interrogated the results of high throughput sequencing of 3,544 genes in RCC Dalgliesh et al. (2010) and our previously reported Illumina Goldengate methylation array profiling results for VHL cRCC analyzed in this study McRonald et al. (2009). Lists of the genes in the nine candidate statistically significant regions (Table 1) are recorded in Supplementary Tables 1 and 2. Strikingly, the identified region for the most frequent copy number abnormality, chromosome arm 3p loss, contained only six genes including the *VHL* TSG. However, none of the genes that had been sequenced by Dalgliesh et al. (2010) and that mapped within other significant regions of copy loss or gain were mutated in >2% of samples (Table 1). Epigenetic inactivation of TSG by promoter region hypermethylation is a frequent finding in human cancer including RCC. We reviewed our previously reported data on the methylation status of 807 genes (assessed by Illumina Goldengate methylation assay) by McRonald et al. (2009) to determine if any genes that showed evidence of frequent tumor specific hypermethylation mapped within significant regions of copy number loss. Three genes had previously been demonstrated to acquire frequent (>10%) tumor-specific promoter region CpG methylation in our previous study of VHL RCC mapped within significant regions of number loss region: *PITX* (within the 4q region) was methylated in 24% of VHL RCC and *TNFRSF10C* (8p22-p21) and *DUSP4* (8p22) were methylated in 24% and 17%, respectively.

GISTIC copy number analysis in VHL wild-type cRCC

GISTIC analysis of the 22 cRCC without detectable *VHL* mutations revealed seven statistically significant peaks for copy number gains: on chromosomes 2 (2q14.3; 18% of tumors), 5 [5p15.31 (32%), 5q13.3 (23%), and 5q35.2 (50%)], 6 (6p21.1; 9%), 8 (8q24.3; 23%), and 12 (12q24.32; 32%) (Table 2 and Fig. 2). GISTIC analysis identified six statistically significant peaks for deletions: on chromosomes 1 (1p22.2; 32%), 3 (3p25.3; 50%), 11 (11q23.3; 18%), 14 (14q11.2; 41%), 16 (16q23.2; 14%), and 17 (17p11.2; 27%).

The median number of significant events (gain or loss) per VHL wild-type cRCC tumor was 3 (range: 0–10) (Fig. 3). Although 3p loss was the joint most frequent event in tumors with only one or two copy number abnormalities, in contrast to the VHL tumors, it was found in only 2/5 such cases and in tumors with three or less copy number

TABLE 1. GISTIC Analysis Results of 57 VHL RCC

	Cytoband	Q value	Residual q value after removing segments shared with higher peaks	Frequency of gain or loss	Wide peak boundaries	Number of genes within wide peak boundaries	Number of genes sequenced in RCC*	Genes mutated (frequency) in RCC*
Regions of copy number gain	2q31.1	0.0013382	0.0013382	21%	chr2: 151155310-179077227	131	19	RAPGEF4 (1%) RIF1 (1%)
	5q34	2.60E-26	2.60E-26	56%	chr5: 162372772-180857866	133	24	–
	7p14.1	0.02291	0.022911	18%	chr7: 1-158821424	862	132	CARD11 (2%); DGK1, LRGUK, NCAPG2, PTPRZ1, TRIM4, ZRF1, PRKAG2, BRAF, CHST12, GLI3, SNX13, TRIM56, GNG11 and PHF14 (all 1%)
	12q12	0.15793	0.15793	11%	chr12: 1-91047873	695	161	AKAP3, ASB8, CCND2, E2F7, GDF11, LRP6, NAV3, NCAPD2, PDZRN4, PFKM, PRKAG1, SPSB2, PLEKHAF5 and ZNF384 (all 1%)
Regions of Copy Number Losses	3p25.3	2.70E-39	2.70E-39	86%	chr3: 10062639-10276299	6	3	VHL (55%)
	4q28.3	0.19804	0.19804	14%	chr4:62126311-132023141	297	51	ADH6, COPS4, HERC6, PTPN3 and USP53 (all 1%)
	8p21.2	0.0066086	0.0066086	21%	chr8: 1-40668448	220	39	ASAM7, ASDAM18, ADAM32, DLC1, PPP2R2A, TNKS and XPO7 (all 1%)
	12q12	0.0017333	0.0017333	5%	chr12: 37843161-37882927	0	–	–
	14q23.3	0.0008957	0.0008957	25%	chr14: 31759308-50375581	67	27	NIN (1%)

*Data derived from Dalgliesh et al. (2010).

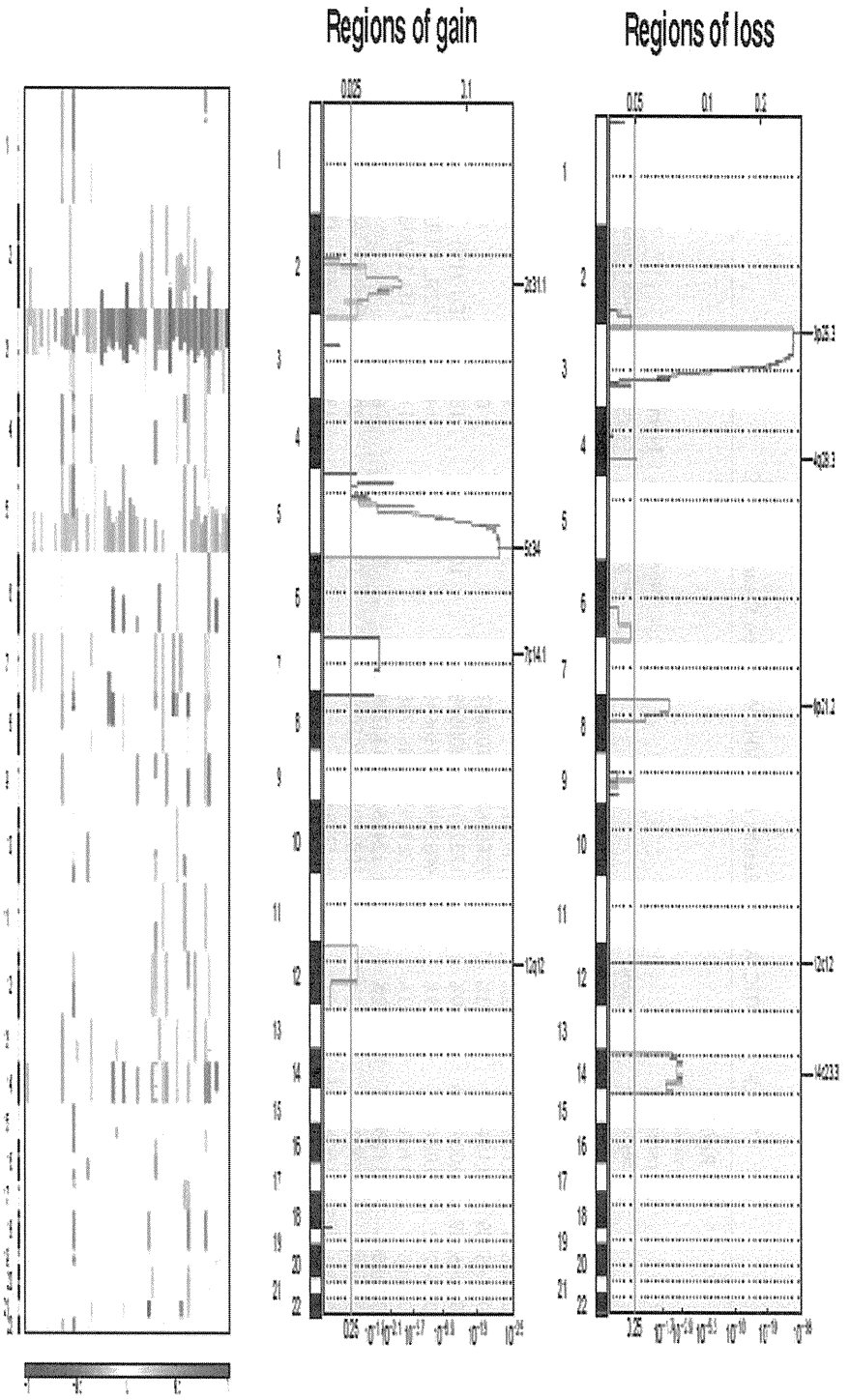

Figure 1. Left panel: GISTIC analysis results for copy number alterations in 57 renal cancers from patients with von Hippel-Lindau disease (see text for details) determined by segmentation analysis of normalized signal intensities from 250K SNP arrays. Amplifications (red) and deletions (blue) are displayed across the genome (chromosome positions, indicated along the *y* axis). Middle panel: the statistical significance of the copy gain aberrations identified is displayed as FDR *q* values to account for multiple-hypothesis testing. Chromosome positions are indicated along the *y* axis with centromere positions indicated by dotted lines. Statistically significant copy gain events exceeded the significance threshold (green line). Right panel: the statistical significance of the copy gain losses identified is displayed. Four statistically significant peaks for copy number gains and five for deletions were detected. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 2. GISTIC Analysis Results of 22 VHL Wild-Type Clear Cell RCC

	Cytoband	Q value	Residual q value after removing segments shared with higher peaks	Frequency of gain or loss	Wide peak boundaries	Number of genes within wide peak boundaries	Number of genes sequenced in RCC*	Genes contained in copy number abnormality regions that are mutated (frequency) in RCC*
Regions of copy number gain	2q14.3	0.041496	0.041496	18%	chr2: 1-216445899	967	170	TPO, RNF144 (each 1%)
	5p15.31	0.011634	0.13857	32%	chr5: 1-6698146	34	9	–
	5q13.3	0.0045289	0.15441	23%	chr5: 73655673-73706663	0	0	–
	5q35.2	0.0045289	0.0045289	50%	chr5: 172211671-180857866	104	18	NSD1 (1%)
	6p21.1	0.034212	0.034212	9%	chr6: 40850964-47366681	92	14	CDC5L, CUL7, XPO5 (each 1%)
	8q24.3	0.16884	0.16884	23%	chr8: 138939588-146274826	99	11	EEF1D, SCRIB (each 1%)
	12q24.32	0.12806	0.12806	32%	chr12: 124163345-132349534	32	6	–
Regions of copy number losses	1p22.2	0.000834	0.000834	32%	chr1: 50860054-92105006	197	30	CDKN2C, and PGM1 (each 1%)
	3p25.3	0.00011935	0.00011935	50%	chr3: 1-13494937	67	13	VHL (55%), ITPR1 and PPARG (each 1%)
	11q23.3	0.18897	0.18897	18%	chr11: 101247817-129938942	255	61	ATM (3%), MLL (3%), ARHGAP20 (2%), MMP10, MMP3, PAFAH1B2, POU2AF1, TRIM29 and UBE4A (each 1%)
	14q11.2	0.019341	0.019341	41%	chr14: 1-20715172	49	5	–
	16q23.2	0.0081492	0.0081491	14%	chr16: 77757916-80539342	14	2	–
	17p11.2	0.10166	0.10166	27%	chr17: 1-22479311	321	61	TP53, NCOR1, NUP88, PER1 and TNFRSF13B (each 1%),

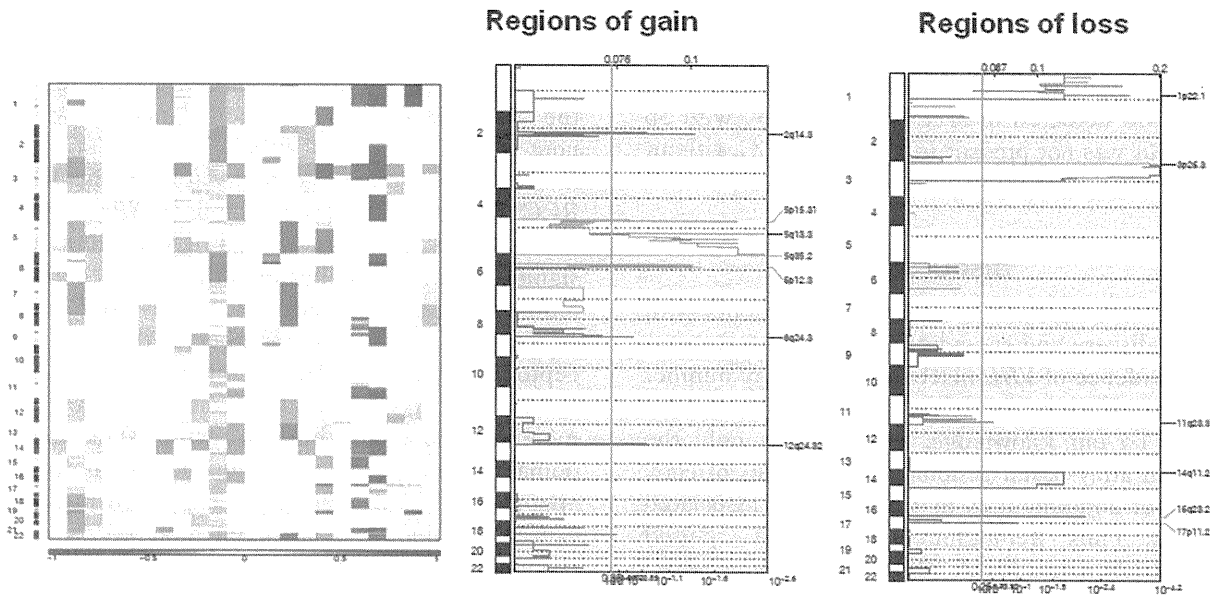

Figure 2. Left panel: GISTIC analysis results for copy number alterations in 22 clear cell renal cell carcinomas with wild-type *VHL* determined by segmentation analysis of normalized signal intensities from 250K SNP arrays. Amplifications (red) and deletions (blue) are displayed across the genome (chromosome positions, indicated along the *y* axis). Middle panel: the statistical significance of the copy gain aberrations identified is displayed as FDR *q* values to account for multiple-hypothesis testing. Chromosome positions are indicated along the *y* axis with centromere positions indicated by dotted lines. Statistically significant copy gain events exceeded the significance threshold (green line). Right panel: the statistical significance of the copy gain losses identified is displayed. Four statistically significant peaks exceed the significance threshold for copy number gains and six for deletions were identified. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

abnormalities gains at 5q35.2 or 12q24.3 and loss at 14q11.2 were equally frequent.

Comparison of the gain/loss patterns in VHL cRCC and *VHL* wild-type cRCC revealed that the most common CNAs (gains on 5q and losses on 3p and 14q) were common to both tumor sets (though the precise GISTIC peaks might vary). Overall, *VHL*-wt cRCC had more significant regions of CNA than VHL RCC. Frequent (>20% of tumors) statistically significant peaks that were detected in only one set of tumors included gains on 8q and losses on 1p and 17p in *VHL*-wt cRCC and gain on 7p and loss on 8p in VHL RCC.

As for VHL RCC, we interrogated the results of high throughput sequencing of 3,544 genes in RCC (Dalgliesh et al., 2010) and our previously reported Illumina Goldengate methylation array profiling results for *VHL*-wt cRCC analyzed in this study by McRonald et al. (2009). Lists of the genes in the 13 candidate regions are recorded in Supplementary Tables 3 and 4. We note that, despite the tumors being selected for the absence of a detectable *VHL* gene mutation, the GISTIC delineated region of chromosome arm 3p loss contained the *VHL* TSG. Sequencing data for sporadic RCC were available for 12 of 66 other genes in the GISTIC defined 3p region but none of these genes were found to be frequently mutated in the study

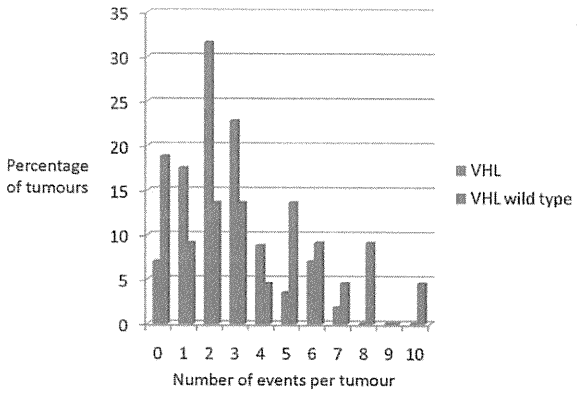

Figure 3. Distribution of copy number abnormalities (in GISTIC defined significant regions) in renal cancers from von Hippel-Lindau disease patients and sporadic renal cancers with wild-type *VHL*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

by Dalgliesh et al. (2010) (Table 3). In addition, none of the 159 genes from other significant regions of copy loss were mutated in >3% of sporadic RCC (Dalgliesh et al., 2010; Table 3). Interrogation of our previous published Illumina Goldengate methylation array analysis (McRonald et al., 2009) revealed that *EFNB3*, which maps within the 17p copy number loss region, was methylated in 20% of sporadic *VHL*-wt cRCC (TP53 was also included in this region).

VHL-wt cRCC with 3p loss had more copy number changes (median: 5, mean: 5.3) than *VHL*-wt cRCC without 3p loss (median: 1, mean: 2). 8q gain was detected in 5/11 *VHL*-wt cRCC with 3p loss but was not present in *VHL*-wt cRCC without 3p loss ($P = 0.035$).

DISCUSSION

We investigated the cRCC from patients with VHL disease and sporadic cRCC without detectable evidence of *VHL* inactivation for copy number abnormalities using high-resolution SNP microarrays. To our knowledge, this study is only the second array-based genome wide analysis of copy number abnormalities in VHL disease associated cRCC. Thus, Beroukhim et al. (2009) reported previously a study of VHL and sporadic RCC using the same microarray platform (Affymetrix 250K SNP array) and this provided us with the opportunity to undertake a GISTIC-based analysis of the combined data for VHL RCC. The combined analysis revealed five significant regions of copy number loss and four significant regions of copy number gain. As expected, the most frequent copy number change (86% of VHL RCC) was 3p loss and it was striking that the GISTIC analysis identified a very small critical region that contained only six genes including the *VHL* TSG. The next most frequent change in VHL cRCC was 5q gain and GISTIC analysis highlighted a ~ 18 Mb interval containing 133 genes. The other significant copy number loss/gain alterations occurred in no more than 25% of tumors and in most cases the critical regions identified were large and contained many candidate genes (though the infrequent 12q12 loss region was very small and did not contain any genes). To identify potential candidate genes that might map within the identified regions the results from the Cancer Genome Project sequencing of 3,544 genes in 101 sporadic RCC (Dalgliesh et al., 2010) were interrogated to identify frequently mutated genes. However, excepting *VHL*, no such genes were identified. Apart from *VHL*, the most commonly mutated genes in cRCC demonstrate mutations in only a minority of tumors (e.g., 7% for *CDKN2A*, 6% for *PTEN* and *SETD2*; Dalgliesh et al., 2010). In contrast, in excess of 50 candidate TSG have been reported to be inactivated by acquired promoter region hypermethylation (see Morris and Maher, 2010 references within) and we have previously reported a methylation profile of 807 genes in VHL RCC using CpG methylation array methodology (McRonald et al., 2009). Although only a fraction of the genes within the five significant regions of copy number loss were represented on the Illumina Goldengate methylation array we note that three genes *PITX*, *TNFRSF10C*, and *DUSP4* were frequently methylated in the VHL RCC samples. *TNFRSF10C* and *DUSP4* map to the 8p region that was deleted in VHL RCC (no significant correlation between the presence of deletion and gene methylation was detected). *TNFRSF10C* encodes a member of the TNF-receptor superfamily (DcR1) that contains an extracellular TRAIL-binding domain and a transmembrane domain, but no cytoplasmic death domain (and so is not capable of inducing apoptosis). The protein is not expressed in many cancer cell lines and has been reported to show promoter hypermethylation and silencing in a variety of cancers including VHL disease associated phaeochromocytomas (Shivapurkar et al., 2004; Margetts et al., 2005). *DUSP4* encodes a dual specificity protein phosphatase (also known as MKP-2) that was recently reported to be frequently epigenetically silenced gene in gliomas (Waha et al., 2010). Hence both *TNFRSF10C* and *DUSP4* would seem to merit further investigation as candidate TSGs in VHL disease associated RCC.

Most RCC in VHL disease patients are detected presymptomatically and surgically removed when the tumor reaches ~3 cm. In contrast, only a minority of sporadic RCC is detected presymptomatically and so, on average, cRCC removed from sporadic patients are larger than those removed from VHL patients. Hence genetic and epigenetic differences between VHL RCC and sporadic *VHL*-wt cRCC might reflect (a) differences in stage of tumorigenesis (i.e., later in sporadic cases), (b) differences in mechanisms of tumorigenesis according to the presence or absence of *VHL* mutations, and/or (c) in view of the smaller number of RCC analyzed, lack of power to detect changes in the sporadic *VHL*-wt cRCC. Copy number gains on chromosomes 2, 5, and 12 were found in both VHL and wild-type *VHL* cRCC (also on chromosome 7 but this did not reach statistical significance in wild-type *VHL* cRCC) but a chromosome 8 peak was only detected in wild-type *VHL* cRCC. Copy number losses on chromosomes 3 and 14 were found in both tumor types but chromosomes 1, 11, 16, and 17 losses were only significant in wild-type *VHL* cRCC. Given that (on average) non-VHL tumors were more advanced this might be expected, but it was interesting that loss on chromosome 8 was only apparent in VHL

cRCC, suggesting that it is likely to be preferentially associated with *VHL*-dependent mechanisms of tumorigenesis. The presence of 3p25 loss in the "*VHL*-wt cRCC" might reflect the presence of undetected non-coding region or mosaic mutations in a "contaminating" subset of tumors or that 3p loss was targeting other 3p TSG or that partial (hemizygous) *VHL* inactivation might promote tumorigenesis in these cases. However, we note that whereas 3p25 loss was present in VHL tumors with very few copy number changes it did not appear to be such an early event in the *VHL*-wt cRCC suggesting that many such tumors are initiated by VHL independent mechanisms (even if 3p loss occurs subsequently). 14q loss has previously been associated with tumor aggressiveness and poor survival in RCC (Alimov et al., 2004). We note that the chromosomes 2 and 17 regions of gain and loss, respectively, in *VHL*-wt cRCC contained the candidate genes *HIF2A* (*EPAS1*) and *TP53*. Inactivation of *VHL* leads to increased expression of HIF-1 and HIF-2 hypoxia inducible transcription factors but several lines of evidence suggest that HIF-2 rather than HIF-1 is critical for driving renal tumorigenesis (Mandriota et al., 2002; Kondo et al., 2003; Raval et al., 2005; Morris et al., 2009), including the recent finding that a genome-wide association study of RCC identifies *HIF2A* as one of two significant susceptibility loci (Purdue et al., 2011); hence, it may be that gains of the *HIF2A* region in *VHL*-wt cRCC might partially mimic the effects of VHL inactivation.

Consistent with the hypothesis that the sporadic non-VHL cRCC were (on average) removed at a more advanced stage, *VHL*-wt cRCC did, on average, harbor more copy number changes than VHL cRCC (Fig. 3). A previous analysis of a very large number of unselected RCC reported that the most frequent cytogenetic changes were loss of 3p (60%), 14q (28%), 8p (20%), 6q (17%), 9p (16%), and 4p (13%), gain of 5q (33%) and trisomy 7 (26%) (Klatte et al., 2009). Copy number analysis studies of sporadic RCC using high resolution SNP arrays have demonstrated recurrent losses on 3p, 4, 6q, 8p, 9p, and 14q and recurrent gains on 1q, 2, 5q, 7, and 12 (Dalgliesh et al., 2010), as did previous smaller studies using lower resolution microarrays (Cifola et al., 2008; Toma et al., 2008). Though the design of these studies differed from ours (sporadic RCC rather than VHL cRCC), as most unselected RCC will be cRCC with *VHL* inactivation, it is apparent that most of the copy number changes observed in VHL cRCC also occur in sporadic cRCC suggesting that VHL RCC could be used as a model to elucidate the timing of genetic changes in the evolution of cRCC (kidneys removed from VHL patients typically contain, in addition to the clinical RCC, a multitude of smaller lesions of varying sizes).

The ultimate aim of cancer geneticists is to understand the precise pathogenetic mechanisms that drive tumorigenesis in individual cancers and so provide a basis for personalized cancer therapies. A comprehensive genomic analysis of RCC requires knowledge of the mutational, transcriptional, epigenetic, and copy number status of individual genes. Further advances in the evaluation of gene copy number analysis (e.g., higher resolution arrays and massive parallel sequencing techniques) will facilitate the investigation on copy number status of individual genes. At present, the most widely detected copy number changes are large (often encompassing a whole chromosome or chromosome arm) but bioinformatic tools such as GISTIC can highlight smaller regions that are apparently most likely point to contain key genes (as exemplified with 3p25 and *VHL*). Our findings suggest that VHL cRCC can provide a paradigm for delineating the evolution of the most common form of sporadic RCC. In addition, although there is overlap between the copy number changes detected in VHL cRCC and sporadic *VHL*-wt cRCC some changes (16q and 17p) are preferentially associated with specific subtypes and further studies are required to determine the potential role of individual genes within these regions.

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