日本財団 The Nippon Foundation 助成事業

財団法人　日中医学協会

2010年度共同研究等助成金報告書－在留中国人研究者－

2010年3月10日

財団法人　日中医学協会　御中

貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：研究報告書

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１．助成金額：　600，000　円

２．研究テーマ

―日中医学協会助成事業―

Dravet症候群患者におけるmodifier遺伝子異常

３．成果の概要

Our results suggest that SCN9A mutations by themselves do not cause Dravet syndrome, on the other hand, some of their mutations role as possible genetic modifiers causing Dravet syndrome when combined with SCN1A mutation.

４．研究業績

（１）学会における発表　　無・有(学会名・演題)

Prevelence and character of SCN1A mutation in genetic diagnosis of Dravet syndrome and Intractable Childhooh Epilepsy　Xiuyu Shi, Jiwen Wang, Kurahashi K, Fukuma G, Ishii A, Higurashi N, Kaneko S, Hirose S.　XIth World Congress Of ICNC 2010.5/2-7 Egypt

（２）発表した論文　　無・有(雑誌名・題名)

Shi X, Huang MC, Ishii A, Yoshida S, Okada M, Morita K, Nagafuji H, Yasumoto S, Kaneko S, Kojima T, Hirose S. Mutational analysis of GABRG2 in a Japanese cohort with childhood epilepsies. J Hum Genet 2010;55(6):375-8.

The developmental changes of Nav1.1 and Nav1.2 expression in the 6 human hippocampus and temporal lobe. Wenze Wang, Takashima S, Segawa Y, Itoh M, Shi X , Su-Kyeong Hwang, Nabeshima K, Takeshita M, Hirose S.　B RES in press

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Dravet syndrome での SCN9A の遺伝子変異分析-

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<要旨>

Background: Dravet syndrome is an intractable epileptic disorder occurring in the first year of life, mutations in SCN1A encoding the sodium channel Nav1.1 are a major cause of it. Recently, concurrent SCN9A and SCN1A mutations in Dravet syndrome have been reported which provide models for potential genetic modifier effects. This was significant as SCN9A is known to cause inherited pain syndromes, and no previous reports have linked it to epilepsy. To clarify their role and association with SCN1A in Dravet syndrome, screening for genetic SCN9A abnormalities was performed using direct sequencing methods.

Materials and methods: We analyzed 492 chromosomes from Dravet syndrome patients and 572 from healthy volunteers. Dravet syndrome patients were divided into two groups; one without, and one with SCN1A mutations (consisted of 256 and 236 chromosomes, respectively). We next analyzed 58 chromosomes from patients with milder forms of SCN1A related epilepsies to evaluate whether SCN9A influences on seizure phenotype.

Results: A sequencing of SCN9A yielded eight missense variants including four novel ones: M787V, S802G, V861E, and Y1175C. All novel variants had relatively high phyloP scores and three of them, M787V, V861E, and Y1175C were predicted to be deleterious. Nevertheless, M787V and V861E were also found in the control group. S802G and Y1175C were found in the SCN1A mutation (+) Dravet syndrome group, but not in milder forms of SCN1A related epilepsies, nor in the controls.

Conclusions: Our results suggest that SCN9A mutations by themselves do not cause Dravet syndrome, on the other hand, some of their mutations role as possible genetic modifiers causing Dravet syndrome when combined with SCN1A.

<Key Words>

Dravet syndrome, SCN1A, SCN9A, mutation, genetics

〈本文〉

緒言：

Dravet syndrome, also known as Severe Myoclonic Epilepsy of Infancy (SMEI; OMIM 607208), is an intractable epileptic disorder occurring in the first year of life in infants who previously had shown normal development. Initial seizures are hemiclonic or generalized tonic-clonic, usually begin with fever, and often lead to status epilepticus. Later episodes begin to occur without fever, and patients manifest other seizure types, including myoclonic, complex-partial, and atypical absences. Prognosis is poor, with psychomotor impairment and intractable seizures. Mutations in SCN1A encoding the sodium channel Nav1.1 are a major cause of Dravet syndrome. To date, nearly 700 mutations of the SCN1A have been found in Dravet syndrome patients, accounting for 50-80% of them. SCN1A mutations show various penetrance and expressivity even within the same family, and have extreme heterogeneity. Thus the disease's pathogenesis is not completely understood by SCN1A alone, and many reports have suggested that it is genetically heterogeneous. Recently, concurrent SCN9A and SCN1A mutations in Dravet syndrome have been reported providing models for potential genetic modifier effects. It was significant because SCN9A is known to cause inherited pain syndromes, and no previous reports suggested it as a contributor to epilepsy. To clarify their role and association with SCN1A mutations in Dravet syndrome, we performed genetic analyses of SCN9A.

対象と方法：

1. Subjects

We analyzed 492 chromosomes from Dravet syndrome patients and 572 from healthy volunteers. Dravet syndrome patients were divided into two groups; one without, and one with SCN1A mutations (consisted of 256 and 236 chromosomes, respectively). We next analyzed 58 chromosomes from patients with milder forms of SCN1A related epilepsies to evaluate whether SCN9A influences on seizure phenotype. Most samples were from Japan although a few were from India. The diagnoses were made at departments of child neurology in various regional tertiary hospitals and detailed clinical reports and questionnaires were completed for each patient. SCN1A mutation analyses for patients and controls were previously examined in our lab.

2. Genetic analysis

Screening for genetic SCN9A abnormalities was performed using direct sequencing methods. Each of the participants or their parent/ guardian signed an informed consent form approved by the Ethics Review Committee of Fukuoka University or similar committees of participating institutions. Genomic DNAs were prepared from ethylenediamineteraacetic acid (EDTA)-treated whole blood samples using a QIAamp DNA Blood kit (Qiagen, Hilden, Germany). Sequencing was carried out in both directions using 28 specific primer pairs amplifying SCN9A's 27 exons. Screening for genetic abnormalities was performed by direct sequencing methods using dye terminator chemistry (Big-Dye, Applied Biosystems, Foster City, CA). Details of PCR conditions and the primers are available upon request. Reference sequences of messenger RNA (mRNA) were based on information available from RefSeq (accession numbers): Human SCN1A, NM 006920; Human SCN9A, NM 002977.

結果：

All patients had a clinical history consistent with Dravet syndrome, and patients with SCN1A mutations carried SCN1A missense or splice site mutations. No SCN1A mutations were found in the controls. A sequencing of SCN9A yielded eight missense variants including four novel ones: M787V, S802G, V861E, and Y1175C. As shown in Table 1, all novel variants had relatively high phyloP scores and three of them (with low SIFT score), M787V, V861E, and Y1175C were predicted to be deleterious. Nevertheless, M787V and V861E were also found in the control group. S802G and Y1175C were found in the

SCN1A(+) Dravet syndrome group, but not in 58 chromosomes with less severe forms of SCN1A related epilepsies, nor in the controls (Table 2). For these two mutations, Fisher's exact two-tailed test yielded p=0.02 (3/236 SCN1A(+) Dravet syndrome group and 0/572 controls), while other six variants including M787V and V861E showed no statistical differences between each groups. Our results suggest that SCN9A mutations by themselves do not cause Dravet syndrome, on the other hand, some of their mutations role as possible genetic modifiers causing Dravet syndrome when combined with SCN1A.

考察：

Our results suggest that SCN9A mutations by themselves do not cause Dravet syndrome, on the other hand, some of their mutations role as possible genetic modifiers causing Dravet syndrome when combined with SCN1A.

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1. Depienne, C., O. Trouillard, et al. (2009). Spectrum of SCN1A gene mutations associated with Dravet syndrome: analysis of 333 patients. J Med Genet **46**(3): 183-91.
2. Singh, N. A., C. Pappas, et al. (2009). A role of SCN9A in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. PLoS Genet **5**(9): e1000649.

Table 1. Overview of all identified SCN9A missense variants.

Exon	EX15		EX16		EX17	EX19	EX27	
Amino acid level	**M787V**	***S802G**	**V861E**	**M932L**	**V991L**	***Y1175C**	**R1893H**	**D1908G**
Nucleotide level	c.2359A>G	c.2404A>G	c.2582T>A	c.2794A>C	c.2971G>T	c.3524A>G	c.5678G>A	c.5723A>G
dbSNP ID	novel	novel	novel	rs12478318	rs4369876	novel	rs79805025	rs3750904
†phyloP	0.96937	2.2588	5.10491	3.44324	0.0215039	1.53396	2.0388	1.16176
‡SIFT score	0.01	0.54	0.00	0.97	0.23	0.01	0.11	0.33
Prediction	Deleterious	Tolerable	Deleterious	Tolerable	Tolerable	Deleterious	Tolerable	Tolerable

Table 2. Allelic frequencies of all identified SCN9A missense variants.

	M787V	***S802G**	**V861E**	**M932L**	**V991L**	***Y1175C**	**R1893H**	**D1908G**
SCN1A(-) Dravet (n=256)	4/256 (1.56%)	0/256 (0%)	0/256 (0%)	9/256 (3.52%)	9/256 (3.52%)	0/256 (0%)	1/256 (0.39%)	18/256 (7.03%)
SCN1A(+) Dravet (n=236)	3/236 (1.27%)	2/236 (0.86%)	0/236 (0%)	7/236 (2.97%)	7/236 (2.97%)	1/236 (0.42%)	1/236 (0.42%)	15/236 (6.36%)
SCN1A(+) Less severe epilepsies (n=58)	1/58 (1.72%)	0/58 (0%)	0/58 (0%)	4/58 (6.90%)	4/58 (6.90%)	0/58 (0%)	1/58 (1.72%)	7/58 (12.07%)
Controls (n=572)	5/572 (0.87%)	0/572 (0%)	1/572 (0.17%)	13/572 (2.27%)	13/572 (2.27%)	0/572 (0%)	2/572 (0.35%)	40/572 (6.99%)