厚生労働科学研究費補助金（難治性疾患克服研究事業）
鰓弓耳腎（BOR）症候群の遺伝子診断法の確立と診療体制モデル構築に関する研究
分担研究報告書

耳鼻科的観点からのBOR症候群の遺伝子診断法の確立と診療体制モデル構築に関する研究

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聴覚障害研究室長

研究要旨

鰓弓耳腎（Branchio-oto-renal（BOR））症候群は、頸瘻・耳瘻孔・外耳奇形などの鰓原性奇形、難聴、腎尿路奇形を３主徴とする症候群である。本年度の研究では、BOR 症候群の遺伝子診断法の確立と診療体制モデル構築を目指して、耳鼻咽喉科的観点からの BOR 症候群に関する情報公開と診療体制モデルの構築を目的とした。このため、難聴の遺伝子診断のためのパンフレットを作成して本症候群の診療にも活用するとともに、国立成育医療研究センター耳鼻科を初めに受診した患者の診療体制モデルの構築について検討した。この結果、パンフレットは遺伝子診断の内容についての理解の促進、遺伝子検査に対する不安の解消、遺伝性疾患についての家族間での話し合いの機会の増加、遺伝について正しく知る機会として有効であった。診療体制モデルの確立は初めの窓口がどの診療科となっても、最終的な診療内容が共通して最適となることが重要と考えられた。

A.　研究目的

鰓弓耳腎（Branchio-oto-renal（BOR））症候群は、頸瘻・耳瘻孔・外耳奇形などの鰓原性奇形、難聴、腎尿路奇形を３主徴とする症候群である。BOR症候群の欧米での頻度は4万人に1人とされ、小児高度難聴の2%を占めるとされている。BOR症候群は、まれな疾患で、小児科と耳鼻咽喉科の境界領域にあり、頭頚部の奇形もそれほど顕著でないために診断がつかないままの患者も少なくないと思われる。また、本症候群は先天性の高度難聴や小児期腎不全の重要な原因であり、小児科医、耳鼻咽喉科医、遺伝専門医らによる総合的な医療を要する。本年度の研究では、BOR症候群の遺伝子診断法の確立と診療体制モデル構築を目指して、耳鼻咽喉科的観点からのBOR症候群に関する情報公開と診療体制モデルの構築を目的とした。

B. 研究方法

1）BOR 症候群の診療に関する情報公開

本症候群は症候群性難聴の代表的な疾患であり、遺伝子診断により遺伝カウンセリングならびに診療に役立つ情報が得られる。このため、難聴の遺伝子診断のためのパンフレッ

トを作成して、本症候群の診療にも活用した。

2）BOR 症候群の診療体制モデルの構築

本症候群では頭頚部奇形、難聴、腎疾患を呈し、優性遺伝するという特徴がある。このため耳鼻咽喉科、小児科（小児腎臓科）、遺伝科による連携が必須となる。大部分の患者は、初めに耳鼻科あるいは小児科を受診する。本研究では、耳鼻科を初めに受診した患者の診療体制モデルの構築について検討した。

（倫理面への配慮）
本研究を含めた研究計画は、国立病院機構東京医療研究センター、国立成育医療研究センター、神戸大学の倫理審査委員会において承認済みである。

C． 研究結果
1）BOR 症候群の診療に関する情報公開

「難聴の遺伝子診断について」のパンフレット（図1-8）を作成し、実際の診療において活用した。その効果としては、遺伝子診断の内容についての理解の促進、遺伝子検査に対する不安の解消、遺伝性疾患についての家族間での話し合いの機会の増加、遺伝について正しく知る機会となる、などであった。

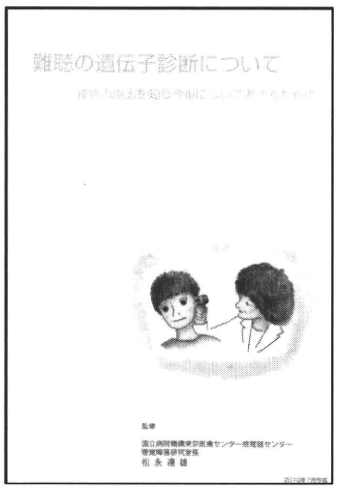

図１

図２

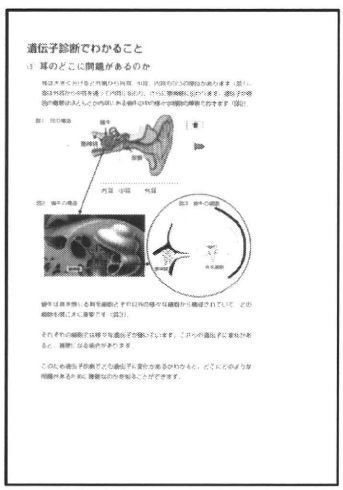

図３

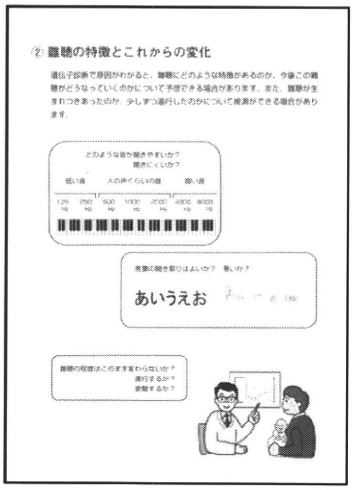

図4

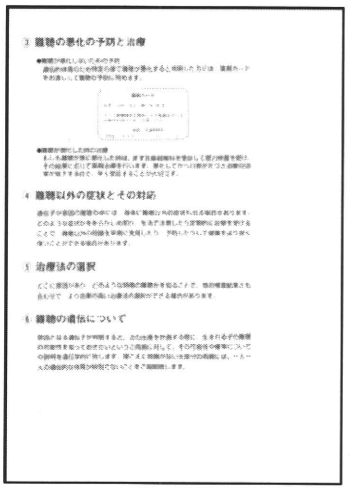

図5

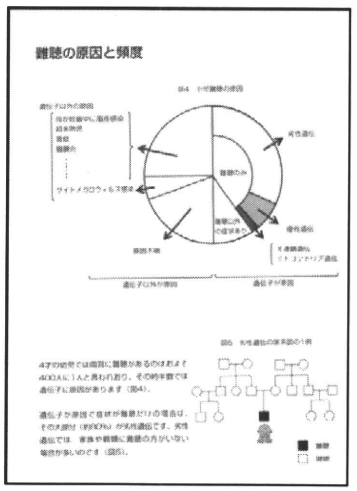

図6

図7

図8

2）BOR 症候群の診療体制モデルの構築

国立成育医療研究センター耳鼻咽喉科の一般診察でBOR症候群の診断となった患者は、遺伝科に紹介されて遺伝子診断の事前説明および遺伝子検査の実施となり、結果の報告と説明は耳鼻咽喉科で行われ、頭頚部奇形、難聴に関する診療は耳鼻科で継続された。併行して、小児科（小児腎臓科）にも紹介されて、腎臓疾患の有無について診察が行われ、必要があればその後の診療も小児科で継続された。遺伝子検査は、EYA1遺伝子のシークエンスを東京医療センターで実施し、異常が認められなかった場合は、神戸大学医学部小

児科でMLPA法によるEYA1遺伝子のエクソン単位の欠失や重複の検索が行われた。それでも異常が認められない場合は、SIX1、SIX5、SALL1など他の候補遺伝子の検索やゲノムワイドCNV　arrayによる解析が行われた。次子の出生前診断などに関する相談、診療は遺伝科に紹介され実施された。

D.考察

1) BOR症候群の診療に関する情報公開

パンフレットの作成は診察室で口頭のみで説明するのと違い、資料が手元に残るので待合室や家庭などで時間をかけて繰り返し読むことができるため、正しい理解が促進される。図解があるため具体的にイメージしやすいことでも、理解や記憶が促進される。また、家族間での話し合うための資料となることから、家族間の相互理解を深めることができる。そして、疑問点があった場合などには、聞きたい内容を次回の診察時に容易に質問できる点も有効である。

2) BOR症候群の診療体制モデルの構築

本症候群のように複数の専門診療科の領域が複合する疾患では、なるべく一施設内で関連する診療科が連携して診療を進めることが望ましい。初めの窓口がどの診療科となっても、最終的な診療内容が共通して最適となるように、あらかじめ診療体制を打ち合わせて決めておくことが重要であると考える。

E.結論

耳鼻咽喉科的観点からのBOR症候群の診療に関する情報公開の１例として「難聴の遺伝子診断について」のパンフレットの有効性を示した。また、耳鼻咽喉科的観点からのBOR症候群の診療体制モデルの構築の1例として、国立成育医療研究センターでの診療体制モデルの実際の運用と有効性を示した。

F．健康危険情報

特になし

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奈良市

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松永達雄
NOG 遺伝子変異による近位指節癒合症を伴う
伝音性難聴を呈した SYM1 の 1 家系
第 55 回日本聴覚医学会総会・学術講演会
2010 年 11 月 11-12 日
奈良市

仲野敦子、有本友季子、大熊雄介、松永達雄、
工藤典代
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の検討
第 55 回日本聴覚医学会総会・学術講演会
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徳丸裕、 進藤彰人、 松崎佐栄子、田中翔子、
角田晃一、 藤井正人
アブミ骨固着症を合併した
Beckwith-Wiedemann 症候群の 1 例
日本耳鼻咽喉科学会東京都地方部会例会　第
190 回学術講演会
2010 年 11 月 13 日
東京都

難波一徳、務台英樹、金子寛生、橋本省、加
我君孝、藤井正人、松永達雄
新規変異型 KCNQ4 蛋白質の立体構造情報によ
る感音性難聴の究明
第 33 回日本分子生物学会年会　第 83 回日本
生化学会大会合同大会
2010 年 12 月 7-10 日
神戸市

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田中翔子、松永達雄、角田晃一、藤井正人、
加我君孝
長期経過後に頬部に転移した嗅神経芽細胞腫
の 1 例
日本耳鼻咽喉科学会東京都地方部会第 191 回
学術講演会
2011 年 3 月 11 日
東京都

H.　知的財産権の出願・登録状況（予定を含
む。）
1. 特許取得
務台英樹、藤井正人、松永達雄
特願２０１１－７５８１
難聴疾患の予防又は治療剤
財団法人ヒューマンサイエンス振興財団
2011年1月18日

2. 実用新案登録　特になし
3. その他　特になし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
鰓弓耳腎（BOR）症候群の遺伝子診断法の確立と診療体制モデル構築に関する研究
分担研究報告書

新規遺伝子変異の網羅的解析手段の確立

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研究要旨

鰓弓耳腎（BOR）症候群の原因遺伝子としてはすでにこれまでに *EYA1*、*SIX1* が報告されている。しかしながらこれまでの変異検出率は両者をあわせても80%足らずであり、決して良好とは言えない。この原因として我々は、遺伝子解析手法の問題が大きいのではないかと推測し、mRNA を用いた RT-PCR 法や Long-PCR 法など、これまでに用いられることのなかった最新の技術を駆使して原因遺伝子変異の同定に努めた。これらの手法により、直接シークエンス法などこれまでの解析手法では明らかにできなかった変異をいくつか同定しえた。しかしこのような最新の手法を用いてもまだ変異を同定し得ない患者が少なからず存在することから、*EYA1*、*SIX1* 以外にも BOR 症候群の原因遺伝子が存在する可能性がある。今後我々は exome 解析、CNV 解析などの新たな手法を用いて新規原因遺伝子の有無につき検討を加える予定である。

A.　研究目的

これまでの報告では、BOR 症候群患者における EYA1 遺伝子変異検出率は 30-70%に過ぎず、決して良好とはいえない。昨年我々は本研究において高い変異検出率を得るための *EYA1* 遺伝子変異網羅的解析方法を確立した。これを用いて、臨床的に BOR 症候群と診断された患者の遺伝子変異の有無について検討を行った。

B.　研究方法

EYA1 遺伝子変異の網羅的解析を行うため、昨年度に我々が確立した以下の方法を用いて検討を行った。

1)　PCR および直接シークエンス法を用いたゲノム DNA の解析

EYA1 の全エクソンおよびエクソン・イントロン境界領域に関して、従来行われてきた直接シークエンス法により解析を行った。

2)　MLPA 法によるゲノム DNA の解析

BOR 症候群は常染色体優性遺伝性疾患である。このためヘテロ接合体の広範囲欠失は上述した直接シークエンス法では検出が不可能である。このような場合には multiplex ligation-dependent probe amplification (MLPA)法によりその変異を検出することが可能となる。

3)　mRNA を用いた RT-PCR 法による解析

ゲノム DNA からの解析を行っても

変異が同定されなかった場合でも、イントロン内の変異によってスプライシング異常がおこり疾患が発症することがある。我々はすでに Gitelman 症候群など他疾患でこのような症例を経験し、報告してきた。EYA1 遺伝子は末梢血単核球でも mRNA 発現を認めるため、RT-PCR 解析が可能である。

C. 結果

臨床的にBOR症候群と診断された6家系において EYA1 遺伝子の解析を行った。その結果、2家系はPCRおよび直接シークエンス法を用いたゲノムDNAの解析により原因遺伝子変異を同定することができた。残る4家系のうち1家系は MLPA 法により数エクソンにわたるヘテロ接合体広範囲欠失が存在することが明らかとなった。この患者の末梢血単核球から mRNA を抽出し、RT-PCR 解析を行うと同様に数エクソンにわたるskippingが確認された。以上の結果から、6家系中3家系においては我々が確立した網羅的解析手法によって *EYA1* 遺伝子変異を同定することができた。しかし残る3家系においては *EYA1* に明らかな変異を認めなかった。

D. 考察

我々が確立した網羅的解析手法を用いてもなお EYA1 遺伝子になんら変異を認めないBOR症候群が存在することが明らかとなった。我々はこの網羅的解析手法をすでに Alport 症候群など他疾患における遺伝子解析ですでに実践し、多数の重要な知見を得てきた。これらの手法を用いても変異が検出されない場合においては、その原因遺伝子は他に存在する可能性が高い。

疾患発症に関与する責任遺伝子の同定にはこれまでに数多くの手法が用いられてきているが、ここ数年で確立された手法としてexome解析がある。exome 解析とは、exon 領域のみをキャプチャーして次世代シーケンサーで解析する方法であり、これまでによく用いられてきた全ゲノム解析に比して効率的でかつ低コストである。また、種々の奇形症候群において、copy number variation (CNV)による遺伝子全体あるいは一部の欠失や重複が原因となることが知られており、ゲノムワイドCNV解析も新規遺伝子同定に有用であると考えられる。BOR 症候群においても今後 exome 解析や CNV 解析を導入し、これまでに報告されている *EYA1*、*SIX1* 以外の新規遺伝子変異の有無について検討を加える必要があるものと考えられる。

E. 結論

我々が確立した網羅的解析手法を用いても *EYA1* に変異が認められないBOR症候群患者が存在することが改めて明らかとなった。BOR 症候群の発症機序や病態解明を明らかにするためには遺伝子変異の同定は必須であり、今後 exome 解析、CNV 解析など最新の遺伝子解析技術を用いてBOR症候群に関与する新規遺伝を同定する必要があるものと思われる。

F. 研究発表

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G. 知的財産権の出願・登録状況

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

IV. 研究成果の刊行物

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル	発表誌	出版年等
Nakayama M, Nozu K, Goto Y, Kamei K, Ito S, Sato H, Emi M, Nakanishi K, Tsuchiya S, Iijima K	HNF1B alterations associated with congenital anomalies of the kidney and urinary tract	Pediatric Nephrology	25(6):1073-9, 2010
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ORIGINAL ARTICLE

HNF1B alterations associated with congenital anomalies of the kidney and urinary tract

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Abstract Hepatocyte nuclear factor 1β (HNF1β) abnormalities have been recognized to cause congenital anomalies of the kidney and urinary tract (CAKUT), predominantly affecting bilateral renal malformations. To further understand the spectrum of HNF1β related phenotypes, we performed *HNF1B* gene mutation and deletion analyses in Japanese patients with renal hypodysplasia (n=31), unilateral multicystic dysplastic kidney (MCDK; n=14) and others (n=5). We identified *HNF1B* alterations in 5 out of 50 patients (10%). De novo heterozygous complete deletions of *HNF1B* were found in 3 patients with unilateral MCDK. Two of the patients showed contralateral hypodysplasia, whereas the other patient showed a radiologically normal contralateral kidney with normal renal function. Copy number variation analyses showed 1.4 Mb microdeletions involving the whole *HNF1B* gene with breakpoints in flanking segmental duplications. We also identified 1 novel truncated mutation (1007insC) and another missense mutation (226G>T) in patients with bilateral hypodysplasia. *HNF1B* alterations leading to haploinsufficiency affect a diverse spectrum of CAKUT. The existence of a patient with unilateral MCDK with normal renal function might provide genetic insight into the etiology of these substantial populations of only unilateral MCDK. The recurrent microdeletions encompassing *HNF1B* could have a significant impact on the mechanism of *HNF1B* deletions.

Keywords Hepatocyte nuclear factor 1β · Congenital anomalies of the kidney and urinary tract · Copy number variation · Heterozygous microdeletion · Unilateral multicystic dysplastic kidney

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Introduction

Congenital anomalies of the kidney and urinary tract (CAKUT), developmental abnormalities of the kidney, occur with a frequency of 1 in 500 neonates and lead to major causes of chronic renal failure in infancy and childhood [1, 2]. To date, several gene mutations have been identified as a cause of human CAKUT, probably affecting the molecular pathogenesis of these disorders [3, 4].

Hepatocyte nuclear factor 1β (HNF1β) is a homeodomain-containing transcription factor that binds DNA and transactivates transcription [5]. HNF1β was initially described as liver-enriched transcription factors, but it was subsequently revealed that this protein is predominantly expressed in renal and pancreatic epithelia. HNF1β is the essential factor for embryogenesis in the kidney, pancreas, and liver, and is

expressed in the Wolffian duct and the Müllerian duct from very early developmental stage of the kidney [6]. In human metanephros, the transcript is strongly detected especially in the fetal medullary and cortical collecting ducts [7].

Alteration of the *HNF1B* gene, which is also known as *TCF2* and encodes HNF1β, originally known to be a gene responsible for the maturity-onset diabetes of the young type 5 (MODY5), has been recognized as a cause of renal structural abnormalities [8]. While a number of *HNF1B* mutations have been identified in individuals with CAKUT, whole-gene deletion of *HNF1B* is the most frequent molecular alteration observed in patients [9]. *HNF1B* gene abnormalities have been reported in a variety of individuals with renal malformations, such as renal hypodysplasia, multicystic dysplastic kidney (MCDK), cystic kidney disease, single kidney, and oligomeganephronia [9–12], suggesting the broad role this transcription factor plays throughout development.

Systematic mutational analyses of *HNF1B* in CAKUT have been carried out in Western countries. However, there have been no such analyses in Japan to date; thus, we have no information on the frequency and characteristics of *HNF1B* mutations in CAKUT in Japan. To address these questions, we analyzed the *HNF1B* gene in 50 children in a Japanese cohort who presented with CAKUT. We found that *HNF1B* alterations involve a diverse spectrum of CAKUT. We also identified *HNF1B* alteration in 1 out of 10 patients with unilateral MCDK and a radiologically normal contralateral kidney resulting in normal renal function, which may provide genetic insight into the etiology of unilateral MCDK. Moreover, using copy number variation (CNV) analyses, we confirmed that the recurrent microdeletions of 17q12 encompassing *HNF1B* could have a significant impact on the etiology of whole exonic deletions of *HNF1B*.

Materials and methods

Patient recruitment

We recruited 50 Japanese individuals with renal abnormalities based on ultrasound findings during the postnatal period or with onset of renal disease in early childhood. Patients selected for this study had at least one of the following renal phenotypes: uni- or bilateral renal hypo/dysplasia with or without cysts, unilateral multicystic dysplasia, single kidney, and uni- or bilateral cystic kidneys. Renal hypoplasia was defined as a kidney length of <2 standard deviations (SD) for age [13]. Renal dysplasia was considered when poor corticomedullary differentiation and/or diffuse hyperechogenicity were found. Patients were excluded if they had other known genetic anomalies, such as autosomal recessive polycystic kidney disease, autosomal dominant polycystic kidney disease, and syndromic forms of renal abnormalities related to mutations of paired-box 2 (*PAX2*), eye-absent homolog 1 (*EYA1*) and sine oculis homeobox homolog 1 (*SIX1*). Written informed consent was obtained from the patients or their parents. The Institutional Review Board of the National Center for Child Health and Development approved this study.

Laboratory assessment

We performed blood tests for characterizing general biochemical parameters, including liver enzymes and fasting blood glucose levels. Serum creatinine levels were measured with an enzymatic assay when patients were in a stable condition. Glomerular filtration rate (GFR) was estimated from the value of serum creatinine levels and height, according to the Schwartz formula. We used the Modified Diet in Renal Disease (MDRD) Study equation for Japanese adult patients. The lower limit of normal estimated GFR was defined as 80 ml/min/1.73 m^2.

Molecular analysis

Genomic DNA was extracted and purified from peripheral leukocytes in whole-blood samples using a QIAamp DNA blood kit (Qiagen, Tokyo, Japan). To detect *HNF1B* gene deletions, we performed semiquantitative polymerase chain reaction (PCR) amplification using capillary electrophoresis (Agilent 2100 Bioanalyzer with DNA 1000 Lab Chips; Agilent Technologies, Palo Alto, CA, USA), as previously described [14]. We applied this method to exons 2, 4, and 9 of the *HNF1B* gene. Probable identified deletions were confirmed by multiple ligation-dependent probe amplification (MLPA) assays [15] using an MLPA kit (SALSA MLPA P241-B1 MODY, Lot 0408; MCR-Holland, Amsterdam, The Netherlands), which contains all 9 exons of *HNF1B*. For patients with whole gene deletion of *HNF1B*, we subsequently performed genome-wide DNA screening for CNVs using deCODE-Illumina CNV chip (57K, i-select format; deCODE genetics, Reykjavik, Iceland) and array-based comparative genomic hybridization (array CGH) analysis (Early Access 400K CNV array; Agilent Technologies, Santa Clara, CA, USA), to identify the boundaries of the deleted region involving *HNF1B*. We identified CNVs by the deCODE-Illumina CNV chip by using DosageMiner software developed by deCODE genetics and loss-of-heterozygosity analysis [16]. For array CGH, we used Agilent Human Whole Genome CNV microarray, consisting of 487,008 probes, which include 392,824 CNV probes. Array CGH experiments were performed according to the manufacturer's instructions [17].

Patients without *HNF1B* deletions were screened for mutations by direct sequencing of all 9 exons and exon–intron boundaries, as previously described [18, 19]. We collected DNA samples from 100 healthy individuals as controls for mutation analysis.

When probands had *HNF1B* alterations, genetic studies were extended to family members whenever possible. For an affected relative whose blood sample was unavailable, we obtained a PCR-ready DNA sample from the autopsy liver tissue embedded in paraffin using a DNA extraction kit (DEXPAT; Takara Bio, Shiga, Japan).

Results

Patient characteristics

We studied 50 patients with renal structural abnormalities who were diagnosed with renal hypodysplasia (n=31), unilateral MCDK (n=14), single kidney (n=4), and glomerulocystic kidney disease (n=1). The mean age at genetic analysis was 10.4 years old (age range, 0.9–31 years) and the ratio of male to female patients was 37 to 13. Cortical cysts were observed in 20 out of 50 patients (40%); 3 patients had unilateral hypodysplasia, 14 patients had unilateral MCDK, and bilateral hypodysplasia, single kidney, and glomerulocystic kidney disease occurred in 1 patient each. Twenty patients (40%) had progressed to non-diabetic end-stage renal disease. Ten out of 14 patients with unilateral MCDK showed radiologically normal or compensatory hypertrophy of the contralateral kidney. Two probands had positive family histories of renal disease. All patients showed normal liver function, except for 1 patient. None of the patients had evidence of diabetes.

HNF1B molecular analysis

We identified *HNF1B* alterations in 5 out of 50 patients (10%); 2 out of 31 patients (7%) had hypodysplastic kidneys and 3 out of 14 patients (21%) had unilateral MCDK. No *HNF1B* alterations were detected in patients with single kidney and glomerulocystic kidney disease. Table 1 shows the clinical findings and *HNF1B* mutations of 5 patients and 2 family members (K7188f and K718s).

De novo heterozygous deletions of *HNF1B* were found in 3 patients with MCDK by semiquantitative PCR (Fig. 1). All deletions were confirmed and found to be complete deletion of *HNF1B* by repeated MLPA analyses in all 3 patients. Two out of 3 patients (S710, S746) showed contralateral renal dysplasia, whereas the other patient (S708) showed a radiologically normal length and appearance of the contralateral kidney with normal renal function. CNV analyses with a deCODE-Illumina CNV chip and array CGH showed 1.4 Mb deletions at 17q12 in all 3 patients with *HNF1B* deletions. Interestingly, the microdeletions found in the 3 patients were flanked by segmental duplications on both sides. The regions flanking the microdeletions in 2 unaffected individuals were polymorphic in copy number. The deleted regions involved *HNF1B* and 14 adjacent genes (Fig. 2).

One frameshift mutation and one missense mutation were identified in patients with bilateral renal hypodysplasia by direct sequencing (K718, S440). These mutations were not detected in 100 healthy controls or in the healthy mother of the affected patient.

A novel frameshift mutation (1007insC) found in a male patient (K718) resulted in a truncation at the transactivation domain. Absence of the vas deferens was discovered at the time of surgery for inguinal hernia. The frameshift mutation identified in the proband was observed in the patient's father (K718f) with a unilateral simple kidney cyst and normal contralateral kidney, and also in a sibling (K718s). The patient's father (K718f) was found to have a high urate level (urate 618 μmol/l, reference range: 220–416 μmol/l). The sibling was diagnosed with bilateral MCDK and the Potter sequence, and was aborted at 21 weeks' gestation. The autopsy specimen showed enlarged kidneys occupied by multiple cysts of various sizes, whereas no abnormalities were observed in the other organs including liver, pancreas, and genital organs.

A heterozygous missense mutation (226G>T) located between the dimerization domain and the DNA binding domain was detected in a male patient (S440). The resulting amino acid change (Gly76Cys) affects a residue highly conserved in the *HNF1B* sequence of different species. This *HNF1B* mutation has also been reported in patients with MCDK [9]. Our patient was diagnosed with bilateral hypodysplastic kidneys at 11 months old, developing end-stage renal disease at the age of 4 years. He received living-related renal transplantation at the age of 10 years. His healthy mother did not carry the same mutation.

Discussion

This study demonstrated, to the best of our knowledge for the first time, the frequency and characteristics of *HNF1B* mutations in CAKUT in Japan, and also in Asian countries. In this study, we identified *HNF1B* alterations comprising 3 whole deletions, 1 truncated mutation, and 1 missense mutation in patients with CAKUT. All of the cases who had whole *HNF1B* deletions presented with unilateral MCDK with/without contralateral hypodysplasia, whereas 1 familial case with a truncated mutation of *HNF1B* presented with various phenotypes between the proband and his family members. Our current study provides compelling evidence

Table 1 Clinical findings and mutation analyses

Patient number	Gender	Age at examination (years)	*HNF1B* gene abnormality	Renal phenotype	eGFR (ml/min/1.73m^2)
S708	Male	2.8	Complete deletion De novo	Right MCDK Left radiologically normal	96.4
S710	Female	2.1	Complete deletion De novo	Right MCDK Left dysplasia	83.5
S746	Female	5.6	Complete deletion De novo	Left MCDK Right dysplasia	94.3
K718	Male	4.0	1007insC	Bilateral hypodysplasia	70.3
K718f	Male	32	1007insC	Right simple renal cyst	45.7
K718s	Female	–	1007insC	Bilateral MCDK Potter syndrome	–
S440	Male	13	226G>T	Bilateral hypodysplasia	ESRD Post-transplantation

eGFR, estimated glomerular filtration rate; f, father; s, sibling; ins, insertion; MCDK, multicystic dysplastic kidney; ESRD, end-stage renal disease

that the clinical spectrum of *HNF1B* abnormalities consists of a wide range of phenotypes with various renal severities [20, 21].

We found that the frequency of *HNF1B* alterations was 10% (5 out of 50 patients), which is similar to that of previous studies (8.9–29%) of CAKUT [9, 12]. This finding indicates that *HNF1B* alterations are a major cause of CAKUT in Japan, as well as in Western countries. Interestingly, with the wide phenotypic variation found in recruited patients, *HNF1B* alterations were clustered in patients with renal cystic malformation including MCDK. Review of all of the individuals with *HNF1B* alterations showed that 5 out of 7 individuals shared a common feature of renal cystic malformation, suggesting that renal cysts seem to be the most frequent outcome when *HNF1B* haploinsufficiency occurs. These findings are in accordance with previous reports showing that *HNF1B* alterations are associated with bilateral renal cysts [9]. These results suggest that patients with renal cysts are good candidates for systematic *HNF1B* screening.

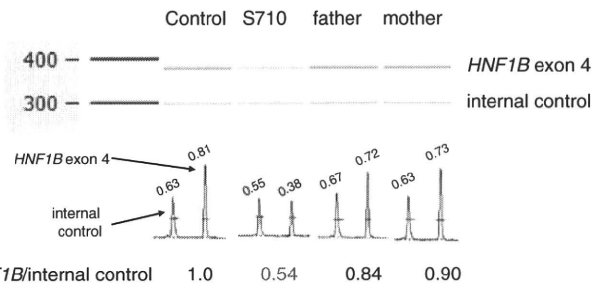

Fig. 1 Semiquantitative polymerase chain reaction (PCR) amplification of *HNF1B* exon 4. Representative result of semiquantitative PCR amplification shows heterozygous deletion of *HNF1B* exon 4 in patient S710. Peak concentration ratio of the patient's PCR product was compared with those of her parents and the normal control, indicating heterozygous deletion of the appropriate exon

In this study, we showed that *HNF1B* abnormalities encompass a wide clinical spectrum with various severities. Three out of 5 patients with *HNF1B* alterations presented with unilateral MCDK, with various phenotypes of contralateral kidney. Interestingly, we identified whole *HNF1B* deletion in 1 patient with unilateral MCDK and a radiologically normal contralateral kidney resulting in normal renal function. While previous studies have reported that *HNF1B* anomalies were only found to be associated with bilateral renal abnormalities [9, 12], various phenotypes in renal diseases also had distinct diagnoses, ranging from bilateral MCDK in autopsy samples [22, 23] to unilateral MCDK with normal renal length in single remaining kidney [24]. Recently, we examined *HNF1B* alterations in an additional 2 patients showing unilateral MCDK with a radiologically normal contralateral kidney and normal renal function. One of the patients showed a whole *HNF1B* deletion detected by MLPA analysis (personal communication (2010), Dr. Kaneko, Kansai Medical University, Japan and Drs. Nozu and Iijima, Kobe University Graduate School of Medicine, Japan), suggesting that *HNF1B* alterations are not rare in this common condition. Further studies are needed to confirm the contribution of *HNF1B* alterations in patients with unilateral MCDK and normal renal function.

Renal function in our affected individuals ranged from normal to dialysis-dependent, which required a renal transplant. A similar variability in renal function has been reported in individuals with *HNF1B* abnormalities [20, 21]. Furthermore, renal function was considerably poorer in one affected family member (K718f), despite the renal morphology of a unilateral simple cyst on repeated ultrasound scans, which was predicted to be the mildest phenotype. Although examination of renal histology was not undertaken in this case, it is reasonable to consider that *HNF1B*

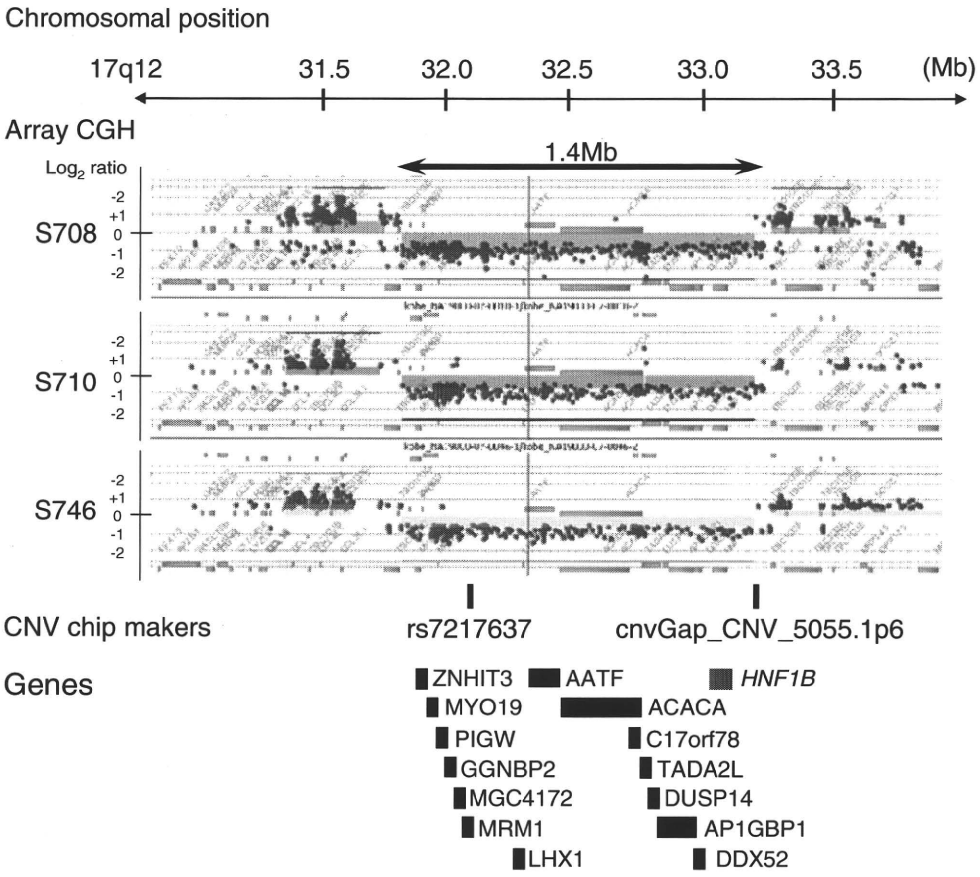

Fig. 2 Recurrent microdeletion at chromosome 17q12 involving the *HNF1B* gene. Agilent array comparative genomic hybridization (CGH) profile shows a heterozygous 1.4-Mb deletion in 3 patients with multicystic dysplastic kidney (MCDK). *Green plots* represent the deleted region and *red dots* indicate the flanking segmental duplication. This region includes *HNF1B* and 14 further genes

dysfunction pathologically affected renal function, which was not detected on renal ultrasound screenings. An important implication from this case is that screening for *HNF1B* alterations for those individuals may provide a better understanding for prognosis of renal function.

One male patient (K718) in our series with *HNF1B* mutation presented with an absence of vas deferens that was incidentally detected. The vas deferens is derived from the Wolffian duct during embryogenesis and is part of the excurrent duct system responsible for the transport, storage, and maturation of sperm. Congenital bilateral absence of the vas deferens is an important cause of male infertility in adulthood. Since the *HNF1B* gene is expressed in the Wolffian duct and Müllerian duct in the mouse embryo, it is possible that *HNF1B* alterations are associated with the genital tract malformation. To date, there have been 5 male patients with anomaly of the genital tract, including 1 case of bilateral agenesis of vas deferens [25, 26]. Although the frequency of male genital abnormalities is reported to be lower than that in females [21], there might be a certain number of potential male individuals carrying congenital genital malformation.

The frameshift mutation and the missense mutation that we found in our study are believed to be pathogenic. The frameshift mutation 1007insC is a novel mutation, which leads to truncation at the transactivation domain, probably affecting HNF1β function. In our study, the position of the missense mutation Gly76Cys was located between the dimerization domain and the DNA binding domain, and this amino acid change affects a residue highly conserved in the *HNF1B* sequence of different species. This *HNF1B* mutation has also been reported in patients with MCDK [9]. Finally, the absence of the same mutation in 200 chromosomes of unrelated Japanese control subjects or in the healthy mother of the affected patient would also support the pathogenetic role of this mutation.

In our present study, screening of *HNF1B* deletions by semiquantitative PCR amplification and MLPA analysis revealed that all 3 cases with *HNF1B* deletions were found to show deletions of whole exons. This tendency toward complete exonic deletions as a major pattern for heterozygous *HNF1B* deletions is similar to that found in previous reports [9, 12, 20]. Furthermore, subsequent CNV analyses of these 3 cases showed that the microdeletions at 17q12 extended to the 1.4-Mb region, including the entire *HNF1B* gene. High resolution mapping of the deleted region by the array CGH showed microdeletions with breakpoints in flanking segmental duplications, indicating that the microdeletions were mediated by flanking segmental duplications. The same mechanism was proposed in patients with

congenital renal abnormalities with or without mental retardation or MODY5 [22, 27], suggesting that recurrent non-allelic homologous recombination occurs in region 17q12. Collectively, this recombination possibly explains the high rate of de novo *HNF1B* deletions detected in previous studies [9, 20], and thus evaluation of this microdeletion by conventional gene dosage analysis should be considered in individuals suspected of having *HNF1B* alterations.

The recurrent microdeletion in the 17q12 region identified in 3 patients in this study involved *HNF1B* and 14 adjacent genes, which is predicted to result in haploinsufficiency of these affected genes. One of the genes in this region is *LHX1*, a limb homeodomain gene important for renal development in mouse studies [28, 29]. It has been proposed that the microdeletion of *LMX1* is associated with an earlier onset of renal pathology, suggesting that haploinsufficiency of *LHX1* as well as *HNF1B* influence this onset variability [22]. In the current study, however, although all 3 patients with microdeletions showed the shared phenotype of unilateral MCDK, no apparent difference was observed in the renal phenotype, severity of renal function or onset of disease between patients with *HNF1B* deletion and those with mutations. Our results suggest that heterozygous deletions of the affected adjacent 14 genes do not seem to influence the core phenotype. It is possible that *HNF1B* is the predominant gene among deleted regions contributing to the renal phenotype. Further studies are needed to confirm our findings.

Copy number variations can be an important source of genetic variation among human populations of different ethnic groups as well as among individuals. It is likely that different location and frequency spectra of CNVs exist for different populations, especially different ethnic groups, such as occurs in cases of single nucleotide polymorphisms and insertion–deletion polymorphisms [30, 31]. It is possible that there are interpopulation differences in the copy number due to non-allelic homologous recombination mediated by flanking segmental duplications [32]. This study demonstrated, for the first time to our knowledge, the existence of the CNV resulting in the 1.4-Mb microdeletion encompassing the *HNF1B* gene in Japanese patients, which has already been shown in several reports performed in the USA and European countries [9, 20, 22, 27].

In conclusion, the current study provides further evidence that *HNF1B* alterations leading to haploinsufficiency affect a wide variety of renal disease spectrum. The existence of an affected patient with unilateral MCDK and a radiologically normal contralateral kidney resulting in normal renal function might provide genetic insight into the etiology of the substantial population of unilateral MCDK. Identifying *HNF1B* deletions and mutations in patients with heterogeneous phenotypes should provide a better understanding of renal function, as well as early detection of extrarenal manifestation related to this gene.

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Disclosure All of the authors declare no competing interests.

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