Table 5　辺縁系脳炎に至った FBDS 症例のまとめ

症例数	26※
健忘に先行した FBDS	20（77%）
FBDS から健忘・混迷までの期間（日）　中央値（レンジ）	36（−150〜730）
てんかん発作（FBDS 以外の）	
総計	18（70%）
全般性強直性間代性発作	13
複雑部分発作	12#
単純部分発作	1
FBDS から側頭葉てんかんに移行した例	10/12（83%）
FBDS から側頭葉てんかんへの移行期間（日）　中央値（レンジ）	12.5（−15〜455）
辺縁系脳炎関連症状	
健忘	26（100%）
混迷	23（88%）
幻覚	9（35%）
睡眠障害	7（31%）
うつ症状	5（19%）
自律神経症状	4（15%）
疼痛	4（15%）
小脳症状	2（8%）
発作間欠期のジストニア	1（4%）
辺縁系脳炎の時期の検査所見	$n=26$
抗 VGKC 複合体抗体価（pM）　中央値（レンジ）	2,281（0〜8,800）*
抗 LGI-1 抗体陽性	23（88%）*
抗 LGI-1 抗体陽性と抗 Caspr-2 抗体/抗コンタクチン 2 抗体のいずれかが陽性	3（12%）
低ナトリウム血症（<135 mmol）	23（88%）
頭部 MRI 正常	12（46%）

※：他の 3 例は健忘や混迷などの辺縁系症状を呈さず，全期間を通じて頭部 MRI に異常を認めなかった。

*：2 例については，発症 4 年以上を経過した後のスクリーニング抗体は陰性。うち 1 例は CBA での抗 LGI-1 抗体は陽性。他の 1 例は免疫グロブリン大量静注療法治療後の測定でスクリーニン抗体は陽性であったが，低値（238 pM）であった。

#：全例が側頭葉内側てんかんであることを脳波で確認した。

〔略語〕FBDS：fasicobrachial dystonic seizures

Irani SR, Michell AW, Lang B, Pettingill P, Waters P, et al: Faciobrachial dystonic seizures precede LGI1 antibody limbic encephalitis. Ann Neurol **69**: 892-900, 2011 を元に作成した。

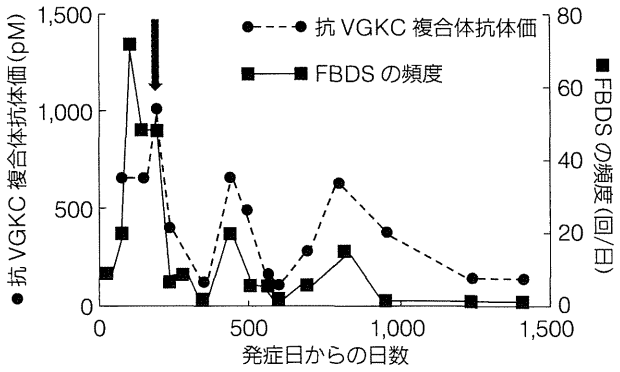


Fig. 3　FBDS と抗 VGKC 複合体抗体

顔面と上肢に頻発するジストニア様の痙攣（faciobrachial dystonic seizure：FBDS）は，抗てんかん薬の治療には不応で，抗 VGKC 複合体抗体価と密接に関連する。免疫療法（矢印が開始日）で臨床症状は抗体価の減少とともに改善する。

Irani SR, Michell AW, Lang B, Pettingill P, Waters P, et al: Faciobrachial dystonic seizures precede LGI1 antibody limbic encephalitis. Ann Neurol **69**: 892-900, 2011 から改変して転載

5．痛みとVGKC関連抗体

先に述べたように筋痙攣を主徴とするアイザックス症候群では感覚障害も呈しており，その中で疼痛も忘れてはいけない大事な徴候である。実際，初期診断が，CRPSである症例も存在する。代表的な症例を提示すると（名古屋市立大学症例），既往歴，家族歴に特記事項のない54歳男性。示指のしびれ感と疼痛が出現し，カルバマゼピンと抗うつ薬で一度は軽快した。1年後，冷やすと改善する両手両足の痛みが出現した。治療を再開したが，難治性で肢端紅痛症としてペインクリニックでの加療が継続された。症状の明らかな改善のないまま2年を経過した後に，全身のピクツキ，全身の筋のこわばりが出現した。神経内科に転科後，アイザックス症候群と診断された。抗VGKC複合体抗体が，2,593 pMと陽性であること（のちに抗CASPR-2抗体であることを確認）から，単純血漿交換5回を行い，ミオキミアや肢端紅痛症の症状は改善した。

聖マリアンナ医大膠原病内科（山野嘉久先生）との共同研究において，診断基準を満たす線維筋痛症患者14例において，抗VGKC複合体抗体を測定したところ，2例において陽性であった。CRPSのみならず，線維筋痛症の一群にも抗VGKC複合体抗体が関与している可能性がある。

メイヨークリニックで行われている抗VGKC複合体抗体の検査サービスでは，25カ月間，54,853検体の4％にあたる1,992検体で陽性であり，うち同施設で神経学的評価を行った症例は316例であった[26]。その半数例にあたる159例が疼痛を有していた。この痛みは亜急性の発症で，経過は慢性であった。または時に線維筋痛症（6％）や心身症（13％）と間違って診断されていた。痛みのコントロールには70％の症例で多剤が必要で，30％の症例で麻薬が必要であった。痛みの治療目的に免疫療法を受けた16例中，14例で痛みの改善が認められた（Table 6）。

慢性特発性疼痛は抗VGKC複合体抗体が関連する1つの徴候であり，侵害受容性経路の過剰興奮が関係する。抗Caspr-2抗体が痛みと強く関連するが，多くの患者で抗VGKC複合体抗体の真の標的抗原は明らかになっていない。しかしながら慢性疼痛の患者で，抗VGKC複合体の自己免疫性をスクリーニングするのは重要である。

6．クロイツフェルト-ヤコブ病とVGKC関連抗体

メイヨークリニックの保存血清約15万例をスクリーニングしたところ，初期臨床診断がCJDであった15例で抗VGKC複合体抗体が陽性であった。いずれの症例も，進行性認知機能障害，ミオクローヌス，錐体外路症状，幻視を認め，WHOのCJDの診断基準を満たしていた[27]。髄液タウ蛋白は，検査をした8例中4例で増加しており，神経細胞特異性エノラーゼ（neuron-specific enolase：NSE）は，2例で陽性であった。脳波異常は13例中9例で認められたが，いずれもびまん性徐波であり，CJDに特徴的な発作性周期性放電（paroxysmal periodic discharge：PSD）は認めなかった。13例中12例で免疫療法が有効であった[28]。

抗VGKC複合体抗体がCJD類似の病態を引き起こす可能性が指摘され，センセーショナルな報告であったが，続報はない。

本邦例では，家族性CJDの診断で，臨床経過および剖検病理所見でもCJDに矛盾しない所見を呈した60歳男性例で，発症から半年後の血清において，抗VGKC複合体抗体が603.5 pMと明らかに上昇していた。しかしながら発症8カ月後（死亡の半月前）の血清では陰性であった[29]。抗体陽性は，CJDの進行に伴うシナプス破壊によって生じた二次的な変化ではないかと考えられる。

自験例では，63歳男性が，6カ月の経過で無言無動になり，CJDが疑われ入院した。髄液14-3-3蛋白陰性で，脳波ではPSDは認めずびまん性徐波のみであった。頭部MRIも拡散強調画像をはじめ異常信号を認めず，CJDを支持する所見はなかった。抗VGKC複合体抗体875 pMと高値であり，メチルプレドニゾロンパルス療法と免疫吸着療法で会話可能となり，日常生活動作も自立するまで回復した。

なお，この症例では，抗LGI-1抗体および抗CASPR-2抗体は陰性であった。CJD様の臨床経過をたどる症例でPSDを認めず非典型例と思われる症例では，抗VGKC複合体抗体の有無を検討し，陽性ならば免疫療法を考慮してもよいのかもしれない。

7．筋萎縮側索硬化症とVGKC関連抗体

ALSにおいて線維束性攣縮は特徴的な所見の1つであり，筋痙攣を呈することも少なくない。一方で，アイザックス症候群の軽症例ともいえるcramp-fasciculation症候群は，筋痙攣と線維束性収縮を主症状とする良性疾患であり，抗VGKC複合体抗体陽性疾患のスペクトラムに入る免疫性神経疾患である。ALSの病初期に，cramp-fasciculation症候群様の臨床症状を呈した場合，その鑑別は困難となる。

Nwosuら[30]は，抗VGKC複合体抗体については，

Table 6　抗 VGKC 複合体抗体陽性の疼痛患者の免疫療法への反応

年齢	性	痛みの局在と種類	神経系・全身性合併症	LGI-1・CASPR-2	免疫療法	転帰
80	男性	四肢，灼熱感，チクチク刺すような疼痛	不安症，体重減少	LGI-1	経口プレドニゾロン，メトトレキサート	疼痛著明軽減，麻薬中止
55	女性	全身性，チクチク/深部	盗汗，線維筋痛症	No	ステロイドパルス，血漿交換	疼痛軽減
39	女性	全身性，深部痛	脊髄症	No	IVIg	疼痛軽減（下肢），筋力低下持続
40	女性	四肢および頭部，チクチク刺すような深部痛	倦怠，筋痙攣，認知機能障害	No	IVIg，ステロイドパルス	疼痛消失
52	男性	四肢　灼熱痛	ミオパチー，ニューロパチー，悪液質	No	経口プレドニゾロン	疼痛消失，ミオパチーとニューロパチーは持続
79	女性	四肢および内臓性，深部の鈍痛	ニューロパチー	No	経口プレドニゾロン	疼痛軽減，ガバペンチン減量
19	女性	四肢の深部鈍痛	統合失調症様，うつ症状，ニューロパチー	No	ステロイドパルス	疼痛軽減
55	男性	脊椎の深部鈍痛	てんかん	LGI-1	ステロイドパルス	疼痛軽減，てんかんと疼痛がステロイド減量で再燃
57	男性	四肢のチクチク刺すような痛み	てんかん	LGI-1	ステロイドパルス，ミコフェノール酸	疼痛消失，てんかん改善
23	女性	四肢の灼熱痛	倦怠，認知機能障害	LGI-1 and CASPR-2	経口プレドニゾロン	疼痛消失，全症状改善
45	男性	全身性の灼熱感を伴う深部鈍痛	スパスム（顎），咳	No	経口プレドニゾロン	疼痛の軽減
76	女性	四肢の灼熱痛	小脳失調	No	ステロイドパルス，血漿交換	明らかな改善なし
77	男性	四肢の灼熱痛	体重減少，自律神経系不安定	No	IVIg	疼痛軽減
36	女性	頭部および眼部のチクチク刺すような痛み	三叉神経障害	LGI-1	メトトレキサート，経口プレドニゾロン	疼痛軽減
55	女性	四肢の灼熱痛	レイノー，ニューロパチー	No	経口プレドニゾロン，クロロキン水酸化物	明らかな改善なし
71	男性	四肢の侵害性深部鈍痛	てんかん，脳症	LGI-1	ステロイドパルス，経口プレドニゾロン，ミコフェノール酸	疼痛・てんかん・筋痙攣の減少，認知機能の改善

〔略語〕IVIg：免疫グロブリン大量静注療法

Klein CJ, Lennon VA, Aston PA, McKeon A, Pittock SJ: Chronic pain as a manifestation of potassium channel-complex autoimmunity. Neurology **79**: 1136-1144, 2012 から改変して転載

ALS 患者血清の抗体価（平均 75.4 pM）は末梢運動神経障害のそれ（平均 42.8 pM）より高値であるとの報告をしているが，その違いは軽度であり病的意義は不明である。

わが国でも，ALS 症例で抗 VGKC 複合体抗体陽性が散見される。新潟市民病院症例は 55 歳男性で，筋痙攣，線維束性攣縮を主訴に受診した。体幹・呼吸筋・四肢の筋力低下，萎縮が進行し ALS が疑われたが，上位運動神経徴候は明らかではなかった。抗 VGKC 複合体抗体が 907.5 pM と著明に上昇していることから，IVIg，メチルプレドニゾロンパルス療法，血漿交換，リツキシマブなど免疫療法が試みられたが，線維束性攣縮や筋痙攣が一時的に軽減するものの筋力低下は進行性で，発症 2 年後に 2 型呼吸不全で死亡した。剖検所見は，脊髄前角，脳幹運動神経核の神経細胞，および大脳運動野のベッツ細胞は軽度脱落し，ブニナ小体，pTDP-43 陽性封入体を認め，下位運動神経優位の ALS の所見であった。

ALS と抗 VGKC 複合体抗体の関連については議論が残っており，多数例での検討が必要である。

おわりに

VGKC関連抗体の抗VGKC複合体抗体について概説した。抗LGI-1抗体と抗CASPR-2抗体の発見により，かなり疾患や症候との関連が解明されたが，わが国のアイザックス症候群症例にみられるように，複数のVGKC関連抗体を有する患者も存在することから，各抗体の量比と臨床徴候の関連など，よりいっそうの検討が望まれる。またVGKC関連抗体研究の端緒となったアイザックス症候群においては，抗VGKC複合体抗体の陽性率は3割程度で，確定診断に至らない多くの症例が存在しており，新たな疾患マーカーの開発が急務である。さらにCJDやALSにおいての抗VGKC複合体抗体の病態への関与についても検討されるべきである。

謝辞

稿を終えるにあたり，ご指導いただきました医療法人三州会大勝病院院長　有村公良先生，および共同研究者の生理学研究所教授　深田正紀先生に深謝いたします。

本稿は，厚生労働省難治性疾患等克服研究事業「免疫性神経疾患に関する調査研究」および「Isaacs症候群の診断，疫学及び病態解明に関する研究」の助成をもとに執筆した。

なお，当科では，抗VGKC複合体抗体の測定（RIA法）を受託している（照会先：vgkckufm@yahoo.co.jp）。

文　献

1）Boronat A, Gelfand JM, Gresa-Arribas N, Jeong HY, Walsh M, et al: Encephalitis and antibodies to dipeptidyl-peptidase-like protein-6, a subunit of Kv4.2 potassium channels. Ann Neurol **73**: 120-128, 2013
2）Sonoda Y, Arimura K, Kurono A, Suehara M, Kameyama M, et al: Serum of Isaacs' syndrome suppresses potassium channels in PC-12 cell lines. Muscle Nerve **19**: 1439-1446, 1996
3）Nagado T, Arimura K, Sonoda Y, Kurono A, Horikiri Y, et al: Potassium current suppression in patients with peripheral nerve hyperexcitability. Brain **122**: 2057-2066, 1999
4）Tomimitsu H, Arimura K, Nagado T, Watanabe O, Otsuka R, et al: Mechanism of action of voltage-gated K^+ channel antibodies in acquired neuromyotonia. Ann Neurol **56**: 440-444, 2004
5）Vincent A, Bien CG, Irani SR, Waters P: Autoantibodies associated with diseases of the CNS: new developments and future challenges. Lancet Neurol **10**: 759-772, 2011
6）Lancaster E, Huijbers MG, Bar V, Boronat A, Wong A, et al: Investigations of Caspr2, an autoantigen of encephalitis and neuromyotonia. Ann Neurol **69**: 303-311, 2011
7）Sinha S, Newsom-Davis J, Mills K, Byrne N, Lang B, et al: Autoimmune aetiology for acquired neuromyotonia (Isaacs' syndrome). Lancet **338**: 75-77, 1991
8）Hart IK, Maddison P, Newsom-Davis J, Vincent A, Mills KR: Phenotypic variants of autoimmune peripheral nerve hyperexcitability. Brain **125**: 1887-1895, 2002
9）Heidenreich F, Vincent A: Antibodies to ion-channel proteins in thymoma with myasthenia, neuromyotonia, peripheral neuropathy. Neurology **50**: 1483-1485, 1998
10）Vernino S, Auger RG, Emslie-Smith AM, Harper CM, Lennon VA: Myasthenia, thymoma, presynaptic antibodies, and a continuum of neuromuscular hyperexcitability. Neurology **53**: 1233-1239, 1999
11）Ishii A, Hayashi A, Ohkoshi N, Oguni E, Maeda M, et al: Clinical evaluation of plasma exchange and high dose intravenous immunoglobulin in a patient with Isaacs' syndrome. J Neurol Neurosurg Psychiatry **57**: 840-842, 1994
12）van den Berg JS, van Engelen BG, Boerman RH, de Baets MH: Acquired neuromyotonia: superiority of plasma exchange over high-dose intravenous human immunoglobulin. J Neurol **246**: 623-625, 1999
13）Hayat GR, Kulkantrakorn K, Campbell WW, Giuliani MJ: Neuromyotonia: autoimmune pathogenesis and response to immune modulating therapy. J Neurol Sci **181**: 38-43, 2000
14）Liguori R, Vincent A, Clover L, Avoni P, Plazzi G, et al: Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage-gated potassium channels. Brain **124**: 2417-2426, 2001
15）Irani SR, Pettingill P, Kleopa KA, Schiza N, Waters P, et al: Morvan syndrome: clinical and serological observations in 29 cases. Ann Neurol **72**: 241-255, 2012
16）Buckley C, Oger J, Clover L, Tüzün E, Carpenter K, et al: Potassium channel antibodies in two patients with reversible limbic encephalitis. Ann Neurol **50**: 73-78, 2001
17）Vincent A, Buckley C, Schott JM, Baker I, Dewar BK, et al: Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. Brain **127**: 701-712, 2004
18）Thieben MJ, Lennon VA, Boeve BF, Aksamit AJ, Keegan M, et al: Potentially reversible autoimmune limbic encephalitis with neuronal potassium channel antibody. Neurology **62**: 1177-1182, 2004

19) Kleopa KA, Elman LB, Lang B, Vincent A, Scherer SS: Neuromyotonia and limbic encephalitis sera target mature Shaker-type K^+ channels: subunit specificity correlates with clinical manifestations. Brain **129**: 1570-1584, 2006
20) Lai M, Huijbers MG, Lancaster E, Graus F, Bataller L, et al: Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. Lancet Neurol **9**: 776-785, 2010
21) Irani SR, Alexander S, Waters P, Kleopa KA, Pettingill P, et al: Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactived 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. Brain **133**: 2734-2748, 2010
22) McKnight K, Jiang Y, Hart Y, Cavey A, Wroe S, et al: Serum antibodies in epilepsy and seizuire-associated disorders. Neurology **65**: 1730-1736, 2005
23) Suleiman J, Brenner T, Gill D, Brilot F, Antony J, et al: VGKC antibodies in pediatric encephalitis presenting with status epilepticus. Neurology **76**: 1252-1255, 2011
24) Irani SR, Michell AW, Lang B, Pettingill P, Waters P, et al: Faciobrachial dystonic seizures precede LGI1 antibody limbic encephalitis. Ann Neurol **69**: 892-900, 2011
25) 村田佳子, 渡邊　修, 谷口　豪, 梁瀬まや, 高木俊輔, 他: Faciobrachial dystonic seizuresを呈した抗電位依存性カリウムチャンネル複合体（LGI-1）抗体関連辺縁系脳炎の1例. てんかん研究 **30**: 43-50, 2012
26) Klein CJ, Lennon VA, Aston PA, McKeon A, Pittock SJ: Chronic pain as a manifestation of potassium channel-complex autoimmunity. Neurology **79**: 1136-1144, 2012
27) Tan KM, Lennon VA, Klein CJ, Boeve BF, Pittock SJ: Clinical spectrum of voltage-gated potassium channel autoimmunity. Neurology **70**: 1883-1890, 2008
28) Geschwind MD, Tan KM, Lennon VA, Barajas RF Jr, Haman A, et al: Voltage-gated potassium channel autoimmunity mimicking Creutzfeldt-Jakob disease. Arch Neurol **65**: 1341-1346, 2008
29) Fujita K, Yuasa T, Watanabe O, Takahashi Y, Hashiguchi S, et al: Voltage-gated potassium channel complex antibodies in Creutzfeldt-Jakob disease. J Neurol **259**: 2249-2250, 2012
30) Nwosu V, Royer J, Stickler D: Voltage gated potassium channel antibodies in amyotrophic lateral sclerosis. Amyotroph Lateral Scler **11**: 392-394, 2010

〈お知らせ〉

第24回日本末梢神経学会学術集会

会　期　2013年8月23日(金)・24日(土)
会　場　朱鷺メッセ　新潟コンベンションセンター（新潟市中央区万代島6-1）
会　長　柴田　実（新潟大学大学院医歯学総合研究科　形成・再建外科学分野）
プログラム
・特別講演
　中田　力（カリフォルニア大学脳神経学・新潟大学脳研究所統合機能研究センター）
　Robert J Spinner（Prof. of Anatomy, Neurosurgery and Orthopedics Mayo Graduate School of Medicine）
・一般演題（口演発表・ポスター）
・パネルディスカッション
・シンポジウム
　「手根幹症候群（CTS）の診断基準」ほか3題を予定
・ワークショップ
・産業医学講座講演
　「業務上疾病としての末梢神経障害」中平浩人（新潟青陵大学看護学科）
・コメディカル・レジデント教育セミナー
　コーディネーター：有村公良（医療法人三州会大勝病院）
・ランチョンセミナー，モーニングセミナー，イブニングセミナー
問合せ　株式会社　新宣　朱鷺メッセ営業所内
　TEL：025-243-7040　E-mail：24jpns@shinsen.biz

II. 各　　論

抗VGKC複合体抗体と免疫性神経疾患

渡邊　修

Neuroimmunological diseases associated with VGKC complex antibodies

Osamu Watanabe
Department of Neurology and Geriatrics,
Kagoshima University Graduate School of Medical and Dental Sciences

Abstract

Antibodies to voltage-gated potassium channels (VGKC) were first identified by radioimmunoassay of radioisotope labeled alpha-dendrotoxin-VGKCs solubilized from rabbit brain. These antibodies were found only in a proportion of patients with acquired neuromyotonia (Isaacs' syndrome). VGKC antibodies were also detected in Morvan's syndrome and in a form of autoimmune limbic encephalitis. Recent studies indicated that the "VGKC" antibodies are mainly directed toward associated proteins (for example LGI-1, Caspr-2) that complex with the VGKCs themselves. The "VGKC" antibodies are now usually known as VGKC-complex antibodies. In general, LGI-1 antibodies are most common in limbic encephalitis with SIADH. Caspr-2 antibodies are present in the majority of patients with Morvan's syndrome. These patients develop combinations of CNS symptoms, autonomic dysfunction, and peripheral nerve hyperexcitability.

Key words: Isaacs' syndrome, Morvan's syndrome, LGI-1, Caspr-2

はじめに

電位依存性カリウムチャネル(voltage-gated potassium channel: VGKC)に対する自己抗体(抗VGKC抗体)は，筋痙攣を主徴とするIsaacs症候群の病態に直接関与する．この抗体は，Morvan症候群や非ヘルペス性辺縁系脳炎の一群でも検出されることが明らかになり，この抗体が関与する疾患のスペクトラムが広がった．同一の抗体が，末梢神経系のみならず，自律神経系や中枢神経系をも含む異なる疾患の原因となる機序については不明であったが，最近の研究でVGKCは種々の分子と複合体を形成しており，自己抗体が標的とする分子が疾患ごとに異なることが明らかになった．末梢神経系，自律神経系，および中枢神経系に及ぶ抗VGKC複合体抗体が関連する一連の免疫性神経疾患について概説する．

1. 抗VGKC複合体抗体

抗VGKC抗体は，Isaacs症候群の疾患マーカーとして同定された自己抗体である．スクリーニング測定は，家兎脳のホモジネートをVGKCのリガンドである^{125}I-αデンドロトキシンで標

鹿児島大学大学院医歯学総合研究科 神経内科・老年病学

0047-1852/13/¥60/頁/JCOPY

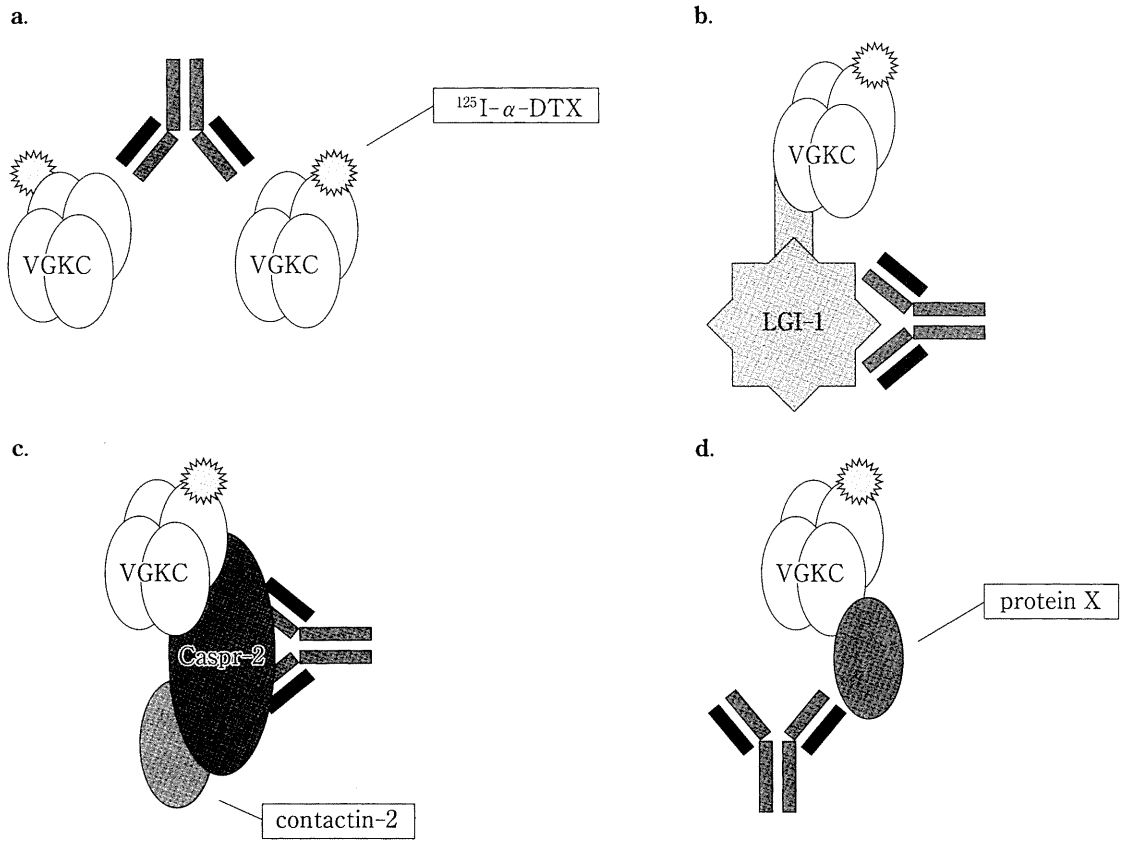


図1 抗VGKC抗体から抗VGKC複合体抗体へ

^{125}I-αデンドロトキシン(^{125}I-α-DTX)を用いた免疫沈降法で検出される抗体は，当初，VGKCそのものに対しての自己抗体と考えられていた(a)．生体内ではVGKCは他の分子と複合体を形成しており，LGI-1(b)やCaspr-2(c)に対する自己抗体をも検出していることが明らかになった．未知の標的抗原の存在も指摘されている(d)．

識するradioimmunoassay(RIA)法で行われる．VGKCそのものに対する抗体を測定するアッセイ系としてデザインされていたが，実際は，VGKCはleucine rich glioma inactivated protein 1(LGI-1)やcontactin associated protein 2(Caspr-2)などと複合体を形成しており，これらに対する自己抗体も検出可能で(**図1**)，このアッセイ系で検出される自己抗体を総称して抗VGKC複合体抗体と呼ぶ．上記のRIA法によるスクリーニングで陽性の場合，LGI-1やCaspr-2の遺伝子を導入したcell lineと被験者血清(IgG)を用いた免疫化学的手法cell-based assayで質的検討が行われる．

Isaacs症候群における抗VGKC抗体によるVGKCの機能障害のメカニズムは，重症筋無力症でみられるような補体介在性のチャネルタンパクの破壊ではない[1]．また抗VGKC抗体は，ブロッキング抗体として個々のチャネルタンパクの機能を損なうのではなく，細胞膜表面上のVGKCの総数を減じ，総和としてVGKCの機能を抑制する[2]．このVGKCの総数の減少は，二価のVGKC抗体と2個のVGKCタンパクとのcross-linkingにより生じる[3]．一方，後述する抗LGI-1抗体や抗Caspr-2抗体については，作用機序は不明である．

抗VGKC複合体抗体の中で，代表的な抗LGI-1抗体と抗Caspr-2抗体について**表1**にまとめた[4,5]．抗LGI-1抗体陽性例は，近時記憶障害やてんかんなど辺縁系症状に関連し，海馬のneuropilに反応を示す．抗LGI-1抗体陽性患者のIgGのみが，視床下部内側のADH分泌細胞に反応する．この抗体が陽性の辺縁系脳炎では，高頻度に抗利尿ホルモン不適合分泌症候群(SIADH)を合併する．抗Caspr-2抗体陽性例に

表1 抗LGI-1抗体と抗Caspr-2抗体のまとめ

	抗VGKC複合体抗体	
	抗LGI-1抗体	抗Caspr-2抗体
よくみられる症候	辺縁系脳炎 健忘 てんかん発作 見当識障害 低ナトリウム血症	Morvan症候群 昏迷 健忘 不眠 自律神経障害 ニューロミオトニア 疼痛
局在	海馬のneuropil	広く分布；海馬，小脳のneuropilに強い
IgG subtype	IgG1＜IgG4	IgG1＜IgG4
髄液異常	41％	~25％
中枢神経内抗体産生	まれ	データ不足
腫瘍随伴	まれ	時に　胸腺腫が主，ほかに肺小細胞癌など
予後	単相性　免疫抑制剤継続は不要	免疫抑制剤に反応を示すが，再燃・再発例も 腫瘍が存在する場合は予後不良例も
発症年齢	30-80歳（中央値60歳）	46-77歳（中央値60歳）
性差	65％が男性	85％が男性
MRI異常	~84％　内側側頭葉FLAIR信号増強	~40％　内側側頭葉FLAIR信号増強
他の自己抗体の合併	~10％ ANA TPO GAD65	~20％ AChR MusK GAD65
相対的発生率 （自己免疫性脳炎）	30％	3％

（文献[4,5]より改変）

比べて，髄液異常の頻度は低く，逆にMRI異常信号の頻度は高い．抗核抗体や抗TPO抗体など他の自己抗体を合併し，自己免疫性の要素が強く，単相性であり，免疫療法の継続は不要と考えられている．一方，抗Caspr-2抗体陽性例は，末梢神経の過剰興奮症状であるニューロミオトニア（neuromyotonia）や神経原性疼痛，および自律神経症状・中枢神経症状を呈するMorvan症候群と関連している．胸腺腫や肺小細胞癌など傍腫瘍性神経症候群の要素が強く，腫瘍に対するアプローチが必要とされている．いずれも神経系外で産生されており，血液脳関門や血液神経関門の破綻がなければ，症状を引き起こさないと考えられている．

2. Isaacs症候群

Isaacs症候群は持続性の四肢・躯幹の筋痙攣，ミオキミア（myokimia），ニューロミオトニアを主徴とする疾患であり，1961年に‘a syndrome of continuous muscle-fibre activity’として初めて記載された．この症候は，末梢運動神経の過剰興奮性によるものであり，臨床的，薬理学的所見から，血液神経関門のない神経終末が主な責任病変部位と考えられている．1990年代，患者血清中に末梢神経の過剰興奮を引き起こす抗体の存在が発見され，本疾患が後天性免疫性神経疾患であることが明らかになり[6]，更にその後，抗VGKC抗体が関与することが明らかになった[7]．

Isaacs症候群の中心となる症候は末梢運動神経の過剰興奮性によるものであり，四肢，躯幹にみられる筋痙攣，筋硬直，ニューロミオトニア(叩打性ミオトニアを認めない神経由来の筋弛緩遅延)と，ミオキミア，線維束攣縮などの不随意運動を特徴とする．持続性の筋痙攣・筋硬直は筋肥大を起こすこともあり，更に強くなると筋力低下がみられることもある．これらの運動症状は運動負荷，虚血，寒冷で増強し，睡眠でも消失しない．また遠位部の神経ブロックでも消失しないことが多く，血液神経関門のない神経終末が主な責任病変部位と考えられている．一部の症例では，神経根ブロックで消失することもあり，同じように血液神経関門の脆弱な神経根に障害部位が存在することもある．運動症状のみならず，疼痛，しびれ感などの感覚異常もしばしばみられる．時にcomplex regional pain syndrome様の激しい痛みと，皮膚色調異常などで発症する例もある．そのほかに自律神経の興奮性異常によると思われる発汗過多，皮膚色調の変化，高体温を示す場合もある．筋痙攣・筋硬直が高度となり，疼痛とともに，歩行や体動が困難となり日常生活に重大な支障を生じる．より軽症の病型として，筋痙攣や筋線維束攣縮が比較的下肢に限局したcramp-fasciculation症候群と呼ばれるものもある．電気生理学的にミオキミア放電(myokymic discharges)の有無で両症候群が鑑別されるが，Isaacs症候群のみならず，cramp-fasciculation症候群でも抗VGKC抗体が認められ，両症候群に質的な相違点はないと考えられている[7]．

筋痙攣，筋硬直が末梢神経起源であることの確認には筋電図検査が有用であり，安静時にdoublet，triplet，multipletなどのミオキミア放電やfasciculation potential，neuromyotonic dischargeを認める．神経伝導検査ではM波やF波に引き続く反復放電がみられることがある．

基本的な治療方針は，日常生活にさほど影響がなければ，まずは，末梢神経のNaチャネルを抑制することで過剰興奮性を抑える抗てんかん薬などによる対症療法を行う．難治症例や，日常生活に著しい支障をきたす場合は，血漿交換による抗VGKC複合体抗体の除去が，有効である[6-9]．免疫グロブリン大量療法(IVIg)については，合計5例の使用経験が報告されているが[10-12]1例のみ著効，3例で無反応，1例では増悪している[10]．

3. Morvan症候群

Morvan症候群は，Isaacs症候群でみられる末梢神経の過剰興奮を特徴とする筋痙攣などの症状に加え，不整脈，重度の便秘，尿失禁，発汗過多，流涙・流涎過多などの多彩な自律神経症状，および重度の不眠，複雑な夜間異常行動，幻覚，記銘力障害などの中枢神経症状を特徴とする免疫性神経疾患で，1890年に'myofibrillary chorea'と記載された．2001年，肺腺癌を伴い，血漿交換で緩解した76歳男性の症例報告を端緒に抗VGKC抗体との関連が明らかになった[13]．Morvan症候群は，極めてまれな疾患で，最初の報告から100年以上を経過しているが，症例報告が散見されるだけで多数例の検討はされていなかった．最近の英国を中心とした29例(我が国の2例を含む)の検討で，以下のことが明らかになった[14]．圧倒的に男性に多く(27/29: 93.1％)，末梢神経の中核症状であるニューロミオトニアは全例で認められ，およそ6割の患者で'足が焼けつくような'神経原性の疼痛が認められた．自律神経系の代表的な症状は，発汗過多(25/29: 86.2％)と血圧変動など心血管系の不安定(14/29: 48.3％)であった．中枢神経系では約9割の患者で不眠が認められた．腫瘍合併は11例(37.9％)で認められ，多くが胸腺腫であった．27例で血清学的な検討を行ったところ，21例で抗Caspr-2抗体が，18例で抗LGI-1抗体が陽性であり，15例では複数の抗体が陽性であった．体重減少，重症筋無力症(MG)，および腫瘍合併は抗Caspr-2抗体に関連し，低ナトリウム血症や妄想，幻覚，情動高揚などの中枢神経系症状は，抗LGI-1抗体に関連していた．

4. VGKC複合体抗体関連脳炎

Morvan症候群で抗VGKC抗体が陽性であることが明らかになって以来，抗VGKC抗体と中

枢神経症状，特に辺縁系症状との関連が注目されていた．2001年，Buckleyら[15]は，筋痙攣など末梢神経の過剰興奮症状を呈さず，辺縁系脳炎の症状を呈し，抗VGKC抗体陽性の2例の症例報告を行った．2004年，Vincentら[16]は，抗VGKC抗体陽性の辺縁系脳炎10例を報告した．その臨床的特徴は，亜急性の経過で進行する近時記憶障害や見当識障害を呈し，極期にてんかんを合併し，両側または片側の側頭葉内側にMRIの信号異常を認め，髄液異常はまれで，高頻度にSIADHによる低ナトリウム血症を合併するもののステロイドや血漿交換，およびIVIgなどの免疫療法によく反応するというものであった．またThiebenら[17]は，抗VGKC抗体陽性の辺縁系脳炎7例について，3例でSIADHによる低ナトリウム血症を認め，全例で末梢神経の過剰興奮症状は認められず，6例で抗VGKC抗体の減少とともに臨床症状が改善したと報告した．これをもって抗VGKC抗体関連辺縁系脳炎(VGKC-LE)の疾患概念が提唱された．

上述したように，同じ抗VGKC抗体が，なぜ末梢神経系，自律神経系および中枢神経系において異なる症状を惹起するのか不明であった．Isaacs症候群，Morvan症候群，およびVGKC-LE患者血清が，異なるVGKCのサブタイプを認識するとの報告[18]があったが，決定的なものではなかった．

2010年，ほぼ同時期に異なる2施設から，これらの疑問を一気に解決するbreakthroughといえる報告があった．Laiら[19]は，57例のVGKC-LEの髄液および血清を検討した結果，57例中55例でLGI-1に対する抗体が陽性であったと報告した．また，Iraniら[20]は，96例の抗VGKC抗体陽性疾患群の血清を検討した結果，55例でLGI-1に対する抗体が陽性であった．VGKCそのものに対する抗体陽性例は3例にすぎなかった．更に19例で，Caspr-2に対する抗体が陽性であった．抗Caspr-2抗体は末梢神経の過興奮性に，抗LGI-1抗体は辺縁系の症状に関与する傾向にあった．また抗Contactin-2抗体陽性例も存在した．この結果から，彼女らは，従来，^{125}I-αデンドロトキシンを用いたRIA法で測定していた自己抗体は，VGKCそのもののみならず，LGI-1，Caspr-2，およびcontactin-2を標的とするポリクローナルな抗体で，抗VGKC複合体抗体と呼ぶべきものであるとし，Isaacs症候群，Morvan症候群，VGKC-LEを包括して，抗VGKC複合体抗体関連症候群という疾患概念を提唱している．

おわりに

抗VGKC複合体抗体は，VGKCそのものに対する抗体と，抗LGI-1抗体や抗Caspr-2抗体などVGKCと複合体を形成する分子に対する抗体の総称である．末梢神経の過剰興奮に起因するIsaacs症候群は，VGKCそのものに対する抗体や抗Caspr-2抗体が，また末梢神経系・自律神経系・中枢神経系の広範な症状を呈するMorvan症候群では，抗Caspr-2抗体が，そして高率にSIADHを合併する亜急性の自己免疫性辺縁系脳炎では抗LGI-1抗体が関連すると理解されている．しかし，複数の抗体を有する症例も多く，今後は自己抗体の量比と症状の解析などの検討が必要である．

謝辞 稿を終えるにあたり，ご指導いただきました医療法人三州会大勝病院院長 有村公良先生，および共同研究者の生理学研究所教授 深田正紀先生に深謝いたします．

本稿は，厚生労働省難治性疾患等克服研究事業‘免疫性神経疾患に関する調査研究’および‘Isaacs症候群の診断，疫学及び病態解明に関する研究’の助成をもとに執筆した．

文献

1) Sonoda Y, et al: Serum of Isaacs' syndrome suppresses potassium channels in PC-12 cell lines. Muscle Nerve **19**: 1439-1446, 1996.
2) Nagado T, et al: Potassium current suppression in patients with peripheral nerve hyperexcitability.

Brain **122**: 2057-2066, 1999.
3) Tomimitsu H, et al: Mechanism of action of voltage-gated K^+ channel antibodies in acquired neuromyotonia. Ann Neurol **56**: 440-444, 2004.
4) Vincent A, et al: Autoantibodies associated with diseases of the CNS: new developments and future challenges. Lancet Neurol **10**: 759-772, 2011.
5) Lancaster E, et al: Investigations of Caspr2, an autoantigen of encephalitis and neuromyotonia. Ann Neurol **69**: 303-311, 2011.
6) Sinha S, et al: Autoimmune aetiology for acquired neuromyotonia(Isaacs' syndrome). Lancet **338**: 75-77, 1991.
7) Hart I, et al: Phenotypic variants of autoimmune peripheral nerve hyperexcitability. Brain **125**: 1887-1895, 2002.
8) Heidenreich F, Vincent A: Antibodies to ion-channel proteins in thymoma with myasthenia, neuromyotonia, peripheral neuropathy. Neurology **50**: 1483-1485, 1998.
9) Vernino S, et al: Myasthenia, thymoma, presynaptic antibodies, and a continuum of neuromuscular hyperexcitability. Neurology **53**: 1233-1239, 1999.
10) Ishii A, et al: Clinical evaluation of plasma exchange and high dose intravenous immunoglobulin in a patient with Isaacs' syndrome. J Neurol Neurosurg Psychiatry **57**: 840-842, 1995.
11) van den Berg J, et al: Acquired neuromyotonia: superiority of plasma exchange over high-dose intravenous human immunoglobulin. J Neurol **246**: 623-625, 1999.
12) Hayat G, et al: Neuromyotonia: autoimmune pathogenesis and response to immune modulating therapy. J Neurol Sci **181**: 38-43, 2000.
13) Liguori R, et al: Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage-gated potassium channels. Brain **124**: 2417-2426, 2001.
14) Irani SR, et al: Morvan syndrome: Clinical and serological observations in 29 cases. Ann Neurol **72** (2): 241-255, 2012.
15) Buckley C, et al: Potassium channel antibodies in two patients with reversible limbic encephalitis. Ann Neurol **50**: 73-78, 2001.
16) Vincent A, et al: Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. Brain **127**: 701-712, 2004.
17) Thieben MJ, et al: Potentially reversible autoimmune limbic encephalitis with neuronal potassium channel antibody. Neurology **62**: 1177-1182, 2004.
18) Kleopa KA, et al: Neuromyotonia and limbic encephalitis sera target mature Shaker-type K^+ channels: subunit specificity correlates with clinical manifestations. Brain **129**: 1570-1584, 2006.
19) Lai M, et al: Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. Lancet Neurol **9**: 776-785, 2010.
20) Irani SR, et al: Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactived 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. Brain **133**: 2734-2748, 2010.

Hereditary sensory and autonomic neuropathy type IID caused by an *SCN9A* mutation

Junhui Yuan, MD
Eiji Matsuura, MD, PhD
Yujiro Higuchi, MD
Akihiro Hashiguchi, MD
Tomonori Nakamura, MD, PhD
Satoshi Nozuma, MD
Yusuke Sakiyama, MD, PhD
Akiko Yoshimura, BS
Shuji Izumo, MD
Hiroshi Takashima, MD, PhD

Correspondence to
Dr. Takashima:
thiroshi@m3.kufm.kagoshima-u.ac.jp

ABSTRACT

Objective: To identify the clinical features of Japanese patients with suspected hereditary sensory and autonomic neuropathy (HSAN) on the basis of genetic diagnoses.

Methods: On the basis of clinical, in vivo electrophysiologic, and pathologic findings, 9 Japanese patients with sensory and autonomic nervous dysfunctions were selected. Eleven known HSAN disease-causing genes and 5 related genes were screened using a next-generation sequencer.

Results: A homozygous mutation, c.3993delGinsTT, was identified in exon 22 of *SCN9A* from 2 patients/families. The clinical phenotype was characterized by adolescent or congenital onset with loss of pain and temperature sensation, autonomic nervous dysfunctions, hearing loss, and hyposmia. Subsequently, this mutation was discovered in one of patient 1's sisters, who also exhibited sensory and autonomic nervous system dysfunctions, with recurrent fractures being the most predominant feature. Nerve conduction studies revealed definite asymmetric sensory nerve involvement in patient 1. In addition, sural nerve pathologic findings showed loss of large myelinated fibers in patient 1, whereas the younger patient showed normal sural nerve pathology.

Conclusions: We identified a novel homozygous mutation in *SCN9A* from 2 Japanese families with autosomal recessive HSAN. This loss-of-function *SCN9A* mutation results in disturbances in the sensory, olfactory, and autonomic nervous systems. We propose that *SCN9A* mutation results in the new entity of HSAN type IID, with additional symptoms including hyposmia, hearing loss, bone dysplasia, and hypogeusia. ***Neurology*® 2013;80:1641-1649**

GLOSSARY

CIP = channelopathy-associated insensitivity to pain; **CMAP** = compound muscle action potentials; **HSAN** = hereditary sensory and autonomic neuropathy; **$Na_V1.7$** = voltage-gated sodium channel; **dbSNP** = single nucleotide polymorphism database; **SCV** = sensory nerve conduction velocity; ***SCN9A*** = sodium channel, voltage-gated, type 9, α; **SNAP** = sensory nerve action potentials.

Hereditary sensory and autonomic neuropathy (HSAN) is a clinically and genetically heterogeneous group of disorders. Until now, HSAN has been classified into 6 main groups on the basis of their mode of inheritance and clinical features, and 11 HSAN disease–causing genes have been identified (table 1[1–17]).

SCN9A encodes the voltage-gated sodium channel ($Na_V1.7$), and the gain-of-function mutations result in several painful disorders, including inherited erythromelalgia,[18] paroxysmal extreme pain disorder,[19] and small nerve fiber neuropathy.[20] Loss-of-function *SCN9A* mutations have been linked to channelopathy-associated insensitivity to pain (CIP), which is characterized by congenital insensitivity to pain perception and anosmia; however, the autonomic dysfunction has been regarded as exclusionary criteria for the diagnosis of CIP.[21] It is noteworthy that no definite abnormalities have been recorded using either nerve conduction studies or sural nerve pathologic examinations in all of the previous cases with homozygous loss-of-function *SCN9A* mutations and part with compound heterozygous mutations.[22–26] However, a slight reduction in sensory nerve action potentials (SNAP) was recorded in two cases with compound heterozygous *SCN9A* mutations.[25,27]

Supplemental data at www.neurology.org

From the Department of Neurology and Geriatrics (J.Y., E.M., Y.H., A.H., T.N., S.N., Y.S., A.Y., H.T.), Kagoshima University, Graduate School of Medical and Dental Sciences, Kagoshima; and Department of Molecular Pathology (S.I.), Center for Chronic Viral Diseases, Kagoshima University School of Medicine, Kagoshima, Japan.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Table 1 **Overview of HSAN disease-causing genes, inheritance pattern, and cardinal phenotypic features**

Gene symbol	HSAN type	Inh	Onset age	Cardinal clinical features
SPTLC1	IA	AD	Adulthood	Loss of pain and temperature sensation; occasional autonomic involvement; variable sensorineural deafness and distal motor involvement[1,2]
SPTLC2	IC	AD		
ATL1	ID	AD	Early adulthood	Severe loss of pain, temperature, and vibration sensation; ulcero-mutilation; spastic paraparesis; rare autonomic involvement[3]
DNMT1	IE	AD	Childhood-adulthood	Severe sensory loss; ulcero-mutilation; sensorineural hearing loss; early-onset dementia; no autonomic symptoms[4-6]
WNK1	IIA	AR	Congenital—early childhood	Severe distal loss of touch, pain, and temperature sensation; mutilations in hands and feet; mild or asymptomatic autonomic dysfunction[7,8]
FAM134B	IIB	AR	Childhood	Severe loss of pain and temperature sensation; ulcero-mutilation; autonomic dysfunctions[9]
KIF1A	IIC	AR	Childhood	Severe loss of pain, temperature, vibration, and position sensation; ulcero-mutilation; distal muscle weakness; developmental delay and short stature[10]
IKBKAP	III	AR	Congenital	Familial dysautonomia; gastrointestinal and respiratory dysfunction; scoliosis; relative indifference to pain and temperature[11-13]
NTRK1	IV	AR	Congenital—early childhood	Loss of pain and temperature sensation; anhidrosis; episodic fever; mild mental retardation; joint deformities[14]
NGF	V	AR	Congenital—adulthood	Reduced sensation of pain and temperature; variable autonomic dysfunctions; painless fractures; joint deformities; mild mental retardation[15,16]
DST	VI	AR	Congenital	Dysautonomia; hypotonia; facial deformity; decreased pain response; joint contractures; retardation; respiratory failure; early death[17]

Abbreviations: AD = autosomal dominant; AR = autosomal recessive; *ATL1* = atlastin GTPase 1; *DNMT1* = DNA (cytosine-5)-methyltransferase 1; *DST* = dystonin; *FAM134B* = family with sequence similarity 134, member B; *IKBKAP* = inhibitor of κ light polypeptide gene enhancer in B cells, kinase complex-associated protein; Inh = inheritance; *KIF1A* = kinesin family member 1A; *NGF* = nerve growth factor (β polypeptide); *NTRK1* = neurotrophic tyrosine kinase, receptor, type 1; *SPTLC1* = serine palmitoyltransferase, long chain base subunit 1; *SPTLC2* = serine palmitoyltransferase, long chain base subunit 2; *WNK1* = WNK lysine deficient protein kinase 1.

In this study, using a next-generation sequencer, in 9 Japanese patients who were diagnosed with HSAN based on their clinical, in vivo electrophysiologic, and pathologic features, 11 known HSAN disease–causing genes and 5 related genes including *SCN9A* were screened. We identified a homozygous frameshift mutation in *SCN9A* of 2 patients/families. Therefore, we demonstrate that loss-of-function *SCN9A* mutation can produce a typical HSAN phenotype, and we propose this new classification as HSAN type IID. This study also broadened the spectrum of clinical phenotypes in patients with *SCN9A*-related disorders. Furthermore, on the basis of clinical, in vivo electrophysiologic, and pathologic findings, we attempted to elucidate the pathogenesis of the mutated $Na_V1.7$.

METHODS All patients who were referred to our department from 2000 to 2012 and who had sensory and autonomic nerve dysfunctions were selected. After excluding patients who had associated multiple motor nerve involvement, 9 patients were enrolled and genotyped in this study. Besides the 11 known HSAN disease-causing genes described above, we also investigated another 5 genes that might also cause sensory and autonomic symptoms, including *SCN9A, CCT5, PRNP, FLVCR1,* and *RNF170.*

The protocol of the following study was reviewed and approved by the Institutional Review Board of Kagoshima University. All patients and family members provided written informed consent to participate in this study.

Pathologic study. Sural nerve biopsies, performed at the age of 42 years in patient 1 and at the age of 25 years in patient 2, were analyzed according to standard morphologic procedures for light and electron microscopy.[28] A portion of the specimen was prepared for teased fiber analysis and classified according to Dyck's criteria.[29] The diameter and density of the myelinated fibers were analyzed with a Luzex AP image analyzer (Nireco Corporation, Tokyo, Japan).

Mutation sequencing. Genomic DNA was extracted from the peripheral blood leukocytes.

Using the Primer 3 program, we designed 375 oligonucleotide primers that covered all 357 coding exons and exon-intron junctions with amplicon lengths of 400–500 base pairs. Briefly, all target fragments of 9 patients were amplified by multiplex PCR (QIAGEN Multiplex PCR Kit; QIAGEN GmbH, Hilden, Germany) and ligated with specified indexes, respectively, then screened on the MiSeq sequencing system simultaneously in

accordance with the manufacturer's protocol. The results were mapped to the genome reference sequence in the CLC Genomics Workbench 4 (CLC Bio, Aarhus, Denmark) and then analyzed with tablet software.[30]

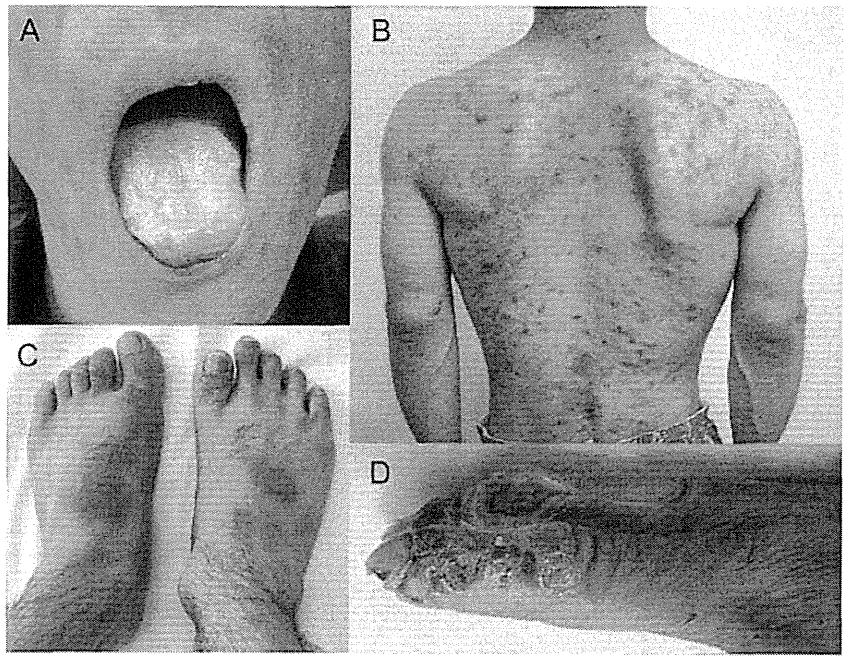
The polymorphic and pathogenic natures of the confirmed variants were checked against the single nucleotide polymorphism database (dbSNP) (http://www.ncbi.nlm.nih.gov/snp/) and the 1000 Genomes database (http://browser.1000genomes.org/index.html). To confirm the suspected pathogenic mutations or low coverage domains (depth less than 10) in the MiSeq sequencing output, Sanger sequencing was also performed using the same methodology as the one employed in a previous study.[31] We screened 100 Japanese population control patients for the c.3993delGinsTT mutation.

RESULTS Patients. ***Patient 1.*** This 50-year-old man was the sixth child from a consanguineous family. No abnormalities were noted at birth or in the early developmental stages except for slight hyposmia since childhood. Since primary school, pain perception started to decrease in his hands and feet. At 30 years of age, he underwent lumbar spinal fixation, but felt no pain. After 40 years of age, the numbness progressed from the distal to the proximal limbs. Furthermore, his toes could not perceive temperature well when he entered his bath, and while walking, his right slipper always slipped off. There was no history of episodes of unexplained vomiting or dysphagia. A detailed physical examination revealed multiple skin lesions, including a burn mark on the right middle finger, which was caused by a cigarette. No pupillary abnormalities were observed. His muscle strength was normal, except grade 4+/5 weakness in the right tibialis anterior muscle. He also had a slight steppage gait. All reflexes were diminished, and his pathologic reflexes were negative. Pain and temperature perception were reduced in the distal limbs and absent in his feet. However, sense of vibration, joint position, and pressure were all preserved. Postural hypotension was excluded. During the sweating test, no sweating was observed in his face or any of his limbs, except in the palm of his left hand. Asymptomatic sensorineural hearing loss with an increase in the 4,000-Hz threshold in the left ear was diagnosed by an otorhinolaryngologist. MRI of the brain was normal.

In nerve conduction study, all motor nerve conduction velocity and compound muscle action potentials (CMAP) values were normal, except for a slightly reduced CMAP in the right tibial nerve at 3.5 mV (normal range, >4.4 mV). Sensory nerve conduction velocity (SCV) was slightly slow in the right median and ulnar nerve at 45.2 m/s (normal range, >47.2 m/s) and 40 m/s (normal range, >46.9 m/s), respectively, and moderately slow in the right sural nerve at 27.5 m/s (normal range, >40.8 m/s). SNAP amplitudes were markedly reduced in the right median nerve (0.9 μV; normal range, >7.0 μV), bilateral ulnar nerves (1.3 and 2.2 μV; normal range, >6.9 μV), and the right sural nerves (1.0 μV; normal range, >5.0 μV). However, the SCV and SNAP in the left median nerve were normal.

Patient 2. This 33-year-old man was from a nonconsanguineous family having no history of neurologic

Figure 1 Clinical pictures of patient 2



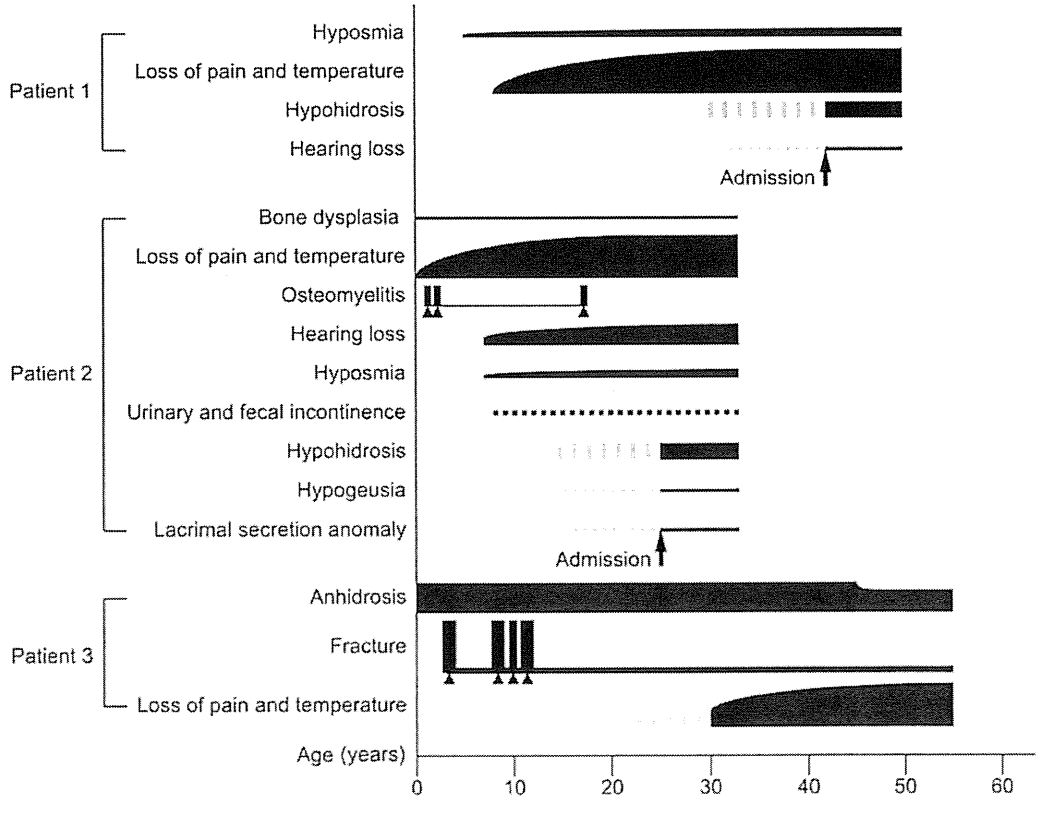
(A) Reduced number of fungiform papilla on the tongue. (B) The back of patient 2, showing scattered rash, pigmentation, and short humerus. (C) Short right hallux. (D) Multiple painless ulcers and deformed joints in the fingers.

disorders. Decreased pain and temperature perception was noted at birth. When he was a year old, his feet were burnt after he walked on asphalt on a hot summer day, and this eventually progressed to osteomyelitis. Subsequently, he underwent operations of the right tibia and both feet, but he could not feel any pain. Before enrolling in primary school, hearing loss in the left ear and hyposmia were detected. He experienced occasional urinary and fecal incontinence, and frequent urination at night. His height was 157 cm, and he had a short humerus (27.5 cm) and right hallux (figure 1, B and C). Left acetabular dysplasia was noticed, which contributed to his left lower limb being 3 cm longer than his right lower limb. Rash and pigmentation were scattered over his chest and back (figure 1B), and several painless ulcers and deformed joints were observed in his fingers (figure 1D). Tendon hyperreflexia was noted in the lower limbs, and pathologic reflexes were negative. Pain perception was impaired in a glove–stocking pattern. Sense of vibration, joint position, and pressure were all normal. The sweating test revealed reduced sweating tendency throughout the body and especially in the trunk, except for his hands and feet. Postural hypotension was excluded, and brain MRI was normal. A lacrimal secretion anomaly was also detected. Otorhinolaryngologic examinations revealed the following: deafness in the left ear and minimal hearing loss in the right ear, glossopharyngeal and chorda tympani nerve abnormalities in the gustatory sensation test, reduced number of fungiform papilla on his tongue (figure 1A), and a decline in olfactory acuity as tested by a jet stream olfactometer. Examinations of the urinary tract excluded any organic disease.

The nerve conduction study revealed all motor nerve conduction velocity and CMAP values within normal ranges. The SCV was slightly slow in the right median and ulnar nerves (45.2 m/s and 46.2 m/s, respectively). However, SNAP was moderately decreased in the bilateral median (7 and 5.7 μV) and ulnar (3.3 and 4.4 μV) nerves. Nevertheless, no abnormalities were detected in sural nerve SCV and SNAP values.

Patient 3. This 55-year-old woman was an elder sister of patient 1. Her pregnancy was uneventful and delivery was normal. She had recurrent fractures and underwent operations for the left thigh (at age of 3 years and 8 years), right thigh (at age 11 years), and left elbow (primary school). When she was 30 years old, she perceived no pain after her feet were burnt on a heater. Anhidrosis was also noted, but after the age of 45 years, occasional sweat was secreted on her back. There was no evident hyposmia or hearing loss. At present, she is able to stand up and walk using

Figure 2 **Clinical courses of the 3 patients**



The solid triangles (▲) indicate surgery.

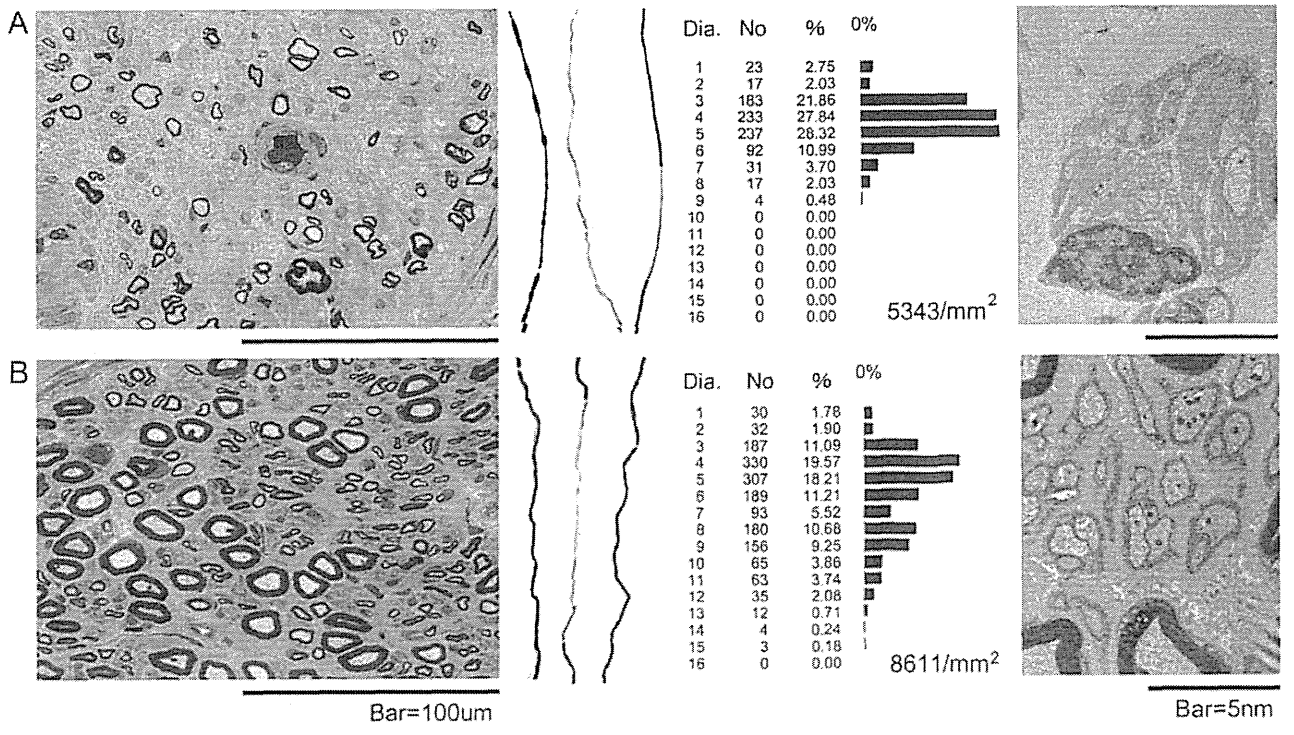
her hands for support. Cranial nerve examinations were normal. Deformities of the left elbow, right foot, and bilateral lower limbs were noted. Muscle strength testing was normal in the upper limbs, whereas the strength in the lower limb muscles decreased to grades 2/5–4/5. Pain and temperature perceptions were reduced in the distal lower limbs and the anterior part of the right thigh, which may have been involved because of damage from surgery. The sense of vibration and joint position were preserved. Reflexes in her lower limbs were absent, and her pathologic reflexes were negative. Osteoporosis was excluded by an orthopedist.

The clinical courses of the 3 patients described above, including the onset and imaged severities of each symptom, are shown in figure 2.

Pathologic studies. In patient 1, the number of myelinated fibers was markedly decreased in all fasciculi, but the changes varied in their scale and extent. A histogram of the fiber diameter indicated a marked loss of large myelinated fibers relative to small myelinated fibers. Some remaining myelinated fibers had thinner myelin sheaths and some exhibited axonal degeneration. An electron microscopic study revealed clusters of Schwann cell processes, which may have been caused by the axonal degeneration of unmyelinated fibers (figure 3A). Contrary to the findings in patient 1, the number of myelinated fibers in patient 2 was slightly decreased, even with marked clinical symptoms. The histogram of fiber diameter showed a normal pattern. Electron microscopy showed that unmyelinated fibers were fairly preserved (figure 3B). No demyelinated fibers or inflammatory cells could be found in either patient.

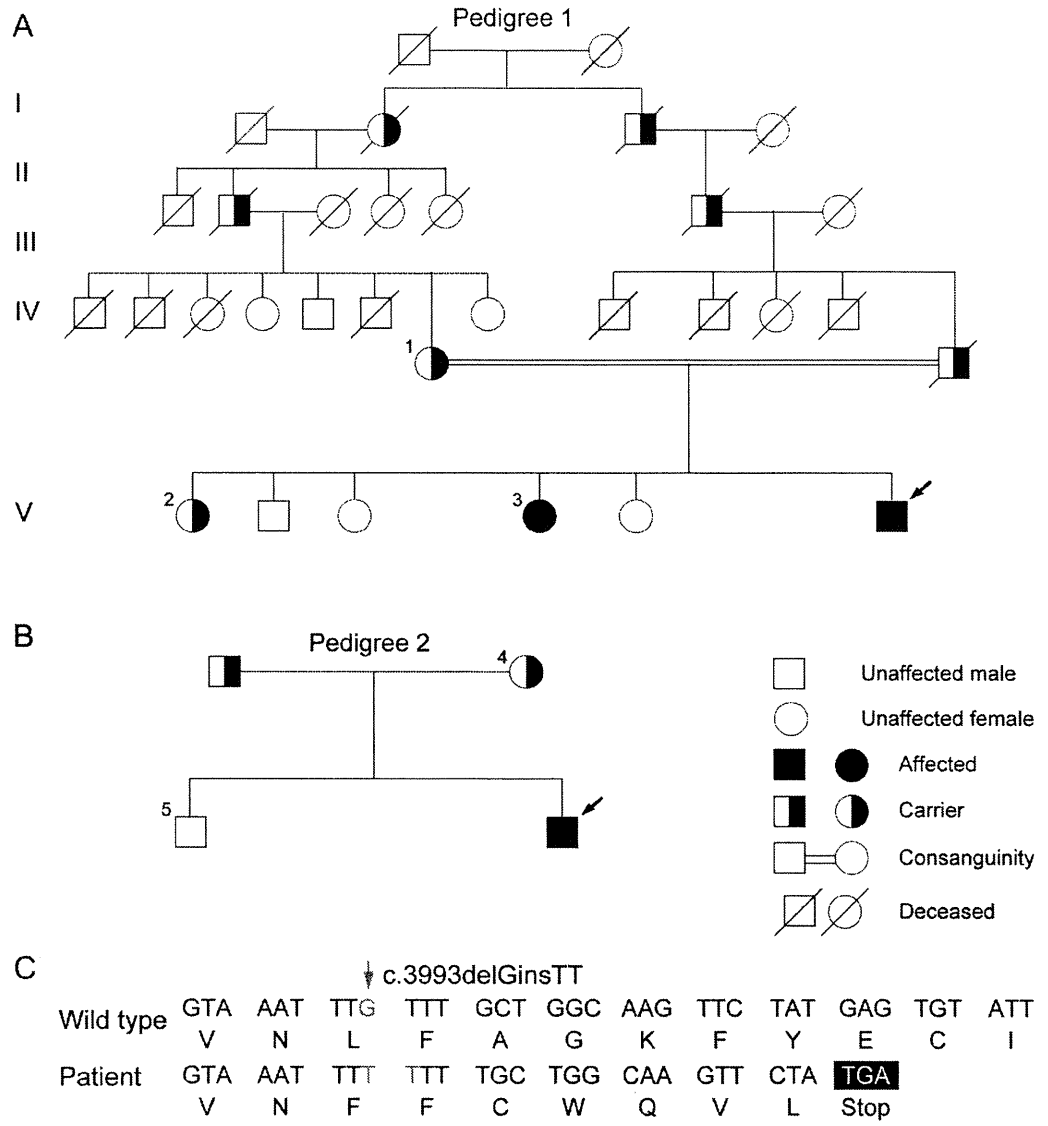
Genetic studies. Using the MiSeq sequencing system, all of the 9 patients were genotyped successfully. Besides patients 1 and 2, no pathogenic mutation was detected. The CLCbio software showed that 96.24% and 93.6% of the data matched the reference sequences in patients 1 and 2, respectively. In the 357 targeted exons, 98.04% and 97.76% covered more than 10 reads. From patients 1 and 2, a total of 41 high-confidence variants were detected (table e-1 on the *Neurology*® Web site at www.neurology.org). In these variants, 39 known SNPs were coincident with the dbSNP or 1000 Genome database. Of the remaining 2 variants, the c.3248A>C in *KIF1A* was found in normal controls and was therefore considered as an SNP. A homozygous mutation in exon 22 of *SCN9A*, c. 3993delGinsTT, remained. This mutation was not observed in 100 Japanese control patient samples,

Figure 3 **Pathologic findings from the sural nerve biopsies**



In patient 1, the density of large and small myelinated fibers is markedly decreased. The remaining myelinated fibers have thinner myelin sheaths. Some teased fibers exhibit axonal degeneration. The histogram of the fiber diameter indicates loss of large myelinated fibers (5,343 fibers/mm^2). Electron microscopic examination shows clusters of Schwann cell processes (A). In patient 2, the densities of large and small myelinated fibers are slightly decreased. Teased fibers exhibit shortened internodal remyelination. The histogram of the fiber diameter shows a normal pattern (8,611 fibers/mm^2). Unmyelinated fibers are fairly preserved, as shown under the electron microscope (B).

Figure 4 **Pedigree diagram and genetic studies**



(A) Pedigree of patient 1. Patients 1 and 3 (V3) harbor the homozygous mutation, c.3993delGinsTT, whereas their mother (IV1) and 1 elder sister (V2) are heterozygous carriers. (B) Pedigree of patient 2. The same mutation, c.3993delGinsTT, can be observed in patient 2. His mother (4) exhibits the carrier genotype, and his elder brother (5) is normal. The black arrows (↙) indicate the proband. All the family members with available DNA samples are labeled with an Arabic numeral. (C) The c.3993delGinsTT mutation (↓) shifts the reading frame and generates a premature stop codon.

nor did we find it on the 1000 Genomes Web site, which catalogs human genetic variations using 2,500 patient samples, including 500 East Asian (100 Japanese) patient samples. DNA samples were then collected from 3 other family members of pedigree 1, and 2 members of pedigree 2. In patient 3, the same genotype was identified. In addition, asymptomatic carriers (mother and another elder sister of patient 1 and the mother of patient 2) and an unaffected member (brother of patient 2) were found (figure 4, A and B). This mutation changes the reading frame during the translation of the mRNA and generates a premature stop codon (figure 4C).

DISCUSSION Among the 16 disease-causing or related genes of HSAN, we identified a homozygous mutation in *SCN9A* from 2 Japanese families. We described 3 patients who presented with new clinical, in vivo electrophysiologic, and pathologic phenotypes.

SCN9A, which is located on chromosome 2q24.3, contains 26 coding exons.[32] It encodes $Na_v1.7$, which is the α-subunit of a tetrodotoxin-sensitive, voltage-gated sodium channel. $Na_v1.7$, composed of 4 domains, each with 6 transmembrane domains and 2 highly conserved pore-forming segments,[33] is preferentially expressed within the dorsal root ganglion and sympathetic ganglion neurons and their small-diameter peripheral

axons.[34] It is crucial for the depolarizing phase of neuronal action potentials, and it seems to determine the excitability and repetitive firing properties of neurons.[35] Gain-of-function *SCN9A* mutations result in hyperexcitable nociceptive neurons and states, such as inherited erythromelalgia,[18] paroxysmal extreme pain disorder,[19] and small nerve fiber neuropathy,[20] whereas loss-of-function *SCN9A* mutations produce no sodium current and generate CIP.[22]

In our study, both families lived in the Kagoshima prefecture, which is located to the south of Kyushu Island, Japan, and both were unrelated to each other. Loss of pain and temperature perceptions began at different ages, appearing as early as birth in patient 2, in the second decade in patient 1, and in the third decade in patient 3. Their ages at the onset of symptoms were different from those reported for patients with CIP who had a congenital onset. Moreover, in these 3 patients, the area of their pain insensitivities was limited mainly within the distal part of the limbs, not the entire body, as is seen in patients with CIP. The sense of vibration and joint position were preserved in our patients. However, the predominant reduction in sweat production in our 3 patients, together with urination and defecation disorder, lacrimal secretion anomaly, and decreased number of fungiform papilla on the tongue in patient 2, suggested autonomic nervous system dysfunction. A recent study indicated that $Na_v1.7$ in sympathetic neurons also contributes to the sensation of neuropathic pain.[36] The severe rash and pigmentation may be due to a post-inflammatory hyperpigmentation, resulting from the disruption of autonomic innervation. Meanwhile, hyposmia/anosmia, which is a common feature in patients with CIP and loss-of-functional *SCN9A* mutation,[23,27,37] was also identified in our patients. Furthermore, in patient 2, hypogeusia was detected using a gustatory sensation test. Bone dysplasia, as an additional symptom in patient 2 (acetabular dysplasia, short humerus, and right hallux), had also been reported in a Dutch kindred.[38] The otorhinolaryngologist confirmed hearing loss, although at different levels, in patients 1 and 2, which has mainly been recorded in patients with HSAN type IE.[4-6] These findings definitely broaden the symptomatic heterogeneity of *SCN9A* mutations.

Although pain insensitivity was symmetrically detected in the distal portion of the limbs of the 2 index patients, nerve conduction studies only revealed asymmetric involvement of the extremities. These findings, except those of the peroneal nerve, were compatible with sensory-predominant axonal multiple mononeuropathy complicated by minimal demyelinating changes, rather than a polyneuropathy.

Interestingly, the pathologic features of the sural nerve in the 2 index cases also varied. Although decreases in the small and large myelinated fibers were observed among the fasciculi in patient 1, the extent was dramatically different, whereas the density of small and large myelinated fibers was fairly preserved in patient 2. These discoveries were consistent with the nerve conduction study findings, which suggested a mismatch between the distribution of affected fibers and the severity of the loss-of-pain sensations. In addition, especially in patient 1, both decreased large myelinated fibers and decreased SNAP/SCV indicated that the large myelinated fibers were affected, whereas cases with gain-of-function *SCN9A* mutations always present with small myelinated and unmyelinated fiber abnormalities.[20] The selective involvement of sensory nerves in these patients is inconsistent with the clinical features and may indicate that the dysfunction of the dorsal root ganglion is more predominant than that of the peripheral nerve. However, the mechanisms underlying the pathologic aberrations in the fasciculi or nerves, caused by the mutated $Na_v1.7$ in the dorsal root ganglion, require further research.

The homozygous mutation, c.3993delGinsTT, which is located in exon 22 of *SCN9A*, is expected to shift the reading frame from amino acid 1331 (p. leu1331phe) and generate a premature stop codon. This will induce an essential alteration in the fifth transmembrane segment of domain 3 in $Na_v1.7$ and eliminate the whole fourth domain. The nonsense-mediated messenger RNA decay mechanism will then be activated, which will induce loss of function of $Na_v1.7$ in nociceptive neurons. Together with the pedigree study that confirmed the cosegregation of the genotype and phenotype, this mutation was believed to be a pathogenic mutation.

In 3 patients from 2 Japanese families who experienced symptoms that were characterized by congenital or adolescence-onset loss of pain and temperature perception and autonomic nervous dysfunction accompanied by hyposmia, hearing loss, hypogeusia, bone dysplasia, and fractures, we identified a novel loss-of-function frameshift *SCN9A* mutation. We demonstrated that this was a new entity on the basis of clinical, in vivo electrophysiologic, and pathologic findings. We introduce this new entity as HSAN type IID, an allelic disorder with CIP, both of which result from loss-of-function mutations in *SCN9A*. Furthermore, on the basis of in vivo electrophysiologic and pathologic findings, we furthered the understanding of the mechanisms induced by the loss of function of $Na_v1.7$. We are able to summarize that a loss-of-function *SCN9A* mutation can produce heterogeneous phenotype, even harboring the same mutation.

AUTHOR CONTRIBUTIONS

Dr. Junhui Yuan: genetic study, analyses and interpretation of data, and drafting the manuscript. Dr. Eiji Matsuura: pathologic study of the sural nerve, analysis and interpretation of data, revising the manuscript. Dr. Yujiro Higuchi: acquisition and analysis of clinical data, revising the manuscript. Dr. Akihiro Hashiguchi: acquisition of clinical data

and case selection. Dr. Tomonori Nakamura: nerve conduction study, analysis and interpretation of data. Dr. Satoshi Nozuma: acquisition of clinical data. Dr. Yusuke Sakiyama and Ms. Akiko Yoshimura: participated in genetic study. Dr. Shuji Izumo: analysis and interpretation of data. Dr. Hiroshi Takashima: study concept and design, interpretation of the data, revising the manuscript, study supervision, obtain funding.

ACKNOWLEDGMENT

The authors thank Ms. Y. Shirahama and A. Nishibeppu of our department for their excellent technical assistance. They also thank the Joint Research Laboratory, Kagoshima University Graduate School of Medical and Dental Sciences, for the use of their facilities.

STUDY FUNDING

Supported by the Intramural Research Grant (23-5) for Neurological and Psychiatric Disorders of NCNP; Research Committee for Neuropathy, Ataxic Disease and Applying Health and Technology of Ministry of Health, Welfare and Labour, Japan; and a research grant (23300201) from the Ministry of Health, Labour, and Welfare of Japan.

DISCLOSURE

Drs. Yuan, Matsuura, Higuchi, Hashiguchi, Nakamura, Nozuma, Sakiyama, and Ms. Yoshimura report no disclosures. Dr. Izumo received an honorarium for lecturing from Bayer Japan, funded by grants from Research Committee for HAM and neuroimmunological diseases of the Ministry of Health, Welfare and Labour of Japan. Dr. Takashima served on the scientific advisory board for Teijin, received a royalty from Athena diagnostics, was on the speakers' bureaus of Astellas, Bayer Group, Kyowa Hakko Kirin Pharma, Takeda Pharmaceutical Company Limited, Biogen Idec, Novartis, Dainippon Sumitomo Pharma, GlaxoSmithKline, Kowa Group, Pfizer Co., Mitsubishi Tanabe Pharma, and Eisai Co., is funded by grants from the Nervous and Mental Disorders and Research Committee for Ataxic Disease, Neuropathy, SMON, HAM, Chronic Pain, Applying Health and Technology of the Japanese Ministry of Health, Welfare and Labour, and received grants from Ministry of Education, Culture, Sports, Science, and Technology of Japan (grant 21591095). Go to Neurology.org for full disclosures.

Received November 2, 2012. Accepted in final form January 18, 2013.

REFERENCES

1. Dawkins JL, Hulme DJ, Brahmbhatt SB, Auer-Grumbach M, Nicholson GA. Mutations in SPTLC1, encoding serine palmitoyltransferase, long chain base subunit-1, cause hereditary sensory neuropathy type I. Nat Genet 2001;27:309–312.
2. Rotthier A, Auer-Grumbach M, Janssens K, et al. Mutations in the SPTLC2 subunit of serine palmitoyltransferase cause hereditary sensory and autonomic neuropathy type I. Am J Hum Genet 2010;87:513–522.
3. Guelly C, Zhu PP, Leonardis L, et al. Targeted high-throughput sequencing identifies mutations in atlastin-1 as a cause of hereditary sensory neuropathy type I. Am J Hum Genet 2011;88:99–105.
4. Wright A, Dyck PJ. Hereditary sensory neuropathy with sensorineural deafness and early-onset dementia. Neurology 1995;45:560–562.
5. Hojo K, Imamura T, Takanashi M, et al. Hereditary sensory neuropathy with deafness and dementia: a clinical and neuroimaging study. Eur J Neurol 1999;6:357–361.
6. Klein CJ, Botuyan MV, Wu Y, et al. Mutations in DNMT1 cause hereditary sensory neuropathy with dementia and hearing loss. Nat Genet 2011;43:595–600.
7. Lafreniere RG, MacDonald ML, Dube MP, et al. Identification of a novel gene (HSN2) causing hereditary sensory and autonomic neuropathy type II through the Study of Canadian Genetic Isolates. Am J Hum Genet 2004;74:1064–1073.
8. Coen K, Pareyson D, Auer-Grumbach M, et al. Novel mutations in the HSN2 gene causing hereditary sensory and autonomic neuropathy type II. Neurology 2006;66:748–751.
9. Kurth I, Pamminger T, Hennings JC, et al. Mutations in FAM134B, encoding a newly identified Golgi protein, cause severe sensory and autonomic neuropathy. Nat Genet 2009;41:1179–1181.
10. Rivière JB, Ramalingam S, Lavastre V, et al. KIF1A, an axonal transporter of synaptic vesicles, is mutated in hereditary sensory and autonomic neuropathy type 2. Am J Hum Genet 2011;89:219–230.
11. Slaugenhaupt SA, Blumenfeld A, Gill SP, et al. Tissue-specific expression of a splicing mutation in the IKBKAP gene causes familial dysautonomia. Am J Hum Genet 2001;68:598–605.
12. Anderson SL, Coli R, Daly IW, et al. Familial dysautonomia is caused by mutations of the IKAP gene. Am J Hum Genet 2001;68:753–758.
13. Axelrod FB, Hilz MJ. Inherited autonomic neuropathies. Semin Neurol 2003;23:381–390.
14. Mardy S, Miura Y, Endo F, et al. Congenital insensitivity to pain with anhidrosis: novel mutations in the TRKA (NTRK1) gene encoding a high-affinity receptor for nerve growth factor. Am J Hum Genet 1999;64:1570–1579.
15. Einarsdottir E, Carlsson A, Minde J, et al. A mutation in the nerve growth factor beta gene (NGFB) causes loss of pain perception. Hum Mol Genet 2004;13:799–805.
16. Carvalho OP, Thornton GK, Hertecant J, et al. A novel NGF mutation clarifies the molecular mechanism and extends the phenotypic spectrum of the HSAN5 neuropathy. J Med Genet 2011;48:131–135.
17. Edvardson S, Cinnamon Y, Jalas C, et al. Hereditary sensory autonomic neuropathy caused by a mutation in dystonin. Ann Neurol 2012;71:569–572.
18. Yang Y, Wang Y, Li S, et al. Mutations in SCN9A, encoding a sodium channel alpha subunit, in patients with primary erythermalgia. J Med Genet 2004;41:171–174.
19. Fertleman CR, Baker MD, Parker KA, et al. SCN9A mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. Neuron 2006;52:767–774.
20. Faber CG, Hoeijmakers JG, Ahn HS, et al. Gain of function Nav1.7 mutations in idiopathic small fiber neuropathy. Ann Neurol 2012;71:26–39.
21. Goldberg YP, Pimstone SN, Namdari R, et al. Human Mendelian pain disorders: a key to discovery and validation of novel analgesics. Clin Genet 2012;82:367–373.
22. Cox JJ, Reimann F, Nicholas AK, et al. An SCN9A channelopathy causes congenital inability to experience pain. Nature 2006;444:894–898.
23. Goldberg YP, MacFarlane J, MacDonald ML, et al. Loss-of-function mutations in the Nav1.7 gene underlie congenital indifference to pain in multiple human populations. Clin Genet 2007;71:311–319.
24. Ahmad S, Dahllund L, Eriksson AB, et al. A stop codon mutation in SCN9A causes lack of pain sensation. Hum Mol Genet 2007;16:2114–2121.
25. Cox JJ, Sheynin J, Shorer Z, et al. Congenital insensitivity to pain: novel SCN9A missense and in-frame deletion mutations. Hum Mutat 2010;31:e1670–e1686.
26. Staud R, Price DD, Janicke D, et al. Two novel mutations of SCN9A (Nav1.7) are associated with partial congenital insensitivity to pain. Eur J Pain 2011;15:223–230.

27. Nilsen KB, Nicholas AK, Woods CG, et al. Two novel SCN9A mutations causing insensitivity to pain. Pain 2009;143:155–158.
28. Schröder JM. Developmental and pathological changes at the node and paranode in human sural nerves. Microsc Res Tech 1996;34:422–435.
29. Dyck PJ, Giannini C, Lais A. Pathologic alterations of nerves. In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF, editors. Peripheral Neuropathy, vol. 1, 3rd ed. Philadelphia: W.B. Saunders; 1993:514–595.
30. Milne I, Bayer M, Cardle L, et al. Tablet—next generation sequence assembly visualization. Bioinformatics 2010;26: 401–402.
31. Okamoto Y, Higuchi I, Sakiyama Y, et al. A new mitochondria-related disease showing myopathy with episodic hypercreatine kinase-emia. Ann Neurol 2011;70:486–492.
32. Michiels JJ, te Morsche RH, Jansen JB, Drenth JP. Autosomal dominant erythermalgia associated with a novel mutation in the voltage-gated sodium channel alpha subunit Nav1.7. Arch Neurol 2005;62:1587–1590.
33. Klugbauer N, Lacinova L, Flockerzi V, Hofmann F. Structure and functional expression of a new member of the tetrodotoxin-sensitive voltage-activated sodium channel family from human neuroendocrine cells. EMBO J 1995;14: 1084–1090.
34. Sangameswaran L, Fish LM, Koch BD, et al. A novel tetrodotoxin-sensitive, voltage-gated sodium channel expressed in rat and human dorsal root ganglia. J Biol Chem 1997;272:14805–14809.
35. Rush AM, Dib-Hajj SD, Liu S, Cummins TR, Black JA, Waxman SG. A single sodium channel mutation produces hyper- or hypoexcitability in different types of neurons. Proc Natl Acad Sci USA 2006;103:8245–8250.
36. Minett MS, Nassar MA, Clark AK, et al. Distinct Nav1. 7-dependent pain sensations require different sets of sensory and sympathetic neurons. Nat Commun 2012;3: 791.
37. Weiss J, Pyrski M, Jacobi E, et al. Loss-of-function mutations in sodium channel Nav1.7 cause anosmia. Nature 2011;472:186–190.
38. Hoeijmakers JG, Han C, Merkies IS, et al. Small nerve fibres, small hands and small feet: a new syndrome of pain, dysautonomia and acromesomelia in a kindred with a novel NaV1.7 mutation. Brain 2012;135:345–358.