厚生労働科学研究費補助金

難治性疾患克服研究事業

孔脳症の遺伝的要因の解明

平成２３～２４年度　総合研究報告書

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研究要旨

孔脳症は、大脳半球内に脳室との交通を有する嚢胞または空洞がみられる先天異常で、脳性麻痺の重要な原因となっている。これまで、IV 型コラーゲン α1 鎖（*COL4A1* 遺伝子）の異常が一部の家系例で報告され、脳血管の構造異常の関与が示唆されていたが、大多数の弧発例における遺伝学的検討・報告は皆無であった。本研究班は、α1 鎖とヘテロトリマーを形成する IV 型コラーゲン α2 鎖（*COL4A2* 遺伝子）の異常が、孔脳症の原因となっていることを世界に先駆けて報告した（Am J Hum Genet, 2012）。更に、孔脳症の 16%（10/61）に、血管基底膜に発現する IV 型コラーゲン α1 鎖（*COL4A1* 遺伝子）の異常を同定し、孔脳症には血管脆弱性という遺伝的要因が大きく関与していることを明らかにした（Ann Neurol, 2012）。また、*COL4A1* 変異は、脳での異常（孔脳症・裂脳症）以外にも、目や筋肉の異常および溶血性貧血など幅広い表現型を引き起こすことが明らかとなった。また、宮城県における検討で、孔脳症のおおよその発生率は出生 10 万対 13.5　(95%CI 6.1-20.9)人と推定され、孔脳症は諸外国に比し本邦での発生率が高いことが示唆された。

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A. 研究目的

孔脳症(porencephaly)は、大脳半球内に脳室との交通を有する嚢胞または空洞がみられる先天異常で、片側の側脳室体部に隣接する脳実質に観察されることが多い。脳性麻痺、特に片麻痺の重要な原因である。胎生期における梗塞や出血といった脳循環障害により発生すると推測されているが、その原因の多くは不明である。これまで、IV 型コラーゲン α1 鎖（*COL4A1* 遺伝子）の異常が一部の家系例で報告されているのみで、弧発例における *COL4A1* を含む遺伝学的検討の報告は皆無であった。本研究では、*COL4A1* 遺伝子とその機能的関連遺伝子の変異解析と並行して、全ゲノム解析用高密度オリゴ DNA マイクロアレイを用いて発症に関与する染色体構造異常を網羅的に検索することにより、孔脳症の遺伝的要因を明らかにすることを目的とする。

B. 研究方法

1) 症例の集積と DNA の抽出

小児神経学会の共同研究支援委員会に申請を行い、宮城県拓桃医療療育センター、山形大学、神奈川県立こども医療センターを中心に、全国の療育センターと協力して症例の集積を行った。

2) *COL4A1* および機能的関連遺伝子（*COL4A2*）の変異解析

COL4A1 および機能的関連遺伝子（*COL4A2*）の変異解析を、High resolution Melt(HRM)法を用いて行った。

3) 染色体構造異常の検出および候補遺伝

子スクリーニング

高密度オリゴ DNA マイクロアレイを用いて、*COL4A1/COL4A2* 遺伝子異常が同定されない症例に対して全ゲノム微細構造異常解析を行い、コピー数異常領域(CNV)を検出した。マイクロアレイで認められた異常については、蛍光 *in situ* hybridization(FISH)法や定量 PCR 法を用いて確認した後、御両親の検体を依頼し、その異常が両親由来かどうかを検査した。健常両親で認められない異常を患者が有していた場合(*de novo* 変異)には、染色体異常が原因となって発症していると考え、異常領域に位置する新規責任候補遺伝子について HRM 法による変異スクリーニングを行う。

4) 責任遺伝子型と孔脳症の臨床病型の比較検討

責任遺伝子の遺伝子型と孔脳症の臨床所見との詳細な比較検討を行い、特定の遺伝子変異による臨床病型を確立する。

(倫理面への配慮)

本研究計画は、三省のヒトゲノム解析研究に関する共通指針(2001 年・2004 年および 2005 年改訂)を遵守して組織された横浜市立大学医学部倫理委員会において、本研究の申請・承認を得、承認された研究計画を遵守し研究を遂行している。個人情報保護法に十分留意しながら研究を施行している。

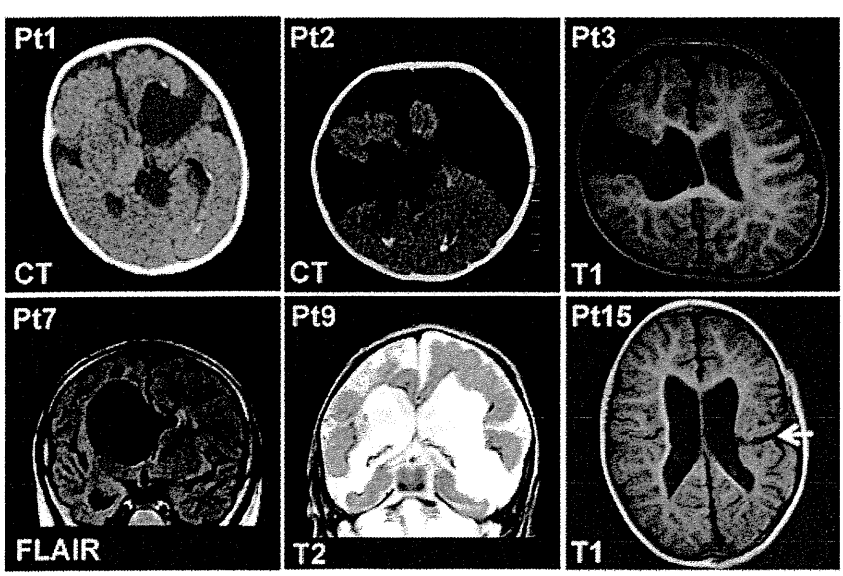
C. 研究結果

1) 症例の集積と DNA の抽出

これまでに孔脳症・裂脳症および周産期脳血管障害が疑われる 160 症例が集積した。集積した検体は詳細な臨床情報が得られる質の高い貴重な研究リソースである。また、宮城県における検討で、孔脳症のおおよその発生率は出生 10 万対 13.5 (95%CI 6.1-20.9)人と推定され、孔脳症は諸外国に比し本邦での発生率が高いことが示唆された。

2) *COL4A1* 遺伝子および *COL4A2* 遺伝子の変異解析

COL4A1 遺伝子および *COL4A2* 遺伝子について HRM 法により、現在までに孔脳症 61 症例・裂脳症 10 症例の変異解析を行った。*COL4A1* 遺伝子変異は孔脳症 61 症例中 10 症例(16%)に認められた。また、孔脳症と同様に、脳循環障害により発生する可能性が示唆されていた裂脳症において、10 症例中 5 例で *COL4A1* 変異を認めた (図 1)。



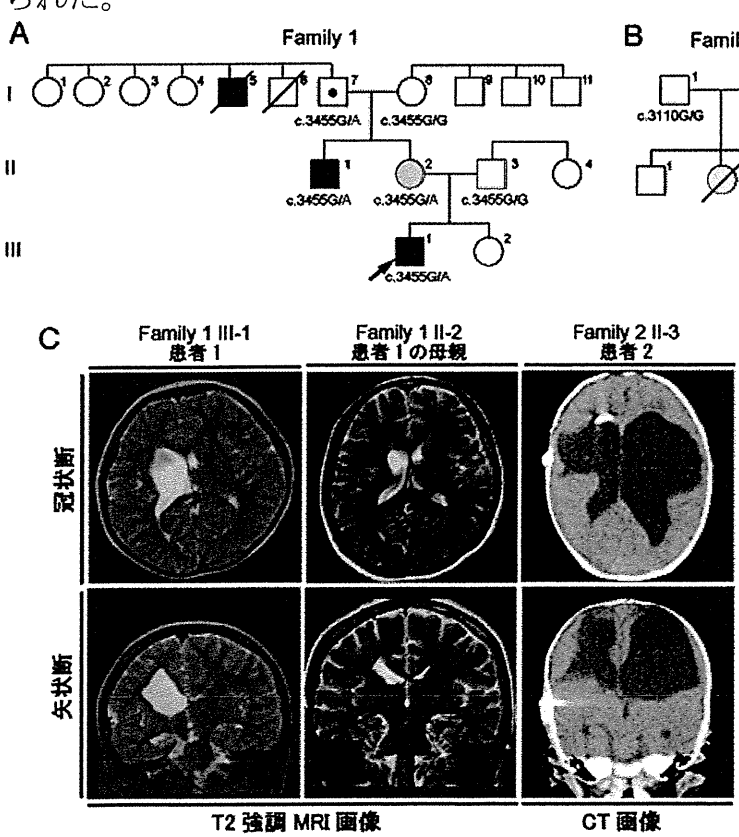
＜図 1＞

COL4A1 変異を有する患者の脳画像。患者 1 (Pt1) および患者 2 の CT 画像では、側脳室周囲の石灰化を認め、TORCH 症候群が疑われた。患者 3, 9, 15 (矢印) はそれぞれ裂脳症を呈している。患者 7 では片側の孔脳症を認めた。

また、*COL4A2* 遺伝子変異は 2 症例で認められた(図 2)。2 名のうちの 1 名は胎児期に脳出血を認めた孤発例であり、変異は de novo であった。もう一方は家族例であり、孔脳症の患児、左上肢の軽微な単麻痺を呈する母親、先天性の片麻痺を呈する母方の伯父、明らかな臨床所見を認めない母方祖父に変異を認めた。孔脳症患者で、*COL4A1/COL4A2* 遺伝子異常が高率に認められるという成果は非常に画期的であり、孔脳症における遺伝子診断の重要性が明らかとなった。

＜図 **2**＞

(A) 患者 1 および(B)患者 2 の家系図。黒塗りは先天性片麻痺を有することを示し、灰色は画像上異常が認められたことを表す。患児を矢印で示す。変異塩基を赤で示している。(C) 患者 1 とその母親の T2 強調 MRI 横断像（左、真ん中）と患者 2 の CT 画像（右）。右側脳室の拡大（左、真ん中）と両側脳室の拡大（右）が認められた。



3) 染色体構造異常の検出および候補遺伝子スクリーニング

29 症例でマイクロアレイ解析および定量 PCR による検証が終了し、遺伝子を含む新規 CNV を複数個検出している。遺伝子のエキソンを含む欠失も複数含まれており、現在、両親検体を依頼して、得られた新規 CNV が *de novo* の変化かどうかの確認を急いでいる。しかし、現在までに、両親検体が得られたケースでは、新規 CNV は健常のご両親由来であり、孔脳症と関連のある CNV の特定には至っていない。

4) 責任遺伝子型と孔脳症の臨床病型の比較検討

COL4A2 変異の 1 例は家族例であり、孔脳症の患児、左上肢の軽微な単麻痺を呈する母親、先天性の片麻痺を呈する母方の伯父、明らかな臨床所見を認めない母方祖父に変異を認めた。頭部 MRI 画像では片側性あるいは両側性の孔脳症が認められ、その程度も様々であった。このことから、*COL4A2* 変異は片側性から両側性まで、また胎児期の脳出血による脳性麻痺から左上肢の軽微な単麻痺や無症候性のキャリアーまで、幅広い表現型を引き起こすと考えられた。*COL4A1* 変異 15 症例の詳細な臨床所見の検討により、1) 孔脳症のみならず、より重症の裂脳症も引き起こす、2) 石灰化を伴うような、TORCH 症候群を疑う症例においても *COL4A1* 変異が関与、3) 既報の目や筋肉の異常のみならず、溶血性貧血も *COL4A1* 変異と関連、といっ

た新たな所見も得られている（表 1）。

＜表 1＞

症例	画像所見	目	筋障害、CK 高値	溶血性貧血	その他
1	両側孔脳症、石灰化	-	-	-	
2	両側裂脳症、石灰化	-	-	-	
3	片側裂脳症、石灰化	-	-	-	
4	両側孔脳症	-	-	-	局所性皮質形成異常
5	両側裂脳症、石灰化	視神経低形成	あり	あり	
6	片側孔脳症	-	あり	-	
7	片側孔脳症	-	あり		
8	片側孔脳症	-			
9	両側孔脳症、石灰化	小眼球症、白内障	-	あり	
10	両側孔脳症	-	-	-	
11	片側孔脳症	白内障	-	-	
12	両側孔脳症と片側裂脳症石灰化	白内障	-	あり	血尿
13	片側孔脳症	-	あり	-	
14	片側孔脳症	-	あり	あり	血尿
15	片側裂脳症	-	あり	あり	

D. 考察

COL4A1 遺伝子は IV 型コラーゲンα1 鎖をコードし、IV 型コラーゲンα2 鎖とヘテロトリマー（α1α1α2）を形成して脈管構造の基底膜に発現している。本研究班は、孔脳症・裂脳症患者 71 例中、実に 15 例（21%）において *COL4A1* 変異を同定した。*COL4A1* 変異に比べて数が少ないながらも、*COL4A2* 変異が孔脳症の原因となっていることもすでに明らかにしており、本研究班によって、孔脳症・裂脳症の遺伝要因として IV 型コラーゲンの異常が深く関与していることが明らかとなった。本研究は、これまで遺伝子異常が想定されていなかった病気に、遺伝子異常が関与していることを明らかにした。この成果は、医療現場での遺伝子診断を推進し、患児あるいは無症候性キャリアーの医療管理に重要な情報を与えるものである。

本研究班は、平成 22 年 7 月 21 日にスタート以降、短時間で高い実績を示せていると考える。これは小児神経の専門家と遺伝医学の専門家の強力なチーム構成によるものと考えている。また、小児神経学会の共同研究支援委員会へ共同研究の申請を行ったこと、これらの研究成果が発表されたことにより、解析検体数は飛躍的に増加している。

孔脳症は、脳性麻痺、特に片麻痺の原因となっており、生活面での長期にわたる支障をきたす疾患である。孔脳症の遺伝子診断や遺伝カウンセリングといった医療現場および厚生労働行政への貢献は、本研究により十分に実現可能と思われる。今後も努力を継続していきたい。

E. 結論

孔脳症・裂脳症の約 2 割に、*COL4A1* あるいは *COL4A2* 変異が関与していることを明らかにした。また、*COL4A1* 変異は、脳での異常（孔脳症・裂脳症）以外にも、目や筋肉の異常および溶血性貧血など幅広い表現型を引き起こすことが明らかとなった。

F. 健康危険情報

特になし

G. 研究発表

1. 論文発表

Tsurusaki Y, Kosho T, Hatasaki K, Narumi Y, Wakui K, Fukushima Y, Doi H, Saitsu H, Miyake N, Matsumoto N. Exome sequencing in a family with an X-linked lethal malformation syndrome: clinical consequences of hemizygous truncating OFD1 mutations in male patients. Clin Genet. Feb;83(2):135-44, 2013

Miyake N, Yano S, Sakai C, Hatakeyama H, Matsushima Y, Shiina M, Watanabe Y, Bartley J, Abdenur JE, Wang RY, Chang R, Tsurusaki Y, Doi H, Nakashima M, Saitsu H, Ogata K, Goto YI, Matsumoto N. Mitochondrial Complex III Deficiency Caused by a Homozygous UQCRC2 Mutation Presenting with Neonatal-Onset Recurrent Metabolic Decompensation. Hum Mutat. 2012 Dec 19. doi: 10.1002/humu.22257.

Yoneda Y, Haginoya K, Kato M, Osaka H, Yokochi K, Arai H, Kakita A, Yamamoto T, Otsuki Y, Shimizu S, Wada T, Koyama N, Mino Y, Kondo N, Takahashi S, Hirabayashi S, Takanashi J, Okumura A, Kumagai T, Hirai H, Nabetani M, Saitoh S, Hattori A, Yamasaki M, Kumakura A, Sugo Y, Nishiyama K, Miyatake S, Tsurusaki Y, Doi H, Miyake N, Matsumoto N, Saitsu H (corresponding author). Phenotypic spectrum of *COL4A1* mutations: porencephaly to schizencephaly. Ann Neurol. Jan;73(1):48-57, 2013

Miyake N, Mizuno S, Okamoto N, Ohashi H, Shiina M, Ogata K, Tsurusaki Y, Nakashima M, Saitsu H, Niikawa N, Matsumoto N. KDM6A point mutations cause Kabuki syndrome. Hum Mutat. Jan;34(1):108-10, 2013

Tsurusaki Y, Kobayashi Y, Hisano M, Ito S, Doi H, Nakashima M, Saitsu H, Matsumoto N, Miyake N. The diagnostic utility of exome sequencing in Joubert syndrome and related disorders. J Hum Genet. 2012 Oct 4. doi: 10.1038/jhg.2012.117.

Miyatake S, Touho H, Miyake N, Ohba C, Doi H, Saitsu H, Taguri M, Morita S, Matsumoto N. Sibling cases of moyamoya disease having homozygous and heterozygous c.14576G>A variant in RNF213 showed varying clinical course and severity. J Hum Genet. Dec;57(12):804-6, 2012

Saitsu H, Kato M, Koide A, Goto T, Fujita T, Nishiyama K, Tsurusaki Y, Doi H, Miyake N, Hayasaka K, Matsumoto N. Whole exome sequencing identifies KCNQ2 mutations in Ohtahara syndrome. Ann Neurol. Aug;72(2):298-300, 2012

Miyatake S, Miyake N, Doi H, Saitsu H, Ogata K, Kawai M, Matsumoto N. A Novel SACS Mutation in an Atypical Case with Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS). Intern Med. 51(16):2221-6, 2012

Tsurusaki Y, Saitoh S, Tomizawa K, Sudo A, Asahina N, Shiraishi H, Ito JI, Tanaka H, Doi H, Saitsu H, Miyake N, Matsumoto N. A DYNC1H1 mutation causes a dominant spinal muscular atrophy with lower extremity predominance. Neurogenetics. Nov;13(4):327-32, 2012

Terao Y, Saitsu H, Segawa M, Kondo Y, Sakamoto K, Matsumoto N, Tsuji S, Nomura Y. Diffuse central hypomyelination presenting as 4H syndrome caused by compound heterozygous mutations in POLR3A encoding the catalytic subunit of polymerase III. J Neurol Sci. Sep 15;320(1-2):102-5, 2012

Saitsu H, Kato M, Osaka H, Moriyama N, Horita H, Nishiyama K, Yoneda Y, Kondo Y, Tsurusaki Y, Doi H, Miyake N, Hayasaka K, Matsumoto N. CASK aberrations in male patients with Ohtahara syndrome and cerebellar hypoplasia. Epilepsia. Aug;53(8):1441-1449, 2012

Nonoda Y, Saito Y, Nagai S, Sasaki M, Iwasaki T, Matsumoto N, Ishii M, Saitsu H. Progressive diffuse brain atrophy in West syndrome with marked hypomyelination due to SPTAN1 gene mutation. Brain Dev. 2012 May 31.

Osaka H, Takagi A, Tsuyusaki Y, Wada T, Iai M, Yamashita S, Shimbo H, Saitsu H, Salomons GS, Jakobs C, Aida N, Toshihiro S, Kuhara T, Matsumoto N. Contiguous deletion of SLC6A8 and BAP31 in a patient with severe dystonia and sensorineural deafness. Mol Genet Metab. 106 (1):43-47, 2012

Writzl K, Primec ZR, Stražišar BG, Osredkar D, Pečarič-Meglič N, Kranjc BS, Nishiyama K, Matsumoto N, Saitsu H. Early onset West syndrome with severe hypomyelination and coloboma-like optic discs in a girl with *SPTAN1* mutation. Epilepsia. Jun;53(6):e106-10, 2012

Tsurusaki Y, Okamoto N, Ohashi H, Kosho T, Imai Y, Hibi-Ko Y, Kaname T, Naritomi K, Kawame H, Wakui K, Fukushima Y, Homma T, Kato M, Hiraki Y, Yamagata T, Yano S, Mizuno S, Sakazume S, Ishii T, Nagai T,

Shiina M, Ogata K, Ohta T, Niikawa N, Miyatake S, Okada I, Mizuguchi T, Doi H, Saitsu H, Miyake N, Matsumoto N. Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome. Nat Genet. Mar 18;44(4):376-8, 2012

Miyatake S, Miyake N, Touho H, Nishimura-Tadaki A, Kondo Y, Okada I, Tsurusaki Y, Doi H, Sakai H, Saitsu H, Shimojima K, Yamamoto T, Higurashi M, Kawahara N, Kawauchi H, Nagasaka K, Okamoto N, Mori T, Koyano S, Kuroiwa Y, Taguri M, Morita S, Matsubara Y, Kure S, Matsumoto N. Homozygous c.14576G>A variant of RNF213 predicts early-onset and severe form of moyamoya disease. Neurology. Mar 13;78(11):803-10, 2012

Yoneda Y, Saitsu H, Touyama M, Makita Y, Miyamoto A, Hamada K, Kurotaki N, Tomita H, Nishiyama K, Tsurusaki Y, Doi H, Miyake N, Ogata K, Naritomi K, Matsumoto N. Missense mutations in the DNA-binding/dimerization domain of NFIX cause Sotos-like features. J Hum Genet. 2012 Feb 2. doi: 10.1038/jhg.2012.7.

Kondo Y, Saitsu H, Miyamoto T, Nishiyama K, Tsurusaki Y, Doi H, Miyake N, Ryoo NK, Kim JH, Yu YS, Matsumoto N. A family of oculofaciocardiodental syndrome (OFCD) with a novel BCOR mutation and genomic rearrangements involving NHS. J Hum Genet. 2012 Feb 2. doi: 10.1038/jhg.2012.4.

Hamdan F*, Saitsu H* (Co-first Author), Nishiyama K, Gauthier J, Dobrzeniecka S, Spiegelman D, Lacaille JC, Décarie JC, Matsumoto N, Rouleau GA, and Michaud JL. Identification of a novel in-frame de novo mutation in SPTAN1 in intellectual disability and pontocerebellar atrophy. Eur J Hum Genet 2012 Jan 18. doi: 10.1038/ejhg.2011.271.

Yoneda Y, Haginoya K, Arai H, Yamaoka S, Tsurusaki Y, Doi H, Miyake N, Yokochi K, Osaka H, Kato M, Matsumoto N, and Saitsu H (corresponding author). De novo and inherited mutations in COL4A2, encoding the type IV collagen α2 chain cause porencephaly. Am J Hum Genet 2012 Jan 13;90(1):86-90.

Saitsu H, Osaka H, Sugiyama S, Kurosawa K, Mizuguchi T, Nishiyama K, Nishimura A, Tsurusaki Y, Doi H, Miyake N, Harada N, Kato M, Matsumoto N. Early infantile epileptic encephalopathy associated with the disrupted gene encoding Slit-Robo Rho GTPase activating protein 2 (SRGAP2). Am J Med Genet A. 2011 Nov 21 online.

Saitsu H, Osaka H, Sasaki M, Takanashi J, Hamada K, Yamashita A, Shibayama H, Shiina M, Kondo Y, Nishiyama K, Tsurusaki Y, Miyake N, Doi H, Ogata K, Inoue K, Matsumoto N. Mutations in POLR3A and POLR3B Encoding RNA Polymerase III Subunits Cause an Autosomal-Recessive Hypomyelinating Leukoencephalopathy. Am J Hum Genet. 2011 89(5):644-51.

Sakai H, Suzuki S, Mizuguchi T, Imoto K, Yamashita Y, Doi H, Kikuchi M, Tsurusaki Y, Saitsu H, Miyake N, Masuda M, Matsumoto N. Rapid detection of gene mutations responsible for non-syndromic aortic aneurysm and dissection using two different methods: resequencing microarray technology and next-generation sequencing. Hum Genet. 2011 Oct 15 online.

Saitsu H, Igarashi N, Kato M, Okada I, Kosho T, Shimokawa O, Sasaki Y, Nishiyama K, Tsurusaki Y, Doi H, Miyake N, Harada N, Hayasaka K, Matsumoto N. De novo 5q14.3 translocation 121.5-kb upstream of MEF2C in a patient with severe intellectual disability and early-onset epileptic encephalopathy. Am J Med Genet A. 2011 155A(11):2879-84.

Doi H, Yoshida K, Yasuda T, Fukuda M, Fukuda Y, Morita H, Ikeda S, Kato R, Tsurusaki Y, Miyake N, Saitsu H, Sakai H, Miyatake S, Shiina M, Nukina N, Koyano S, Tsuji S, Kuroiwa Y, Matsumoto N. Exome sequencing reveals a homozygous SYT14 mutation in adult-onset, autosomal-recessive spinocerebellar ataxia with psychomotor retardation. Am J Hum Genet. 2011 89(2):320-7.

Miyake N, Yamashita S, Kurosawa K, Miyatake S, Tsurusaki Y, Doi H, Saitsu H, Matsumoto N. A novel homozygous mutation of DARS2 may cause a severe LBSL variant. Clin Genet. 2011 80(3):293-6.

Saitsu H, Osaka H, Nishiyama K, Tsurusaki Y, Doi H, Miyake N, Matsumoto N. A girl with

early-onset epileptic encephalopathy associated with microdeletion involving CDKL5. Brain Dev. 2011 Jul 27 Online.

Tsurusaki Y, Okamoto N, Suzuki Y, Doi H, Saitsu H, Miyake N, Matsumoto N. Exome sequencing of two patients in a family with atypical X-linked leukodystrophy. Clin Genet. 2011 80(2):161-6.

Saitsu H, Kato M, Shimono M, Senju A, Tanabe S, Kimura T, Nishiyama K, Yoneda Y, Kondo Y, Tsurusaki Y, Doi H, Miyake N, Hayasaka K, Matsumoto N. Association of genomic deletions in the STXBP1 gene with Ohtahara syndrome. Clinical Genetics 2012 81(4):399-402, 2012

Tsurusaki Y, Okamoto N, Suzuki Y, Doi H, Saitsu H, Miyake N, Matsumoto N. Exome sequencing of two patients in a family with atypical X-linked leukodystrophy. Clinical Genetics, 2011 Jun 3.

Saitsu H, Matsumoto N. De novo mutations in epilepsy. Dev Med Child Neurol. 2011 53(9):806-7.

Tsurusaki Y, Osaka H, Hamanoue H, Shimbo H, Tsuji M, Doi H, Saitsu H, Matsumoto N, Miyake N. Rapid detection of a mutation causing X-linked leucoencephalopathy by exome sequencing. J Med Genet. 2011 Sep;48(9):606-9.

Tadaki H, Saitsu H, Kanegane H, Miyake N, Imagawa T, Kikuchi M, Hara R, Kaneko U, Kishi T, Miyamae T, Nishimura A, Doi H, Tsurusaki Y, Sakai H, Yokota S, Matsumoto N. Exonic deletion of CASP10 in a patient presenting with systemic juvenile idiopathic arthritis, but not with autoimmune lymphoproliferative syndrome type IIa. Int J Immunogenet. 2011 38(4):287-93.

Tadaki H, Saitsu H, Nishimura-Tadaki A, magawa T, Kikuchi M, Hara R, Kaneko U, Kishi T, Miyamae T, Miyake N, Doi H, Tsurusaki Y, Sakai H, Yokota S, Matsumoto N. De novo 19q13.42 duplications involving NLRP gene cluster in a patient with systemic-onset juvenile idiopathic arthritis. J Hum Genet. 2011 May;56(5):343-7.

学会発表

H. Saitsu, M. Kato, H. Osaka, N. Moriyama, H. Horita, K. Nishiyama, T. Yoshinori, H. Doi, N. Miyake, K. Hayasaka, N. Matsumoto. CASK aberrations in males with Ohtahara syndrome and cerebellar hypoplasia. 62th Annual Meeting of The American Society of Human Genetics, San francisco, Nov 9 2012, USA

才津　浩智、加藤　光広、小坂　仁、森山　伸子、堀田　秀樹、西山　精視、鶴崎　美徳、三宅　紀子、早坂　清、松本　直通. CASK の null 変異は小脳低形成を伴う大田原症候群をひきおこす. 日本人類遺伝学会第 57 回大会 2012 年 10 月 25 日、東京

才津　浩智、米田　祐梨子、萩野谷和裕 荒井洋、山岡繁夫、松本直通. De novo and inherited mutations in COL4A2, encoding type IV collagen α2 chain cause porencephaly. 第 52 回日本先天異常学会学術集会,　2012 年 7 月 7 日 東京

才津　浩智. 発達期の脳神経疾患における遺伝子解析～次世代シークエンサーの活用～ 第 5 回がん・ゲノム重点セミナー　2012 年 6 月 29 日長崎大学

才津浩智.　シンポジウム「染色体異常をめぐって－ダウン症の病態解明－」　ダウン症候群の分子遺伝学的解析. 第 51 回日本先天異常学会学術集会,　2011 年 7 月 23 日、東京

H. 知的所有権の出願・取得状況(予定を含む)

1) 特許取得
無し

2) 実用新案登録
無し

3) その他
特願 2011-226488
び慢性大脳白質形成不全症患者又は保因者の検出方法

特願 2011-247457
孔脳症又は脳出血のリスクを予測する方法

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tsurusaki Y. et al., Saitsu H. et al.	Exome sequencing in a family with an X-linked lethal malformation syndrome: clinical consequences of hemizygous truncating OFD1 mutations in male patients.	Clin Genet	83(2)	135-144	2013
Miyake N. et al., Saitsu H. et al.	Mitochondrial Complex III Deficiency Caused by a Homozygous UQCRC2 Mutation Presenting with Neonatal-Onset Recurrent Metabolic Decompensation.	Hum Mutat	印刷中	未確定	2013
Yoneda Y., Haginoya K., Kato M., Osaka H., et al., Saitsu H.	Phenotypic spectrum of COL4A1 mutations: porencephaly to schizencephaly.	Ann Neurol	73(1)	48-57	2013
Miyake N. et al., Saitsu H. et al.	KDM6A point mutations cause Kabuki syndrome.	Hum Mutat	34(1)	108-110	2013
Tsurusaki Y. et al., Saitsu H. et al.	The diagnostic utility of exome sequencing in Joubert syndrome and related disorders.	J Hum Genet	印刷中	未確定	2013
Miyatake S. et al., Saitsu H. et al.	Sibling cases of moyamoya disease having homozygous and heterozygous c.14576G>A variant in RNF213 showed varying clinical course and severity.	J Hum Genet	57(12)	804-806	2012
Saitsu H., Kato M., et al.,	Whole exome sequencing identifies KCNQ2 mutations in Ohtahara syndrome.	Ann Neurol	72(2)	298-300	2012
Miyatake S. et al., Saitsu H. et al.	A Novel SACS Mutation in an Atypical Case with Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS).	Intern Med.	51(16)	2221-6	2012

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tsurusaki Y. et al., Saitsu H. et al.	A DYNC1H1 mutation causes a dominant spinal muscular atrophy with lower extremity predominance.	Neurogene-tics.	13(4)	327-332	2012
Terao Y., Saitsu H. et al.	Diffuse central hypomyelination presenting as 4H syndrome caused by compound heterozygous mutations in POLR3A encoding the catalytic subunit of polymerase III.	J Neurol Sci.	320(1-2)	102-105	2012
Saitsu H. Kato M. Osaka H. et al.	CASK aberrations in male patients with Ohtahara syndrome and cerebellar hypoplasia.	Epilepsia	53(8)	1441 -1449	2012
Nonoda Y. et al, Saitsu H.	Progressive diffuse brain atrophy in West syndrome with marked hypomyelination due to SPTAN1 gene mutation.	Brain Dev	印刷中	未確定	2013
Osaka H. et al, Saitsu H. et al.	Contiguous deletion of SLC6A8 and BAP31 in a patient with severe dystonia and sensorineural deafness.	Mol Genet Metab.	106(1)	43-47	2012
Writzl K. et al., Saitsu H.	Early onset West syndrome with severe hypomyelination and coloboma-like optic discs in a girl with SPTAN1 mutation.	Epilepsia	53(6)	106-110	2012
Tsurusaki Y. et al., Kato M. et al., Saitsu H. et al.	Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome.	Nat Genet	44(4)	376-378	2012
Miyatake S. et al., Saitsu H. et al.	Homozygous c.14576G>A variant of RNF213 predicts early-onset and severe form of moyamoya disease.	Neurology	78(11)	803-810	2012
Iwasaki M., et al., Haginoya K. et al.	Parental satisfaction and seizure outcome after corpus callosotomy in patients with infantile or early childhood onset epilepsy.	Seizure	印刷中	未確定	2013
Shiraishi H. Haginoya K. et al.	Magnetoencephalography localizing spike sources of atypical benign partial Epilepsy.	Brain Dev	印刷中	未確定	2013

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Wakusawa K. et al., Haginoya K.	A girl with Cardio-facio-cutaneus syndrome complicated with status epileptics and acute encephalopathy.	Brain Dev	印刷中	未確定	2013
Kobayasi S. et al, Haginoya K.	A case of atypical benign partial epilepsy with action myoclonus.	Seizure	印刷中	未確定	2013
Abe Y. et al, Haginoya K.	Bilateral Periventricular Nodular Heterotopia with Megalencephaly: A Case Report.	J Child Neurol	印刷中	未確定	2013
Kakisaka Y. Haginoya K., et al	Additional Evidence That the Ryanodine Receptor Gene (RYR1) Causes Malignant Hyperthermia and Severe Skeletal Malformations.	Am J Med Genet	161	234-235	2013
Numata Y. et al., Haginoya K.	Brain magnetic resonance imaging, and motor and intellectual functioning in 86 patients born at term with spastic diplegia.	Dev Med Child Neurol.	55	167-172	2013
Yokoyama H, et al., Haginoya K. et al.	Ketotifen overdose in infancy associated with development of epilepsy and mild mental retardation.	Pediatr Int.	54(6)	963	2012
Zhao YJ, et al. Haginoya K.	Protective effects of glutamine in a rat model of endotoxemia.	Mol Med Report.	6	739-744	2012
Iwasaki M, et al. Haginoya K. et al.	Complete remission of seizures after corpus callosotomy.	J Neurosurg Pediatr.	10	7-13	2012
Watanabe S, et al. Haginoya K. et al.	Schinzel–Giedion syndrome: A further cause of early myoclonic encephalopathy and vacuolating myelinopathy.	Brain Dev	34	151-155	2012
Kakisaka Y, et al. Haginoya K.	Abdominal migraine revi ewed from both central and peripheral aspects.	World J Exp Med.	2	75-77	2012
Tsuburaya R, et al. Haginoya K. et al.	Unusual ribbon-like periventricular heterotopia with congenital cataract in a Japanese girl.	Am J Med Genet	158A	674-677	2012

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Uematsu M. Haginoya K. et al.	Hypoperfusion in caudate nuclei in patients with brain–lung–thyroid syndrome.	J Neurol Sci.	315	77-81	2012
Nakamura K. Kato M. et al.	Congenital dysplastic microcephaly and hypoplasia of the brainstem and cerebellum with diffuse intracranial calcification.	J Child Neurol	27	218-221	2012
Tanigawa J. et al. Osaka K	Two Japanese patients with Leigh syndrome caused by novel SURF1 mutations.	Brain Dev	印刷中	未確定	2013
Koizume S. et al., Osaka H. et al.	HIF2α-Sp1 interaction mediates a deacetylation-dependent FVII-gene activation under hypoxic conditions in ovarian cancer cells.	Nucleic Acids Res.	印刷中	未確定	2013
Yu LH. et al., Osaka H. Inoue K.	Effect of curcumin in a mouse model of Pelizaeus-Merzbacher disease.	Mol Genet Metab	106(1)	108-114	2012
Tomiyasu M. et al., Osaka H.	Monitoring the brain metabolites of children with acuteencephalopathy caused by the H1N1 virus responsible for the 2009 influenzapandemic: a quantitative in vivo (1)H MR spectroscopy study.	Magn Reson Imaging.	30(10)	1527-1533	2012
Kouga T., et al. Osaka H.	A Child with Three Episodes of Reversible Splenial Lesion.	Neuropediatrics.	印刷中	未確定	2013
Yoshihara N. et al. Osaka H.	Idiopathic cranial polyneuropathy with unilateral IX and X and contralateral XI nerve palsy in a 4-year-old boy.	Pediatr Neurol.	47(3)	198-200	2012
Kimura-Ohba S, et al. Osaka H. et al.	A case of cerebral hypomyelination with spondylo-epi-metaphyseal dysplasia.	Am J Med Genet	161(1)	203-207	2013

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yoneda Y. et al., Haginoya K., Kato M., Osaka H. et al., Saitsu H.	De novo and inherited mutations in *COL4A2*, encoding the type IV collagen α2 chain cause porencephaly.	Am J Hum Genet	90(1)	86-90	2012
Yoneda Y., Saitsu H. et al.	Missense mutations in the DNA-binding/dimerization domain of NFIX cause Sotos-like features.	J Hum Genet	57(3)	207-211	2012
Kondo Y., Saitsu H. et al.	A family of oculofaciocardiodental syndrome (OFCD) with a novel BCOR mutation and genomic rearrangements involving NHS.	J Hum Genet	57(3)	197-201	2012
Hamdan F*, Saitsu H* (Co-first Author) et al.	Identification of a novel in-frame de novo mutation in *SPTAN1* in intellectual disability and pontocerebellar atrophy.	Eur J Hum Genet	20(7)	197-201	2012
Saitsu H., Osaka H., et al. Kato M. et al.	Early infantile epileptic encephalopathy associated with the disrupted gene encoding Slit-Robo Rho GTPase activating protein 2 (*SRGAP2*).	Am J Med Genet A	158A(1)	199-205	2012
Saitsu H., Osaka H. et al.	Mutations in POLR3A and POLR3B Encoding RNA Polymerase III Subunits Cause an Autosomal-Recessive Hypomyelinating Leukoencephalopathy.	Am J Hum Genet	89(5)	644-651	2011
Sakai H. et al., Saitsu H. et al.	Rapid detection of gene mutations responsible for non-syndromic aortic aneurysm and dissection using two different methods: resequencing microarray technology and next-generation sequencing.	Hum Genet	131(4)	591-599	2012
Saitsu H. et al. Kato M. et al.	De novo 5q14.3 translocation 121.5-kb upstream of MEF2C in a patient with severe intellectual disability and early-onset epileptic encephalopathy.	Am J Med Genet A	155A (11)	2879-84	2011

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Doi H. et al. Saitsu H. et al.	Exome sequencing reveals a homozygous SYT14 mutation in adult-onset, autosomal -recessive spinocerebellar ataxia with psychomotor retardation.	Am J Hum Genet	89(2)	320-327	2011
Miyake N. et al. Saitsu H, Matumoto N.	A novel homozygous mu tation of DARS2 may ca use a severe LBSL vari ant.	Clin Genet	80(3)	293-296	2011
Saitsu H. Osaka H. et al.	A girl with early-onset e pileptic encephalopathy associated with microdel etion involving CDKL5	Brain Dev	34(5)	364-367	2012
Tsurusaki Y. et al, Saitsu H. et al.	Exome sequencing of tw o patients in a family w ith atypical X-linked leu kodystrophy.	Clin Genet.	80(2)	161-166	2011
Saitsu H., Matsumoto N.	De novo mutations in ep ilepsy.	Dev Med Child Neurol.	53(9)	806-807	2011
Tsurusaki Y. Os aka H. et al., Sa itsu H. et al.	Rapid detection of a mu tation causing X-linked l eucoencephalopathy by e xome sequencing.	J Med Genet.	48(9)	606-609	2011
Tadaki H., Saits u H. et al.	Exonic deletion of CASP 10 in a patient presenti ng with systemic juvenil e idiopathic arthritis, bu t not with autoimmune lymphoproliferative synd rome type IIa.	Int J Immunogenet.	38(4)	287-293	2011
Tadaki H., Saits u H. et al.	De novo 19q13.42 duplic ations involving NLRP g ene cluster in a patient with systemic-onset juve nile idiopathic arthritis.	J Hum Genet	56(5)	343-347	2011
Tsuburaya R., et al., Haginoya K. et al.	Unusual ribbon-like peri ventricular heterotopia with congenital cataract in a Japanese girl.	Am J Med Genet A	158A(3)	674-677	2012
Uematsu M, Hag inoyaK. et al.,	Hypoperfusion in caudat e nuclei in patients with brain–lung–thyroid syn drome.	J Neurol Sci.	315(1-2)	77-81	2012

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Sakamoto O. et al., Haginoya K. et al.	Neonatal lactic acidosis with methylmalonic aciduria by novel mutations in the SUCLG1 gene.	Pediatr Int.	53	921-925	2011
Iwasaki M. et al., Haginoya K. et al.	Lateralization of interictal spikes after corpus callosotomy.	Clin Neurophysiol.	122(21)	21-27	2011
Hirose M, Haginoya K. et al. Kato M. et al.	Progressive atrophy of the cerebrum in 2 Japanese sisters with microcephaly with simplified gyri and enlarged extraaxial space.	Neuropediatrics.	42	163-166	2011
Watanabe S. et al., Haginoya K., et al	Schinzel–Giedion syndrome: A further cause of early myoclonic encephalopathy and vacuolating myelinopathy.	Brain Dev	34	151-155	2012
Kakisaka Y. et al., Haginoya K., et al	Somatotopic distribution of peri-rolandic spikes may predict prognosis in pediatric-onset epilepsy with sensorimotor seizures	Clin Neurophysiol.	122	869-873	2011
Komaki H, et al Kato M. et al.	Progressive atrophy of the cerebrum in 2 Japanese sisters with microcephaly with simplified gyri and enlarged extraaxial space.	Neuro-pediatrics	42	163-166	2011
Arai M, Osaka H.	Acute leukoencephalopathy possibly induced by phenytoin intoxication in an adult patient with methylenetetrahydrofolate reductase deficiency.	Epilepsia	52(7)	58-61	2011
Tsuji M, et al. Osaka H.	Acute encephalopathy in a patient with Dravet syndrome.	Neuro-pediatrics	42(2)	78-81	2011
Tsuyusaki Y, et al. Osaka H.	Paradoxical increase in seizure frequency with valproate in nonketotic hyperglycinemia.	Brain Dev	34(1)	72-75	2012
Tanoue K, et al. Osaka H.	Acute encephalopathy in two cases with severe congenital hydrocephalus.	Brain Dev	33(7)	616-619	2011

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Wada T, et al., Osaka H.	A simple screening method using ion chromatography for the diagnosis of cerebral creatine deficiency syndromes.	Amino Acids	43(2)	993-997	2012
Tomiyasu M, et al. Osaka H.	Acute hemicerebellitis in a pediatric patient: a case report of a serial MR spectroscopy study.	Acta Radiol.	53(2)	223-227	2012

ORIGINAL ARTICLE

Phenotypic Spectrum of *COL4A1* Mutations: Porencephaly to Schizencephaly

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Objective: Recently, *COL4A1* mutations have been reported in porencephaly and other cerebral vascular diseases, often associated with ocular, renal, and muscular features. In this study, we aimed to clarify the phenotypic spectrum and incidence of *COL4A1* mutations.

Methods: We screened for *COL4A1* mutations in 61 patients with porencephaly and 10 patients with schizencephaly, which may be similarly caused by disturbed vascular supply leading to cerebral degeneration, but can be distinguished depending on time of insult.

Results: *COL4A1* mutations were identified in 15 patients (21%, 10 mutations in porencephaly and 5 mutations in schizencephaly), who showed a variety of associated findings, including intracranial calcification, focal cortical dysplasia, pontocerebellar atrophy, ocular abnormalities, myopathy, elevated serum creatine kinase levels, and hemolytic anemia. Mutations include 10 missense, a nonsense, a frameshift, and 3 splice site mutations. Five mutations were confirmed as de novo events. One mutation was cosegregated with familial porencephaly, and 2

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mutations were inherited from asymptomatic parents. Aberrant splicing was demonstrated by reverse transcriptase polymerase chain reaction analyses in 2 patients with splice site mutations.
Interpretation: Our study first confirmed that *COL4A1* mutations are associated with schizencephaly and hemolytic anemia. Based on the finding that *COL4A1* mutations were frequent in patients with porencephaly and schizencephaly, genetic testing for *COL4A1* should be considered for children with these conditions.
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Type IV collagens are basement membrane proteins that are expressed in all tissues, including the vasculature. COL4A1 (α1 chain) and COL4A2 (α2 chain) are the most abundant type IV collagens, and form heterotrimers with a 2:1 stoichiometry (α1α1α2).[1] Mutations in *COL4A1* and *COL4A2* cause sporadic and hereditary porencephaly, a neurological disorder characterized by fluid-filled cysts in the brain that often cause hemiplegia or tetraplegia.[2–4] In addition, a variety of clinical phenotypes, including small vessel disease affecting the brain, eyes, and kidneys, are associated with *COL4A1* abnormality[5,6]: neonatal porencephaly and adult stroke,[7] sporadic extensive bilateral porencephaly resembling hydranencephaly,[8] periventricular leukomalacia with intracranial calcification,[9] HANAC (hereditary angiopathy with nephropathy, aneurysm, and muscle cramps) syndrome,[10,11] Axenfeld–Rieger anomaly with leukoencephalopathy, and adult stroke and intracerebral hemorrhage.[12–14] Notably, *COL4A1* mutations were present in 2 patients with muscle–eye–brain/Walker–Warburg syndrome (MEB/WWS), which is characterized by ocular dysgenesis, neuronal migration defects, and congenital muscular dystrophy, suggesting that *COL4A1* is also involved in normal cortical and muscular development in humans.[15] Consistent with this hypothesis, a mouse model of a heterozygous *COL4A1* mutation (*Col4a1*$^{+/\Delta ex40}$) showed ocular dysgenesis, cortical neuronal localization defects, and myopathy, along with cerebral hemorrhage and porencephaly.[2,15] The phenotypic spectrum of *COL4A1* mutations is expanding; however, the whole spectrum of systemic phenotypes and the incidence of *COL4A1* mutations associated with porencephaly has not been systemically examined.

In this study, we screened for *COL4A1* mutations in 61 patients with porencephaly and 10 patients with schizencephaly, which may be similarly caused by disturbed vascular supply leading to cerebral degeneration, but can be distinguished depending on time of insult.[2–4,16,17] *COL4A1* mutations were identified in 10 patients with porencephaly and 5 patients with schizencephaly, who showed a variety of associated findings, including intracranial calcification, focal cortical dysplasia (FCD), ocular abnormalities, pontocerebellar atrophy, myopathy, elevated serum creatine kinase levels, and hemolytic anemia. Our study demonstrated the importance of genetic testing for *COL4A1* in children with porencephaly or schizencephaly.

Patients and Methods

Patients

A total of 61 patients with porencephaly including a previous cohort with porencephaly,[4] and 10 patients with schizencephaly including a patient who also had porencephaly were analyzed for *COL4A1* mutations. Schizencephaly is defined as transmantle clefts bordered by polymicrogyria in adjacent cortex.[18] The clefts extended through the entire hemisphere, from the ependymal lining of the lateral ventricles to the pial covering of the cortex.[19] The clefts are further divided into those with closed lips and those with open lips. In the clefts with closed lips, the walls affix each other directly, obliterating the cerebrospinal fluid space within the cleft at that point.[20] *COL4A2* mutations were negative for these patients. Genomic DNA was isolated from blood leukocytes according to standard methods, and amplified using an illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Buckinghamshire, UK). The DNA of familial members of patient 6 was isolated from saliva samples using Oragene (DNA Genotek, Kanata, Ontario, Canada). Experimental protocols were approved by the committee for ethical issues at Yokohama City University School of Medicine. All patients were investigated in agreement with the requirements of Japanese regulations.

Mutation Analysis

Exons 1 to 52, covering the entire *COL4A1* coding region, were examined by high-resolution melting (HRM) curve analysis. Samples showing an aberrant melting curve pattern in the HRM analysis were sequenced. Polymerase chain reaction (PCR) primers and conditions are shown in Supplementary Table S1. All novel mutations were verified using original genomic DNA, and screened in 200 Japanese normal controls by HRM analysis. For the family showing de novo mutations, parentage was confirmed by microsatellite analysis, as previously described.[21] Biological parents were confirmed if >4 informative markers were compatible and other markers showed no discrepancy.

Reverse Transcriptase-PCR

Reverse transcriptase (RT)-PCR using total RNA extracted from lymphoblastoid cell lines (LCL) was performed essentially as previously described.[22] Briefly, total RNA was extracted using RNeasy Plus MiniKit (Qiagen, Tokyo, Japan) from LCL with or without 30μM cycloheximide (CHX; Sigma, Tokyo, Japan) incubation for 4 hours. Four micrograms total RNA was subjected to reverse transcription, and 2μl cDNA was used for PCR. Primer sequences are ex20-F (5′-CCCAAAAGGTTTCC CAGGACTACCA-3′) and ex22-R (5′-GTCCGGGCTGACAT TCCACAATTC-3′; for patient 4); and ex22-F (5′-GAATTC-CAGGGCAGCCAGGATTTAT-3′) and ex24-R (5′-CATCTCT GCCAGGCAAACCTCTGT-3′; for patient 7). DNA of each

PCR band was purified by QIAEXII Gel extraction kit (Qiagen; for patient 4) and E.Z.N.A. poly-Gel DNA Extraction kit (Omega Bio-Tek, Norcross, GA; for patient 7), respectively.

Results

Mutation and RT-PCR analysis

COL4A1 abnormalities were identified in 15 patients (Fig 1 and Table). Nine mutations occurred at highly conserved Gly residues in the Gly-X-Y repeat of the collagen triple helical domain. Interestingly, a missense mutation (c.4843G>A [p.Glu1615Lys]) at an evolutionary conserved amino acid and a nonsense mutation (c.4887C>A [p.Tyr1629X]) were found in the carboxy-terminal noncollagenous (NC1) domain. The other 4 mutations include a frameshift mutation (c.2931dupT [p.Gly978TrpfsX15]) and 3 splice site mutations (c.1121-2dupA, c.1382-1G>C, and c.1990+1G>A). None of these mutations was present in 200 Japanese normal controls, and Web-based prediction tools suggested that these mutations are pathogenic (Supplementary Table S2). The c.2842G>A (patient 1), c.3976G>A (patient 2), c.4887C>A (patient 8), c.2689G>A (patient 13), and c.1990+1G>A (patient 14) mutations occurred de novo. The c.3995G>A mutation (patient 3) was not found in the mother's DNA (the father's DNA was unavailable). The c.1121-2dupA (patient 4) and c.2931dupT (patient 6) mutations were found in the asymptomatic fathers. c.1963G>A (patient 10) was found in familial members affected with porencephaly as well as asymptomatic carriers, suggesting incomplete penetrance of the mutation (Supplementary Fig S1). The remaining patients' parental DNA was unavailable.

To examine the mutational effects of the 2 splice acceptor site mutations (c.1121-2dupA and c.1382-1G>C), RT-PCR and sequencing were performed (see Fig 1). c.1121-2dupA caused the deletion of exon 21 from the wild-type *COL4A1* mRNA, resulting in an in-frame 55-amino acid deletion (p.Gly374_Asn429delinsAsp). The effect of c.1382-1G>C was more complicated. There were 3 PCR products amplified from LCL treated with CHX, which inhibits nonsense-mediated mRNA decay (NMD). The middle band corresponded to the wild-type allele. The sequence of the lower mutant band showed a 33bp insertion of intron 22 and an 84bp deletion of all of exon 23 from the use of cryptic splice acceptor and donor sites within intron 22. The change of amino acid sequence from this mutant transcript was a deletion of 29 amino acids and an insertion of 12 amino acids (p.Gly461_Gly489delinsValHisCysGlyAspPheTrpSerHisValThrArg). The upper band was only observed in CHX-treated LCL, but was not evident in the untreated LCL, suggesting that this mutant transcript may undergo NMD. Sequencing of the upper band showed a 61bp insertion of intron 22 from the use of a cryptic splice acceptor site within intron 22, as mentioned above. The product of this mutant transcript leads to a frameshift, creating a premature stop codon (p.Gly461ValfsX31), which is consistent with degradation of the mutant transcript by NMD.

Clinical Features

The clinical information for individuals with *COL4A1* mutations is summarized in the Table, and their representative brain images are shown in Figure 2 and Supplementary Fig S2. *COL4A1* mutations were identified in 10 of 61 patients with porencephaly (16.4%). Of note, *COL4A1* mutations were identified in 5 of 10 patients with schizencephaly (50.0%), revealing a novel association between *COL4A1* mutations and schizencephaly. Thirteen patients were born at term, and 2 patients (patients 1 and 12) were born at preterm. Their body weight was normal at birth except for 5 patients (patients 3, 4, 9, 12, and 15) who were below −2.0 standard deviations. The occipitofrontal circumference was available in 12 patients, and 6 patients (patients 2, 3, 6, 13, 14, and 15) were below −2.0 standard deviations. Two patients (patients 11 and 12) were confirmed to have an antenatal hemorrhage as previously reported.[23,24] Among associated findings with *COL4A1* mutations, a patient showed FCD that was histologically demonstrated (Fig 3A–F). In addition, hemolytic anemia was found in 5 of 15 patients, suggesting that hemolytic anemia may be a novel feature associated with *COL4A1* mutation. Pontocerebellar atrophy along with severe bilateral porencephaly was observed in 2 patients, and a patient showed cerebellar hypoplasia. Previously reported magnetic resonance imaging and systemic findings associated with *COL4A1* mutations were also observed, including intracranial calcification (7 of 15), myopathy (1 of 15; see Fig 3G, H), ocular abnormalities (4 of 15), and elevated serum creatine kinase levels (6 of 15), confirming that these features are useful signs for *COL4A1* testing. Case reports are available in the Supplementary Data.

Discussion

We found a total of 15 novel mutations in this study. Nine mutations occurred at highly conserved Gly residues in the Gly-X-Y repeat of the collagen triple helical domain, suggesting that these mutations may alter the collagen IV $\alpha1\alpha1\alpha2$ heterotrimers.[1,25] We reported for the first time 2 mutations (a nonsense and a missense change) in the NC1 domain. The nonsense mutation