69. 塚口裕康.【腎臓症候群(第2版)上-その他の腎臓疾患を含めて-】 Galloway-Mowat 症候群(脳・腎糸球体異形成). 日本臨床別冊 2012;17:411-9
70. 塚口裕康. ゲノムからみた慢性腎臓病の発症分子機序解明へのアプローチ 細胞 2012;44(5):20-6
71. 五十嵐隆.【腎臓症候群(第2版)上-その他の腎臓疾患を含めて-】特発性尿細管性たんぱく尿症（Dent病）.日本臨床別冊 2012;17:856-9
72. 五十嵐隆. 遺伝性腎尿路疾患の原因遺伝子リスト 最新版、小児内科 2012;44: 324-7
73. 五十嵐隆. デント病、今日の小児治療指針 第15版 2012:pp587-588、医学書院、東京
74. 五十嵐隆. Fanconi症候群、Dent病および他の近位尿細管疾患、カラー版内科学 2012:pp1526-1528、西村書店、東京
75. 松尾洋孝, 四ノ宮成祥. 腎性低尿酸血症の遺伝学. Annual Review 糖尿病・代謝・内分泌 2012. 2012; P145-54
76. 松尾洋孝, 市田公美, 高田龍平, 中山昌喜, 四ノ宮成祥. 尿酸動態の支配要因としての尿酸トランスポーター. 細胞工学, 2012;31:553-7
77. 千葉俊周, 松尾洋孝, 市田公美, 四ノ宮成祥. テーマ:A.診断8.低尿酸血症の頻度(人種差も含め), 原因, 分類を教えてください. 腎と透析. 2012 ; P301-4.
78. 中山昌喜, 松尾洋孝, 市田公美, 四ノ宮成祥.【腎疾患治療マニュアル 2012-13】 尿細管疾患 尿細管機能異常症 腎性低尿酸血症. 腎と透析, 2012 ; P370-3.

＜学会発表＞

1. Ninchoji T, Kaito H, Matsunoshita N, Nozu K, Nakanishi K, Yoshikawa N, Iijima K. The relation between steroid responsiveness and immunofluorescence findings in infant idiopathic nephrotic syndrome. European Renal Association-European Dialysis and Transplant Association Istanbul 2013.
2. Iijima K, Mayumi Sako, Nozu K, Tsuchida N, Tanaka R,enji Ishikura K, Ito Y, Ohashi Y. Micenter, Double-Blind, Placebo-Controlled, Randomized Trial of Rituximab for the Treatment of Childhood-Onset Refractory. The Sixteenth Congress of the International Pediatric Nephrology Association, Shanghai, 2013.
3. Matsunoshita N, Kaito H, Shigeo Hara, Ishimori S, Ninchoji T, Hamahira K, Yoshikawa N, Iijima K. A case of C3 glomerulonephritis in a 6-month-old infant accompanied by glomerular endothelial injury : the expanding spectrum of complement-related kidney diseases. The Sixteenth Congress of the International Pediatric Nephrology Association Shanghai, 2013.
4. Ninchoji T, Kaito H, Matsunoshita N, Ishimori S, Yoshikawa N, Iijima K. Actual outcome and problem of cyclosporine treatment in children with steroid-sensitive nephrotic syndrome. The Sixteenth Congress of the International Pediatric Nephrology Association, Shanghai, 2013.
5. Morisada N, Taniguchi-Ikeda M, Ishimori S, Ninchoji T, Kaito H, Nozu K, Adach Mi, Takeshima Y, SekineT, Iijima K. Heterozygous microdeletion of 16q covering SALL1 and RPGRIP1L could be a novel contiguous gene syndrome with renal impairment. American Society of Human Genetics, Boston, 2013
6. Kaito H, Nozu K, Oka M, Morisada N, Ninchoji T, Nakanishi K, Yoshikawa N, Iijima K. Natural History and Protein Expression Pattern in Autosomal Recessive Alport Syndrome Based on the Comprehensive Strategy for Genetic Analysis. American Society of Nephrology KIDNEY WEEK, Atlanta, 2013.
7. Ninchoji T, Kaito H, Nozu K, Hama T, Nakanishi

K, Yoshikawa N, Iijima K. Investigation of Principal Mechanism for Renal Sodium Retention in Children with Idiopathic Nephrotic Syndrome. American Society of Nephrology KIDNEY WEEK, Atlanta, 2013

8. Miura K, Sekine T, Takahashi K, Takita J, Harita Y, Tajima A, Ishihara M, Hisano M, Murai M, Igarashi T. Mutational analyses of the ATP6V1B1 and ATP6V0A4 genes in patients with primary distal renal tubular acidosis. International Pediatric Nephrology Association 2013, Shanghai, China, September 1, 2013
9. Matsuo H, Takada T, Nakayama A, Shimizu T, Kasuga H, Nakashima H, Nakamura T, Takada Y, Kawamura Y, Utsumi Y, Ogata H, Nakamura M, Sakurai Y, Hosoya T, Shinomiya N, Suzuki H, Ichida K. Common dysfunctional variants of ABCG2 decrease extra-renal urate excretion and cause hyperuricemia, 2012 American Society of Human Genetics 62nd Annual Meeting, San Francisco, USA, 2012.11.6-2012.11.10.
10. Chiba T, Matsuo H, Nakayama A, Kawamura Y, Sakiyama M, Hosoyamada M, Hamajima N, Shinomiya N: Identification of a hypouricemia patient with SLC2A9 R380W, a RHUC2 mutation, Madrid, Spain, 2013.6.9-2013.6.13.
11. Matsuo H, Takada T, Nakayama A, Shimizu T, Sakiyama M, Hosoya T, Shinomiya N, Ichida K: ABCG2 dysfunction increases the risk of renal overload hyperuricemia, Madrid, Spain, 2013.6.9-2013.6.13.
12. Ichida K, Matsuo H, Takada T, Nakayama A, Shimizu T, Kasuga H, Nakashima H, Nakamura T, Takada Y, Kawamura Y, Utsumi Y, Ogata H, Nakamura M, Sakurai Y, Shinomiya N, Suzuki H, Hosoya T: Common dysfunctional variants of ABCG2 decrease extra-renal urate excretion and cause hyperuricemia, Up Close and Personalized, International Congress on Personalized Medicine, Paris, France, 2013.7.25-2013.7.28.
13. Matsuo H, Takada T, Nakayama A, Shimizu T, Nakashima H, Nakamura T, Takada Y, Hosoya T, Shinomiya N, Ichida K: Common dysfunctional variants of ABCG2 cause renal overload hyperuricemia, Bali, Indonesia, 2013.8.30-2013.9.1.
14. Matsuo H, Takada T, Ichida K, Nakayama A, Shinomiya N: Genetic analyses of renal hypouricemia type 1 and 2 (RHUC1&2), Molecular basis of uric acid transport and gout, Prague, Czech, 2013.10.15.
15. Nakayama A, Matsuo H, Ichida K, Takada T, Sakiyama M, Shinomiya N: Urate transporter gene ABCG2, a major causative gene for gout, Molecular basis of uric acid transport and gout, Prague, Czech, 2013.10.15.
16. Takada T, Matsuo H, Ichida K, Nakayama A, Shinomiya N, Suzuki H: Molecular functional analysis of urate transporters related to "urate transport disorders", Molecular basis of uric acid transport and gout, Prague, Czech, 2013.10.15.
17. Ichida K, Matsuo H, Takada T, Nakayama A, Hosoyamada M, Shinomiya N, Hosoya T: Mechanism of hyperuricemia induced by ABCG2 dysfunction, Molecular basis of uric acid transport and gout, Prague, Czech, 2013.10.15.
18. Matsuo H, Ichida K, Takada T, Nakayama A, Nakashima H, Nakamura T, Kawamura Y, Takada Y, Yamamoto K, Shimizu S, Sakiyama M, Chiba T, Hamajima N, Sakurai Y, Shinomiya N: Common dysfunctional variants of urate exporter gene ABCG2/BCRP are a major cause of early-onset gout, The American Society of Human Genetics 63rd Annual Meeting, Boston, USA, 2013.10.22-2013.10.26.
19. Sakiyama M, Matsuo H, Chiba T, Nakayama A,

Kawamura Y, Shimizu S, Hamajima N, Shinomiya N: Identification of a new hypouricemia patient with a pathogenic GLUT9/SLC2A9 mutation (R380W), The American Society of Human Genetics 63rd Annual Meeting, Boston, USA, 2013.10.22-2013.10.26.

20. Yoneda K, Era T, Fusaki N, Takami Y, Nakazato H, Endo F: Establishing of exogenous factors-free iPS cells from patients with intractable diseases. The 18th Annual Meeting of Japan Society of Gene Therapy, June 28-30, 2012 (Kuamoto, Japan)
21. Hashimura Y, Nozu K, Kaito H, Ohtsubo H, Hashimoto F, Ishimori S, Ninchoji T, Morisada N, Nakanishi K, Yoshikawa N, Iijima K. Genetical and Clinical Aspects of X-Linked Alport Syndrome in Males with Positive Staining of the alpha5(IV) Chain. 45th Annual Meeting of the American Society of Nephrology, 2012.11.1-4, San Diego, USA
22. Miura K, Sekine T, Takahashi K, Takita J, Harita Y, Ishihara M, Hisano M, Igarashi T. Detection of novel interstitial large deletions in the ATP6V0A4 gene and mutations in the ATP6V1B1 gene in patients with distal renal tubular acidosis. 45th Annual Meeting of the American Society of Nephrology, 2012.11.1-4, San Diego, USA
23. Tsukaguchi H. Molecular Genetics of Proteinuric Disorders. 8th Congress of Asian Society for Pediatric Research., 2012.5.16-18, Seoul, Korea
24. Goto S, Hosomichi K, Tsukaguchi H , Narita I. Exome sequencing identifies a novel EEA1 variant in Japanese familial IgA nephropathy. 45th Annual Meeting of the American Society of Nephrology, 2012.11.1-4, San Diego, USA)
25. Matsuo H, Takada T, Nakayama A, Shimizu T, Kasuga H, Nakashima H, Nakamura T, Takada Y, Kawamura Y, Utsumi Y, Ogata H, Nakamura M, Sakurai Y, Hosoya T, Shinomiya N, Suzuki H, Ichida K. Common dysfunctional variants of ABCG2 decrease extra-renal urate excretion and cause hyperuricemia, 2012 American Society of Human Genetics 62nd Annual Meeting, 2012.11.6-10, San Francisco, USA
26. 石森真吾，松野下夏樹，橋本総子，忍頂寺毅史，貝藤裕史，飯島一誠。小児ステロイド感受性ネフローゼ症候群患者の成人期における現状と問題点、第 116 回日本小児科学会学術集会　広島　2013
27. 飯島一誠，佐古まゆみ，大橋靖雄。小児期発症難治性ネフローゼ症候群に対するリツキシマブ他施設共同二重盲検プラセボ対照ランダム化比較試験、第 56 回日本腎臓学会学術総会　東京　2013
28. 忍頂寺毅史，貝藤裕史，松野下夏樹，石森真吾，吉川徳茂，飯島一誠。小児特発性ネフローゼ症候群におけるステロイド反応性と蛍光免疫染色所見との関連、第 56 回日本腎臓学会学術総会　東京　2013
29. 飯島一誠。遺伝性腎疾患の最近の知見、第 48 回日本小児腎臓病学会学術集会　徳島　2013
30. 忍頂寺毅史，貝藤裕史，石森真吾，松野下夏樹，吉川徳茂，飯島一誠。ステロイド感受性ネフローゼ症候群におけるシクロスポリン投与の現状と問題点、第 48 回日本小児腎臓病学会学術集会　徳島　2013
31. 野津寛大，Williams Sweeney，Emma Schwasingar，Nicholas Kampa，飯島一誠，Ellis Avner。常染色体優性多発性嚢胞腎(ADPKD)の間質線維化における上皮間葉系形質転換（ＥＭＴ）の関与に関する研究、第 48 回日本小児腎臓病学会学術集会　徳島　2013
32. 松野下夏樹，貝藤裕史，石森真吾，忍頂寺毅史，野津寛大，吉川徳茂，飯島一誠。ネフローゼ症候群を呈したＸ染色体連鎖型 Alport 症

候群の 2 例、第 48 回日本小児腎臓病学会学術集会　徳島　2013

33. 飯島一誠。CAKUT の分子遺伝学的アプローチ、第 22 回日本小児泌尿器科学会総会　東京 2013
34. 神吉直宙，貝藤裕史，松野下夏樹，忍頂寺毅史，大坪裕美，野津寛大，久松千恵子，飯島一誠。腹膜透析中に急性虫垂炎を発症した 3 歳女児例、第 35 回日本小児腎不全学会学術集会　福島　2013
35. 森貞直哉, 野田俊輔, 降?めぐみ, 池田真理子, 竹島泰弘，飯島一誠。総排泄腔遺残をともなった 1q21.1 欠失症候群の 1 女児例、日本人類遺伝学会第 58 回大会　仙台　2013
36. 後藤 眞, 細道 一善, 塚口裕康, 渡辺 博文, 井ノ上 逸朗, 成田 一衛 次世代シークエンサーが開く新しい腎臓基礎研究の展開: 家族内集積を示す腎疾患のエクソーム解析 第 56 回 日本腎臓学会学術総会シンポジウム (日本腎臓学会誌 55(3): 283, 2013)
37. 高田龍平、市田公美、松尾洋孝、中山昌喜、村上啓造、山梨義英、春日裕志、四ノ宮成祥、鈴木洋史: ABCG2/BCRP による腸管への尿酸排泄の低下は腎外排泄低下型高尿酸血症を引き起こす. 日本薬剤学会第 25 年会, 神戸, 2012.5.24-2012.5.26.
38. 内海由貴、松尾洋孝、高田龍平、中山昌喜、清水徹、春日裕志、中島宏、中村好宏、高田雄三、河村優輔、中村真希子、櫻井裕、細谷龍男、四ノ宮成祥、鈴木洋史、市田公美: 高尿酸血症の新たな発症機序の解明: ABCG2 の機能低下による腸管からの尿酸排泄低下. 第 7 回日本トランスポーター研究会年会, 京都, 2012.6.9-2012.6.10.
39. 高田龍平、市田公美、松尾洋孝、中山昌喜、村上啓造、山梨義英、春日裕志、四ノ宮成祥、鈴木洋史: ABCG2/BCRP による腸管への尿酸排泄の低下は腎外排泄低下型高尿酸血症の原因となる. 医療薬学フォーラム 2012/第 20 回クリニカルファーマシーシンポジウム, 福岡, 2012.7.14.-15.
40. 高田龍平、市田公美、松尾洋孝、中山昌喜、村上啓造、山梨義英、春日裕志、四ノ宮成祥、鈴木洋史: ABCG2/BCRP による腸管への尿酸排泄の低下は腎外排泄低下型高尿酸血症をもたらす. 第 6 回トランスポーター研究会九州部会, 福岡, 2012.9.1.
41. 松尾洋孝、高田龍平、中山昌喜、清水徹、春日裕志、中島宏、中村好宏、高田雄三、河村優輔、内海由貴、中村真希子、櫻井裕、細谷龍男、四ノ宮成祥、鈴木洋史、市田公美: ABCG2 遺伝子変異に基づく高尿酸血症の病態解明と新規病型分類. 日本人類遺伝学会第 57 回大会, 東京, 2012.10.25-2012.10.27.
42. 高田雄三、松尾洋孝、中山昌喜、中島宏、松村耕治、鈴木康司、浜島信之、櫻井裕、四ノ宮成祥、小林靖: 全自動 SNP タイピング装置を利用した痛風の遺伝子タイピング. 日本 DNA 多型学会第 21 回学術集会, 京都, 2012.11.7-2012.11.9.
43. 高田龍平、市田公美、松尾洋孝、中山昌喜、村上啓造、山梨義英、春日裕志、四ノ宮成祥、鈴木洋史: 尿酸トランスポーター ABCG2/BCRP の機能低下型遺伝子変異多型は腎外排泄低下型高尿酸血症をもたらす, 第 33 回日本臨床薬理学会, 沖縄, 2012.11.29-2012.12.1.
44. 松尾洋孝、高田龍平、中山昌喜、清水徹、春日裕志、中島宏、中村好宏、高田雄三、中村真希子、櫻井裕、四ノ宮成祥、鈴木洋史、市田公美: 痛風病因遺伝子 ABCG2 の解析による高尿酸血症の新規病態の解明, 第 23 回日本疫学会学術総会, 大阪, 2013.1.24-2013.1.26.
45. 中山昌喜、松尾洋孝、高田雄三、中島宏、佐藤弘樹、中村好宏、森厚嘉、内藤真理子、菱田朝陽、若井建志、清水聖子、及川雄二、丹

羽和紀、櫻井裕、市田公美、浜島信之、四ノ宮成祥: 痛風病因遺伝子 ABCG2 の変異が血清尿酸値におよぼす影響, 第 23 回日本疫学会学術総会, 大阪, 2013.1.24-2013.1.26.

46. 小縣開、松尾洋孝、中山昌喜、高田雄三、櫻井裕、四ノ宮成祥: ABCG2 の機能低下に基づく高尿酸血症の病態解明と新規病型「腎負荷型」の提唱, 第 58 回防衛衛生学会, 東京, 2013.1.31.
47. 高田雄三、松尾洋孝、高田龍平、中山昌喜、清水徹、四ノ宮成祥、市田公美: ABCG2 遺伝子タイピングに基づく高尿酸血症における新規病型分類の提唱, 第 23 回生物試料分析科学会年次学術集会, 大阪, 2013.2.10-2013.2.11.
48. 松尾洋孝、高田龍平、中山昌喜、清水徹、高田雄三、井上寛規、岡田千沙、中村真希子、細山田真、四ノ宮成祥、細谷龍男、市田公美: ABCG2 の機能低下による腎外の尿酸排泄低下：高尿酸血症の症例解析と動物モデル解析, 第 46 回日本痛風・核酸代謝学会総会, 東京, 2013.2.14-2013.2.15.
49. 中山昌喜、松尾洋孝、市田公美、高田龍平、岡田千沙、清水聖子、千葉俊周、崎山真幸、細谷龍男、四ノ宮成祥: 腎性低尿酸血症の実態把握調査について（第 1 報）, 第 46 回日本痛風・核酸代謝学会総会, 東京, 2013.2.14-2013.2.15.
50. 松尾洋孝、高田龍平、中山昌喜、清水徹、春日裕志、中島宏、中村好宏、高田雄三、河村優輔、内海由貴、中村真希子、櫻井裕、細谷龍男、四ノ宮成祥、鈴木洋史、市田公美: 尿酸の腸管排泄低下は高尿酸血症の主要な新規機序である, 第 99 回日本消化器病学会総会, 鹿児島, 2013.3.21-2013.3.23.
51. Takada T, Ichida K, Matsuo H, Nakayama A, Murakami K, Yamanashi Y, Kasuga H, Shinomiya N, Suzuki H: Decreaased ABCG2-mediated urate excretion from intestine is a common cause of hyperuricemia, 第 86 回日本薬理学会年会, 福岡, 2013.3.21-2013.3.23.
52. 市田公美、松尾洋孝、高田龍平、中山昌喜、清水徹、春日裕志、中島宏、中村好宏、高田雄三、河村優輔、内海由貴、中村真希子、櫻井裕、細谷龍男、四ノ宮成祥、鈴木洋史. 尿酸の腸管排泄低下は高尿酸血症の主要な新規機序である. 日本消化器学会第 99 回総会, 鹿児島, 2013.3.21.-2013.3.23.
53. 松尾洋孝、高田龍平、中山昌喜、清水徹、中島宏、中村好宏、高田雄三、櫻井裕、細谷龍男、四ノ宮成祥、鈴木洋史、市田公美: 腸管における尿酸排泄の低下は高尿酸血症の新規機序である, 第 90 回日本生理学会大会, 東京, 2013.3.27-2013.3.29.
54. 市田公美、松尾洋孝、高田龍平、中山昌喜、四ノ宮成祥、鈴木洋史、細谷龍男: 高尿酸血症の主要な新規機序の発見—腎外排泄低下型高尿酸血症, 第 110 回日本内科学会総会・講演会, 東京, 2013.4.12-2013.4.14.
55. 中山昌喜、松尾洋孝、細谷龍男、四ノ宮成祥、市田公美: 痛風・高尿酸血症の新たな発症機序の解明：腸管における尿酸排泄低下, 第 57 回日本リウマチ学会総会・学術集会/第 22 回国際リウマチシンポジウム, 京都, 2013.4.18-2013.4.20.
56. Nakayama A, Matsuo H, Hosoya T, Shinomiya N, Ichida K: A common mechanism of gout/hyperuricemia with decreased urate excretion from intestine, 第 57 回日本リウマチ学会総会・学術集会/第 22 回国際リウマチシンポジウム, 京都, 2013.4.18-2013.4.20.
57. 松尾洋孝、四ノ宮成祥: 痛風・高尿酸血症と尿酸トランスポーター, 第 31 回日本ヒト細胞学会, 所沢, 2013.8.10-2013.8.11.
58. 清水聖子、松尾洋孝、千葉俊周、高田雄三、河村優輔、崎山真幸、中山昌喜、永森收志、金井好克、浜島信之、市田公美、四ノ宮成祥:

GLUT9/SLC2A9 遺伝子の R380W 変異を認める低尿酸血症の新規症例の同定, 第 31 回日本ヒト細胞学会, 所沢, 2013.8.10-2013.8.11.

59. 千葉俊周、松尾洋孝、市田公美、高田龍平、中山昌喜、清水徹、中島宏、中村好宏、高田雄三、河村優輔、崎山真幸、井上寛規、岡田千沙、櫻井裕、細谷龍男、鈴木洋史、四ノ宮成祥: ABCG2 の分子機序解明による新規高尿酸血症分類の提唱, 第 31 回日本ヒト細胞学会, 所沢, 2013.8.10-2013.8.11.
60. 崎山真幸、松尾洋孝、市田公美、高田龍平、中山昌喜、中島宏、中村好宏、河村優輔、高田雄三、井上寛規、清水聖子、千葉俊周、浜島信之、鈴木洋史、金井好克、櫻井裕、細谷龍男、清水徹、四ノ宮成祥: 若年発症痛風の主たる要因としての ABCG2 遺伝子の機能低下型変異, 第 31 回日本ヒト細胞学会, 所沢, 2013.8.10-2013.8.11.
61. 高田雄三、松尾洋孝、崎山真幸、中山昌喜、清水聖子、千葉俊周、及川雄二、松村耕治、鈴木康司、浜島信之、小林靖、宮平靖、四ノ宮成祥: 痛風・高尿酸血症の主要病因遺伝子である ABCG2 の遺伝子検査法の検討, 第 31 回日本ヒト細胞学会, 所沢, 2013.8.10-2013.8.11.
62. 松尾洋孝、市田公美、高田龍平、中山昌喜、中島宏、中村好宏、高田雄三、山本健、清水聖子、崎山真幸、千葉俊周、浜島信之、鈴木洋史、櫻井裕、細谷龍男、清水徹、四ノ宮成祥: ABCG2 遺伝子の機能低下型変異は若年発症の痛風の主要な病因である, 日本人類遺伝学会第 58 回大会, 仙台, 2013.11.20-2013.11.23.
63. 高田雄三、松尾洋孝、中山昌喜、清水聖子、崎山真幸、千葉俊周、中島宏、松村耕治、宮平靖、櫻井裕、清水徹、四ノ宮成祥: 痛風・高尿酸血症のリスク評価のための迅速遺伝子検査, 日本DNA多型学会第22回学術集会, 仙台, 2013.11.21-2013.11.22.
64. 松尾洋孝、市田公美、高田龍平、中山昌喜、中島宏、中村好宏、河村優輔、高田雄三、及川雄二、内藤真理子、菱田朝陽、若井建志、清水聖子、崎山真幸、千葉俊周、森厚嘉、浜島信之、櫻井裕、清水徹、四ノ宮成祥: 尿酸輸送体遺伝子 ABCG2 の機能低下型変異は若年性痛風の主要な原因である,第 24 回日本疫学会学術総会, 仙台, 2014.1.23-2014.1.25.
65. 中山昌喜、松尾洋孝、清水卓也、高田雄三、中島宏、中村好宏、清水聖子、千葉俊周、崎山真幸、高田龍平、井上勝央、川合紗世、菱田朝陽、若井建志、浜島信之、市田公美、櫻井裕、加藤将夫、清水徹、四ノ宮成祥: 輸送体遺伝子 MCT9 のミスセンス変異は腎負荷型の痛風と関連する,第 24 回日本疫学会学術総会, 仙台, 2014.1.23-2014.1.25.
66. 貝藤裕史, 野津寛大, 飯島一誠. 尿中落下細胞の遺伝子解析と尿細管疾患の診断の実際 salt-losing tubulopathy を例に. 第 47 回日本小児腎臓病学会学術集会（教育講演）2012.6.29-30, 東京
67. 忍頂寺毅史, 貝藤裕史, 大坪裕美, 橋本総子, 石森真吾, 橋村裕也, 野津寛大, 森貞直哉, 飯島一誠. Gitelman 症候群と偽性 Gitelman 症候群の臨床的差異に関する検討. 第 55 回日本腎臓学会学術集会 2012.6.1-3, 横浜
68. 森貞直哉, 関根孝司, 橋本総子, 大坪裕美, 付学軍, 石森真吾, 忍頂寺毅史, 橋村裕也, 貝藤裕史, 飯島一誠. 16q12.1-q12.2 ヘテロ欠失は鰓弓耳腎（BOR）症候群の原因となる. 47 回日本小児腎臓病学会学術集会 2012.6.29-30, 東京
69. 森貞直哉, 貝藤裕史, 伊藤秀一, 奥山虎之, 松永達雄, 関根孝司, 飯島一誠. 本邦における鰓弓耳腎（BOR）症候群の全国診療実態調査と原因遺伝子解析. 第 57 回日本人類遺伝学会 2012.10.24-27, 東京
70. 竹村司. ネフロン癆の末期腎不全への進行と

腎尿細管基底膜の異常. 2.　　第２９回中国四国小児腎臓病研究会 2012.11.18, 米子

71. 三浦健一郎, 張田豊, 高橋和浩, 滝田順子, 五十嵐隆：常染色体劣性遠位尿細管性アシドーシスの臨床像と遺伝子変異の解析. 第 55 回日本腎臓学会学術総会 2012.6.2, 横浜
72. 後藤眞, 成田一衛, 塚口裕康, 井ノ上逸朗, 成田一衛. 次世代シークエンサーを用いた腎臓病研究. 第 55 回 日本腎臓学会学術総会 会長主導企画シンポジウム. 2012.6.1, 横浜
73. 仲里仁史. よくわかるシリーズ 21　遺伝性腎疾患. 第 55 回日本腎臓学会学術総会 2012.6.3, 横浜
74. 内海由貴、松尾洋孝、高田龍平、中山昌喜、清水徹、春日裕志、中島宏、中村好宏、高田雄三、河村優輔、中村真希子、櫻井裕、細谷龍男、四ノ宮成祥、鈴木洋史、市田公美: 高尿酸血症の新たな発症機序の解明: ABCG2 の機能低下による腸管からの尿酸排泄低下. 第 7 回日本トランスポーター研究会年会, 2012.6.9-10, 京都
75. 松尾洋孝、高田龍平、中山昌喜、清水徹、春日裕志、中島宏、中村好宏、高田雄三、河村優輔、内海由貴、中村真希子、櫻井裕、細谷龍男、四ノ宮成祥、鈴木洋史、市田公美: ABCG2 遺伝子変異に基づく高尿酸血症の病態解明と新規病型分類. 日本人類遺伝学会第 57 回大会, 2012.10.25-27, 東京
76. 中山昌喜、松尾洋孝、高田龍平、清水徹、春日裕志、中島宏、中村好宏、高田雄三、河村優輔、内海由貴、中村真希子、櫻井裕、細谷龍男、四ノ宮成祥、鈴木洋史、市田公美: ABCG2 機能低下による「腎外排泄低下型」高尿酸血症. 日本薬理学会関東部会, 2012.10.20-27, 東京

H. 知的所有権の取得状況

1. 特許取得

特許（出願中）：発明の名称：尿酸トランスポーター，並びに，尿酸輸送関連疾患素因及び炎症関連疾患素因の評価方法及び評価キット，検査体及び薬.　特許出願中，発明者：松尾洋孝，高田龍平，鈴木洋史，池淵祐樹，伊藤晃成，市田公美，中村好宏，四ノ宮成祥.

2. 実用新案登録

該当無し

II.　研究成果の刊行に関する一覧表

III.　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
Miura K, Sekine T, Takahashi K, Takita J, Harita Y, Ohki K, Park MJ, Hayashi Y, Tajima A, Ishihara M, Hisano M, Murai M, Igarashi T.	Mutational analyses of the ATP6V1B1 and ATP6V0A4 genes in patients with primary distal renal tubular acidosis.	Nephrol Dial Transplant	2013;28(8):2123-30
Hashimura Y, Nozu K, Kaito H, Nakanishi K, Fu XJ, Ohtsubo H, Hashimoto F, Oka M, Ninchoji T, Ishimori S, Morisada N, Matsunoshita N, Kamiyoshi N, Yoshikawa N, Iijima K.	Milder clinical aspects of X-linked Alport syndrome in men positive for the collagen IV α5 chain.	Kidney Int	2013 Dec 4. Epub ahead of print
Sekine T, Komoda F, Miura K, Takita J, Shimadzu M, Matsuyama T, Ashida A, Igarashi T.	Japanese Dent disease has a wider clinical spectrum than Dent disease in Europe/USA: genetic and clinical studies of 86 unrelated patients with low-molecular-weight proteinuria.	Nephrol Dial Transplant	2014 ;29(2):376-84
Miura K, Kurihara H, Horita S, Chikamoto H, Hattori M, Harita Y, Tsurumi H, Kajiho Y, Sawada Y, Sasaki S, Igarashi T, Kunishima S, Sekine T.	Podocyte expression of nonmuscle myosin heavy chain-IIA decreases in idiopathic nephrotic syndrome, especially in focal segmental glomerulosclerosis.	Nephrol Dial Transplant	2013;28(12):2993-3003
Chiba T, Matsuo H, Nagamori S, Nakayama A, Kawamura Y, Shimizu S, Sakiyama M, Hosoyamada M, Kawai S, Okada R, Hamajima N, Kanai Y, Shinomiya N	Identification of a hypouricemia patient with SLC2A9 R380W, A pathogenic mutation for renal hypouricemia type 2	Nucleosides, Nucleotides and Nucleic Acids	2013; 0:1-5
Matsuo H, Nakayama A, Sakiyama M, Chiba T, Shimizu S, Kawamura Y, Nakashima H, Nakamura , Takada Y, Oikawa Y, Takada T, Nakaoka H, Abe J, Inoue H, Wakai K, Kawai S, Guang Y, Nakagawa H, Ito T, Niwa K, Yamamoto K, Sakurai Y, Suzuki H, Hosoya T, Ichida K, Shimizu T, Shinomiya N.	ABCG2 dysfunction causes hyperuricemia due to both renal urate underexcretion and renal urate overload.	Sci Rep	2014;4:3755

Matsuo H, Ichida K, Takada T, Nakayama A, Nakashima H, Nakamura T, Kawamura Y, Takada Y, Yamamoto K, Inoue H, Oikawa Y, Naito M, Hishida A, Wakai K, Okada C, Shimizu S, Sakiyama M, Chiba T, Ogata H, Niwa K, Hosoyamada M, Mori A, Hamajima N, Suzuki H, Kanai Y, Sakurai Y, Hosoya T, Shimizu T, Shinomiya N.	Common dysfunctional variants in ABCG2 are a major cause of early-onset gout.	Sci Rep	2013;3:2014
竹村司	ネフロン癆	先天代謝異常ハンドブック 中山書店 東京	2013; 334-335
Ogawa A, Kitamura S, Nakayama K, Sugiyama H, Morisada N, Iijima K, Makino H.	Right hypoplastic kidney.	Kidney Int	2012;82(9):1037
Iijima K, Someya T, Ito S, Nozu K, Nakanishi K, Matsuoka K, Ohashi H, Nagata M, Kamei K, Sasaki S.	Focal segmental glomerulosclerosis in patients with complete deletion of one WT1 allele.	Pediatrics	2012;129(6):e1621-5
Ohtsubo H, Morisada N, Kaito H, Nagatani K, Nakanishi K, Iijima K.	Alport-like glomerular basement membrane changes with renal-coloboma syndrome.	Pediatr Nephrol	2012;27(7):1189-92
竹村 司	学校検尿の限界	日本小児科医会報(日本小児科医会編集)	2012;44;123-5
竹村 司	ネフロン癆・腎髄質嚢胞	小児腎臓病学(日本小児腎臓病学会編集)診断と治療社 東京	2012;245-8
Ichida K, Matsuo H, Takada T, Nakayama A, Murakami K, Shimizu T, Yamanashi Y, Kasuga H, Nakashima H, Nakamura T, Takada Y, Kawamura Y, Inoue H, Okada C, Utsumi Y, Ikebuchi Y, Ito K, Nakamura M, Shinohara Y, Hosoyamada M, Sakurai Y, Shinomiya N, Hosoya T, Suzuki H.	Decreased extra-renal urate excretion is a common cause of hyperuricemia.	Nat Commun	2012;3:764
松尾洋孝、市田公美、高田龍平、中山昌喜、四ノ宮成祥	尿酸動態の支配因子としての尿酸トランスポーター	細胞工学	2012;31;553-7
千葉俊周、松尾洋孝、市田公美、中山昌喜、四ノ宮成祥	膜輸送系の異常 遺伝性腎性低尿酸血症	日本臨床	2012;807-11
千葉俊周、松尾洋孝、市田公美、四ノ宮成祥	低尿酸血症の頻度、原因、分類を教えてください	腎と透析	2012;73:301-4
中山昌喜、松尾洋孝、市田公美、四ノ宮成祥	尿細管疾患 尿細管機能異常症 腎性低尿酸血症	腎と透析	2012;72:370-373
松尾洋孝、四ノ宮成祥	腎性低尿酸血症の遺伝学	Annual Review 糖尿病・代謝・内分泌 2012	2012;145-54
Nishina S, Kosaki R, Yagihashi T, Azuma N, Okamoto N, Hatsukawa Y, Kurosawa K, Yamane T, Mizuno S, Tsuzuki K, Kosaki K.	Ophthalmic features of CHARGE syndrome with CHD7 mutations.	Am J Med Genet A	2012;158A(3):514-8

Nephrol Dial Transplant (2013) 28: 2123–2130
doi: 10.1093/ndt/gft216
Advance Access publication 31 May 2013

Mutational analyses of the *ATP6V1B1* and *ATP6V0A4* genes in patients with primary distal renal tubular acidosis

Kenichiro Miura[1], Takashi Sekine[1,2], Kazuhiro Takahashi[1], Junko Takita[1,3], Yutaka Harita[1], Kentaro Ohki[4], Myoung-Ja Park[4], Yasuhide Hayashi[4], Asako Tajima[5], Masayuki Ishihara[6], Masataka Hisano[7], Miki Murai[8] and Takashi Igarashi[1]

[1]Department of Pediatrics, Faculty of Medicine, The University of Tokyo, Tokyo, Japan,
[2]Department of Pediatrics, Toho University Graduate School of Medicine, Ohashi Hospital, Tokyo, Japan,
[3]Department of Cell Therapy and Transplantation Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan,
[4]Department of Hematology/Oncology, Gunma Children's Medical Center, Gunma, Japan,
[5]Department of Pediatrics, Jikei University School of Medicine, Tokyo, Japan,
[6]Department of Pediatrics, Kochi Medical School, Kochi University, Nankoku, Kochi, Japan,
[7]Department of Pediatric Nephrology, Chiba Children's Hospital, Chiba, Japan and
[8]Department of Pediatrics, Seirei Numazu Hospital, Numazu, Shizuoka, Japan

Correspondence and offprint requests to: Kenichiro Miura; E-mail kmiu-tky@umin.ac.jp

Keywords: autosomal-recessive distal renal tubular acidosis, *ATP6V1B1*, *ATP6V0A4*, hyperammonemia, large deletions

ORIGINAL ARTICLE

ABSTRACT

Background. Mutations in the *ATP6V1B1* and the *ATP6V0A4* genes cause primary autosomal-recessive distal renal tubular acidosis (dRTA). Large deletions of either gene in patients with dRTA have not been described.

Methods. The *ATP6V1B1* and *ATP6V0A4* genes were directly sequenced in 11 Japanese patients with primary dRTA from nine unrelated kindreds. Large heterozygous deletions were analyzed by quantitative real-time polymerase chain reaction (PCR). The clinical features of the 11 patients were also investigated.

Results. Novel mutations in the *ATP6V1B1* gene were identified in two kindreds, including frameshift, in-frame insertion and nonsense mutations. Large deletions in the *ATP6V0A4* gene were identified in two kindreds. Exon 15 of *ATP6V0A4* was not amplified in one patient, with a long PCR confirming compound heterozygous deletions of 3.7- and 6.9-kb nucleotides, including all of exon 15. Direct DNA sequencing revealed a heterozygous frameshift mutation in *ATP6V0A4* in another patient, with quantitative real-time PCR indicating that all exons up to exon 8 were deleted in one allele. Clinical investigation showed that four of the six patients with available clinical data presented with hyperammonemia at onset.

Conclusions. To our knowledge, these dRTA patients are the first to show large deletions involving one or more entire exons of the *ATP6V0A4* gene. Quantitative PCR amplification may be useful in detecting heterozygous large deletions. These results expand the spectrum of mutations in the *ATP6V0A4* and *ATP6V1B1* genes associated with primary dRTA and provide insight into possible structure–function relationships.

INTRODUCTION

Primary distal renal tubular acidosis (dRTA) is a rare genetic disease caused by impaired excretion of hydrogen ions (H^+) by intercalated cells in the collecting ducts [1, 2]. Its clinical

features include hyperchloremic acidosis with inappropriately alkaline urine, hypokalemia, hypercalciuria, nephrocalcinosis and nephrolithiasis. Affected infants present with polyuria, dehydration and failure to thrive [3]. Both autosomal-dominant and autosomal-recessive forms of dRTA have been described. Mutations in the *ATP6V1B1* gene, which encodes the B1 subunit of the H^+-ATPase, have been associated with autosomal-recessive dRTA accompanied by severe sensorineural hearing loss (SNHL), whereas mutations in the *ATP6V0A4* gene, which encodes the a4 subunit of the H^+-ATPase, have been associated with autosomal-recessive dRTA without SNHL [1, 4, 5]. Recent genetic analyses, however, have revealed that some individuals with mutations in the *ATP6V0A4* gene also have early-onset severe SNHL [6, 7]. To expand knowledge of mutations in patients with dRTA, we analyzed the *ATP6V1B1* and *ATP6V0A4* genes in 11 Japanese patients from nine unrelated kindreds with dRTA.

MATERIALS AND METHODS

Patients

We analyzed 11 Japanese patients with dRTA belonging to nine unrelated kindreds; their clinical features and representative biochemical data are shown in Table 1. The diagnosis of dRTA was based on metabolic acidosis with a normal anion gap and overly high urinary pH, with or without nephrocalcinosis and SNHL. The parents of the patients in families 1 and 5 were consanguineous. Patients 1-1 and 1-2, and patients 7-1 and 7-2 were siblings. Detailed clinical data were not available for patient 5-1 at initial presentation, but she presented with full-blown symptoms of dRTA, including nephrocalcinosis and SNHL.

The study protocol was approved by the Ethics Committee of The University of Tokyo (Approval No. 2204). Informed consent was obtained from each patient and his or her parents.

Mutation analysis

Genomic DNA was extracted from the peripheral blood of the patients and their family members using QuickGene DNA whole-blood S kits (Fujifilm). Thirteen and 20 pairs of oligonucleotide primers were generated to amplify all exons of the *ATP6V1B1* and *ATP6V0A4* genes, respectively (Supplemental Tables 1 and 2). Polymerase chain reactions (PCRs) were performed in 20 μL of solution containing AmpliTaq Gold 360 Master Mix (Applied Biosystems), ~30-ng genomic DNA and 10 pmol of each primer. The amplification protocol consisted of an initial denaturation step at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at the temperatures shown in Supplemental Tables 1 and 2 for 30 s and elongation at 72°C for 30 s. PCR samples were subjected to bidirectional sequencing on an ABI 3130*xl* Genetic Analyzer (Applied Biosystems).

Quantitative real-time PCR amplification

The heterozygous large deletion in the *ATP6V0A4* gene of patient 4-1 was analyzed by quantitative genomic real-time PCR using SYBR Green (Bio-Rad Laboratories, Hercules, CA) and the primer sequences shown in Supplemental Table 2. The standard curve method was used to calculate the target genome numbers in patient 4-1, with the target copy number normalized to normal human genomic DNA. Statistical significance was assessed using Student's *t*-tests.

RESULTS

Clinical findings

A total of 11 patients from nine unrelated kindreds were enrolled in this study (Table 1). Consanguinity was noted in two families. Among the 10 patients with available data at onset, all but 1 (patient 2-1) presented with metabolic acidosis with a normal anion gap and abnormally high urinary pH. Although patient 2-1 had a near normal serum bicarbonate concentration, she presented with hypokalemia, hypercalciuria, growth retardation and bilateral SNHL. Supplementation with bicarbonate improved her growth. Urine anion gap was positive or near-zero in all patients with available data, which is consistent with reduced NH4+ excretion as seen in dRTA [8].

Serum creatinine concentrations in all patients were within normal range throughout the follow-up period. Growth retardation in infancy occurred in 9 of the 11 patients, with alkali therapy improving their growth to almost normal range. Of the two patients without growth retardation at onset, one (patient 7-2) was diagnosed with dRTA by screening tests before presenting with any symptoms because her older sister had been diagnosed with dRTA. The other patient with normal growth during infancy (patient 9-1) presented with severe weight loss, metabolic acidosis, abnormally high urinary pH and hypercalciuria at age 7 years.

Abdominal ultrasonography showed nephrocalcinosis in nine patients. Unilateral or bilateral SNHL, as determined by audiograms, were observed in four patients. Hypokalemia was observed in six of the nine patients with available data and hypercalciuria in seven of nine. The chart of an additional patient (patient 3-1) mentioned hypercalciuria, although precise data were not available.

Hyperammonemia was observed in four of the six patients with available data, improving in all four after correction of metabolic acidosis. Of the two patients without hyperammonemia, one (patient 4-1) had high-normal (60 μg/dL) serum ammonia concentration, whereas the other (patient 2-1) had normal serum ammonia, a finding probably associated with the absence in this patient of profound metabolic acidosis at onset.

Patient 7-2, a younger sister of patient 7-1, was diagnosed with dRTA 10 days after birth by screening blood and urine tests. Since then, she has been treated with citrate and her growth is normal. At age 8 months, she presented with transient metabolic acidosis resulting from insufficient supplementation with citrate. A higher dosage of citrate improved her symptoms. Of note, she is free from nephrocalcinosis at age 2 years. In contrast, her older sister, patient 7-1, presented with prominent nephrocalcinosis at age 1 month.

Table 1. Clinical features of the patients

	Consanguinity	Age/sex	Age at onset	FTT	NC	SNHL	pH	HCO_3^- (19–23) mmoL/L	Potassium (3.5–6.0) mEq/L	Serum ammonia (12–66) μg/dL	Urine pH	Urine anion gap, mEq/L	Urine calcium excretion (<4 mg/kg/day, Ca/Cr <0.21)
Patients with mutations in the *ATP6V1B1* gene													
1-1	+	46 years/M	Infancy	+	+	+rt	7.230	11.8	3.4	NA	7.0	4	7.2 mg/kg/day
1-2	+	38 years/F	Infancy	+	+	–	7.215	12.4	1.8	NA	7.0	2	6.5 mg/kg/day
2-1	–	1 year/F	9 months	+	+	+bil	7.325	19.6	1.8	27	8.0	NA	Ca/Cr 0.42
Patients with mutations in the *ATP6V0A4* gene													
3-1	–	12 years/M	2 months	+	+	–	7.293	14.1	NA	NA	8.0	NA	(high)[a]
4-1	–	1 month/M	1 month	+	+	+rt	7.167	12.3	3.3	60	7.5	17	Ca/Cr 0.77
Patients with no mutations													
5-1	+	35 years/F	1 month	+	+	+bil	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]
6-1	–	15 years/M	1 month	+	+	–	7.322	14.1	4.1	94	7.3	NA	Ca/Cr 0.19
7-1	–	6 years/F	1 month	+	+	–	7.220	11.2	4.1	97	7.0	NA	Ca/Cr 1.42

Continued

Table 1. Continued

	Consanguinity	Age/sex	Age at onset	FTT	NC	SNHL	pH	HCO_3^- (19–23) mmoL/L	Potassium (3.5–6.0) mEq/L	Serum ammonia (12–66) μg/dL	Urine pH	Urine anion gap, mEq/L	Urine calcium excretion (<4 mg/kg/day, Ca/Cr <0.21)
7-2	–	3 years/F	10 days	–[c]	–	–	7.354	14.1	3.8	144	8.0	28	Ca/Cr 0.27
8-1	–	3 years/F	1 year 0 month	+	+	–	6.999	9.2	2.2	NA	7.5	–2	Ca/Cr 0.06
9-1	–	7 years/F	7 years	–[d]	–	–	7.120	9.0	2.2	78	7.5	15	Ca/Cr 0.59

Normal values for infants are shown in parentheses.
FTT, failure to thrive; NC, nephrocalcinosis; SNHL, sensorineural hearing loss; rt, right; NA, not available; bil, bilateral; Ca/Cr, urine calcium to creatinine ratio (mg/mgCr).
[a]Although specific data were not available, hypercalciuria was noted in the chart.
[b]Detailed clinical data at initial presentation were not available in patient 5-1, but she presented with full-blown symptoms of dRTA, including nephrocalcinosis and SNHL.
[c]Patient 7-2 was diagnosed by screening tests when she had no symptoms.
[d]Although no growth retardation was noted in patient 9-1 in infancy, she presented with weight loss at the age of 7.

Genetic analysis

Three novel mutations in the *ATP6V1B1* gene were identified in two of the nine kindreds (Figure 1). Patients 1-1 and 1-2 (family 1) presented with novel homozygous single nucleotide deletions (c.33delG) in exon 1, causing a frameshift that resulted in the premature termination of the protein at codon 19 (L19X) (Figure 1A). In addition, patient 2-1 presented with novel compound heterozygous mutations, consisting of an in-frame insertion (c.978_979insGCC or p.A326insA) and a nonsense mutation (c.1251C>G or p.Y417X). Genotyping of family members of patient 2-1 revealed that this patient had inherited the p.Y417X mutated allele from her mother (Figure 1B).

We observed novel aberrations of the *ATP6V0A4* gene in two of the nine kindreds. In patient 3-1, the genomic PCR product of exon 15 was not amplified (Figure 2A), suggesting homozygous deletions that included exon 15. To confirm this finding, we performed long genomic PCR using primers for exons 14 (forward) and 16 (reverse), which amplified a 7.9-kb product derived from wild-type *ATP6V0A4* allele in the healthy control (Figure 2B). In patient 3-1, however, these primers amplified two PCR products, of 4.2 and 1.0 kb, suggesting that deletions involving exon 15 were responsible for the aberrant *ATP6V0A4* transcripts. Subsequent sequencing disclosed the presence of 3.7- and 6.9-kb deletions within introns 14 and 15, respectively, in each allele. These deletions caused a frameshift that resulted in premature termination of the protein at codon 536 (L536X) in exon 16 (Figure 2C).

We also observed a novel heterozygous large deletion of the *ATP6V0A4* gene in patient 4-1. This was accompanied by a novel heterozygous single nucleotide insertion (c.1185insC) causing a frameshift that resulted in premature termination of the protein at codon 429 (p.E429X) in exon 13, as shown by direct DNA sequencing (Figure 3A). The quantities of the PCR products up to exon 8 seemed to be reduced (data not shown), suggesting the presence of an interstitial heterozygous deletion of an *ATP6V0A4* allele in patient 4-1. To determine the *ATP6V0A4* gene copy numbers in this patient, we performed quantitative genomic PCR of exons 3, 5, 6, 7, 8, 9, 10 and 18 using the same amounts of genomic DNA (1 μg/tube) from the patient and a healthy control. We found that the signal intensities of exons 3, 5, 6, 7 and 8 were significantly reduced compared with those of exons 9, 10 and 18 ($P < 0.05$), indicating that the N-terminal region up to exon 8 was deleted in one of the *ATP6V0A4* alleles of patient 4-1 (Figure 3B).

Given that large deletions including one or more entire exons were frequently identified, multiplex ligation-dependent probe amplification analyses for both the *ATP6V0A4* and *ATP6V1B1* genes were performed in the five patients without any alterations of these two genes. No abnormalities in relative copy numbers were detected in these patients, suggesting that

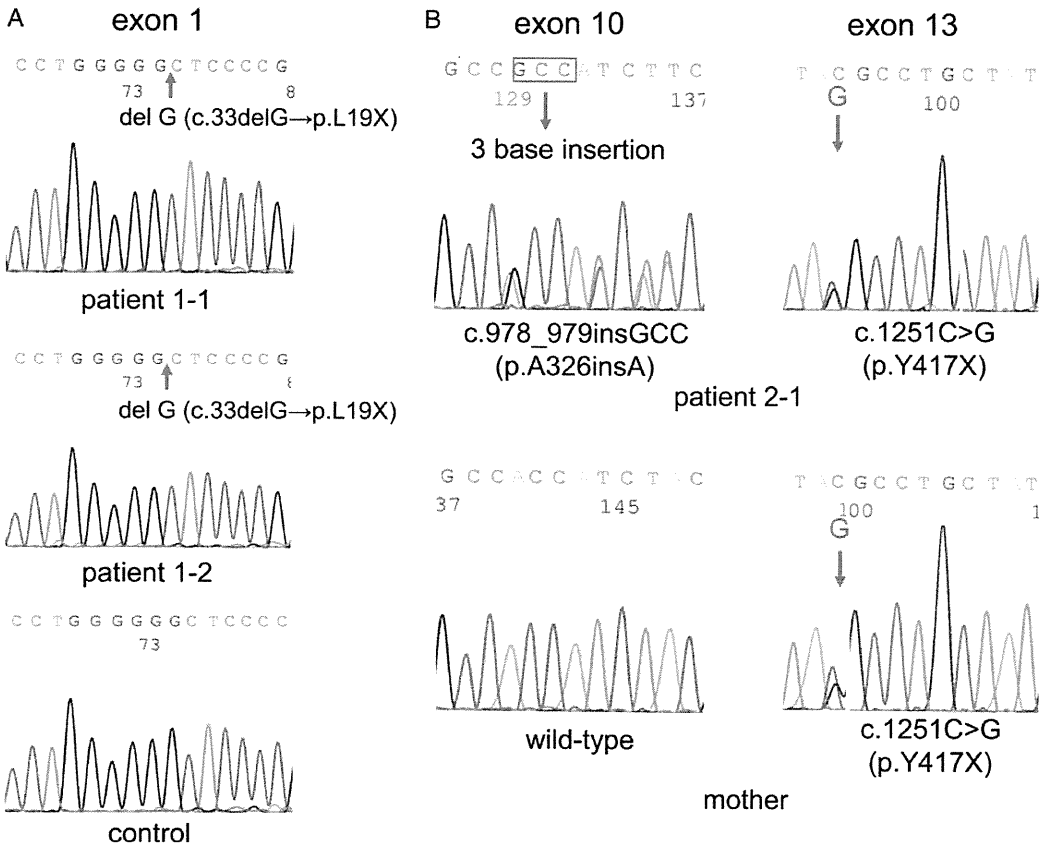

FIGURE 1: Novel mutations in the *ATP6V1B1* gene. (**A**) Genomic sequences of novel homozygous mutations in patients 1-1 and 1-2 (upper panels), as well as normal DNA from a healthy control (lower panel). (**B**) Novel compound heterozygous mutations in patient 2-1 (upper panels). Mother also carries a heterozygous mutation p.Y417X (lower panels). Mutated nucleotides are indicated by arrows.

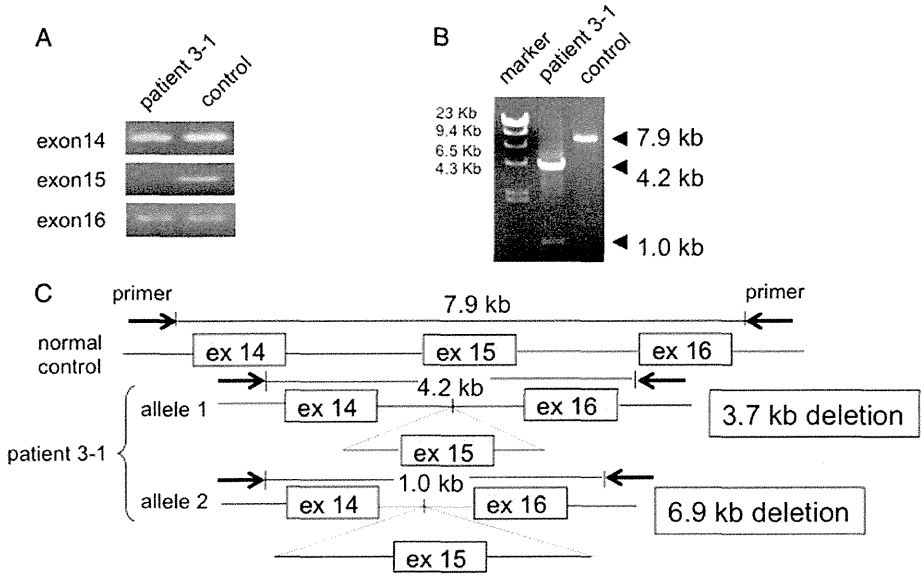

FIGURE 2: Novel large interstitial deletions in the *ATP6V0A4* gene in patient 3-1. (**A**) Genomic PCR product of exon 15 was absent, whereas DNA fragments of exons 14 and 16 were amplified in patient 3-1. (**B**) Long genomic PCR using primers for exon 14 (forward) and exon 16 (reverse) in patient 3-1. Two aberrant sizes of the PCR products, 4.2 and 1.0 kb, were observed in patient 3-1, while a 7.9-kb product was detected in the healthy control. (**C**) Schematic presentation of large deletions in the *ATP6V0A4* gene in patient 3-1. Deletions of 3.7- and 6.9-kb nucleotides including whole exon 15 were confirmed by subsequent sequencing analysis.

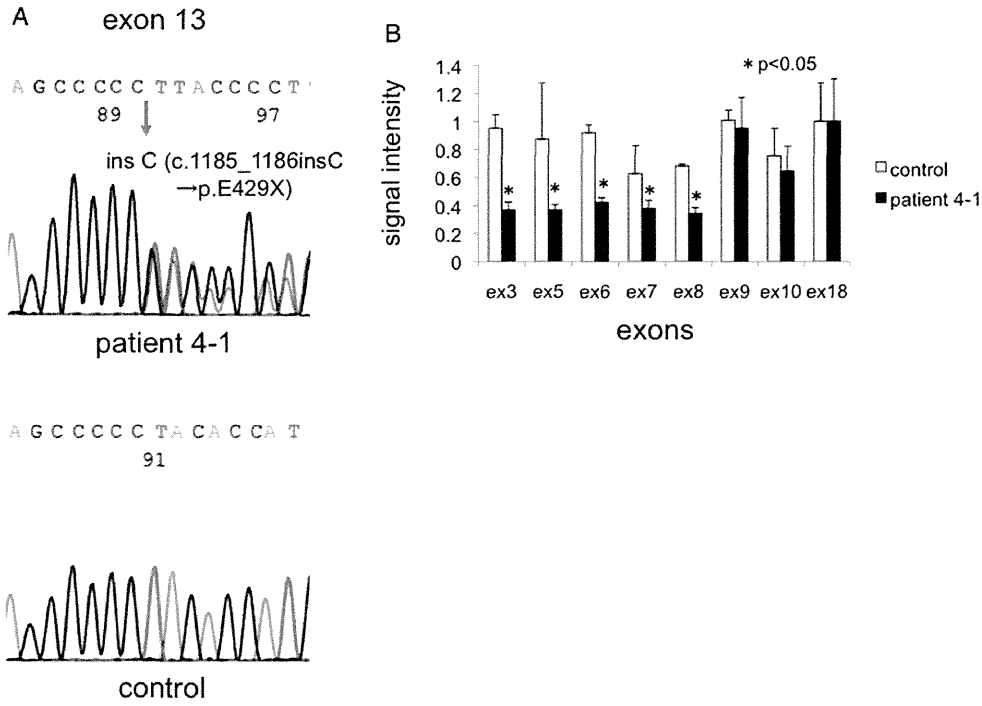

FIGURE 3: Novel compound heterozygous mutations comprising an insertion and a large deletion in the *ATP6V0A4* gene in patient 4-1. (**A**) Genomic sequences of a novel heterozygous mutation in patient 4-1 (upper panel) as well as normal DNA from a healthy control (lower panel) are shown. (**B**) Quantitative genomic PCR of the *ATP6V0A4* gene in patient 4-1. The signal intensity of exons 3, 5, 6, 7 and 8 was significantly reduced as compared with exons 9, 10 and 18 in patient 4-1, while the signal intensity was not significantly reduced in any exons in healthy control. The signal intensity of each exon was adjusted so that the intensity score was 1 in exon 18.*P < 0.05. P-value was assessed by Student's *t*-test. ex: exon.

they carry no homozygous or heterozygous large deletions of these two genes (data not shown).

Clinical features of the patients with mutations in the *ATP6V1B1* and *ATP6V0A4* genes

All the patients with mutations in the *ATP6V1B1* or *ATP6V0A4* gene presented with growth retardation in infancy and nephrocalcinosis accompanied by hypercalciuria. Three patients from three unrelated families presented with early-onset SNHL. Of these, two had mutations in the *ATP6V1B1* gene (patients 1-1 and 2-1), and the third had a truncating mutation in one allele of the *ATP6V0A4* gene and a large deletion that included several exons in the other allele (patient 4-1). Patients 1-1 and 1-2 are siblings and possess the same mutation in the *ATP6V1B1* gene. However, only the brother (patient 1-1) presented with early-onset unilateral SNHL, which was confirmed by audiogram at age 9 years, while his 38-year-old sister (patient 1-2) has had no audiographic evidence of SNHL to date.

DISCUSSION

We have identified three *ATP6V1B1* mutations (one deletion, one insertion and one nonsense mutation) and four *ATP6V0A4* mutations (one insertion and three large deletions involving one or more exons) in patients with primary dRTA. These findings expand the spectrum of mutations in the *ATP6V0A4* and *ATP6V1B1* genes associated with primary dRTA, and provide insight into possible structure–function relationships.

To date, about 30 mutations in the *ATP6V1B1* gene and about 40 in the *ATP6V0A4* gene have been identified, including missense, nonsense, frameshift and splicing site mutations [1, 3, 6, 7, 9–12]. Prior to this study, however, large interstitial deletions involving one or more exons in either gene had not been reported. Thus, to our knowledge, this is the first report of patients with large deletions in one or both alleles of the *ATP6V0A4* gene. Homozygous large interstitial deletions can be revealed by the absence of PCR products of particular exons. Although heterozygous large deletions are much harder to detect, quantitative real-time genomic PCR can be useful when PCR products of particular exons show relatively weak bands.

One of the compound heterozygous mutations in patient 2-1 was an in-frame insertion mutation (c.978_979insGCC/p.A326insA), the functional consequence of which should be addressed. A326 and T327 are highly conserved among species. Three-dimensional structural analysis shows that these amino acids are located near the hydrophobic residues at the interface between subunits A and B, which is critical for the catalytic activity [13].

In five of the nine kindreds, we observed no mutations in the *ATP6V1B1* and *ATP6V0A4* genes. In contrast, 80% of patients with dRTA were found to have a mutation in either gene [5, 14]. The low mutation frequency of these genes in our patients may be due to our small sample size and/or to differences in ethnicity, suggesting that dRTA is genetically heterogeneous. The clinical diagnosis seemed accurate in all of our patients, as all had metabolic acidosis with a normal anion gap and abnormally high urinary pH, accompanied by either nephrocalcinosis or hypercalciuria. Although hypokalemia was not noted at onset in three patients, it developed later in their clinical courses. H^+-ATPase is composed of at least 13 different subunits, including the B1 and a4 subunits. The expression of the isoforms C2, G3 and d2, which are encoded by the *ATP6V1C2*, *ATP6V1G3* and *ATP6V0D2* genes, respectively, is restricted to the kidneys and a few other tissues [2,15]. Although these genes were assessed as candidate causative genes in patients with recessive dRTA, no mutations were identified [15].

Our findings have important clinical implications. Administration of citrate salts is recommended for the prevention of nephrocalcinosis in dRTA, because they correct hypercalciuria and hypocitraturia [16]. Patient 7-2 was diagnosed with dRTA at age 10 days by screening blood samples, because her older sister, patient 7-1, had previously been diagnosed with dRTA. Alkali therapy was instituted immediately after diagnosis in patient 7-2, and this patient has shown no evidence of nephrocalcinosis at age 2 years. In contrast, her older sister, patient 7-1, presented with marked nephrocalcinosis at age 1 month. This difference in clinical manifestation between these two sisters, who are theorized to carry the same genetic abnormalities, suggests that nephrocalcinosis may be prevented by alkali therapy. As for early-onset SNHL, it was present in four patients, two with mutations in *ATP6V1B1*, one with a mutation in *ATP6V0A4*, and one without any mutations in either gene. This finding and the fact that only the brother (patient 1-1) in kindred one presented with unilateral SNHL confirm that intrafamilial variations in clinical manifestations can occur and that some individuals with mutations in the *ATP6V0A4* gene also have early-onset SNHL [6, 7]. The mechanism of intrafamilial variations in hearing disabilities remains unclear. Although the younger sister (patient 1-2) was diagnosed with dRTA earlier than her older brother (patient 1-1), earlier initiation of alkali therapy is reported to have no effect on hearing loss [17]. In regard to laterality in SNHL (patient 1-1), asymmetrical progression of hearing disabilities has been previously described, the mechanism of which is also unknown [18]. We found that four of six patients with available data presented with hyperammonemia. To our knowledge, a high frequency of hyperammonemia in patients with dRTA has not been previously described, although a few patients were found to have hyperammonemia secondary to dRTA [19–21]. Hyperammonemia may result from an imbalance between increased synthesis and reduced excretion of ammonia. Chronic metabolic acidosis and hypokalemia can increase renal ammoniagenesis. However, in our cases, hypokalemia was noted in only one of four patients presenting with hyperammonemia. The precise mechanism by which hyperammonemia develops in dRTA remains to be determined.

In conclusion, we have described patients with dRTA and large interstitial deletions involving one or more exons in the *ATP6V0A4* gene. Long genomic PCR as well as quantitative genomic PCR analyses can be useful in detecting large homozygous or heterozygous deletions. Clinically, nephrocalcinosis may be prevented by early and adequate alkali therapy.

Hyperammonemia is a frequent finding at onset in dRTA, suggesting that the latter condition be included as a miscellaneous cause of hyperammonemia.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

ACKNOWLEDGEMENTS

We thank Masayo Matsumura and Naoko Hoshino for their excellent technical assistance.

FUNDING

This work was supported by the Japan Society for the Promotion of Science.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Karet FE, Finberg KE, Nelson RD *et al.* Mutations in the gene encoding B1 subunit of H+-ATPase cause renal tubular acidosis with sensorineural deafness. Nat Genet 1999; 21: 84–90
2. Batlle D, Haque SK. Genetic causes and mechanisms of distal renal tubular acidosis. Nephrol Dial Transplant 2012; 27: 3691–3704
3. Rodriguez-Soriano J. New insights into the pathogenesis of renal tubular acidosis—from functional to molecular studies. Pediatr Nephrol 2000; 14: 1121–1136
4. Smith AN, Skaug J, Choate KA *et al.* Mutations in ATP6N1B, encoding a new kidney vacuolar proton pump 116-kD subunit, cause recessive distal renal tubular acidosis with preserved hearing. Nat Genet 2000; 26: 71–75
5. Karet FE. Inherited distal renal tubular acidosis. J Am Soc Nephrol 2002; 13: 2178–2184
6. Vargas-Poussou R, Houillier P, Le Pottier N *et al.* Genetic investigation of autosomal recessive distal renal tubular acidosis: evidence for early sensorineural hearing loss associated with mutations in the ATP6V0A4 gene. J Am Soc Nephrol 2006; 17: 1437–1443
7. Andreucci E, Bianchi B, Carboni I *et al.* Inner ear abnormalities in four patients with dRTA and SNHL: clinical and genetic heterogeneity. Pedatr Nephrol 2009; 24: 2147–2153
8. Batlle DC, Hizon M, Cohen E *et al.* The use of the urinary anion gap in the diagnosis of hyperchloremic metabolic acidosis. N Engl J Med 1988; 318: 594–599
9. Ruf R, Rensing C, Topaloglu R *et al.* Confirmation of the ATP6B1 gene as responsible for distal renal tubular acidosis. Pediatr Nephrol 2003; 18: 105–109
10. Gil H, Santos F, Garcia E *et al.* Distal RTA with nerve deafness: clinical spectrum and mutational analysis in five children. Pediatr Nephrol 2007; 22: 825–828
11. Carboni I, Andreucci E, Caruso MR *et al.* Medullary sponge kidney associated with primary distal renal tubular acidosis and mutations of the H+−ATPase genes. Nephrol Dial Transplant 2009; 24: 2734–2738
12. Vivante A, Lotan D, Pode-Shakked N *et al.* Familial autosomal recessive renal tubular acidosis: importance of early diagnosis. Nephron Physiol 2011; 119: 31–39
13. Maher MJ, Akimoto S, Iwata M *et al.* Crystal structure of A3B3 complex of V-ATPase from Thermus thermophilus. EMBO J 2009; 28: 3771–3779
14. Stover EH, Borthwick KJ, Bavalia C *et al.* Novel ATP6V1B1 and ATP6V0A4 mutations in autosomal recessive distal renal tubular acidosis with new evidence for hearing loss. J Med Genet 2002; 39: 796–803
15. Smith AN, Borthwick KJ, Karet FE. Molecular cloning and characterization of novel tissue-specific isoforms of the human vacuolar H+-ATPase C, G and d subunits, and their evaluation in autosomal recessive distal renal tubular acidosis. Gene 2002; 297: 169–177
16. Domrongkitchaiporn S, Khositseth S, Stitchantrakul W *et al.* Dosage of potassium citrate in the correction of urinary abnormalities in pediatric distal renal tubular acidosis patients. Am J Kidney Dis 2002; 39: 383–391
17. Bajaj G, Quan A. Renal tubular acidosis and deafness: report of a large family. Am J Kidney Dis 1996; 27: 880–882
18. Joshua B, Kaplan DM, Raveh E *et al.* Audiometric and imaging characteristics of distal renal tubular acidosis and deafness. J Laryngol Otol 2008; 122: 193–198
19. Seracini D, Poggi GM, Pela I. Hyperammonaemia in a child with distal renal tubular acidosis. Pediatr Nephrol 2005; 20: 1645–1647
20. Pela I, Seracini D. Hyperammonaemia in distal renal tubular acidosis: is it more common than we think? Clin Nephrol 2007; 68: 109–114
21. Saito T, Hayashi D, Shibata S *et al.* Novel compound heterozygous ATP6V0A4 mutations in an infant with distal renal tubular acidosis. Eur J Pediatr 2010; 169: 1271–1273

Received for publication: 16.10.2012; Accepted in revised form: 2.4.2013

Milder clinical aspects of X-linked Alport syndrome in men positive for the collagen IV α5 chain

Yuya Hashimura[1], Kandai Nozu[1], Hiroshi Kaito[1], Koichi Nakanishi[2], Xue Jun Fu[1], Hiromi Ohtsubo[1], Fusako Hashimoto[1], Masafumi Oka[1], Takeshi Ninchoji[1], Shingo Ishimori[1], Naoya Morisada[1], Natsuki Matsunoshita[1], Naohiro Kamiyoshi[1], Norishige Yoshikawa[2] and Kazumoto Iijima[1]

[1]*Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan and* [2]*Department of Pediatrics, Wakayama Medical University, Wakayama, Japan*

X-linked Alport syndrome is caused by mutations in the *COL4A5* gene encoding the type IV collagen α5 chain (α5(IV)). Complete absence of α5(IV) in the renal basal membrane is considered a pathological characteristic in male patients; however, positive α5(IV) staining has been found in over 20% of patients. We retrospectively studied 52 genetically diagnosed male X-linked Alport syndrome patients to evaluate differences in clinical characteristics and renal outcomes between 15 α5(IV)-positive and 37 α5(IV)-negative patients. Thirteen patients in the α5(IV)-positive group had non-truncating mutations consisting of nine missense mutations, three in-frame deletions, and one splice-site mutation resulting in small in-frame deletions of transcripts. The remaining two showed somatic mutations with mosaicism. Missense mutations in the α5(IV)-positive group were more likely to be located before exon 25 compared with missense mutations in the α5(IV)-negative group. Furthermore, urinary protein levels were significantly lower and the age at onset of end-stage renal disease was significantly higher in the positive group than in the negative group. These results help to clarify the milder clinical manifestations and molecular characteristics of male X-linked Alport syndrome patients expressing the α5(IV) chain.

Kidney International advance online publication, 4 December 2013; doi:10.1038/ki.2013.479

KEYWORDS: Alport syndrome; *COL4A5*; genetic renal disease; somatic mosaic mutation

Correspondence: *Kandai Nozu, Department of Pediatrics, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo, Kobe, Hyogo 6500017, Japan. E-mail: nozu@med.kobe-u.ac.jp*

Received 10 July 2013; revised 16 September 2013; accepted 19 September 2013

Alport syndrome (AS) is a hereditary disorder of type IV collagen, characterized by chronic kidney disease progressing to end-stage renal disease (ESRD), sensorineural hearing loss, and ocular abnormalities.[1] Approximately 85% of AS patients show X-linked inheritance (XLAS), and mutations in the *COL4A5* gene, which encodes the type IV collagen α5 (α5(IV)) chain, can be detected.[1] Disease-causing mutations in *COL4A5* result in abnormal α5(IV) expression and typically in complete absence of α5(IV) in the glomerular basement membrane (GBM) and Bowman's capsule (BC). However, a previous review suggested that 20% of male XLAS sufferers showed complete or partial staining for this collagen chain,[2] although the genetic and clinical backgrounds of male XLAS patients presenting with such atypical immunohistological findings have not yet been elucidated. The aim of this study was to clarify the genetic and clinical backgrounds of XLAS patients with positive expression of α5(IV), and to determine if expression of this chain correlated with renal phenotype in men with XLAS.

RESULTS

Patient clinical features and pathological findings

A total of 139 patients were referred to our hospital for mutational analysis between January 2006 and January 2011. Among these, 63 female patients were excluded from this study. Of the remaining 76 male patients, 62 were genetically defined as male XLAS sufferers. α5(IV) Staining was not carried out in 10 patients, and 52 men with α5(IV) expression data were therefore included in this study (Figure 1). Thirty-seven (71%) patients showed no α5(IV) staining in kidney tissue (negative group), whereas the remaining 15 (29%) showed positive staining for α5(IV) (positive group). Thirteen patients in the positive group showed normal α5(IV) distribution, although some showed reduced expression levels, and two showed a mosaic expression pattern (Supplementary Table 2 online). The characteristics of the two groups are summarized in Table 1. Although the ages at kidney biopsy and mutational analysis were similar in both groups, the time between age at biopsy and age at mutational analysis was significantly shorter in the positive group. This

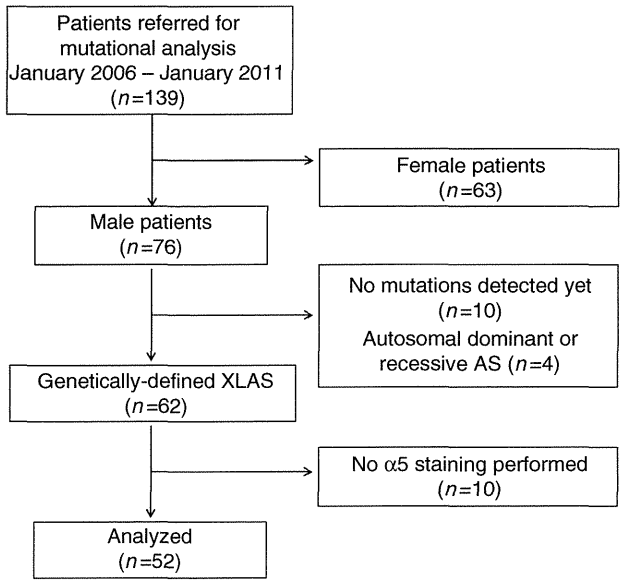

Figure 1 | Flow diagram showing patient recruitment and analysis. Fifty-two proven XLAS males were finally enrolled in this study. AS, Alport syndrome; XLAS, AS patients showing X-linked inheritance.

Table 1 | Clinical and laboratory data and treatments in α5(IV)-negative and α5(IV)-positive patients

	α5(IV)-Negative group (n = 37)	α5(IV)-Positive group (n = 15)	*P*-value
Age at analysis (years)	14.0 ± 9.2	12.8 ± 7.0	0.88
Age at kidney biopsy (years)	8.0 ± 6.2	10.5 ± 6.6	0.13
Time between kidney biopsy and mutational analysis (years)	5.8 ± 7.5	2.3 ± 3.0	0.041
Age at the detection of urinary protein (years)	4.0 ± 3.2	6.3 ± 4.8	0.036
Urinary protein/creatinine ratio (g/g Cr)[a]	1.7 ± 2.4	0.78 ± 1.0	0.027
Estimated GFR (ml/min per 1.73m^2)	111.2 ± 31.6	122.9 ± 20.6	0.43
Number of patients developing ESRD	5	1	0.66
Hearing loss (%)[b]	54.3	0	0.0002
Ocular abnormality (%)[c]	15.6	6.7	0.65
No medication	3	4	
ACEI/ARB only	29	11	
CyA only	0	0	
ACEI/ARB + CyA	5	0	

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CyA, cyclosporine A; ESRD, end-stage renal disease; GFR, glomerular filtration rate; α5(IV), type IV collagen α5.
[a]Values for patients with ESRD were excluded from this analysis.
[b]Hearing test was not conducted in one patient in the positive group.
[c]Ophthalmologic exams were not conducted in five patients in the positive group.

could be because atypical cases require genetic diagnosis, and doctors may tend to request mutational analysis sooner in these patients than in typical cases. The shorter duration of follow-up in positive patients could help to explain the smaller number of patients developing ESRD.

Table 2 | Mutation types in α5(IV)-negative and α5(IV)-positive patients

Genotype	α5(IV)-Negative group (n = 37)	α5(IV)-Positive group (n = 15)	Total
Missense	11	9	20
Nonsense	5	0	5
Insertion	7 (0)	0 (0)	7
Deletion	6 (0)	3 (3)	9
Splice site	8 (3)	1 (1)	9
Somatic mosaicism	0	2[a]	2
Total	37	15	52
Truncating mutation	23	0	
	62%	0%	

Abbreviation: α5(IV), type IV collagen α5.
Number of patients with in-frame mutation are shown in parenthesis.
[a]Both patients showed α5(IV) expression with mosaic pattern.

Five (13.5%) patients developed ESRD in the negative group, compared with one (6.7%) in the positive group. Three patients in the negative group and four in the positive group received no medications. Twenty-nine in the negative group and 11 in the positive group received angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers, whereas five in the negative group received angiotensin-converting enzyme inhibitor/angiotensin receptor blocker and cyclosporin treatment. There were no significant differences in medications between the two groups ($P = 0.091$).

Genotypes

All mutations detected in the positive and negative groups are shown in Supplementary Tables 1 and 2 online, respectively. Regarding *COL4A5* genotype, 23 (62%) of the 37 patients in the negative group had truncating mutations, including nonsense ($n = 5$), insertion ($n = 7$), deletion ($n = 6$), and splice-site mutations ($n = 5$). All these insertion, deletion, and splice-site mutations led to out-of-frame mutations. In contrast, no patients in the positive group had truncating mutations. Mutations in the positive group included missense ($n = 9$), large in-frame deletion ($n = 1$, exons 2–8, 384-bp deletion), small in-frame deletions ($n = 2$, 9-bp and 36-bp deletions), splice-site mutation ($n = 1$, leading to exon 9, 81-bp skipping), and somatic mosaic mutations ($n = 2$; Table 2). Twenty (38%) patients in this study had missense mutations in *COL4A5*; 11 in the negative and 9 in the positive groups. All the missense mutations in this study resulted in glycine substitutions. The positions of the missense mutations are shown in Figure 2. Receiver operating characteristic curve analysis of patients with missense mutations showed a cutoff value at exon 25 in both groups (sensitivity 0.778, specificity 0.8, Figure 2). Seven patients in the positive group and two in the negative group had missense mutations in the exons between 1 and 25, whereas two and nine patients, respectively, had mutations between exons 26 and 51 ($P = 0.02$). Missense mutations in the