5.病因・病態

1) GPIアンカーの生合成およびタンパク質への付加に必要な遺伝子の変異によるもの（PIG遺伝子欠損症）
PIGA, PIGQ, PIGL, PIGW, PIGM, PIGV, PIGN, PIGO, PIGT欠損症など
2) GPIアンカーの修飾に必要な遺伝子の変異によるもの（PGAP遺伝子欠損症）
PGAP1, PGAP3, PGAP2欠損症など
3) その他の遺伝子の変異によるもの

GPIアンカー型タンパク質の欠損と病態

アルカリホスファターゼ(ALP)の発現低下とてんかん

GPIアンカー型タンパク質であるALPは、細胞表面でビタミンB6の１つピリドキサールリン酸を脱リン酸化して細胞内に取り込める形のピリドキサールにし、細胞内に入ったピリドキサールは再びリン酸化されてピリドキサールリン酸となり、抑制性ニューロンにおいてγ-アミノ酪酸（GABA）合成酵素の補酵素として働く。細胞膜上のALPが発現が低下すると、細胞内のピリドキサールリン酸が不足し、GABA合成が抑制され、けいれん発作が誘発されると考えられる。ビタミンB6はGABA合成だけでなく様々なアミノ酸代謝に関わっているので、ビタミンB6欠乏による代謝異常が起こる可能性がある。

6.検査所見

- 高ALP血症
高ALP血症を伴う知的障害、運動発達障害はIGDである可能性が高いが変異遺伝子のステップによっては正常の場合もある。逆にPIGT欠損症では低値になり共通の症状に加えて骨の形成異常を示す。
- 手指・足趾のX線写真で末節骨欠損
- 聴性脳幹反応（ABR）の異常、脳波の異常
- 脳MRIの拡散強調画像（DWI）にて基底核に高信号、小脳萎縮、
- 顆粒球のフローサイトメトリーにてCD16の発現低下

7.重症度分類

Barthel Indexが85点(%)以下を重症とする。

8.治療

てんかんを合併している症例では、通常の抗てんかん薬や治療では難治に経過することが多い。しかし、ビタミンB6（ピリドキシン）の投与がてんかん発作に著効する症例が報告されている（文献8）。注意すべきは、ビタミンB6製剤の選択である。ピリドキサールリン酸はGPIアンカー型タンパク質であるALPを介して細胞内に取り込まれるため、IGDでは細胞内への取り込みが低下し無効であると考えられる。一方、ピリドキシンは前述のALPを介さずに細胞内に取り込まれるために有効と考えられる。

商品名：アデロキシン(鳥居薬品株式会社、ゾンネボード製薬株式会社)
投与量：Pyridoxine 10-15mg/kg/day分3で投与を開始。副作用等確認した後に　20-30mg/kg/day分3まで増量

現在大阪大学小児科にて臨床研究が行われているので投与をお考えの先生は下記までご連絡ください。

連絡先
青天目　信
大阪大学大学院医学研究科小児科
〒565-0871　大阪府吹田市山田丘 2-2

疾患ホームページ　http://igd.biken.osaka-u.ac.jp

付記）本ガイドラインにご意見がある方はご連絡ください。

大阪大学微生物病研究所　村上良子
〒565-0871　大阪府吹田市山田丘 3-1
e-mail: yoshiko@biken.osaka-u.ac.jp

参考文献

1. Almeida, A1., Y. Murakami1, M. Layton, P. Hillmen, G. S. Sellick, Y. Maeda, S. Richards, S. Patterson, I. Kotsianidis, L. Mollica, D. Crawford, A. Baker, M. Ferguson, Roberts, R. Houlston, T. Kinoshita and A. Karadimitris. Hypomorphic promoter mutation in the mannosyltransferase-encoding PIG-M gene causes inherited glycosylphosphatidylinositol deficiency. Nat. Med., 2006; 12: 846-851. 1Equal contribution

2. Almeida, A.M1., Y. Murakami1, A. Baker, Y. Maeda, I.A.G. Roberts, T. Kinoshita, D. M. Layton, and A. Karadimitris.Targeted therapy for inherited GPI deficiency. N. Engl. J. Med., 2007; 356: 1641-1647. 1Equal contribution

3. .Krawitz, P. M., M. R. Schweiger, C. Rödelsperger, C. Marcelis, U. Kölsch, C, Meisel, F. Stephani, T. Kinoshita, Y. Murakami, S. Bauer, M. Isau, A. Fischer, A. Dahl, M. Kerick, J. Hecht, S. Köhler, M. Jaeger, J. Grünhagen, B. Jonske de Condor, S. Doelken, H. G. Brunner, P. Meinecke, E. Passarge, M. Thompson, D. E. Cole, D. Horn, T. Roscioli, S. Mundlos and P. N. Robinson.Identity-by-descent filtering of exome sequence data identifies PIGV mutations in hyperphosphatasia mental retardation syndrome. Nat. Genet., 2010;42:827-829.

4. Murakami, Y., N. Kanzawa, K. Saito, P. M. Krawitz, S. Mundlos, P. N. Robinson, A. Karadimitris, Y. Maeda and T. Kinoshita.
Mechanism for release of alkaline phosphatase caused by glycosylphosphatidylinositol deficiency in patients with hyperphosphatasia-mental retardation syndrome.
J. Biol. Chem., 2012;287:6318-6325.

5. Krawitz, P. M., Y. Murakami, J. Hecht, U. Krüger, S. E. Holder, G. R. Mortier, B. delle Chiaie, M. D. Thompson, T. Roscioli, S. Kielbasa, T. Kinoshita, S. Mundlos, P. N. Robinson and D. Horn.
Mutations in *PIGO*, a member of the GPI anchor synthesis pathway, cause hyperphosphatasia with mental retardation.
Am. J. Hum. Genet., 2012;91:146-151.

6. Hansen, L., H. Tawamie, Y. Murakami, Y. Mang, S. ur Rehman, R. Buchert, S. Schaffer, S. Muhammad, M. Bak, M. M. Noethen, E. P. Bennett, Y. Maeda, M. Aigner, A. Reis, T. Kinoshita, N. Tommerup, S. M. Baig, R. A. Jamra.
Hypomorphic mutations in *PGAP2*, encoding a GPI-anchor-remodeling protein, cause autosomal-recessive intellectual disability.
Am. J. Hum. Genet., 2013;92:575-583.

7. Krawitz, P. M., Y. Murakami, A. Riess, M. Hietala, U. Krueger, N. Zhu, T. Kinoshita, S. Mundlos, J. Hecht, P. N. Robinson and D. Horn.
PGAP2 mutations, affecting the GPI-anchor-synthesis-pathway, cause hyperphosphatasia with mental retardation syndrome.
Am. J. Hum. Genet., 2013;92:584-589.

8. Kuki, I., Y. Takahashi, Okazaki, Ebara, N. Inoue, T. Kinoshita and Y. Murakami.
Case report with vitamin B6 responsive epilepsy due to inherited GPI deficiency.
Neurology, 2013; 81:1467-1469.

9. Ohba, C., N. Okamoto, Y. Murakami, Y. Suzuki, Y. Tsurusaki, M. Nakashima, N. Miyake, F. Tanaka, T. Kinoshita, N. Matsumoto and H. Saitsu. *PIGN* mutations cause congenital anomalies, developmental delay, hypotonia, epilepsy, and progressive cerebellar atrophy. *Neurogenet.*, 2013; 15:85-92.

10. Chiyonobu, T., N. Inoue, M. Morimoto, T. Kinoshita and Y. Murakami. Glycosylphosphatidylinositol (GPI) anchor deficiency caused by mutations in *PIGW* is associated with West syndrome and hyperphosphatasia with mental retardation syndrome. *J. Med. Genet.*, 2014; 51:203-207.

11. Howard, M. F[1]., Y. Murakami[1], A. T. Pagnamenta[1], C. Daumer-Haas, B. Fischer, J. Hecht, D. A. Keays, S. J. L. Knight, U. Kölsch, U. Krüger, S. Leiz, Y. Maeda, D. Mitchell, S. Mundlos, J. A. Phillips III, P. N. Robinson, U. Kini, J. C. Taylor, D. Horn, T. Kinoshita, P. M. Krawitz. Mutations in PGAP3 impair GPI-anchor maturation causing a new subtype of hyperphosphatasia with intellectual disability. *Am. J. Hum. Genet.*, 2014; 94:278-287. [1]Equal contribution

12. Nakamura, K., H. Osaka, Y. Murakami, R. Anzai, K. Nishiyama, H. Kodera, M. Nakashima, Y. Tsurusaki, N. Miyake, T. Kinoshita, N. Matsumoto, H. Saitsu. *PIGO* mutations in intractable epilepsy and severe developmental delay with mild elevation of alkaline phosphatase levels. *Epilepsia*, 2014; 55(2):e13-e17.

13. Martin, H. C., G. Kim, A. T. Pagnamenta, Y. Murakami, G. Carvill, E. Meyer, R. Copley, A. Rimmer, G. Barcia, M. Fleming, J. Kronengold, M. R. Brown, K. A. Hudspith, J. Broxholme, A. Kanapin, J.-B. Cazier, T. Kinoshita, R. Nabbout, The WGS600 Consortium, D. Bentley, G. McVean, S. Heavin, Z. Zaiwalla, T. McShane, H. Mefford, D. Shears, H. Stewart, M. A. Kurian, I. E. Scheffer, E. Blair, P. Donnelly, L. K. Kaczmarek and J. Taylor.
Clinical whole-genome sequencing in severe early-onset epilepsy reveals new genes and improves molecular diagnosis.
Hum. Mol. Genet., 2014; 23: 3200-3211.

14. Kato, M[1]., H. Saitsu[1], Y. Murakami[1], K. Kikuchi, S. Watanabe, M. Iai, K. Miya, R. Matsuura, R. Takayama, C. Ohba, M. Nakashima, Y. Tsurusaki, N. Miyake, S. Hamano, H. Osaka, K. Hayasaka, T. Kinoshita and N. Matsumoto.
PIGA mutations cause early-onset epileptic encephalopathies and distinctive features.
Neurology, 2014.; 82:1587-1596. [1]Equal contribution
(In Focus の論文に採択、同号の表紙に掲載)

15. Murakami, Y., H. Tawamie, Y. Maeda, C, Buttner, R. Buchert, F. Radwan, S. Schaffer, H. Sticht, M. Aigner, A. Reis, T. Kinoshita and R. A. Jamra.
Null mutation in *PGAP1* impairs GPI-anchor maturation and causes severe non-syndromic recessive intellectual disability.
PLoS Genet., 2014.;10(5):e1004320.

16. Nakashima, M., H. Kashii, Y. Murakami, M. Kato, Y. Tsurusaki, N. Miyake, M. Kubota, T. Kinoshita, H. Saitsu, N. Matsumoto.
Novel compound heterozygous *PIGT* mutations caused multiple congenital anomalies-hypotonia-seizures syndrome 3.
Neurogenet., 2014; 15: 193-200.

17. Fujiwara I, Murakami Y, Niihori T, Kanno J, Hakoda A, Sakamoto O, Okamoto N, Funayama R, Nagashima T, Nakayama K, Kinoshita T, Kure S, Matsubara Y, Aoki Y. Mutations in PIGL in a patient with Mabry syndrome. *Am J Med Genet A.* 2015 Apr;167A(4):777-85.

18. 村上良子　木下タロウ 知的障害とてんかんを主症状とする新しい疾患—先天性GPI欠損症 脳と発達誌 第47巻1号 2015

Ⅱ．　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

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
Inoue, N., Kinoshita, T.	Pathogenesis of Clonal Dominance in PNH; Growth Advantage in PNH.	Kanakura, Y., Kinoshita, T., Nishimura, J.	Paroxysmal Nocturnal Hemoglobinuria-From bench to bedside	Springer-Verlag	Tokyo	2016	In press
井上徳光	PNH型細胞のクローン性拡大の機序	金倉譲、西村純一	発作性夜間ヘモグロビン尿症（PNH）～遺伝子異常の解析から新規治療の臨床・開発まで～	医薬ジャーナル社	大阪	2016	69-82
高橋幸利、西田拓司 山口解冬	自己免疫性脳炎	編集：辻省次、吉良潤一	アクチュアル脳・神経疾患の臨床、免疫性神経疾患病態と治療のすべて	中山書店	東京	2016	270-279
高橋幸利 東本和紀	てんかん	編集:山崎麻美 坂本博昭	小児脳神経外科学（改定2版）	金芳堂	東京		印刷中
高橋幸利	診断（免疫・その他の診断）		「てんかん白書」～てんかん医療・研究のアクションプラン				印刷中
高橋幸利	臨床研究（小児期）		「てんかん白書」～てんかん医療・研究のアクションプラン				印刷中

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
Ohba C, Okamoto N, Murakami Y, Suzuki Y, Tsurusaki Y, Nakashima M, Miyake N, Tanaka F, Kinoshita T, Matsumoto N, Saitsu	H. PIGN mutations cause congenital anomalies, developmental delay, hypotonia, epilepsy, and progressive cerebellar atrophy.	*Neurogenetics.*	15(2)	85-92	2014

Martin, H. C., G. Kim, A. T. Pagnamenta, Y. Murakami, G. Carvill, E. Meyer, R. Copley, A. Rimmer, G. Barcia, M. Fleming, J. Kronengold, M. R. Brown, K. A. Hudspith, J. Broxholme, A. Kanapin, J.-B. Cazier, T. Kinoshita, R. Nabbout, The WGS600 Consortium, D. Bentley, G. McVean, S. Heavin, Z. Zaiwalla, T. McShane, H. Mefford, D. Shears, H. Stewart, M. A. Kurian, I. E. Scheffer, E. Blair, P. Donnelly, L. K. Kaczmarek and J. Taylor. Clinical	whole-genome sequencing in severe early-onset epilepsy reveals new genes and improves molecular diagnosis.	*Hum. Mol. Genet.*	23	3200-3211	2014
Kato, M[1]., H. Saitsu[1], Y. Murakami[1], K. Kikuchi, S. Watanabe, M. Iai, K. Miya, R. Matsuura, R. Takayama, C. Ohba, M. Nakashima, Y. Tsurusaki, N. Miyake, S. Hamano, H. Osaka, K. Hayasaka, T. Kinoshita and N. Matsumoto.	*PIGA* mutations cause early-onset epileptic encephalopathies and distinctive features.	*Neurology,*	82	1587-1596	2014
Murakami, Y., H. Tawamie, Y. Maeda, C, Buttner, R. Buchert, F. Radwan, S. Schaffer, H. Sticht, M. Aigner, A. Reis, T. Kinoshita and R. A. Jamra.	Null mutation in *PGAP1* impairs GPI-anchor maturation and causes severe non-syndromic recessive intellectual disability.	*PLoS Genet.*	10(5)	e1004320.	2014
Nakashima, M., H. Kashii, Y. Murakami, M. Kato, Y. Tsurusaki, N. Miyake, M. Kubota, T. Kinoshita, H. Saitsu, N. Matsumoto.	Novel compound heterozygous *PIGT* mutations caused multiple congenital anomalies-hypotonia-seizures syndrome 3.	*Neurogenet.,*	15	193-200	2014
Ueda, Y., J. Nishimura, Y. Murakami, S, Kajigaya, T. Kinoshita, Y. Kanakura and N. S. Young.	Paroxysmal nocturnal hemoglobinuria with copy number-neutral 6pLOH in GPI (+) but not in GPI (-) granulocytes.	*Eur. J. Haematol.,*	92:	450-453 DOI: 10.1111/ejh.12253	2014.

Stokes, M., Y. Murakami, Y. Maeda, T. Kinoshita and Y. S. Morita.	New insights to the functions of PIGF, a protein involved in the ethanolamine phosphate transfer steps of glycosylphosphatidylinositol biosynthesis.	*Biochem. J.*,	463(2)	249-256	2014
Fujiwara I, Murakami Y, Niihori T, Kanno J, Hakoda A, Sakamoto O, Okamoto N, Funayama R, Nagashima T, Nakayama K, Kinoshita T, Kure S, Matsubara Y, Aoki Y.	Mutations in PIGL in a patient with Mabry syndrome.	*Am J Med Genet A.*		doi: 10.1002/ajmg.a.36987	2015
村上良子　木下タロウ	知的障害とてんかんを主症状とする新しい疾患—先天性 GPI 欠損症	脳と発達誌	第 47 巻 1 号	5-13	2015
Inoue N, Akazawa T.	IL17A (interleukin 17A).	Atlas of Genetics and Cytogenetics in Oncology and Haematology	19	18-27	2014
Akazawa T, Ohashi T, Nakajima H, Nishizawa Y, Kodama K, Sugiura K, Inaba T, Inoue N.	Development of a dendritic cell-targeting lipopeptide as an immunoadjuvant that inhibits tumor growth without inducing local inflammation.	*Int. J. Cancer*	135	2847-2856	2014
Nobusuke Kimura, Yukitoshi Takahashi, Hideo Shigematsu, Katsumi Imai, Hiroko Ikeda, Hideyuki Ootani, Rumiko Takayama, Yukiko Mogami, Noriko Kimura, Koichi Baba, Kazumi Matsuda, Takayasu Tottori, Naotaka Usui, Yushi Inoue	Developmental outcome after surgery in focal cortical dysplasia patients with early-onset epilepsy.	Epilepsy Research	108(10)	1845-1852	2014

Hiroyuki Fujita Hiroyuki, Matsukura S, Watanabe T, Komitsu N, Watanabe Y, Takahashi Y, Kambara T, Ikezawa Z, Aihara M	The serum level of HMGB1 (high mobility group box 1 protein) is preferentially high in drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms.	British Journal of Dermatology	171(6)	1585-1588	2014
Chiba Yuhei, Katsuse Omi, Fujishiro Hiroshige,Kamada Ayuko, Saito Tomoyuki, Ikura Takahiro, Takahashi Yukitoshi, Kunii Misako, Takeno Mitsuhiro Hirayasu, Yoshio	Lymphopenia Helps Early Diagnosis of Systemic Lupus Erythematosus forPatients with Psychosis as an Initial Symptom	Psycho-somatics.	56 (1)	85-8.	2015
Takahiro Furukawa, Naoko Matsui, Koji Fujita, Ai Miyashiro, Yuishin Izumi, Fumitaka Shimizu, Katsuichi Miyamoto, Yukitoshi Takahashi, Takashi Kanda, Susumu Kusunoki, Ryuji Kaj	Increased proinflammatory cytokines in sera of patients with multifocal motor neuropathy.	J Neurol Sci.	15;346 (1-2)	75-9	2014
Hiroshi Sakuma, NaoyukiTanuma, Ichiro Kuki, Yukitoshi Takahashi, Masashi Shiomi, Masaharu Hayashi	Intrathecal overproduction of pro-inflammatory cytokines and chemokines in febrile infection related refractory status epilepticus.	Journal of Neurology, Neurosurgery,and Psychiatry	86	820-822	2015
Masaki Yoshimura, Zhang Shouwen, Yuki Ueda, Kazumi Matsuda, Katsumi Imai, Yukitoshi Takahashi, Yushi Inoue	An analysis of epileptic negative myoclonus by magnetoencephalography.	Epilepsy Research	110	139-45	2015
高橋幸利、森達夫、大星大観、東本和紀、渡辺陽和、吉富晋作、山口解冬	神経疾患と NMDA 型グルタミン酸受容体抗体.	日本小児科学会誌	118(12)	1695-1707	2014

Fujiwara I, Murakami Y, Niihori T, Kanno J, Hakoda A, Sakamoto O, Okamoto N, Funayama R, Nagashima T, Nakayama K, Kinoshita T, Kure S, Matsubara Y, Aoki Y.	Mutations in PIGL in a patient with Mabry syndrome.	Am J Med Genet A.	167A(4)	777-85	2015
Hirata T, Fujita M, Nakamura S, Gotoh K, Motooka D, Murakami Y, Maeda Y, Kinoshita T.	Post-Golgi anterograde transport requires GARP-dependent endosome-to-TGN retrograde transport.	Mol Biol Cell.	26(17)	3071-84	2015
Ilkovski B, Pagnamenta AT, O'Grady GL, Kinoshita T, Howard MF, Lek M, Thomas B, Turner A, Christodoulou J, Sillence D, Knight SJ, Popitsch N, Keays DA, Anzilotti C, Goriely A, Waddell LB, Brilot F, North KN, Kanzawa N, Macarthur DG, Taylor JC, Kini U, Murakami Y, Clarke NF.	Mutations in PIGY: expanding the phenotype of inherited glycosylphosphatidylinositol deficiencies.	Hum Mol Genet.	24(21)	6146-59	2015
Bosch DG, Boonstra FN, Kinoshita T, Jhangiani S, de Ligt J, Cremers FP, Lupski JR, Murakami Y, de Vries BB.	Cerebral visual impairment and intellectual disability caused by PGAP1 variants.	Eur J Hum Genet.	23(12)	1689-93	2015
Nakagawa T, Taniguchi-Ikeda M[1], Murakami Y[1], Nakamura S, Motooka D, Emoto T, Satake W, Nishiyama M, Toyoshima D, Morisada N, Takada S, Tairaku S, Okamoto N, Morioka I, Kurahashi H, Toda T, Kinoshita T, Iijima K. ([1]Correspondence)	A novel *PIGN* mutation and prenatal diagnosis of inherited glycosylphosphatidylinositol deficiency.	Am J Med Genet A	170(1)	183-8	2016
Makrythanasis P, Kato M, Zaki MS, Saitsu H, Nakamura K, Santoni FA, Miyatake S, Nakashima M, Issa MY, Guipponi M, Letourneau A, Logan CV, Roberts N, Parry DA, Johnson CA, Matsumoto N, Hamamy H, Sheridan E, Kinoshita T, Antonarakis SE, Murakami Y.	Pathogenic Variants in PIGG Cause Intellectual Disability with Seizures and Hypotonia.	Am J Hum Genet.	98(4)	615-26	2016
Roy, N., Ohtani, K., Matsuda, Y., Mori, K., Hwang, I., Suzuki, Y., Inoue, N., Wakamiya, N.	Collectin CL-P1 utilizes C-reactive protein for complement activation.	*Biochim Biophys Acta*	1860	1118-1128	2016

Nakanishi, K., Kukita, Y., Segawa, H., Inoue, N., Ohue, M., and Kato, K.	Characterization of the T cell receptor beta chain repertoire in tumor infiltrating lymphocytes	***Cancer Medicine***			In press
Norimichi Higurashi, Yukitoshi Takahashi, Ayako Kashimada, Yuji Sugawara, Hiroshi Sakuma, Yuko Tomono, Takahito Inoue, Megumi Hoshina, Ruri Satomi, Masaharu Ohfu, Kazuya Itomi, Kyoko Takano, Tomoko Kirino, Shinichi Hirose	Immediate suppression of seizure clusters by corticosteroids in PCDH19 female epilepsy.	Seizure	27	1-5	2015
Tetsuhiro Fukuyama, Yukitoshi Takahashi, Yuko Kubota, Yukiko Mogami, Katsumi Imai, Yoshiyuki Kondo, Hiroshi Sakuma, Koji Tominaga, Hirokazu Oguni, Shigeko Nishimura	Semi-quantitative analyses of antibodies to N-methyl-D-aspartate type glutamate receptor subunits (GluN2B & GluN1) in the clinical course of Rasmussen syndrome.	Epilepsy Research	113	34-43	2015
Shinsaku Yoshitomi, Yukitoshi Takahashi, Mamiko Hotate Ishizuka, Tokito Yamaguchi, Akito Watanabe, Hirosato Nasu, Yuki Ueda, Hideyuki Ohtani, Hiroko Ikeda, Katsumi Imai, Hideo Shigematsu, Yushi Inoue, Yoshihiro Tanahashi, Kaori Aiba, Hodaka Ohta, Shino Shimada, Toshiyuki Yamamoto	Three patients manifesting early infantile epileptic spasms associated with 2q24.3 microduplications.	Brain & Development	37(9)	874-879	2015
Mori T, Imai K, Oboshi T, Fujiwara Y, Takeshita S, Saitsu H, Matsumoto N, Takahashi Y, Inoue Y.	Usefulness of ketogenic diet in a girl with migrating partial seizures in infancy.	Brain & Development	Jan 11		2016
Hiroko Ikeda, Katsumi Imai, Hitoshi Ikeda, Hideo Shigematsu, Yukitoshi Takahashi, Yushi Inoue, Norimichi Higurashi, Shinichi Hirose	Characteristic phasic evolution of convulsive seizure in PCDH19-related epilepsy.	Epileptic Disord.	18	26-33	2016
高橋幸利	特別企画シリーズ：てんかんを分かり易く理解するための神経科学、６：免疫	てんかん研究	33	683-687	2016

Ⅲ. 研究成果の刊行物・別刷

Neurogenetics (2014) 15:85–92
DOI 10.1007/s10048-013-0384-7

ORIGINAL ARTICLE

PIGN mutations cause congenital anomalies, developmental delay, hypotonia, epilepsy, and progressive cerebellar atrophy

Chihiro Ohba · Nobuhiko Okamoto · Yoshiko Murakami · Yasuhiro Suzuki · Yoshinori Tsurusaki · Mitsuko Nakashima · Noriko Miyake · Fumiaki Tanaka · Taroh Kinoshita · Naomichi Matsumoto · Hirotomo Saitsu

Received: 2 November 2013 / Accepted: 10 November 2013 / Published online: 20 November 2013

Abstract Defects of the human glycosylphosphatidylinositol (GPI) anchor biosynthetic pathway show a broad range of clinical phenotypes. A homozygous mutation in *PIGN*, a member of genes involved in the GPI anchor-synthesis pathway, was previously reported to cause dysmorphic features, multiple congenital anomalies, severe neurological impairment, and seizure in a consanguineous family. Here, we report two affected siblings with compound heterozygous *PIGN* mutations [c.808T >C (p.Ser270Pro) and c.963G >A] showing congenital anomalies, developmental delay, hypotonia, epilepsy, and progressive cerebellar atrophy. The c.808C >T mutation altered an evolutionarily conserved amino acid residue (Ser270), while reverse transcription-PCR and sequencing demonstrated that c.963G >A led to aberrant splicing, in which two mutant transcripts with premature stop codons (p.Ala322Valfs*24 and p.Glu308Glyfs*2) were generated. Expression of GPI-anchored proteins such as CD16 and CD24 on granulocytes from affected siblings was

Chihiro Ohba, Nobuhiko Okamoto, and Yoshiko Murakami contributed equally

Electronic supplementary material The online version of this article (doi:10.1007/s10048-013-0384-7) contains supplementary material, which is available to authorized users.

C. Ohba · Y. Tsurusaki · M. Nakashima · N. Miyake · N. Matsumoto · H. Saitsu (✉)
Department of Human Genetics, Graduate School of Medicine, Yokohama City University, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan
e-mail: hsaitsu@yokohama-cu.ac.jp

C. Ohba
e-mail: t116017g@yokohama-cu.ac.jp

Y. Tsurusaki
e-mail: tsurusak@yokohama-cu.ac.jp

M. Nakashima
e-mail: mnakashi@yokohama-cu.ac.jp

N. Miyake
e-mail: nmiyake@yokohama-cu.ac.jp

N. Matsumoto
e-mail: naomat@yokohama-cu.ac.jp

C. Ohba · F. Tanaka
Department of Clinical Neurology and Stroke Medicine, Yokohama City University, Yokohama 236-0004, Japan

F. Tanaka
e-mail: ftanaka@yokohama-cu.ac.jp

N. Okamoto
Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi 594-1101, Japan
e-mail: okamoto@osaka.email.ne.jp

Y. Murakami · T. Kinoshita
Department of Immunoregulation, Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan

Y. Murakami
e-mail: yoshiko@biken.osaka-u.ac.jp

T. Kinoshita
e-mail: tkinoshi@biken.osaka-u.ac.jp

Y. Murakami · T. Kinoshita
World Premier International Immunology Frontier Research Center, Osaka University, Osaka 565-0871, Japan

Y. Suzuki
Department of Pediatric Neurology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan
e-mail: yasuzuki@mch.pref.osaka.jp

significantly decreased, and expression of the GPI-anchored protein CD59 in *PIGN*-knockout human embryonic kidney 293 cells was partially or hardly restored by transient expression of p.Ser270Pro and p.Glu308Glyfs*2 mutants, respectively, suggesting severe and complete loss of PIGN activity. Our findings confirm that developmental delay, hypotonia, and epilepsy combined with congenital anomalies are common phenotypes of *PIGN* mutations and add progressive cerebellar atrophy to this clinical spectrum.

Keywords Cerebellar atrophy · Compound heterozygous mutation · Glycosylphosphatidylinositol anchor · *PIGN*

Introduction

Defects of the biosynthetic pathway of the glycosylphosphatidylinositol (GPI) anchor cause broad clinical phenotypes [1]. The products of more than 20 genes in the phosphatidylinositol glycan (PIG) family are involved in GPI biosynthesis, whereas post-GPI-attachment to proteins (PGAP) gene products play a role in the structural remodeling of GPI glycan and lipid portions [2]. Mutations in eight genes involved in GPI biosynthesis and remodeling (*PIGA*, *PIGM*, *PIGN*, *PIGV*, *PIGL*, *PIGO*, *PIGT*, and *PGAP2*) have been identified in individuals with neurological abnormalities [1, 3–5], of which *PIGN* controls the addition of phosphoethanolamine to the first mannose in GPI [6]. To date, only one homozygous *PIGN* mutation has been reported to cause dysmorphic features, multiple congenital anomalies, severe neurological impairment, and seizures in a consanguineous family [7]. Here, we report a family with two affected siblings, possessing compound heterozygous *PIGN* mutations. Detailed clinical information and molecular and functional analyses are presented.

Patients and methods

Patients

We analyzed two affected siblings and their parents. Experimental protocols were approved by the Institutional Review Board of Yokohama City University School of Medicine. Clinical information and peripheral blood samples were acquired from the family members after obtaining written informed consent. Patient clinical features are summarized in Table 1. They showed dysmorphic facial features, developmental delay, intellectual disability, hypotonia, vertical nystagmus, and epilepsy.

Patient 1

This 9-year-old girl was born to nonconsanguineous healthy parents as a second child after 39 weeks of gestation (Fig. 1a). Her birth weight was 3,390 g [+1.40 standard deviation (SD)], body length of 49 cm (−0.03 SD), and head circumference of 35 cm (+1.35 SD). At 1 month of age, she showed vertical nystagmus without eye pursuit. She was hypotonic, and severe developmental delay was evident from early infancy. She was unable to control her head or utter words at 9 years of age. Abdominal echogram revealed bilateral vesicoureteral reflux as a cause of repeated urinary tract infections. Complex partial seizures developed at 8 months of age and were controlled by antiepileptic drugs. Tube feeding by gastrostomy was necessary for poor appetite at the age of 2 years.

Several dysmorphic features (prominent occiput, bitemporal narrowing, epicanthal folds, open mouth, tented upper lip, high arched palate, micrognathia, and deep plantar groove) were noted (Fig. 1b), but hypoplasia was absent from fingers and fingernails. Initial brain magnetic resonance imaging (MRI) at 6 months of age was normal, but cerebellar atrophy was observed at 2 and 6 years of age (Fig. 1c–f).

At present, her height is 122 cm (+1.1 SD), weight of 18.4 kg (−1.2 SD), and head circumference of 51.4 cm (−0.3 SD). Generalized muscle weakness and nystagmus were neurologically recognized. Laboratory examination showed a normal profile, including blood cell count and blood smear, renal and liver function, total bilirubin, uric acid, albumin, serum electrolytes, lactate, pyruvate, ammonia, amino acids, blood gasses, thyroid function, and cerebrospinal fluid study. Her serum alkaline phosphatase (ALP) activity has been normal for her age since infancy. Metabolic disorder screening including organic acid analysis, lysosomal enzymes, and mass spectrometry of transferrin was normal. G-banded analysis showed a normal karyotype (46, XX).

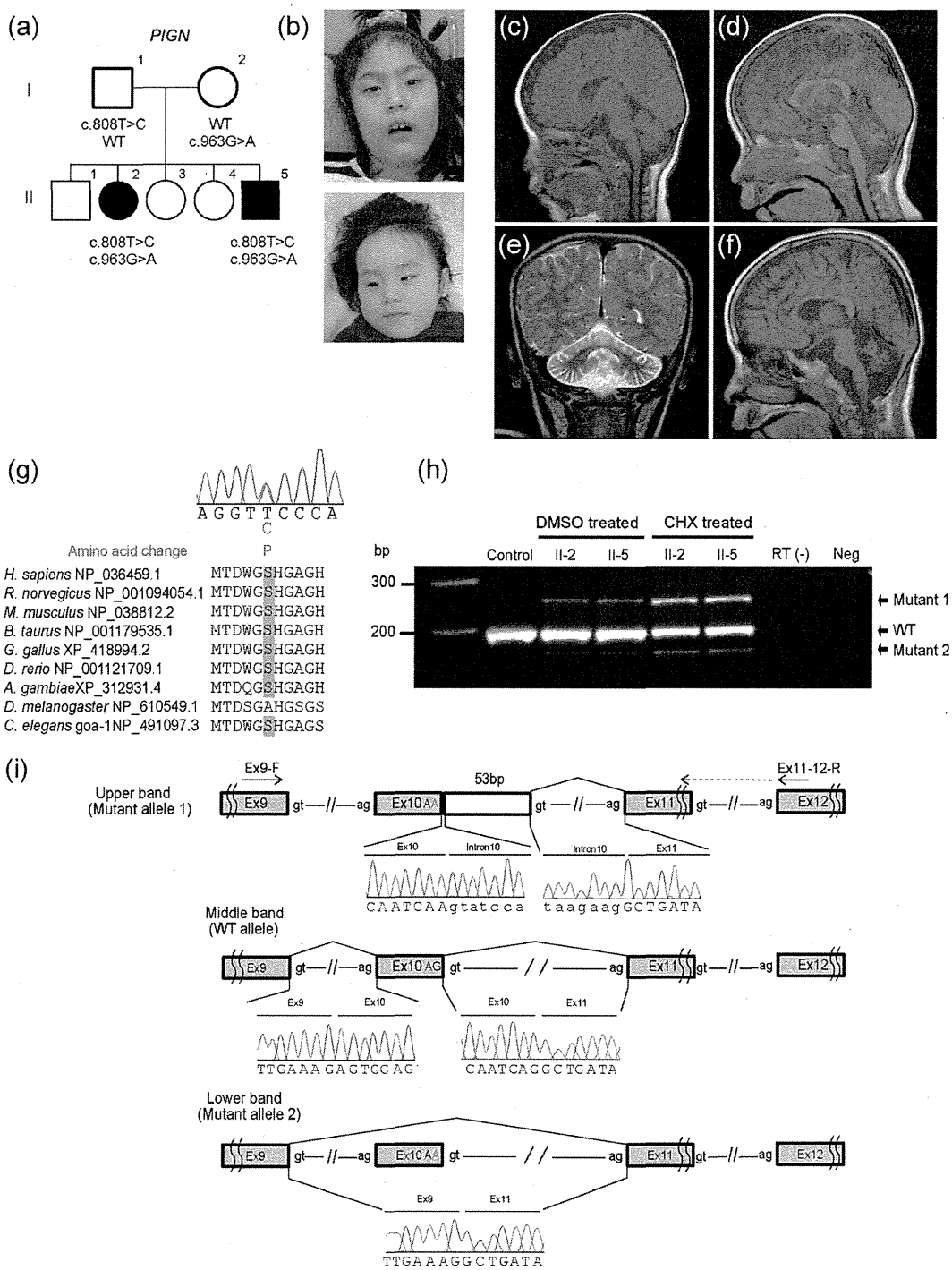
Patient 2

This 2-year-old boy was born after 37 weeks of gestation as a younger brother to patient 1 (Fig. 1a). His birth weight was 3,252 g (+1.3 SD), body length of 50 cm (+1.2 SD), and head circumference of 35 cm (+1.6 SD). He also showed vertical nystagmus at 1 month of age. Complex partial seizures developed at 5 months of age. He was hypotonic, and his developmental milestones were severely delayed with no head control at 1 year and 10 months of age. At present, his height is 92.3 cm (+2.5 SD), weight of 10.9 kg (−0.6 SD), and head circumference of 48.8 cm (+0.4 SD). He showed similar dysmorphic features to patient 1. Brain MRI at 2 months of age revealed no significant abnormalities.

Table 1 Clinical features of patients with *PIGN* mutations

Patient	1	2	Reported by Maydan et al. [7]							
			V-1	V-2	V-4	V-5	V-8	V-9	V-10	Total
Age (years)	9	2	N.D.	Diseased at 14	Diseased at 1	Diseased at 5	Diseased at 3	Diseased at 17	Diseased at 39	
Sex	Female	Male	Male	Male	Male	Female	Female	Female	Male	
Size at birth (percentile)										
Weight (g)	3,390 (90–97)	3,252 (90–97)	3,566 (95)	4,065 (97)	3,850 (95)	3,410 (40)	4,250 (99)	4,300 (98)	4,800 (>99)	
Head circumference (cm)	35 (90)	35 (90–97)	37 (>97)	37 (97)	35.5 (75)	34.5 (10)	N.D.	N.D.	N.D.	
Abnormalities										
Facial features	+	+	+	+	+	+	+	+	+	9/9
Fingers/foot	+	+	+	+	+	-	–	+	+	6/9
Heart	–	–	+	+	+	+	–	+	–	5/9
Urinary tract	+	–	+	+	+	–	–	–	–	4/9
Gastrointestinal tract	GER	–	GER	GER	Anal stenosis	Imperforate anus, ano-vestibular fistula, GER	–	Feeding and swallowing difficulties	Feeding and swallowing difficulties	7/9
Neurological features										
Developmental delay	+	+	+	+	+	+	+	+	+	9/9
Hypotonia	+	+	+	+	+	+	+	+	+	9/9
Nystagmus	+	+	+	+	–	+	–	+	+	7/9
Tremor	+	+	+	+	+	+	+	–	–	7/9
Seizure	+	+	+	–	+	+	+	+	+	8/9
Brain CT	Normal	Normal	Normal	N.D.	Normal	Multiple small subdural hematomas	N.D.	N.D.	N.D.	1/5
Brain MRI				N.D.	N.D.		N.D.	N.D.	N.D.	
Delayed myelination	+	–	–			+				2/4
Thin corpus callosum	–	–	–			+				1/4
Cerebellar atrophy	+	–	Minimal loss of vermis parenchyma			–				1/4
Enlargement of the ventricle	+	–	–			+ (Mild)				2/4

GER Gastroesophageal reflux, *N.D.* not determined



Whole exome sequencing (WES)

Genomic DNA was isolated from peripheral blood leukocytes, captured using the SureSelect Human All Exon v4 Kit (51 Mb; Agilent Technologies, Santa Clara, CA), and sequenced on an Illumina HiSeq2000 (Illumina, San Diego, CA) with 101 bp paired-end reads. Data processing, variant calling, and variant annotation were performed as previously described [8].

◀ **Fig. 1** **a** Familial pedigree and mutations. **b** Photographs of the faces of patient 1 (*upper*) and patient 2 (*lower*). Frontal narrow temporal, frontal bossing, hypertelorism, epicanthal folds, down-slanting palpebral fissures, high nasal bridge, bilateral low set ears, thin philtrum, downturned mouth, and microretrognathia are noted in both patients. **c**, **d**, **f** T1-weighted midline sagittal images and **e** T2-weighted coronal images of patient 1 (**c** at 6 months, **d** and **e** at 2 years, and **i** at 6 years). Progressive vermis atrophy (**c**, **d**, **f**) and hemispheres atrophy (**e**) were observed. **g** Sequence chromatography showing heterozyogous c.808C >T mutation, which alters an evolutionarily conserved amino acid. Homologous sequences were aligned using CLUSTALW. **h** RT-PCR analysis using cDNA of LCLs derived from two patients (II-2 and II-5) and a control. **i** Schematic representation of wild-type (WT) and mutant transcripts and primers used for the analysis. Primer of ex11-12-R spans exons 11 and 12. A single band (200 bp), corresponding to the WT allele, was amplified using control cDNA. Upper and lower bands were detected from patient cDNA. The upper band (253 bp) has a 53-bp insertion of intron 10 sequences, leading to a frameshift mutation. The lower band has a 41-bp deletion of the entire exon 10, also leading to a frameshift mutation

Reverse transcriptase-PCR

Lymphoblastoid cell lines (LCLs) were established from the two patients. RT-PCR using total RNA extracted from LCLs was performed as previously described [9].

Briefly, total RNA was extracted using the RNeasy Plus Mini kit (Qiagen, Tokyo, Japan) from LCLs with or without incubation in 30 μM cycloheximide (CHX; Sigma, Tokyo, Japan) for 4 h. Four micrograms of total RNA was subjected to reverse transcription, and 2 μl cDNA was used for PCR. Primer sequences were ex9-F (5′-TCCTTTAGTCACTTGG GGAGCTGGA-3′) and ex11-12-R (5′-AATCCACAGGAA GGATTCCCACTGA-3′) (Supplementary Table 1). PCR products were electrophoresed on a 10 % polyacrylamide gel and sequenced. PCR bands were purified by the E.Z.N.A. poly-Gel DNA Extraction kit (Omega Bio-Tek, Norcross, GA).

Fluorescence-activated cell sorting (FACS) analysis

Surface expression of GPI-anchored proteins (GPI-APs) was determined by staining cells with Alexa 488-conjugated inactivated aerolysin [fluorescently-labeled inactive toxin aerolysin (FLAER); Protox Biotech, Victoria, BC, Canada] and appropriate primary antibodies: mouse anti-decay accelerating factor (DAF; IA10), −CD16 (3G8), −CD24 (ML5), −CD59 (5H8), and −CD48 (BJ40) followed by a PE-conjugated anti-mouse IgG antibody (3G8, ML5, BJ40, and secondary antibodies; BD Biosciences, Franklin Lakes, NJ). Cells were analyzed by flow cytometry (Cant II; BD Biosciences) with Flowjo software (v9.5.3, Tommy Digital, Tokyo, Japan).

Functional analysis in HEK293 cells

PIGN-knockout cells were generated from HEK293 cells using the CRISPR/Cas System [10]. We obtained the human codon-optimized *Streptococcus pyogenes* Cas9 and chimeric guide RNA expression plasmid pX330 from Addgene (Cambridge, MA). The seed sequence for the SpCas9 target site in *PIGN* exon 4 (CCA-GGTCATGTAGCTCTGATAGC) was selected and a pair of annealed oligos designed according to this sequence and cloned into the *Bbs* I sites of pX330. HEK293 cells were transfected with pX330 containing the target site using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Cells were stained with anti-CD59 antibody 14 days after transfection, and *PIGN*-knockout clones were obtained by limiting dilution.

PIGN-knockout HEK293 cells (clone PIGNKO2-12) were transiently transfected with a wild-type or mutant (S290P or exon 10 skipping) *PIGN* cDNA cloned into the SRα promoter-driven expression vector pME HA-PIGN. Restoration of the surface expression of CD59, DAF, and GPI-APs was assessed 2 days later by flow cytometry.

Results

WES detected 288 and 292 rare protein-altering and splice-site variants in patients 1 and 2, respectively. We filtered out common single nucleotide polymorphisms (SNPs) that met the following two criteria: variants showing minor allele frequencies ≥1 % in dbSNP 135 and variants found in more than two of our in-house 406 control exomes (Supplementary Table 2). All genes were surveyed for compound heterozygous or homozygous mutations consistent with an autosomal recessive trait, and only *PIGN* (GenBank accession number NM_176787.4) met this criterion, possessing compound heterozygous mutations in two patients. The missense mutation c.808T >C (p.Ser270Pro) was inherited from the patients' father, while c.963G >A is a synonymous mutation inherited from their mother but located at the last base of exon 10 (Fig. 1i). Neither of the two mutations was present in the 6,500 exomes sequenced by the National Heart, Lung, and Blood Institute exome project. In our 406 in-house control exomes, c.808T >C was absent, but c.963G >A was found in one, as a heterozygous mutation. c.808T >C occurred at evolutionary conserved amino acids (Fig. 1g) and was predicted to be pathogenic using online software (Supplementary Table 3). To examine the actual effects of c.963G >A on splicing, RT-PCR was performed (Fig. 1h, i) and a single band (200 bp) corresponding to the wild-type *PIGN* allele was amplified from control LCL cDNA template (Fig. 1h). By contrast, two aberrant faint bands were detected in addition to a wild-type band from patient cDNA (Fig. 1h). Sequencing of the upper aberrant band indicated a 53-bp insertion of intron 10 sequences that had

used a cryptic splice donor site within intron 10, producing a premature stop codon (p.Ala322Valfs*24). Sequencing of the lower band demonstrated the deletion of exon 10 from wild-type *PIGN* mRNA, also producing a premature stop codon (p.Glu308Glyfs*2). Therefore, these two mutant transcripts are likely to be degraded by nonsense-mediated mRNA decay (NMD). In fact, CHX treatment, which inhibits NMD, increased the intensity of the aberrant bands, suggesting that NMD was indeed involved.

To examine the functional impairment of *PIGN* caused by compound heterozygous mutations, the surface expression of various GPI-APs was analyzed by flow cytometry. CD16 and CD24 expression on blood granulocytes was decreased to 26–54 % of normal levels in both patients (Fig. 2a). No abnormal GPI-APs expression was observed on LCLs from either patient (Supplementary Fig. 1).

Transient expression of p.Ser270Pro and exon 10 skipping (p.Glu308Glyfs*2) mutants in *PIGN*-knockout HEK293 cells, in which expression of GPI-APs CD59 was decreased, was confirmed by immunoblotting. Expression of the exon 10 skipping mutant was decreased compared with that of wild-type and p.Ser270Pro mutant (Supplementary Fig. 2). CD59 expression was only partially or hardly restored by the transient expression of p.Ser270Pro and exon 10 skipping mutants, respectively, suggesting severe or complete loss of PIGN activity (Fig. 2b).

Discussion

Herein, we report a second family with *PIGN* mutations that showed clinical features common to a previous affected family, including congenital anomalies, developmental delay, hypotonia, and epilepsy [7]. In addition, nystagmus was an early symptom of patients in this study and was also observed in five of seven patients previously [7]. Of note, progressive cerebellar atrophy was observed in the current patient 1, and this appears to be a novel phenotype associated with *PIGN*

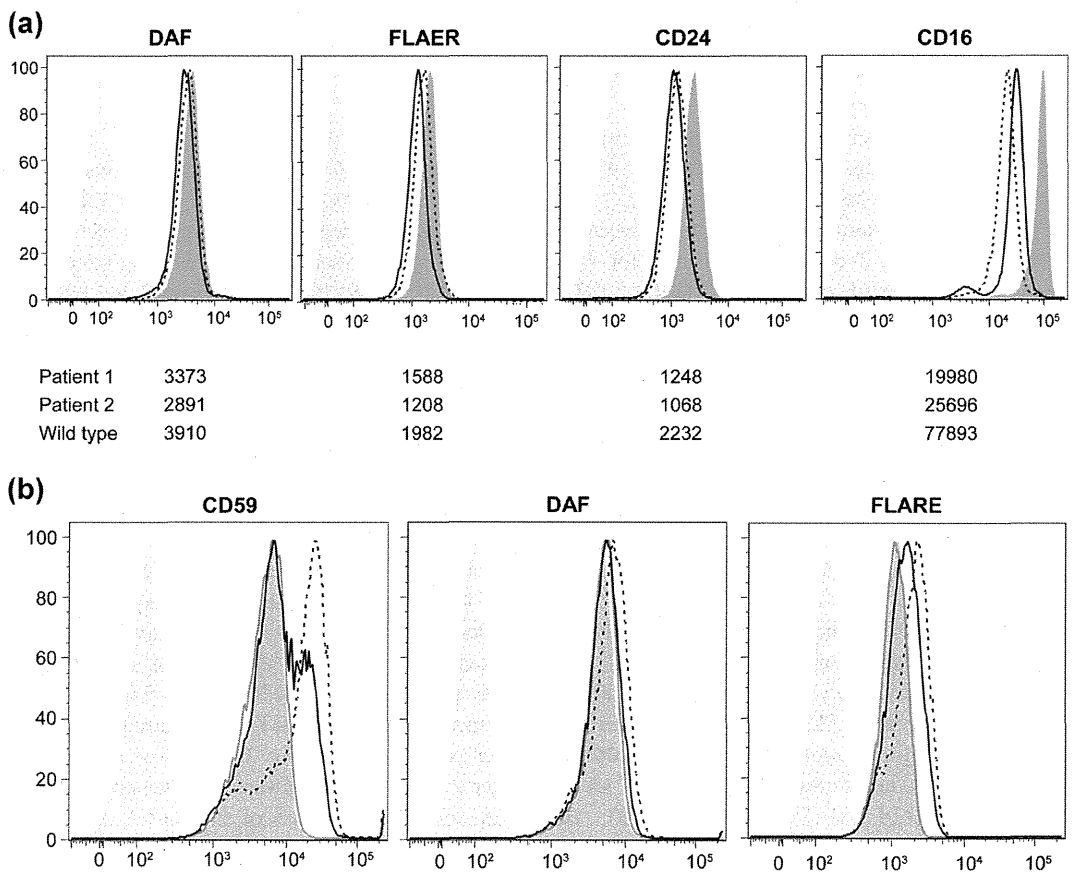


Fig. 2 **a** Surface expression of various GPI-APs on patient granulocytes (patient 1: *dotted lines*, patient 2: *solid lines*), a normal control (*dark shadow*) and an isotype control (*light shadows*). Numbers represent mean fluorescent intensities. Expression of DAF and FLAER in both patients did not significantly change compared with the control. However, CD16 and CD24 expression decreased to 26–54 % of normal levels. **b** *PIGN*-knockout HEK293 cells were transiently transfected with wild type (*dotted lines*) or mutants (exon 10 skipping: *gray line*, p.Ser270Pro: *black line*) of pME HA-PIGN vectors. Empty vector: *dark shadow*, isotype control: *light shadow*. Expression of CD59 was only partially or hardly restored by p.Ser270Pro and exon 10-skipping vectors, respectively

mutations as two patients examined by MRI showed no cerebellar atrophy but only minimal loss of vermis parenchyma in one patient (patient V-1) in the previous report [7].

PIGN is involved in the addition of phosphoethanolamine to the first mannose in GPI [6]. In *Pign*-knockout mouse F9 embryonal carcinoma cells, the first mannose in GPI precursors is not modified by phosphoethanolamine. Nevertheless, further biosynthetic steps continue and the cell surface expression of GPI-anchored proteins is only partially affected [6], suggesting that this modification may not be essential for GPI-anchored protein biosynthesis [6]. By contrast, the *gonzo* mouse line, which harbors the splice donor site mutation in *Pign*, showed abnormal forebrain development resembling holoprosencephaly [11], and patients with *PIGN* mutations show severe phenotypes such as multiple congenital anomalies, neurological impairment, and even lethality [7]; this indicates that particular defects of *PIGN*/*Pign* cause abnormal development both in mice and humans. Although the overall amount of GPI-anchored proteins might not be significantly affected by *PIGN* defects, as revealed by the minimal decrease in DAF and FLAER expression on patient granulocytes in the present study, changes to a subset of GPI-anchored proteins such as CD16 and CD24 can be sufficient to cause severe neurological phenotypes. Additionally or alternatively, GPI-APs expressed on PIGN-defective cells lack the phosphoethanolamine-side branch, and this abnormal structure of the glycan part of the anchor might affect functions of GPI-APs. Functional analysis using neuronal cells may provide novel insights into the pathogenesis of neurological phenotypes caused by *PIGN* mutations.

Seven genes (*PIGA*, *PIGM*, *PIGN*, *PIGV*, *PIGL*, *PIGO*, and *PGAP2*) have been identified as being mutated in patients with neurological abnormalities. Mutations in three of these (*PIGV*, *PIGO*, and *PGAP2*) cause hyperphosphatasia [3, 12–14], suggesting that ALP is a useful marker for suspected GPI anchor-synthesis pathway deficiencies. However, mutations of the other four genes (*PIGA*, *PIGM*, *PIGN*, and *PIGL*) did not cause hyperphosphatasia [7, 14, 15], so clinical diagnosis might be difficult in the absence of specific biomarkers. Even in clinically unsuspected patients, WES may identify mutations in genes involved in the GPI anchor-synthesis pathway. Current advances in next generation sequencing should find more comprehensive answers for unsolved GPI anchor-related diseases.

Acknowledgments We would like to thank patients and their parents for their participation in this study. We also thank Nobuko Watanabe and Kana Miyanagi for technical assistance. This work was supported by the Ministry of Health, Labour, and Welfare of Japan; a Grant-in-Aid for Scientific Research (A), (B), and (C) from the Japan Society for the Promotion of Science (A: 24249019, B: 25293085 25293235, C: 23590363); the Takeda Science Foundation; the Japan Science and Technology Agency; the Strategic Research Program for Brain Sciences (11105137); and a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle, Exploring molecular basis for brain diseases based on personal genomics) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (12024421, 25129705).

References

1. Freeze HH, Eklund EA, Ng BG, Patterson MC (2012) Neurology of inherited glycosylation disorders. Lancet Neurol 11(5):453–466. doi:10.1016/s1474-4422(12)70040-6
2. Maeda Y, Kinoshita T (2011) Structural remodeling, trafficking and functions of glycosylphosphatidylinositol-anchored proteins. Prog Lipid Res 50(4):411–424. doi:10.1016/j.plipres.2011.05.002
3. Hansen L, Tawamie H, Murakami Y, Mang Y, ur Rehman S, Buchert R, Schaffer S, Muhammad S, Bak M, Nothen MM, Bennett EP, Maeda Y, Aigner M, Reis A, Kinoshita T, Tommerup N, Baig SM, Abou Jamra R (2013) Hypomorphic mutations in *PGAP2*, encoding a GPI-anchor-remodeling protein, cause autosomal-recessive intellectual disability. Am J Hum Genet 92(4):575–583. doi:10.1016/j.ajhg.2013.03.008
4. Krawitz PM, Murakami Y, Riess A, Hietala M, Kruger U, Zhu N, Kinoshita T, Mundlos S, Hecht J, Robinson PN, Horn D (2013) *PGAP2* mutations, affecting the GPI-anchor-synthesis pathway, cause hyperphosphatasia with mental retardation syndrome. Am J Hum Genet 92(4):584–589. doi:10.1016/j.ajhg.2013.03.011
5. Kvarnung M, Nilsson D, Lindstrand A, Korenke GC, Chiang SC, Blennow E, Bergmann M, Stodberg T, Makitie O, Anderlid BM, Bryceson YT, Nordenskjold M, Nordgren A (2013) A novel intellectual disability syndrome caused by GPI anchor deficiency due to homozygous mutations in *PIGT*. J Med Genet 50(8):521–528. doi:10.1136/jmedgenet-2013-101654
6. Hong Y, Maeda Y, Watanabe R, Ohishi K, Mishkind M, Riezman H, Kinoshita T (1999) Pig-n, a mammalian homologue of yeast Mcd4p, is involved in transferring phosphoethanolamine to the first mannose of the glycosylphosphatidylinositol. J Biol Chem 274(49):35099–35106
7. Maydan G, Noyman I, Har-Zahav A, Neriah ZB, Pasmanik-Chor M, Yeheskel A, Albin-Kaplanski A, Maya I, Magal N, Birk E, Simon AJ, Halevy A, Rechavi G, Shohat M, Straussberg R, Basel-Vanagaite L (2011) Multiple congenital anomalies-hypotonia-seizures syndrome is caused by a mutation in *PIGN*. J Med Genet 48(6):383–389. doi:10.1136/jmg.2010.087114
8. Saitsu H, Nishimura T, Muramatsu K, Kodera H, Kumada S, Sugai K, Kasai-Yoshida E, Sawaura N, Nishida H, Hoshino A, Ryujin F, Yoshioka S, Nishiyama K, Kondo Y, Tsurusaki Y, Nakashima M, Miyake N, Arakawa H, Kato M, Mizushima N, Matsumoto N (2013) *De novo* mutations in the autophagy gene *WDR45* cause static encephalopathy of childhood with neurodegeneration in adulthood. Nat Genet. doi:10.1038/ng.2562
9. Saitsu H, Kato M, Okada I, Orii KE, Higuchi T, Hoshino H, Kubota M, Arai H, Tagawa T, Kimura S, Sudo A, Miyama S, Takami Y, Watanabe T, Nishimura A, Nishiyama K, Miyake N, Wada T, Osaka H, Kondo N, Hayasaka K, Matsumoto N (2010) *STXBP1* mutations in early infantile epileptic encephalopathy with suppression-burst pattern. Epilepsia 51(12):2397–2405. doi:10.1111/j.1528-1167.2010.02728.x
10. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339(6121):819–823. doi:10.1126/science.1231143
11. McKean DM, Niswander L (2012) Defects in GPI biosynthesis perturb Cripto signaling during forebrain development in two new mouse models of holoprosencephaly. Biology open 1(9):874–883. doi:10.1242/bio.20121982
12. Freeze HH (2013) Understanding human glycosylation disorders: biochemistry leads the charge. J Biol Chem 288(10):6936–6945. doi:10.1074/jbc.R112.429274

13. Krawitz PM, Murakami Y, Hecht J, Kruger U, Holder SE, Mortier GR, Delle Chiaie B, De Baere E, Thompson MD, Roscioli T, Kielbasa S, Kinoshita T, Mundlos S, Robinson PN, Horn D (2012) Mutations in *PIGO*, a member of the GPI-anchor-synthesis pathway, cause hyperphosphatasia with mental retardation. Am J Hum Genet 91(1):146–151. doi:10.1016/j.ajhg.2012.05.004
14. Almeida AM, Murakami Y, Layton DM, Hillmen P, Sellick GS, Maeda Y, Richards S, Patterson S, Kotsianidis I, Mollica L, Crawford DH, Baker A, Ferguson M, Roberts I, Houlston R, Kinoshita T, Karadimitris A (2006) Hypomorphic promoter mutation in *PIGM* causes inherited glycosylphosphatidylinositol deficiency. Nat Med 12(7):846–851. doi:10.1038/nm1410
15. Johnston JJ, Gropman AL, Sapp JC, Teer JK, Martin JM, Liu CF, Yuan X, Ye Z, Cheng L, Brodsky RA, Biesecker LG (2012) The phenotype of a germline mutation in *PIGA*: the gene somatically mutated in paroxysmal nocturnal hemoglobinuria. Am J Hum Genet 90(2):295–300. doi:10.1016/j.ajhg.2011.11.031